

# Prevalence and determinants of hypertension in apparently healthy schoolchildren in India: A multi-center study

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## Abstract

**Background:** Hypertension in children is often under recognized, especially in developing countries. Data from rural areas of developing countries is particularly lacking.

**Objectives:** To study prevalence of hypertension and its determinants in apparently healthy school children from predominantly rural populations of India.

**Methods:** Apparently healthy schoolchildren ( $n = 14,957$ ) aged 5–15 years (mean (standard deviation) age 10.8 (2.8) years; 55.5% boys) at four predominantly rural sites in separate states of India were studied. Systolic and diastolic blood pressures were recorded by trained staff in addition to age, gender, height, weight, type of school and season. Waist circumference was also recorded in 12,068 children. Geographic location and type of school (government, government-aided or private) were used to determine socio-economic status.

**Results:** Systolic and/or diastolic hypertension was present in 3443 (23%) children. Systolic hypertension was present in 13.6%, diastolic hypertension in 15.3% and both in 5.9%. Isolated systolic hypertension was present in 7.7% while isolated diastolic hypertension was present in 9.4%.

On univariate analysis, age, gender, geographical location, socio-economic status, season and anthropometric parameters (z-scores of height, weight and waist circumference, waist/height ratio and body mass index) were all significantly related to risk of hypertension ( $p < 0.0001$  for each). Similar association was observed with weight group (normal, overweight and obese). Multiple regression analysis showed lower age, female gender, richer socio-economic status, certain geographical locations, higher weight and larger waist circumference to be independently associated with a greater risk of hypertension.

**Conclusion:** There is a high prevalence of hypertension in apparently healthy schoolchildren even in predominantly rural areas of India. Screening and management programs targeted to high risk groups identified may prove cost-effective.

## Keywords

Hypertension, children, blood pressure, epidemiology

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## Introduction

There is increasing interest in blood pressure (BP) and hypertension (HT) in children.<sup>1–3</sup> HT in children has been shown to be associated with HT in adulthood and with cardiovascular risk in later life.<sup>4–8</sup> The adverse effects are shown to increase in the presence of obesity,

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of which the world is currently witnessing an epidemic.<sup>9</sup> The recording of BP has also become easier with widespread availability of reliable oscillometric (digital) BP recording instruments. These can be used by paramedical and even lay people, including parents, to record BP at home or at school.

There is also increasing interest in regional variation in BP and in the prevalence of HT since reports from different parts of world have shown wide variations.<sup>10–13</sup> The pattern may be different in developing countries since obesity is less of a problem there as compared to the Western world. Moreover, children from rural areas are less well studied than their urban counterparts. It will also be important to determine the prevalence of HT in undernourished (rather than overnourished obese) children. We report here the prevalence and factors determining HT in schoolchildren in four predominantly rural areas located in different parts of India.

## Methods

This cross-sectional epidemiological study was conducted in primary and secondary schools in four states in India (Haryana, Goa, Gujarat and Manipur) as part of a rheumatic heart disease screening program. Details of the method of the study are already published.<sup>14</sup> Informed consent for the project was gained from each school principal as well as the parents. The study was approved by institutional ethics committees at all sites. Children with known major hepatic, renal, cardiac, or respiratory diseases were excluded. For the current analysis, apparently healthy schoolchildren with completed age of 5–15 years were included ( $n=14957$ ). The children were screened by clinical examination and echocardiography and those found to have significant heart disease were excluded.

During the survey, trained paramedical staff documented demographic and anthropometric data, including height, weight, and waist circumference. Waist circumference was recorded in 12,068 of the total of 14,957 children (it was not recorded at one site). Children sat and rested for five minutes before measurement of BP. BP was measured in a sitting position by trained medical attendants. It was measured using oscillometric instrument in Haryana (Omron HEM 7080) and Gujarat, anaeroid instrument in Manipur and mercury sphygmomanometer (Diamond(R)) at Goa. The digital and aneroid instruments were calibrated against a mercury sphygmomanometer. Children with a high BP reading underwent a repeat BP measurement by research physicians after an interval of about five minutes and the second reading was taken as the final one. All children with BP values which were normal for their age did not have a repeat

BP check. Two cuff sizes were used in this study according to the size of the child. No child was excluded based on BP reading.

Socioeconomic status (SES; “richer,” “poorer,” or “mixed”) was assigned to each school depending on type of school and development of the area where the school was located. This SES was applied to all participating children from that school. The season when BP was measured was taken to be ‘summer’ for April–September months and ‘winter’ for other months. HT was defined as given by the established Fourth report using BP according to age and gender tables but with 50th centile for height taken for all children.<sup>7,12,15</sup> We have earlier reported simplified percentile tables and charts derived from the BP measurements of 7761 children from one of these sites (Haryana).<sup>11</sup>

## Statistical analysis

Data is presented as mean (standard deviation (SD)) unless specified otherwise. Ninety-five percent confidence intervals were calculated whenever appropriate. The z-scores were used for anthropometric parameters (height, weight, and waist circumference) as standardized values (using age, gender, and site).

For univariate analysis, the Student’s *t*-test was used for comparison of continuous variables and the Chi-square test was used to compare categorical variables between hypertensive and non-hypertensive children. Multiple logistic regression analysis was performed to identify independent predictors of HT. For this purpose, weight, height were divided by 10 to obtain the odds ratio for every 10-unit change rather than one-unit change. Similarly, waist circumference was divided by five for multiple regression. Children were divided into weight categories of normal, overweight, and obese. Overweight was defined as weight in the 85th–95th percentile, while obesity was defined as weight greater than the 95th percentile for age and gender of the child. Graphs of prevalence of HT versus continuous variables like age were plotted using the LOESS curve fitting (local polynomial regression) technique. The shaded areas indicate 95% confidence intervals unless stated otherwise. Values of  $p < 0.05$  were considered to be statistically significant. All statistical analyses were done using R version 3.3.3.

## Results

Table 1 shows age and gender distribution of children included in the study. Mean (SD) age was 10.8 (2.8) years and 55.5% were males. The mean (SD) height was 136.7 (16.6) cm, weight was 31.5 (11.8) kg, waist circumference was 55.0 (9.8) cm, and body mass index (BMI) was 16.3 (3.1) kg/m<sup>2</sup>.

**Table 1.** Age and gender distribution of children participating in the study.

Age (Completed years)	Girls		Boys		Total	
	n	% Of whole group	n	% Of whole group	n	% Of whole group
5	133	0.9%	188	1.3%	321	2.1%
6	442	3.0%	526	3.5%	968	6.5%
7	539	3.6%	628	4.2%	1167	7.8%
8	611	4.1%	690	4.6%	1301	8.7%
9	653	4.4%	675	4.5%	1328	8.9%
10	740	4.9%	930	6.2%	1670	11.2%
11	629	4.2%	817	5.5%	1446	9.7%
12	788	5.3%	1051	7.0%	1839	12.3%
13	749	5.0%	1023	6.8%	1772	11.8%
14	726	4.9%	919	6.1%	1645	11.0%
15	640	4.3%	860	5.7%	1500	10.0%
Total	6650	44.5%	8307	55.5%	14957	100.0%

Number (proportion) of children studied at four sites, i.e. Gujarat, Goa, Haryana, and Manipur, were 2324 (15.5%), 1939 (12.9%), 7761 (51.9%) and 2933 (19.6%), respectively. The SES was “richer” for 47.6%, “poorer” for 39.4%, and “mixed” for 13% children. Baseline characteristics are summarized in Table 2.

### Prevalence of different types of HT

The overall prevalence of HT (systolic, diastolic, or both) was 23%. Systolic HT was present in 13.6%, diastolic HT in 15.3%, and both systolic and diastolic HT were present in 5.9% children. Isolated systolic HT was present in 7.7% while isolated diastolic HT was present in 9.4% (Table 2).

### Univariate analysis (Table 3)

**Geographic location vs prevalence of HT.** The prevalence was 9.9%, 13.6%, 26.5%, and 29.9% at the four sites of Goa, Gujarat, Haryana, and Manipur, respectively ( $p < 0.0001$ ). The prevalence was much higher at the two northern sites than the southern sites which are also closer to the ocean (see map in Figure 1).

**Prevalence of HT vs age and gender.** The overall prevalence of HT in boys was 20.2% and that in girls was 26.5% ( $p < 0.0001$ ). Figure 2(a) shows the relationship of prevalence of HT with age in boys and girls. Prevalence of HT was seen to decline with age, especially in boys. From age of 5–15 years, the prevalence of HT reduced from 33% to 16.3% in boys and from 30.1% to 23% in girls.

**Anthropometric parameters versus prevalence of HT.** The prevalence of HT was found to be positively related to z-scores of height, weight, and waist ( $p < 0.0001$  for each). There was also a positive relation with waist-height ratio ( $p < 0.0001$ ) and body mass index ( $p < 0.0001$ ). The relationships with various anthropometric parameters in boys and girls are shown in Figure 3. Waist z-score and waist height ratio have the most linear relationship with the prevalence of HT.

The prevalence of HT in normal, overweight, and obese children was 20.7%, 32.6%, and 42%, respectively ( $p = 0.0001$ ). Figure 2(b) shows the prevalence of HT at different ages in obese and overweight children as compared with the rest. The risk was found to be highest in the obese but there was considerable overlap with overweight children.

**Socio-economic factors versus prevalence of HT.** Analyzing for socio-economic status, the prevalence of HT was 29.9%, 19%, and 9.9% in richer, poorer, and mixed groups, respectively ( $p < 0.0001$ ). Figure 2(c) shows the prevalence of HT in each of these three groups to be consistently different at most ages.

**Season versus prevalence of HT.** The prevalence of HT was higher (29.4%) in the group whose BP was measured in winter (October–March) as compared with those where it was measured in summer (April–September) months (18.7%;  $p < 0.0001$ ). Figure 2(d) shows the prevalence of HT to start rising at the end of summer, reaching a peak in the first two months of the year, with a smaller rise during the peak of summer as well. This pattern is seen in both boys and girls.

**Table 2.** Baseline characteristics and prevalence of hypertension.

Parameter	Mean $\pm$ SD	n	%
Age (completed years)	10.8 $\pm$ 2.8	14,957	
Age group			
9–12 years		6283	42.0%
<9 years		3757	25.1%
>12 years		4917	32.9%
Gender			
Female		6650	44.5%
Male		8307	55.5%
Site			
Goa		1939	13.0%
Gujarat		2324	15.5%
Haryana		7761	51.9%
Manipur		2933	19.6%
Socio-economic status			
Richer		7122	47.6%
Poorer		5896	39.4%
Mixed		1939	13.0%
Weight (kg)	31.5 $\pm$ 11.8		
Height (cm)	136.7 $\pm$ 16.6		
Body mass index (BMI)	16.3 $\pm$ 3.1		
Waist (cm)	55.0 $\pm$ 9.8		
Waist/height ratio	0.4 $\pm$ 0.05		
Weight group			
Normal		12,696	84.9%
Overweight		1501	10.0%
Obese		760	5.1%
Season			
Summer		8884	59.4%
Winter		6073	40.6%
Pulse rate	89.3 $\pm$ 14.4		
Systolic pressure	108 $\pm$ 12.3		
Diastolic pressure	68.6 $\pm$ 9.8		
Hypertension (systolic or diastolic)		3443	23.0%
Systolic hypertension		2036	13.6%
Diastolic hypertension		2289	15.3%
Both systolic and diastolic hypertension		882	5.9%
Isolated systolic hypertension		1154	7.7%
Isolated diastolic hypertension		1407	9.4%

SD: standard deviation.

### Multiple regression analysis

Since waist circumference was not recorded at one site (Gujarat), multiple regression analysis was performed both excluding and including this variable. Age, gender, site, SES, height, weight, and season were other predictor variables in the model. Height and weight were divided by 10 to obtain odds ratios for each 10-unit

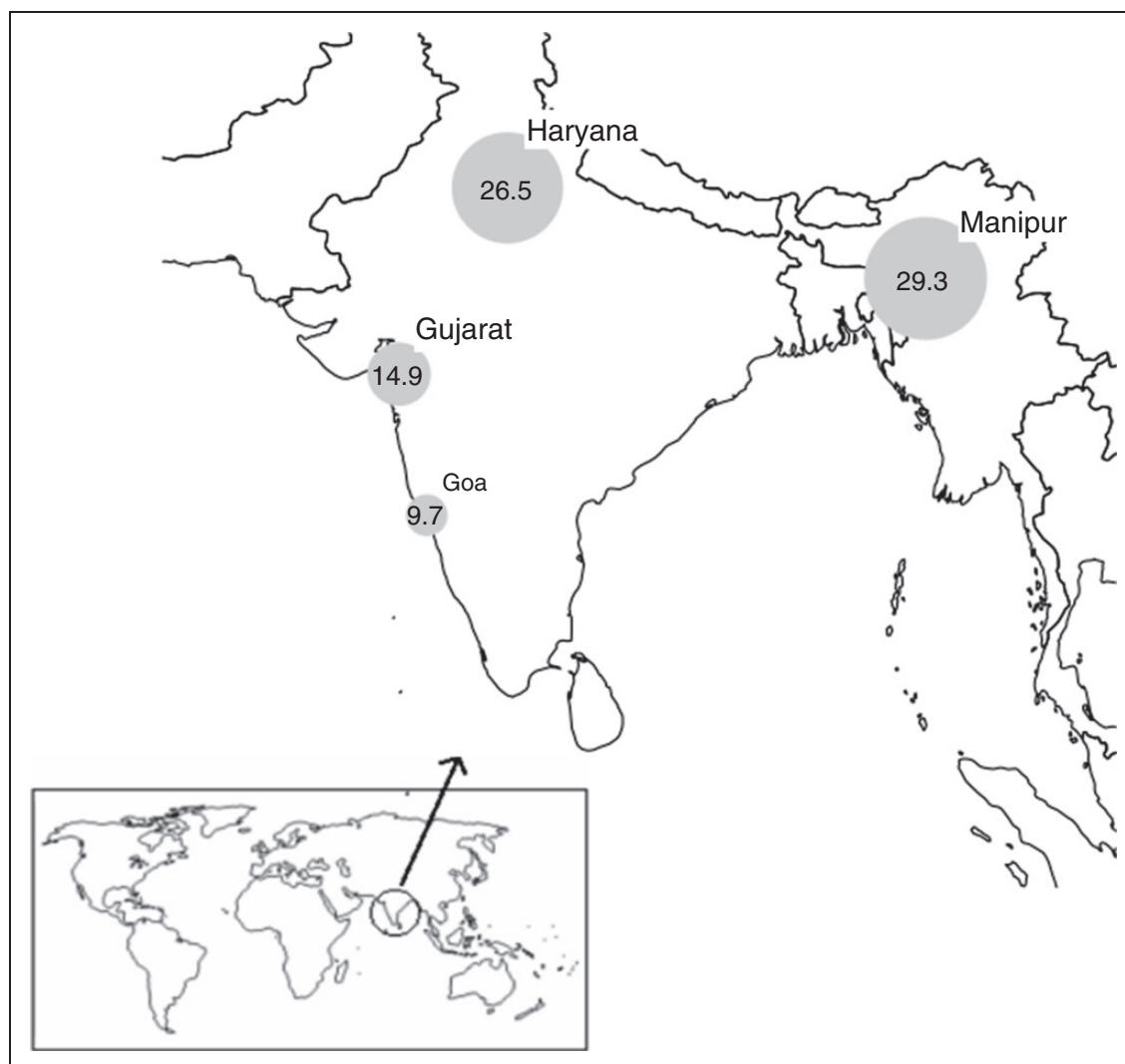
change in parameter. For similar reasons, waist circumference was divided by five.

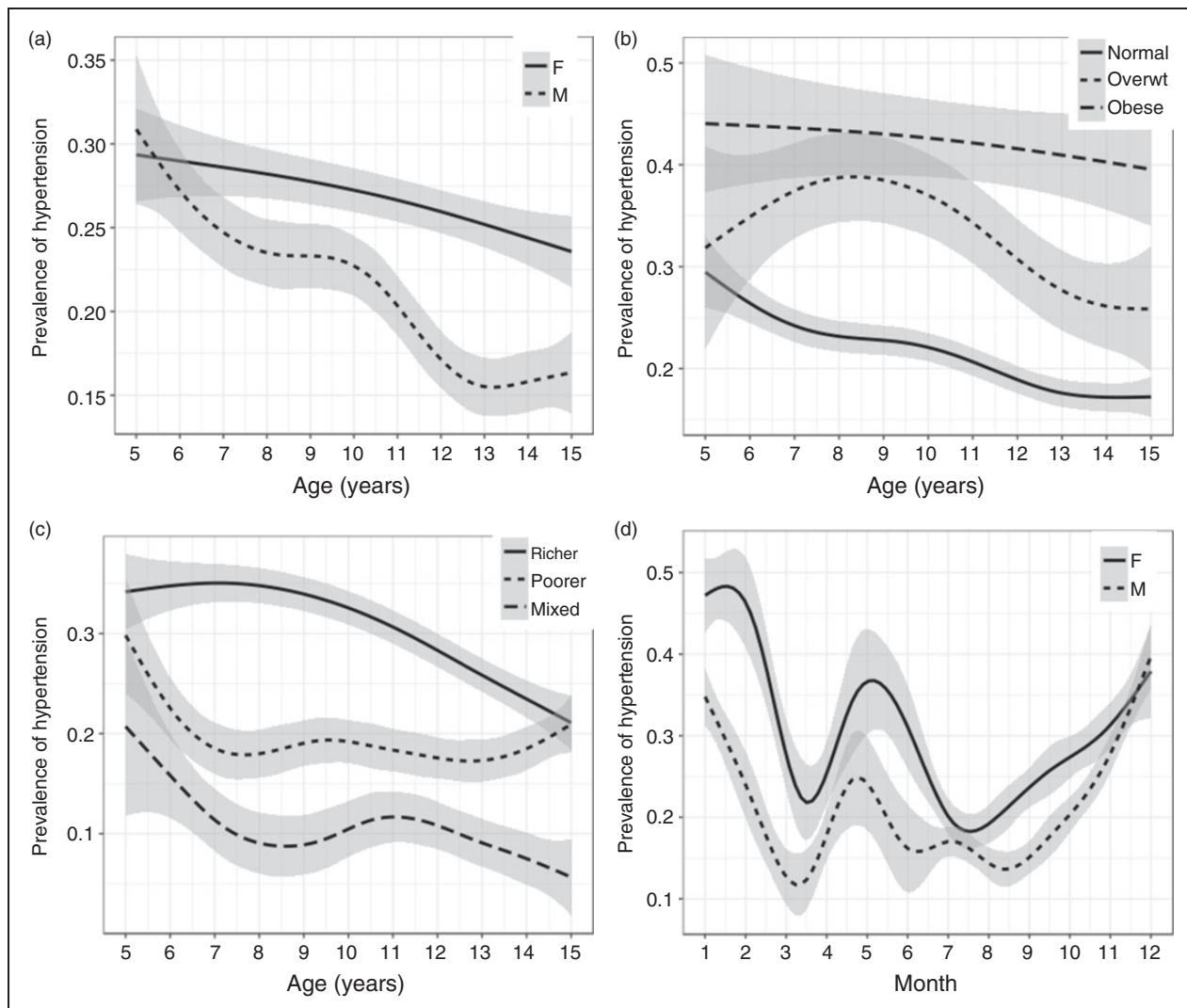
In the first analysis without waist circumference, complete data was available for 14,957 children from all four sites (Figure 4(a)). Age, male gender, lower SES, sites of Goa and Gujarat were independently associated with lower risk while weight, site of Manipur, and winter season were independent predictors of a

**Table 3.** Univariate analysis for relation between prevalence of hypertension and different parameters.

Variable	Mean (SD) for numeric variables		ORs for categorical variables			<i>p</i> Value
	Normotensive group	Hypertensive group	OR	Lower 95% CI	Upper 95% CI	
Age	10.89 (2.82)	10.39 (2.85)				<0.0001
Male gender			0.7	0.65	0.76	<0.0001
Site (four sites)			a			<0.0001
Socio-economic status (three groups)			a			<0.0001
Height z-score	-0.06 (0.98)	0.21 (1.01)				<0.0001
Weight z-score	-0.09 (0.93)	0.32 (1.14)				<0.0001
Waist z-score	-0.08 (0.94)	0.26 (1.11)				<0.0001
Waist/height ratio (WHR)	0.4 (0.05)	0.41 (0.06)				<0.0001
Body mass index (BMI)	16.07 (2.99)	17.01 (3.43)				<0.0001
Obese			2.56	2.21	2.98	<0.0001
Obese or overweight			2.13	1.93	2.34	<0.0001
Winter season			1.81	1.68	1.96	<0.0001

CI: confidence interval; OR: odds ratio.

<sup>a</sup>OR not shown since multiple categories present.**Figure 1.** Map of India showing prevalence of hypertension at different locations. Circle sizes are proportional to prevalence.



**Figure 2.** Relationship of prevalence of hypertension (prevHT) with (a) age and gender, (b) age and overweight/obese status, (c) age and socio-economic status and (d) month of the year in boys and girls. Shaded areas indicate 95% confidence intervals around locally weighted scatterplot smoothing or local regression (LOESS) lines. F: female; M: male.

higher risk of HT. The value of  $p$  was  $< 0.0001$  for all these except for lower SES ( $p = 0.002$ ). For height the value of  $p$  was of borderline significance ( $p = 0.047$ ).

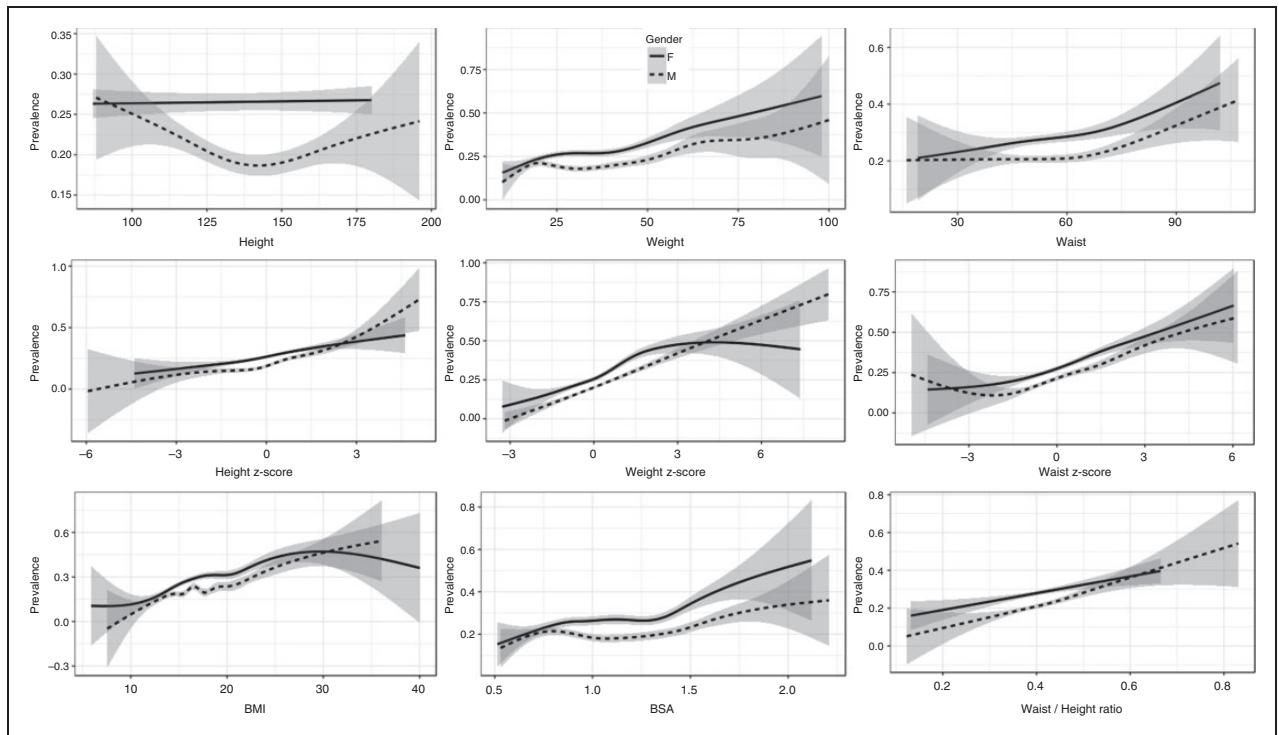
In the second analysis which included waist circumference, complete data was available for 12,068 children from three sites. The same predictor variables as above were taken in addition to waist. Higher age, male gender, and site of Goa were independently associated with a lower risk of HT while weight, waist, and winter season were independently associated with increased risk of HT. The odds ratios are shown in Figure 4(b).

## Discussion

Bassareo et al. called pediatric HT a “burning problem” and emphasized that management of HT in

children should be considered a preventive measure (p. 257).<sup>16</sup> The problem of obesity HT in children has reached epidemic proportions in the western world and is rising rapidly in the developing world.<sup>9</sup> A disturbingly high cumulative incidence of HT has been reported in Chinese children (50.1% and 70% in overweight and obese children, respectively).<sup>11</sup> A major finding of our study is also a high prevalence of HT amongst Indian children. Studies on newer aspects of HT in children, such as the relation to adult-derived genetic BP scores, prenatal exposure to maternal stress, and the effect of traffic-related air pollution, are also being reported.<sup>17–19</sup>

Highly significant associations ( $p < 0.0001$ ) found in our univariate analysis are likely related to the large sample size of this study. A significant variation of



**Figure 3.** Relation between various anthropometric parameters and prevalence of hypertension in boys and girls. Parameters are (from left to right and top to bottom) height, weight, waist circumference, height z-score, weight z-score, waist z-score, body mass index (BMI), body surface area (BSA), and waist/height ratio. Shaded areas indicate 95% confidence intervals around locally weighted scatterplot smoothing or local regression (LOESS) lines. F: female; M: male.

the prevalence of HT between different geographical locations was observed. This variation could be due to local diet (especially salt intake) as well as other environmental factors (including temperature, humidity, exercise habits etc.). The prevalence was higher at two sites which are located more in the north and have lower ambient temperatures over the year. The two sites with lower prevalence are also closer to the ocean, where higher humidity could cause increased perspiration and hence salt and water loss from body. Additionally, ethnic variation and genetic factors could also be important.

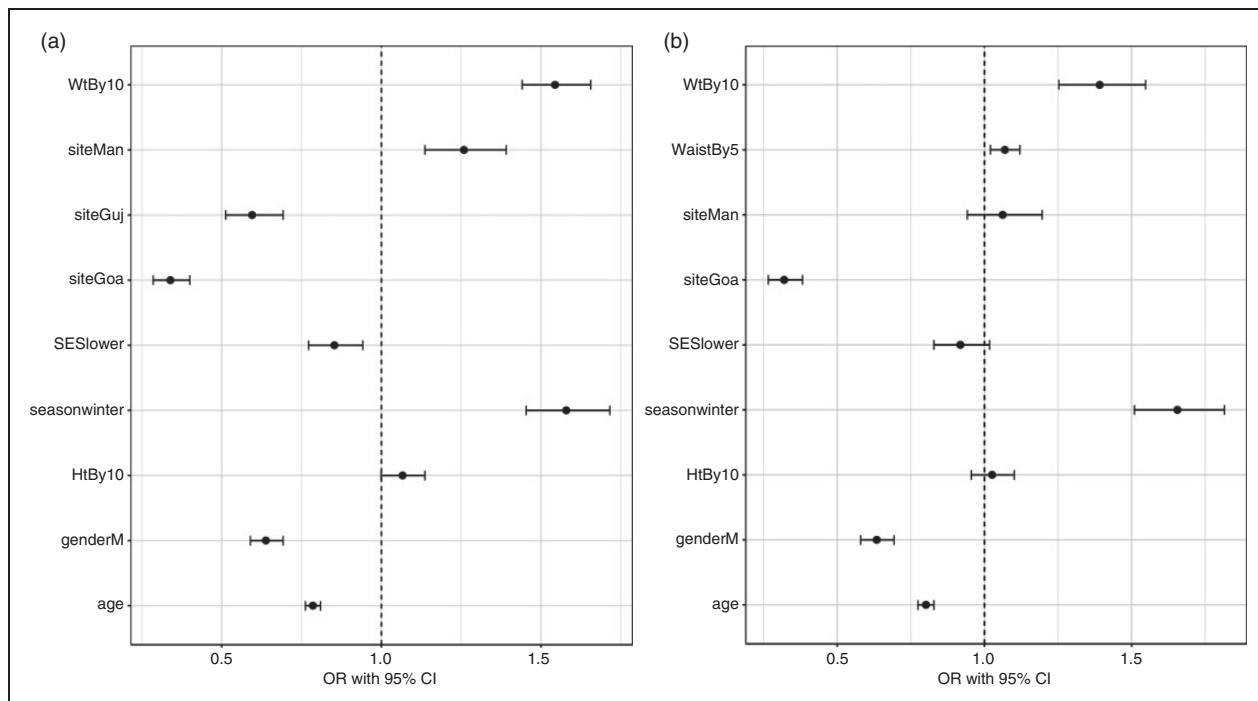
Contrary to expectation, we found the prevalence to be higher in younger children and in females, though this has been observed in other studies as well.<sup>10,20</sup> The prevalence of HT at the upper end of our age group could be affected by the onset of puberty, though we did not study this aspect.

A clear relationship between anthropometric variables and the prevalence of HT found in our study is consistent with earlier reports. Multiple regression analysis including waist circumference showed that weight, and to a lesser extent waist, were independent predictors, while height was not. Our data strengthens the evidence that obesity is an important risk factor for the prevalence of HT. The association

of richer SES with higher prevalence of HT was also expected. Since this relationship is independent of obesity in our analysis, other factors such as psychosocial stress and greater intake of salt and processed foods in the higher SES group could be contributing mechanisms.

There was a significant association of prevalence of HT with season of the year, and higher prevalence was found in winter months. Increase of BP in winter months has also been reported in several earlier studies.<sup>21–23</sup> It is most likely due to increased salt and water loss with perspiration during hot weather that occurs during the summer months in most parts of the country.

Measurement of BP on a single day raises the possibility that the prevalence observed in our study represents a falsely high value. Chiolero et al. found the prevalence to be lower if measurements were repeated over a period of a few weeks.<sup>24</sup> The possibility of overestimation of the prevalence of HT was also highlighted by Wirix et al.<sup>25</sup> Another confounding factor could be using the 50<sup>th</sup> percentile of height values for all children to classify HT using Fourth report tables. However, a number of reports have stressed the need for simplification of the current definition of HT for children.<sup>26–29</sup> A recent update of



**Figure 4.** Forest plot of odds ratios (ORs) from multiple regression analysis performed (a) without and (b) with waist circumference in model. Age, gender, winter season, Goa site, and weight are significant and independent predictors of hypertension in both models. Waist was significant predictor in second model. Height was of border significance in first and not significant in the second model. Lower socio-economic status (SES), Gujarat (Guj) and Manipur (Man) sites were significant predictors in one model only. For numeric parameters, ORs are shown for one-year change in age, 5 cm change in waist circumference and 10 cm change in height or weight. CI: confidence interval.

the Fourth report has also included a simplified screening BP table based on age and gender only.<sup>30</sup>

The high prevalence of HT seen in our study also raises the possibility that Fourth report definition (which is based on children in different areas of the USA) may not be applicable to Indian children. Normative data is being reported from different parts of the world.<sup>31–35</sup> A recently published update to the Fourth report has thresholds which are generally, though slightly, even lower.<sup>30</sup> There have also been attempts to develop a more representative international definition.<sup>1</sup>

There are many studies supporting the association of childhood HT with cardiovascular risk in adulthood. Chen et al. documented evidence of BP tracking from childhood into adulthood in a meta-regression analysis.<sup>4</sup> Theodore et al. found that preHT and HT in childhood are associated with the development of more cardiovascular risk factors over time and predict adult cardiovascular risk.<sup>5</sup> In the Bogalusa Heart Study, Berenson et al. showed an association between multiple cardiovascular risk factors and atherosclerosis in children and young adults.<sup>8</sup> In addition, systolic BP was found to be an independent predictor of arterial stiffness

in young adults, providing evidence that target organ damage also occurs as a result of childhood HT.<sup>8</sup> In a recent further analysis, they reported BP trajectories from childhood to adult life and found puberty to be a critical stage in development of adult HT.<sup>36</sup> Systolic BP was also one of the predictors of carotid artery intima-media thickness (CIMT) in the Cardiovascular Risk in Young Finns Study.<sup>6</sup> Similarly, in the International Childhood Cardiovascular Cohort Consortium study, of 4210 subjects, individuals with persistently elevated BP were found to be at higher risk of carotid atherosclerosis, as measured directly by CIMT, than those in whom high BP in childhood had resolved by adulthood.<sup>37</sup> The difference persisted after controlling for age, gender, adiposity, and definition of HT used. Hence, there is a great deal of evidence that elevated BP in childhood causes target organ damage and an active approach to screening and managing childhood HT is likely to be rewarding.

We have recorded blood pressure twice only in children in whom the first reading was high. It is possible that the prevalence may be higher if the mean of two recordings was taken for all children, as recommended by some published guidelines.<sup>38–40</sup>

## Conclusions

Even in the predominantly rural populations of India, the prevalence of HT is high in childhood. Both systolic and diastolic HT are common. Richer SES, high weight (obesity), and increased waist circumference (abdominal obesity) are important independent predictors and efforts should be made to contain the obesity epidemic. The relationship of higher risk of HT with lower age, female gender, certain geographical locations, and winter months needs further studies for confirmation and to clarify underlying pathogenetic mechanisms. Factors identified in this study can help plan cost-effective strategies of screening and management programs to control this widespread public health problem.

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## Author contribution

AS and SR contributed to the conception or design of the work. RN, AnD, RST, SK, KN, AKJC, AmD, and RS contributed to the acquisition, analysis, or interpretation of data for the work. RN and AS drafted the manuscript. AnD and JC critically revised the manuscript. All authors gave final approval. RN, AS, AnD, SR, RST, and SK agree to be accountable for all aspects of work ensuring integrity and accuracy.

## Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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# Prevalence and Predictor of Nonalcoholic Steatohepatitis (NASH) in Nonalcoholic Fatty Liver Disease (NAFLD)

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## Summary:

Fatty liver is a common cause of chronic liver disease in developed as well as developing countries. We have designed this study to estimate the prevalence and predictors for non alcoholic steatohepatitis (NASH) in non alcoholic fatty liver disease (NAFLD). We have included 493 patients with sonographic evidence of fatty change in liver and 177 of them had done liver biopsy for histopathological study. Other causes of liver disease and alcohol consumption were excluded. Metabolic syndrome and biochemical and anthropometric evaluation was done. Females were predominating 250 (57.0 %). Centrally obese 422 (96.2 %) was more than over all obesity 330 (75.1%). NASH was absent in 10 (5.6%) cases and diagnostic of NASH was 75

(42.4 %). Presence of diabetes could significantly ( $p = 0.001$ ) predicted NASH. Age, sex, BMI, waist circumference, Serum HDL, triglyceride, insulin resistance index, hypertension, metabolic syndrome could not predict NASH. Serum GGT level was significantly ( $p = 0.05$ ) higher in NASH with a sensitivity of 45 % and specificity of 68 % only. Serum ALT and AST level could not detect NASH. Females were predominant sufferer of NAFLD in Bangladesh. Prevalence of NASH was much higher 42.4%. Diabetes was the main predictor of NASH. GGT was the only biochemical indicator of NASH. We recommend liver biopsy in NAFLD with diabetes and raised GGT.

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## Introduction:

Nonalcoholic fatty liver disease (NAFLD) is a clinico-histopathological entity with histological features that resemble alcohol-induced liver injury. By definition, occurs in patients with little or no history of alcohol consumption<sup>1</sup>. NAFLD is the most common liver disease in western countries, affecting 20-30% of the general population<sup>2,3</sup>. It encompasses a histological spectrum that ranges from fat accumulation in hepatocytes without concomitant inflammation or fibrosis (simple hepatic steatosis) to hepatic steatosis with a necro-inflammatory component (steatohepatitis)

that may or may not have associated fibrosis. The latter condition, referred to as nonalcoholic steatohepatitis (NASH), may progress to cirrhosis in up to 20% of patients<sup>4</sup>. Reports have also suggested that the prevalence of NAFLD among Asian Indians is comparable to that seen in the West<sup>5,6</sup>. Average age for NASH patients is 40-50 years and for NASH-related cirrhosis it is 50-60 years. NASH probably causes around 80% of cases of cryptogenic cirrhosis which accounts for 10-20% of all cirrhosis and progresses to advanced fibrosis in 32 to 37% of patients<sup>7</sup>.

In parallel with the epidemic of obesity and metabolic syndrome worldwide, the prevalence of NAFLD in Asian countries has increased rapidly with a trend to younger patients during the last two decades. The prevalence of NAFLD was about 15% in adults in Shanghai and Hong Kong<sup>8</sup>. NAFLD has been associated with insulin resistance and hyperinsulinaemia, even in lean subjects with normal glucose tolerance<sup>9</sup>. Diabetes mellitus may be an independent predictor of NASH, including cirrhosis and hepatocellular carcinoma<sup>10</sup>. NAFLD is now recognized as the hepatic component of the metabolic syndrome, which includes hyperlipidemia, glucose intolerance, obesity, and systemic hypertension. Predictors of NASH increase with the number of components of the metabolic syndrome<sup>11</sup>. The contrasting clinical course of NASH versus non NASH fatty liver (NNFL) indicates that these

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two conditions diverge early in the course of NAFLD although some patients probably transition from NNFL to NASH. Progression to cirrhosis is usually preceded by longstanding histological NASH and is infrequent in NNFL. Longitudinal studies with serial biopsies have shown that about one-third of NASH patients develop advanced fibrosis (stage 3 or 4 fibrosis) over 5–10 years from the time of the initial diagnosis<sup>12</sup>. Although usually relatively slow, progression to cirrhosis can occur in as little as 2–3 years. NASH is a common cause of ‘cryptogenic’ cirrhosis, which accounts for 10 – 20% of all cirrhosis<sup>13</sup>. Among patients diagnosed with NASH-related cirrhosis, the risk of developing a major complication of portal hypertension is 17, 23 and 52% at 1, 3 and 10 years, respectively. Among patients with early stage NASH, overall mortality over 10–15 years is about 10–12%, being significantly higher in NASH versus NNFL, compared to the general population. The risk of developing decompensated cirrhosis is 5–10% and for hepatocellular cancer it is 1–2%. There is a tenfold risk of cirrhosis relative to the general population<sup>14</sup>.

A complete diagnosis of fatty liver disease ideally should define the histology, including the stage and grade of the disease as well as its etiology. In Bangladesh NAFLD is never been or insufficiently addressed in the field of medical research and practice. NASH is a potentially dangerous condition which requires medical intervention. The prevalence of NASH and potential risk factors for it is not yet explored here. We have designed this study protocol to estimate the prevalence of NASH in NAFLD and predictor of NASH in the perspective of Bangladesh which will be helpful future scientific knowledge and intervention.

### Materials and Methods:

#### Study population:

We have included initially 439 patients at outpatient department of Hepatology in the University Hospital during the period of March 2010- December 2012 for fatty filtration in liver with ultrasonography. Exclusion criteria consisted of significant alcohol abuse (< 20g daily), evidence of hepatitis B and C and of drug induced fatty liver and other specific liver diseases: Hemochromatosis, Wilson’s disease or autoimmune liver disease. These patients underwent clinical evaluation, anthropometric measurements, and blood

tests. Liver biopsy was done after randomization in 190 patients but 4 biopsy samples were inadequate to comment for histopathology 4 patients withdrawn themselves from the study. The study was approved by the Institutional Review Board and all individuals provided written informed consent prior to enrollment in the study. Metabolic syndrome was defined according to Asian criteria,<sup>[15]</sup> and three of the five listed criteria were considered: waist circumference (WC) ≥ 80 cm for women and ≥ 90 cm for men, serum triglyceride ≥ 150 mg /dl (1.7 mmol/l), serum HDL cholesterol < 50 mg/dl (1.3 mmol/l) for women and < 40 mg/dl (1 mmol/l) for men, elevated blood pressure (systolic blood pressure ≥ 130 and/or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and plasma glucose concentration ≥ 100 mg/dl (5.6 mmol/l) or drug treatment for diabetes.

#### Clinical and Biochemical evaluation:

All the patients were clinically evaluated: Blood pressure, Body mass index (BMI) and waist circumference was recorded for every patient. Liver function tests were performed prior to the liver biopsy. Blood samples were obtained under fasting conditions and the following tests were performed using standard laboratory methods: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, Gamma glutamyltranspeptidase (GGT) international normalized ratio (INR), blood glucose fasting and 2 hours after breakfast, lipid profile, Insulin level was assessed using the method of indirect chemiluminescence (MEIA). Insulin resistance was calculated according to the HOMA index (Homeostatic Metabolic Assessment).

#### Histological assessment

Liver biopsy specimens of 182 were analyzed by pathologist blinded to the patients’ clinical and biochemical results. Histopathology was done in the department of Pathology BSMMU. The diagnosis of NASH was based on the Brunt et al criteria,<sup>[16]</sup> modified by Kleiner et al<sup>[17]</sup>. In this scoring system, the degree of disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and hepatocyte ballooning (0–2) and thus ranged from 0 to 8. A NAS of 5 or more was diagnosed as “definitive NASH”, NAS of 2 or

less as “non-NASH,” and 3 or 4 as “borderline NASH.” Other than NASH, was considered as NNFL. Hepatic fibrosis staging was as follows: 0 = no fibrosis; 1 = zone 3 fibrosis only; 2 = zone 3 and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

### Statistical analysis

Results are presented as mean  $\pm$  standard deviation (SD) for quantitative data and as numbers or percentages for categorical or qualitative data. Statistical differences in quantitative data were determined using t test or one way Anova test. Qualitative data were compared using the  $\chi^2$  test. Multivariate regression analysis was done to explore the strongest predictor of NASH including the variables with significance in univariate analysis. For all tests, significance was achieved at  $p < 0.05$ .

### Results:

#### Patient Characteristics:

Total of 439 patients were included in this study. Females were 250 (57.0 %) and males were 189 (43.0 %). Mean age of the sample was  $40.8 \pm 10.2$  years. Most of the population was house wife 217 (50.3 %), others were service holder 84 (19.5 %), business man 69 (16.0 %) and students were 59 (13.7 %). Hypertension and diabetes were prevailing in 83 (18.8 %) and 74 (16.8 %) respectively but metabolic syndrome was 188 (42.9 %). Triglyceride was high in 320 (72.8 %). BMI was normal in 51 (11.7 %), over weight 58 (13.2 %), Obese I 237 (53.9 %) and obese II 93 (21.2 %) according to criteria for Asian<sup>18</sup>. Most of the patients were centrally obese 422 (96.2 %) having waist circumference above normal. ALT, AST and GGT level were  $54.1 \pm 54.4$ ,  $45.1 \pm 51.8$  and  $46.6 \pm 33.7$  u/l respectively. Insulin resistance index were higher than normal in 218 (49.6 %).

**Histological Changes:** Histopathological reports of 182 patients were available but 5 of them did not have fatty change on microscopy. We have included 177 patients for further analysis. There was no significant difference between biopsied and non- biopsied patient regarding clinical, anthropometric and biochemical variables. Steatosis of < 33% was 73(41.2%), 33 – 66 % was 82 (46.4 %) and > 66 % was 22 (12.4%). Lobular inflammation was absent in 10 (5.6 %), mild in 93 (52.5 %), moderate in 70 (39.5 %) and severe in 4 (2.3 %). Ballooning was absent in 5 (2.8 %), few ballooning in 138 (78.0 %) and prominent ballooning in 34 (19.2%) (Figure I). No fibrosis was seen in 28 (15.8%), stage I in 94 (53.3%), stage II in 40 (22.5 %) and stage III in 15 (8.3%). None had stage IV fibrosis (Table I).

According to NAS scoring system NASH was absent in 10 (5.6%) cases, borderline NASH was 92 (52.6%) and diagnostic of NASH was 75 (42.4 %). So NNFL was 102 (57.6%) and NASH was 75 (42.4%).

### Predictors of NASH:

Prevalence of NASH in NAFLD was 75 (42.4%). There were no significant difference of age, BMI, waist circumference, Serum HDL and triglyceride level, insulin resistance index, sex, hypertension, metabolic syndrome did not differ in NASH and Non NASH. Mean age, BMI and waist circumference was similar in NNFL and NASH patients. Mean triglyceride was higher in NASH and mean HDL was lower in NASH but could not establish statistically significant value. Presence of diabetes could significantly ( $p = 0.001$ ) differentiate NASH from NNFL. Serum ALT and AST level could not detect NASH in NAFLD. But serum GGT level was significantly ( $p = 0.05$ ) higher in NASH than that of NNFL (Table II). GGT level for NASH was  $(51.7 \pm 32.8)$  U/L and for NNFL was  $(40.4 \pm 22.6)$  U/L. Multivariate regression analysis also explore that presence of diabetes could influence the development of NASH ( $p=0.04$ ) and GGT could differentiate NASH from NNFL ( $p=0.01$ ) (table III). But area under the curve is 59.3 % for GGT to differentiate NASH, with a sensitivity of 45 % and specificity of 68 % only for 44.5 U/L (Figure II).

**Table-I**

<i>Histopathological features of biopsied patients</i>		
Variable	Number	Percent
Lobular inflammation		
Absent	10	5.6
Mild	93	52.5
Moderate	70	39.5
Severe	4	2.3
Ballooning		
Absent	5	2.8
Few	138	78.0
Prominent	34	19.2
Fibrosis		
Absent	28	15.8
Stage I	94	53.3
Stage II	40	22.5
Stage III	15	8.3
NASH	75	42.4

**Table-II***Clinical, anthropometric and biochemical differences of NNFL and NASH*

Variable	NNFL N=102	NASH N=75	Pvalue
Age (yr)Mean ± SD	39.3 ± 9.4	41.0 ± 9.7	0.24
Sex: Male/ female	42/60	31/44	1.00
Body Mass Index (Kg/m <sup>2</sup> )	27.8 ± 3.9	27.8 ± 4.6	0.998
Waistcircumference in cm	Male 93.0 ± 5.5 Female 95.8 ± 9.9	93.0 ± 9.8 95.6 ± 11.0	0.081 0.927
HDL in mg/dl	Male 36.3 ± 8.9 Female 39.8 ± 10.3	34.2 ± 6.5 39.2 ± 10.3	0.337 0.801
Serum Triglyceride mg/dl	225.2 ± 165.8	239.8 ± 111.6	0.509
Insulin Resistance Index	1.8 ± 1.3	1.5 ± 0.7	0.337
Diabetes Present / Absent	13/86	25/48	0.001
Hypertension Present / Absent	17/65	17/48	0.555
Metabolic SyndromePresent/ Absent	41/41	39/32	0.328
ALT U/L	56.9 ± 38.8	56.3 ± 31.8	0.603
AST U/L	46.9 ± 63.7	46.1 ± 22.2	0.916
GGT U/L	40.4 ± 22.6	51.7 ± 32.8	0.05

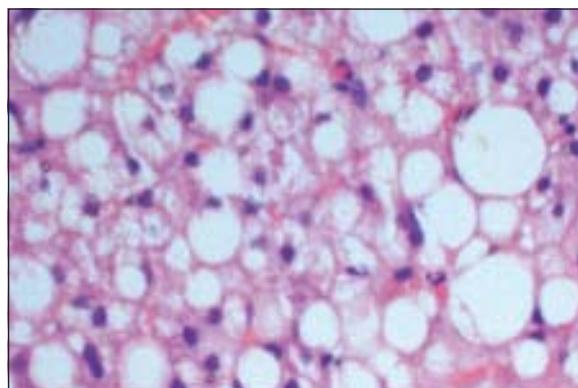
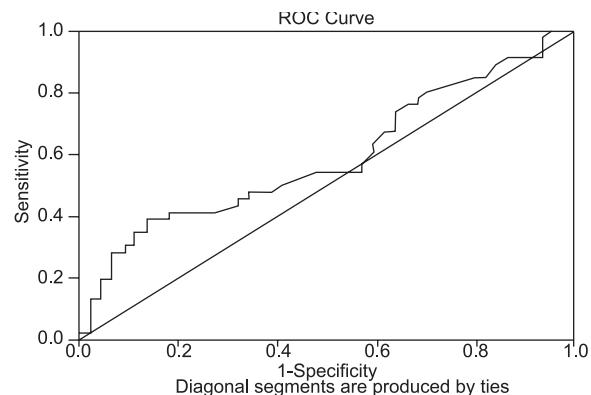
NASH; Non alcoholic steatohepatitis, NNFL; Non nash fatty liver

**Table-III***Multivariate regression analysis for variable detecting NASH*

Model	Unstandardized Coefficients		Standardized Coefficients Beta	t	Sig
	B	Std. Error			
(Constant)	1.247	.517		2.411	.018
BMI	.014	.018	.124	.780	.438
Diabetes	.260	.125	.227	2.084	.040
Serum Triglyceride	.000	.000	-.105	-.919	.361
GGT	.005	.002	.289	2.473	.015
Waist Circumference	-.004	.008	-.077	-.491	.624

a. Dependent Variable: nash and nnfl

NASH; Non alcoholic steatohepatitis, NNFL; Non nash fatty liver

**Fig.-1:** Microscopic feature of Nonalcoholic steatohepatitis:steatosis and ballooning degeneration.**Fig.-2:** Receiver Operating Characteristic curve for GGT to differentiate NASH from NNFL.

**Discussion:**

This study is the largest series from Bangladesh on NAFLD. Report of biopsy proven NASH and NNFL is also rare. University hospital is a tertiary care “center of excellence” hospital only and patients are referred from whole over the country. So this study may be the representative of prevalence of NASH in NAFLD of the country. Population based prevalence of NAFLD was not yet done in Bangladesh. Most of our NAFLD patients are of 30 to 50 years; this is similar to several reports from Asia<sup>6,19-20</sup>. But age could not influence the development of NASH. Female preponderance in NAFLD is dissimilar from reports from developed counties. Many recent studies have reported that male gender is a risk factor for fatty liver disease<sup>21</sup>. For example, in a study of 26,527 subjects undergoing medical checkups; the prevalence of NAFLD was 31% in men and 16% in women<sup>22</sup>. This female preponderance 250 (57.0 %) in our study may be the social conservative attitude which bounded most of our ladies to stay home for house hold activities without job leading to sedentary life style. Similar female preponderance was observed in one population studies from India<sup>23</sup>. But in accordance with previous studies sex did not influenced the development of NASH in NAFLD<sup>22</sup>.

Centrally obese was 422 (96.2 %) outnumbered the overall obesity 330 (75.1%). The prevalence of NAFLD was increased according to the increase of BMI or abdominal circumference reported from Japan<sup>24</sup>. But other report concluded that waist circumference is an independent predictor of advance histological changes in NAFLD than BMI<sup>25,26</sup>. But waist circumference was similar in NASH and NNFL in our series. It could be explained by that waist circumference indicate visceral obesity but no influence on pathogenesis of NASH at the stage of 2<sup>nd</sup> hit. Hypertriglyceridemia was very common 320 (72.8 %) in this study with no difference between NASH and NNFL. TG was long been considered as major factor in the development of NAFLD,<sup>5-8</sup> but there is mounting evidence that such non-TG lipid molecules are implicated in the pathogenesis of NASH by the process of lipotoxicity. Conversely, formation of TG may actually be a cytoprotective mechanism in liver<sup>27, 28</sup>. Our study revealed similar role of TG in NAFLD.

Our study explored that prevalence of NASH was 75 (42.4%) in NAFLD which is much higher. It is alarming

for the country like Bangladesh. It was neither addressed previously nor considered anyway. In previous review, NAFLD was found highly prevalent (15% to 45%) in modern societies, only 10% to 25% of cases develop NASH, hepatic fibrosis leading to cirrhosis, end-stage liver disease or hepatocellular carcinoma<sup>29</sup>. In other studies prevalence of NASH was 10 to 30 % in NAFLD<sup>30</sup> and it is less in Asian than that of European<sup>31,32</sup>. We were unbiased in selecting patient for liver biopsy and it was irrespective of clinical, biochemical and anthropometric status of the study population. So it is the representative of prevailing situation in the society. This finding warrants further extensive study on prevalence of NASH in Bangladesh and awareness of clinician is essential to diagnose NASH and to advice possible intervention as early as possible.

Presence of diabetes signified the presence of NASH in our study population ( $p= .001$ ). Metabolic syndrome was prevailing in 188 (42.9%) population. NAFLD is strongly associated with insulin resistance (IR) and other components of the metabolic syndrome, like T2DM, central obesity, hyperlipidemia, and hypertension<sup>33</sup>. The pathogenesis of NASH appears to be a multiple hit process. The initial insult is the development of macrovesicular steatosis with the accumulation of hepatic fat from decreased hepatic free fatty acid oxidation and D or increased hepatic de novo lipogenesis, and D or decreased lipid export from the liver. Although IR can contribute to this dysregulation of lipid metabolism, once fatty liver develops, it can worsen hepatic IR and diabetes, contributing to a vicious cycle<sup>34</sup>.

Serum ALT and AST levels were similar in NASH and NNFL in this study. But GGT were significantly ( $P= 0.05$ ) higher in NASH than that of NNFL. NASH has been associated with slight elevation of liver enzymes mostly ALT<sup>35</sup>. In other reports NAFLD patient typically present with asymptomatic serum aminotransferase elevations of 2-3 times the normal<sup>36</sup>. This difference was due to different selection criteria. GGT is a sensitive indicator of liver damage<sup>37</sup>. Excess deposition of fat in the liver is associated with an elevated serum GGT<sup>38</sup>. Recent reports suggest that an increased GGT level is a risk factor for advanced fibrosis in NAFLD and, with weight loss, a decrease in GGT activity is predictive of improved lobular inflammation and fibrosis of liver<sup>39</sup>.

The limitation of the study was that we had not done it at the community level rather at a tertiary level hospital of the country.

In conclusion, Females were predominant sufferer of NAFLD in Bangladesh. Prevalence of NASH was much higher in NAFLD. Diabetes was the main culprit in developing NASH in NAFLD. GGT was the only biochemical predictor of NASH but with low sensitivity and specificity. We recommend liver biopsy in NAFLD with diabetes and raised GGT.

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Article

# Prevalence and Correlates of Hypertension among Japanese Adults, 1975 to 2010

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**Abstract:** We investigated the prevalence and factors associated with hypertension, its treatment, and control using individual-level data from 300,249 respondents aged 20 years and older from the Japanese National Health and Nutrition Survey for the period of 1975–2010. We applied multivariate random effects logistic regression to assess associations between the risk factors and the prevalence of hypertension, the proportion of uncontrolled hypertension, and the proportions of respondents seeking treatment and controlling hypertension. The trends in the effect of the birth cohort on uncontrolled hypertension were also examined. Having hypertension was associated with being male, older, obese, drinking alcohol, and working in the primary industry and a higher proportion of middle-aged men than women were found being obese and drinking alcohol. Seeking treatment was associated with being older, obese, drinking alcohol, working in a primary industry and exercising. Controlling hypertension was associated with being younger, underweight and exercising. The proportion of individuals with uncontrolled hypertension declined for cohorts born in later years with a steeper decline for women than men. Raising awareness in the hypertensive population, especially among men, could help further reduce the prevalence of hypertension in Japan.

**Keywords:** Japan epidemiology; hypertension epidemiology; health surveys; public health

## 1. Introduction

Hypertension remains a leading global risk factor for death and disability. In 2011 the World Health Organization (WHO, Geneva, Switzerland) targeted a 25% global reduction in prevalence of hypertension by 2025 [1], but progress varies widely between countries. Over the past 50 years, the mean blood pressure of the Japanese population has seen a steady decline, but hypertension and smoking still remain the two biggest risk factors for non-communicable diseases, in particular, cardiovascular disease [2–4]. Understanding the trends in hypertension prevalence and management is essential to preparing strategies to target this risk factor in Japan. We used individual-level data from the Japanese National Health and Nutrition Survey (NHNS) from 1986 to 2010, and, where available, from 1975 to 2010, to explore the trends and risk factors for hypertension in Japan. We thus expand on previous investigations of blood pressure in the Japanese population [5–7] and aimed to:

- Analyze trends in and risk factors for uncontrolled hypertension over 35 years and for hypertension over 25 years
- Identify factors associated with seeking treatment and controlling hypertension

- Investigate changing patterns in the proportion of individuals with uncontrolled hypertension by the birth cohort over 35 years.

Japan has made progress in reducing hypertension and improving management of this condition, but further work is needed. The results of this study may be used to plan interventions in specific population groups at risk of hypertension, and to raise awareness of the need for the identification, treatment, and control of hypertension.

## 2. Method

### 2.1. Data Sources

This study used data from the NHNS between 1975 and 2010. The NHNS is a repeated cross-sectional survey conducted annually on a random sample of Japanese citizens in all 47 Japanese prefectures. The sample size was about 28,000 persons in 1975, 20,000 persons between 1976 and 1986, and gradually decreased to about 10,000 or less after 2000 [8,9]. In addition to answering a questionnaire, the respondents were invited to undergo a physical examination that included blood pressure measurements. The response rate to the physical examination was reported as around 50 to 60% [9,10]. Stratified randomization by prefecture and household in a two-stage cluster sampling design is used for participant selection [8,9,11]. Here, we restricted the analysis to respondents who were at least 20 years old, not pregnant, and whose systolic and diastolic blood pressure data were available. Fifteen-year age brackets were chosen to ensure sufficient numbers of respondents in each age category.

### 2.2. Blood Pressure Measurement and Hypertension

Data on blood pressure values were available from 1975 to 2010 and data on hypertension medication were available from 1986 to 2010. Until 1999, the survey included one measurement of blood pressure in the sitting position, and from 2000 it included two measurements of blood pressure measured in the sitting position with a Riva-Rocci mercury sphygmomanometer after resting [5,11,12]. The two measurements were averaged for analysis, consistent with the Japanese Ministry of Health, Labour and Welfare guidelines [13].

Hypertension was defined as a systolic blood pressure of at least 140 mmHg or a diastolic blood pressure of at least 90 mmHg or the use of medication for hypertension, in line with the current World Health Organization (WHO, Geneva, Switzerland) guidelines [14,15]. Uncontrolled hypertension was defined in the same way except that respondents who successfully controlled their blood pressure to the normal range by medication were counted as controlled. Values of 50 to less than 300 mmHg for systolic blood pressure and 40 to less than 140 mmHg for diastolic blood pressure were considered valid. Values outside these extremes were omitted. Medicated hypertension was defined as taking medication for hypertension regardless of successful control or not. Controlled hypertension was defined as medicated hypertension with a measured blood pressure in the normal range. Since information on medication was only available after 1986, the prevalence of hypertension and the proportions of medicated hypertension and controlled hypertension were analyzed starting from the survey year 1986.

### 2.3. Correlates of Hypertension

In its guidelines, the Japanese Society of Hypertension defines the male sex, advanced age, high body mass index (BMI), not exercising, high salt intake, low vegetable and fruit intake, regularly drinking alcohol and smoking as risk factors for hypertension [16]. Job strain, defined as a low control over work-related decisions and having a high workload, has also been reported as a risk factor [17,18] as has socioeconomic status [19]. Risk factors available in the NHNS were sex, age, BMI (1975–2010), salt intake (2000–2010), exercising (1986–2010, 2008 and 2009 missing), drinking alcohol (1986–2010), current smoking status (1986–2010), and occupation (1975–2010). The BMI was calculated as weight

in kilograms divided by the square of height in meters, omitting values of weight below 20 kg and of height below one meter. The BMI values of 10 to less than  $70 \text{ kg/m}^2$  were retained as these have been defined elsewhere as plausible limits [20,21]. The BMI was classified following WHO guidelines: underweight was defined as BMI less than  $18.5 \text{ kg/m}^2$ ; normal as the BMI values from  $18.5$  to  $25 \text{ kg/m}^2$ ; overweight as the BMI values over  $25 \text{ kg/m}^2$  and obese as  $30 \text{ kg/m}^2$  or more [22]. For the risk factor analysis, overweight and obese subjects were collapsed into one category of obese defined as  $25 \text{ kg/m}^2$  BMI or more because Asian populations have been found to be at risk for metabolic disease at lower BMI values than Western populations [23]. In the NHNS, the dietary sodium intake is estimated retrospectively from self-reported food intake [9]. We converted sodium intake to salt intake by multiplication with a factor of 2.54 [24]. Drinking alcohol was defined as regularly drinking more than one glass of Japanese sake (180 mL), one bottle of beer (500 mL), or one double whiskey (60 mL) at least four times a week [5,11]. Exercising was defined as physical activity for more than 30 min at least three times a week for over one year.

Occupations were classified into primary, secondary, and tertiary industries, in line with the Japanese Ministry of Internal Affairs and Communication [25]. This classification would to some extent reflect socioeconomic status, as the primary industries include workers in farms and fisheries, the secondary industries include a large proportion of blue collar workers and the tertiary industries include the largest proportion of white-collar workers and persons with high education levels among the three industries.

#### 2.4. Statistical Analysis

All statistical analyses were performed with a commercially available software package (Stata14, StataCorp LP, College Station, TX, USA). We applied multivariate random effects logistic regression to assess the association of hypertension, uncontrolled hypertension, treated hypertension, and controlled hypertension with the covariates, including a random intercept term for prefecture to account for clustering within and unmeasured variations between prefectures. Graphs of the proportion of individuals with uncontrolled hypertension by birth year for birth cohorts were smoothed using a kernel-weighted local polynomial regression smoother.

### 3. Results

#### 3.1. Demographic Characteristics of the Sample

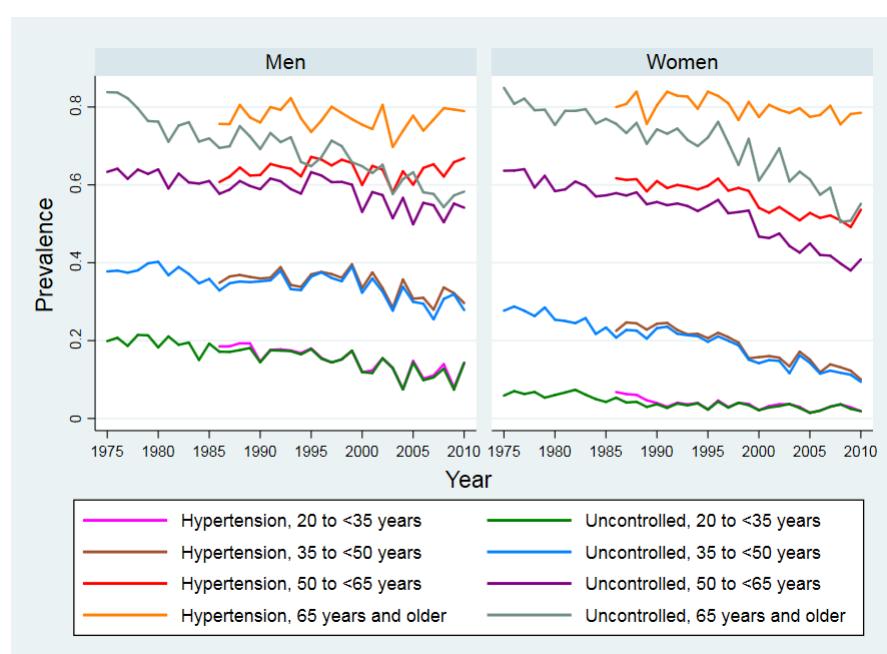
There were 554,544 observations available after the exclusion of pregnant women. After excluding 5 respondents because of anomalies between gestational age and pregnancy variables, 149,846 respondents because they were less than 20 years old, and 104,210 missing and 54 unrealistic blood pressure measurements, the final sample consisted of 300,429 respondents sampled between 1975 and 2010. The mean age was  $49.9 \pm 16.2$  years. Among the respondents, 124,871 were men and 175,558 women with mean ages of  $49.6 \pm 16.2$  years and  $49.4 \pm 16.1$  years, respectively. The mean BMI was  $22.9 \pm 3.3 \text{ kg/m}^2$  for all respondents,  $23.2 \pm 3.1 \text{ kg/m}^2$  for men and  $22.7 \pm 3.5 \text{ kg/m}^2$  for women. The mean salt intake was  $11.7 \pm 4.8 \text{ g/day}$  for all respondents,  $12.7 \pm 4.8 \text{ g/day}$  for men and  $11.0 \pm 4.8 \text{ g/day}$  for women. Table 1 gives an overview of the years of data availability, and the frequencies and proportions of characteristics of the sample.

**Table 1.** The characteristics of the sample.

		Frequency	Proportion in %	Years of Available Data
Sex	Male	124,871	41.56	1975 to 2010
	Female	175,558	58.44	
Age (years)	20 to 35	63,208	21.04	1975 to 2010
	35 to 50	91,366	30.41	
	50 to 65	125,305	41.71	
	At least 65	20,550	6.84	
BMI	Underweight	22,317	7.44	1975 to 2010
	Normal	211,419	70.50	
	Overweight	58,842	19.62	
	Obese	7296	2.43	
Smoking	Non smoker	123,042	73.82	1986 to 2010
	Current smoker	43,640	26.18	
Drinking alcohol	Less than 4 times a week	125,411	75.34	1986 to 2010
	At least 4 times a week	41,040	24.66	
Exercise	Less than 3 times a week	119,814	76.18	1986 to 2010 (2008, 2009 missing)
	At least 3 times a week	37,462	23.82	
Occupation	Primary industry	25,782	9.73	1975 to 2010
	Secondary industry	52,622	19.87	
	Tertiary industry	186,485	70.40	

### 3.2. Prevalence of Hypertension and Proportions of Uncontrolled Hypertension, Treatment, and Control

Figure 1 shows the trends in the prevalence of hypertension (1986–2010) and the proportion of individuals with uncontrolled hypertension (1975–2010) by sex and age. Between 1986 and 2010, the prevalence of hypertension has not changed much in men and women of 65 years and older but appears to increase in middle-aged men of 50 to less than 65 years old. In contrast, for men younger than 50 and women younger than 65 years, the prevalence of hypertension and the proportion of individuals with uncontrolled hypertension both showed decreasing trends.



**Figure 1.** The prevalence of hypertension (1986–2010) and the proportion of individuals with uncontrolled hypertension (1975–2010) in Japan by sex and age.

Table 2 shows the prevalence of hypertension (1986–2010), the proportion of individuals with uncontrolled hypertension (1975–2010), the proportion of hypertensive respondents receiving medication (1986–2010), and the proportion of individuals with controlled hypertension (1986–2010) among medicated respondents stratified by sex and age. The prevalence of hypertension increased with age and was higher among men than women, except for the age group of 65 and older. The proportion of hypertensive respondents seeking treatment increased with age, as did the proportion of respondents who controlled their hypertension with higher proportions of women than men for both. Men of 65 years and older were, however, equally or more successful in controlling their hypertension than women.

### 3.3. Risk Factors Associated with Hypertension

The means and proportions of the risk factors of age, salt intake, BMI, smoking, drinking alcohol, exercising and occupation are shown in Table 3 for one year in the beginning, in the middle and at the end of the period of analysis. Differences between men and women were seen in trends of BMI, smoking, and drinking. The proportion of women who smoke and regularly drink alcohol was still much lower in 2010 than that of men although the difference has narrowed in more recent years compared to the year 1975. Table 4 shows the odds ratios of having hypertension for each factor adjusted for all other factors. Older age was the strongest predictor ( $OR\ 42.85$ , 95% CI 39.93–46.00, for at least 65 compared to 20 to less than 35 years old). The strongest modifiable risk factor was BMI ( $OR\ 2.55$ , 95% CI 2.48–2.62 for obese compared to normal weight people). Salt intake and exercise showed no effect. Occupation had a weak association with hypertension.

### 3.4. Factors Associated with the Treatment and Control of Hypertension

Table 4 also shows the association of each risk factor with the treatment and control of hypertension adjusted for all other covariates. The odds of taking medication ( $OR\ 1.02$ , 95% CI 1.02–1.02) and the odds of controlling hypertension ( $OR\ 1.05$ , 95% CI 1.04–1.05) increased year by year. The odds of taking medication increased with age. The odds of controlling hypertension among those who sought treatment was lower for those aged 50 and older than for those younger than 50 years. Obese people had higher odds of seeking treatment than normal weight people ( $OR\ 2.07$ , 95% CI 1.99–2.14), but lower odds of controlling their hypertension ( $OR\ 0.75$ , 95% CI 0.70–0.81). In contrast, underweight people had lower odds of seeking treatment than people of normal weight ( $OR\ 0.58$ , 95% CI 0.53–0.64), but higher odds of controlling their hypertension ( $OR\ 1.32$ , 95% CI 1.10–1.59).

**Table 2.** The prevalence of hypertension (1986–2010) and the proportions of individuals with uncontrolled hypertension (1975–2010), medicated hypertension (1986–2020) and controlled hypertension (1986–2010).

		Hypertension (1986–2010, n = 167,506)		Uncontrolled Hypertension (1975–2010, n = 300,429)		Medicated Hypertension (1986–2010, n = 73,758)		Controlled Hypertension (1986–2010, n = 28,519)	
Age (years)		Frequency in Each Age Group (Age Group Total)	Proportion (%) in Each Age Group (95% CI)	Frequency in Each Age Group (Age Group Total)	Proportion (%) in Each Age Group (95% CI)	Frequency in Each Age Group (Age Group Total)	Proportion (%) in Each Age Group (95% CI)	Frequency in Each Age Group (Age Group Total)	Proportion (%) in Each Age Group (95% CI)
Men	20 to 34	1886 (11,638)	16.2 (15.5–16.9)	4737 (26,539)	17.8 (17.4–18.3)	100 (1886)	5.3 (4.3–6.3)	65 (100)	65.0 (55.7–74.3)
	35 to 49	6409 (18,057)	35.5 (34.8–36.2)	13,379 (37,055)	36.1 (35.6–36.6)	826 (6409)	12.9 (12.1–13.7)	213 (826)	25.8 (22.8–28.8)
	50 to 64	21,193 (33,253)	63.7 (63.2–64.2)	31,559 (52,918)	59.6 (59.2–60.0)	8236 (21,193)	38.9 (38.2–39.5)	1884 (8236)	22.9 (22.0–23.8)
	At least 65	4712 (6104)	77.2 (76.1–78.2)	5707 (8359)	68.3 (67.3–69.3)	2611 (4712)	55.4 (54.0–56.8)	741 (2611)	28.4 (26.7–30.1)
Women	20 to 34	670 (16,301)	4.1 (3.8–4.4)	1811 (36,669)	4.9 (4.7–5.2)	118 (670)	17.6 (14.7–20.5)	102 (118)	86.4 (80.3–92.6)
	35 to 49	5638 (27,642)	20.4 (19.9–20.9)	12,274 (54,311)	22.6 (22.2–23.0)	993 (5638)	17.6 (16.6–18.6)	331 (993)	33.3 (30.4–36.3)
	50 to 64	26,121 (45,586)	57.3 (56.8–57.8)	39,539 (72,387)	54.6 (54.3–55.0)	11,217 (26,121)	42.9 (42.3–43.5)	2836 (11,217)	25.3 (24.5–26.1)
	At least 65	7129 (8925)	79.9 (79.0–80.7)	8517 (12,191)	69.9 (69.0–70.7)	4418 (7129)	62.0 (60.8–63.1)	1205 (4418)	27.3 (26.0–28.6)

**Table 3.** The means and proportions of risk factors by sex for the survey years of 1975, 1990, 2000, and 2015.

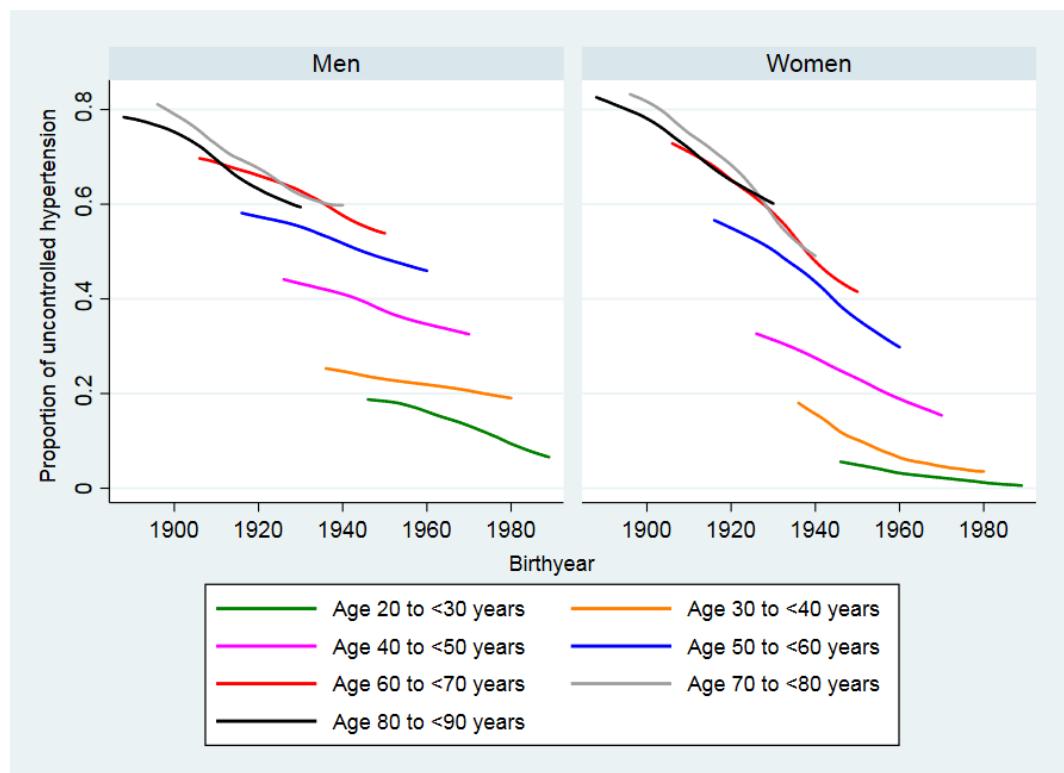
Survey Year	1975		1990		2000		2010	
	Men	Women	Men	Women	Men	Women	Men	Women
Mean age in years (SD)	44.6 (15.2)	44.5 (15.3)	50.1 (15.8)	50.1 (15.9)	54.1 (16.2)	53.4 (15.9)	59.4 (16.0)	58.1 (16.0)
Mean salt in g/day (SD)	NA	NA	NA	NA	14.3 (6.0)	12.5 (5.0)	11.8 (4.6)	9.7 (3.8)
Mean BMI in kg/m <sup>2</sup> (SD)	22.2 (2.9)	22.4 (3.3)	22.9 (3.0)	22.7 (3.3)	23.5 (3.2)	22.9 (3.5)	23.8 (3.2)	22.7 (3.5)
BMI frequency (%)	Underweight	680 (7.6%)	1224 (9.7%)	246 (6.2%)	439 (8.1%)	97 (4.0%)	290 (8.5%)	61 (3.6%)
	Normal weight	6758 (76.0%)	8948 (70.7%)	2822 (70.9%)	3783 (69.7%)	1617 (66.7%)	2311 (67.5%)	1068 (63.8%)
	Obese	1456 (16.4%)	2476 (19.6%)	913 (22.9%)	1205 (22.2%)	711 (29.3%)	824 (24.1%)	546 (32.6%)
Smoking frequency (%)	Non-smoker	NA	NA	1755 (44.0%)	4890 (90.0%)	1310 (54.0%)	3045 (89.0%)	440 (26.3%)
	Current smoker	NA	NA	2230 (56.0%)	542 (10.0%)	1115 (46.0%)	378 (11.0%)	1230 (73.7%)
Drinking alcohol frequency (%)	Less than 4 times a week	NA	NA	1777 (44.6%)	5089 (93.7%)	1170 (48.3%)	3113 (91.0%)	483 (28.9%)
	More than 4 times a week	NA	NA	2208 (55.4%)	343 (6.3%)	1254 (51.7%)	309 (9.0%)	1187 (71.1%)
Exercise frequency (%)	Less than 3 times a week	NA	NA	3063 (76.9%)	4414 (81.3%)	1626 (67.1%)	2454 (71.7%)	1063 (63.6%)
	More than 3 times a week	NA	NA	922 (23.1%)	1018 (18.7)	796 (32.9%)	967 (28.3%)	609 (36.4%)
Occupation frequency (%)	Primary industry	1218 (14.9%)	1418 (12.4%)	425 (12.5%)	415 (8.5%)	184 (10.1%)	142 (4.8%)	145 (8.8%)
	Secondary industry	2378 (29.1%)	1676 (14.7%)	951 (27.9%)	914 (18.8%)	457 (25.2%)	467 (15.8%)	231 (14.0%)
	Tertiary industry	4572 (56.0%)	8312 (72.9%)	2028 (59.6%)	3532 (72.7%)	1176 (64.7%)	2344 (79.4%)	1277 (77.3%)
								2137 (92.1%)

**Table 4.** The adjusted odds ratios for the prevalence of hypertension and the proportion of medicated and controlled hypertension for the survey years 1986 to 2010.

		Prevalence of Hypertension (n = 144,299)		Proportion of Medicated Hypertension (n = 109,229)		Proportion of Controlled Hypertension (n = 28,301)	
		OR (95%CI)	p-Value	OR (95%CI)	p-Value	OR (95%CI)	p-Value
Survey year	Per increase of one year	0.99 (0.99–0.99)	<0.001	1.02 (1.02–1.02)	<0.001	1.05 (1.04–1.05)	<0.001
Sex	Male	1.35 (1.31–1.39)		0.96 (0.92–1.00)	0.05	1.06 (0.97–1.15)	0.22
	Female	1	NA	1	NA	1	NA
Age (years)	20 to 34	1	NA	NA	NA	NA	NA
	35 to 49	3.22 (3.07 to 3.37)	<0.001	1	NA	1	NA
	50 to 64	13.49 (12.89–14.13)	<0.001	6.47 (6.13–6.83)	<0.001	0.56 (0.50–0.63)	<0.001
	at least 65	42.85 (39.93–46.00)	<0.001	19.09 (17.75–20.53)	<0.001	0.54 (0.47–0.62)	<0.001
BMI	Underweight	0.54 (0.51–0.57)	0.04	0.58 (0.53–0.64)	<0.001	1.32 (1.10–1.59)	0.003
	Normal	1	NA	1	NA	1	NA
	Obese	2.55 (2.48–2.62)	<0.001	2.07 (1.99–2.14)	<0.001	0.75 (0.70–0.81)	<0.001
Smoking	Non-smoker	1	NA	1	NA	1	NA
	Current smoker	0.92 (0.89–0.94)	<0.001	0.75 (0.72–0.79)	<0.001	0.97 (0.87–1.07)	0.49
Drinking alcohol	Less than 4 times a week	1	NA	1		1	NA
	At least 4 times a week	1.52 (1.47–1.57)	<0.001	1.21 (1.15–1.27)	<0.001	0.69 (0.63–0.77)	<0.001
Exercise	Less than 3 times a week	NA	NA	1	NA	1	NA
	At least 3 times a week	NA	NA	1.09 (1.04–1.13)	<0.001	1.09 (1.01–1.17)	0.03
Salt intake	Per 1 g/day	NA	NA	NA	NA	NA	NA
Occupation	Primary industry	1.14 (1.09–1.19)	<0.001	1.13 (1.07–1.19)	<0.001	0.99 (0.89–1.10)	0.83
	Secondary industry	1.00 (0.97–1.03)	0.99	0.83 (0.79–0.87)	<0.001	0.86 (0.77–0.96)	0.007
	Tertiary industry	1	NA	1	NA	1	NA

### 3.5. Effect of Birth Cohort on Uncontrolled Hypertension

Figure 2 shows that the proportion of individuals with uncontrolled hypertension by birth year for birth cohorts declined for cohorts born in later years in both men and women of all age groups. The decline appears to be steeper for women than men for all age groups.



**Figure 2.** Uncontrolled hypertension in Japan by birth year (1886 to 1989) and sex for birth cohorts.

## 4. Discussion

This study investigated the prevalence of hypertension and risk factors, the proportions of treatment and control among Japanese adults from 1986 to 2010, the proportion of individuals with uncontrolled hypertension, and the influence of birth cohorts from 1975 to 2010 using a large annual cross-sectional survey. Our study expanded on previous studies based on NHNS data on blood pressure levels [5,7] and on the prevalence of hypertension in Japan [7].

In the time period preceding our data, an analysis of NHNS data collected between 1956 and 1980 showed that the proportion of individuals with uncontrolled hypertension defined as systolic blood pressure over 180 mmHg declined in the age groups over 50 but did not markedly change in the younger population [7]. The proportion of individuals with uncontrolled hypertension, defined as diastolic blood pressure over 100 mmHg, also declined for age groups over 50 but did not markedly change in the younger population [7]. In our analysis of NHNS data between 1975 and 2010, we found that the proportion of individuals with uncontrolled hypertension declined for men and women of all ages with steeper declines in women and in the older population.

The risk factors for having hypertension identified in our study were consistent with those previously reported as being male, of older age, obese, drinking alcohol, and working in the primary industry. These risk factors, except occupation-related factors, were also investigated in a previous analysis of blood pressure levels based on NHNS data between 1986 and 2002 [5] and correlated with having a systolic blood pressure of at least 140 mmHg. Drinking alcohol has consistently been reported as a risk factor in other reports [5,7,26]. In contrast to other investigators [27,28], we did not

find evidence of an effect of salt intake on the prevalence of hypertension. One possible reason is that salt intake was estimated from self-reported food intake [8], which may not be precise enough for a quantitative analysis.

In our analysis, smoking appears to have a blood pressure lowering effect, which has also been reported elsewhere [29]. This effect has been a subject of controversy because smoking is a known risk factor for atherosclerosis, which may lead to more severe forms of hypertension [29]. There is agreement that smoking is overall harmful regardless of its potential impact on blood pressure [29].

Our results show that primary industry workers are at increased risk of having hypertension. Although specific information on income or socioeconomic status was not collected in the NHNS, the classification of occupations into industries could serve as a proxy indicator of socioeconomic status in Japan, because more people of lower education and lower socioeconomic status work in the primary and secondary industries. Similar results have been found in Western Europe where the odds of hypertension was inversely associated with the education level [30] and for the US where people of lower socioeconomic status had a higher incidence of hypertension [31].

Our analysis revealed that the prevalence of hypertension declined for men and women except for men between 50 and less than 65. The steepest declines were seen in women of 35 and older. Additionally, the prevalence of hypertension was consistently lower in women than in men of the same age except for women in the age group of 65 and older. Possible explanations for the lower prevalence of hypertension in Japanese women are that they consume less alcohol than men and have a lower BMI than men of the same age [32]. Japan has been successful in managing obesity in women, but obesity prevalence in Japanese men is still increasing [5,32]. We found that the decline in the proportion of individuals with uncontrolled hypertension seemed to have resulted from successful control by medication. A previous report also concluded that control of hypertension by medication was the main reason for a decline of systolic blood pressure in all people, except in young women [5]. Furthermore, we found that birth cohorts of women born between 1920 and 1960 experienced a steeper decrease in the proportion of individuals with uncontrolled hypertension than men born in the same years.

Our data suggest that the proportion of hypertensive respondents who sought treatment and of those who successfully controlled their hypertension has been increasing with time. This confirmed a result from the survey “National Integrated Project for Prospective Observation of Non-communicable Disease and its Trends in the Aged” (NIPPON DATA) 1980 to 2010, a large survey that is undertaken every 10 years [6]. We found that the adjusted odds of seeking treatment were higher for older and obese respondents, alcohol consumers, workers in the primary industry and individuals who exercised. The adjusted odds of controlling hypertension were higher for underweight and younger respondents and individuals who exercised, suggesting that respondents who are more health conscious are also more likely to control their blood pressure.

In Japan, every employee is required to undergo a yearly health examination that includes blood pressure measurements [33] and every resident 40 years and older regardless of employment can receive an optional inexpensive yearly health examination that includes a blood pressure measurement [34]. Health examinations in middle age have been shown to be associated with a lower utilization of hospital beds by the elderly [34]. Although the early detection of hypertension was not specifically investigated, it may be one of the factors contributing to the lower utilization of hospital beds.

The global targets for non-communicable diseases for 2025 set by the WHO in 2011 include a reduction in the prevalence of age-standardized hypertension by 25% [1]. In 2009 it was 36.8% (95% CI 35.4–38.3%) in Japan [35] and would thus need to decrease to the level of about 28% to meet the WHO target. The Japanese Society of Hypertension recommends the following public healthcare measures to lower the systolic blood pressure of the Japanese: improved nutrition, more exercise, lower alcohol consumption for men, and seeking treatment [36]. Our results provide further evidence for these measures, in particular, that efforts should be made to ensure that men achieve the same decrease in the prevalence of hypertension as women by addressing obesity and drinking, especially targeting

middle-aged men. The prevalence of hypertension among Japanese people of 50 years and older remains high, so raising awareness for seeking treatment and controlling blood pressure should be a priority, in particular, among men.

There were limitations in this analysis. The blood pressure measurements were changed from one to two measurements in the year 2000. We used the averaged blood pressure values which may have resulted in lower blood pressure than using only the first measurement. The NHNS contained consistent information on hypertension risk factors only for sex, age, BMI and occupation over the whole time period of 1975 to 2010. Data on hypertension medication, smoking, drinking alcohol and exercising started being collected from 1986, data on exercising were missing for the years of 2008 and 2009 and data on individual level salt intake were recorded from 2000. The participation rate of the physical NHNS examination was about 50 to 60% [9,10], which may have introduced selection bias. Randomization schemes and probability weights were not available, thus, we could not carry out a rigorous survey analysis [9,10,37]. We could not quantitatively compare our results with those of previous reports because of differences in definitions and statistical methods.

## 5. Conclusions

The prevalence of hypertension has been declining in Japan in adults except recently in middle-aged men. The proportion of individuals with uncontrolled hypertension has been declining in all adults. Japanese women consume less alcohol than men and have a lower BMI than men which may partly explain the lower prevalence of hypertension in women than in men except in the oldest age group. The proportion of individuals with uncontrolled hypertension declined for cohorts born in later years in both men and women of all age groups with a steeper decline for women than men. Raising awareness for seeking treatment and controlling hypertension among the Japanese hypertensive population, in particular, those over 50 years old and lifestyle changes among men could bring Japan nearer the goal of reducing the burden of hypertension, with considerable positive health impacts in Japan's aging population.

**Author Contributions:** Conceptualization, K.O. and S.G.; Methodology, K.O. and S.G.; Software, Stata14, StataCorp LP, College Station, USA; Validation, K.O., R.H. and S.G.; Formal Analysis, K.O.; Investigation, K.O., R.H. and S.G.; Resources, S.G.; Data Curation, K.O., R.H. and S.G.; Writing-Original Draft Preparation, K.O.; Writing-Review & Editing, K.O., R.H. and S.G.; Supervision, S.G.; Project Administration, S.G.; Funding Acquisition, S.G.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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## ORIGINAL ARTICLE

## EPIDEMOIOLOGY, CLINICAL PRACTICE AND HEALTH

# Prevalence and predictors of co-occurring diabetes and hypertension in community-dwelling older adults

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**Aim:** The objectives of the present study were to estimate the prevalence of co-occurring diabetes and hypertension among older adults, examine predictors of co-occurring diabetes and hypertension, and ascertain whether predictors varied by race.

**Methods:** A retrospective analysis was carried out using a statewide survey of Alabama community-dwelling older adults ( $n = 1204$ ). Measures of central tendency and frequency distributions were used for univariate analysis. Logistic regression was used to predict co-occurring diabetes and hypertension.

**Results:** The prevalence of co-occurring diabetes and hypertension among older adults was 17%. African American race (OR 2.28, 95% CI 1.596–3.255), body mass index  $\geq 30$  (OR 2.45, 95% CI 1.732–3.463), heart disease (OR 1.93, 95% CI 1.355–2.756) and eye disease (OR 1.44, 95% CI 1.018–2.024) were associated positively with co-occurring diabetes and hypertension.

**Conclusions:** The prevalence of co-occurring diabetes and hypertension among older adults was alarmingly high. The notable difference in the likelihood of co-occurring diabetes and hypertension is representative of a racial health disparity that largely disfavors African American older adults. Findings from the present study highlight a need for identification of older adults who have and who are at risk of co-occurring diabetes and hypertension in the general population and in clinical settings, and the development and implementation of suitable interventions, particularly targeting older African American adults. *Geriatr Gerontol Int* 2018; **•••**: **•••**.

**Keywords:** diabetes, hypertension, older adults, predictors, prevalence.

## Introduction

According to recent estimates, the prevalence of diabetes and of hypertension among older adults in the USA is 33% and 65% respectively.<sup>1,2</sup> Although two distinct disease states, risk factors for diabetes and hypertension are quite similar. Consequently, diabetes and hypertension are commonly associated with each other and frequently co-occur. Commonly shared risk factors include, but are not limited to, physical inactivity, obesity, unhealthy diet, heavy alcohol use and smoking; those who live in rural areas are more likely to have diabetes and hypertension compared with those who live in urban areas.<sup>3–6</sup>

Among older adults, both diabetes and hypertension are independent risk factors for frailty, cardiovascular disease and chronic kidney disease;<sup>7–9</sup> and co-occurring diabetes and hypertension has synergistic effects on morbidity and mortality. Co-occurring diabetes and hypertension increases all-cause mortality by 38% and cardiovascular disease by 70%.<sup>10</sup> Furthermore, persons with co-occurrence have 3.67 higher odds of frailty.<sup>11</sup> Hence, the accelerated pathophysiology associated with co-occurring diabetes and hypertension has been coined “an unholy alliance”.<sup>12</sup>

To date, the majority of studies on older adults characterize and examine diabetes or hypertension alone, or highlight ways in which one is associated with the other,<sup>13–17</sup> whereas only Balogun and Salako 2011 have sought to characterize and examine co-occurrence.<sup>18</sup> However, this examination was limited to clinical and metabolic differences between hypertensive patients who developed diabetes and patients with diabetes who developed hypertension. Thus, the prevalence of co-occurring diabetes and hypertension among older adults has not been well-established, and predictors of co-occurring diabetes and hypertension have been largely unexamined. This is an important public health and clinical consideration given the adverse effects of co-occurring diabetes and hypertension on the survival and well-being of older adults. To fill this knowledge gap, using a sample of community-dwelling older adults living in the state of Alabama, the present study estimated the prevalence of co-occurring diabetes and hypertension among older adults, examined predictors of co-occurring diabetes and hypertension, and ascertained whether predictors varied by race.

## Methods

The present study analyzed data from an Alabama Medicaid Agency-funded long-term care needs assessment carried out by the Comprehensive Center for Healthy Aging at the University of Alabama at Birmingham known as “Charting the Course.” The purpose of Charting the Course was to assess current and emerging medical conditions and social needs of older Alabama residents. To recruit a sample representative of the state of Alabama, counties were selected based on the size of the county and geographic distribution, and it was determined a sample size of 1200 would give adequate power to detect differences by age, race, sex and area of residence. A stratified random sampling design was used to ensure that the proportion of participants would represent the state population aged >55 years.

Of residents randomly selected for participation, a letter was mailed to inform and recruit potential participants for in-home interview assessments. A toll free number was provided for persons to inquire about the study. In-home face-to-face interviews were scheduled for respondents confirmed to be aged ≥55 years, able to communicate with an interviewer and living in the community.

The survey questionnaire was developed and approved by the State of Alabama Long-Term Care Task Force and the University of Alabama at Birmingham’s Comprehensive Center for Healthy Aging. Survey questions included, but were not limited to, socio-demographics, general health, health services utilization, medical history, cognitive functioning and emotional health, spirituality, social care, retirement planning, and long-term care planning. Interviewers for Charting the Course interviewed 1204 community-dwelling older adults. All participants provided signed informed consent before being interviewed. The analysis for the present study included the total sample of participants. The current study was approved by the institutional review boards of the University of Alabama at Birmingham and the University of Alabama.

## Outcomes

### **Co-occurring diabetes and hypertension**

The main outcomes were prevalence and predictors of co-occurring diabetes and hypertension. Co-occurring diabetes and hypertension was defined for participants who reported having medical diagnoses of diabetes and hypertension.

## Predictors

### **Demographics**

Demographic data included sex, race (African American and white), age, education (reported in years of education), income (reported categorically as ≤\$19 999, \$20 000–\$49 999, ≥\$50 000) and residence (urban vs non-urban).

### **Self-rated general health and physical activity**

Self-rated general health was assessed by a single-item Likert scale question that assessed overall health, “In general, how would you say your health is?”: 1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent. Self-rated physical activity was a single-item Likert scale question that assessed physical activity, “How would you describe your level of physical activity?”: 1 = not active, 2 = minimally active, 3 = moderately active and 4 = active.

## Obesity

Body Mass Index (BMI) was calculated using self-reported weight and height. Obesity was defined as BMI ≥30.

### **Tobacco use**

Participants who reported current cigarette use were defined as current cigarette smokers.

### **Medical conditions**

Medical conditions included heart disease and eye disease.

### **Depression**

Scores from the Geriatric Depression Scale (Short Form), a 15-item self-report assessment of depression, were used to identify older adults with depressive symptomatology (a score >5).<sup>19</sup>

### **Statistical analysis**

Measures of central tendency and frequency distributions were used for univariate analysis. Student’s *t*-tests and the  $\chi^2$ -test were used to

**Table 1** Characteristics of older adults

Variables (Categories)	Frequency (n)	Percent (%)	Mean (SD)
<b>Demographics</b>			
Sex			
Male	514	42.7	
Female	690	57.3	
Race			
White	877	72.8	
African American	327	27.2	
Age (range 55–90)			68.52 (8.70)
Education			
1–11 years	306	25.4	
High school	365	30.3	
College or Higher	532	44.2	
Income			
Less than \$19 999	460	44.0	
\$20 000–\$49 999	360	34.4	
\$50 000 or more	225	21.5	
Residence			
Urban	720	59.8	
Non-urban	484	40.2	
<b>Self-rated Health and Physical Activity</b>			
General health (range 1–5)			2.89 (1.19)
Physical activity level (range 1–4)			2.01 (0.97)
Obesity			
No (BMI < 30)	803	68.9	
Yes (BMI ≥ 30)	363	31.1	
<b>Tobacco Use</b>			
Smoke cigarettes			
No	1035	86.0	
Yes	169	14.0	
<b>Medical Conditions</b>			
Diabetes			
No	950	78.9	
Yes	254	21.1	
Hypertension			
No	452	37.5	
Yes	752	62.5	
Heart disease			
No	774	64.3	
Yes	430	35.7	
Eye disease			
No	613	50.9	
Yes	591	49.1	
Depression			
No	565	46.9	
Yes	639	53.1	

compare numerical and dichotomous variables between those with and without co-occurring diabetes and hypertension. Logistic regression was used to determine which predictors were associated with co-occurring diabetes and hypertension. The correlations ( $-0.003 \leq r \leq 0.193$ ) and variance inflation factor statistics ( $\geq 1.256$ ) were checked during preliminary analysis. No multicollinearity problem was observed in the multivariate analysis. SPSS Statistics 23 (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses.

## Results

More than half of the sample was female (58%) and white (73%; Table 1). The average age was 69 years (range 55–90 years). Nearly half of the sample (44%) had an income of <\$19 999, and the majority resided in an urban area (60%). Self-rated general health was fair (2.89, SD 1.19), and physical activity was minimally active (2.01, SD 0.97). One-third of participants were obese, and 14% smoked cigarettes. A total of 49% reported eye disease, 36% reported heart disease and 53% reported depressive symptomatology.

The prevalence of co-occurring diabetes and hypertension among older adults was 17% (Table 2). Compared with older adults without co-occurring diabetes and hypertension, a higher

proportion of older adults with co-occurring diabetes and hypertension had BMI  $\geq 30$  ( $\chi^2 = 50.96$ ,  $P = 0.000$ ), heart disease ( $\chi^2 = 46.45$ ,  $P = 0.000$ ), eye disease ( $\chi^2 = 14.33$ ,  $P = 0.000$ ) and depression ( $\chi^2 = 10.75$ ,  $P = 0.001$ ), and lower levels of self-rated general health ( $t = 13.26$ ,  $P = 0.000$ ) and physical activity ( $t = 5.17$ ,  $P = 0.000$ ). Those with and without co-occurring diabetes and hypertension did not differ in sex, age, residence or smoking status.

In the logistic regression analysis (Table 3), African American race (OR 2.28, 95% CI 1.596–3.255), BMI  $\geq 30$  (OR 2.45, 95% CI 1.732–3.463), heart disease (OR 1.93, 95% CI 1.355–2.756), and eye disease (OR 1.44, 95% CI 1.018–2.024) were associated positively with co-occurring diabetes and hypertension. Low levels of self-rated general health were associated negatively with co-occurring diabetes and hypertension (OR 0.50, 95% CI 0.415–0.610).

Predictors of co-occurring diabetes and hypertension did not vary by race (Table 4). Obesity and heart problems were associated positively with co-occurring diabetes and hypertension for both African American and white participants, and low levels of self-rated general health were associated negatively with co-occurring diabetes and hypertension for both racial groups.

**Table 2** Comparison of older adults with and without co-occurring diabetes and hypertension

	Co-occurring diabetes and hypertension		Significance
	No (n = 994)	Yes (n = 210)	
<b>Demographics</b>			
Sex, n (%)			$\chi^2 (1) = 2.19$ , ( $P = 0.138$ )
Male	434 (43.7%)	80 (38.1%)	
Female	560 (56.3%)	130 (61.9%)	
Race, n (%)			$\chi^2(1) = 39.83$ , ( $P = 0.000$ )*
White	761 (76.6%)	116 (55.2%)	
African American	233 (23.4%)	94 (44.8%)	
Mean age, years (SD)	68.52 (8.86)	68.51 (7.95)	$t = -0.017$ , ( $P = 0.986$ )
Education			$\chi^2(2) = 25.30$ , ( $P = 0.000$ )*
1–11 years	230 (23.2%)	76 (36.2%)	
High school	293 (29.5%)	72 (34.3%)	
College or higher	470 (47.3%)	62 (29.5%)	
Income			$\chi^2(2) = 25.93$ , ( $P = 0.000$ )*
<\$19 999	349 (40.7%)	111 (59.4%)	
\$20 000–49 999	304 (35.4%)	56 (29.9%)	
≥\$50 000	205 (23.9%)	20 (10.7%)	
Residence, n (%)			$\chi^2(1) = 3.21$ , ( $P = 0.073$ )
Urban	606 (61.0%)	114 (54.3%)	
Non-urban	388 (39.0%)	96 (45.7%)	
<b>Self-rated health and physical activity</b>			
Mean general health (SD)	3.06 (1.17)	2.09 (0.916)	$t = -13.26$ , ( $P = 0.000$ )*
Mean physical activity level (SD)	2.08 (0.953)	1.70 (0.998)	$t = -5.17$ , ( $P = 0.000$ )*
Obesity, n (%)			$\chi^2(1) = 50.96$ , ( $P = 0.000$ )*
No (BMI <30)	706 (73.3%)	97 (47.8%)	
Yes (BMI $\geq 30$ )	257 (26.7%)	106 (52.2%)	
<b>Tobacco use</b>			
Smoke cigarettes, n (%)			$\chi^2(1)=0.98$ , ( $P = 0.328$ )
No	850 (85.5%)	185 (88.1%)	
Yes	144 (14.5%)	25 (11.9%)	
<b>Medical and psychiatric conditions</b>			
Heart disease, n (%)			$\chi^2(1) = 46.45$ , ( $P = 0.000$ )*
No	682 (68.6%)	92 (43.8%)	
Yes	312 (31.4%)	118 (56.2%)	
Eye disease, n (%)			$\chi^2(1) = 14.33$ , ( $P = 0.000$ )*
No	531 (53.4%)	82 (39.0%)	
Yes	463 (46.6%)	128 (61.1%)	
Depression, n (%)			$\chi^2(1) = 10.75$ , ( $P = 0.001$ )
No	488 (49.1%)	77 (36.7%)	
Yes	506 (50.9%)	133 (63.3%)	

\* $P < 0.05$ . Age range 55–90 years. Bold indicates statistical significance.

**Table 3** Logistic regression predicting co-occurring diabetes and hypertension

Variables	Categories	OR	95% CI	P-values
Race	White	1		
	African American	2.279	1.596–3.255	0.000*
Sex	Male	1		
	Female	1.171	0.828–1.656	0.372
Residence	Urban	1		
	Rural	1.078	0.766–1.518	0.666
General health		0.503	0.415–0.610	0.000*
Physical activity level		1.178	0.974–1.425	0.092
Obesity	No (BMI <30)	1		
	Yes (BMI ≥30)	2.449	1.732–3.463	0.000*
Smoke cigarettes	No	1		
	Yes	0.733	0.445–1.206	0.221
Heart disease	No	1		
	Yes	1.932	1.355–2.756	0.000*
Eye disease	No	1		
	Yes	1.435	1.018–2.024	0.039*
Depression	No	1		
	Yes	1.247	0.873–1.781	0.225

\*P < 0.05. CI, confidence interval; OR, odds ratio.

**Table 4** Logistic regression predicting co-occurring diabetes and hypertension by race

Variables	Categories	White OR (95% CI)	African American OR (95% CI)
Sex	Male	1	1
	Female	1.236 (0.787–1.940)	1.136 (0.652–1.979)
Residence	Urban	1	
	Rural	1.350 (0.869–2.097) <b>0.504 (0.395–0.644)</b>	0.725 (0.411–1.279) <b>0.507 (0.366–0.702)</b>
General health			
Physical activity level		1.125 (0.878–1.442)	1.256 (0.929–1.697)
Obesity	No (BMI <30)	1	
	Yes (BMI ≥30)	<b>3.092 (1.979–4.829)</b>	<b>1.744 (1.001–3.040)</b>
Smoke cigarettes	No	1	
	Yes	0.785 (0.397–1.549)	0.692 (0.333–1.438)
Heart disease	No	1	
	Yes	<b>1.994 (1.255–3.169)</b>	<b>1.925 (1.094–3.388)</b>
Eye disease	No	1	
	Yes	1.314 (0.835–2.067)	1.714 (1.000–2.937)
Depression	No	1	
	Yes	1.267 (0.800–2.007)	1.178 (0.666–2.084)

\*P < 0.05. CI, confidence interval; OR, odds ratio. Bold indicates statistical significance.

## Discussion

Several main findings emerged from the present study. The prevalence of co-occurring diabetes and hypertension was 17%. Older adults who were African American, obese, and had heart disease or eye disease had greater odds of co-occurring diabetes and hypertension. Predictors of co-occurring diabetes and hypertension did not vary by race.

The prevalence of co-occurring diabetes and hypertension among older adults was alarmingly high. Given the multiplicative effects of co-occurrence on frailty, cardiovascular disease, renal disease and renal failure, identification and management of older adults with co-occurring diabetes and hypertension in the general population and in clinical settings are of public health importance. Public health efforts are required to educate older adults of increased health risks associated with having both disease states, and more clinical research is needed to assist clinicians with proper management of co-occurrence.

The notable difference in the likelihood of co-occurring diabetes and hypertension is representative of a racial health disparity that largely disfavors African American older adults. If suitable interventions to prevent these diseases are not designed to target this

population, the potential is for an increase in annual incident cases of co-occurring diabetes and hypertension-related morbidity and mortality among the growing numbers of African American older adults.

Predictors of co-occurring diabetes and hypertension did not vary by race. Although there are no prior studies that have examined whether predictors of co-occurring diabetes and hypertension vary by race, this finding suggests that independent risk factors for co-occurring diabetes and hypertension are not race specific. More importantly, of the independent risk factors, obesity had the highest odds of predictive association with co-occurring diabetes and hypertension. This finding is not surprising, given that obesity is a leading risk factor for both diabetes and hypertension. The global prevalence of obesity has nearly doubled in the past three decades;<sup>20</sup> in the USA, 36% of adults have a BMI ≥30,<sup>21</sup> it is estimated nearly 90% of diabetes is attributable to excess weight<sup>22</sup> and excess weight accounts for 65–75% of the risk for hypertension.<sup>21</sup> This vicious synergistic combination of obesity, diabetes, and hypertension also increases the risk for cardiovascular disease and chronic kidney disease.<sup>9</sup> Altogether, this is an important issue for public health professionals to address for the state of Alabama, because Alabama has the second highest rate for diabetes<sup>23</sup> and the third highest rate for hypertension in the USA.<sup>24</sup>

Obesity and heart disease were risk factors across both racial groups. Health professionals and clinicians should closely monitor older adults with these conditions, and recommend strategies to prevent onset and exacerbation of both diabetes and hypertension. Physical activity as an evidence-based treatment modality for the prevention and treatment of both diabetes and hypertension has been well established.<sup>22</sup> In addition, lifestyle changes, such as healthy dieting, and smoking and alcohol cessation, have been shown to be therapeutic for both diabetes and hypertension.<sup>25,26</sup> Although understudied in co-occurring diabetes and hypertension populations, it is likely lifestyle changes will have similar, if not greater, therapeutic benefits.

The present study had some noteworthy limitations and strengths. The limitations of this study were its cross-sectional nature; the absence of sufficient data on alcohol use, anxiety and diet; and reliance on self-reported data. Given the geographic context of the study, the prevalence estimate of co-occurring diabetes and hypertension might not be generalizable to other states. Notwithstanding, the study had a relatively large number of older adults ( $n = 1204$ ) and African American older adults ( $n = 233$ ). The stratified random sampling strategy permitted analysis of a sample that was closely representative of community-dwelling older adults in the state of Alabama. To the authors' knowledge, this is the first study to estimate the prevalence of co-occurring diabetes and hypertension among older adults in the UAS, and to examine predictors of co-occurrence.

In summary, more research is required to prevent older adults with diabetes from developing hypertension, and hypertensive older adults from developing diabetes. Targeted public health screening and educational efforts can raise public awareness of risk factors and adverse outcomes associated with co-occurring diabetes and hypertension. The findings of the present study highlight a need for identification of older adults who have and who are at risk of co-occurring diabetes and hypertension in the general population and in clinical settings, and development and implementation of suitable interventions, particularly targeting older African American adults. Based on the findings of this study, it is advisable that intervention development purposefully includes health behavior and medical approaches to negate the pathophysiological role of obesity in co-occurring diabetes and hypertension.

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## Disclosure statement

The authors declare no conflict of interest.

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RESEARCH ARTICLE

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# Metabolic syndrome in Russian adults: associated factors and mortality from cardiovascular diseases and all causes

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## Abstract

**Background:** Metabolic syndrome (MetS) is a cluster of four major obesity-related risk factors for cardiovascular disease (CVD). Russia has one of the highest CVD mortality in the world, but its association with MetS remains unknown. Also little is known about factors associated with MetS and its components in Russia.

**Methods:** Data on 3555 adults aged 18-90 years were collected in a cross-sectional study in 2000. MetS was defined by the International Diabetes Federation (IDF) and National Cholesterol Education Program (NCEP) criteria. Sex-specific associations between the IDF-defined MetS, its components, and life-style, socio-economic factors and laboratory indicators, were analysed using multivariable Poisson regression. Vital status of the study participants was identified by July 2009. Sex-specific associations between MetS and stroke, Coronary Heart Disease (CHD), CVD and all-cause death, were studied by Poisson regression adjusted for age, smoking, alcohol and history of CVDs.

**Results:** After adjustment for all studied factors except BMI, age, serum GGT, C-reactive protein and AST-to-ALT ratio were associated with MetS in both genders. Additionally, MetS was associated with sedentary lifestyle in women and with smoking in men. In the same regression model drinking alcohol 2-4 times a month and consumption of five or more alcohol units at one occasion in men, and drinking alcohol 5 times or more a month in women were inversely associated with MetS. After a 9-year follow-up, MetS was associated with higher risk of death from stroke (RR = 3.76, 95% CI:1.35-10.46) and from either stroke or myocardial infarction (MI, RR = 2.87, 95% CI:1.32-6.23) in men. No associations between MetS and any of the studied causes of death were observed in women.

**Conclusion:** Factors associated with MetS in both genders were age, GGT, C-reactive protein, and AST-to-ALT ratio. Moderate frequency of alcohol consumption and binge drinking in men and higher leisure time physical activity in women, were inversely associated with MetS.

Positive associations between MetS and mortality were only observed for deaths from stroke and either stroke or MI in men.

## Background

The metabolic syndrome (MetS) is a cluster of four major cardiovascular disease (CVD) risk factors; obesity, insulin resistance (hyperglycemia), arterial hypertension and dyslipidemia where obesity and insulin resistance are the core elements [1]. Other important characteristics of MetS include low-grade inflammation, endothelial dysfunction, plasma hypercoagulability and atherosclerosis [2].

MetS is associated with increased CVD and all-cause mortality [3,4]. Moreover, it may be used as an alternative to the classic coronary heart disease (CHD) risk assessment scale such as the Framingham Risk Score [5]. The prevalence of MetS varies greatly between countries and ethnic groups [6]. Among Europeans and white Americans it varies between 20% and 30% with similar gender distribution [7,8]. Due to its high prevalence, MetS is considered as the major public health problem in Europe, and, particularly in the USA, where obesity and overweight are the second leading cause of preventable death accounting for 300.000 deaths per year [9].

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The prevalence of MetS is associated with life-style, demographic, socio-economic, and genetic factors. Age, body mass index, postmenopausal status, diet rich in saturated fats, carbohydrates, and smoking have been positively associated with MetS, while inverse associations have been shown for physical activity, education, income, and alcohol intake [7,10-12].

Cardiovascular mortality in Russia is about four times higher than in Western Europe and the gap is the largest among middle aged men [13]. Although there is evidence for a high contribution of hazardous level of alcohol consumption to high death rates in Russia [14-16], other factors also need to be investigated. As MetS represents a cluster of the four of six major cardiovascular risk factors strongly associated with CVD mortality, one might expect similar high rates of MetS or its components in Russia.

Despite the fact that determinants of MetS and its contribution to mortality in Europe and North America receive much attention by the research community, it remains one of the least studied factors in Russia. In an earlier study we showed that while the prevalence of MetS among Russian women in 2000 was comparable with findings from other European countries, among men it was a half of that [17].

The aim of this study was to further explore the data collected in 2000 by studying socio-demographic and lifestyle correlates of MetS and associations between the MetS and CVD-and all-cause mortality after 9 years of follow-up.

## Methods

### Study sample

The data were obtained from a cross-sectional population-based study conducted in 2000 in Arkhangelsk, Northwest Russia. Detailed information on study design, sampling procedure and data collection is presented elsewhere [17-19]. In brief, we invited 3745 subjects aged  $\geq 18$  years from the patient register at the Semashko outpatient clinic in Arkhangelsk. Most of the participants were consecutively recruited when they came for their obligatory annual medical examinations. Others, particularly pensioners from the area served by the Semashko clinic, were specifically invited to participate in this study. Only 40 individuals refused to participate (response rate 98.9%).

### Data collection

Data on education, occupational status, use of medications, history of myocardial infarction (MI), diabetes mellitus, and stroke as well as typical patterns of leisure time physical activity, smoking, frequency and amount of alcohol consumption, frequency of fresh fruits and vegetables intake with no specified time-frames were

collected using a 6-page comprehensive questionnaire. Blood pressure (BP) was measured three times. The average of the two last readings was used in the study. Waist circumference (WC) was measured at the umbilical level. Weight and height were measured with subjects in light clothing and without shoes. Venous blood samples were drawn and centrifuged within 15-25 min. Most of the participants fasted prior to testing.

### Laboratory analyses

Enzymatic colorimetric tests were used to measure TC (cholesterol esterase, cholesterol oxidase) and TG (lipoprotein lipase, glycerokinase, and glycerophosphate oxidase). HDL-C was measured by a homogenous enzymatic colorimetric test (PEG cholesterol esterase, and PEG peroxidase). All biochemical analyses of serum lipids were performed using a Hitachi 737 analyzer. Gamma-glutamyltransferase (GGT) was measured by an enzymatic colorimetric test (standardized method, Roche). Aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) were measured photometrically by Hitachi 917 analyzer. Serum C-reactive protein (CRP) was measured by particle-enhanced immunoturbidimetric assay in a Roche Modular P analyzer (Roche Diagnostics GmbH, D-68298 Mannheim). Glycated hemoglobin (HbA1c), which reflects the mean glucose level over the preceding 2-3 months, was assessed by Bio-Rad Variant II HPLC system with reagents from Bio-Rad Laboratories (Inc., Hercules, CA 94547, USA).

The inter-assay and intra-assay coefficients of variation for all laboratory tests were under 3%.

### Definition of the metabolic syndrome

MetS was defined according to the International Diabetes Federation (IDF) [6] and National Cholesterol Education Program (NCEP) [20] criteria. We applied cut-offs for WC as it was recommended for europids (men  $\geq 94$  cm, women  $\geq 80$  cm) and (men  $\geq 102$  cm, women  $\geq 88$  cm), respectively, for IDF and NCEP. We used HbA1c as the measurement of hyperglycemia (defined as HbA1c  $\geq 6.1\%$ , and/or self-reported diabetes, and/or receiving treatment for diabetes).

### Description of the variables

Education was divided into 3 categories: low (primary or secondary school), average (vocational school or incomplete university education) and high (complete university education). As income level was difficult to determine due to high inflation and collapsing economy due to the crisis and default in 1998-99, we used data on self-reported occupational status as a "surrogate" measure for income. Income level was defined according to official data on average salary levels in different sectors of the economy for year 2000 [21]. Five categories were

generated: very low, low, medium, high and unknown. Occasional and daily smokers comprised the group of smokers, while non-smokers and ex-smokers comprised the non-smoking group. Leisure-time physical activity was dichotomized as "inactive" or "sedentary lifestyle" (predominantly sitting activity like reading, watching TV) and "active" (walking or bicycling or yard working at least 4 hours per week, regular training and professional sport). Intake of fresh fruits or vegetables was dichotomized as "low" (once a week or less) and "high" (2 times a week or more). Alcohol consumption was presented by two variables: frequency of alcohol intake, and number of alcohol units (AU) consumed on one occasion. One AU was equal to 13.8 g of pure alcohol. The frequency of alcohol consumption was divided into 4 groups: abstainers, ≤1 times a month, 2-4 times a month, ≥5 times a month. The number of AU consumed at one drinking session was divided into 3 categories: abstainers, 1-4 AU and ≥5 AU (later referred to as binge drinking). Normal weight, overweight and obesity were defined as a BMI <25, 25-29.9, and ≥30 kg/m<sup>2</sup>, respectively. As the distribution of the liver enzymes and CRP was right-skewed, we used logarithmically transformed values in the regression models.

Altogether 150 individuals had missing data on one or more variables and were excluded from the analyses. The final sample consisted of 3555 individuals (1918 men and 1637 women) aged 18-90 years or 96% of the initial sample.

#### Statistical analyses

Differences in the distribution of the studied characteristics between genders were studied by Pearson's chi-squared tests and unpaired t-tests for categorical and continuous data, respectively. Gender-specific associations between MetS defined by the IDF criteria, its individual components and socio-demographic, lifestyle and metabolic factors were calculated using Poisson regression with robust variance estimates as recommended by Barros and Hirakata [22], and are presented as crude and adjusted prevalence ratios (PR) with 95% confidence intervals (CI).

#### Follow-up study

In July 2009 we collected data on the vital status of all study participants, using the mortality register of the Arkhangelsk Regional Healthcare Department which is based on data from medical death certificates. Causes of death were coded using the International Classification of Diseases, 10th Revision (ICD-10). The study endpoints were: death from coronary heart disease (CHD) (I20-25); death from stroke (I60-64); death from either myocardial infarction (MI) or stroke (I21-23; I60-64); CVD death (I00-99); and all-cause death. By July 2009,

200 subjects of the 3555 participants had died and in 97 of the cases (48%) the diagnosis was verified by autopsy. To study associations between MetS in 2000 and mortality by 2009, we used both IDF and NCEP definitions of MetS to increase comparability of the findings with other studies. Gender-specific risk ratios (RR) were calculated by Poisson regression.

All analyses were performed using STATA 10 (STATA Corp, TX, USA). The study was approved by the Regional Ethical Committee in Norway.

## Results

### Sample characteristics

Participants' background characteristics and the prevalence of MetS are presented in Table 1. Men were younger, had higher income, but lower education than women. They were more physically active, smoked more, drank alcohol more often and had higher levels of alcohol intake at one drinking session. About 50% of the men reported binge drinking, by contrast to 15% among women. Vodka/liquor constituted about 66% and 45% of the total consumption in men and women, respectively (data not shown). Men also had higher levels of liver enzymes and CRP. The prevalence of MetS in men was half of that in women (Table 1).

### Correlates of the metabolic syndrome

Among men, MetS was positively associated with age, BMI, sedentary lifestyle, GGT and CRP; and inversely associated with income, smoking, frequency and amount of alcohol intake as well as the AST-to-ALT ratio in the crude analysis. After adjustment for all studied factors except BMI, the associations between MetS and income disappeared. Additional adjustment for BMI attenuated most of the associations except the positive association with age, and inverse associations with the AST-to-ALT ratio, frequency and amount of alcohol consumption (Table 2).

In women, MetS was positively associated with BMI, age, very low income, sedentary lifestyle, GGT and CRP, and inversely associated with education, unknown income category, smoking, frequency and amount of alcohol consumption as well as AST-to-ALT ratio in crude analysis. After adjustment for all study factors except BMI, the associations between MetS and income, education, smoking and alcohol disappeared. After further adjustment for BMI, only age, sedentary lifestyle, GGT and CRP remained associated with MetS.

### Correlates of the individual metabolic components

In the multivariable analysis of the MetS components (Table 3), frequency and volume of alcohol intake were inversely associated with prevalence of hypertriglyceridemia (high-TG), low levels of high density lipoproteins (low-HDL-C) and hyperglycemia in men. Similar

**Table 1 Prevalence of the metabolic syndrome stratified by gender, age, BMI, laboratory tests, socio-demographic and the life-style characteristics**

Socio-demographic and the life-style characteristics	Men		Women		P-value <sup>2</sup>
	N (%)	MetS, % with (95% CI) <sup>1</sup>	N (%)	MetS, % with (95% CI) <sup>1</sup>	
Age, years					0.002
18-29	515 (26.9)	1.75 (0.9-3.4)	347 (21.2)	3.8 (2.1-6.5)	
30-39	352 (18.4)	6.25 (4.1-9.5)	303 (18.5)	8.6 (5.8-12.5)	
40-49	441 (23.0)	11.3 (8.6-14.8)	400 (24.4)	22.8 (18.8-27.2)	
50-59	298 (15.5)	14.8 (11.0-19.4)	290 (17.7)	41.4 (35.7-47.3)	
60+	312 (16.3)	18.3 (14.2-23.1)	297 (18.1)	45.5 (39.7-51.3)	
BMI, kg/m <sup>2</sup>					< 0.001
< 25.0	989 (51.5)	0.3 (0.1-1.0)	781 (47.7)	4.0 (2.8-5.7)	
25.0-29.9	707 (36.9)	9.3 (7.4-11.8)	515 (31.5)	28.9 (25.1-33.1)	
≥30.0	222 (11.6)	50.9 (44.1-57.6)	341 (20.8)	60.1 (54.7-65.3)	
Education					< 0.001
Secondary school	435 (22.7)	10.3 (7.7-13.7)	426 (26.0)	31.9 (27.6-36.6)	
College	1170(61.0)	7.9 (6.4-9.6)	774 (47.3)	21.3 (18.5-24.4)	
University	313 (16.3)	14.4 (10.8-18.9)	437 (26.7)	19.2 (15.7-23.3)	
Income					< 0.001
Very low	283 (14.8)	17.0 (12.9-22.0)	379 (23.2)	43.8 (38.8-49.0)	
Low	136 (7.1)	14.7 (9.4-22.0)	740 (45.2)	20 (17.2-23.1)	
Medium	144 (7.5)	6.9 (3.6-12.7)	189 (11.5)	23.8 (18.1-30.7)	
High	1058(55.2)	9.3 (7.6-11.2)	34 (2.1)	17.7 (7.4-35.2)	
Unknown	297 (15.5)	2.0 (0.8-4.6)	295 (18.0)	6.8 (4.3-10.4)	
Sedentary lifestyle					< 0.001
Yes	437 (22.8)	14.0 (10.9-17.7)	656 (40.1)	32.2 (28.6-35.9)	
No	1481(77.2)	8.2 (6.9-9.7)	981 (59.9)	17.7 (15.4-20.3)	
Current smoking					< 0.001
Yes	1085(56.6)	7.5 (6.0-9.2)	348 (21.3)	13.2 (9.9-17.3)	
No	833 (43.4)	12.1 (10.0-14.6)	1289(78.7)	26.3 (23.9-28.8)	
Low fresh fruits/vegetables intake					0.01
Yes	779 (40.6)	8.2 (6.4-10.4)	599(36.6)	25.5 (22.1-29.3)	
No	1139(59.4)	10.4 (8.7-12.3)	1038(63.4)	22.4 (19.9-25.0)	
Frequency of alcohol intake					< 0.001
Abstainers	230 (12.0)	15.2 (11.0-20.7)	445 (27.2)	34.2 (29.8-38.8)	
≤ 1 times a month	434 (22.6)	12.4 (9.6-16.0)	542 (33.1)	25.3 (21.7-29.2)	
2-4 times a month	979 (51.0)	7.2 (5.7-9.0)	571 (34.9)	15.9 (13.1-19.3)	
≥5 times a month	275 (14.3)	8.4 (5.5-12.4)	79 (4.8)	6.3 (2.4-14.8)	
Number of AU on occasion					< 0.001
Abstainers	230 (12.0)	15.2 (11.0-20.7)	445 (27.2)	34.2 (29.8-38.8)	
1-4 AU	780 (40.5)	9.5 (7.6-11.8)	946 (57.7)	20.0 (17.5-22.7)	
≥ 5 AU	912 (47.5)	8.0 (6.4-10.0)	248 (15.1)	17.7 (13.3-23.2)	
Self-reported MI or stroke					0.768
GGT, U/l, mean (SD)	43.7 (60.8)		28.4 (39.8)		< 0.001
AST, U/l, mean (SD)	29.5 (22.7)		23.6 (13.7)		< 0.001
ALT, U/l, mean (SD)	20.7 (20.8)		12.9 (12.8)		< 0.001
AST/ALT, mean (SD)	1.8 (0.9)		2.1 (0.8)		< 0.001
CRP, mg/l, mean (SD)	3.2 (9.1)		2.6 (5.6)		0.02
Metabolic syndrome <sup>3</sup>	182/1918	9.5 (8.2-10.9)	385/1637	23.5 (21.5-25.7)	< 0.001

<sup>1</sup> 95% CI for proportions calculated using Wilson procedure

<sup>2</sup> p-values for the differences between genders

<sup>3</sup> Metabolic syndrome defined according to the modified IDF criteria

**Table 2 Sex-specific crude and multivariate adjusted PRs for metabolic syndrome<sup>1</sup>**

Factor	Men			Women		
	Model 1 <sup>2</sup>	Model 2	Model 3	Model 1 <sup>2</sup>	Model 2	Model 3
Age						
18-29	Reference	Reference	Reference	Reference	Reference	Reference
30-39	3.58 (1.67-7.68)	2.24 (0.82-6.18)	1.4 (0.57-3.43)	2.29 (1.20-4.38)	1.55 (0.80-2.98)	1.42 (0.78-2.58)
40-49	6.49 (3.23-13.04)	3.75 (1.44-9.78)	2.03 (0.88-4.68)	6.07 (3.46-10.67)	3.43 (1.90-6.19)	2.50 (1.45-4.33)
50-59	8.45 (4.18-17.06)	4.98 (1.89-13.14)	2.91 (1.25-6.75)	11.1 (6.37-19.16)	5.39 (2.93-9.89)	3.76 (2.12-6.67)
60+	10.45 (5.25-20.82)	6.58 (2.34-18.49)	5.06 (2.09-12.21)	12.1 (7.02-20.98)	5.09 (2.69-9.65)	3.97 (2.17-7.26)
P for trend	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Education						
Secondary school	Reference	Reference	Reference	Reference	Reference	Reference
College	0.76 (0.54-1.07)	1.03 (0.73-1.47)	1.23 (0.86-1.76)	0.67 (0.55-0.81)	0.96 (0.78-1.18)	0.89 (0.73-1.09)
University	1.39 (0.94-2.05)	1.10 (0.73-1.65)	1.18 (0.79-1.77)	0.60 (0.48-0.76)	0.80 (0.62-1.03)	0.89 (0.69-1.13)
P for trend	0.190	0.660	0.375	< 0.001	0.085	0.329
Income						
Very low	1.15 (0.71-1.86)	0.87 (0.48-1.59)	0.81 (0.46-1.44)	2.19 (1.82-2.63)	0.99 (0.77-1.26)	0.97 (0.76-1.23)
Low	Reference	Reference	Reference	Reference	Reference	Reference
Medium	0.47 (0.23-0.97)	0.67 (0.32-1.40)	0.62 (0.28-1.33)	1.19 (0.89-1.60)	1.11 (0.82-1.50)	1.03 (0.75-1.41)
High	0.62 (0.40-0.98)	0.84 (0.52-1.33)	0.81 (0.50-1.31)	0.88 (0.42-1.85)	0.97 (0.47-1.98)	1.01 (0.53-1.92)
Unknown	0.14 (0.06-0.33)	0.83 (0.26-2.62)	1.22 (0.45-3.31)	0.34 (0.22-0.53)	0.69 (0.45-1.07)	0.79 (0.52-1.19)
Fresh fruits/vegetab. intake; high vs. low	1.26 (0.94-1.69)	1.21 (0.90-1.63)	1.17 (0.85-1.58)	0.88 (0.73-1.05)	1.05 (0.88-1.25)	1.06 (0.90-1.25)
Current smoking	0.62 (0.47-0.81)	0.74 (0.56-0.97)	1.08 (0.81-1.45)	0.50 (0.38-0.67)	0.98 (0.74-1.30)	1.04 (0.80-1.35)
Sedentary lifestyle	1.7 (1.28-2.28)	1.33 (0.99-1.81)	1.13 (0.84-1.52)	1.81 (1.52-2.16)	1.31 (1.11-1.55)	1.19 (1.01-1.40)
Frequency of alcohol intake						
Abstainers	Reference	Reference	Reference	Reference	Reference	Reference
≤1 times a month	0.82 (0.55-1.21)	0.90 (0.60-1.35)	0.66 (0.45-0.98)	0.74 (0.61-0.90)	1.13 (0.92-1.39)	1.04 (0.85-1.27)
2-4 times a month	0.47 (0.32-0.67)	0.62 (0.42-0.93)	0.59 (0.41-0.85)	0.47 (0.37-0.59)	0.96 (0.75-1.22)	0.90 (0.71-1.14)
≥5 times a month	0.55 (0.33-0.90)	0.76 (0.46-1.26)	0.61 (0.37-1.00)	0.19 (0.08-0.44)	0.42 (0.19-0.97)	0.58 (0.26-1.30)
P for trend	< 0.001	0.045	0.030	< 0.001	0.202	0.190
Body Mass Index						
< 25	Reference	Reference	Reference	Reference	Reference	Reference
25.0-29.9	30.78 (9.71-97.51)	-	22.0 (6.61-73.24)	7.3 (5.0-10.6)	-	4.4 (3.0-6.5)
30.0-34.9	163.1 (52.2-509.6)	-	105.0 (31.6-349.1)	14.5 (10.1-20.9)	-	7.0 (4.6-10.5)
≥35	195.7 (61.0-628.1)	-	132.9 (39.1-451.5)	16.8 (11.5-24.5)	-	7.3 (4.8-11.3)
Number of AU on one occasion <sup>3</sup>						
Abstainers	Reference	Reference	Reference	Reference	Reference	Reference
1-4 AU	0.61 (0.42-0.89)	0.78 (0.53-1.15)	0.69 (0.48-0.99)	0.58 (0.49-0.70)	1.04 (0.85-1.26)	0.96 (0.79-1.17)
≥ 5 AU	0.52 (0.36-0.75)	0.61 (0.40-0.93)	0.52 (0.35-0.76)	0.52 (0.38-0.70)	1.07 (0.79-1.46)	1.06 (0.78-1.43)
P for trend	0.003	0.017	0.001	< 0.001	0.631	0.893
Log GGT	4.3 (3.18-5.81)	1.83 (1.22-2.75)	1.28 (0.82-2.0)	4.08 (3.28-5.07)	1.69 (1.27-2.26)	1.62 (1.22-2.15)
Log AST/Log ALT	0.11 (0.05-0.27)	0.09 (0.04-0.22)	0.19 (0.08-0.46)	0.34 (0.21-0.56)	0.56 (0.33-0.94)	0.82 (0.51-1.32)
Log CRP	2.28 (1.9-2.73)	1.63 (1.28-2.09)	1.27 (0.93-1.72)	3.16 (2.73-3.65)	2.17 (1.82-2.59)	1.61 (1.09-1.12)

<sup>1</sup> Metabolic syndrome is defined according to modified IDF criteria.

<sup>2</sup> Model 1: crude PRs. Model 2 estimates for the MetS adjusted for age, education, income, frequency of fresh fruit and vegetable intake, smoking habits, physical activity and the frequency of alcohol consumption. Model 3: estimates for the MetS adjusted for all covariates in Model 2 plus BMI.

<sup>3</sup> The PRs for "Number of AU on one occasion" in Model 2 and Model 3 adjusted as before excluding variable "Frequency of alcohol consumption".

results were found for frequency of alcohol consumption in women. The volume of consumed alcohol increased HDL-C levels, but showed weaker association in women than in men. Current smoking in men and sedentary lifestyle in women, were related to unfavorable lipid status. It was associated with 40% lower

rates of central adiposity in men, as compared to non-smokers.

Serum levels of GGT and CRP in women were positively related to all five metabolic components (Table 3). High serum levels of GGT were the strongest metabolic marker in men, in whom it was related to increased prevalence of

**Table 3 Gender-specific multivariable adjusted PRs<sup>1</sup> for individual components of the metabolic syndrome defined according to modified IDF criteria by frequency and volume of alcohol consumption, other life-style factors, levels of GGT, AST-to-ALT ratio and C-reactive protein**

	Metabolic abnormalities				
	High TG	Low HDL-C	Central obesity <sup>2</sup>	Hypertension	Hyperglycemia
<b>Men (1918)</b>					
Frequency of alcohol intake					
Never	Reference	Reference	Reference	Reference	Reference
1 time a month	0.85(0.65-1.12)	0.94(0.75-1.18)	1.11(0.83-1.49)	1.01(0.90-1.14)	0.52(0.26-1.02)
2-4 times a month	0.76(0.59-0.97)	0.80(0.65-0.98)	0.95(0.71-1.26)	0.96(0.86-1.08)	0.59(0.33-1.05)
≥5 times a month	1.02(0.76-1.36)	0.85(0.64-1.12)	0.89(0.62-1.29)	0.97(0.83-1.13)	0.42(0.13-1.37)
P for trend	0.743	0.059	0.243	0.410	0.090
Number of AU on one occasion <sup>3</sup>					
Abstainers	Reference	Reference	Reference	Reference	Reference
1-4 AU	0.90(0.71-1.16)	0.86(0.70-1.06)	1.00(0.76-1.33)	0.97(0.87-1.09)	0.56(0.30-1.02)
≥5 AU	0.73(0.57-0.95)	0.79(0.63-0.98)	0.96(0.71-1.29)	0.96(0.86-1.08)	0.50(0.27-0.95)
P for trend	0.005	0.034	0.682	0.603	0.053
Current smoking	1.12(0.95-1.33)	1.25(1.07-1.46)	0.61(0.50-0.74)	0.95(0.87-1.03)	0.87(0.52-1.46)
Sedentary lifestyle	0.98(0.82-1.19)	1.11(0.93-1.31)	1.17(0.96-1.44)	1.04(0.95-1.15)	1.14(0.69-1.90)
LogAST-to-LogALT	0.39(0.22-0.67)	0.90(0.62-1.32)	0.19(0.11-0.33)	1.14(0.95-1.37)	0.82(0.22-3.04)
Log GGT	2.32(1.80-2.98)	0.80(0.59-1.08)	1.78(1.32-2.40)	1.21(1.05-1.40)	1.45(0.68-3.07)
Log CRP	0.92(0.76-1.11)	1.52(1.33-1.75)	1.57(1.30-1.89)	1.00(0.91-1.10)	1.51(0.95-2.41)
<b>Women (1637)</b>					
Frequency of alcohol intake					
Never	Reference	Reference	Reference	Reference	Reference
1 time a month	0.96(0.76-1.22)	0.90(0.78-1.04)	1.12(1.00-1.26)	0.96(0.85-1.09)	1.0(0.57-1.75)
2-4 times a month	0.83(0.63-1.10)	0.75(0.64-0.88)	1.03(0.91-1.18)	0.96(0.82-1.12)	0.42(0.17-1.04)
≥5 times a month	0.52(0.25-1.09)	0.48(0.32-0.72)	0.75(0.52-1.08)	0.76(0.45-1.30)	-
P for trend	0.065	< 0.001	0.605	0.346	0.030
Number of AU on one occasion <sup>3</sup>					
Abstainers	Reference	Reference	Reference	Reference	Reference
1-4 AU	0.88(0.71-1.11)	0.82(0.71-0.94)	1.06(0.95-1.18)	0.94(0.84-1.06)	0.77(0.44-1.35)
≥5 AU	0.98(0.70-1.37)	0.84(0.69-1.02)	1.15(0.98-1.36)	1.03(0.84-1.27)	0.79(0.28-2.20)
P for trend	0.684	0.039	0.087	0.915	0.442
Current smoking	1.40(1.08-1.82)	1.16(1.01-1.35)	0.94(0.81-1.09)	0.87(0.71-1.08)	1.08(0.37-3.18)
Sedentary lifestyle	1.24(1.03-1.50)	1.11(0.99-1.25)	1.06(0.97-1.16)	0.96(0.87-1.06)	0.98(0.60-1.60)
LogAST-to-LogALT	0.55(0.31-0.99)	1.17(0.88-1.55)	0.51(0.37-0.68)	0.92(0.70-1.21)	0.78(0.21-2.96)
Log GGT	1.93(1.42-2.62)	1.25(1.01-1.55)	1.27(1.08-1.50)	1.19(0.98-1.44)	2.18(0.99-4.83)
Log CRP	1.49(1.21-1.82)	1.42(1.25-1.61)	1.68(1.51-1.86)	1.29(1.14-1.45)	1.98(1.27-3.07)

<sup>1</sup> The regression models are adjusted for: age, intake of fresh fruits or vegetables, level of leisure time physical activity, income, education, smoking, frequency of alcohol intake and body mass index (BMI).

<sup>2</sup> PRs for central obesity are given for the regression model excluding BMI.

<sup>3</sup> PRs for "Number of alcohol units (AU) on one occasion" are given for the regression model excluding "Frequency of alcohol intake".

hypertriglyceridemia, central obesity and hypertension. The AST-to-ALT ratio was inversely associated with hypertriglyceridemia and central obesity in both genders, although the strength of association was larger in men.

#### Metabolic syndrome and mortality

MetS as defined by the IDF criteria was associated with more than 6 times higher risk of death from stroke among men after 9 years of observation in (Table 4, Model 1). Adjustment for age, history of CVD, smoking

and alcohol attenuated the association, but the risk of death from stroke was still more than 3 times higher for men with MetS. Death from either stroke or myocardial infarction occurred twice as common among the men with IDF-defined MetS (Model 3). In women, the association between MetS and death from the former causes was much less pronounced (Model 1) and disappeared after adjustment for other covariates.

The risk of cardiovascular death was almost 2.5 times higher both in men and women with MetS (Table 4,

**Table 4 Risk ratios (RR) with 95% confidence intervals (CI) for death from CHD, stroke, myocardial infarction or stroke, CVD and all causes associated with metabolic syndrome during the 9-year follow-up**

Models <sup>1</sup>	RR (95% CI)			
	Men (1918)		Women (1637)	
	IDF	NCEP	IDF	NCEP
N (%)	182 (9.5)	191 (10.0)	385 (23.5)	343 (21.0)
CHD death, N		44		18
Model 1	1.84(0.81-4.18)	1.74(0.76-3.95)	1.64(0.61-4.39)	3.07(1.20-7.83)
Model 2	0.97(0.41-2.26)	0.87(0.37-2.03)	0.99(0.34-2.94)	1.53(0.54-4.34)
Model 3	0.78(0.32-1.91)	0.73(0.30-1.76)	0.86(0.27-2.67)	1.45(0.49-4.33)
Stroke death, N		15	17	
Model 1	6.36(2.29-17.67)	7.91(2.90-21.58)	1.77(0.66-4.77)	2.64(1.01-6.89)
Model 2	3.32(1.25-8.83)	4.07(1.55-10.72)	0.95(0.36-2.53)	1.23(0.48-3.14)
Model 3	3.16(1.11-9.00)	3.76(1.35-10.46)	0.92(0.36-2.33)	1.18(0.48-2.90)
Stroke/MI death, N		25		23
Model 1	4.49 (1.96-10.26)	6.03 (2.75-13.23)	1.15 (0.46-2.89)	2.01 (0.86-4.71)
Model 2	2.40 (1.09-5.31)	3.12 (1.45-6.73)	0.63 (0.25-1.56)	0.92 (0.40-2.11)
Model 3	2.22 (1.02-4.94)	2.87 (1.32-6.23)	0.60 (0.25-1.43)	0.89 (0.40-1.97)
CVD death, N		66		42
Model 1	2.34(1.30-4.21)	2.66(1.53-4.64)	2.00(1.09-3.69)	3.43(1.89-6.21)
Model 2	1.25(0.73-2.15)	1.38(0.83-2.28)	1.11(0.63-1.96)	1.58(0.91-2.73)
Model 3	1.08(0.64-1.82)	1.23(0.76-2.00)	1.09(0.63-1.89)	1.54(0.91-2.61)
All-cause death, N		124		76
Model 1	1.41(0.86-2.34)	1.95(1.26-3.02)	2.12(1.36-3.31)	2.90(1.87-4.49)
Model 2	0.80(0.51-1.27)	1.07(0.71-1.59)	1.15(0.76-1.72)	1.40(0.94-2.09)
Model 3	0.76(0.48-1.18)	1.01(0.69-1.49)	1.13(0.76-1.68)	1.38(0.94-2.04)

<sup>1</sup>Model 1 presents crude estimates.

Model 2 presents data adjusted for age.

Model 3 presents data adjusted for age, history of cardiovascular diseases, smoking status and alcohol intake (number of AU taken on one occasion).

Model 1). However, in both genders these associations disappeared after adjustment for age, and reduced even further after adjustment for other factors. Similar associations were found between all-cause death and MetS in crude analysis, but were attenuated after adjustment for age. The adjusted risk ratio for women was about 40% higher, but did not reach the level of statistical significance (Model 3). No consistent associations between CHD death and MetS were found. Associations between mortality and MetS defined by the NCEP criteria were in the same direction.

## Discussion

To the best of our knowledge this is the first study in Russia on determinants of MetS and its association with all-cause and cardiovascular mortality. The main findings suggest that frequency of alcohol consumption and amount of alcohol consumed at one drinking episode are important correlates of MetS in Northwest Russia. Age, sedentary lifestyle and liver enzymes were also associated with MetS independently of all other studied factors. Moreover, MetS was associated with increased risk of death from stroke and either stroke or myocardial

infarction among men during the 9-year observation period. The study discloses sex-specific adjusted relationships between frequency and volume of alcohol consumption in Russia (where these factors are considered to be very important correlates [15,16,23] of cardiovascular death) and all other major cardiovascular risk factors (except smoking) taken both individually and in frames of the MetS concept. GGT, AST, ALT and, particularly, C-reactive protein and AST-to-ALT ratio were associated with MetS and its individual components as expected from the current knowledge [2,24-27].

However, the results should be interpreted cautiously taking into account several limitations of the study. Unemployed and marginalized subjects are likely to be underrepresented. There were 150 participants with missing data on one or several characteristics, although, they did not differ systematically from those included in the analyses by characteristics for which the data were available. Application of modified IDF and NCEP criteria where we used HbA1c serum levels instead of plasma glucose could result in some underestimation of the prevalence of MetS, since the HbA1c is less sensitive. Other limitations related to study design including

glycemia measurement have been discussed in details elsewhere [17-19]. Given that diabetes is a risk factor for CVD, the sample was re-analyzed without those who reported diabetes in 2000, but the results were virtually identical.

During the 9-year follow-up we were not able to differentiate those who died in other regions and those who migrated, but did not die. This problem can be attributed to virtually all large Russian longitudinal studies, since there is no national population and mortality registers available for medical research. As a result, the participants, who moved from the Arkhangelsk region during the period of observation, could not be traced and those who died outside the region couldn't be registered. The approximate estimates of loss due to migration during the 9-year period is estimated to be between 15 and 17.5% [28]. Young people ( $\leq 30$  years) were more likely to migrate to other regions, presumably looking for better work or education. Cardiovascular mortality in this age-group was lowest, compared to the other age-groups, and deaths from external causes accounted for more than 75%. Therefore, it seems unlikely that this loss to follow-up strikingly affected the observed associations between MetS and CVD mortality.

Both frequency of alcohol drinking and amount of alcohol consumed on one occasion were inversely associated with MetS, particularly among men. Interestingly, the crude association between the amount of alcohol consumed on one occasion and MetS was similar for men and women. However, after adjustment, the association persisted only among men. This association seems to be mediated by favorable changes in the lipid profile, but also by improvements in insulin action and lower risk for hyperglycemia (Table 3). A consumption of five or more AU on occasion (about  $\geq 75$  g of ethanol) was independently related to 50% lower prevalence of MetS (Table 2, Model 3), and, respectively, 25, 20 and 50% lower prevalence of hypertriglyceridemia, low-HDL-C levels and hyperglycemia (Table 3) among men.

Moderate alcohol consumption is known to increase serum triglycerides level, mainly because of alcohol-stimulated lipolysis [29]. There is evidence that a large part of this TG increase is mediated by contemporary fat consumption [30]. In western communities alcohol intake is often moderate and followed by affluent ingestion of foods, rich in polyunsaturated fats, whereas Russian men still widely combine a pattern of vodka binge drinking with low food intake [15,31]. These cultural peculiarities may explain the decrease of the TG level in response to higher amounts of alcohol consumed among Russian men. Taking into account that more than 50% of men in the study sample reported that they drink at least 5 AU (about one 200 ml glass of vodka) on occasion, and two thirds reported intake of mainly vodka at least two times

a month (much the same findings were reported in other studies [32,33] from Russia), we consider that the life-style associated with such a pattern of alcohol intake plays an important role in metabolic risk reduction among Russian men. Thus, gender-specific pattern of alcohol intake and the type of alcohol consumed (high single occasion consumption of strong alcohol by men) together with a confirmed effect of alcohol on serum lipids and insulin resistance might at least partly explain lower rates of MetS among men. It is also possible that this mechanism might also explain the lower metabolic risk in Russian men compared to their Western counterparts. Higher metabolic risk among subjects who abstained from alcohol relative to moderate drinkers, has also been described in longitudinal [34] and cross-sectional [10,35,36] studies. Our results are in line with these findings, suggesting that the pattern of alcohol consumption we found, improves the lipid spectrum by increasing the HDL-C concentration and lowering the low-density lipoprotein cholesterol (LDL-C). Similar results were obtained in another study from Russia where the levels of HDL-C and LDL-C were, respectively, directly and inversely associated with alcohol consumption[37]. Our finding that a consumption of  $\geq 5$  AU on occasion is associated with improved glycemic profile, possibly, due to reduction of the insulin resistance, agrees also well with the existing knowledge [29,38].

Low education and low income has been consistently associated with MetS in the US [7,34]. In our study we found no clear effect of these factors. In crude analysis we found slightly lower prevalence of MetS in women with university education and in men with high income. This association disappeared after adjustment. This discrepancy with the findings from other countries may be due to the fact that in Russia the distribution of health outcomes is less strongly linked to socio-economic status compared to the US or the UK. Higher education in Russia does not guarantee high socio-economic status. Moreover, subjects included in the high income category were relatively poor by international standards with an average monthly salary of about 500 USD.

Several studies have reported sedentary life-style as a risk factor for MetS [7,11,12]. We also observed that low leisure-time physical activity was associated with higher prevalence of MetS in both genders, independently of other studied factors.

We observed an inverse association between smoking and MetS (crude analysis), but this association disappeared after adjustment. However, smoking was positively associated with dyslipidemia in both genders and inversely with central adiposity in men. These findings are consistent with previous research suggesting that nicotine increases the energy expenditure, reduces the appetite and stimulates the lipolysis, thus decreasing the

risk for obesity [39]. On the other hand, smoking negatively affects the coronary heart system through elevation of blood pressure and development of arteriosclerosis.

Increased serum levels of GGT, CRP and low AST-to-ALT ratio turned out to be independently associated with high metabolic risk, similarly to what has been observed in previous research[2,24-26]. The association of GGT and CRP with MetS was stronger in women, whereas the effect of AST-to-ALT ratio was more pronounced in men (Model 3, Table 2). Several studies have reported that both GGT and CRP synergistically increase with the risk of both metabolic syndrome and obesity as well as with a high alcohol intake [24,25,40]. The pattern of association of the AST-to-ALT ratio is totally different; the ratio tends to be lower (often  $\leq 1$ ) in obese and subjects with the MetS, and higher (often  $\geq 2$ ) in those with high alcohol consumption [27]. One possible explanation of this gender difference is that the association of BMI with MetS in men was much stronger. Another explanation is that the adjusted effects of AST-to-ALT ratio constitute a proxy of the protective action of alcohol which was not fully reflected by self-report [19]. This effect is not evident for GGT and CRP since they are synergistically related to both MetS and alcohol consumption, but it is apparent for the AST-to-ALT ratio (antagonistic association). This suggests that the gender-dependent strength of association for GGT and CRP levels and the AST-to-ALT ratio with MetS and its individual components underlines the protective effect of alcohol intake on MetS we found in our study.

MetS was associated with an increased risk of stroke-, either stroke or MI-, CVD-and all-cause death during the 9 year follow-up. After adjustment for age and other potential confounders, the risk was still more than 3 times higher for a fatal stroke and more than 2 times higher for death from either stroke or MI among men with MetS. The lack of significant associations between MetS and, CVD-, and, particularly, CHD-death in the adjusted analyses, might be due to heterogeneity of these diagnostic groups. The CHD, for example, included not only diagnoses of fatal myocardial infarction (I21-23) which are clinically well-distinguishable, characterized by progressing atherosclerosis and pathogenetically close to relatively homogenous and clinically well-defined group of cerebral strokes (I60-I64), but also such vaguely defined conditions as "other forms of acute or sub-acute ischemia" (I24) and "chronic ischemic heart disease" (I25). The latest evidences from Russia suggest that alcohol is an important factor implicated in the pathophysiology of the former two causes of death, and that some deaths within these subgroups are actually caused by acute alcohol intoxication[16] or alcoholic cardiomyopathy due to chronic toxic effects of alcohol on the myocardium[41,42]. We suggest that these CHD-

subcategories should be included in future longitudinal analyses as separate end-points. However, it will require more statistical power which we lacked in the study. We also emphasize the need for larger population-based studies from Russia to either replicate or refute our results.

Thus, as a cluster of four major CVD risk predictors, MetS represents one of the factors contributing to the high cardiovascular mortality in Russia, but it is unlikely that it plays a central role at present. Following the improvements of living conditions during the last decade, the latest state's anti-alcohol initiatives launched in 2006, and the recent increase in life-expectancy [43], the prevalence of MetS is likely to increase in the nearest future, thereby enhancing the proportion of MetS-mediated cardiovascular and all-cause deaths in Russia.

## Conclusion

Age, GGT and C-reactive protein, and AST-to-ALT ratio were associated with MetS in both men and women. High leisure time physical activity in women and moderate frequency of alcohol consumption and binge drinking in men were inversely associated with MetS. Differences between men and women in alcohol consumption may explain gender variation in the MetS prevalence. MetS increased the risk of death from stroke and from either myocardial infarction or stroke during the 9-year follow-up period in men while no associations with mortality were found in women.

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## Authors' contributions

OS and ON planned the study and were responsible for collection of data. OS and AG performed data analysis. OS drafted the manuscript, which was further elaborated by AG and ON. All authors read and approved the final version of the manuscript.

## Competing interests

The authors declare that they have no competing interests.

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## Gamma-Glutamyltransferase, Atherosclerosis, and Cardiovascular Disease

### Triggering Oxidative Stress Within the Plaque

Michele Emdin, MD, PhD; Alfonso Pompella, MD, PhD; Aldo Paolicchi, MD, PhD

**T**he serum determination of gamma-glutamyltransferase ( $\gamma$ -GT) activity is a low-cost, highly sensitive and accurate, and frequently used laboratory test. Although it is considered to be an index of hepatobiliary dysfunction and alcohol abuse,<sup>1</sup> recent epidemiology and pathology studies have suggested its independent role in the pathogenesis and clinical evolution of cardiovascular diseases brought on by atherosclerosis.<sup>1,2</sup>

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The prospective study by Ruttman and colleagues in 163 944 Austrian adults studied for 17 years shows that  $\gamma$ -GT is independently associated with cardiovascular mortality.<sup>3</sup> Serum  $\gamma$ -GT activity had a prognostic impact on fatal events of chronic forms of coronary heart disease, congestive heart failure, and ischemic or hemorrhagic stroke. This was found to be true in both sexes, with a clear dose-response relationship, and with a stronger prognostic significance of  $\gamma$ -GT in younger participants.

These findings from a large unselected cohort unequivocally confirm previous observations that  $\gamma$ -GT is associated with overall mortality and cardiovascular events, in both unselected populations<sup>4–7</sup> and patients with ascertained coronary artery disease, independent of all confounders including liver function and alcohol use.<sup>8,9</sup>

#### Well-Known Versus “Unknown” $\gamma$ -Glutamyltransferase

$\gamma$ -GT is the enzyme responsible for the extracellular catabolism of glutathione (GSH,  $\gamma$ -glutamyl-cisteinyl-glycine), the main thiol intracellular antioxidant agent in mammalian cells.<sup>1</sup> It is present, linked through a small lipophilic sequence of its larger subunit, on the cell surface membrane of most cell types; although the same protein is produced in all tissues, differences in the sugar moieties allow that only the liver  $\gamma$ -GT is detectable in serum.<sup>10</sup> Most serum  $\gamma$ -GT is bound to carriers, such as  $\alpha$ - and  $\beta$ -lipoproteins and albumin.<sup>1</sup>

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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This association is likely to occur within hepatocytes, before  $\gamma$ -GT releases in serum, through still-unknown mechanisms.

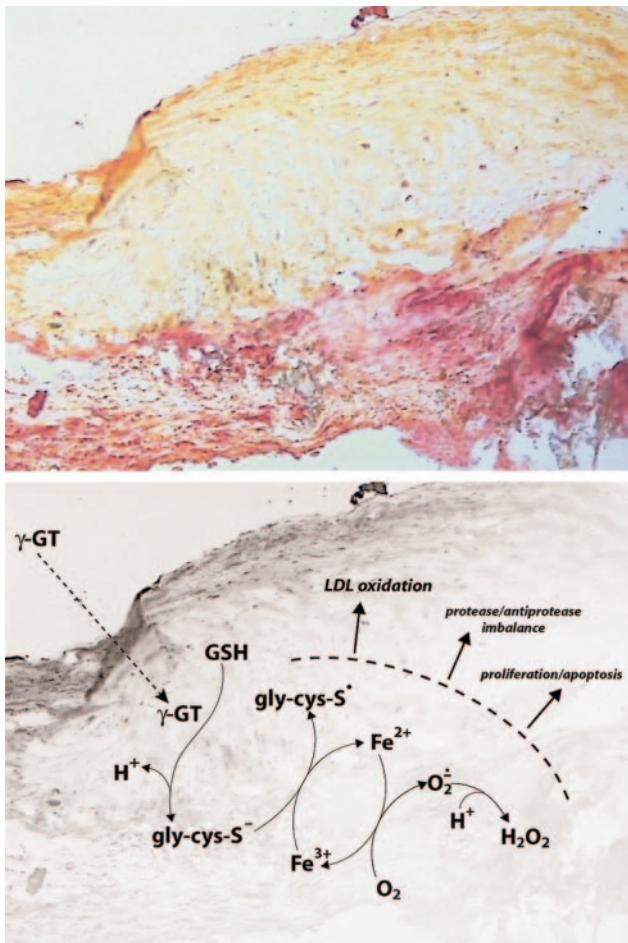
Serum  $\gamma$ -GT activity is affected by genetic and environmental factors, with heritability estimated at 0.52.<sup>11</sup> Within its normal range, it has many other determinants, even stronger than liver function or alcohol consumption.<sup>1</sup> The findings of the Austrian Vorarlberg Health Monitoring and Promotion Program,<sup>3</sup> a large unselected Norwegian population,<sup>12</sup> and a prospective study of 7613 middle-age British men<sup>6</sup> show a strong positive association between serum  $\gamma$ -GT level and body mass index, alcohol use, smoking, total lipoprotein and HDL, serum cholesterol, uric acid, serum triglycerides, heart rate, systolic and diastolic blood pressure, antihypertensive medication, preexisting ischemic heart disease, diabetes mellitus, and blood glucose use of oral contraceptives and menopause; pregnant women had lower values.<sup>3,5,6,12</sup>  $\gamma$ -GT showed a negative association in men in regard to physical activity and lung function (forced expiratory volume in 1 second) and coffee consumption.<sup>3,5,6</sup>

#### Prooxidant Effects of Glutathione Hydrolysis by $\gamma$ -Glutamyltransferase

Catalytically active  $\gamma$ -GT has been found within atherosclerotic cerebral, carotid, and coronary plaques from autopic studies and surgical endarterectomy, colocalized with oxidized density lipoproteins (LDL) and CD68<sup>+</sup> foam cells.<sup>13–15</sup> As concerns the possible association between  $\gamma$ -GT and inflammatory process, it should also be considered that  $\gamma$ -GT has a key role in the interconversion of the glutathione-containing inflammatory mediator leukotriene C4 into leukotriene D4.<sup>16</sup>

Although the exact mechanism leading to accumulation of  $\gamma$ -GT within the plaque is unknown, the association of  $\gamma$ -GT to lipoproteins suggests that LDL lipoproteins can carry  $\gamma$ -GT activity inside the plaque,<sup>17</sup> where free iron is also present.<sup>18</sup> In the extracellular milieu,  $\gamma$ -GT is the only enzyme responsible for GSH catabolism by hydrolysis of its  $\gamma$ -glutamyl bond between glutamate and cysteine. This reaction produces cysteinyl-glycine moieties, which are usually taken within intracellular milieu by the action of membrane dipeptidases, as precursors for GSH resynthesis.<sup>1</sup>

Cysteinyl-glycine is a powerful reducer of Fe<sup>3+</sup> in the extracellular milieu—and likely at the plaque level—that is able to simultaneously generate Fe<sup>2+</sup> and a free thiyl radical; subsequent reactions lead to the formation of superoxide anion radical and hydrogen peroxide.<sup>2</sup> These  $\gamma$ -GT-mediated reactions have been shown to catalyze the oxidation of LDL lipoproteins,<sup>13</sup> likely contributing to oxidative events influ-



**A**, Histochemical demonstration of  $\gamma$ -GT enzyme activity within a frozen section of coronary atheroma from endoarterectomy *in vivo*. Histochemical reaction for  $\gamma$ -GT enzyme activity was performed with the specific substrate  $\gamma$ -glutamyl-4-methoxy-2-naphthylamide and the diazonium salt Fast Garnet GBC as a chromogen. A strong  $\gamma$ -GT activity (red stain) is selectively present in correspondence of the core of the atheroma, whereas the fibrous cap stains negative (original magnification  $\times 20$ ). Adapted with permission from Paolicchi et al.<sup>15</sup> **B**,  $\gamma$ -GT metabolism of glutathione (GSH) within the plaque. The hydrolysis of GSH originates cisteinyl-glycine, which is a powerful reductant of  $Fe^{3+}$ , able to simultaneously generate  $Fe^{2+}$  and a free thiol radical. Thereafter, oxygen reactive species, by the same reaction, contribute to a prooxidant effect, leading to LDL oxidation and likely contributing to other processes, such as metalloproteinase activation, cell proliferation, and apoptosis.

encing plaque evolution and rupture (Figure, A and B). Because of the iron dependence of  $\gamma$ -GT mediated reactions, the described association between increased body iron stores and excess risk of acute myocardial infarction suggests that iron metabolism could influence the predictive value of serum  $\gamma$ -GT.<sup>19</sup> The oxidative stress mediated by  $\gamma$ -GT could thus play a relevant role in the evolution of atherosclerotic plaque and its destabilization: apoptosis of cellular elements of the lesion, plaque erosion and rupture, enhanced platelet aggregation, and thrombosis.<sup>20</sup>

### $\gamma$ -GT from Bench to Bedside

Ruttmann and colleagues established the prognostic value of  $\gamma$ -GT for cardiovascular events at serum levels that lie within

normal values. The receiver operating characteristics analysis suggested  $\gamma$ -GT cutoff values of 15.5 U/L for men and 10.5 U/L for women, corresponding to 27.6 U/L and 18.7 U/L, respectively, for measurements made at 37°C.<sup>3</sup> An increasing number of population studies have evaluated the relationship between serum  $\gamma$ -GT activity and mortality, since the observation of Conigrave et al in 1993<sup>4</sup> that indicated the predictive value of  $\gamma$ -GT for mortality, irrespective of hepatic disease or alcohol consumption.

Thereafter, Wannamethe confirmed the negative prognostic value of  $\gamma$ -GT:<sup>6</sup> After adjustment for all confounders, elevated  $\gamma$ -GT (highest quintile  $\geq 24$  U/L versus the rest) was associated with a significant increase in mortality from all causes and from ischemic heart disease, namely in individuals with a history of ischemic heart disease. This suggested a link with underlying atherosclerotic coronary artery disease. Another more recent study aimed at evaluating the long-term prognosis among 714 patients with a small or unconfirmed acute myocardial infarction indicated  $\gamma$ -GT as an independent risk factor for death in association with age, previous myocardial infarction, smoking, and glucose.<sup>9</sup>

The link with underlying atherosclerotic coronary artery disease has been demonstrated by a prospective study of our group. During a 6-year follow-up of 469 patients with ischemic syndrome and angiographically documented coronary artery disease,<sup>8</sup> and after correcting for other cardiovascular risk factors (eg, age, smoking habit, serum cholesterol, left ventricular ejection fraction, body mass index, diabetes mellitus) or confounding factors, such as serum alanine-aminotransferase level and self-reported alcohol consumption, the prognostic value of serum  $\gamma$ -GT activity for cardiac death and nonfatal infarction has been confirmed in a subset of patients corresponding to 36% of the whole population, characterized by the association of multivessel disease and history of myocardial infarction. The risk increased with different cutoff values of 25 and 40 U/L, however, within the normal range, and the event excess was concentrated within the first 3-year period.<sup>8</sup> The prognostic significance of  $\gamma$ -GT seems thus correlated not only with the extent of coronary artery disease but also with the instability of the plaque.

As concerns stroke mortality, the finding by Ruttmann's group confirm earlier reports from a large Finnish study in unselected populations in both sexes, which demonstrated that  $\gamma$ -GT is associated with an independent prognostic value for ischemic stroke, independent of self-reported alcohol consumption, whereas another common marker of alcohol consumption, carbohydrate-deficient transferrin, was inversely associated with risk.<sup>3,7</sup>

Ruttmann's group does not report any information about the diagnosis of cardiovascular disease. This makes it impossible to understand whether the prognostic role of  $\gamma$ -GT is associated either with evolution or final complications of the atherosclerotic process.<sup>3</sup> History of previous ischemic heart disease, in particular of previous myocardial infarction, regardless of ventricular function, strengthened  $\gamma$ -GT predictive value, indicating that considering coronary patients as a whole, those with higher  $\gamma$ -GT activity may constitute a subset at highest risk of repetitive events.<sup>4,8</sup> The extent of coronary atherosclerotic disease enhances the  $\gamma$ -GT prognos-

tic significance, which may act on a larger substratum.<sup>8</sup> Interestingly, both percutaneous and surgical revascularization were able to abolish the  $\gamma$ -GT prognostic value, confirming its intrinsic link with the evolution of the plaque.<sup>8</sup>

The pathogenetic mechanism proposed for the role of  $\gamma$ -GT in promoting the atherosclerotic process should be considered independent, complementary, and synergistic to conventional determinants. In fact, level of serum  $\gamma$ -GT is significantly genetically determined;<sup>6</sup> serum  $\gamma$ -GT activity holds an independent prognostic value within reference level range in all epidemiological studies after adjustment for confounders such as indicators of hepatic function and alcohol consumption (the latter has often been found to exert a rather protective effect);<sup>3–8,13</sup>  $\gamma$ -GT maintains its predictive value after adjustment for other established cardiovascular risk factors, which, however, affect at least in part its concentration, such as obesity, smoking, total serum cholesterol, arterial hypertension, diabetes, reduced physical activity, with particular emphasis in patients with history of preexisting ischemic heart disease.<sup>3–8,13</sup>

### Do We Need Another Risk Factor?

In conclusion, the recent insights into the role of thiol metabolism in atherosclerosis not only increase our understanding of the disease but also have practical clinical applications in risk stratification and targeting of therapy for a clinical challenge of growing worldwide importance. Elevation in serum  $\gamma$ -GT activity predicts outcomes in unsselected populations and in patients with ascertained ischemic heart disease, independently of myocardial damage, thus adding to prognostic information provided by traditional risk factors.

As for ischemic cerebral and heart disease,  $\gamma$ -GT serum assay seems to have all of the main features of a true prognostic marker: the diagnostic assay has optimal sensitivity-specificity,<sup>1,2</sup> epidemiological evidence of its presence before the event in apparently healthy people and patients with clinical overt disease increases our ability to predict it,<sup>3,5–9,13</sup> it has additive and independent predictive value in comparison with established risk factors.<sup>3,5–9</sup> Finally, as concerns ischemic heart disease, the prognostic impact of  $\gamma$ -GT can be abolished by therapeutic interventions, such as coronary revascularization.<sup>8</sup>

The physiological function of  $\gamma$ -GT enzyme activity as a source of peptide precursors for intracellular GSH resynthesis, as well as the current clinical concept of its serum activity as the consequence of a compensatory overexpression in response to hepatobiliary dysfunction or alcohol toxic effect, is challenged. The evidence is growing in favor of a detrimental role, triggering a prooxidant action within the atherosclerotic plaque. Additional investigation would permit the identification among subjects with higher  $\gamma$ -GT value those with a higher risk of developing clinical disease, allowing definition of the interrelationships with iron metabolism alterations, markers of inflammatory process, of glucose and metabolic disease, and with presence, features, and extent of atherosclerotic vessel disease, to better define the most risky combination for the vulnerable plaque and the best medical

strategies for the stabilization of lesions, rather than percutaneous or surgical procedures.

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# Body Fat Distribution, Liver Enzymes, and Risk of Hypertension

## Evidence From the Western New York Study

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**Abstract**— $\gamma$ -Glutamyltransferase (GGT) has been associated with hypertension (HTN); however, the nature of this association remains unclear. GGT is a marker of alcohol consumption, but it is also related to the infiltration of fat in the liver (fatty liver). The association between GGT and HTN was examined in a 6-year longitudinal investigation among 1455 men and women who returned for the follow-up visit. Baseline variables included serum GGT, blood pressure, and anthropometric measures. Incident HTN was defined as blood pressure  $\geq 140/90$  or on antihypertensive medication at the follow-up visit. To eliminate individuals with potential liver pathology, analyses focused only on individuals with GGT within its normal range ( $n=897$ ). Participants were divided in quintiles (Q) based on their baseline GGT levels. Multiple logistic regression analyses [odds ratio (95% confidence intervals)] revealed a significant association of GGT with incident hypertension [2.1 (1.1 to 4.0) Q5 versus Q1]. In subgroup analyses, GGT and HTN were significantly associated among both noncurrent and current drinkers, but only for participants above the median of anthropometric measures [eg, body mass index  $> 26.4$ , 2.3 (0.9 to 5.7), waist circumference  $> 86.1$  cm, 3.7 (1.4 to 9.9), and abdominal height  $> 19.8$  cm, 3.1 (1.2 to 8.5), for Q5 versus Q1, in fully adjusted models]. These findings suggest that the association between GGT and hypertension is not caused solely by alcohol consumption and indicate that serum GGT, within its normal range, may predict hypertension among individuals with increased central fat distribution, suggesting that fatty liver may represent an important underlying mechanism for this association. (*Hypertension*. 2005;46:1186-1193.)

**Key Words:** adipose tissue ■ blood pressure ■ epidemiology ■ hypertension ■ liver

Recent epidemiologic and clinical studies have reported a strong association between  $\gamma$ -glutamyltransferase (GGT), a commonly used biochemical liver test, and several cardiovascular risk factors and diseases including hypertension.<sup>1-15</sup> The mechanism underlying this association is still not well understood. Specifically, it is unclear whether these observations are not confounded by use of alcohol or obesity, especially central (visceral) fat, and may reflect an underlying condition, such as hepatic insulin resistance or nonalcoholic fatty liver (NAFL). It is known that GGT has a protective function in maintaining appropriate hepatic glutathione levels, which are crucial in antioxidant defenses.<sup>16</sup> In addition, GGT has been widely used as a biological marker of alcohol consumption.<sup>17,18</sup> Recent findings have shown as well that regional body fat distribution, with abdominal accumulation, may represent a stronger predictor of elevated liver enzymes including GGT than relative weight, as assessed by body mass index (BMI).<sup>19,20</sup> Furthermore, central adiposity can be an independent predictor of NAFL.<sup>21-23</sup> This common clinical and histological condition has been recently related to insulin

resistance and has been suggested as an additional feature of the metabolic syndrome.<sup>24-26</sup> There is evidence that both fatty liver and central obesity are associated with free radical generation thus enhancing oxidative stress.<sup>16,27,28</sup> Therefore, it is possible that NAFL may represent the link in the association of GGT with hypertension and other components of the metabolic syndrome.

We examined this question in a 6-year longitudinal investigation of the Western New York Study, a population-based study of diabetes and cardiovascular risk factors among residents of Erie and Niagara Counties, New York.

## Methods

### Study Population

Participants in this report were originally enrolled as healthy control participants in the Western New York Health Study, an epidemiologic case-control investigation of patterns of alcohol intake and coronary heart disease in Erie and Niagara Counties, New York, conducted from 1986 to 2001 (59.5% initial response rate). The details of the methodology have been previously described.<sup>29</sup> The initial cohort was selected from drivers' license lists and Health Care

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Finance Administration lists. Eligible participants for the current study were men and women aged 39 to 79 years selected from the baseline examination without known clinical cardiovascular disease (self-report) or type 2 diabetes (fasting plasma glucose  $\geq 125$  mg/dL or self-report) and who were capable of completing the current study protocol ( $n=2652$ ). Exclusion criteria included self-report of any medical condition that would prohibit participation (eg, all cancers except skin cancer, type 1 diabetes, physical or mental impairment, permanent change in residence out-of-state, deceased, or inability to contact and determine eligibility). This left 2139 persons eligible, of whom 1455 completed the full clinic examination (68.0%) at the follow-up visit (6.0 years  $\pm 0.8$ ). Participants with prevalent hypertension (blood pressure  $\geq 140/90$  or on antihypertensive treatment) at baseline were further excluded ( $n=448$ ). Finally, to eliminate individuals with potential liver pathology, we excluded 110 individuals with GGT values above the normal reference range of the laboratory (5 to 55 U/L). The remaining 897 participants are included in this analysis.

The protocol was approved by the University at Buffalo Health Science institutional review board and all participants provided written informed consent before participation.

Compared with those who refused, participants in the current report were less likely to be smokers at the baseline and somewhat more educated (14.4 years versus 13.1 years of formal education;  $P<0.001$ ). There were no significant differences in race, sex ratio, BMI, fasting glucose concentration or blood pressure values between participants and refusals.

**TABLE 1. Baseline Characteristics of Participants According to the Subsequent Development of Hypertension\*: The Western New York Study, 1995–2001**

Variable	Normotensive (n=702) Mean (SD)	Hypertensive (n=195) Mean (SD)	P†
Age (years)	53.2 (11.0)	58.3 (10.5)	<0.0001
Education (years)	14.3 (2.5)	13.6 (2.3)	<0.0001
BMI (kg/m <sup>2</sup> )	26.5 (4.7)	28.1 (5.0)	<0.0001
Waist circumference (cm)	85.8 (12.0)	89.9 (14.6)	0.001
Abdominal height (cm)	19.8 (3.1)	20.9 (3.5)	<0.0001
Physical activity (metabolic equivalent unit · h)	262.4 (47.4)	263.4 (49.1)	0.804
Drinks per day	0.4 (0.8)	0.5 (1.0)	0.378
Total cholesterol (mg/dL)	211.3 (37.8)	222.4 (38.2)	<0.0001
Triglycerides (mg/dL)	119.2 (80.0)	132.9 (74.2)	0.030
Systolic blood pressure (mm Hg)	111.3 (10.9)	122.5 (9.5)	<0.0001
Diastolic blood pressure (mm Hg)	68.9 (7.9)	74.3 (8.0)	<0.0001
GGT (U/L)	21.6 (10.3)	25.4 (10.9)	<0.0001
	%	%	
Women	67.3	59.0	0.036
White	96.6	94.7	0.222
Smoking status			
Never-smokers	54.1	42.2	
Former-smokers	35.1	45.5	
Current smokers	10.8	12.3	0.014
Drinking status			
Lifetime abstainers	9.7	8.0	
Former drinkers	18.9	23.0	
Current drinkers	71.4	69.0	0.412

\*Systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mm Hg or on medication for hypertension.

†P values for comparison between normotensive and hypertensive participants at the follow-up visit.

BMI indicates body mass index; GGT,  $\gamma$ -glutamyltransferase; SD, standard deviation.

## Study Protocol

All participants received a clinical examination that included resting blood pressure, measures of height, weight, waist circumference, and abdominal height. In addition, several questionnaires that were first administered at the baseline examination were re-administered. These assessed lifestyle and health habit information including: cigarette use, physical activity, alcohol use, general health and well-being, personal and family health history, medication use, and socioeconomic status.

Anthropometric measurements were determined by trained and certified interviewers on participants who wore light clothing and no shoes. Waist circumference was determined with participants standing erect with the abdomen relaxed, arms at the side, and feet together. The tape was horizontally placed between the bottom of the rib cage and the top of the iliac crest (hip bones) around the smallest circumference between these 2 reference points. The measurement was taken at the end of a normal expiration, without the tape compressing the skin, to the nearest 0.1 cm. Abdominal height was measured using the Holtain-Kahn abdominal caliper.<sup>30</sup> Three separate measurements were made to the nearest 0.1 cm of the sagittal (eg, antero-posterior) abdominal diameter. If the 3 readings were not within 0.5 cm of each other, the 3 readings were repeated until they were all within 0.5 cm of each other. The mean of the 3 readings were used in these analyses. During the study we examined the intra- and inter-observer variability of the abdominal height measurement. The intra-observer variability, evaluated by the intra-class correlation (ICC) coefficient, was 0.99. The inter-observer variability was

**TABLE 2. Mean (SD) of Selected Covariates According to GGT Quintiles at Baseline: The Western New York Study, 1995–2001**

U/L	GGT at Baseline					<i>P*</i> for Trend
	≤14	15–19	20–25	26–38	39–55	
No.	220	209	178	196	94	
Variable						
Age (years)	51.7 (10.8)	53.4 (10.3)	54.8 (11.1)	55.3 (11.2)	55.1 (10.5)	0.006
Education (years)	14.4 (2.5)	14.2 (2.4)	14.3 (2.4)	14.0 (2.4)	13.8 (2.5)	0.268
Body mass index (kg/m <sup>2</sup> )	25.5 (4.4)	26.6 (4.8)	26.8 (4.9)	27.7 (4.6)	29.1 (5.3)	<0.0001
Waist circumference (cm)	80.4 (11.9)	84.6 (12.1)	87.8 (12.1)	91.1 (13.9)	93.8 (14.5)	<0.0001
Abdominal height (cm)	18.6 (2.8)	19.7 (3.0)	20.1 (3.0)	20.8 (3.1)	22.1 (3.8)	<0.0001
Physical activity (metabolic equivalent unit/h)	257.1 (36.9)	262.4 (47.0)	265.8 (49.2)	264.0 (48.5)	274.3 (68.4)	0.068
Drinks per day	0.3 (0.6)	0.4 (0.8)	0.5 (0.8)	0.6 (1.1)	0.6 (0.9)	0.048
Total cholesterol (mg/dL)	205.0 (34.5)	214.0 (39.4)	213.8 (40.6)	218.5 (40.3)	220.8 (32.0)	0.001
Triglycerides (mg/dL)	102.0 (58.3)	111.6 (69.2)	125.2 (71.7)	137.8 (95.7)	150.8 (92.6)	<0.0001
Systolic blood pressure (mm Hg)	110.0.9 (11.6)	111.8 (10.7)	113.7 (11.7)	116.7 (11.7)	117.2 (10.7)	<0.0001
Diastolic blood pressure (mm Hg)	69.3 (7.7)	69.0 (7.8)	70.2 (9.0)	70.9 (7.7)	72.4 (8.8)	0.005

\**P* values for linear trend.

0.99. Both waist circumference and abdominal height have been shown to be highly correlated with the volume of visceral fat as determined by multi-scan tomography.<sup>31–33</sup> Height was measured on a permanently mounted vertical board (Perspective Enterprises, Kalamazoo, Mich), according to a standardized protocol. Weight was measured to the nearest tenth of a pound on a calibrated balance beam scale (Detecto, Inc, Webb City, Mo). BMI was calculated as weight (kg) divided by height in meters<sup>2</sup>.

At both examinations, blood pressure was measured 3 times using a standard mercury manometer by trained and certified technicians. The onset of the first phase (systolic) and fifth phase (diastolic) Korotkoff sounds were recorded. The mean of the second and third measures were used in the analyses. At both examinations, hypertension was defined as blood pressure ≥140/90 or use of antihypertensive medications.<sup>34</sup>

At the baseline, a blood sample was obtained for determination of routine chemistry between 7:30 and 9:30 AM after a fasting for 8 to 12 hours. Immediately after phlebotomy, tubes were wrapped in aluminum foil to protect them from light and kept at room temperature for 30 minutes and allowed to clot. Blood tubes were centrifuged at 3000g for 10 minutes and 1.5 mL of serum was transferred to polypropylene screw cap vials and placed in a cooler with a cold pack. Samples were delivered by courier to Millard Fillmore Center for Laboratory Medicine (Amherst, NY) for analysis the same day. Hepatic enzymes alanine amino transferase (ALT), aspartate aminotransferase (AST), serum γ-glutamyl transferase (SGGT), and alkaline phosphatase (ALP) were measured by kinetic enzyme assays as part of a chemistry profile on a Paramax Automated Chemistry System.<sup>35,36</sup>

### Statistical Analysis

All analyses were conducted using the Statistical Package for Social Sciences (SPSS-12.0; SPSS Inc, Chicago, Ill). Differences in baseline characteristics between participants who remained normotensive and those who became hypertensive at the follow-up visit were evaluated using independent sample *t* tests for continuous variables and χ<sup>2</sup> test for categorical variables. Participants were divided into quintiles (Q) of GGT concentration according to the baseline distribution. Differences in baseline characteristics were also evaluated across GGT quintiles. Tests for interaction between GGT and gender were not significant; therefore, all analyses were conducted without stratifying for gender.

Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CI) of incident hypertension across

baseline GGT quintiles. The lowest quintile was used as the reference category. Covariates included: the baseline values of age, gender, race, average amount of alcohol, smoking status, BMI, physical activity, and systolic blood pressure. Subgroup analyses were performed to assess the association between GGT and incident hypertension across baseline categories of drinking status (nondrinkers and current drinkers) and anthropometric measures, including BMI, waist circumference, and abdominal height, categorized by the median values.

### Results

Table 1 shows the baseline characteristics of the study participants according to the subsequent development of hypertension. Mean values of age, anthropometric measures, concentrations of total cholesterol and triglycerides, blood pressure, and GGT were significantly higher among participants who became hypertensive than among those who remained normotensive, whereas no significant difference between the 2 groups was found in mean values of physical activity and alcohol consumption. Participants who became hypertensive were also significantly less educated and characterized at baseline by significantly lower percentage of women and higher percentage of smokers (both former and current), whereas no significant difference between the 2 groups was found in the baseline distribution of race and drinking status.

The mean values of the continuous characteristics at baseline by GGT quintiles are shown in Table 2. For all but education and physical activity, a significant linear trend was found across quintiles of GGT.

Table 3 displays the ORs of incident hypertension across baseline GGT quintiles. Model 1 is adjusted for age, gender, and race. Compared with the bottom quintile, the ORs of incident hypertension increased monotonically from quintile 2 through quintile 5: 0.8, 1.6, 1.8, and 2.7 (*P* for trend <0.0001). After further adjustment for baseline average amount of alcohol, smoking status, BMI, and physical activity (model 2), these risk estimates were only slightly attenuated.

**TABLE 3. Odds Ratio (95% CI) of Incident Hypertension\* by GGT Quintiles at Baseline: The Western New York Study, 1995–2001**

U/L	GGT at Baseline					<i>P</i> for Trend
	≤14	15–19	20–25	26–38	39–55	
N	220	209	178	196	94	
Model 1†	1.0	0.8 (0.5–1.4)	1.6 (0.9–2.8)	1.8 (1.1–3.1)	2.7 (1.5–4.9)	<0.0001
Model 2‡	1.0	0.8 (0.4–1.4)	1.6 (0.9–2.7)	1.8 (1.0–3.0)	2.1 (1.1–4.0)	<0.0001
Model 3§	1.0	0.9 (0.5–1.6)	1.7 (0.9–3.0)	2.0 (1.1–3.4)	2.1 (1.1–4.0)	0.002
By drinking status						
Nondrinkers						
N	77	64	39	56	31	
Model 1	1.0	0.8 (0.3–2.0)	1.0 (0.3–2.9)	1.6 (0.6–3.8)	3.9 (1.4–10.4)	0.006
Model 2	1.0	0.7 (0.3–1.9)	1.0 (0.3–2.9)	1.5 (0.6–3.8)	3.4 (1.2–9.4)	0.011
Model 3	1.0	0.8 (0.3–2.2)	1.0 (0.3–3.0)	1.8 (0.7–4.8)	3.5 (1.2–10.0)	0.010
Current drinkers						
N	143	145	139	140	63	
Model 1	1.0	0.8 (0.4–1.7)	1.8 (0.9–3.5)	2.0 (1.0–3.8)	1.9 (0.9–4.3)	0.008
Model 2	1.0	0.8 (0.4–1.7)	1.8 (0.9–3.6)	2.0 (1.0–3.9)	1.5 (0.6–3.4)	0.035
Model 3	1.0	0.9 (0.4–2.0)	2.1 (1.0–4.4)	2.2 (1.0–4.5)	1.4 (0.6–3.4)	0.057

\*Systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mm Hg or on medication for hypertension.

†Adjusted for age, gender, and race.

‡Adjusted as above plus average amount of alcohol (except among categories of drinking status), smoking status, body mass index, and physical activity.

§Adjusted as above plus systolic blood pressure.

ated: 0.8, 1.6, 1.8, and 2.1 (*P* for trend <0.0001). Model 3 is adjusted further for baseline systolic blood pressure with little notable change: 0.9, 1.7, 2.0, and 2.1 (*P* for trend 0.002).

To examine any confounding by drinking status, we stratified the results by baseline drinking status. A significant linear relationship between GGT quintiles and incident hypertension was found among both nondrinkers (including lifetime abstainers and former drinkers) and current drinkers; however, the association was stronger among nondrinkers than among current drinkers with an OR of 3.5 (1.2 to 10.0) comparing Q5 versus Q1 in the fully adjusted model.

We also examined the impact of obesity and visceral fat on the results (Table 4). After stratification by the median values of baseline anthropometric measures, such as BMI, waist circumference, and abdominal height, GGT and incident HTN were significantly associated only among participants above the median of all anthropometric measures [eg, for BMI  $>26.4$ , 2.3 (0.9 to 5.7), for waist circumference  $>86.1$  cm, 3.7 (1.4 to 9.9), and for abdominal height  $>19.8$  cm, 3.1 (1.2 to 8.5), comparing Q5 versus Q1, in fully adjusted models]. However, it should be noted that the association between GGT and HTN, generally, appeared to be stronger among participants who were above the median of waist circumference and abdominal height compared with participants who were above the median of BMI in the fully adjusted model (model 3). These findings suggest that GGT may be differentially related to these anthropometric measures. To further examine this, we cross-classified tertiles of waist circumference and BMI. A direct and statistically significant relation between the age-adjusted mean values of

GGT and waist circumference persisted within each tertile of BMI (Figure 1). By contrast, no significant association was found between GGT and BMI across tertiles of waist circumference (Figure 2). Thus, these figures indicate that mean values of GGT vary as a function of waist circumference, independently of BMI.

## Discussion

In this prospective population-based study GGT, within the physiological range, was a strong predictor of incident hypertension during 6 years of follow-up in a dose-response relationship. This association was independent of the effects of alcohol consumption and was present in both nondrinkers and drinkers; however, it appeared stronger among nondrinkers than among drinkers. When we evaluated the association between GGT and incident hypertension according to anthropometric measures of either relative weight, ie, BMI, or body fat distribution, ie, waist circumference and abdominal height, GGT was a significant predictor of incident hypertension only among the overweight and especially among persons with increased central fat distribution. The latter is a novel finding and is consistent with the hypothesis that fatty liver may represent an important underlying mechanism for the observed associations between GGT and hypertension.

Over the past 20 years, many cross-sectional studies and fewer longitudinal investigations have reported a positive association of GGT with blood pressure and risk of hypertension.<sup>2–7,12,13</sup> This association has been shown to be independent of alcohol consumption and to be present among both drinkers and nondrinkers.<sup>2,7,13</sup> Our findings are consis-

**TABLE 4.** Odds Ratio (95% CI) of Incident Hypertension\* by GGT Quintiles at Baseline: The Western New York Study, 1995–2001

U/L	GGT at Baseline					<i>P</i> for Trend	
	≤14	15–19	20–25	26–38	39–55		
By median of BMI							
≤26.4							
N	143	115	90	78	33		
Model 1†	1.0	0.8 (0.4–1.8)	1.3 (0.6–2.8)	1.3 (0.6–2.9)	1.4 (0.5–4.1)	0.342	
Model 2‡	1.0	0.8 (0.4–1.7)	1.1 (0.5–2.5)	1.3 (0.6–2.9)	1.2 (0.4–3.8)	0.457	
Model 3§	1.0	1.0 (0.5–2.2)	1.2 (0.5–2.8)	1.5 (0.6–3.5)	1.6 (0.5–5.4)	0.243	
>26.4							
N	77	94	88	118	61		
Model 1	1.0	0.8 (0.3–1.9)	2.1 (0.9–4.5)	2.2 (1.0–4.7)	3.3 (1.4–7.6)	<0.0001	
Model 2	1.0	0.8 (0.3–1.8)	2.0 (0.9–4.4)	2.1 (1.0–4.6)	3.0 (1.3–6.9)	0.001	
Model 3	1.0	0.8 (0.3–1.9)	2.1 (0.9–4.8)	2.2 (1.0–4.9)	2.3 (0.9–5.7)	0.006	
By median of waist circumference							
≤86.1 (cm)							
N	159	122	79	78	31		
Model 1	1.0	0.9 (0.4–1.7)	1.1 (0.5–2.3)	1.3 (0.6–2.7)	1.4 (0.5–3.9)	0.349	
Model 2	1.0	0.9 (0.4–1.7)	1.0 (0.5–2.3)	1.3 (0.6–2.8)	1.2 (0.4–3.5)	0.426	
Model 3	1.0	1.1 (0.5–2.3)	1.1 (0.5–2.7)	1.5 (0.7–3.4)	1.1 (0.4–3.3)	0.434	
>86.1 (cm)							
N	61	87	99	118	63		
Model 1	1.0	0.9 (0.3–2.5)	2.8 (1.1–6.8)	2.9 (1.2–6.9)	4.4 (1.7–11.1)	<0.0001	
Model 2	1.0	0.9 (0.3–2.4)	2.7 (1.1–6.5)	2.9 (1.2–7.0)	4.0 (1.5–10.2)	<0.0001	
Model 3	1.0	1.0 (0.3–2.7)	2.8 (1.1–7.1)	3.1 (1.2–7.6)	3.7 (1.4–9.9)	<0.0001	
By median of abdominal height							
≤19.8 (cm)							
N	164	117	85	75	27		
Model 1	1.0	1.0 (0.5–2.0)	1.2 (0.6–2.6)	1.6 (0.8–3.4)	0.8 (0.2–2.9)	0.450	
Model 2	1.0	1.0 (0.5–2.0)	1.1 (0.5–2.5)	1.7 (0.8–3.5)	0.6 (0.1–2.5)	0.521	
Model 3	1.0	1.3 (0.6–2.6)	1.3 (0.6–2.9)	1.8 (0.8–3.9)	0.6 (0.1–2.9)	0.459	
>19.8 (cm)							
N	56	92	93	121	67		
Model 1	1.0	0.8 (0.3–2.1)	2.3 (0.9–5.8)	2.0 (0.8–5.1)	3.7 (1.4–9.4)	<0.001	
Model 2	1.0	0.8 (0.3–2.1)	2.3 (0.9–5.8)	2.1 (0.9–5.3)	3.4 (1.3–8.9)	0.001	
Model 3	1.0	0.8 (0.3–2.4)	2.6 (1.0–6.8)	2.6 (1.0–6.7)	3.1 (1.2–8.5)	0.002	

\*Systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or on medication for hypertension.

†Adjusted for age, gender, and race.

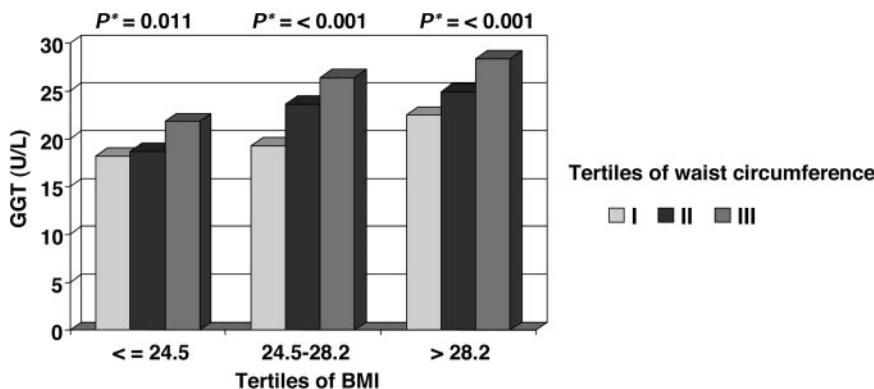
‡Adjusted as above plus average amount of alcohol, smoking status, and physical activity.

§Adjusted as above plus systolic blood pressure.

tent with previous work, further supporting the conclusion that the association between GGT and blood pressure is not mediated by alcohol consumption. Unfortunately, we were not able to assess this association separately in lifetime abstainers and former drinkers, because our sample size precluded us from performing meaningful comparisons within these subsets of drinkers. However, other studies have shown that GGT is associated with blood pressure even among lifetime abstainers.<sup>2</sup>

By contrast, the association of GGT with blood pressure has been shown to be affected by variation in body fat

distribution and parameters of insulin resistance. For example, in a study of 38-year-old Dutch men GGT was not associated with either systolic or diastolic blood pressure in multiple regression analysis including waist-to-hip circumference ratio, as a measure of body fat distribution, whereas the latter was significantly associated with diastolic blood pressure.<sup>1</sup> Similarly, in a large population-based Italian study the significant univariate correlations between GGT and both systolic and diastolic blood pressures were no longer significant in multiple regression analysis including blood lipids.<sup>9</sup> A study of Japanese male workers showed that blood pressure



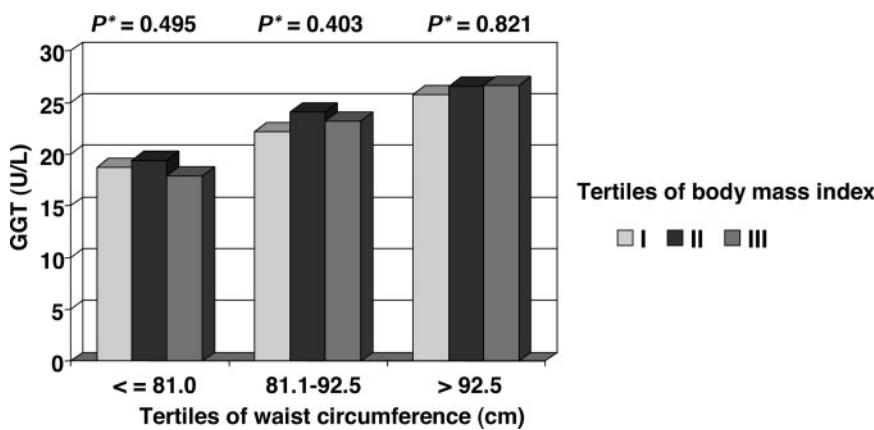
**Figure 1.** Age-adjusted GGT mean values across tertiles of waist circumference within each tertile of BMI. The Western New York Study, 1995 to 2001.  
\*P values for linear trend across tertiles of waist circumference within each tertile of BMI.

was more strongly related to plasma insulin levels after a glucose tolerance test than to GGT, and that GGT was no longer significantly associated with blood pressure after adjustment for insulin levels.<sup>6</sup> Our findings extend previous work and indicate that the association of GGT with hypertension risk is strongly affected by variation in relative weight and, above all, body fat distribution. Specifically, we found that GGT was a significant predictor of incident hypertension only among overweight or individuals with increased central fat distribution. In addition, the association between GGT and HTN appeared to be stronger among participants who were above the median of waist circumference and abdominal height than among those who were above the median of BMI. Our results also indicate that mean values of GGT vary as a function of waist circumference independent of BMI, supporting the notion that central adiposity may represent a stronger predictor of elevated liver enzymes including GGT than relative weight, as assessed by BMI.<sup>19,20</sup> Because central adiposity can correlate with the development of fatty liver,<sup>21-23</sup> our findings further support the hypothesis that NAFL may represent an important underlying mechanism for the observed associations between GGT and hypertension. Moreover, the association between hepatic insulin resistance and fatty liver has been shown in several clinical studies and some authors have suggested that fatty liver should be considered part of the metabolic syndrome.<sup>24-26</sup> In addition, there is evidence that both fatty liver and central obesity are associated with increased free radical generation.<sup>27,28</sup> It is known that GGT has a protective function in maintaining appropriate hepatic glutathione levels, which are crucial in antioxidant

defenses.<sup>16</sup> Therefore, it is possible that the generation of free radicals, which can occur in fatty liver and central obesity, may deplete intracellular glutathione and thus induce the activity of GGT to enhance glutathione levels. The increase in GGT at the sinusoidal membrane of hepatocytes can lead to an increased release of GGT into the circulation. Unfortunately, in our study we did not assess at baseline plasma insulin levels and could not further investigate the association between GGT and parameters of insulin resistance.

Consistently with previous work,<sup>13</sup> in our study no association was found between hypertension risk and other hepatic enzymes including ALT, AST, and ALP (data not shown). The lack of association between hypertension risk and more specific enzymes of liver damage (ALT and AST) further suggests that the association of GGT, within its normal range, and hypertension may be caused by an increased condition of oxidative stress produced by either central adiposity or fatty liver rather than to merely liver damage. Additionally, there is evidence that GGT can act as a pro-oxidant and lead to formation of free radicals and lipid peroxidation,<sup>16,37</sup> which are pathologic mechanisms commonly associated with hypertension and other cardiovascular risk factors.<sup>38</sup>

When we performed analyses including participants with elevated GGT (>55 U/L), the point estimates of hypertension risk among these participants were somewhat attenuated and not significant (data not shown). Although these findings indicate that the predictive value of GGT for hypertension may decrease in persons with potential liver damage, they further support the hypothesis that GGT, within its normal range, may represent an early and sensitive biomarker for the



**Figure 2.** Age-adjusted GGT mean values across tertiles of BMI within each tertile of waist circumference. The Western New York Study, 1995 to 2001. \*P values for linear trend across tertiles of BMI within each tertile of waist circumference.

development of hypertension as well as of other components of the metabolic syndrome.<sup>10,13,14</sup>

Several limitations of this study deserve mention. First, the suboptimal initial participation rate (59.5%) and reexamination rate (68.0%) may leave the possibility for selection bias and restrict the generalization of our findings to the general public. However, this would not affect the internal validity of our results. Second, we cannot rule out the presence of additional unknown confounding variables that we were unable to control for in our analyses, and the potential of residual confounding that, in the absence of a known physiological link, may have contributed to our findings. The strengths of this study include the very detailed information elicited on several covariates known to be related to either GGT or blood pressure elevation including alcohol consumption and several measures of body fatness. A further strength is that we enrolled participants randomly selected from a community-wide population.

## Perspectives

Our study adds new and important information to the current body of evidence about the association of GGT with hypertension and other components of the metabolic syndrome. Our findings indicate that the association between GGT and hypertension is not caused solely by alcohol consumption; in addition, they further support the hypothesis that NAFL, and its metabolic consequences (eg, insulin resistance), may represent an important link between GGT and components of the metabolic syndrome. These findings may have both clinical and public health implications if we consider that fatty liver is the most common cause of liver injury in the United States.<sup>39</sup> Population-based studies are necessary to further investigate the association between fatty liver and hepatic insulin resistance. Moreover, experimental studies are needed also to better understand the physiological functions of GGT with respect to oxidative stress and to support the epidemiologic and clinical evidence regarding the association between metabolic abnormalities and fatty liver.

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## ORIGINAL ARTICLE

# Morning blood pressure surge is associated with serum gamma-glutamyltransferase activity in essential hypertensive patients

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The phenomenon that blood pressure rises sharply in the morning is called 'Morning Blood Pressure Surge' (MBPS). Serum gamma-glutamyltransferase (GGT) is a proinflammatory marker involved in the pathogenesis of cardiovascular diseases. Although both are novel cardiovascular risk factors associated with inflammation and atherosclerosis, the specific relationship between MBPS and serum GGT is unknown. This study investigates the relationship between MBPS and serum GGT activity in essential hypertensive patients. Totally, 320 hypertensive patients were recruited. Mean MBPS was  $17.0 \pm 12.9$  mm Hg. MBPS was positively correlated with age ( $r = +0.222$ ,  $P < 0.0001$ ), body mass index ( $r = +0.132$ ,  $P = 0.018$ ), GGT ( $r = +0.271$ ,  $P < 0.0001$ ), daytime augmentation index adjusted for heart rate (Alx@75) ( $r = +0.140$ ,  $P = 0.014$ ), 24-h pulse wave velocity (PWV) ( $r = +0.143$ ,  $P = 0.014$ ) and daytime PWV ( $r = +0.158$ ,  $P = 0.007$ ). From the 25th to 75th quartile of serum GGT, MBPS increased significantly ( $P_{trend} < 0.0001$ ). In multivariate linear regression analysis, MBPS was independently associated with age ( $P = 0.002$ ), dipping status ( $P < 0.0001$ ) and logGGT ( $P < 0.0001$ ). In conclusion, MBPS is independently associated serum GGT activity in essential hypertensive patients. This is the first study in the literature to demonstrate an independent and a dose-response relationship between the two novel cardiovascular risk factors, MBPS and serum GGT, in this patient population.

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## INTRODUCTION

The importance of hypertension as a risk factor of target organ damage and cardiovascular disease is widely acknowledged. Although clinic blood pressure (BP) is used as the primary tool for the diagnosis and management of hypertension, out of clinic BP measurements, by the use of ambulatory BP monitoring, is considered more reliable than clinic BP measurements because they are more reproducible.<sup>1</sup>

BP exhibits a diurnal variation, reaching the highest level in the morning and then declining to reach a trough level at night. The phenomenon that BP rises sharply in the morning is called 'Morning Blood Pressure Surge' (MBPS). MBPS has been reported to occur in response to sudden activation of the sympathetic nervous system and increased α-mediated sympathetic activity,<sup>2</sup> and can be measured reliably using ambulatory BP monitoring. MBPS is considered to be a cardiovascular risk factor.<sup>1</sup> The study by Kario *et al.*<sup>3</sup> was the first to show that an excessive MBPS was a predictor of subsequent stroke in elderly hypertensive patients, independent of ambulatory BP levels and target organ damage. Subsequently, in the Ohasama study, an increased risk of cerebral hemorrhage was observed in subjects with a large MBPS ( $\geq 25$  mm Hg).<sup>4</sup> Recently, Li *et al.*<sup>5</sup> reported that MBPS exceeding the 90th percentile was a significant and independent predictor of mortality and cardiovascular events.

Serum gamma-glutamyltransferase (GGT), a plasma membrane-bound enzyme, has been used as a biological marker for alcohol intake or liver cell damage.<sup>6</sup> GGT has a pro-oxidative effect, as it is involved in the degradation of the antioxidant glutathione, and has an indirect pro-oxidative effect by causing low-density

lipoprotein cholesterol oxidation in the presence of iron. GGT is also considered as a proinflammatory marker, and serum GGT activity found within atherosclerotic lesions directly contributes to atherosclerosis progression.<sup>7</sup> Studies have shown that serum GGT might have a role in the pathogenesis of cardiovascular disease, diabetes mellitus, stroke and metabolic syndrome.<sup>6,7</sup> Shankar *et al.*<sup>8</sup> reported that higher serum GGT levels were positively associated with prehypertension in US adults free of hypertension and cardiovascular disease. Stranges *et al.*<sup>9</sup> showed that serum GGT, within the physiological range, was a strong predictor of incident hypertension in a dose-response relationship. Kawamoto *et al.*<sup>10</sup> reported that both systolic and diastolic BPs increased significantly with increasing GGT levels among community-dwelling men in Japan. Most recently, Chun *et al.*<sup>6</sup> and Kim *et al.*<sup>7</sup> disclosed that serum GGT was independently associated with incident prehypertension and hypertension in Korean adults, respectively.

In this study, we investigated whether MBPS, which is a cardiovascular risk factor, is associated with serum GGT in patients with essential hypertension.

## MATERIALS AND METHODS

The study had a single-center, cross-sectional design. Totally, 320 consecutive patients from outpatient clinics with essential hypertension who agreed to participate in this study were recruited. The study protocol complied with the Helsinki Declaration of 1975, as revised in 2000, and was approved by the Institutional Ethics Committee. All participants gave informed consent. The exclusion criteria were the presence of secondary

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hypertension, cardiac arrhythmias, congestive heart failure, inflammatory diseases (acute infection, autoimmune diseases), malignancy, sleep apnea, positive HBsAg and/or positive anti-HCV antibody tests and shift working. None of the patients had alcohol abuse. Patients with known coronary artery disease, who were free from acute coronary syndrome, myocardial infarction, angina pectoris or coronary revascularization procedure within the last 3 months, were included. All patients underwent the following procedures; anamnesis, physical examination, routine biochemical testing, office and ambulatory BP monitoring and calculation of sleep-through MBPS.

Body mass index (BMI) was calculated as (weight (kg))/(height (m))<sup>2</sup>. Hypertension was defined as systolic BP (SBP) of 140 mm Hg or more, diastolic BP (DBP) of 90 mm Hg or more or both. The diagnosis of type 2 diabetes mellitus was based on the American Diabetes Association criteria.<sup>11</sup> Coronary artery disease was defined as a history of acute coronary syndrome, myocardial infarction, angina pectoris or coronary revascularization procedure (coronary stent replacement and coronary artery by-pass graft surgery). Cerebrovascular disease was defined as a history of stroke, transient ischemic attack or carotid revascularization procedure. Peripheral arterial disease was defined as a history of intermittent claudication, ischemic leg ulcer, peripheral revascularization or amputation for critical limb ischemia.

Level of kidney function was assessed by estimated glomerular filtration rate (eGFR) calculated by Modification of Diet in Renal Disease formula,<sup>12</sup> as follows;

$$\text{eGFR} (\text{ml min}^{-1} \text{ per } 1.73 \text{ m}^2) = 186 \times \text{serum creatinine} (\text{mg dl}^{-1})^{-1.154} \times \text{age (years)}^{-0.203} \times (1.210 \text{ if African-American}) \times (0.742 \text{ if female}).$$

#### Office BP measurement

Office BP measurements were recorded by Omron MZ model (Omron Health Care, Mukou City, Kyoto, Japan) sphygmomanometer. BPs were measured according to European Society of Hypertension Guidelines.<sup>13</sup>

#### Ambulatory BP measurement

Ambulatory BP measurement was performed by the validated Mobil-O-Graph Arteriograph (I.E.M. GmbH, Stolberg, Germany) device based on the method described in elsewhere.<sup>14</sup> All patients were instructed to rest or sleep between 2200 and 0700 hours (nighttime) and to continue their usual activities between 0700 and 2200 hours (daytime). By using Mobil-O-Graph arteriograph device with an ARC solver method (Austrian Institute of Technology, Vienna), pulse wave forms from brachial artery were recorded during 24 h. This new BP monitor oscillometrically captures pulse wave form from brachial artery by an upper-arm cuff and measures pulse wave velocity (PWV). Within a single measurement cycle, cuff pressure is held for a time period of 10 s at diastolic value during cuff deflation. Recording time of oscillometric signal at diastolic level allows derivation of augmentation index adjusted for heart rate (Alx@75). Measurements were automatically calculated during 24-h, daytime and nighttime.<sup>14,15</sup>

Morning BP was defined as the average of BPs during the first 2 h after wake-up time (four BP readings). The lowest BP was defined as the average BP of three readings centered on the lowest nighttime reading (that is, the lowest reading plus the readings immediately before and after). The sleep-through MBPS was calculated as the morning SBP minus the lowest SBP.<sup>3</sup>

#### Biochemical analysis

Blood samples were obtained after participants had fasted overnight. Complete blood counts were made by using automated blood counting device. Serum uric acid was determined by enzymatic method. Other biochemical parameters were measured by standard methods.

#### Statistical analysis

Statistical analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Normality of data was evaluated by Kolmogorov-Smirnov test (Lilliefors modification). Data are shown as mean  $\pm$  s.d. for normally distributed continuous variables, median (range) for non-normally distributed continuous variables, and as a percentage (%), where appropriate. Spearman nonparametric correlation analysis was run between the MBPS and the clinical, laboratory and ambulatory BP parameters and eGFR. Kendall's Tau correlation analysis was used to assess the correlation between MBPS and the dipping status. Scatterplot graphic between MBPS and logGGT was run with Pearson correlation analysis. Comparison of the MBPS among GGT quartiles was carried out by

one-way analysis of variance test. For the posthoc comparison, Tukey test was used. Multivariate linear regression analysis was performed with stepwise method to determine the possible factors (including age, gender, BMI, smoking, 24-h SBP, 24-h DBP, dipping status, diabetes mellitus, coronary artery disease, use of acetylsalicylic acid, statin and antihypertensive drugs, uric acid, alanine aminotransferase, logGGT and logeGFR as variables) independently related to MBPS. Because GGT and eGFR were not normally distributed, logarithmic conversion was performed before linear regression analysis. Results were considered statistically significant if two-tailed *P* is less than 0.05.

## RESULTS

Totally, 320 hypertensive patients (male/female: 133/187) were included. Table 1 demonstrates the basal demographic and clinical characteristics of the study population. Overall, 79.7% of the patients were nondippers. The laboratory characteristics and eGFR of the study population are shown in Table 2.

**Table 1.** The demographic and clinical characteristics of the study population

Parameter	N = 320
Age (years)	54.4 $\pm$ 14.4
Gender (male/female)	133/187
Body mass index ( $\text{kg m}^{-2}$ )	29.1 $\pm$ 5.6
Smoking (smoker/nonsmoker)	92/228
Diabetes mellitus (present/absent; n)	125/195
Coronary artery disease (present/absent; n)	107/213
Cerebrovascular disease (present/absent; n)	11/309
Peripheral arterial disease (present/absent; n)	5/315
Statin, n (%)	58 (18.1)
Acetylsalicylic acid, n (%)	96 (30.0)
ACEI, n (%)	67 (20.9)
ARB, n (%)	106 (33.1)
Calcium channel blocker, n (%)	124 (38.8)
$\alpha$ -Blocker, n (%)	60 (18.8)
$\beta$ -Blocker, n (%)	72 (22.5)
Thiazide diuretics, n (%)	57 (17.8)
Loop diuretic, n (%)	17 (5.3)
Dippers/nondippers, n (%)	65 (20.3)/255 (79.7)

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

**Table 2.** The laboratory characteristics and eGFR of the study population

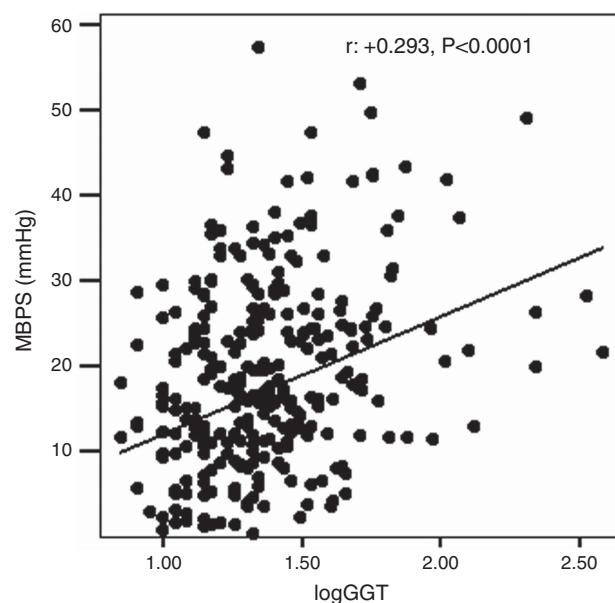
Parameter	N = 320
Hemoglobin ( $\text{g l}^{-1}$ )	134.3 $\pm$ 19.2
Hematocrit (%)	40.3 $\pm$ 5.2
Albumin ( $\text{g l}^{-1}$ )	40.7 $\pm$ 4.9
Urea ( $\text{mmol l}^{-1}$ )	14.4 $\pm$ 8.9
Creatinine ( $\mu\text{mol l}^{-1}$ )	70.7 (44.2–468.5)
Sodium ( $\text{mmol l}^{-1}$ )	138.4 $\pm$ 3.1
Potassium ( $\text{mmol l}^{-1}$ )	4.4 $\pm$ 0.4
Calcium ( $\text{mmol l}^{-1}$ )	2.4 $\pm$ 1.4
Phosphorus ( $\text{mmol l}^{-1}$ )	1.1 $\pm$ 0.2
Total cholesterol ( $\text{mmol l}^{-1}$ )	4.9 $\pm$ 1.1
HDL-C ( $\text{mmol l}^{-1}$ )	1.1 $\pm$ 0.3
LDL-C ( $\text{mmol l}^{-1}$ )	3.1 $\pm$ 0.9
Triglyceride ( $\text{mmol l}^{-1}$ )	1.7 $\pm$ 0.9
Gamma-glutamyltransferase ( $\text{U l}^{-1}$ )	21.0 (7.0–382.0)
Alanine aminotransferase ( $\text{U l}^{-1}$ )	21.4 $\pm$ 12.6
Uric acid ( $\mu\text{mol l}^{-1}$ )	333.7 $\pm$ 115.6
eGFR ( $\text{ml min}^{-1} \text{ per } 1.73 \text{ m}^2$ )	80.8 (11.8–158.7)

Abbreviations: eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

**Table 3.** The office and ambulatory blood pressure parameters, PWV and Alx@75 of the study population

Parameter	N = 320
Office SBP (mm Hg)	133.5 ± 18.5
Office DBP (mm Hg)	84.6 ± 11.4
24-h SBP (mm Hg)	125.7 ± 17.4
Daytime SBP (mm Hg)	127.1 ± 17.2
Nighttime SBP (mm Hg)	121.4 ± 19.5
24-h DBP (mm Hg)	79.1 ± 11.7
Daytime DBP (mm Hg)	80.7 ± 11.6
Nighttime DBP (mm Hg)	74.4 ± 12.9
24-h MAP (mm Hg)	100.4 ± 13.3
Daytime MAP (mm Hg)	101.9 ± 13.3
Nighttime MAP (mm Hg)	95.8 ± 15.1
24-h pulse pressure (mm Hg)	46.6 ± 11.3
Daytime pulse pressure (mm Hg)	46.4 ± 11.3
Nighttime pulse pressure (mm Hg)	47.0 ± 12.4
24-h heart rate (mm Hg)	75.6 ± 10.9
Daytime heart rate (mm Hg)	78.1 ± 11.5
Nighttime heart rate (mm Hg)	68.4 ± 10.6
Maximum daytime SBP (mm Hg)	157.8 ± 26.2
Maximum nighttime SBP (mm Hg)	141.0 ± 25.1
Maximum daytime DBP (mm Hg)	102.2 ± 15.1
Maximum nighttime DBP (mm Hg)	89.2 ± 14.8
Minimum daytime SBP (mm Hg)	99.5 ± 16.4
Minimum nighttime SBP (mm Hg)	104.5 ± 18.7
Minimum daytime DBP (mm Hg)	57.1 ± 12.4
Minimum nighttime DBP (mm Hg)	59.7 ± 12.9
MBPS (mm Hg)	17.0 ± 12.9
24-h Alx@75 (%)	23.1 ± 9.9
Daytime Alx@75 (%)	23.1 ± 9.6
Nighttime Alx@75 (%)	22.9 ± 12.4
24-h PWV ( $m s^{-1}$ )	7.5 ± 1.9
Daytime PWV ( $m s^{-1}$ )	7.5 ± 1.9
Nighttime PWV ( $m s^{-1}$ )	7.4 ± 1.9

Abbreviations: Alx@75, augmentation index adjusted for heart rate; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; MBPS, morning blood pressure surge; PWV, pulse wave velocity; SBP, systolic blood pressure.

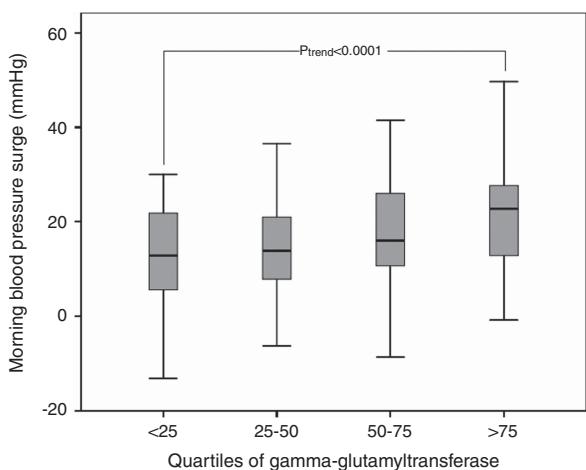
**Figure 1.** The scatterplot graphic between morning BP surge and logarithmically converted GGT. logGGT, logarithmically transformed GGT.**Table 4.** The correlation of morning blood pressure surge with clinical, laboratory and ambulatory blood pressure parameters and estimated glomerular filtration rate of the study population

Correlation coefficient (r)	MBPS (r)	P-value
Age (years)	+0.222	< 0.0001
Body mass index ( $kg m^{-2}$ )	+0.132	0.018
Hemoglobin ( $g l^{-1}$ )	+0.084	0.135
Hematocrit (%)	+0.090	0.108
Albumin ( $g l^{-1}$ )	+0.020	0.732
Urea ( $mmol l^{-1}$ )	+0.067	0.242
Creatinine ( $\mu mol l^{-1}$ )	+0.004	0.942
Sodium ( $mmol l^{-1}$ )	-0.016	0.777
Potassium ( $mmol l^{-1}$ )	+0.012	0.829
Calcium ( $mmol l^{-1}$ )	-0.034	0.558
Phosphorus ( $mmol l^{-1}$ )	-0.040	0.510
Total cholesterol ( $mmol l^{-1}$ )	+0.001	0.995
HDL-C ( $mmol l^{-1}$ )	-0.027	0.645
LDL-C ( $mmol l^{-1}$ )	+0.022	0.706
Triglyceride ( $mmol l^{-1}$ )	+0.073	0.204
Gamma-glutamyltransferase ( $U l^{-1}$ )	+0.271	< 0.0001
Alanine aminotransferase ( $U l^{-1}$ )	+0.142	0.012
Uric acid ( $\mu mol l^{-1}$ )	+0.075	0.214
24-h Alx@75 (%)	+0.111	0.051
Daytime Alx@75 (%)	+0.140	0.014
Nighttime Alx@75 (%)	-0.010	0.864
24-h PWV ( $m s^{-1}$ )	+0.143	0.014
Daytime PWV ( $m s^{-1}$ )	+0.158	0.007
Nighttime PWV ( $m s^{-1}$ )	+0.101	0.084
eGFR ( $ml min^{-1} per 1.73 m^2$ )	+0.013	0.819

Abbreviations: Alx@75, augmentation index adjusted for heart rate; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MBPS, morning blood pressure surge; PWV, pulse wave velocity.

## DISCUSSION

Our results showed that MBPS was positively correlated with age, BMI, serum GGT, daytime Alx@75, 24-h PWV and daytime PWV in hypertensive individuals. From the 25th to 75th quartile of serum



**Figure 2.** The distribution of morning BP surge according to quartiles of GGT.

**Table 5.** Multivariate linear regression analysis of factors associated with morning blood pressure surge in the study population

Parameter	$\beta$	95% confidence interval	P-value*
Age	0.156	0.056 to 0.257	0.002
Dipping status	-8.595	-12.289 to -4.901	< 0.0001
logGGT	10.749	5.435 to 16.063	< 0.0001

Abbreviations: GGT, gamma-glutamyltransferase; logGGT, logarithmic-transformed GGT. \*Adjusted for gender, body mass index, smoking, 24-h systolic blood pressure, 24-h diastolic blood pressure, diabetes mellitus, coronary artery disease, use of acetylsalicylic acid, statin and antihypertensive drugs, uric acid, alanine aminotransferase and logarithmic-transformed estimated glomerular filtration rate.

GGT, MBPS increased significantly. In adjusted analysis, MBPS was independently associated only with age, dipping status and logGGT. This is the first study in the literature to demonstrate an independent association between MBPS and serum GGT, which is a proinflammatory marker, in patients with essential hypertension.

BP fluctuates in a daily pattern of peaks and troughs known as the 'Circadian Rhythm'.<sup>16</sup> In the early morning, an abrupt and steep acceleration in BP occurs, coincident with arousal and rising from overnight sleep. A slow but steady increase in BP is then observed over the early morning hours.<sup>17</sup> This is known as the 'Morning Blood Pressure Surge', and is thought to result from increased physical activity and endogenous factors, such as sympathetic nervous system and renin–angiotensin–aldosterone system activities, and endothelial function.<sup>16–18</sup> Nitric oxide production rises during morning hours in normotensives, a rise that can be disrupted in patients with hypertension, further potentiating vasoconstriction.<sup>17</sup> MBPS, which is determined by a sum of physiologic and unphysiologic factors, is associated with increased cardiovascular risk.<sup>19</sup> MBPS contributes to target organ damage<sup>2</sup> and is positively correlated with left ventricular mass and increased carotid intima–media thickness.<sup>20</sup> The landmark study by Kario *et al.*<sup>3</sup> showed an association between the MBPS and cardiovascular risk. The authors reported that the top MBPS decile had a threefold greater risk of multiple infarcts or stroke after adjustment for 24-h BP and dipping status.

Lee *et al.*<sup>21</sup> reported that in never-treated essential hypertensive patients, with no other cardiovascular risk factors, age was an independent risk factor for MBPS.<sup>21</sup> Neutel *et al.*<sup>16</sup> found that increasing age was associated with a higher SBP MBPS. However,

in the multivariate analysis, age was not significantly associated with MBPS and the authors suggested that the age effect is fully accounted for by changes in other variables, such as BP variability in elderly patients. On the other hand, Sun *et al.*<sup>22</sup> reported that in hypertensive individuals, MBPS was correlated with age. Similarly, we found that MBPS was positively correlated with age in hypertensive individuals, and the association remained significant even after adjusting for potential confounders.

Lee *et al.*<sup>21</sup> disclosed a weak and not statistically significant association between MBPS and BMI in never-treated hypertensive patients. In the study by Neutel *et al.*,<sup>16</sup> MBPS was not affected by BMI in hypertensive patients. In the present study, although we showed a positive correlation between MBPS and BMI, the association lost significance in adjusted analysis.

Cross-sectional studies have indicated that MBPS is associated with vascular remodeling such as atherosclerosis, arterial stiffening and small vessel disease. Inflammation also has an important role in the pathogenesis of atherosclerosis. Studies have reported that inflammatory status is associated with MBPS and MBPS-related atherosclerotic lesions.<sup>23</sup> GGT is an enzyme expressed in serum and most cell surfaces. Emerging evidence has shown that serum GGT might be an important enzyme in the pathogenesis of cardiovascular diseases and GGT has been suggested as a novel biomarker of cardiovascular risk.<sup>6,24</sup> The role of GGT in cardiovascular disease is partly explained by its correlation with conventional cardiovascular risk factors such as dyslipidaemia, hypertension, diabetes mellitus and metabolic syndrome, irrespective of alcohol consumption. However, the exact mechanism linking GGT and cardiovascular disease is still unclear.<sup>25</sup> GGT has a direct role in the generation of reactive oxygen species in the presence of iron or other transition metals, including lipid peroxidation in human biological membranes and is an indirect marker of antioxidant systems, with the primary function of maintaining the intracellular concentration of glutathione in response to oxidative stress.<sup>10</sup> GGT has also been used as a proinflammatory marker because of its indirect involvement in the generation of cysteinyl-glycine, which results in low-density lipoprotein oxidation. The enzyme activity of serum GGT found within atherosclerotic lesions directly contributes to atherosclerosis progression.<sup>7</sup> The current evidence suggests that MBPS and serum GGT are the two novel cardiovascular risk factors associated with inflammation and atherosclerosis. However, the specific relationship between MBPS and serum GGT activity is a matter of interest.

Recent studies disclosed that serum GGT activity is related with BP changes. Chun *et al.*<sup>6</sup> reported that serum GGT was positively associated with the incident prehypertension in healthy Korean men. Karakurt *et al.*<sup>26</sup> found that serum GGT level was higher in patients with prehypertension group than in the control group in Turkish men and women. Stranges *et al.*<sup>9</sup> showed that serum GGT was a significant predictor of incident hypertension among overweight persons with increased central fat distribution. Kotani *et al.*<sup>27</sup> disclosed that the degree of increase in SBP (and DBP only in non-diabetic subjects) was significantly, independently and positively correlated to that of GGT in both diabetic and non-diabetic subjects. In this study, we specifically investigated the relationship between MBPS and serum GGT in patients with essential hypertension. We firstly demonstrated that MBPS was positively correlated with serum GGT (in a dose-response relationship), which remained significant in adjusted analysis along with age and dipping status. The mechanisms underlying the relationship between serum GGT and BP changes are not fully understood. First, serum GGT has been interpreted as a reliable marker of oxidative stress, which increases BP by direct vasoconstriction and sodium retention in the vascular smooth muscle and endothelial cells. Second, insulin resistance could have a role in the association between serum GGT and BP changes, because GGT might be interpreted as a marker for hepatic steatosis and hepatic insulin resistance.<sup>6</sup> Nonetheless, this issue warrants further prospective and interventional studies.

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Increasing arterial stiffness is closely associated with atherosclerotic cardiovascular disease.<sup>28</sup> Because pulse wave travels faster in arteries with decreased elasticity, arterial stiffness could be measured by PWV.<sup>2</sup> Augmentation index (Alx) is a composite index that integrates the amount of wave that is reflected back to aorta and the velocity of incident and reflected wave.<sup>29</sup> Aortic PWV and Alx are indices of arterial stiffness and are predictors for cardiovascular diseases.<sup>30</sup> There is a paucity of studies investigating the relationship between arterial stiffness and MBPS. Suh et al.<sup>2</sup> studied the association of MBPS with PWV in healthy Korean women. They showed that although arterial stiffness did not show significant association with MBPS, it was associated with higher morning BP. However, their participants were nonhypertensive and they used maximum morning BP<sub>power</sub> for evaluation of MBPS. Contrarily, Polonia et al.<sup>31</sup> reported that MBPS was correlated significantly with PWV. In our study, we showed that MBPS was positively correlated with daytime Alx@75, 24-h PWV and daytime PWV in hypertensive individuals.

This study has limitations that deserve mention. First, our study had a cross-sectional design, which precludes deriving a cause–effect relationship between MBPS and serum GGT activity. Second, although patients were instructed to rest or sleep between predefined hours, the precise sleeping patterns of the patients are unknown. Third, we excluded patients with known viral hepatitis, but as we did not perform routine hepatic ultrasonography, we cannot completely rule out parenchymal liver diseases (fatty liver, cirrhosis, hepatocellular carcinoma and so on). Fourth, different kidney function levels may influence the relationship between MBPS and GGT. However, in the current study, we had limited number of patients with loss of renal function; only 78 patients had eGFR  $\leq 60 \text{ ml min}^{-1} \text{ per } 1.73 \text{ m}^2$ , which precluded us performing subgroup analysis according to stages of chronic kidney disease due to low statistical power. This issue merits further investigation. Lastly, as being hypertensive, our patients were on antihypertensive treatment and this may have been disadvantageous during MBPS assessment. Lastly, we relied on a single serum GGT measurement. However, on the other hand, our patient population is composed of patients with somewhat high cardiovascular risk. Therefore, our findings deserve critical attention.

In conclusion, MBPS is independently associated serum GGT activity in essential hypertensive patients. This is the first study in the literature to demonstrate an independent and a dose–response relationship between the two novel cardiovascular risk factors, MBPS and serum GGT, in this patient population.

## What is known about topic

- Morning blood pressure surge is a novel cardiovascular risk factor.
- Gamma-glutamyltransferase is an enzyme involved in the pathogenesis of cardiovascular diseases and a novel biomarker of cardiovascular risk.

## What this study adds

- Morning blood pressure surge is independently associated with serum gamma-glutamyltransferase activity in essential hypertensive patients.
- The interplay between morning blood pressure surge and serum gamma-glutamyltransferase activity complies with a dose–response relationship.
- This is the first study in the literature to demonstrate an independent association between morning blood pressure surge and serum gamma-glutamyltransferase activity, which is a proinflammatory marker, in patients with essential hypertension.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

# Platelet Membrane $\gamma$ -Glutamyl Transferase-Specific Activity and the Clinical Course of Acute Coronary Syndrome

Angiology

I-8

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## Abstract

$\gamma$ -Glutamyl transferase (GGT) participates in oxidative and inflammatory reactions inside the atheroma plaque and platelets. We evaluated whether platelet membrane  $\gamma$ -glutamyl transferase (Plt-GGT) activity is a predictor of major adverse cardiac events (MACEs) during 3 months follow-up of patients with acute coronary syndrome (ACS; MACE-3M). We included 105 patients who were hospitalized consecutively with the diagnosis of ACS. Patients with an MACE-3M were older, more likely to have hypertension, hyperlipidemia, family history of coronary artery disease(CAD), thrombolysis in myocardial infarction (TIMI) risk score >4, higher Plt-GGT and serum GGT activities, serum C-reactive protein level, and lower left ventricular ejection fraction (LVEF) when compared to those without MACE-3M (all *P* values  $\leq .05$ ). By receiver-operator characteristic (ROC) curve analysis, 265 mU/mg for Plt-GGT, 30 U/L for serum GGT, and 45% for LVEF were determined as cutoff values to discriminate MACEs. Platelet GGT activity >265 mU/mg, TIMI risk score >4, and family history of CAD were independent predictors of MACE-3M (all *P* values  $< .05$ ). Platelet GGT activity was as an independent predictor for MACEs in patients with ACS during the 3 months follow-up.

## Keywords

acute coronary syndrome, MACE, platelet,  $\gamma$ -glutamyl transferase

## Introduction

Coronary artery disease (CAD), which accounts for the majority of cardiovascular diseases (CVDs), causes >7 million deaths per year worldwide.<sup>1</sup> The most important cause of acute coronary syndrome (ACS) is plaque rupture.<sup>2</sup> The lipid content released by the rupture of the fibrous capsule possesses thrombogenic activity and contains high amounts of tissue factor.<sup>2</sup> After the erosion of endothelial cells, collagen exposure leads to adhesion and activation of platelets via von Willebrand factor (deposited with P-selectin in platelets).<sup>3</sup> Platelet activation stimulates the thrombotic cascade intertwined with inflammatory pathways.<sup>3</sup>

Serum  $\gamma$ -glutamyl transferase (GGT) levels are used as a marker of alcohol consumption and impaired liver function and have been shown to correlate with oxidative and inflammatory events.<sup>4</sup>  $\gamma$ -Glutamyl transferase activity has been confirmed within atherosomatous plaques by histopathological examination.<sup>5</sup>  $\gamma$ -Glutamyl transferase is also known to have prognostic significance in patients with myocardial infarction (MI).<sup>6</sup>

However, the tissue and cellular sources of elevated serum GGT in CVDs have not yet been established. The different isoforms of GGT have been determined based on relative mobility on electrophoresis (GGT1-GGT5).<sup>7</sup> These isoforms can be

found in serum in different amounts in various clinicopathological conditions.<sup>8</sup>

$\gamma$ -Glutamyl transferase 4 isoforms have been found in the platelets and neutrophils.<sup>7</sup> It is known that GGT converts leukotriene C4 to leukotriene D4 in the platelet cell membrane, and these products are also prothrombotic.<sup>9</sup> The concentration of GGT in the platelet membrane may reflect the level of platelet activation.<sup>9</sup> The activity of GGTs in the platelets and their electrophoretic behaviors are different from those in the serum.<sup>7</sup>  $\gamma$ -Glutamyl transferase in the platelets produces a distinct electrophoretic band and has different substrate interactions than those seen in the serum. It is understood that the

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activity measured in the serum is due to the composition of GGT isoforms originating from many tissues.<sup>7,9,10</sup>

The aim of this study was to investigate the predictive value of platelet membrane GGT (Plt-GGT) activity for the development of major adverse cardiac events (MACEs; recurrent angina pectoris, MI, rehospitalization due to ACS, and death) after a 3-month follow-up (MACE-3M) in patients hospitalized with ACS.

## Methods

Patients hospitalized (n = 105) with a prediagnosis of ACS at Baskent University Cardiology Clinic, between May 2007 and July 2007, were included in the study consecutively after obtaining written informed consents. The follow-up continued till the end of October 2007. The study was approved by Baskent University Faculty of Medicine Clinical Research Ethics Committee (approval dated February 05, 2007, and number 07/90). Patients diagnosed with an increased concentration of liver enzymes, active hepatobiliary disease, active use of alcohol, who underwent major surgery (vascular, pulmonary, cerebral, hepatobiliary, or genitourinary) within the last 4 weeks, definite diagnosis of pulmonary embolism, deep vein thrombosis, cerebrovascular event, peripheral arterial embolism and thrombosis, disseminated intravascular coagulation, sepsis and active malignancy, active tuberculosis, AIDS, and other immunodeficiency were excluded from the study.

Three months after discharge, the patients were investigated for recurrent angina or angina equivalent symptoms, hospitalization due to ACS and death (MACE-3M), either during follow-up examinations or via telephone calls. If any death was reported within this 3-month period, the cause of death (cardiac or non-cardiac) was recorded.

Hypertension (HT) was defined as documentation of a systolic blood pressure of  $\geq 140$  mm Hg and/or a diastolic blood pressure of  $\geq 90$  mm Hg in at least 2 measurements or active use of any antihypertensive agent. Diabetes mellitus was diagnosed as a fasting plasma glucose level over 126 mg/dL or glucose level over 200 mg/dL at any measurement or active use of an antidiabetic agent. Hyperlipidemia (HL) was defined according to the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report.<sup>11</sup>

Venous blood samples were obtained from the patients using a thick needle (20G = 0.9 mm) and were placed in tubes containing acid–citrate–dextrose (ACD; composition: 38 mmol/L citric acid, 75 mmol/L sodium citrate, and 124 mmol/L dextrose) in a ratio of 1.5 mL ACD/8.5 mL blood. After the sample was “rested” for 15 minutes, it was centrifuged at 1500 rpm for 8 minutes to obtain platelet-rich plasma.<sup>9,12</sup> This plasma was then centrifuged at 4000 rpm for 30 minutes to remove the remaining platelet cell pellet. The precipitate thus obtained was washed 3 times with cell washing solution (0.003 mmol/L Tris–HCl buffer+0.12 mmol/L NaCl+0.005 mmol/L EDTA, adjusted to pH 7.4) and was

recentrifuged at 4000 rpm for 30 minutes. The washed platelet precipitate was mixed with distilled water. It was then frozen and thawed 4 times with addition of distilled water for cell lysis. Frozen and thawed platelets were centrifuged for 15 minutes at 10 000 rpm. The precipitate was suspended in 0.01 mmol/L Tris-HCl buffer containing 1% Triton X-100, aiming a cool incubation for 5 hours at 4°C with gentle stirring, and then centrifuged at 10 000 rpm for 15 minutes at 4°C to give a clear supernatant.<sup>13</sup>

The enzymatic colorimetric test was performed for measuring GGT activity at 37°C in patients’ serum and obtained supernatant. L- $\gamma$ -glutamyl-3-carboxy-4-nitroaniline was used as the substrate. The analysis was performed using a Roche-Hitachi analyzer (Mannheim, Germany).  $\gamma$ -Glutamyl transferase values were measured in units per liter (U/L). Total protein was determined in the obtained supernatant according to the Bradford method<sup>13</sup> and also with enzymatic colorimetric assay at 37°C, using a Roche-Hitachi analyzer (Mannheim, Germany). Because of the identical measurements of protein amounts by these 2 methods, colorimetric assay had been used in entire cases. Serum high-density lipoprotein cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride values were measured using an enzymatic colorimetric assay at 37°C, using a Roche-Hitachi analyzer (Mannheim, Germany), and the concentrations were measured in mg/dL.

The following formula was used to calculate the “Plt-GGT-specific activity” and expressed in mU/mg for easy comparison of the small numbers (Plt-GGT: U/mg = 1000 mU/mg):

$$\text{Plt - GGT } \frac{\text{U}}{\text{mg}} = \frac{\text{GGT activity in platelet membrane protein solution U/L}}{\text{total protein in platelet membrane protein solution } \frac{\text{mg}}{\text{L}}}.$$

## Statistical Analysis

All calculations were performed using Statistical Program for the Social Services Version (13.0) software. The continuous variables were expressed as mean (standard deviation) and median (interquartile range). The categorical variables were reported as frequency and percentage. The data were tested for normal distribution by Kolmogorov-Smirnov test. The comparison of differences between the groups was done using  $\chi^2$  (chi-square) test for categorical variables and independent sample *t*-test for continuous variables. The cutoff values for the parameters investigated were calculated using the receiver-operator characteristic (ROC) curve analysis. Single Cox regression analysis was used to analyze parameters that were determinant for MACE-3M. Independent determinants of MACE-3M were recorded using the multiple Cox regression analysis. All *P* values were 2 sided, and *P*  $\leq .05$  was considered significant.

## Results

Of all the patients, 77 (73.3%) were male, 28 (26.7%) were female, and the mean age of the patients was 61.9 (10.5) years. The median Plt-GGT level of the patients was 170.3 (88.3-355.0) mU/mg. A total of 105 patients were followed

**Table I.** Baseline Clinical and Laboratory Characteristics of the Study Population and the Comparison of Groups With and Without MACE-3M.<sup>a</sup>

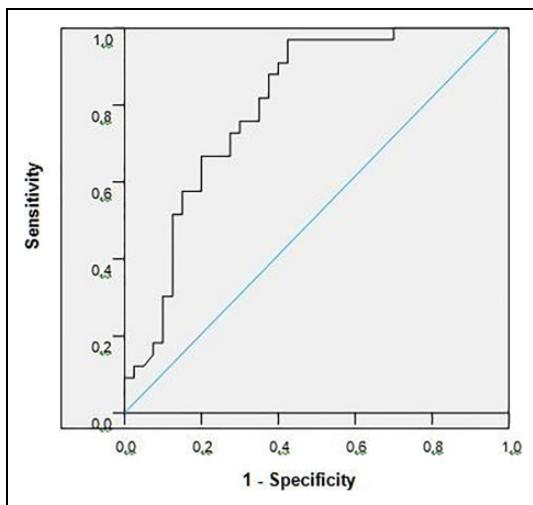
Clinical Characteristics	Total Population, n = 105	No MACE-3M, n = 75	MACE-3M, n = 30	P Values
Age, years	61.86	60.2 (10)	66 (11)	.010
Age ≥70 years	28 (26%)	14 (18.7%)	14 (46.7%)	.003
Male gender	77 (73%)	52 (69.3%)	25 (83.3%)	.143
Traditional risk factors				
Hypertension	67 (63.8%)	43 (57.3%)	24 (80%)	.029
Diabetes mellitus	52 (49.5%)	40 (53.3%)	12 (40%)	.217
Hyperlipidemia	69 (65.7%)	45 (60%)	24 (80%)	.050
BMI ≥30	37 (35.2%)	23 (30.7%)	14 (46.7%)	.121
History of CAD	50 (47.6%)	29 (38.7%)	21 (70%)	.004
Family history of premature CAD	49 (46.7%)	28 (37.3%)	21 (70%)	.002
History of PAD	21 (20%)	8 (10.7%)	13 (43.3%)	<.001
History of CVE	4 (3.8%)	2 (2.7%)	2 (6.7%)	.322
Smoking	77 (73.3%)	53 (70.7%)	24 (80%)	.329
TIMI score ≥4	43 (41%)	17 (22.7%)	26 (86.7%)	<.001
Mean Days of Hospitalization	4.64 (3.6)	3.3 (2.3)	8 (4.5)	<.001
Hospitalization Time ≥5 days	42 (40%)	16 (21.3%)	26 (86.7%)	<.001
Medications				
ASA	94 (89%)	67 (89.3%)	27 (90%)	.920
Clopidogrel	37 (35%)	25 (33.3%)	12 (40.0%)	.518
Intravenous Heparin	45 (43%)	28 (37.3%)	17 (56.7%)	.071
Low-molecular-weight heparins	43 (40.9%)	26 (34.7%)	17 (56.7%)	.038
Tirofiban	15 (14.3%)	10 (13.3%)	5 (16.7%)	.659
Statin	82 (78%)	58 (77.3%)	24 (80%)	.765
Beta-Blocker	66 (62.8%)	44 (58.7%)	22 (73.3%)	.160
Calcium antagonists	26 (24.7%)	19 (25.3%)	7 (23.3%)	.830
ACE inhibitor/ARB	62 (59%)	46 (61.3%)	16 (53.3%)	.451
Nitrates	50 (47.6%)	29 (38.7%)	21 (70%)	.004
Laboratory variables				
LVEF, %	47.82 (9.5)	49.9 (8.4)	42.5 (10.3)	<.001
Plt-GGT, mU/mg	170 (88-355)	125 (72-300)	267 (192-641)	<.001
Serum GGT, U/L	29 (15)	27 (13)	33 (17)	.050
Serum CRP, mg/L	9.3 (12.2)	7.8 (11)	13 (14.2)	.044
HbA <sub>1c</sub> , %	6.5 (1.4)	6.6 (1.5)	6.2 (1.1)	.283
Serum HDL cholesterol, mg/dL	41 (9)	41 (10)	41 (7)	.802
Serum LDL cholesterol, mg/dL	115 (34)	117 (35)	109 (30)	.275
Serum Triglyceride, mg/dL	186 (113)	180 (112)	199 (117)	.445
Serum AST, U/L	27 (15)	27 (11)	30 (22)	.064
Serum ALT, U/L	24 (10)	23.4 (11)	26 (9)	.578
Serum ALP, U/L	184 (54)	182 (46)	187 (71)	.157

Abbreviations: ACE: angiotensin converting enzyme; ALT: alanine aminotransferase; ALP: alkaline phosphatase; ASA: acetylsalicylic acid; AST: aspartate aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CAD: coronary artery disease; CRP: C-reactive protein; CVE: cerebrovascular event; GGT: γ-glutamyl transferase; HDL: high density lipoprotein; LDL: low density lipoprotein; LVEF: left ventricular ejection fraction; MACE-3M: major adverse cardiac events at 3 months; PAD: peripheral artery disease; Plt-GGT: platelet γ-glutamyl transferase; TIMI: Thrombolysis In Myocardial Infarction.

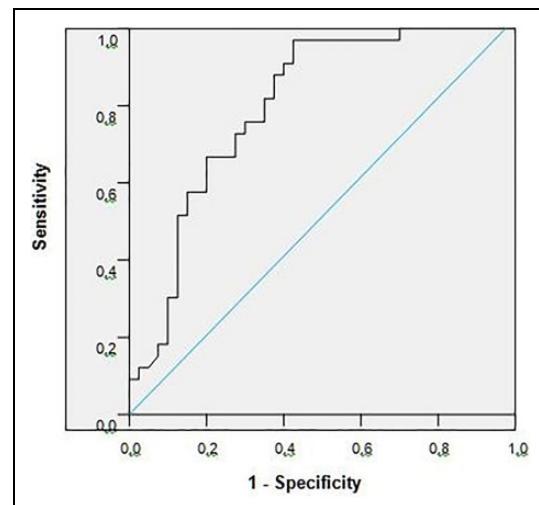
<sup>a</sup>Data are given as mean (SD), n (%), or median (interquartile range).

up to investigate the development of MACE-3M after discharge. In all, 60 (57.1%) patients were diagnosed as unstable angina pectoris (USAP), 28 (26.7%) patients were diagnosed as non-ST-segment elevation myocardial infarction (NSTEMI), and 17 (16.2%) patients were diagnosed as ST-segment elevation myocardial infarction (STEMI). Of these patients, 30 (28.6%) developed MACE-3M (recurrent angina in 18 patients, MI in 10 patients, hospitalization due to ACS in 14 patients, and cardiac death in 4 patients). Clinical characteristics, medications used, and laboratory findings of the patients are shown in Table 1.

A significant difference was found between the patients with and without MACE-3M in terms of the mean age (66.2 [11.0] and 60.2 [10] years;  $P = .01$ ), being older than 70 years (46.7% and 18.7%;  $P = .003$ ), HT (80.0% and 57.3%;  $P = .029$ ), and HL (80% and 60%;  $P = .05$ ). The laboratory parameters that showed significant differences between the groups were Plt-GGT, 267 (191-640) mU/mg and 125 (72-300) mU/mg;  $P < .001$ , serum GGT (33 [17] and 27[13] U/L;  $P = .05$ , and serum C-reactive protein (CRP) levels (13 [8.2] and 7.8 [4.9] mg/L;  $P = .044$ ). There were no significant differences between 2 groups about the other liver function tests such as aspartate



**Figure 1.** Receiver–operator characteristic (ROC) curve of platelet membrane  $\gamma$ -glutamyl transferase (Plt-GGT) activity and prediction of major adverse cardiac events-3 months (MACE-3M). Area under the curve: 0.800,  $P = .001$ .

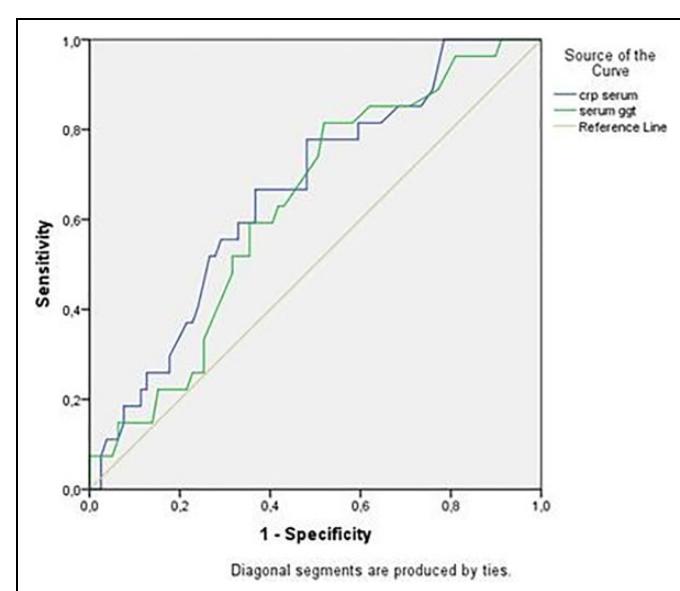


**Figure 2.** Receiver–operator characteristic (ROC) curve of left ventricular ejection fraction (LVEF) and prediction of major adverse cardiac events-3 months (MACE-3M). Area under the curve: 0.727,  $P = .001$ .

aminotransferase, alanine aminotransferase, and alkaline phosphatase levels. There were no significant differences between 2 groups regarding the medication used such as acetylsalicylic acid, clopidogrel, and statins which could affect platelet function and atherosclerotic plaque stabilization. Among the study population, 77 (73.3%) patients were smoking. There was no significant difference regarding smoking between the patients with and without MACE-3M, 24 (80%) and 53 (70.7%);  $P = .329$ . Platelet GGT and serum GGT levels were significantly higher in the smoking group compared to nonsmokers (for Plt-GGT: 245 [111-415] mU/mg vs 111 [60-240] mU/mg;  $P = .006$ ; for Serum GGT: 32 [15] vs 25 [13] U/L;  $P = .036$ , respectively). The clinical characteristics, medications used, and the laboratory parameters of patients with and without MACE-3M are given in Table 1.

It was seen that the Plt-GGT-specific activity at the time of admission increased proportional to the clinical severity of ACS. The analysis showed a statistically significant difference between the median Plt-GGT activities in each diagnostic step from USAP to NSTEMI and STEMI, USAP 126 (71-273) mU/mg, NSTEMI 226 (106-353) mU/mg, and STEMI 365 (287-516) mU/mg;  $P = .01$ ,  $P = .021$ , and  $P < .001$ .

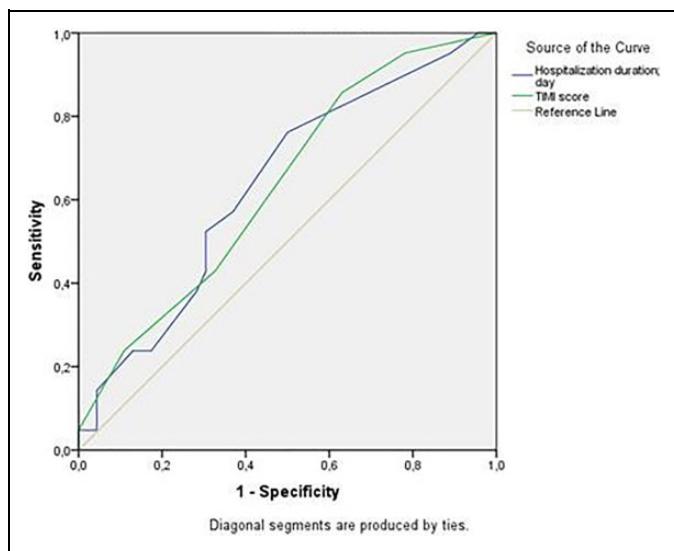
The cutoff values as calculated by ROC curve analysis were used in single and multiple regression analyses and are as follows: LVEF <45%, Plt-GGT >265 mU/mg, serum GGT activity >30 U/L, serum CRP levels >4 mg/L, and length of hospitalization >5 days. The ROC curves of these variables are provided in Figures 1 to 4 with the area under the curve (AUC) and  $P$  values. The parameters that determine the development of MACE-3M in single Cox regression analysis were LVEF >45%, Plt-GGT >265 mU/mg, thrombolysis in myocardial infarction (TIMI) risk score >4, the presence of HT and CAD, family history of CAD and peripheral artery disease (PAD), and the length of hospitalization >5 days (Table 2). For the



**Figure 3.** Receiver–operator characteristic (ROC) curve of serum  $\gamma$ -glutamyl transferase (GGT) and serum C-reactive protein (CRP) and prediction of major adverse cardiac events-3 months (MACE-3M). Area under the curve; GGT: [0.631,  $P = .043$ ] CRP: [0.661,  $P = .013$ ].

prediction of MACE-3M, the sensitivity and specificity were 83.3% and 82.7% for a Plt-GGT value >265 mU/mg, 74.7% and 63.3% for an LVEF >45, and 86.7% and 77.3% for a TIMI Risk Score >4, respectively.

After multiple regression analysis, Plt-GGT >265 mU/mg, length of hospitalization >5 days, TIMI risk score >4, and family history of CAD were found to be independent predictors of MACE-3M with a positive predictive value of 65.8%, 61.9%, 60.5%, and 43% and negative predictive value of 92.5%, 94%, 93.5%, and 83.9%, respectively. We found that the risk of MACE-3M is 4.8 times higher in patients having a



**Figure 4.** Receiver-operator characteristic (ROC) curve of hospitalization duration and thrombolysis in myocardial infarction (TIMI) score and prediction of major adverse cardiac events-3 months (MACE-3M). Area under the curve; Hospitalization day: [0.637 P = .074] TIMI score: [0.634 P = .081].

**Table 2.** Independent Variables Related MACE-3M Determination With Multivariate Cox Regression Analysis.

Characteristics of Patients	Hazard Ratio (95% Confidence Interval)	P Values
Hospitalization Time $\geq$ 5 days	5.9 (1.6-22.8)	.002
Plt-GGT $\geq$ 265 mU/mg	4.8 (1.5-14.7)	.006
TIMI score $\geq$ 4	3.5 (1.0-12.3)	.046
Family history of premature CAD	3.0 (1.1-8.1)	.028
Age $\geq$ 70 years	2.6 (0.8-8.5)	.114
History of PAD	1.8 (0.6-5.3)	.277
Serum GGT $\geq$ 30 U/L	1.7 (0.8-4.0)	.190
History of CAD	1.6 (0.6-4.2)	.307
CRP $\geq$ 4 mg/L	1.6 (0.6-4.2)	.311
Hypertension	1.5 (0.5-4.4)	.379
LVEF (%) $\leq$ 45	1.4 (0.6-3.4)	.374

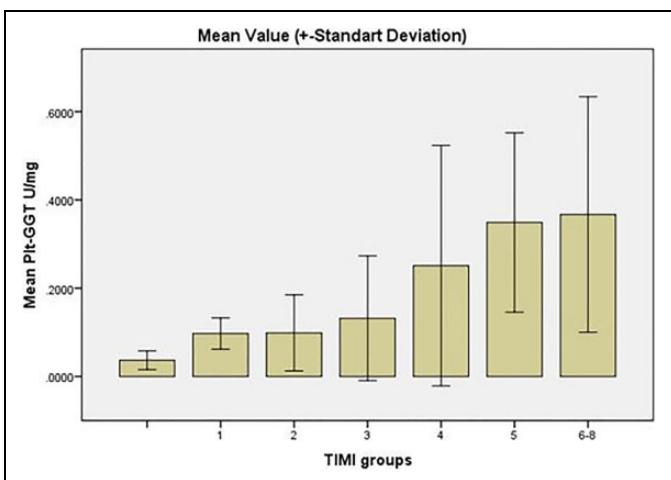
Abbreviations: CAD: coronary artery disease; CRP: C-reactive protein; GGT:  $\gamma$ -glutamyl transferase; LVEF: left ventricular ejection fraction; MACE-3M: major adverse cardiac events at 3 months; PAD: peripheral artery disease; Plt-GGT: platelet  $\gamma$ -glutamyl transferase; TIMI: Thrombolysis In Myocardial Infarction.

Plt-GGT-specific activity  $>$ 265 mU/mg at the time of admission (Table 2).

When the mean Plt-GGT-specific activities were compared using the clinical TIMI score groups, it was found that Plt-GGT-specific activity showed a steady increase toward the high-risk groups with a strong statistical significance (TIMI 1 to TIMI 6-8 analysis of variance;  $P < .0001$ ; Figure 5).

## Discussion

The most valuable finding of our study was that Plt-GGT activity seems to be a strong predictor of MACE-3M in patients



**Figure 5.** Mean platelet  $\gamma$ -glutamyl transferase (Plt-GGT) activities according to thrombolysis in myocardial infarction (TIMI) clinical score groups.

hospitalized with ACS, correlating with known prognostic indicator TIMI risk score. The level of Plt-GGT specific activity was associated with the clinical severity of ACS.

Our study population consisted of high-risk patients, so the TIMI risk score was  $>$ 4 in 41% of them. An increase in TIMI risk score was associated with Plt-GGT activity; a TIMI risk score  $>$ 4 was associated with a 3.5-fold increase in MACE-3M. Therefore, the specific activity of Plt-GGT measured at hospital admission may help to identify and manage high-risk patients, since it was found to be an independent variable for the development of MACEs and to provide similar results with the globally accepted TIMI risk score.

Approximately 20% of all patients presenting to the emergency department with acute chest pain are diagnosed with ACS.<sup>14</sup> During admission and in-hospital follow-up in patients with an ACS, risk stratification is of paramount importance. We intend to investigate the Plt-GGT as a novel biochemical marker for risk stratification of ACSs.

The MACE-3M was significantly higher in the patients having a Plt-GGT-specific activity  $>$ 265 mU/mg. Platelet GGT  $>$ 265 mU/mg was found to be an independent predictor for MACE-3M by multiple regression analysis. Until now, no study has been published that emphasizes the prognostic significance of Plt-GGT for any CVD. Therefore, it is not possible to exactly compare with similar studies. However, previous studies have shown that high serum GGT activity has a prognostic value in patients hospitalized with a diagnosis of ACS<sup>15</sup> or after MI.<sup>6</sup>

$\gamma$ -Glutamyl transferase has been shown to directly participate in oxidative events related to the formation of the atheromatous plaque.<sup>16</sup> It is considered that the migration of GGT into the plaque occurs via LDL.<sup>16</sup>  $\gamma$ -Glutamyl transferase is also present in the platelet membrane and converts leukotriene C4 to leukotriene D4.<sup>9</sup> An increased excretion of leukotriene D4 and E4 has been found in patients after MI and coronary artery bypass surgery.<sup>17</sup>

An important issue that determines long-term MACEs in the ACS clinic is multiple vulnerable lesions that produce greater risk for recurrent ischemic events.<sup>18-20</sup> In angiographic studies, it has been observed that even if the culprit lesion is successfully treated with an interventional approach, the remaining unstable plaques are responsible for recurrent events.<sup>18-20</sup> Platelet GGT activity, which is claimed to be a marker of platelet function,<sup>9</sup> may be thought to be mediated from platelets during plaque rupture thrombus formation in the ACS clinic. The view that the cumulative GGT activities in serum and platelets are related to the amount of thrombus supports our finding that Plt-GGT activity is associated with severity of ACS. It can be argued that the Plt-GGT-specific activity increased due to the excess amount of inflamed plaques (vulnerable plaques), which is not responsible for the ACS presentation at admission; however, it is constantly in contact with platelets in the coronary circulation and increases the tonic reactivity of platelets as a trigger for transient micro-thrombosis, while accelerating the platelet cycle. This also explains the fact that a Plt-GGT level  $>265$  mU/mg was determined to be an independent variable for the development of MACEs in the following days and months.

Left ventricular ejection fraction (LVEF) is the most important determinant of MACEs in the patients with acute MI. Several studies have shown that the LVEF measured in intensive care unit is predictive of 1-year survival after acute MI.<sup>21-24</sup> It has been shown in previous studies that the rate of death increases within 6 months after acute MI when LVEF falls below 40%.<sup>25</sup> The mean LVEF values of patients included in the present study were higher than those in similar studies (mean [SD] = 47.9 [9.5]). However, the rate of the development of adverse events within the 3-month follow-up period was significantly higher in patients with an LVEF  $<45\%$  compared to those with an LVEF  $>45\%$ . Unfortunately, LVEF  $<45\%$  was not found to be an independent predictor of the development of MACE-3M in this study. This may be due to the short duration of the follow-up (3-months) and small sample size. This result could also be due to the fact that the LVEF values of patients in this study were better than those in the other studies.

The patients older than 70 years of age who are admitted with ACS are always at a high risk in the long term.<sup>26,27</sup> The age of  $>70$  years was found to be the most important predictor of prognosis in patients with NSTEMI in a study<sup>28</sup> and STEMI in another study.<sup>29</sup> The rate of the development of adverse events within the 3-month follow-up period was significantly higher in patients older than 70 years when compared to those younger than 70 years of age in the present study, and this was in accordance with the literature. The age  $>70$  years alone was found to be a predictor of MACE-3M.

Cigarette smoking induces inflammation and oxidative stress.<sup>30,31</sup> In this study, we found an increase in serum GGT and Plt-GGT in smokers confirming previous studies.<sup>32</sup> Nevertheless, smoking could not predict MACE-3M during our follow-up probably due to our small sample size. It has been shown that smoking increases serum GGT activity in a dose-dependent manner and with co-use of alcohol. Whitehead et al

found that the effect of cigarette on serum GGT was significant in amounts up to 3 to 4 units/d of alcohol consumption.<sup>33</sup> They showed that cigarette smoking significantly increases the activity of GGT in drinkers. Those who consume alcohol at this level were not included in our study group. Our patients are usually teetotalers and occasional drinkers. These conditions might have weakened the relation between smoking and MACE-3M in our study. In addition, as a limitation of our study, the total amount of cigarettes smoked daily by our patients was not recorded. We think that smoking as a risk factor for coronary atherosclerosis is associated with increased serum GGT and Plt-GGT activities in patients with NSTEMI and STEMI with high thrombotic activity. In these patients, we tried to measure the Plt-GGT which we thought could be used as a platelet activation indicator and to investigate the relationship with MACE-3M. Further research should compare the amount of cigarette consumption with Plt-GGT in patients with ACS and distinguish which GGT isoenzyme is affected by smoking. The effect of various treatments also needs to be assessed.

Besides, significant statistical correlation was found between serum GGT level and MACE-3M in the present study, the risk coefficient and the predictive value of Plt-GGT-specific activity for MACE-3M were higher than the values obtained with serum GGT. This suggests that platelets might be a source of serum GGT activity.

It is not known whether the negative impact of Plt-GGT-specific activity on prognosis can be reversed by the drugs or surgical and percutaneous revascularization methods used in the treatment of atherosclerotic disease. It must be assessed in future studies whether any drug decreases the Plt-GGT activity; if found so, the effect of this decline on the prognosis must also be evaluated.

Our study is single centered, small scaled, and short termed in follow-up. Therefore, examination of the effects of risk-reducing drug treatments on MACE development was not possible. Also, the course of Plt-GGT-specific activity in patient groups during follow-up was not investigated.

Platelet GGT-specific activity was found to be an independent predictor of MACE-3M and a stronger prognostic marker than serum GGT activity. Platelet GGT  $>265$  mU/mg, TIMI risk score  $>4$ , family history of CAD, duration of stay in coronary care unit (CCU)  $>5$  days, and age  $>70$  years were considered as independent predictors of the development of MACE-3M. In addition, the activity of Plt-GGT was increased with the clinical severity of ACS. New confirmative large-scale studies will be noteworthy for investigating the Plt-GGT (GGT4) as a novel biochemical marker for risk stratification of ACSs in a practical manner like giving way to a new Elisa kit design measuring Plt-GGT (GGT4) in serum.

## Author contribution

All authors contributed to: (1) conception and design, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and, (3) final approval of the version to be published.

## ORIGINAL ARTICLE

# Gamma-glutamyltransferase: an effect modifier in the association between age and hypertension in a 4-year follow-up study

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We performed a prospective study to assess whether the relationship of age with hypertension was stronger in men with high normal serum gamma glutamyltransferase (GGT) than in those with lower GGT levels. The study population included 8170 healthy male workers in a steel manufacturing company who had undergone health examinations in both 1994 and 1998. The higher the baseline GGT level, the effect of age on the development of hypertension was stronger. The incidence of hypertension among those aged 25–34, 35–44 and 45–50 years was 0.9, 2.2, 3.8% in those with GGT <20 U/l; 1.0, 4.1, 12.5% in those with GGT between 20 and 39 U/l; and 1.9, 6.3, 17.2% in those with GGT ≥40 U/l,

respectively. All relationships persisted after adjusting for baseline values of body mass index, alcohol intake, smoking, exercise, family history of hypertension, systolic and diastolic blood pressure, and changes of body mass index during 4 years (*P* for interaction = 0.03). Our data supported the hypothesis that the effect of age on the development of hypertension differed by baseline GGT level, although the underlying mechanism for this interaction is unclear.

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**Keywords:** gamma glutamyltransferase; age

## Introduction

In our previous longitudinal studies,<sup>1,2</sup> we reported a strong dose-response relationships between serum gamma glutamyltransferase (GGT) at baseline and development of type II diabetes. Although GGT has been widely used as a marker of alcohol consumption or liver disease,<sup>3</sup> neither alcohol nor hepatic dysfunction explained the observed relationships between GGT and diabetes. In addition, the typical relationships between age or obesity and type II diabetes were shown only among those with high normal GGT level at baseline.<sup>1,2</sup>

In the same cohorts,<sup>2,4</sup> GGT was a modest risk factor for hypertension. Furthermore, a strong interaction was found between GGT and alcohol consumption on the development of hypertension.<sup>4</sup> In this interaction, a positive association between

alcohol consumption and hypertension was observed only in those with high normal GGT level. Among subjects with low GGT level, no matter how much alcohol the subjects drank, the risk of hypertension in drinkers was similar to that of nondrinkers.

Although serum and cellular GGT may not have the same biological meanings, it is interesting to note experimental studies in which GGT at a cellular level plays an important role in antioxidant systems through the maintenance of intracellular levels of glutathione.<sup>5–7</sup> Paradoxically, recent studies<sup>8–11</sup> have shown that GGT is itself able to play a pro-oxidant role, particularly in the presence of iron.

Emerging evidence suggests that hypertension and type II diabetes share pathophysiological mechanisms, especially oxidative stress.<sup>12–15</sup> So, by analogy with type II diabetes, the association between age and hypertension might also depend on GGT level. Therefore, we performed this prospective study with the hypothesis that the relationship between age and hypertension was modified by baseline GGT level.

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## Materials and methods

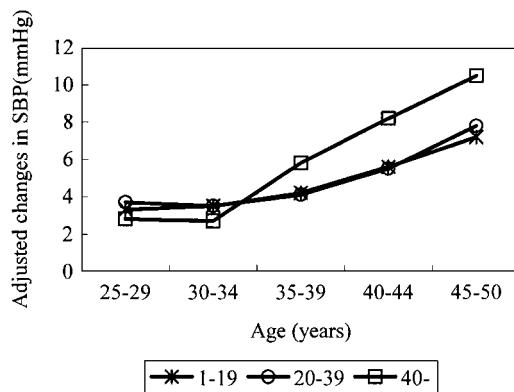
This study was based on the same cohort as our previous study of GGT and hypertension.<sup>4</sup> Study design, recruitment of participants, and methods have been described in detail elsewhere.<sup>4</sup> Briefly, our study population was 8170 male workers at one steel company in Korea, who were between 25 and 50 years without hypertension (systolic BP (SBP)  $\geq 140$  mmHg, diastolic BP (DBP)  $\geq 90$  mmHg, and/or taking antihypertensive medication) at baseline and examined in both 1994 and 1998 (follow-up rate: 73.7%). No specific informed consent for this study was obtained. Data are analysed pursuant to the Korean health regulation pertaining to factories, which states that the factory physician has an obligation to analyse health examination data to educate workers.

SBP and DBP were recorded oscillometrically with an automatic device (TM-2650A; A&D Company, Japan) in the sitting position after the subjects rested on a chair for 5 min or longer. For employees with SBP  $\geq 160$  mmHg or  $\geq 95$  mmHg, BP were measured again with an ordinary sphygmomanometer by an experienced nurse after another 5 min of rest. Serum GGT concentrations were measured at 37°C with an automatic analyzer (normal range 0–50 U/L, Hitachi 7170, Japan).

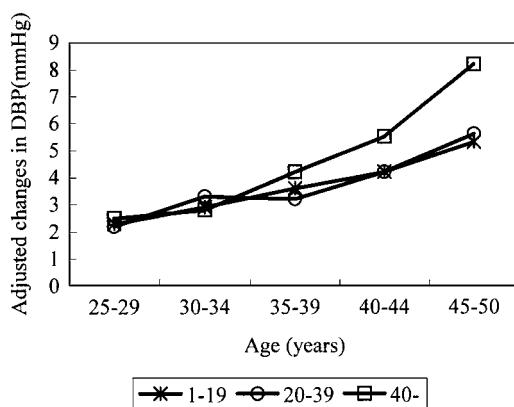
In this study, 169 men met the definition of hypertension was SBP  $\geq 160$  mmHg or DBP  $\geq 95$  mmHg or under antihypertensive medication. First, we examined the relationship between age (25–29, 30–34, 35–39, 40–44, 45–50 years) and SBP or DBP changes within three GGT categories at baseline GGT (0–19, 20–39,  $\geq 40$  U/l) by an analysis of covariance. Next, the relationship between age and incidence of hypertension was examined within the each category of GGT. We performed logistic regression analyses including interaction terms for GGT (0–19, 20–39,  $\geq 40$  U/l) and age (25–34, 35–44, 45–50 years). Covariates were the baseline values of body mass index (BMI) ( $\text{kg}/\text{m}^2$ ), cigarette smoking (pack years), alcohol consumption (g/week), exercise (frequency/week), family history of hypertension, either SBP (mmHg) or DBP (mmHg), and the changes in BMI during 4 years. The SAS statistical program, version 8.02, was used in all analyses, the *P*-values quoted are two-sided, and those values  $<0.05$  are regarded as statistically significant.

## Results

Relationships between age and changes in SBP or DBP varied by baseline GGT level. There were stronger dose-response relationships between age and changes in both SBP and DBP for baseline GGT level  $\geq 40$  U/l than for lower GGT levels (Figures 1 and 2) (*P* for multiplicative interaction,  $<0.01$  for SBP; 0.04 for DBP). In addition, the associations of GGT with both SBP and DBP varied by age. There were positive associations between GGT and



**Figure 1** Changes in SBP by age and baseline GGT levels, adjusted for the baseline values of BMI, smoking, alcohol consumption, exercise, family history of hypertension, SBP, and the changes of BMI during 4 years.



**Figure 2** Changes in DBP by age and baseline GGT levels, adjusted for the baseline values of BMI, smoking, alcohol consumption, exercise, family history of hypertension, DBP, and the changes of BMI during 4 years.

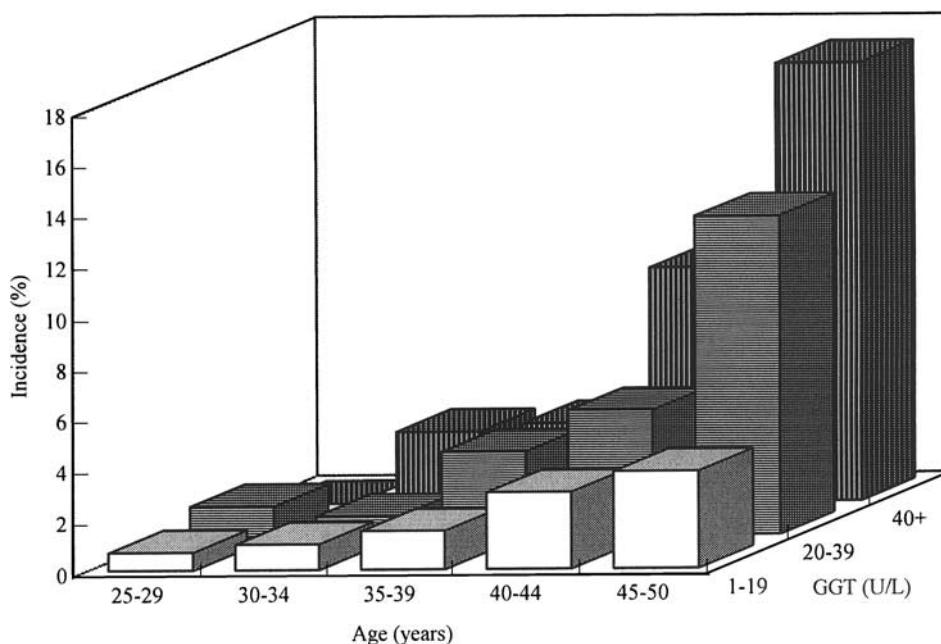
changes in SBP or DBP among those with age  $\geq 35$ , whereas there was no association among those with age  $< 35$ .

The association of age with incidence of hypertension also varied by baseline GGT level. As baseline GGT level increased, the association between age and incidence of hypertension strengthened (*P* = 0.03 for multiplicative interaction) (Figure 3). Using those aged  $< 35$  years and GGT  $< 20$  as a reference group, adjusted relative risks for those age  $< 35$ , 35–44, and  $\geq 45$  years were 1.0, 2.7, 4.5 in those with GGT  $< 20$  U/l, 0.6, 3.0, 10.9 in those with GGT between 20 and 39 U/l, and 0.9, 3.9, 19.7 in those with GGT  $\geq 40$  U/l (Table 1). The interaction was similarly observed among nondrinkers, drinkers, and subjects with normal GGT (data not shown). On the other hand, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) did not show any relationship with age in the development of hypertension. For example, adjusted relative risks for those aged  $\geq 45$  years compared to those aged  $< 35$  years was 8.8 (unadjusted inci-

**Table 1** Adjusted<sup>a</sup> relative risks (aRR) (95% confidence interval (CI)) for incidence of hypertension during the follow-up period by age and gamma-glutamly transferase (GGT) in 1994 among 8170 male workers

GGT	Age (years)					
	25–34		35–44		45–50	
	Cases/pop (%)	aRR (95% CI)	Cases/pop (%)	aRR (95% CI)	Cases/pop (%)	aRR (95% CI)
≤19	30/3433 (0.9%)	1.0 <sup>b</sup>	54/2506 (2.2%)	2.7 (1.7–4.5)	10/264 (3.8%)	4.5 (2.1–9.7)
	6/629 (1.0%)	0.6 (0.3–1.5)	30/732 (4.1%)	3.0 (1.7–5.2)	10/80 (12.5%)	10.9 (4.7–25.1)
	3/161 (1.9%)	0.9 (0.3–3.1)	21/336 (6.3%)	3.9 (2.1–7.3)	5/29 (17.2%)	19.7 (6.3–61.3)

<sup>a</sup>Adjusted for the baseline values of BMI, smoking, alcohol consumption, exercise, family history of hypertension, SBP and the changes of BMI during 4 years; <sup>b</sup>Reference group.

**Figure 3** Crude incidence of hypertension by age and baseline GGT level.

dence: 6.0% (10/168) vs 0.8% (18/2240) in those with ALT <20 U/l and 8.7 (unadjusted incidence: 8.7% (4/46) vs 1.5% (7/479)) in those with ALT ≥40 U/l, very similar between low-normal ALT group and high-normal ALT group. Neither exclusion of participants who had abnormal ALT or AST nor additional adjustment for ALT or AST attenuated the associations among incident hypertension, GGT, and age shown in Table 1 and Figure 3.

## Discussion

In agreement with our hypothesis, this study found that the association of age with the risk of hypertension varied by baseline GGT level. Among those

with high normal GGT, the effect of age on the development of hypertension was much greater than among those with low GGT. Another way to view the findings of this study is that the effect of GGT on the development of hypertension differed by age. Among young subjects, there was no effect of GGT for the risk of hypertension, whereas among old subjects, GGT showed a strong association. However, other liver enzymes such as ALT or AST did not show a relationship with incident hypertension, suggesting that the association between GGT, age and hypertension is not mediated by liver damage. This finding is consistent with the findings shown in the relationship between age and type II diabetes,<sup>1</sup> supporting the concept that underlying mechanisms of hypertension and type II diabetes

are closely related, which was suggested by other studies.<sup>12–15</sup>

Although GGT has been used clinically as a marker of alcohol consumption or liver disease, GGT plays an important role in antioxidant systems at a cellular level.<sup>5–7</sup> The maintenance of intracellular levels of glutathione is critical for antioxidant defence mechanisms of the cell, but intact extracellular glutathione is poorly transported across cell membranes. GGT is a key enzyme for transport of glutathione into cells. Furthermore, recent experimental studies<sup>8–11</sup> indicated that under physiological conditions, especially in the presence of iron, GGT is involved directly in reactive oxygen species generation as a pro-oxidant. Thus, it may be that GGT plays an antioxidant or pro-oxidant role, depending on the presence of iron or similar oxidative stress.

On the other hand, aging itself is also related to oxidative stress.<sup>16,17</sup> For example, aging has been proposed to be related to accumulation of mutations in DNA, damage to mitochondrial DNA, or advanced glycation end products, all of which could result from overproduction of reactive oxygen species. Hypertension and diabetes are major risk factors for cardiovascular disease, and the mechanisms underlying these disorders are not completely clear.<sup>12</sup> Recent studies suggest that excessive production of reactive oxygen species, outstripping endogenous antioxidant defence mechanisms, may be involved in the pathogenesis and complications of both conditions.<sup>13–15</sup> Therefore, the interaction between age and serum GGT level might be interpreted as a synergic action of two markers of oxidative stress. Alternatively, we are interested in the possibility that body iron storage plays a role in this interaction between GGT and age, because the cellular role of GGT differs depending on iron storage.<sup>8–11</sup> In this study, among young subjects who probably had low body iron storage,<sup>18</sup> GGT showed no relationship with hypertension, whereas among old age subjects who probably had high body iron storage,<sup>18</sup> GGT was a strong risk factor for hypertension.

The use of a single reading of BP in our study may have served as a drawback. A single reading is generally considered inadequate for determining the individual's usual BP level because of large random fluctuations in casual readings. However, although random errors due to single determinations weaken the association, they should not cause a spurious association. Moreover, our diagnosis of hypertension was based on two measurements of BP. In addition, because 42.6% of all hypertension cases ( $n=77$ ) were taking antihypertensive medication at follow-up, use of such medication could lead to misclassification of changes in BP. To assess this possibility, the changes in BP analyses were repeated with the 77 treated hypertensives excluded or including medication as a possible confounder. The results were unchanged. It should be also

stressed that the present study was conducted among healthy male workers only, and should be replicated among female before any generalizations can be made.

In conclusion, our data supported our hypothesis that the effect of age on the development of hypertension differed markedly by baseline GGT level, similarly to our findings with incidence of type II diabetes. However, at this point, the underlying mechanism is unclear. Further study on the role of GGT in the development of hypertension and type II diabetes is needed.

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# Serum $\gamma$ -Glutamyltransferase Was Differently Associated with Microalbuminuria by Status of Hypertension or Diabetes: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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**Background:** We hypothesized that serum  $\gamma$ -glutamyltransferase (GGT) would positively predict the risk of microalbuminuria, a frequent consequence of both diabetes and hypertension, because serum GGT predicted diabetes and hypertension in dose-response relationships.

**Methods:** In this prospective study, 2478 black and white men and women without microalbuminuria at year 10 provided urine samples 5 years later. Year 10 GGT cutpoints were 12, 18, and 29 U/L.

**Results:** The incidence of microalbuminuria across year 10 GGT categories was U-shaped. Adjusted odds ratios across quartiles of serum GGT were 1.0, 0.39, 0.54, and 0.94 ( $P < 0.01$  for quadratic term), but the shape of association depended on the status of hypertension or diabetes ( $P < 0.01$  for interaction). Among individuals who ever had hypertension or diabetes, year 10 serum GGT showed a clear positive dose-response association with incident microalbuminuria ( $P < 0.01$  for trend), whereas among individuals with neither hypertension nor diabetes during the study, year 10 GGT showed a U-shaped association with it ( $P = 0.01$  for quadratic term). When the long-term risk was evaluated in 3895 participants based on serum GGT at year 0 and prevalence of microalbuminuria at year 10 or year 15, the

trends were similar but weaker than those of short-term incidence risk.

**Conclusions:** Serum GGT within the physiologic range predicted microalbuminuria among patients with hypertension or diabetes and may act as a predictor of microvascular and/or renal complications in these vulnerable groups. GGT showed a U-shaped association with microalbuminuria among persons who did not develop either hypertension or diabetes.

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Serum  $\gamma$ -glutamyltransferase (GGT)<sup>5</sup> concentrations within the physiologic range have been strongly associated with most cardiovascular disease risk factors and predicted the development of heart disease, hypertension, stroke, and type 2 diabetes (1–6). In particular, serum GGT concentrations have shown a strong graded relationship with incident diabetes, suggesting a role in the pathogenesis of diabetes (2,3). Although serum GGT activity has commonly been used as a marker for excessive alcohol consumption or liver diseases (7), neither alcohol consumption nor liver dysfunction likely explain the association between serum GGT and diabetes (2,3). A series of Coronary Artery Risk Development in Young Adults (CARDIA) studies (3,8,9) suggested that oxidative stress might explain these associations because serum GGT within the physiologic range had dose-response relationships with serum and/or dietary antioxidant vitamins and markers of oxidative stress such as F<sub>2</sub>-isoprostanes. Although the relationship between cellular GGT and serum GGT is not known, cellular GGT has been

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<sup>5</sup> Nonstandard abbreviations: GGT,  $\gamma$ -glutamyltransferase; CARDIA, Coronary Artery Risk Development in Young Adults; OR, odds ratio; and GSH, glutathione.

known to play an important role in antioxidant defense systems (10–12); paradoxically, cellular GGT may also be involved in the generation of reactive oxygen species in the presence of transition metals (13–16). Recently, a role of serum GGT as an early and sensitive marker of oxidative stress was reviewed (17).

Microalbuminuria, slightly increased albumin excretion in the urine, is now considered to be a predictor of atherosclerotic diseases (18, 19). Recent evidence strongly suggested that microalbuminuria is an independent predictor of cardiovascular disease in diabetic or hypertensive patients, in elderly patients, and in the general population (18, 19). The mechanisms linking microalbuminuria and risk for cardiovascular disease are not fully understood; a recent concept is that microalbuminuria is a marker of endothelial dysfunction (18, 19). Generalized endothelial dysfunction has been hypothesized to be the underlying factor for microalbuminuria on the one hand and the underlying factor for increased cardiovascular risk on the other. Accumulating evidence suggests that oxidative stress alters many functions of the endothelium, including modulation of vasomotor tone (20).

We therefore performed a prospective study to examine whether GGT, possibly as a marker of oxidative stress or a generator of oxidative stress itself, is a predictor of microalbuminuria among young adult black and white men and women.

## Materials and Methods

### STUDY POPULATION

CARDIA is a longitudinal, multicenter epidemiologic study of the impact of lifestyle and other factors on evolution of coronary heart disease risk factors during young adulthood. The study design, recruitment of participants, and methods have been described elsewhere (21). In 1985–1986, at total of 5115 black and white men and women 18–35 years of age were recruited and examined at 4 clinical sites in the United States: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Participants were reexamined at 2, 5, 7, 10, and 15 years after baseline, with reexamination rates among surviving cohort members of 91%, 86%, 81%, 79%, and 74%, respectively.

In this study, we examined (*a*) the association between year 0 GGT and microalbuminuria at year 10 or year 15 and (*b*) the association between year 10 GGT and microalbuminuria at year 15. For this study, a total of 139 persons were excluded because they were pregnant at year 10 or year 15, had any kidney disease at year 10 or year 15, or had macroalbuminuria ( $A/kC \geq 250\text{mg/g}$ ; see below for definition of  $A/kC$ ) at year 10 or year 15. For the analysis of year 0 GGT in relation to year 10 or 15 microalbuminuria, we excluded 61 study participants in whom GGT was not measured at year 0 and 1038 who dropped out before year 10 or who did not provide a urine sample at both year 10 and 15, leaving 3895 participants. For the analysis of year 10 GGT in relation to year

15 microalbuminuria, among 3817 participants who attended a year 10 examination, we excluded 75 in whom GGT was not measured at year 10, 1116 who did not return for a year 15 follow-up examination or who did not provide a urine sample at either year 10 or 15 measurements, and 263 who had prevalent microalbuminuria at year 10, leaving 2478 participants for analysis. In both sets of analyses, some individuals satisfied more than 1 exclusion criterion.

### QUESTIONNAIRES

Standard questionnaires were used to maintain consistency in the assessment of demographic and behavioral information across CARDIA examination visits. Sex, race, date of birth, weekly alcohol consumption, and cigarette smoking were determined by structured interview or by self-administered questionnaire. A physical activity score was derived from the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire (22). Alcohol intake (mL/day) was computed from the self-reported frequency of beer, wine, and liquor consumed per week.

### CLINICAL MEASUREMENTS

All participants were asked to fast at least 12 h and to avoid smoking and heavy physical activity at least 2 h before the examination. After a 5-min rest, blood pressure was measured on the right arm in the sitting position. First- and fifth-phase Korotkoff sounds were recorded 3 times at 1-min intervals by use of a random zero sphygmomanometer (WA Baum Company). The mean of the second and third measurements was used in the analyses. Blood was then collected, with minimal stasis, for GGT and glucose. After plasma or serum separation, aliquots were stored at  $-70^{\circ}\text{C}$  until shipped on dry ice to a central laboratory.

The methods for measuring serum GGT were not comparable between year 0 and year 10. At year 0, liver-related enzymes, including GGT, were measured with a SMAC 12 continuous-flow analyzer (Technicon Instruments Corp.) at American Bio-science Laboratories (now Smith-Kline Beecham). At year 10, GGT was measured colorimetrically by a nitroanilide methodology at Linco Research Inc. Therefore, to identify an appropriate recalibration formula, GGT was remeasured at Linco Research Inc. with the year 10 methodology in 103 baseline samples with original GGT values ranging from 3 to 228 U/L that had been stored at  $-70^{\circ}\text{C}$  for 17 years (since 1985–1986). The correlation between measurements made at year 0 and those measured with year 10 methodology was 0.995; accordingly, the year 0 values reported here are 2.7618 plus 1.9004 times the original year 0 values. Year 0 and year 10 glucose was measured by the hexokinase-ultraviolet method at Linco, Inc. Year 0 and year 10 lipids were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Total triglycerides and total HDL-cholesterol were measured by enzymatic

methods. HDL-cholesterol was measured after dextran sulfate-magnesium precipitation. LDL-cholesterol was calculated by use of the Friedewald equation. Body weight with light clothing was measured to the nearest 0.09 kg (0.2 pounds), and body height without shoes was measured to the nearest 0.5 cm. Body mass index was computed as weight divided by height squared ( $\text{kg}/\text{m}^2$ ).

#### MEASUREMENT OF URINE ALBUMIN AND CREATININE

A single, untimed (spot) urine sample was collected at the year 10 examination when convenient during the clinic visit, usually shortly after arrival at the clinic. Albumin (A) and creatinine (C) were measured, and the term  $A/kC$  adjusted for race and gender bias, which was reported previously (23). Urinary creatinine concentration (mg/dL) in men was multiplied by  $k = 0.68$  (23, 24) and in blacks by  $k = 0.88$ ; the constant in black men is therefore  $0.68 \times 0.88$  (23, 25). Applying these adjustments allowed the use of 25 mg/g  $A/kC$  as the cutpoint for microalbuminuria in each of the 4 race-gender groups of CARDIA (23).

#### STATISTICAL ANALYSIS

Year 0 serum GGT concentrations were first classified into 4 groups based on cutpoints of 12, 18, and 26 U/L (the 25th, 50th, and 75th percentiles computed over the entire sample) for study of GGT as a predictor of microalbuminuria 10 or 15 years later. Because there was no earlier measure of microalbuminuria, we could not be sure that all cases were incident; we will therefore use the term "prevalent microalbuminuria" for this analysis. Logistic regression models were used to calculate multivariate-adjusted odd ratios (ORs). Covariates in the minimally adjusted model were the values of study center, sex, race, and baseline age. The second model added baseline body mass index, alcohol consumption, cigarette smoking, physical exercise, and education. The third model added LDL-cholesterol, HDL-cholesterol, and triglycerides. Given the strong possibility that diabetes and hypertension are intermediate in the causal pathway between GGT and microalbuminuria (26), we examined associations

after stratification by status of diabetes or hypertension (never experiencing diabetes or hypertension during follow-up compared with having diabetes or hypertension during follow-up). The definition of diabetes incidence was serum fasting glucose  $\geq 1260$  mg/L or taking diabetes medication, and the definition of hypertension was systolic blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg, or the use of antihypertensive medication. In tests for trend, the quartile number of the serum GGT value was treated as a continuous variable.

We next examined the shorter term risk of GGT measured at year 10 when participants were 28–40 years of age. Year 10 serum GGT cutpoints of 12, 18, and 29 U/L (the 25th, 50th, and 75th percentiles computed over the entire sample) and year 10 values of all covariates were used for study of association of year 10 GGT with year 15 incident microalbuminuria (omitting participants who had microalbuminuria at year 10).

## Results

#### YEAR 0 GGT AND YEAR 10 OR 15 PREVALENT MICROALBUMINURIA

The risk of prevalent microalbuminuria was increased in the highest quartile of year 0 GGT (Table 1). After minimal adjustment for study center, race, sex, and age, the ORs of year 10 or year 15 prevalent microalbuminuria across quartiles of year 0 GGT were 1.0, 0.97, 1.02, and 1.82 (model 1;  $P < 0.01$  for trend;  $P < 0.01$  for quadratic term). After further adjustment for alcohol consumption, cigarette smoking, physical activity, education, body mass index, education, LDL-cholesterol, HDL-cholesterol, and triglycerides, the ORs were 1.0, 0.93, 0.92, and 1.44 ( $P = 0.03$  for trend;  $P = 0.01$  for quadratic term).

The shape of association, however, depended on the status of hypertension or diabetes ( $P < 0.01$  for interaction). Among the 777 participants who ever had either hypertension or diabetes, the distribution of first diagnosis was 14.0% at year 0, 6.1% at year 2, 10.3% at year 5, 10.6% at year 7, 18.9% at year 10, and 40.2% at year 15. Thus, 59% of the diagnoses of hypertension or diabetes were seen at year 10 or later. Of the participants with

**Table 1. Adjusted ORs for prevalent microalbuminuria at year 10 or year 15 by quartile of serum GGT at baseline (year 0) in the CARDIA Study: 1985–1986.<sup>a</sup>**

	Quartile of GGT at year 0, U/L				<i>P</i> for trend <sup>b</sup>	<i>P</i> for quadratic term
	<12	12 to <18	18 to <26	≥26		
Cases/No. of participants	61/682	106/1196	115/1191	138/826		
Relative risk, %	8.9	8.9	9.7	16.7		
OR <sup>c</sup>						
Model 1	1.0	0.97 (0.69–1.35)	1.02 (0.73–1.43)	1.82 (1.28–2.57)	<0.01	<0.01
Model 2	1.0	0.93 (0.67–1.30)	0.95 (0.67–1.34)	1.59 (1.11–2.27)	<0.01	<0.01
Model 3	1.0	0.93 (0.66–1.30)	0.92 (0.65–1.30)	1.44 (1.00–2.08)	0.03	0.01

<sup>a</sup> Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

<sup>b</sup> *P* for trend was calculated in a model without a quadratic term.

<sup>c</sup> 95% confidence limits in parentheses.

hypertension, 43.9% took antihypertensive medication, and 21.4% of participants took antidiabetes medication. Among participants who ever had hypertension or diabetes, the association between year 0 GGT and year 10 or 15 prevalent microalbuminuria was positive in a dose-response pattern, even after full adjustment (Table 2); adjusted ORs across quartiles of serum GGT were 1.0, 1.08, 1.50, and 1.94 ( $P = 0.02$  for trend). Separate analyses for participants with diabetes but no hypertension and those with hypertension but no diabetes had results very similar to those for participants with either diabetes or hypertension (data not shown). However, among participants with neither hypertension nor diabetes during the study, year 0 GGT showed a shallow, nonsignificant, U-shaped association; adjusted ORs across quartiles of serum GGT were 1.0, 0.94, 0.80, 1.05 ( $P = 0.26$  for quadratic term).

#### YEAR 10 GGT AND YEAR 15 INCIDENT MICROALBUMINURIA

Year 10 serum GGT showed a clear U-shaped association with year 15 incident microalbuminuria (Table 3;  $P < 0.01$  for quadratic term in all models). The risk of incident microalbuminuria among participants in the second or third quartile of GGT was less than one-half of that in the lowest year 10 GGT quartile. Similar with year 0 serum GGT, the shape of association was different depending on the status of hypertension or diabetes ( $P < 0.01$  for interaction). Among participants with neither hypertension nor diabetes during the study, year 10 GGT also showed a U-shaped association with year 15 incident microalbuminuria (Table 4). However, among participants who ever had hypertension or diabetes, the association between

year 10 GGT and year 15 incident microalbuminuria was positive in a dose-response pattern. The short-term relative risk (between year 10 GGT and year 15 incident microalbuminuria) appeared to be stronger than the long-term relative risk (year 0 GGT and year 10 or 15 prevalent microalbuminuria) irrespective of the shape of association, linear or U-shaped.

#### Discussion

We performed this study with the hypothesis that serum GGT concentrations within the physiologic range positively predict future development of microalbuminuria because serum GGT has been strongly associated with most cardiovascular disease risk factors and predicted the development of heart disease, hypertension, stroke, and type 2 diabetes (1–6). However, in general, the shape of association between serum GGT and microalbuminuria was closer to a U-shaped association than a linear positive association. This finding was much clearer in the association between year 10 GGT and year 15 incident microalbuminuria than in the association between year 0 GGT and year 10 or 15 prevalent microalbuminuria.

Interestingly, the shape of association was clearly different depending on the status of hypertension or diabetes. Consistent with our previous hypothesis, serum GGT was positively associated with microalbuminuria among participants who were ever diagnosed with hypertension or diabetes during the 15 years of study. Also similar to our finding predicting diabetes in a previous CARDIA study (3), the 5-year short-term risk of serum GGT was stronger than the 10- to 15-year long-term risk of serum GGT. However, serum GGT showed a U-shaped association with microalbuminuria among participants with nei-

**Table 2. Adjusted ORs for prevalent microalbuminuria at year 10 or year 15 by quartile of serum GGT at baseline (year 0) stratified by the status of hypertension or diabetes in CARDIA.<sup>a</sup>**

	Quartile of GGT at year 0, U/L				<i>P</i> for trend <sup>b</sup>	<i>P</i> for quadratic term
	<12	12 to <18	18 to <26	≥26		
Neither hypertension nor diabetes						
Cases/No. of participants	52/610	81/1041	62/941	46/526		
Relative risk, %	8.5	7.8	6.6	8.8		
OR <sup>c</sup>						
Model 1	1.0	0.93 (0.64–1.34)	0.80 (0.53–1.19)	1.07 (0.68–1.68)	0.93	0.20
Model 2	1.0	0.93 (0.64–1.35)	0.80 (0.53–1.20)	1.09 (0.69–1.74)	0.98	0.19
Model 3	1.0	0.94 (0.65–1.37)	0.80 (0.53–1.20)	1.05 (0.66–1.68)	0.82	0.26
Either hypertension or diabetes						
Cases/No. of participants	9/72	25/155	53/250	92/300		
Relative risk, %	12.5	16.1	21.2	30.7		
OR						
Model 1	1.0	1.32 (0.58–3.00)	1.79 (0.83–3.88)	2.92 (1.36–6.28)	<0.01	0.55
Model 2	1.0	1.12 (0.49–2.59)	1.60 (0.83–3.88)	2.25 (1.02–4.94)	<0.01	0.70
Model 3	1.0	1.08 (0.47–2.50)	1.50 (0.68–3.29)	1.94 (0.87–4.31)	0.02	0.80

<sup>a</sup> Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline serum triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

<sup>b</sup> *P* for trend was calculated in a model without a quadratic term.

<sup>c</sup> 95% confidence limits in parentheses.

**Table 3. Adjusted ORs for incident microalbuminuria at year 15 by quartile of GGT at year 10 in CARDIA.<sup>a</sup>**

	Quartile of GGT at year 10, U/L				<i>P</i> for trend <sup>b</sup>	<i>P</i> for quadratic term
	<12	12 to <18	18 to <29	≥29		
Cases/No. of participants	29/489	18/694	28/684	46/611		
Relative risk, %	5.9	2.6	4.1	7.5		
OR <sup>c</sup>						
Model 1	1.0	0.44 (0.24–0.81)	0.68 (0.38–1.20)	1.36 (0.79–2.36)	0.06	<0.01
Model 2	1.0	0.39 (0.21–0.72)	0.54 (0.30–0.99)	0.95 (0.53–1.71)	0.51	<0.01
Model 3	1.0	0.39 (0.21–0.73)	0.54 (0.29–0.99)	0.94 (0.51–1.75)	0.60	<0.01

<sup>a</sup> Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

<sup>b</sup> *P* for trend was calculated in a model without a quadratic term.

<sup>c</sup> 95% confidence limits in parentheses.

ther hypertension nor diabetes during the study, especially in the 5-year short-term risk. These different findings by status of hypertension or diabetes may have arisen by chance, particularly given the small numbers of participants who were both in the lowest quartile of serum GGT and had either hypertension or diabetes, but this seems unlikely in light of the high degree of statistical significance for the positive trends seen among hypertensive or diabetic participants and for the U-shape for 5-year risk of incident microalbuminuria starting with year 10 GGT.

In this study, we used a single, untimed spot urine sample for measurement of albumin in urine. This may have led to some participants being misclassified; generally, nondifferential misclassification leads to a null association rather than a spurious association. If we had used a more rigorous design with urine samples collected over

24 h or collected in the first morning urine, we might have expected stronger associations.

We speculate that the current findings are interpretable from the perspective of the cellular role of GGT, which may connect serum GGT to oxidative stress. Although the relationship between cellular GGT and serum GGT is not known, experimental studies have shown that cellular GGT activity plays a role in maintaining intracellular glutathione (GSH) as an antioxidant defense mechanism (10–12). Cellular GGT is widely distributed in the human body and is frequently localized to the plasma membrane with its active site directed into the extracellular space (27). The highest activity was in the kidneys, where GGT was localized to the luminal surface of the proximal tubule cells; the distal tubules and glomeruli gave negative results. Although serum GGT is known as one of the liver enzymes, cellular GGT activity in homogenates of

**Table 4. Adjusted ORs for incident microalbuminuria at year 15 by quartile of serum GGT at year 10 stratified by the status of hypertension or diabetes in CARDIA.<sup>a</sup>**

	Quartile of GGT at year 10, U/L				<i>P</i> for trend <sup>b</sup>	<i>P</i> for quadratic term
	<12	12 to <18	18 to <29	≥29		
Neither hypertension nor diabetes						
Cases/No. of participants	28/462	14/585	12/533	14/420		
Relative risk, %	6.1	2.4	2.3	3.3		
OR <sup>c</sup>						
Model 1	1.0	0.43 (0.22–0.84)	0.42 (0.20–0.89)	0.70 (0.34–1.47)	0.20	<0.01
Model 2	1.0	0.41 (0.21–0.81)	0.40 (0.19–0.84)	0.60 (0.28–1.32)	0.12	0.01
Model 3	1.0	0.40 (0.21–0.80)	0.39 (0.18–0.83)	0.56 (0.25–1.27)	0.09	0.01
Either hypertension or diabetes						
Cases/No. of participants	1/27	4/109	16/151	32/191		
Relative risk, %	3.7	3.7	10.6	16.8		
OR						
Model 1		1.0 <sup>d</sup>	2.88 (1.01–8.23)	5.24 (1.92–14.32)	<0.01	0.98
Model 2		1.0 <sup>d</sup>	2.65 (0.88–7.95)	3.97 (1.38–11.43)	<0.01	0.46
Model 3		1.0 <sup>d</sup>	2.66 (0.88–8.09)	4.38 (1.48–12.93)	<0.01	0.54

<sup>a</sup> Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

<sup>b</sup> *P* for trend was calculated in a model without a quadratic term.

<sup>c</sup> 95% confidence limits in parentheses.

<sup>d</sup> First and second quartiles are combined because there was only 1 case in the first quartile.

liver was approximately one-fifth that in kidney. The primary role of GGT ectoactivity is to metabolize extracellular reduced GSH, allowing precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis; in this way, a continuous "GSH cycling" across the plasma membrane occurs in many cell types (28). Thus, cellular GGT favors the intracellular supply of GSH, the most important nonprotein antioxidant of the cell, suggesting that increased serum GGT activity might eventually minimize oxidative stress and the consequent pathologic changes attributable to oxidative stress. Small increases in serum GGT might therefore reflect a successful defense response and, particularly in the short run (e.g., 5 years, second and third quartiles of year 10 GGT), might lead to less endothelial dysfunction and microalbuminuria than would have been the case if the GGT concentration had remained low (first quartile of year 10 GGT). However, larger increases in serum GGT suggest an environment in which there is more oxidative stress, in which the primary role of GGT in the antioxidant defense of cells might be overwhelmed, leading to a U-shaped association.

However, recent experimental studies (13–16) indicate that cellular GGT can also be involved in the generation of reactive oxygen species. This effect of cellular GGT occurs when it is produced in the presence of free iron or other transition metals. In vitro experimental studies have reported that free iron can be released from iron storage proteins such as ferritin by superoxide radicals or nitric oxide (29–31). It is well known that substantial oxidative stress exists in diabetes (32, 33) and hypertension (34, 35). Therefore, patients with diabetes or hypertension might have a potential to have free iron released from iron storage protein, and in this case, cellular GGT might act as a prooxidant.

In conclusion, this study showed that serum GGT within the physiologic range was differently associated with the risk of microalbuminuria depending on the status of diabetes or hypertension. We speculate that these complicated associations might be related to the dual roles of cellular GGT as antioxidant or prooxidant, depending on the presence of iron or other transition metals.

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## Research Article

# Gamma-Glutamyl Transferase Levels in Patients with Acute Ischemic Stroke

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**Objective.** The aim of this study was to investigate the relationship between gamma-glutamyl transferase (GGT) levels, cerebrovascular risk factors, and distribution of cerebral infarct areas in patients with acute ischemic stroke (AIS). **Patients and Methods.** Sixty patients with AIS and 44 controls who had not cerebrovascular disease were included in the study. The patients were divided into four groups according to the location of the infarct area and evaluated as for GGT levels and the presence of diabetes mellitus (DM), hypertension (HT), and hyperlipidemia (HL). **Results.** The frequency of DM, HT, and HL and gender distributions were similar. The mean GGT levels were significantly higher in patients with AIS and those with relatively larger areas of infarction ( $P < 0.05$ ). Increased mean GGT levels were found in the subgroup with hypertension, higher LDL-cholesterol, and triglyceride levels among cases with AIS ( $P < 0.05$ ). **Conclusion.** Higher GGT levels in AIS patients reinforce the relationship of GGT with inflammation and oxidative stress. The observation of higher GGT levels in patients with relatively larger areas of infarction is indicative of a positive correlation between increases in infarct areas and elevated GGT levels.

## 1. Introduction

Gamma-glutamyl transferase (GGT) mediates intracellular intake of extracellular glutathione which is an important component of antioxidant mechanisms. Glutathione is produced during normal metabolic processes and plays an important role in the protection of cells against oxidative stress. GGT has been used for years as an index of hepatic dysfunction and marker of alcohol use [1, 2]. In population-based studies, after exclusion of alcohol consumption, a positive correlation has been demonstrated between higher GGT levels and advanced age, male gender, increases in body mass index (BMI), smoking, sedentary life style, hypertension, tachycardia, hyperglycemia, increased LDL-cholesterol, and decreased HDL-cholesterol levels, hypertriglyceridemia, menopause, and oral contraceptive use [3, 4].

In this study, our aim was to investigate the relationship between serum GGT levels and several risk factors for cerebrovascular disease (CVD) and also distribution of cerebral infarct areas in patients with acute ischemic stroke (AIS).

## 2. Material and Method

Sixty patients hospitalized with the diagnosis of AIS and 44 CVD-naïve individuals who consulted to neurology polyclinic for other reasons were investigated. Patients with a history of chronic liver or renal disease, endocrine, and autoimmune diseases other than diabetes, alcoholics, smokers, those who had undergone surgical interventions related to coronary, carotid, or extremity arteries, or users of drugs which might alter GGT test results (lipid-lowering drugs, antibiotics) were excluded from the study. However diabetic and/or hypertensive patients continued to use the drugs for regulation of their glycemic state and blood pressure levels. The patients with newly onset angina, myocardial infarction, and advanced heart failure were not enrolled in the study. Levels of liver enzymes, bilirubin, and hepatic markers of all cases were within normal limits. Blood samples of AIS patients were obtained within twenty-four hours after stroke.

According to Bamford classification, stroke patients were divided into 4 groups based on the infarct area as total

TABLE 1: Demographic data of the cases with AIS and the control group.

	AIS n = 60	Control n = 44	P
Age (mean ± SD)	71.7 ± 9.9	69.5 ± 8.7	0.065
Age range	51–90	50–82	
	AIS n (%)	Control n (%)	P
Gender			
Male	32 (53%)	19 (43%)	0.273
Female	28 (47%)	25 (57%)	

Student's *t*-test.

AIS: acute ischemic stroke.

anterior circulation infarcts (TACI), partial anterior circulation infarcts (PACI), lacunar infarcts (LACI), and posterior circulation infarcts (POCI). In the patient and the control groups, study participants were evaluated as for the presence of diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), and alterations in serum GGT levels. GGT was analyzed using a spectrophotometric method, and values ranging between 7 and 60 U/L were considered to be within physiologic limits.

In the statistical analysis Student's *t*-test, independent samples *t*-test, Mann Whitney *U* test, chi-square test, and Kruskal-Wallis test were performed. The significance level was evaluated at  $P < 0.05$ .

### 3. Results

Demographic data are seen in Table 1. A significant difference was not found between groups regarding mean ages and gender distribution. The frequencies of hypertension, diabetes mellitus, and hyperlipidemia in the cases with AIS and controls were comparable (88.3 versus 88.6%, 38.3 versus 38.6%, and 36.6 versus 40.9%, resp.). Mean GGT level in the AIS group was found to be significantly higher relative to the control group ( $23.3 \pm 11.8$  versus  $15.0 \pm 5.7$  IU/L;  $P < 0.000$ ). The cut-off value for GGT was calculated as 26.4 IU/L. Mean GGT level in the AIS group did not differ between female and male cases ( $23.1 \pm 13.9$  versus  $23.5 \pm 9.9$  IU/L, resp.;  $P > 0.05$ ). The lowest and the highest GGT levels were detected in the LACI and PACI groups, respectively. Mean GGT levels were significantly higher in TACI, PACI, and POCI groups than in LACI group (Table 2). Increased GGT level was found in the subgroup with hypertension, higher LDL-cholesterol, and triglyceride levels among cases with AIS (Table 3).

### 4. Discussion

Although gamma-glutamyl transferase is mostly found within cytosoles, it is also present on cellular membrane in considerable amounts and plays a role in intracellular ingress of amino acids and peptides in the form of  $\gamma$ -glutamyl peptides. Glutathione is its most important substrate. This tripeptide is a thiol derivative which is the most important non-protein intracellular component and functions as a primary

TABLE 2: Mean GGT levels in cases with AIS according to infarction area.

Infarction area	n (%)	GGT mean ± SD (IU/L)	P
LACI	15 (25.0%)	16.0 ± 6.1	
PACI	21 (35.0%)	26.7 ± 15.3	0.044*
POCI	16 (26.7%)	24.4 ± 11.1	
TACI	8 (13.3%)	25.8 ± 3.1	

Kruskal-Wallis test.

LACI: lacunar infarctions, PACI: partial anterior circulation infarctions, POCI: posterior circulation infarctions, and TACI: total anterior circulation infarctions.

\*Statistically significant (mean GGT level in the LACI group was significantly lower than other groups).

TABLE 3: Mean GGT levels in cases with AIS according to gender and risk factors.

	n	GGT mean ± SD (IU/L)	P
Gender			
Female	28	23.18 ± 13.98	0.918
Male	32	23.50 ± 9.910	
LDL cholesterol			
<129	24	17.25 ± 8.330	0.00*
>129	36	27.42 ± 12.21	
Triglyceride			
>150	27	27.00 ± 8.350	0.03*
<150	33	20.36 ± 13.50	
HDL cholesterol			
>40	15	27.13 ± 17.87	0.155
<40	45	22.09 ± 8.970	
Total cholesterol			
>200	22	23.86 ± 7.580	0.801
<200	38	23.05 ± 13.83	
Diabetes mellitus			
(-)	37	21.49 ± 8.620	0.124
(+)	23	26.35 ± 15.50	
Hypertension			
(-)	7	14.14 ± 2.670	0.028*
(+)	53	24.57 ± 12.08	

Chi-square test.

\*Statistically significant.

determinant of cellular redox mechanisms. In conditions giving rise to cellular stress, intracellular glutathione levels decrease. Decreased intracellular glutathione levels induce formation of GGT enzyme so as to maintain preexisting levels. Increased oxidative stress enhances requirement for glutathione. In the presence of inadequate amounts of glutathione, oxidative stress exerts more harmful effects [5–7]. The mechanism of the relationship between cardiovascular and cerebrovascular risk factors and GGT level is not fully known. According to a currently entertained theory, oxidative stress and related decrease in glutathione levels induce

activity of GGT. Independent of alcohol consumption and presence of a liver disease, the predictive role of GGT activity in the development of new cases of diabetes, hypertension, and ischemic stroke has been established [8–12].

The Vorarlberg Health Monitoring and Promotion Program (VHM&PP) study conducted by Ruttman et al. in Austria is the largest scale prospective study performed up to date [13]. This epidemiologic study has investigated the association between GGT and cardiovascular mortality. In this survey study an independent but significant association between GGT levels and cardiovascular mortality in both female and male cohorts was found. In the male patient cohort, a significant correlation between GGT and cardiovascular disease and ischemic and hemorrhagic stroke was present. In the same study, the correlation between GGT and cardiovascular disease was observed, but a statistically significant correlation between GGT levels and stroke (both hemorrhagic and ischemic types) could not be detected. Besides, prognostic significance of GGT was more prominently observed in patients younger than 60 years of age. Also in our study GGT levels were statistically significantly higher in the ischemic stroke group relative to the control group. However distribution of increased GGT values in the female and male patient groups did not differ significantly.

A multicenter prospective epidemiologic study (CARDIA, The Coronary Artery Risk Development in Young Adults) investigated 5115 individuals aged between 17 and 35 years. CARDIA study revealed correlations among normal GGT levels, diabetes, and hypertension [14]. The investigators also reported potential role of oxidative stress as a risk factor for the development of diabetes and hypertension and concluded that GGT is a sensitive early stage predictor of oxidative stress. In our study, a statistically significant difference was not observed between patients with or without diabetes; however, significant increases in GGT levels were noted in hypertensive patients. Besides significantly higher GGT levels were detected in patients with increased LDL-cholesterol and triglyceride levels.

D'Ambrosio et al. reported that increased gamma-glutamyl transferase levels predict functional impairment in elderly adults after ischemic stroke [15]. Korantzopoulos et al. also found positive correlation between serum gamma-glutamyl transferase and acute ischemic nonembolic stroke in the elderly subjects [16]. The role played by oxidative stress, subclinical inflammation in the pathophysiology of cardiovascular diseases, and development of stroke is already acknowledged. Mechanisms related to oxidative stress and subclinical inflammation can account for the role of GGT in the development of cerebrovascular disease [5, 17–20]. Increased GGT levels can play a pathogenetic role in the evolution and instability of atherosclerotic plaques in different vascular regions [21].

In our study, significantly higher GGT levels were detected in the AIS group relative to the control group. Besides, statistically significantly higher levels of GGT in cases with hypertension, increased LDL-cholesterol, and triglyceride levels suggest the role of oxidative stress. GGT might increase secondary to arterial wall inflammation and

resultant arterial wall thickening, and higher GGT levels might protect arterial wall against oxidative stress as well.

In conclusion, higher GGT levels in AIS patients relative to the control group reinforce the relationship of GGT with inflammation and oxidative stress. Detection of relatively higher levels of GGT in AIS patients with hypertension, increased LDL-cholesterol, and triglyceride levels indicates the presence of a positive correlation between GGT levels, oxidative stress, and inflammation. When compared with the LACI group of patients with relatively smaller lacunar infarcts, observation of higher GGT levels in TACI, PACI, and POCI groups with relatively larger areas is indicative of a positive correlation between increases in infarct areas and elevated GGT levels. Future studies can better reveal the possible role of GGT in the prediction of oxidative stress and mild degrees of chronic inflammation.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## The Relationship between Serum Gamma-Glutamyl Transpeptidase Levels and Hypertension: Common in Drinkers and Nondrinkers

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A significant association between elevations of serum gamma-glutamyl transpeptidase ( $\gamma$ -GTP) levels and those of blood pressure and hypertension has been reported separately in drinkers and nondrinkers. The aim of the present study is to evaluate whether the relationship between serum  $\gamma$ -GTP and the prevalence of hypertension is the same or similar in both drinkers and nondrinkers. The study subjects comprised 4,920 male nondrinkers, 9,390 male daily drinkers, 8,081 female nondrinkers, and 278 female daily drinkers, who were aged 40 to 59 years. The prevalence of hypertension in the male and female daily drinkers was 1.5 and 1.3 times, respectively, higher than in the nondrinkers. Mean systolic blood pressure in the male and female drinkers was 4.4 and 3.1 mmHg, respectively, higher than in the nondrinkers. After adjusting for age, body mass index, and serum  $\gamma$ -GTP levels, the differences in the prevalence of hypertension and the mean systolic blood pressure level between the drinkers and nondrinkers decreased to 1.2 times and 2.7 mmHg, respectively. Although these small differences remained statistically significant, the association between serum  $\gamma$ -GTP and hypertension appears to be quite similar in both drinkers and nondrinkers, suggesting that hepatic steatosis may play a common, pathogenetic role in the development of hypertension. (*Hypertens Res* 1995; 18: 295-301)

**Key words:** serum  $\gamma$ -glutamyl transpeptidase, hypertension, alcohol, obesity, hepatic steatosis

A large number of epidemiological studies have confirmed an association between the volume of alcohol consumed and blood pressure levels (1, 2). The reliability of the reported alcohol consumption data in epidemiological studies has been questioned, but a close association between the level of serum gamma-glutamyl transpeptidase ( $\gamma$ -GTP), which is a well-known biological indicator of alcohol consumption, and blood pressure has also been observed by many researchers (3-7).

The mechanism underlying the elevation of  $\gamma$ -GTP in the sera of alcohol consumers has been debated, but hepatic cell-membrane damage (8-10), rather than enzyme-induction (10, 11), has been suggested to be the major mechanism. The association of serum  $\gamma$ -GTP with blood pressure elevations in alcohol consumers was also suggested to be a reflection of hepatic cell damage rather than enzyme induction, since serum angiotensin-converting enzyme (ACE), which is elevated in alcoholic liver disease but not induced in the liver cells (12, 13), also showed an association with blood pressure in alcohol consumers (14). The hepatic cell damage must be related to hepatic steatosis since it is the earliest liver manifestation in alcohol consumers (15, 16).

On the other hand, a significant association of serum  $\gamma$ -GTP with blood pressure and hypertension has been found even in nondrinkers (17-19). In people without a drinking habit, elevations of serum  $\gamma$ -GTP, as well as those of other serum hepatic enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are thought to reflect the progression of hepatic steatosis with increasing body weight (20, 21). Hepatic steatosis may play an important role in the development of insulin resistance and hyperinsulinemia, resulting in hypertension (22, 23).

These previous findings suggest that elevations of serum  $\gamma$ -GTP levels, which could be a reflection of hepatic steatosis, may relate to blood pressure elevations and hypertension in both alcohol drinkers and nondrinkers. However, drinkers and nondrinkers have been evaluated for the relationship separately, and the similarity of the relationship has not been fully evaluated. In the present study, we compared the relationship between serum  $\gamma$ -GTP levels and blood pressure and hypertension in middle-aged men and women who consumed alcohol or did not to determine if that the relationship is similar in alcohol drinkers and nondrinkers.

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**Table 1.** Means and Standard Deviations of Age, Body Mass Index, Serum  $\gamma$ -GTP Level and Blood Pressure in Middle-Aged Men and Women with or without Alcohol Consumption

Variables	Men		Women	
	ND* (n=4920) Mean (SD)	DD (n=9390) Mean (SD)	ND (n=8081) Mean (SD)	DD (n=278) Mean (SD)
Age (y. o)	49.7 (6.6)	49.6 (6.4)	48.3 (6.0)	48.4 (6.1)
BMI ( $\text{kg}/\text{m}^2$ )	23.1 (2.9)	23.0 (2.7)	22.7 (2.9)	22.3 (2.9)
$\gamma$ -GTP (U/l) <sup>†</sup>	19.5 (2.0)	33.0 (2.2)	10.4 (1.6)	15.2 (1.9)
SBP (mmHg)	123.3 (15.1)	127.7 (15.9)	119.9 (15.6)	123.0 (16.6)
DBP (mmHg)	77.7 (10.1)	81.1 (10.4)	74.3 (10.2)	76.4 (10.5)

\* Drinking habit - ND: nondrinkers, including persons who consume no alcohol or small volumes of alcohol only or several social occasions. DD: daily drinkers, persons who consume alcoholic beverages almost every day.

<sup>†</sup> Geometric means (GM) and geometric standard deviations (GSD). The 95% confidence limits are calculated as  $\text{GM} \div (\text{GSD})^2$  to  $\text{GM} \times (\text{GSD})^2$ .

### Methods

Male and female subjects, who were between 40 to 59 years of age and either consumed alcohol or did not, were recruited from among all participants in this age group, (21,873 men and 10,449 women) who underwent a health screening program conducted by an occupational health service facility during the one-year period of 1992. The participants who stated in a self-report questionnaire that they had not drunk at all, or had drunk only a small volume (< 10 ml) of alcohol not more often than once a month during the preceding one-year period, were regarded as essentially nondrinkers.

On the other hand, the participants who had drunk alcoholic beverages almost every day were defined as daily drinkers. The male nondrinkers and daily drinkers numbered 4,920 and 9,390, respectively, and the female nondrinkers and daily drinkers 8,081 and 278, respectively. The remaining 9,653 male and female participants who had drunk alcoholic beverages sometimes but not every day, and thus had consumed a smaller volume than the daily drinkers, were excluded from the present study to facilitate comparison between the nondrinkers and daily drinkers.

In the health screening program, body weight was measured with only the jacket removed, and the value of body weight was determined as the measured weight minus 1 kg. Blood pressure was measured once using an automatic oscillometric monitor, BP-103N (Nippon Colin, Japan), following the recommendations of the Japanese Association for Cerebro-cardiovascular Disease Control for the mass screening of hypertension using automatic equipment (24, 25). Namely, blood pressure was measured after the subjects had rested on a chair for five minutes or longer, using cuffs 13 cm wide and 24 cm long. After the measurement of blood pressure, fasting venous blood was obtained from the cubital vein, and serum  $\gamma$ -GTP level was determined using an automatic analyzer, Hitachi 7250 (Hitachi, Japan).

The subjects were divided into four categories of

body mass index (BMI:  $\text{kg}/\text{m}^2$ ): slender ( $\text{BMI} < 20 \text{ kg}/\text{m}^2$ ), medium ( $20\text{--}24 \text{ kg}/\text{m}^2$ ), overweight ( $25\text{--}27 \text{ kg}/\text{m}^2$ ), and obese ( $\text{BMI} \geq 28 \text{ kg}/\text{m}^2$ ). The subjects were then divided into five categories of serum  $\gamma$ -GTP levels: less than 15, 15 to 29, 30 to 59, 60 to 119, and above 120 U/l. This categorization of serum  $\gamma$ -GTP level was based on the fact that the geometric mean value of serum  $\gamma$ -GTP in the male participants aged 40 to 59 years in the health check-ups was around 30 U/l, and the geometric standard deviation was 2.0.

The prevalence of hypertension was calculated in each of the categories, and compared among the different categories of serum  $\gamma$ -GTP levels for each category of BMI in both drinkers and nondrinkers, and in both men and women. Hypertension was defined here as being present in persons who showed blood pressure levels above 160/95 mmHg at the health check-ups or those being treated with hypotensive agents. After excluding the subjects receiving hypotensive agents, the means of blood pressure were then compared among the categories.

The differences in the relationships of serum  $\gamma$ -GTP to the prevalence of hypertension or to blood pressure levels between drinkers and nondrinkers, and between men and women, after adjusting for age and BMI, were statistically evaluated by a multiple logistic regression analysis and a generalized linear model analysis. These statistical analyses were performed using an SAS program package distributed by SAS Japan for a personal computer, PC-98 RL (NEC, Japan). Statistical significance was defined as  $p < 0.05$ .

### Results

Means and standard deviations of age, BMI, serum  $\gamma$ -GTP, and blood pressure in the male and female subjects with or without alcohol consumption are shown in Table 1. Geometric means and geometric standard deviations were obtained for serum  $\gamma$ -GTP levels. The means of all these variables were significantly higher in the men than in the women. Mean age and BMI were 1.3 year and  $0.3 \text{ kg}/\text{m}^2$  respectively higher in the men than in the women.

**Table 2.** Numbers of Middle-Aged Men and Women with or without Alcohol Consumption According to Body Mass Size and Serum  $\gamma$ -GTP Levels

Serum $\gamma$ -GTP levels (U/l)	SEX	Slender*		Medium		Overweight		Obese	
		ND <sup>†</sup>	DD	ND	DD	ND	DD	ND	DD
I: -14	M	390	285	1,232	883	173	101	46	11
	F	1,083	31	4,251	99	770	14	235	2
II: 15-29	M	208	481	1,186	2,238	386	489	145	121
	F	145	18	700	57	240	7	187	10
III: 30-59	M	50	219	406	1,624	214	585	119	186
	F	16	4	152	15	57	3	70	4
IV: 60-119	M	11	121	133	846	85	347	36	132
	F	6	0	36	7	20	1	15	2
V: 120+	M	11	94	58	434	20	136	11	47
	F	0	1	3	3	2	0	2	0

\* Body mass size - slender: BMI < 20 kg/m<sup>2</sup>, medium: BMI 20-24, overweight: BMI 25-27, obese: BMI ≥ 28.

† Drinking habit - ND: nondrinkers, including persons who consume no alcohol or small volumes of alcohol only on several social occasions. DD: daily drinkers, persons who consume alcoholic beverages almost every day.

The geometric mean of serum  $\gamma$ -GTP was 1.5 times higher in the men than in the women. Differences in systolic and diastolic blood pressure between the men and women were 5.9 and 4.8 mmHg, respectively.

On the other hand, there were no significant differences in age and BMI between the drinkers and nondrinkers, either in the men or women. However, geometric means of serum  $\gamma$ -GTP in the men and women with alcohol consumption were 1.7 and 1.5 times, respectively, higher than in those without alcohol consumption. Mean systolic and diastolic blood pressure levels in the male drinkers were 4.4 and 3.4 mmHg, respectively, higher than those in the male nondrinkers. Respective differences between the female drinkers and nondrinkers were 3.1 and 2.1 mmHg.

Although not shown in the table, the prevalence of hypertension in the combined total of male and female subjects was 16.6 and 10.6%, respectively, *i.e.* 1.6 times more prevalent in men as compared to women. Hypertension was found in 626 of 4,920 male nondrinkers (12.7%) but in 1,748 of 9,390 male drinkers (18.6%), and in 844 of 8,081 female nondrinkers (10.4%) and 38 of 278 female drinkers (13.7%). Thus, hypertension in men and women with alcohol consumption was 1.5 and 1.3 times, respectively, more frequent than in those without it.

The numbers of male and female subjects with or without alcohol consumption in each category of BMI and serum  $\gamma$ -GTP are shown in Table 2. In women, the numbers of subjects with higher serum  $\gamma$ -GTP levels were small in both nondrinkers and drinkers. Thus, the prevalences of hypertension in each of the categories are summarized in Table 3, excluding the categories with numbers less than ten in women. The prevalences of hypertension were increased with increased BMI, but were higher at higher serum  $\gamma$ -GTP levels at all levels of body

mass size, in both drinkers and nondrinkers, and in both men and women.

The mean of blood pressure was then calculated in each of the categories, after excluding the subjects being treated with hypotensive agents. Table 4 shows the mean systolic blood pressure levels in the male and female subjects with or without alcohol consumption according to body mass size and serum  $\gamma$ -GTP levels. The mean values were elevated with increased BMI, but were also higher at higher serum  $\gamma$ -GTP levels. Similar results were obtained for diastolic blood pressure, although not shown in the table.

Multiple logistic regression analysis was performed to evaluate the association of hypertension with the variables of age, BMI, and serum  $\gamma$ -GTP levels, separately in the male and female drinkers and nondrinkers. All three variables were significantly related to the prevalence of hypertension in both sexes and in both drinkers and nondrinkers. The results of the analysis including sex and the difference in alcohol consumption as independent variables are shown in Table 5. Among daily drinkers the odds ratio of hypertension was 1.24 as compared with nondrinkers; and among female daily drinkers the odds ratio of hypertension was 1.24 as compared with male daily drinkers, which meant that the prevalence of hypertension among male daily drinkers was 0.81 times that among female daily drinkers.

Generalized linear model analysis of systolic blood pressure and related variables in the male and female drinkers and nondrinkers also showed that age, BMI, and serum  $\gamma$ -GTP levels, were all significantly related to blood pressure in both sexes and in both drinkers and nondrinkers. Table 6 shows the results of the analysis including sex and the difference in alcohol consumption as independent variable. The daily drinkers showed a systolic

**Table 3. Prevalence of Hypertension in Middle-Aged Men and Women with or without Alcohol Consumption According to Body Mass Size and Serum  $\gamma$ -GTP Levels**

Serum $\gamma$ -GTP levels (U/l)	Sex	Slender		Medium		Overweight		Obese	
		ND <sup>#</sup>	DD	ND	DD	ND	DD	ND	DD
I: -14	M	5%	9	8	11	15	12	17	18
	F	6	0	7	8	15	29	18	— <sup>§</sup>
II: 15-29	M	7	14	12	13	15	20	23	28
	F	10	0	13	18	20	—	30	30
III: 30-59	M	20	16	16	17	24	27	23	30
	F	19	—	15	20	25	—	34	—
IV: 60-119	M	18	21	20	24	28	27	33	37
	F	—	—	11	—	20	—	53	—
V: 120-	M	27	26	22	33	25	29	18	47
	F	—	—	—	—	—	—	—	—

\* Hypertension was defined as  $BP \geq 160/95$  mmHg or being treated with antihypertensive agents.

† Body mass size - slender:  $BMI < 20$  kg/m<sup>2</sup>, medium:  $BMI 20-24$ , overweight:  $BMI 25-27$ , obese:  $BMI \geq 28$ .

# Drinking habit - ND: nondrinkers, including persons who consume no alcohol or small volumes of alcohol only on several social occasions. DD: daily drinkers, persons who consume alcoholic beverages almost every day.

§ Because of the small number of subjects (<10), the prevalence is not shown.

**Table 4. Means of Systolic Blood Pressure (mmHg) in Middle-Aged Men and Women with or without Alcohol Consumption According to Body Mass Size and Serum  $\gamma$ -GTP Levels**

Serum $\gamma$ -GTP levels (U/l)	Sex	Slender*		Medium		Overweight		Obese	
		ND <sup>#</sup>	DD	ND	DD	ND	DD	ND	DD
I: -14	M	111	121	121	123	125	126	128	129
	F	117	115	119	120	122	125	125	— <sup>‡</sup>
II: 15-29	M	120	124	123	125	126	129	129	131
	F	117	122	121	124	126	—	127	132
III: 30-59	M	127	129	125	129	126	130	129	132
	F	114	—	123	127	121	—	129	—
IV: 60-119	M	127	131	128	130	130	133	135	135
	F	—	—	121	—	123	—	136	—
V: 120-	M	136	130	131	134	135	134	133	133
	F	—	—	—	—	—	—	—	—

\* Body mass size - slender:  $BMI < 20$  kg/m<sup>2</sup>, medium:  $BMI 20-24$ , overweight:  $BMI 25-27$ , obese:  $BMI \geq 28$ .

† Drinking habit - ND: nondrinkers, including persons who consume no alcohol or small volumes of alcohol only on several social occasions. DD: daily drinkers, persons who consume alcoholic beverages almost every day.

‡ Because of the small number of subjects (<10), the mean value is not shown.

blood pressure 2.7 mmHg higher than the nondrinkers, and this difference was statistically significant. On the other hand, systolic blood pressure was shown to be 0.4 mmHg higher in the men than in the women, but this difference not statistically significant.

The results of multiple logistic regression analysis indicated that an increased prevalence of hypertension was associated with increased BMI in both male and female subjects with or without alcohol consumption, for the three levels of serum  $\gamma$ -GTP of 20, 50 and 100 U/l (Fig. 1; age fixed at 50 years).

## Discussion

Hypertension was more prevalent in male and female daily drinkers than in the nondrinkers of similar age and body mass index, i.e., 1.5 times more prevalent in male drinkers than in male nondrinkers and 1.3 times more prevalent in female drinkers than in female nondrinkers. Also, systolic blood pressure was higher in the daily drinkers than in the nondrinkers in both sexes, i.e., 4.4 mmHg higher in male drinkers than in male nondrinkers and 3.1 mmHg higher in female drinkers than in

**Table 5. Results of Multiple Logistic Regression Analysis for Variables Related to Hypertension in Middle-Aged 14,310 Men and 8,359 Women**

Variable	Parameter	Estimate	SE ( $\beta$ )	Probability	Odds ratio (95% range) <sup>†</sup>
Intercept	$\beta_0$	-10.82	0.289		
$X_1$ : Age (y. o)	$\beta_1$	0.083	0.003	<0.0001	
$X_2$ : BMI (kg/m <sup>2</sup> )	$\beta_2$	0.113	0.007	<0.0001	
$X_3$ : $\gamma$ -GTP (U/l) <sup>‡</sup>	$\beta_3$	1.182	0.061	<0.0001	
$X_4$ : Alcohol <sup>§</sup>	$\beta_4$	0.217	0.052	<0.0001	1.24 (1.12-1.38)
$X_5$ : Sex <sup>¶</sup>	$\beta_5$	0.216	0.058	<0.0002	1.24 (1.11-1.39)

\* Log e (pX/qX) =  $\beta_0 + \sum \beta_i X_i$ . pX: probability of hypertension, qX = 1 - pX, pX/qX means the odds of the probability,  $\beta_i$ : parameter estimate, SE ( $\beta$ ): standard error of estimate.

† Odds ratios were calculated only for differences in sex (women/men) and in alcohol consumption (drinkers/nondrinkers).

‡ Logarithmically transformed.

§ "Nondrinker" was coded as 0 and "daily drinker" as 1.

¶ "Man" was coded as 0 and "woman" as 1.

**Table 6. Results of Generalized Linear Model Analysis for Variables Related to Systolic Blood Pressure in Middle-Aged 13,118 Men and 7,753 Women**

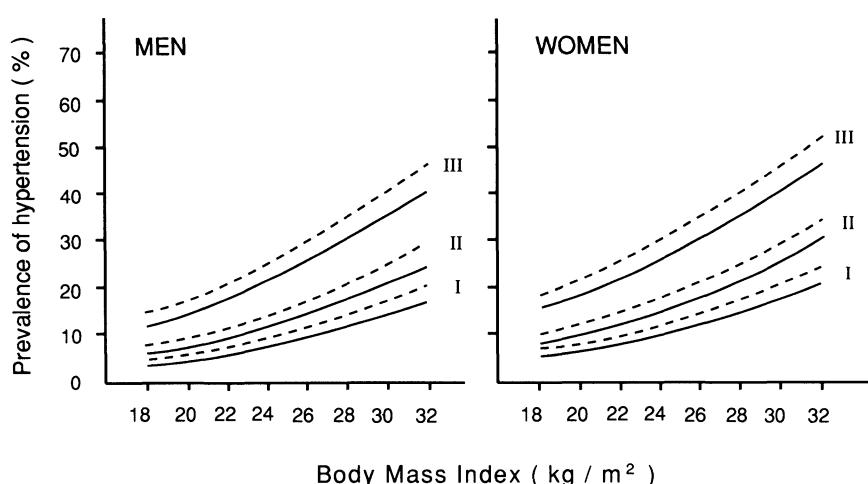
Variables	Parameter	Estimate	SE( $\beta$ )	Probability
Intercept	$\beta_0$	75.79	1.223	
$X_1$ : Age (y. o)	$\beta_1$	0.473	0.017	<0.0001
$X_2$ : BMI (kg/m <sup>2</sup> )	$\beta_2$	0.693	0.038	<0.0001
$X_3$ : $\gamma$ -GTP (U/l) <sup>†</sup>	$\beta_3$	7.927	0.362	<0.0001
$X_4$ : Alcohol <sup>‡</sup>	$\beta_4$	2.671	0.277	<0.0001
$X_5$ : Sex <sup>§</sup>	$\beta_5$	-0.413	0.288	0.1510

\*  $Y = \beta_0 + \sum \beta_i X_i$ . Y=systolic blood pressure (mmHg),  $\beta_i$ : parameter estimate, SE ( $\beta$ ): standard error of estimate.

† Logarithmically transformed.

‡ "Nondrinker" was coded as 0 and "daily drinker" as 1.

§ "Man" was coded as 0 and "woman" as 1.



**Fig. 1. Relationships between body mass index, serum  $\gamma$ -glutamyl transpeptidase levels and hypertension in men and women aged 50 years with or without alcohol consumption. Illustrated from the results of a multiple logistic analysis. Serum  $\gamma$ -GTP levels - I: 20 U/l, II: 50 U/l, III: 100 U/l. Solid lines denote nondrinkers, and dashed lines denote daily drinkers.**

female nondrinkers. Since about 75 percent of the male daily drinkers consumed 30 to 60 ml of alcohol per day, and most of the female daily drinkers consumed up to 30 ml of alcohol per day, these differences in the prevalence of hypertension and blood pressure levels between the male and female drinkers and nondrinkers were consistent with the dose-response relation observed in previous epidemiological studies (1, 2).

Prevalences of hypertension and levels of blood pressure were positively correlated with the levels of serum  $\gamma$ -GTP in both male and female drinkers and nondrinkers, as shown in Tables 3 and 4, although the numbers of female drinkers and nondrinkers who had high serum  $\gamma$ -GTP levels were small. These findings were also consistent with those in our previous studies conducted separately in drinkers (5, 6) and nondrinkers (17-19).

When adjustments were done for serum  $\gamma$ -GTP levels, in addition to age and BMI, the difference in the prevalence of hypertension between daily drinkers and nondrinkers was decreased to 1.2 times, and that in systolic blood pressure was decreased to 2.7 mmHg. These differences were still statistically significant, suggesting that the relationship of serum  $\gamma$ -GTP with blood pressure and hypertension may differ in drinkers and nondrinkers.

However, as shown in Fig. 1, these differences were very small, and it remains open to question whether these differences were truly related to the pressor effects of alcohol itself. Other factors might have influenced the results in drinkers and nondrinkers. For example, alcohol consumers may be detected to be hypertensive more often than non-consumers, either because physicians may pay more attention to hypertension in alcohol consumers, or because alcohol consumers may visit physicians more often. Further, lifestyle and behavioral factors other than alcohol consumption in daily drinkers, such as psychological stress, may contribute to blood pressure elevation. The effects of these possible confounding factors, however, were not analyzed in the present study.

It is difficult to draw definite conclusions from the present cross-sectional observations owing in part to limitations in the study design. For example, we cannot deny a possible bias in this study associated with the selection of subjects based on a self-administered questionnaire of alcohol consumption. Our nondrinker group might have included many alcohol consumers who had elevated blood pressure and serum  $\gamma$ -GTP, although a significant association between serum  $\gamma$ -GTP and blood pressure has been observed in a smaller nondrinker population (17) in which the subjects were carefully evaluated for alcohol consumption by interviews.

At present, however, the small differences in the prevalence of hypertension and the levels of blood pressure between drinkers and nondrinkers after adjusting for serum  $\gamma$ -GTP levels indicate that the relationship between serum  $\gamma$ -GTP and blood pressure and hypertension is very similar in both drinkers and nondrinkers. This similar relation suggests a pathogenetic role of elevated serum  $\gamma$ -GTP, which

may reflect hepatic steatosis (20, 21, 23), in the development of hypertension in middle-aged people, regardless of alcohol consumption.

Decreases in serum  $\gamma$ -GTP have been observed to precede those in blood pressure in interventional studies of alcohol moderation. This is consistent with the contention that elevated levels of serum  $\gamma$ -GTP may be causally related to blood pressure elevations and the development of hypertension in drinkers. A close association between changes in serum  $\gamma$ -GTP and those in blood pressure during alcohol moderation has been detected by some researchers (26-29). A similar association was also observed in obese men during weight reduction by dieting (30).

The exact biological link between elevations of serum  $\gamma$ -GTP and those of blood pressure and the development of hypertension in drinkers and nondrinkers remains unknown and should be elucidated by further studies.

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## The Association between an Increased Level of Gamma-Glutamyl Transferase and Systolic Blood Pressure in Diabetic Subjects

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Gamma-glutamyl transferase (GGT) is an enzyme present in serum and on most cell surfaces and serves as an oxidative stress marker. Although serum GGT is associated with hypertension development, little data are available on the associations between GGT and hypertension among populations with diabetes mellitus (DM). Our aim was to investigate the potential association between the changes in systolic or diastolic blood pressure (SBP/DBP) and the GGT level in type 2 DM subjects, in comparison with non-DM subjects. In 179 non-DM and 177 DM subjects, SBP/DBP, body mass index (BMI), fasting plasma glucose, serum aspartate aminotransferase, alanine aminotransferase and GGT were measured at the baseline and after a 1-year period. Between these 2-measurement points, in non-DM subjects, SBP and DBP levels were significantly increased, while GGT tended to increase. In contrast, in DM subjects, the mean levels of SBP, DBP and GGT remained unchanged. Multivariate analysis revealed that in non-DM subjects the degree of increase in SBP was significantly and positively correlated to that of GGT ( $\beta = 0.165$ ), along with age and BMI. Likewise, the increase in DBP was correlated to that of GGT in non-DM subjects ( $\beta = 0.170$ ). In contrast, in DM subjects, the degree of increase in SBP was significantly correlated to that of only GGT ( $\beta = 0.166$ ). These results suggest that the presence of DM may attenuate the effects of GGT on DBP. ——— liver enzyme; gamma-glutamyl transferase; body mass index; weight change.

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Gamma-glutamyl transferase (GGT) is an enzyme that is present in serum and on the surfaces of most cell types, and serum GGT is clinically used as a marker of alcohol consumption in general. Recently, serum GGT has been recognized

as a marker of oxidative stress (OS) (Lee et al. 2004). Although the mechanism on the associations between GGT and OS remains largely unknown, some possible explanations exist: e.g., its direct involvement in the generation of reac-

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tive oxygen species, its indirect role to maintain intracellular antioxidant glutathione (in a response to OS, increased transport of glutathione into cells by increased GGT activity), its relation to chronic inflammation, or its relation to the pathophysiology of insulin resistance (Lee et al. 2003; Shankar and Li 2007).

OS is involved in the pathophysiology of various diseases such as cardiovascular and/or metabolic regulation (Lee et al. 2004). In fact, increased serum GGT levels are implicated in an increased blood pressure (BP) and the progression of hypertension (HT) (Nilssen et al. 1990; Ikai et al. 1994; Lee et al. 2002, 2003; Stranges et al. 2005; Shankar and Li 2007). However, little data have been available on the associations between GGT and HT among populations with diabetes mellitus (DM) specifically, still more data regarding GGT change levels (over a period of at least 1 year to see a chronic influence) are needed. DM and HT are interrelated diseases predisposing to atherosclerotic cardiovascular disease: OS-related mechanisms can be hypothesized in this interrelationship (Lee et al. 2003). Accordingly, the association between GGT and BP levels among DM populations may have some specific characteristics, but there are not any data with a comparison to non-DM populations.

With these backgrounds in mind, we conducted a 1-year observational study to investigate the following outcome-of-interest: whether systolic/diastolic BP (SBP/DBP) level differences could be potentially associated with those of GGT in addition to other hepatic enzymes in type 2 DM subjects, and whether the association between BP and GGT was different between type 2 DM and non-DM subjects.

#### SUBJECTS AND METHODS

In total, 356 asymptomatic Japanese subjects were studied during a 1-year study period between 2006 and 2007: there were 179 non-DM subjects (79 males and 100 females; mean age:  $48.7 \pm 6.2$  [range: 36-64] years) and 177 type 2 DM subjects (78 males and 99 females; mean:  $50.5 \pm 6.5$  [35-65] years). This study was approved by the Tottori University Ethics Committee and each subject gave informed consent. These subjects were

recruited from a general population for health check-ups. Type 2 DM was diagnosed through repeated doctor's checks for subjects with plasma glucose (PG) of  $\geq 7$  mmol/l (World Health Organization criteria) in blood examinations both at the baseline and after a 1-year study period. Non-DM subjects showed persistent normal PG levels in similar examinations, and were apparently of normal health.

All subjects had had no medical history of cardiovascular, thyroid, renal or malignant disorders. They were negative for both hepatitis B surface antigen and hepatitis C virus antibody, had serum levels less than 2-folds the upper limit of the reference range of each hepatic enzyme, did not take any continuous medication, and had not had an alcohol intake and smoking habits. They were untreated during the study period. For each subject, seated SBP/DBP was measured 3-times with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Komaki) and the 3 measurements were averaged. At the baseline, in addition to SBP/DBP and body mass index (BMI), fasting PG, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and GGT were measured. After the 1-year period, the same variables were reexamined. PG was assayed with an automatic analyzer (JCA-BM2250, JEOL Co. Ltd., Tokyo), and AST, ALT and GGT were also assayed with an automatic analyzer (TBA-200FR, Toshiba, Tokyo). These intraassay-coefficients of variation were 0.8% in PG, 0.5% in AST, 0.5% in ALT and 0.6% in GGT, respectively.

All values were expressed as mean  $\pm$  S.D. (regarding AST, ALT and GGT, geometric mean). Level differences between the data at the baseline and after a 1-year period were analyzed by paired *t*-test. A multiple regression analysis on level differences of SBP/DBP was used to analyze the correlation to those of GGT after adjusting for measured confounders (age, gender, BMI, AST and ALT). Because of their skewed distributions, AST, ALT and GGT were log-transformed and included in the analysis model. We used SBP/DBP as continuous variables and gender simply as an explanatory variable, since no threshold effects of GGT on BP levels and no clear gender-differences in the association between GGT and BP have been confirmed (Shankar and Li 2007). A level of  $p < 0.05$  was considered significant.

#### RESULTS

The data at baseline (pre-study period) and after 1 year (post-study period) on each measured

variable in the non-DM and type 2 DM group were respectively listed in Table 1. During a 1-year period, in non-DM subjects, SBP and DBP levels were significantly increased, while GGT slightly increased. In contrast, in type 2 DM subjects, the mean levels of SBP, DBP and GGT remained unchanged.

In non-DM subjects, the degree of increase in SBP was significantly, independently and posi-

tively correlated to that of GGT, along with age and BMI (Table 2). Similarly, DBP were significantly, independently and positively correlated to GGT. On the other hand, in type 2 DM subjects, the degree of increase in SBP was significantly, independently and positively correlated to that of only GGT. Although a correlated tendency to GGT was observed, DBP did not show any relative significance.

TABLE 1. Pre- and post-study characteristics of each variable in the non-DM and type 2 DM group.

Variable	Pre-study levels	Post-study levels	Level differences
<b>Non-DM group (<i>n</i> = 179)</b>			
Systolic blood pressure (mmHg)	116.8 ± 15.1 (83 – 155)	120.0 ± 15.3** (91 – 168)	2.9 ± 9.7 (–24 – +26)
Diastolic blood pressure (mmHg)	75.8 ± 10.6 (52 – 99)	76.6 ± 10.4* (50 – 101)	1.4 ± 7.7 (–17 – +19)
Body mass index (kg/m <sup>2</sup> )	22.3 ± 2.4 (17.1 – 29.3)	22.5 ± 2.5** (16.8 – 29.5)	0.2 ± 0.7 (–2.9 – +2.8)
Glucose (mmol/l)	4.8 ± 0.2 (4.0 – 5.5)	4.8 ± 0.3 (4.2 – 5.5)	–0.02 ± 0.23 (–0.6 – +1.1)
AST (U/l)	18.6 ± 1.4 (10 – 52)	20.1 ± 1.4** (8 – 49)	1.3 ± 1.3 (–15 – +27)
ALT (U/l)	18.2 ± 1.6 (10 – 64)	19.3 ± 1.6** (7 – 69)	1.1 ± 1.3 (–20 – +26)
GGT (U/l)	30.0 ± 1.5 (11 – 70)	31.0 ± 1.5 (12 – 79)	1.0 ± 1.4 (–24 – +44)
<b>Type 2 DM group (<i>n</i> = 177)</b>			
Systolic blood pressure (mmHg)	122.9 ± 19.1 (80 – 169)	122.1 ± 17.9 (82 – 169)	–0.8 ± 12.4 (–38 – +27)
Diastolic blood pressure (mmHg)	79.3 ± 11.6 (51 – 99)	79.2 ± 10.9 (50 – 99)	–0.1 ± 8.8 (–25 – +20)
Body mass index (kg/m <sup>2</sup> )	23.2 ± 3.2 (16.1 – 29.6)	23.3 ± 3.1 (16.0 – 29.9)	0.0 ± 0.7 (–3.7 – +1.9)
Glucose (mmol/l)	7.2 ± 0.3 (7.0 – 9.8)	7.4 ± 0.5** (7.0 – 11.1)	0.19 ± 0.30 (–0.9 – +1.7)
AST (U/l)	20.4 ± 1.4 (10 – 65)	21.2 ± 1.4 (11 – 61)	1.0 ± 1.3 (–18 – +30)
ALT (U/l)	20.4 ± 1.6 (11 – 57)	21.1 ± 1.5 (10 – 58)	1.0 ± 1.5 (–42 – +47)
GGT (U/l)	31.4 ± 1.5 (12 – 62)	30.0 ± 1.6 (10 – 67)	–1.0 ± 1.6 (–43 – +41)

Data are shown as mean ± s.d. and ranges are in parentheses. DM, diabetes mellitus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

Level differences between pre- and post-study data were analyzed by paired *t*-test.

Significance levels: \* *p* < 0.05, \*\* *p* < 0.01.

TABLE 2. Multiple regression analysis of variables correlated to blood pressure level differences.

Variable	Non-DM group	Type 2 DM group
	$\beta$ -coefficient ( <i>p</i> value)	$\beta$ -coefficient ( <i>p</i> value)
For $\Delta$ Systolic blood pressure		
Age	0.183 (0.011 *)	0.003 (0.968)
Gender, male	0.029 (0.683)	0.060 (0.421)
$\Delta$ Body mass index	0.179 (0.027 *)	0.069 (0.378)
$\Delta$ Glucose	-0.032 (0.664)	0.001 (0.997)
$\Delta$ AST <sup>†</sup>	0.078 (0.328)	-0.037 (0.699)
$\Delta$ ALT <sup>†</sup>	0.049 (0.552)	0.159 (0.099)
$\Delta$ GGT <sup>†</sup>	0.165 (0.045 *)	0.166 (0.040 *)
For $\Delta$ Diastolic blood pressure		
Age	0.139 (0.061)	-0.022 (0.773)
Gender, male	0.088 (0.238)	-0.038 (0.616)
$\Delta$ Body mass index	0.026 (0.755)	0.087 (0.276)
$\Delta$ Glucose	-0.076 (0.321)	-0.012 (0.879)
$\Delta$ AST <sup>†</sup>	-0.093 (0.263)	-0.009 (0.925)
$\Delta$ ALT <sup>†</sup>	0.144 (0.096)	0.105 (0.283)
$\Delta$ GGT <sup>†</sup>	0.170 (0.047 *)	0.147 (0.071)

DM, diabetes mellitus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.  $\Delta$  (difference levels) means the values by subtracting the pre-study values from the post-study values.

<sup>†</sup> AST, ALT and GGT were analyzed after log-transformation because of their skewed distributions.

Significance level: \* *p* < 0.05.

## DISCUSSION

Our study during a 1-year period found a significant and positive association between GGT and SBP level differences, but DBP, in a population of type 2 DM, independent of age, gender, BMI and other hepatic enzymes. This finding extends to type 2 DM in that GGT may be a predictive biochemical marker at least for SBP changes. Notably, the impact of measured variables on SBP/DBP might be different between type 2 DM and non-DM: while in non-DM, the effects of age and BMI on SBP and the associations of GGT with both SBP and DBP are in lines with prior reports (Nilssen et al. 1990; Ikai et al. 1994; Lee et al. 2002, 2003; Whelton et al. 2002; Stranges et al. 2005; Shankar and Li 2007), lack of age- or BMI-related effects and attenuation on the association of GGT with DBP, observed in

type 2 DM, may be reflective of some specific pathophysiology of type 2 DM. Because DM is usually found together with OS (Ceriello 2006), the presence of OS may modify GGT functions unlike those in non-DM subjects. Furthermore, for example, premature aging or weight reduction, which is linked with OS, is known during the progression of DM (Preuss 1997; Wakabayashi and Masuda 2004), and this may partly explain our results on type 2 DM.

In summary, our study showed a positive association between GGT and SBP level differences in type 2 DM, similar to non-DM. However, the impact of GGT on measured variables including DBP among type 2 DM subjects can be different from that of non-DM subjects, suggesting that the presence of DM may attenuate the effects of GGT on increases in DBP. These results call for further study.

## Association between Serum Gamma-Glutamyl Transferase Level and Prehypertension among Community-Dwelling Men

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Serum gamma-glutamyl transferase (GGT) activity is a general clinical marker of excessive alcohol consumption, and GGT reflects changes in oxidative stress and implicated in the progression of hypertension. Recent guidelines classify persons with above-optimal blood pressure (BP) but not clinical hypertension as having prehypertension for a systolic BP (SBP) of 120 to 139 mmHg and/or a diastolic BP (DBP) of 80 to 89 mmHg; however, only limited data are available on the association between serum GGT and this entity among community-dwelling men in Japan. We performed a cross-sectional study to examine whether serum GGT was associated with prehypertension. Study participants (754 men, age  $56 \pm 15$  years) without a clinical history of stroke, transient ischemic attack, myocardial infarction, angina, or renal failure were recruited from a single community. Thirty-seven percent of participants had prehypertension and 39.3% had hypertension. Multiple regression analysis using SBP and DBP as objective variables, adjusted for risk factors as explanatory variables, showed that log GGT was significantly and independently associated with elevated SBP ( $\beta = 0.109$ ,  $P = 0.006$ ) and DBP ( $\beta = 0.238$ ,  $P < 0.001$ ). Compared with participants in the lowest tertile of serum GGT (< 29 IU/L), the multivariate-adjusted odds ratio (OR) (95% CI) for prehypertension was 1.73 (1.06-2.81) for the middle tertile (29-53 IU/L) and 2.37 (1.31-4.31) for the highest tertile (> 53 IU/L). Moreover, the respective ORs for hypertension were 1.82 (1.04-3.18) and 3.11 (1.61-6.03). These results suggest that higher serum GGT levels are associated with prehypertension or hypertension in the general male population. ——— gamma-glutamyl transferase; hypertension; prehypertension; risk factor; Japanese men.

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Serum gamma-glutamyl transferase (GGT) is a general clinical marker of alcohol consumption, although it is documented that other factors are also associated with serum levels of GGT. Serum GGT activity reflects changes in oxidative

stress, perhaps via a direct role for GGT in generating reactive oxygen species (Lee et al. 2004; Lim et al. 2004) and an indirect role in increasing the transport of glutathione precursors into cells. Thus, several large epidemiological studies have

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shown that elevated GGT levels are associated with cardiovascular disease (CVD). (Lee et al. 2006; Meisinger et al. 2006; Shankar et al. 2007), whereas others have demonstrated that GGT reflects other concomitant risk factors, such as obesity, insulin resistance, diabetes (Lee et al. 2003), hypertension (Shanklar and Li 2007), dyslipidemia, and metabolic syndrome (Lee et al. 2007).

Hypertension is one of the most common diseases in Japan and is strongly associated with an increased risk of CVD. Increased CVD mortality risk occurs when blood pressure (BP) is as low as 115 mmHg systolic BP (SBP) and 70 mmHg diastolic BP (DBP), and the risk increases steadily with elevating BP (Lewington et al. 2002). The most recent references for BP classification in adults are the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) in 2003. In this guideline, people with SBP of 120 to 139 mmHg and/or DBP of 80 to 89 mmHg are categorized as having prehypertension (Chobanian et al. 2003). Prehypertension and even normal BP frequently progress to clinical hypertension over several years, especially in older adults (Vasan et al. 2001). The incremental relationship between BP and CVD risk is continuous and consistent (Miura et al. 1994; Lee et al. 2002). Although prehypertension is associated with an increased risk of major CVD events (Chobanian et al. 2003), only limited data are available on the association between GGT and the prevalence of prehypertension among community-dwelling men in Japan.

Here, we evaluated the distribution of BP and GGT, as well as associated risk factors such as age, using cross-sectional data from community-dwelling men.

## MATERIALS AND METHODS

### *Subjects*

Participants were recruited at the time of their annual health examination in a rural town: Nomura-cho, Seiyo-city, which has a total male population of 5,357 (as of April 2002) and located in Ehime prefecture, Japan, in 2002. Among 4,395 male adults aged 19 to 90 years in

this population, 1,284 (29.2%) took part in the program and agreed to join the study. Information on medical history, present conditions, and drug usage was obtained by interview. Subjects with a clinical history of stroke, transient ischemic attack, myocardial infarction, or angina were excluded. The final study sample included 754 eligible men. This study was approved by the ethics committee of Ehime University School of Medicine and all participants gave written informed consent.

### *Evaluation of Risk Factors*

Information on demographic characteristics and risk factors was collected using clinical files. Body mass index was calculated by dividing weight (in kilograms) by the square of the height (in meters). We measured BP with an appropriate-sized cuff on the right upper arm of participants in a sedentary position using an automatic oscillometric BP recorder (BP-103i; Colin, Aichi, Japan) while the subjects were seated after having rested for at least 5 min. Normotension was defined as SBP < 120 mmHg and DBP < 80 mmHg. Prehypertension was defined as SBP 120 to 139 mmHg and/or DBP 80 to 89 mmHg. Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg (Chobanian et al. 2003). Cigarette smoking was quantified based on daily consumption and duration of smoking. Fasting total cholesterol (T-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose (FBG), creatinine (enzymatic method), uric acid and GGT were measured during fasting. Serum GGT concentration was assayed with an automatic analyzer (TBA-c16000, TOSHIBA, Tokyo) and this intraassay-coefficients of variation was 0.87 to 2.11% in GGT. Low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald formula (Friedewald et al. 1972). Participants with TG levels ≥ 400 mg/dL were excluded (24 cases). The presence of diabetes was defined as a history of treatment for diabetes. Estimated glomerular filtration ratio (eGFR) was calculated with the following equation:  $eGFR = 194 \times Cr^{-1.094} \times Age^{-0.287}$  (The Japanese Society of Nephrology: Japanese version of GFR estimation. <http://www.jsn.or.jp/>: Updated Oct 29, 2007.). Participants with an eGFR of < 30 mL/min/1.73 m<sup>2</sup> were excluded (1 cases).

### *Statistical Analysis*

Statistical analyses were performed using SPSS 10.0J (Statistical Package for Social Science, Inc., Chicago, IL, USA). All values are expressed as mean ±

standard deviation (s.d.), unless otherwise specified. The differences among groups categorized by serum GGT levels were analyzed by Mann-Whitney U test or chi-square ( $\chi^2$ ) test. Correlations between various characteristics and GGT were determined using Spearman's correlation. Multiple linear regression analysis was used to evaluate the contribution of risk factors for SBP or DBP and logistic regression analyses were used to test significant determinants of prehypertension or hypertension status serving as the dichotomous outcome variable. To examine the consistency of the observed association between serum GGT levels and prehypertension, we performed subgroup analyses by age (< 60,  $\geq$  60 years), BMI (< 25,  $\geq$  25 kg/m<sup>2</sup>), drinking status (absent, present), TG (< 150,  $\geq$  150 mg/dL), HDL-C ( $\geq$  40, < 40 mg/dL), and uric acid (< 7.0,  $\geq$  7.0 mg/dL). A *p*-value < 0.05 was considered significant.

## RESULTS

### *Subject background factors categorized by body mass index*

The subjects consisted of 754 men, age 56 ± 15 (mean ± s.d.; range, 20-87) years. Table 1 shows subject characteristics categorized by serum GGT levels. Participants in higher GGT tertiles were younger, had higher BMI, and were more likely to have elevated DBP, TG, and uric acid. Smoking status, T-C, and FBG were significantly higher only in the middle tertile compared with the low tertile of serum GGT. Drinking status, SBP, HDL-C, and eGFR were significantly higher only in the high tertile compared with middle tertile of serum GGT. There were no inter-group differences in LDL-C and serum creatinine.

TABLE 1. Characteristics of subjects categorized by serum GGT levels.

Characteristics	Serum GGT tertiles Men (N = 754)				
			<i>P</i> -value*	<i>N</i> = 250	<i>P</i> -value**
	Tertile 1 < 29 IU/L <i>N</i> = 262	Tertile 2 29-53 IU/L <i>N</i> = 242			
Age (years)	60 ± 16	58 ± 14	<b>0.005</b>	51 ± 12	<b>&lt; 0.001</b>
Body mass index <sup>†</sup> (kg/m <sup>2</sup> )	22.2 ± 2.5	23.1 ± 3.0	<b>&lt; 0.001</b>	24.2 ± 3.3	<b>&lt; 0.001</b>
Smoking status <sup>‡</sup> (pack year)	15 ± 20	20 ± 21	<b>0.005</b>	22 ± 20	0.109
Drinking status, g/day	18 ± 8	19 ± 8	0.567	21 ± 4	<b>&lt; 0.001</b>
Systolic blood pressure (mmHg)	132 ± 21	134 ± 20	0.060	138 ± 18	<b>0.020</b>
Diastolic blood pressure (mmHg)	78 ± 11	82 ± 11	<b>&lt; 0.001</b>	85 ± 11	<b>&lt; 0.001</b>
Total cholesterol (mg/dL)	182 ± 32	189 ± 34	<b>0.034</b>	193 ± 35	0.537
Triglycerides (mg/dL)	91 ± 39	121 ± 63	<b>&lt; 0.001</b>	138 ± 68	<b>0.001</b>
HDL cholesterol (mg/dL)	58 ± 14	56 ± 14	0.132	61 ± 16	<b>0.004</b>
LDL cholesterol (mg/dL)	106 ± 29	109 ± 31	0.468	104 ± 36	0.110
Fasting blood glucose (mg/dL)	97 ± 20	99 ± 20	<b>0.014</b>	102 ± 23	0.085
Serum creatinine (mg/dL)	0.77 ± 0.12	0.78 ± 0.12	0.482	0.77 ± 0.13	0.207
Serum uric acid (mg/dL)	5.3 ± 1.2	5.8 ± 1.4	<b>&lt; 0.001</b>	6.4 ± 1.3	<b>&lt; 0.001</b>
eGFR (mL/min/1.73 m <sup>2</sup> )	83.0 ± 16.2	82.9 ± 15.4	0.927	87.0 ± 16.5	<b>0.002</b>

Data presented are mean ± s.d. GGT, gamma-glutamyl transferase; HT, hypertension; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate. <sup>†</sup>Body mass index was calculated using weight in kilograms divided by the square of the height in meters.

<sup>‡</sup>Smoking status: daily consumption (pack) × duration of smoking (year). eGFR = 194 × Cr<sup>1.094</sup> × Age<sup>-0.287</sup>.

\**P*-value for comparison between lowest tertile of and middle tertile of GGT subjects; \*\**P*-value for comparison between middle and highest tertile of GGT subjects; Mann-Whitney U test.

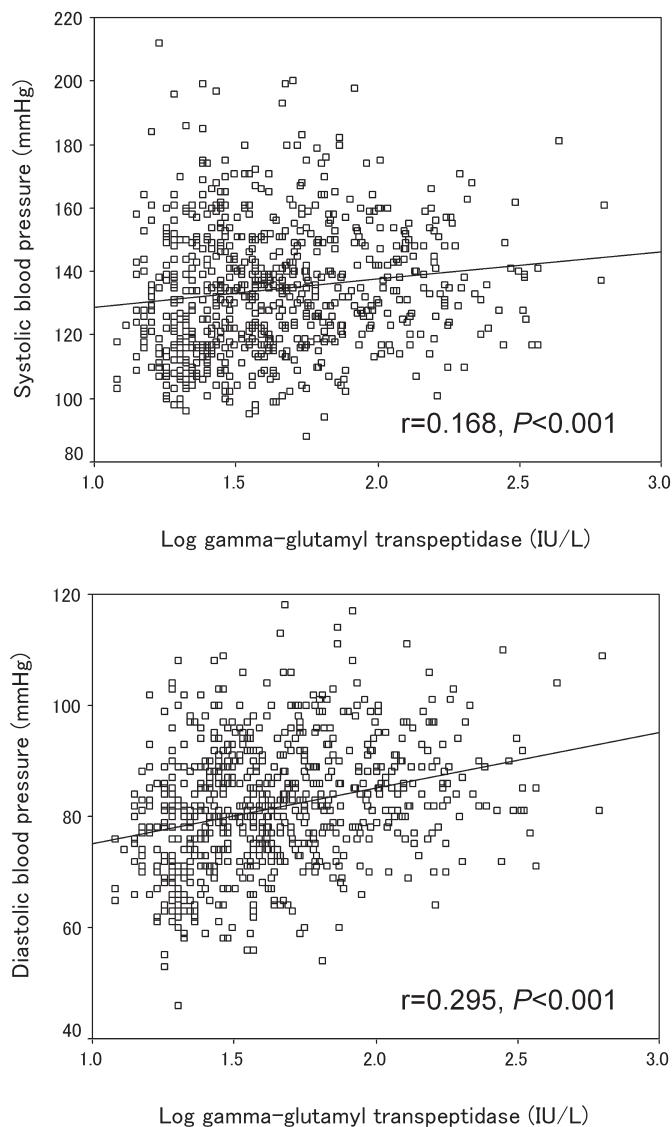


Fig. 1. Relationship between serum gamma-glutamyl transferase (GGT) and blood pressure status. Both Systolic blood pressure ( $r = 0.168, P < 0.001$ ) and diastolic blood pressure ( $r = 0.295, P < 0.001$ ) significantly increased progressively with increasing log GGT.  $P$ -value: Spearman's correlation.

#### *Association between various characteristics and BP status*

Both SBP ( $r = 0.168, P < 0.001$ ) and DBP ( $r = 0.295, P < 0.001$ ) significantly increased progressively with increasing log GGT (Fig. 1). Multiple regression analysis using SBP and DBP as an objective variable, adjusted for risk factors as explanatory variables, showed that SBP independently associated with log GGT ( $\beta = 0.109$ ) with age ( $\beta = 0.399$ ), BMI ( $\beta = 0.251$ ), drinking status ( $\beta = 0.079$ ), HDL-C ( $\beta = 0.153$ ), and FBG

( $\beta = 0.116$ ), and log GGT ( $\beta = 0.238$ ) was also independently associated with DBP.

#### *Association between GGT categories and risk for prehypertension or hypertension*

Thirty-seven percent of participants had prehypertension and 39.3% had hypertension. Compared with the lowest tertile of serum GGT, the non-adjusted odds ratio for prehypertension was 1.84 (95% CI, 1.18-2.88) for the middle tertile and 3.05 (95% CI, 1.87-4.97) for the highest ter-

TABLE 2. Relationship between various characteristics and blood pressure status.

Characteristics	Systolic blood pressure $\beta$ -coefficient ( <i>P</i> -value)	Diastolic blood pressure $\beta$ -coefficient ( <i>P</i> -value)
Age (years)	0.399 (< 0.001)	0.261 (< 0.001)
Body mass index <sup>†</sup> (kg/m <sup>2</sup> )	0.251 (< 0.001)	0.219 (< 0.001)
Smoking status <sup>‡</sup> (pack year)	-0.016 (0.625)	0.010 (0.776)
Drinking status, g/day	0.079 (0.022)	0.065 (0.064)
Triglycerides (mg/dL)	0.054 (0.171)	0.094 (0.019)
HDL cholesterol (mg/dL)	0.153 (< 0.001)	0.146 (< 0.001)
LDL cholesterol (mg/dL)	-0.047 (0.171)	0.014 (0.693)
Fasting blood glucose (mg/dL)	0.116 (0.001)	0.036 (0.292)
Serum uric acid (mg/dL)	0.009 (0.813)	-0.008 (0.833)
Estimated GFR (mL/min/1.73 m <sup>2</sup> )	0.037 (0.327)	-0.029 (0.446)
Log GGT (IU/L)	0.109 (0.006)	0.238 (< 0.001)
R <sup>2</sup>	0.222 (< 0.001)	0.194 (< 0.001)

<sup>†</sup>Body mass index was calculated using weight in kilograms divided by the square of the height in meters. <sup>‡</sup>Smoking status: daily consumption (pack)  $\times$  duration of smoking (year). HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; GGT, Gamma-glutamyl transferase. Gamma-glutamyl transferase was analyzed after log-transformation because of their skewed distribution. eGFR = 194  $\times$  Cr<sup>1.094</sup>  $\times$  Age<sup>-0.287</sup>.

tile (Table 4). The age-adjusted odds ratio for prehypertension was 1.95 (95% CI, 1.24-3.07) for the middle tertile and 3.75 (95% CI, 2.25-6.26) for the highest tertile, with multivariate-adjusted odds ratios of 1.73 (95% CI, 1.06-2.81) for the middle tertile and 2.37 (95% CI, 1.31-4.31) for the highest tertile. Moreover, multivariate-adjusted odds ratios for hypertension were 1.82 (1.04-3.18) for the middle tertile and 3.11 (95% CI, 1.61-6.03) for the highest tertile.

#### Association between serum GGT levels and prehypertension

The OR of prehypertension associated with increasing levels of log-transformed serum GGT did not change within subgroups of age, BMI, drinking status, TG, HDL-C or uric acid (Table 4).

#### DISCUSSION

In this cross-sectional, population-based study, we determined the prevalence of prehypertension and hypertension, as defined by JNC-7 criteria (Chobanian et al. 2003), and their

relationship to GGT levels. In our study, participants were only 754 eligible men because of sex differences in GGT (Skurtveit and Tverdal 2002). This study showed that prehypertension is extremely common, affecting 37.1% of male subjects, and both SBP and DBP significantly increased with increasing GGT levels. Furthermore, higher GGT levels were significantly associated with risk for prehypertension or hypertension, even after adjusting for age, BMI, smoking status, drinking status, TG, HDL-C, LDL-C, FBG, uric acid, and eGFR. Our data are in agreement with the results of previous prospective studies (Miura et al. 1994; Lee et al. 2002; Lee et al. 2003) showing that baseline serum GGT was an independent confounding factor for hypertension development, and we further suggest that serum GGT levels are also related to clinical prehypertension, a disease state when primary prevention is possible.

Our study found an overall prehypertension prevalence rate of 34.5% in rural adult male Japanese, similar to levels in Taiwanese adults (Tsai et al. 2005) and American adults (Greenlund

TABLE 3. Association between serum GGT levels and blood pressure status.

	Serum GGT tertiles			Men (N = 754)	<i>P</i> -value
	Tertile 1 < 29 IU/L <i>N</i> = 262	Tertile 2 29-53 IU/L <i>N</i> = 242	Tertile 3 > 53 IU/L <i>N</i> = 250		
Normotension (NTN)	90 (34.9)	54 (38.4)	34 (13.6)		
Prehypertension (Pre-HTN)	86 (32.8)	95 (39.3)	99 (39.6)		
Hypertension (HTN)	86 (32.8)	93 (38.4)	117 (46.8)		
Pre-HTN vs. NTN					<b>&lt; 0.001</b>
Non-adjusted OR (95% CI)	1.00	1.84 (1.18-2.88)	3.05 (1.87-4.97)		
Age-adjusted OR (95% CI)	1.00	1.95 (1.24-3.07)	3.75 (2.25-6.26)		
Multivariate-adjusted OR (95% CI)	1.00	1.73 (1.06-2.81)	2.37 (1.31-4.31)		
HTN vs. Pre-HTN					<b>0.583</b>
Non-adjusted OR (95% CI)	1.00	0.98 (0.65-1.48)	1.18 (0.79-1.77)		
Age-adjusted OR (95% CI)	1.00	1.19 (0.77-1.83)	2.11 (1.33-3.34)		
Multivariate-adjusted OR (95% CI)	1.00	0.99 (0.63-1.58)	1.34 (0.80-2.25)		
HTN vs. NTN					<b>&lt; 0.001</b>
Non-adjusted OR (95% CI)	1.00	1.80 (1.15-2.82)	3.60 (2.22-5.84)		
Age-adjusted OR (95% CI)	1.00	2.42 (1.47-4.00)	7.00 (3.96-12.4)		
Multivariate-adjusted OR (95% CI)	1.00	1.82 (1.04-3.18)	3.11 (1.61-6.03)		

Data presented are number (%). GGT, gamma-glutamyl transferase; OR, odds ratio; CI, confidence interval. Multivariate-adjusted for age, body mass index, drinking status, smoking status, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting blood glucose, uric acid and estimated glomerular filtration rate. *P*-value:  $\chi^2$  test.

et al. 2004). We found that higher GGT levels were positively associated with prehypertension or hypertension, independent of other confounders. Similar results were found in a community-based cross-sectional study in US adults (Shankar et al. 2007), with a multivariate-OR (95% CI) of 1.84 (1.37-2.46) comparing quartile 4 of GGT (> 29 U/L) to quartile 1 (< 13 U/L). This association persisted in separate analyses in men and women. Moreover, the results were consistent in subgroup analyses by race-ethnicity, age, smoking status, drinking status, BMI, waist circumference, and diabetes. Also in our study, the OR of GGT for prehypertension did not change within subgroups of age, BMI, drinking status, TG, HDL-C and uric acid. Furthermore, serum GGT levels correlated with relative changes in BP in individuals with normal GGT concentrations, a finding consistent with previous reports looking at hypertension (Miura et al. 1994; Lee et al. 2003).

Miura et al. (1994) suggest that the serum GGT levels may predict the future development of hypertension among drinkers after adjustment for baseline BP level and the amount of alcohol consumption, and Yamada et al. (1995) suggest that the association between serum GGT and hypertension appears to be quite similar in both drinker and nondrinker.

Serum GGT is a marker of drinking alcohol and/or liver dysfunction such as fatty liver (Teschke et al. 1977). Although the association between serum GGT and prehypertension was only present in alcohol drinkers, it strongly persisted after adjusting for grams of alcohol drinking. This suggests that subjects with alcohol-induced serum GGT increases may have increased susceptibility to high BP (Lee et al. 2006). There were no significant differences in the incidence of prehypertension or serum GGT between drinkers and nondrinkers. Yamada et al. (1995) showed

TABLE 4. Association between serum GGT levels and Prehypertension, within selected subgroups.

Stratified subgroups	N	PHT Cases (%)	Multivariate OR (95% CI)*	P -interaction
ALL	458	280 (61.1)	2.96 (1.28-6.85)	-----
Age (years)				
< 60 years	287	169 (58.9)	3.08 (1.09-8.66)	0.295
≥ 60 years	171	111 (64.9)	2.89 (0.54-15.4)	
Body mass index <sup>†</sup>				
< 25 kg/m <sup>2</sup>	359	205 (57.1)	3.29 (1.26-8.59)	0.749
≥ 25 kg/m <sup>2</sup>	99	75 (75.8)	1.20 (0.15-9.44)	
Drinking status				
Absent	66	37 (56.1)	2.51 (0.94-66.9)	0.913
Present	392	243 (62.0)	2.72 (1.12-6.60)	
Triglycerides				
< 150 mg/dL	362	209 (57.7)	3.32 (1.21-9.11)	0.350
≥ 150 mg/dL	96	71 (74)	1.27 (0.24-6.61)	
HDL cholesterol				
≥ 40 mg/dL	407	249 (61.2)	2.63 (1.08-6.44)	0.543
< 40 mg/dL	51	31 (60.8)	2.91 (0.14-61.8)	
Uric acid				0.315
< 7.0 mg/dL	367	215 (58.6)	3.14 (1.20-8.27)	
≥ 7.0 mg/dL	91	65 (71.4)	3.38 (0.48-23.6)	

Data presented are number (%). GGT, gamma-glutamyl transpeptidase; OR, odds ratio; CI, confidence interval. <sup>†</sup>Body mass index was calculated using weight in kilograms divided by the square of the height in meters. Multivariate-adjusted for age, body mass index, drinking status, smoking status, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting blood glucose, uric acid and estimated glomerular filtration rate. \*Multivariate OR (95% CI) of prehypertension associated with log-transformed GGT, IU/L.

that the association between serum GGT and BP status was present in both drinkers and nondrinkers, suggesting drinking status does not dramatically affect the usefulness of GGT as a biomarker for hypertension risk.

The mechanisms that lead to increased BP in individuals with increased GGT are not completely understood. Serum GGT is associated with hypertension, dyslipidemia, and abnormal glucose tolerance, suggesting that it is related to hepatic insulin resistance rather than non-alcoholic fatty liver disease (Ikai et al. 1994; Nilssen and Førde 1994; Kang et al. 2007). GGT plays a direct role in the generation of reactive oxygen species in the

presence of iron or other transition metals (Brown et al. 1998), inducing lipid peroxidation in human biological membranes (Paolicchi et al. 1997), and is an indirect marker of antioxidant systems, with the primary function of maintaining the intracellular concentration of glutathione in response to oxidative stress (Karp et al. 2001). Higher c-reactive protein or other inflammatory parameters, indicating sub-clinical inflammation, correlate with GGT levels, as do levels of nitrotyrosine, an oxidative stress maker (Bo et al. 2005). These findings suggest that GGT could be an early marker of oxidative stress and sub-clinical inflammation, perhaps related to the pathology of in-

creased BP as an oxidative stressor.

Some limitations of this study must be considered. First, our cross-sectional study design does not eliminate potential causal relationships between GGT and increased BP. There still remain important problems on the cumulative effects of these CVD risk factors over several decades and the interactions with other risk factors. The prevalence of various BP categories is based on a single assessment of BP, which may introduce a misclassification bias. Moreover, a single measurement of GGT levels represents a limitation of the present study because 12% of adults with initially elevated GGT levels had normal levels at the second examination in the American general population (Lazo et al. 2008). Therefore the demographics and referral source may limit generalizability.

In conclusion, the present study showed that GGT levels correlate with prehypertension or hypertension in the general male population. The underlying mechanism seems to be independent from traditional cardiovascular risk factors such as age, BMI, dyslipidemia, and diabetes. For community-dwelling healthy persons, prospective population-based studies are needed to investigate the mechanisms underlying this association to determine whether intervention, such as effective lifestyle modifications that decrease GGT in adult male populations, will decrease risks.

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## Original Article

# Elevated Serum Gamma-glutamyl Transpeptidase Levels and Fatty Liver Strongly Predict the Presence of Carotid Plaque

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**Aim:** There is a strong relationship between carotid atherosclerosis and future cardiovascular disease (CVD). This study sought to clarify the association of fatty liver and an elevated serum gamma-glutamyl transpeptidase (GGT) level with carotid atherosclerosis.

**Methods:** We reviewed the medical records of subjects who underwent medical checkups at our institute. Carotid atherosclerosis and fatty liver were assessed using ultrasound (US), and predictors of increased carotid intima-media thickness (IMT) and carotid plaque were identified using a logistic regression model.

**Results:** In total, 958 subjects (564 men, 394 women; median age, 59 years) were enrolled. The median value of the mean carotid IMT was 0.713 mm, and the frequency of carotid plaque was 19.5%. For the highest quartile of the mean carotid IMT ( $\geq 0.863$  mm), a male sex, older age, hypertension (HT), dyslipidemia (DL) and type 2 diabetes mellitus (DM) were identified as independent predictors. A male sex, older age, HT and elevated serum GGT level were found to be significant predictors of the presence of carotid plaque. In addition, fatty liver correlated with the existence of carotid plaque. When the combination of the serum GGT level and presence or absence of fatty liver was included as a variable in the analysis, a male sex, older age, HT and fatty liver with a serum GGT level of  $\geq 83$  IU/L (90th percentile) (odds ratio 3.21, 95% confidence interval 1.27–8.12,  $p=0.014$ ) were identified to be significantly associated with carotid plaque.

**Conclusions:** This study suggests that the simultaneous presence of an elevated serum GGT level and fatty liver is highly predictive of carotid plaque.

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**Key words:** Fatty liver, Gamma-glutamyl transpeptidase, Carotid plaque

## Introduction

Atherosclerosis underlies the major pathogenesis of cardiovascular disease (CVD), which is the leading cause of death worldwide and responsible for approximately 30% of all deaths<sup>1,2)</sup>. Therefore, assessing atherosclerosis is essential for predicting and preventing

CVD in apparently healthy subjects. Due to its accessibility, simplicity and reproducibility, carotid ultrasound (US) is the most widely used tool for evaluating the degree of generalized atherosclerosis<sup>3)</sup>, and there is a strong relationship between the detection of carotid atherosclerosis and future CVD events<sup>4-6)</sup>.

Previous studies have demonstrated that multiple factors, such as aging, hypertension (HT), dyslipidemia (DL), type 2 diabetes mellitus (DM) and smoking, are associated with carotid atherosclerosis<sup>7)</sup>. Recently, other associated factors have been identified. The number of individuals with fatty liver has been increasing worldwide, and fatty liver has become the most common

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liver disorder, with an estimated prevalence of 20% to 30% in the general population<sup>8)</sup>. Furthermore, epidemiological studies have revealed a strong association between fatty liver and carotid atherosclerosis<sup>9, 10)</sup>, and accumulating data indicate that elevated serum gamma-glutamyl transpeptidase (GGT) levels are correlated with carotid atherosclerosis<sup>11, 12)</sup> and that GGT per se may play a direct role in the development of atherosclerosis<sup>13)</sup>. In this context, studies have been conducted to determine whether an elevated serum GGT level and fatty liver are predictive of future CVD events. Some studies have examined the impact of elevated serum GGT levels on all-cause and CVD mortality and showed positive results<sup>14, 15)</sup>, and similar results have been reported regarding the relationship between fatty liver and CVD events<sup>16)</sup>. Notably, one study found that the serum GGT levels are significantly associated with all-cause and CVD mortality in men and that this association is stronger among those with increased echogenicity of the liver parenchyma, known as the US sign of fatty liver<sup>17)</sup>.

### Aim

In the current study, we hypothesized that the simultaneous presence of fatty liver and an elevated serum GGT level may therefore be more strongly associated with atherosclerosis than the presence of either variable alone. Hence, we determined predictors of carotid atherosclerosis using a logistic regression model that included these two parameters.

### Methods

#### Subjects

We reviewed the medical records of subjects who consecutively underwent medical checkups at our health evaluation center between 2011 and 2013. For subjects who underwent repeated medical checkups, we used their most recent records. The exclusion criteria included cases of insufficient recorded data, hepatitis B or C virus infection, a past and/or current history of cardiovascular or liver disease and past and/or current medications capable of influencing the course of atherosclerosis, such as anti-diabetic agents, anti-hypertensive agents, DL agents and antiplatelet/anticoagulant agents. The study protocol was approved by the Ethics Committee of Kanazawa Medical University (approval no. 144) and conducted in accordance with the Declaration of Helsinki.

#### Laboratory Tests

The serum total cholesterol levels were measured

using the cholesterol oxidase method (Kyowa Medex Co., Ltd, Tokyo, Japan). The serum low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol levels were measured according to the direct method (Sekisui Medical Co., Ltd, Tokyo, Japan), and the serum triglyceride levels were measured using an enzymatic color test (Kyowa Medex Co., Ltd). Additionally, the fasting blood glucose levels were assessed using the hexokinase method (Shinoh Test Corporation, Tokyo, Japan), the hemoglobin A1c (HbA1c) levels were determined via high-performance liquid chromatography (HPLC) (Tosoh Corporation, Tokyo, Japan) and the serum alanine aminotransferase and GGT levels were measured using the Japan Society of Clinical Chemistry (JSCC) standardization method (Kanto Chemical Co., Inc., Tokyo, Japan).

#### Definitions of HT, DL, DM and Metabolic Syndrome

HT was defined according to the Japanese Society of Hypertension guidelines for the management of HT, as follows: a systolic blood pressure of  $\geq 140$  mmHg or diastolic blood pressure of  $\geq 90$  mmHg<sup>18)</sup>. DL was defined as a serum LDL cholesterol level of  $\geq 140$  mg/dL, serum HDL cholesterol level of  $< 40$  mg/dL or serum triglyceride level of  $\geq 150$  mg/dL, according to the criteria of the Japan Atherosclerosis Society<sup>19)</sup>. DM was defined according to the guidelines of the Japan Diabetes Society, as follows: a fasting blood glucose level of  $\geq 126$  mg/dL and HbA1c level of  $\geq 6.5\%$ <sup>20)</sup>. Metabolic syndrome was defined according to the Japanese criteria, as follows: a waist circumference of  $\geq 85$  cm for men and  $\geq 90$  cm for women and two or more of the following items, a serum triglyceride level of  $\geq 150$  mg/dL and/or serum HDL cholesterol level of  $< 40$  mg/dL, systolic blood pressure of  $\geq 130$  mmHg and/or diastolic blood pressure of  $\geq 85$  mmHg or a fasting blood glucose level of  $\geq 110$  mg/dL<sup>21)</sup>.

#### Assessment of Carotid Atherosclerosis and Fatty Liver

Experienced ultrasonographers who were blinded to the medical checkup data of each subject performed the carotid and abdominal US examinations. High-resolution US machines (Aplio XG/500/80, Toshiba Medical Systems Corporation, Tochigi, Japan) and a 5-MHz probe were used for abdominal US; the same US machines and an 8.4- or 9-MHz probe were used for carotid US. The mean carotid intima-media thickness (IMT) was calculated by averaging six or eight measurements obtained from both sides of the far walls of the common carotid arteries. Carotid plaque

**Table 1.** Study subject characteristics ( $n=958$ )

Variable	
Sex, male/female	564/394
Age, years	59 (44, 71)
BMI, kg/m <sup>2</sup>	22.8 (19.2, 26.9)
BMI $\geq 25$ kg/m <sup>2</sup> , n (%)	212 (22.1)
Systolic blood pressure, mmHg	123 (102, 145)
Diastolic blood pressure, mmHg	74 (60, 89)
Hypertension, n (%)	171 (17.8)
TC, mg/dL	213 (171, 257)
LDL, mg/dL	122 (84, 159)
HDL, mg/dL	56 (40, 79)
TG, mg/dL	99 (55, 209)
Dyslipidemia, n (%)	443 (46.2)
FBG, mg/dL	95 (85, 111)
HbA1c, %	5.6 (5.2, 6.1)
Type 2 diabetes mellitus, n (%)	19 (2.0)
Metabolic syndrome, n (%)	110 (11.5)
ALT, IU/L	20 (13, 41)
GGT, IU/L	27 (14, 83)
Fatty liver, n (%)	206 (21.5)
Current smoking, n (%)	187 (19.5)
Alcohol consumption, $\leq 20$ / $> 20$ , $< 60$ / $\geq 60$ , g/day	602/307/49
Carotid IMT, mm	0.713 (0.563, 0.963)
Carotid plaque, n (%)	187 (19.5)

Variables are expressed as the median (10, 90 percentiles).

BMI, body mass index; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; IMT, intima-media thickness.

was defined as an IMT  $> 1.5$  mm in any portion of the carotid arteries<sup>22</sup>. The diagnosis of fatty liver was made based on the detection of increased echogenicity of the liver parenchyma compared to that of the right renal cortex<sup>23</sup>. All US results were double checked by experienced ultrasonographers.

### Statistical Analysis

The variables are expressed as the median (10, 90 percentile). The chi-square test or Fisher's exact probability test were used to compare categorical variables, and Student's *t*-test, the Mann-Whitney *U* test and the Kruskal-Wallis test were used to compare continuous variables. We further examined predictors of carotid atherosclerosis using a multiple logistic regression model; the objective variables included an increased mean carotid IMT (highest quartile) and the presence of carotid plaque, and the explanatory variables included sex (female/male), age (years), body mass index (BMI) (kg/m<sup>2</sup>), HT (absence/presence), DL (absence/presence), DM (absence/presence),

serum alanine aminotransferase level (IU/L), serum GGT level (IU/L), fatty liver (absence/presence), smoking status (nonsmoking and past smoking/current smoking) and alcohol consumption ( $\leq 20$  g/day /  $> 20$ ,  $< 60$  g/day /  $\geq 60$  g/day). Following a univariate analysis of each variable, we performed a multivariate analysis using all variables. A *p* value of  $< 0.05$  was considered to be statistically significant. All statistical analyses were performed using the STATA version 11.1 software program (STATA Corp, College Station, TX, USA).

### Results

During the study period, 1,599 subjects underwent medical checkups at least once (813 subjects, once; 786 subjects, twice or more). Of these subjects, 56 were excluded due to insufficient data and 567 were excluded due to a past and/or current history of cardiovascular or liver disease and a past and/or current history of medications capable of influencing the

**Table 2.** Factors correlated with the highest quartile of the mean carotid IMT ( $\geq 0.863$  mm) ( $n=958$ )

Variable	Univariate			Multivariate		
	OR	95% CI	p value	OR	95% CI	p value
Male (vs. female)	2.89	2.07–4.02	<0.0001	2.61	1.71–3.97	<0.0001
Age, years	1.12	1.10–1.15	<0.0001	1.13	1.11–1.16	<0.0001
BMI, kg/m <sup>2</sup>	1.11	1.06–1.16	<0.0001	1.07	0.99–1.15	0.054
Hypertension	2.40	1.69–3.40	<0.0001	2.05	1.36–3.09	0.001
Dyslipidemia	1.75	1.30–2.35	<0.0001	1.62	1.13–2.34	0.009
Type 2 diabetes mellitus	8.73	3.11–24.51	<0.0001	5.37	1.70–16.92	0.004
ALT, IU/L	1.01	0.99–1.02	0.247	0.99	0.98–1.01	0.548
GGT, IU/L	1.002	0.99–1.005	0.110	1.001	0.99–1.01	0.542
Fatty liver	1.64	1.17–2.29	0.004	1.42	0.89–2.26	0.133
Current smoking (vs. nonsmoking and past smoking)	0.86	0.59–1.25	0.427	0.84	0.53–1.32	0.445
Alcohol consumption, >20, <60 g/day (vs. ≤ 20 g/day)	1.27	0.93–1.72	0.119	0.87	0.58–1.31	0.506
Alcohol consumption, ≥60 g/day (vs. ≤ 20 g/day)	1.07	0.56–2.06	0.642	0.65	0.28–1.51	0.318

IMT, intima-media thickness; OR, odds ratio; CI, confidence interval; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

course of atherosclerosis. Of the remaining 976 subjects, eight were excluded due to hepatitis B virus infection and 10 were excluded due to hepatitis C virus infection. Therefore, 958 subjects (men 564, women 394; median age 59 years) were enrolled in this study. **Table 1** lists the subject characteristics. The median value of the mean carotid IMT was 0.713 mm, and 19.5% (187/958) of the subjects had plaque. In total, 21.5% (206/958) of the subjects had fatty liver.

In order to determine the predictors of an increased mean carotid IMT, we used the highest quartile of the mean carotid IMT ( $\geq 0.863$  mm) as the objective variable. In the univariate analysis, a male sex, older age, increased BMI, HT, DL, DM and fatty liver were identified as significant variables. In the multivariate analysis, a male sex, older age, HT, DL and DM were found to be independently associated with an increased mean carotid IMT (**Table 2**). Neither fatty liver ( $p=0.133$ ) nor an elevated serum GGT level ( $p=0.542$ ) were significant predictors. When the analysis was performed for each sex, there were differences with respect to the predictors of an increased mean carotid IMT. In men, an older age [odds ratio (OR) 1.12, 95% confidence interval (CI) 1.09–1.16,  $p<0.0001$ ], HT (OR 1.99, 95% CI 1.21–3.27,  $p=0.007$ ), DL (OR 1.72, 95% CI 1.07–2.75,  $p=0.025$ ) and DM (OR 6.09, 95% CI 1.93–19.26,  $p=0.002$ ) were significant predictors. Furthermore, fatty liver (OR 2.26, 95% CI 1.31–3.91,  $p=0.003$ ) was significantly associated with an increased mean carotid IMT. In contrast, in women, an older age (OR

1.16, 95% CI 1.11–1.21,  $p<0.0001$ ) was the only significant predictor.

In the analysis of predictors of carotid plaque, a male sex, older age, HT, DL, elevated serum GGT level and increased alcohol consumption ( $>20, <60$  g/day) were found to be significant in the univariate analysis. In the multivariate analysis, a male sex, older age, HT and elevated serum GGT level were identified as independent predictors of the presence of carotid plaque (**Table 3**). Fatty liver ( $p=0.093$ ) and DL ( $p=0.059$ ) trended with the presence of carotid plaque. When the analysis was performed for each sex, there were differences with respect to the predictors of carotid plaque. For example, in men, an older age (OR 1.09, 95% CI 1.06–1.11,  $p<0.0001$ ), HT (OR 1.95, 95% CI 1.21–3.14,  $p=0.006$ ) and elevated serum GGT level (OR 1.005, 95% CI 1.001–1.009,  $p=0.025$ ) were significant predictors, whereas in women, an older age (OR 1.12, 95% CI 1.07–1.17,  $p<0.0001$ ) was the only significant predictor.

To obtain the cut-off value of the serum GGT level for predicting carotid plaque, we calculated the odds ratios in the 50th, 75th and 90th percentiles. Consequently, the 90th percentile (83 IU/L) was chosen as the cut-off threshold ( $\geq 83$  IU/L, OR 1.83, 95% CI 1.03–3.24,  $p=0.038$ ). When the enrolled subjects were divided into two groups according to the cut-off value of the serum GGT level, the rates of a male sex (89.7% vs. 55.4%), DL (63.9% vs. 44.3%), fatty liver (34.0% vs. 20.1%), current smokers (33.0% vs. 18.0%), alcohol consumption ( $>20$  g/day, 78.4% vs. 32.5%) and carotid plaque (32.0% vs. 18.1%)

**Table 3.** Factors associated with the presence of carotid plaque ( $n=958$ )

Variable	Univariate			Multivariate		
	OR	95% CI	p value	OR	95% CI	p value
Male (vs. female)	2.70	1.87–3.90	<0.0001	1.99	1.28–3.08	0.002
Age, years	1.08	1.06–1.11	<0.0001	1.09	1.07–1.12	<0.0001
BMI, kg/m <sup>2</sup>	1.04	0.99–1.09	0.101	0.98	0.91–1.05	0.606
Hypertension	2.03	1.40–2.96	<0.0001	1.71	1.13–2.59	0.011
Dyslipidemia	1.51	1.10–2.09	0.011	1.43	0.99–2.09	0.059
Type 2 diabetes mellitus	2.46	0.95–6.34	0.062	1.32	0.48–3.64	0.588
ALT, IU/L	1.00	0.99–1.01	0.347	0.99	0.98–1.01	0.506
GGT, IU/L	1.006	1.002–1.009	<0.0001	1.005	1.001–1.009	0.014
Fatty liver	1.44	0.99–2.08	0.053	1.50	0.93–2.42	0.093
Current smoking (vs. nonsmoking and past smoking)	1.25	0.85–1.84	0.259	1.19	0.76–1.86	0.460
Alcohol consumption, >20, <60 g/day (vs. ≤ 20 g/day)	1.60	1.15–2.23	0.005	1.17	0.78–1.75	0.442
Alcohol consumption, ≥60 g/day (vs. ≤ 20 g/day)	1.53	0.79–2.94	0.207	0.96	0.43–2.16	0.927

OR, odds ratio; CI, confidence interval; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

were significantly higher among the subjects with a serum GGT level of  $\geq 83$  IU/L ( $n=97$ ) compared to those observed in the subjects with a serum GGT level of  $<83$  IU/L ( $n=861$ ). Furthermore, the BMI (median 23.7 kg/m<sup>2</sup> vs. 22.7 kg/m<sup>2</sup>) and serum alanine aminotransferase level (median 31 IU/L vs. 19 IU/L) were also higher in the former group than in the latter group.

The subjects were divided into four groups based on the cut-off value of the serum GGT and the presence or absence of fatty liver (Table 4). The ratios of male to female subjects were higher in the groups with a serum GGT level of  $\geq 83$  IU/L, fatty liver and both compared to that seen in the group without these two factors. BMI and the rates of HT and DL were also higher in the subjects with fatty liver than in the subjects without fatty liver. The number of current smokers was highest in the group without fatty liver and a serum GGT level of  $\geq 83$  IU/L, followed by the groups with fatty liver and a serum GGT level of  $\geq 83$  IU/L, fatty liver and a serum GGT level of  $<83$  IU/L and non-fatty liver and a serum GGT level of  $<83$  IU/L. The number of subjects with moderate to severe alcohol consumption was highest in the group with non-fatty liver and a serum GGT level of  $\geq 83$  IU/L, followed by the groups with fatty liver and a serum GGT level of  $\geq 83$  IU/L, non-fatty liver and a serum GGT level of  $<83$  IU/L and fatty liver and a serum GGT level of  $<83$  IU/L. The mean carotid IMT was higher in the subjects with fatty liver than in those without fatty liver, and the ratio of patients with carotid plaque was highest in the group with fatty liver and a serum GGT level of  $\geq 83$  IU/L, followed by the groups with non-fatty liver and a serum GGT level of

$\geq 83$  IU/L, fatty liver and a serum GGT level of  $<83$  IU/L and non-fatty liver and a serum GGT level of  $<83$  IU/L.

**Table 5** shows the predictors of carotid plaque when the combination of the serum GGT level and the presence or absence of fatty liver was included as an explanatory variable. In the univariate analysis, a male sex, older age, HT, DL, the combination of fatty liver and serum GGT  $\geq 83$  IU/L and increased alcohol consumption ( $>20, <60$  g/day) were identified as significant variables. In the multivariate analysis, a male sex, older age, HT, fatty liver and serum GGT level of  $\geq 83$  IU/L (OR 3.21, 95% CI 1.27–8.12, p = 0.014) were independent predictors of carotid plaque.

## Discussion

The current findings suggest that an elevated serum GGT level and fatty liver are closely associated with carotid atherosclerosis, although this association was stronger for carotid plaque than for an increased mean carotid IMT. Moreover, when the serum GGT levels and fatty liver were combined in the analysis of predictors of carotid plaque, the simultaneous presence of an elevated serum GGT level and fatty liver was significant. In contrast, an elevated serum GGT level without fatty liver and fatty liver without an elevated serum GGT level were not significant.

In the current study, we used fatty liver and the serum GGT level as two distinct variables. Although fatty liver and the serum GGT level are positively correlated with each other<sup>24)</sup>, the relationship between these two parameters is not necessarily constant. Alco-

**Table 4.** Comparison of the characteristics of the subjects stratified according to the fatty liver status and serum GGT level ( $n=958$ )

Variable	Non-fatty liver and GGT $< 83 \text{ IU/L}$ ( $n=688$ )	Fatty liver and GGT $< 83 \text{ IU/L}$ ( $n=173$ )	Non-fatty liver and GGT $\geq 83 \text{ IU/L}$ ( $n=64$ )
Sex, male/female	348/340	129/44	57/7
Age, years	59 (44, 71)	59 (44, 67)	57 (46, 69)
BMI, $\text{kg}/\text{m}^2$	22.1 (18.6, 25.4)	25.2 (22.4, 29.7)	23.0 (19.8, 26.1)
Hypertension, n (%)	113 (16.4)	35 (20.2)	11 (17.2)
Dyslipidemia, n (%)	259 (37.6)	122 (70.5)	38 (59.4)
Type 2 diabetes, n (%)	10 (1.5)	7 (4.0)	1 (1.6)
ALT, $\text{IU}/\text{L}$	18 (12, 29)	28 (16, 59)	27.5 (18, 49)
GGT, $\text{IU}/\text{L}$	22 (13, 52)	32 (18, 67)	104 (86, 221)
Current smoking n (%)	114 (16.6)	41 (23.7)	23 (35.9)
Alcohol consumption, $\leq 20/ > 20, < 60/ \geq 60, \text{g/day}$	455/211/22	126/45/2	11/33/20
Carotid IMT, mm	0.700 (0.563, 0.963)	0.738 (0.575, 1.000)	0.688 (0.517, 0.988)
Carotid plaque, n (%)	119 (17.3)	37 (21.4)	18 (28.1)
Variable	Fatty liver and GGT $\geq 83 \text{ IU/L}$ ( $n=33$ )	<i>p</i> value*	
Sex, male/female	30/3	<0.0001	
Age, years	59 (41, 66)	0.051	
BMI, $\text{kg}/\text{m}^2$	26.0 (23.7, 30.4)	<0.0001	
Hypertension, n (%)	12 (36.4)	0.046	
Dyslipidemia, n (%)	24 (72.7)	<0.0001	
Type 2 diabetes, n (%)	1 (3.0)	0.170	
ALT, $\text{IU}/\text{L}$	42 (23, 124)	<0.0001	
GGT, $\text{IU}/\text{L}$	108 (83, 203)	<0.0001	
Current smoking n (%)	9 (27.3)	0.0001	
Alcohol consumption, $\leq 20/ > 20, < 60/ \geq 60, \text{g/day}$	10/18/5	<0.0001	
Carotid IMT, mm	0.740 (0.613, 1.060)	0.047	
Carotid plaque, n (%)	13 (39.4)	0.003	

All variables are expressed as the median (10, 90 percentiles).

\*The chi-square test or Fisher's exact probability test were used to compare categorical variables. The Kruskal-Wallis test was used to compare continuous variables.

GGT, gamma-glutamyl transpeptidase; BMI, body mass index; ALT, alanine aminotransferase; IMT, intima-media thickness.

hol consumption<sup>25)</sup>, smoking<sup>26)</sup>, an older age<sup>27)</sup> and obesity<sup>28)</sup> can increase the serum GGT levels and thereby modify the relationship between fatty liver and the serum GGT level. Therefore, our analyses were conducted to clarify the manner in which fatty liver and an elevated serum GGT level are associated with carotid atherosclerosis in the same logistic regression model.

We compared our results with those of previous studies investigating the association of fatty liver and an elevated serum GGT level with carotid atherosclerosis. In a systematic review analyzing the association between non-alcoholic fatty liver disease and carotid atherosclerosis, liver disease was positively correlated with both an increased carotid IMT and carotid plaque<sup>9)</sup>. In contrast, a recent study of a Japanese pop-

ulation demonstrated a positive correlation between the serum GGT levels and increased carotid IMT values (although the analyses were univariate)<sup>12)</sup>. Another large-scale study by Kozakova *et al.*, in which the subjects were free of CVD, HT, DL, DM and metabolic syndrome, examined the association between the fatty liver index (FLI) (an index used to predict the presence of fatty liver) and carotid atherosclerosis. These authors reported the following conclusions: 1) FLI is a significant predictor of an increased mean carotid IMT, but not an elevated serum GGT level, when FLI is replaced by parameters used in its equation (BMI, waist circumference, serum triglyceride level and serum GGT level) and 2) FLI is a significant predictor of carotid plaque, with an elevated serum GGT level also being identified as a significant predictor when

**Table 5.** Association of fatty liver and the serum GGT level with carotid plaque ( $n=958$ )

Variable	Univariate			Multivariate		
	OR	95% CI	p value	OR	95% CI	p value
Male (vs. female)	2.70	1.87–3.90	<0.0001	2.07	1.34–3.21	0.001
Age, years	1.08	1.06–1.11	<0.0001	1.09	1.07–1.12	<0.0001
BMI, kg/m <sup>2</sup>	1.04	0.99–1.09	0.101	0.98	0.91–1.05	0.575
Hypertension	2.03	1.40–2.96	<0.0001	1.73	1.14–2.63	0.010
Dyslipidemia	1.51	1.10–2.09	0.011	1.40	0.97–2.04	0.077
Type 2 diabetes mellitus	2.46	0.95–6.34	0.062	1.32	0.47–3.68	0.595
ALT, IU/L	1.00	0.99–1.01	0.347	0.99	0.98–1.01	0.576
Fatty liver and GGT < 83 IU/L (vs. Non-fatty liver and GGT < 83 IU/L)	1.15	0.77–1.73	0.494	1.48	0.89–2.45	0.133
Non-fatty liver and GGT ≥ 83 IU/L (vs. Non-fatty liver and GGT < 83 IU/L)	1.68	0.95–2.97	0.075	1.67	0.84–3.32	0.141
Fatty liver and GGT ≥ 83 IU/L (vs. Non-fatty liver and GGT < 83 IU/L)	2.81	1.37–5.75	0.005	3.21	1.27–8.12	0.014
Current smoking (vs. Nonsmoking and Past smoking)	1.25	0.85–1.84	0.259	1.22	0.78–1.91	0.385
Alcohol consumption, >20, <60 g/day (vs. ≤ 20 g/day)	1.60	1.15–2.23	0.005	1.21	0.82–1.81	0.340
Alcohol consumption, ≥ 60 g/day (vs. ≤ 20 g/day)	1.53	0.79–2.94	0.207	1.09	0.49–2.40	0.837

IMT, intima-media thickness; OR, odds ratio; CI, confidence interval; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

FLI is replaced by other parameters<sup>11</sup>). The results of our study and the study by Kozakova *et al.* are similar in terms of the close association between an elevated serum GGT level and carotid plaque. Our results also suggest that the presence of fatty liver reinforces this association. Conversely, an elevated serum GGT level enhances the association between fatty liver and carotid plaque. In our cohort, traditional factors, including an older age, HT, DL and DM, were more strongly associated with an increased mean carotid IMT than fatty liver.

In the current study, the results revealed sex differences with respect to the predictors of carotid atherosclerosis. For example, in men, fatty liver and an elevated serum GGT level were identified to be predictors of an increased mean carotid IMT and carotid plaque, whereas in women, neither of these two parameters were significantly associated with carotid atherosclerosis. These findings are in agreement with those of previous studies in which the association of an elevated serum GGT level with oxidative stress<sup>12</sup> and that of an elevated serum GGT level and fatty liver with CVD mortality<sup>17</sup> were found to be stronger in men than in women. Future studies are needed to clarify the reasons underlying these sex differences.

Accumulating data suggest that GGT per se may play a role in the progression of atherosclerosis. Previous studies have demonstrated the presence of GGT-positive foam cells in atherosclerotic plaques<sup>29, 30</sup>. In addition, another study by Franzini *et al.* examined human carotid plaques and revealed the presence of

glutathione, cysteinyl-glycine, cysteine and LDL/GGT complexes<sup>31</sup>. Cysteinyl-glycine, a dipeptide derived from GGT-mediated glutathione degradation, promotes LDL oxidation via an iron reduction reaction that results in the production of superoxide radicals. This GGT-induced LDL oxidation may be a possible underlying mechanism of atherosclerotic progression<sup>13</sup>. The study by Franzini *et al.* also demonstrated a positive correlation between the serum GGT level and GGT activity in atherosclerotic plaques<sup>31</sup>. Our results showing an elevated serum GGT level to be associated with carotid plaque are in agreement with the above findings, and this association supports the concept of the presumptive pathological mechanism of atherosclerosis.

The role of fatty liver in atherosclerosis, particularly the causal relationship between fatty liver and atherosclerosis, remains to be fully elucidated. Some researchers believe fatty liver is a cause of atherosclerosis, whereas some researchers believe that fatty liver is only a bystander to atherosclerosis. Various evidence supports the first hypothesis, as the putative underlying mechanisms of atherosclerosis in non-alcoholic fatty liver disease include insulin resistance, atherogenic DL, chronic inflammation (as represented by elevated serum levels of C-reactive protein, interleukin-6 and tumor necrosis factor  $\alpha$ ), hypercoagulation and hypofibrinolysis (as represented by elevated serum levels of fibrinogen, factor VII and plasminogen activator inhibitor 1)<sup>32</sup>. In particular, one study examined the associations between non-alcoholic steatohepatitis

and conditions related to atherosclerosis in overweight men with non-alcoholic steatohepatitis and those without steatosis but with similar levels of visceral adiposity. That study demonstrated higher serum levels of high-sensitivity C-reactive protein, fibrinogen and plasminogen activator inhibitor 1 and lower levels of adiponectin (a parameter of insulin sensitivity) in the former group than in the latter group, which suggests that non-alcoholic steatohepatitis may be directly associated with atherosclerosis<sup>33)</sup>. Given these previous findings and our current results, an elevated serum GGT level and fatty liver may synergistically contribute to the development of atherosclerotic plaque.

This study is associated with some limitations. First, because this was a cross-sectional study, we cannot prove the causal links between carotid atherosclerosis and its predictors. Second, fatty liver was diagnosed based on US findings. Studies have shown that the diagnostic accuracy of the US method is excellent for moderate to severe fatty liver (sensitivity, 82% to 100%; specificity, 98%), although it is somewhat lower for mild fatty liver (sensitivity, 53% to 67%; specificity, 77% to 93%)<sup>23)</sup>. Therefore, the application of US might have affected our results. Third, we did not determine the degree of steatosis or the presence or absence of steatohepatitis. Previous studies have shown a positive relationship between the stage of non-alcoholic fatty liver disease and the carotid IMT<sup>34)</sup>. A recent study demonstrated a close association between an elevated serum GGT level and the stage of non-alcoholic steatohepatitis in DM subjects<sup>35)</sup>. Future analyses are required to clarify whether the combination of fatty liver diagnosed on US and the serum GGT level can be used as a surrogate marker of the severity and stage of fatty liver disease. Fourth, the present study population included only Japanese subjects, thereby limiting the extrapolation of our results to other ethnic groups.

## Conclusion

This study suggests that the simultaneous presence of an elevated serum GGT level and fatty liver is highly predictive of carotid plaque and can serve as an effective marker of the risk of future CVD events. Therefore, routine carotid US should be performed in subjects with these conditions. Future clinical and experimental studies will help to reveal the causal relationship between an elevated serum GGT level, fatty liver and atherosclerosis and clarify the underlying pathological mechanisms.

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## Conflicts of Interest

The authors have no conflicts of interest to declare regarding the current manuscript.

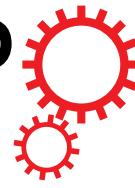
## Author Contributions

Dr. Toshikuni, Dr. Nagasawa, Dr. Uenishi, Dr. Asaji and Dr. Tsutsumi contributed to the study design. Dr. Nakanishi, Dr. Uenishi, Dr. Nagasawa and Dr. Asaji performed the data collection. Dr. Toshikuni performed the data analysis. Dr. Toshikuni wrote the manuscript. Dr. Tsutsumi supervised the work. All authors approved the final version of the manuscript.

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# SCIENTIFIC REPORTS



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## Association between plasma gamma-glutamyltransferase fractions and metabolic syndrome among hypertensive patients

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Among the risk factors associated to metabolic syndrome (MetS), hypertension shows the highest prevalence in Italy. We investigated the relationship between the newly identified serum  $\gamma$ -glutamyltransferase (GGT) fractions, b- s- m- f-GGT, and risk factors associated to MetS in hypertensive patients. A total of ninety-five consecutive hypertensive patients were enrolled. GGT fractions were analysed by gel-filtration chromatography, and hepatic steatosis was evaluated by ultrasound. MetS was diagnosed in 36% of patients. Considering the whole group, b- and f-GGT showed the highest positive correlation with BMI, glucose, triglycerides and insulin, and the highest negative correlation with HDL cholesterol. While both serum triglycerides and insulin were independently associated with b-GGT levels, only triglycerides were independently associated with f-GGT. The values of b-GGT activity increased with steatosis grade ( $g_0 = 1.19$ ;  $g_2 = 3.29$ ; ratio  $g_2/g_0 = 2.75$ ,  $p < 0.0001$  linear trend). Patients with MetS showed higher levels of b-GGT, m-GGT and f-GGT [median (25<sup>th</sup>–75<sup>th</sup>) U/L: 3.19 (1.50–6.59); 0.55 (0.26–0.81); 10.3 (9.1–13.6); respectively] as compared to subjects presenting with one or two MetS criteria [1.75 (0.95–2.85),  $p < 0.001$ ; 0.33 (0.19–0.60),  $p < 0.05$ ; 8.8 (7.0–10.6),  $p < 0.001$ ]. Our data point to a potential role for b- and f-GGT fractions in identifying MetS patients among hypertensive subjects, thus providing a minimally invasive blood-based tool for MetS diagnosis.

Metabolic syndrome (MetS) is a complex clinical condition represented by a cluster of five interconnected risk factors including abdominal obesity, insulin resistance, high levels of serum triglycerides, low high-density lipoprotein (HDL) cholesterol and hypertension. The presence of three or more of these factors allows the clinical diagnosis of MetS<sup>1</sup>. MetS is associated with a 5-fold increased risk of type 2 diabetes mellitus, 3-fold increased risk of cardiovascular disease and also with an increased risk of developing some types of cancers<sup>2,3</sup>. MetS is a growing problem worldwide and substantial efforts have been made in the last years to identify early, minimally invasive blood-based biomarkers for MetS diagnosis. Indeed, a large number of biomarkers have been reported to be associated – even not exclusively – with MetS<sup>4</sup> and recent studies have aimed at the identification of early biomarkers of MetS – such as specific levels/types of extracellular microvesicles, DNA, RNAs or proteins<sup>5</sup>.

Serum  $\gamma$ -glutamyltransferase (GGT; EC 2.3.2.2) has been proposed as a useful predictive biomarker for MetS, as its levels were found to correlate with an increased risk of metabolic syndrome and type 2 diabetes<sup>6</sup>. The Framingham Heart Study demonstrated that GGT is positively associated with body mass index, blood pressure, LDL cholesterol, triglycerides, and blood glucose (*i.e.* all the major components of MetS) and that the risk of metabolic syndrome increases with higher GGT levels<sup>7</sup>. Other large population studies confirmed the association between GGT and MetS<sup>8,9</sup> further supporting the potential role of GGT for a better classification of patients diagnosed with MetS.

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Nevertheless, serum GGT activity is firstly recognized as a sensitive marker of liver dysfunction and alcohol abuse, though with a low specificity. GGT levels increase in various physiological and pathological conditions including hepatobiliary disorders such as steatosis and viral hepatitis<sup>10</sup>.

A high sensitivity clinical laboratory method allowing the simultaneous detection of four different fractions of GGT in human plasma has been developed in our laboratories<sup>11</sup>. These fractions consist of three GGT-containing molecular complexes, *i.e.* b-GGT, m-GGT, and s-GGT, with molecular weight >2000, 940, 140 kDa, respectively, and the free enzyme, f-GGT (70 kDa). We found that f-GGT is the most abundant fraction in healthy subjects<sup>12</sup>, while s-GGT increases to become the main GGT fraction in chronic viral hepatitis C and alcoholic-liver disease<sup>13,14</sup> and that b-GGT levels are elevated in non-alcoholic fatty liver disease (NAFLD)<sup>13</sup>. The b-GGT fraction is also positively associated with several cardiovascular risk factor, including atherogenic dyslipidemia<sup>15</sup>. Patients from the Framingham Heart Study showed a positive correlation between markers of MetS (BMI, DBP, glucose, triglycerides) and the b- and f-GGT fractions, with an increase of the b/s ratio<sup>15</sup>.

As regards Italy, among the risk factors associated to MetS hypertension shows the highest prevalence<sup>16,17</sup>. Hypertension is known to be associated with insulin resistance and thus with alterations in glucose homeostasis which are the main features of MetS<sup>18,19</sup>.

The aim of the present investigation was to establish if a specific GGT fraction pattern is associated with MetS in a population of hypertensive patients at high risk for cardiovascular disease.

## Patients and Methods

**Patient selection.** Ninety-five consecutive Caucasian patients presenting with hypertension and one or more additional risk factors for the diagnosis of MetS were enrolled in the study at the O.U. of Cardiovascular Disease (University Hospital of Pisa; Department of Surgery, Medical, Molecular, and Critical Area Pathology). MetS was defined using modified National Cholesterol Education Program (NCEP) criteria<sup>1,7</sup>, which requires at least three of the following: elevated blood pressure ( $\geq 130$  mmHg systolic,  $\geq 85$  mmHg diastolic) or anti-hypertensive drug treatment; elevated fasting blood glucose ( $\geq 100$  mg/dL) or drug treatment for elevated glucose; high triglyceride levels ( $\geq 150$  mg/dL), reduced HDL cholesterol levels ( $< 50$  mg/dL for women and  $< 40$  mg/dL for men), high body mass index (BMI  $\geq 27.2$  for women and  $\geq 29.5$  for men). Grading of diffuse hepatic steatosis was evaluated by liver ultrasound and patients were grouped depending on their steatosis grade (0 = no steatosis; 1 = low; 2 = medium; 3 = high).

The following represented exclusion criteria: presence of liver disease (*i.e.* viral hepatitis, cholestasis, hepatocellular carcinoma, cirrhosis); excessive alcohol consumption (more than 45 g/day for men or 30 g/day for women, according to World Health Organization Recommendations 2014); use of hepatotoxic drugs.

The Institutional Ethics Committee of the University Hospital of Pisa approved the study (n° 3865; date: 12/11/2013) and all subjects gave informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

**Liver ultrasound.** Ultrasound (US) examinations were performed by using a US unit (ACUSON S2000TM Siemens), with a 3–4.5 MHz convex array transducer. Patients were examined following an overnight fasting period. Hepatic steatosis on US appears as a diffuse increase in hepatic echogenicity, or “bright liver”, due to increased reflection of US from the liver parenchyma, which is caused by intracellular accumulation of fat vacuoles. US evaluation of hepatic steatosis typically consists of a qualitative visual assessment of hepatic echogenicity, measurements of the difference between the liver and kidneys in echo amplitude, evaluation of echo penetration into the deep portion of the liver, and determination of the clarity of blood vessel structures in the liver<sup>20</sup>.

The alteration of echogenicity was graded as follows: grade 0, normal echogenicity; grade 1, slight, diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders; grade 2, moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm; grade 3, marked increase in fine echoes with poor or no visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver<sup>21</sup>.

**Laboratory analyses.** Standard assay of all blood tests were simultaneously performed according to the standard clinical laboratory procedures by automated analyzers, and included: creatinine, glucose, insulin, total cholesterol; low density lipoprotein (LDL) cholesterol; high density lipoprotein (HDL) cholesterol, triglycerides (TG); total and direct bilirubin; aspartate aminotransferases (AST); alanine aminotransferases (ALT); alkaline phosphatases (ALP), C-reactive protein (CRP), complete blood count. Analysis were performed at the Clinical Laboratory of the University Hospital of Pisa; quality control was ensured by the participation to external quality assessment of the Tuscany Region (Italy).

**Gamma-glutamyltransferase fraction analysis.** Analysis of total and fractional GGT was performed, as previously described<sup>11,12</sup>, on plasma-EDTA samples using a fast protein liquid chromatography system (AKTA purifier; GE Healthcare Europe, Milan, Italy) equipped with a gel-filtration column (Superose 6 HR 10/300 GL; GE Healthcare Europe) and a fluorescence detector (Jasco FP-2020; Jasco Europe, Lecco, Italy). Separation of fractional GGT was obtained by gel-filtration chromatography and the enzymatic activity was quantified by post-column injection of the fluorescent substrate for GGT, gamma-glutamyl-7-amido-4-methylcoumarin (gGlu-AMC). Enzymatic reaction, in the presence of gGluAMC 0.030 mmol/L and glycylglycine 4.5 mmol/L, proceeded for 4.5 min in a reaction coil (PFA, 2.6 mL) kept at the 37 °C in a water bath. The fluorescence detector operating at excitation/emission wavelengths of 380/440 nm detected the AMC signal; the intensity of the fluorescence signal was expressed in arbitrary fluorescence units. Under this reaction conditions, area under curve (AUC) is proportional to GGT activity. Fractional GGT activity was quantified as previously described<sup>12</sup>.

Number of patients, n (M/W)	95 (60/35)
Age, years	56 (12)
BMI, kg/m <sup>2</sup>	27.4 (25.4–30.0)
Creatinine, mg/dL	0.96 (0.80–1.06)
Glucose, mg/dL	101 (94–109)
Insulin, µU/mL	8.6 (5.4–11.7)
Total cholesterol, mg/dL	187 (166–208)
HDL cholesterol, mg/dL	56 (46–67)
LDL cholesterol, mg/dL	117 (101–138)
Triglycerides, mg/dL	95 (69–126)
Total bilirubin, mg/dL	0.51 (0.40–0.67)
Direct bilirubin, mg/dL	0.20 (0.16–0.25)
AST, U/L	21(17–25)
ALT, U/L	21(15–29)
ALP, U/L	64 (54.0–77.5)
CRP, mg/dL	1.6 (0.77–3.30)
Leucocyte count ( $10^3/\mu\text{L}$ )	6.4 (5.5–7.4)
Total GGT, U/L	17.8 (12.1–22.9)
b-GGT, U/L	1.9 (1.1–3.8)
m-GGT, U/L	0.4 (0.2–0.6)
s-GGT, U/L	4.3 (3.1–7.2)
f-GGT, U/L	9.6 (7.7–11.4)
b/s ratio	0.4 (0.3–0.6)

**Table 1.** Characteristics of the study patients. Data are presented as mean (SD) or as median (25<sup>th</sup>–75<sup>th</sup> percentile). BMI, body mass index; TG, triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein.

**Statistical analysis.** Data are presented as mean (standard deviation, SD) or median (25<sup>th</sup>–75<sup>th</sup> percentile) as appropriate. GGT fractions, the b-GGT/s-GGT ratio, glucose, TG, ALT and insulin data were ln-transformed to reduce the distribution skewness. The statistical comparisons between two or more groups were carried out with the Student's t-test or one-way analysis of variance (ANOVA), followed by post test for linear trend, respectively. Two-way ANOVA was used to test the effect of MetS presence (categorized as presence/absence) and the grade of steatosis (categorized as grade 0, 1, 2, 3). Univariate linear correlations between variables and fractional GGT activity were evaluated with the Pearson's correlation coefficient; multivariable linear regression analysis was performed applying a stepwise model including the following variables: gender, age, BMI, glucose, insulin, TG, HDL-cholesterol, ALT; variables entered the model if  $p < 0.05$  and were removed if  $p > 0.1$ . An alpha level of  $p < 0.05$  was considered significant for all statistical tests. Statistical analysis has been performed with the MedCalc Statistical Software version 14.12.0.

**Data Availability.** All data generated or analyzed during this study are included in this published article.

## Results

**Patients' characteristics.** All the patients enrolled in the study presented with elevated blood pressure (hypertension): Table 1 shows a detailed list of the baseline characteristics of the study patients. About 36% of patients presented with three or more criteria used for MetS diagnosis according to the modified NCEP criteria, whereas the remaining part of the patients presented with one (hypertension) or two risk factors. As far as GGT is concerned, total and fractional GGT values were all within the normal reference values<sup>12</sup>. Table 2 shows the percentage distribution of MetS risk factors in the study population; the percentage of patients undergoing drug treatments relevant for MetS are also reported.

**Fractional gamma-glutamyltransferase analysis and MetS risk factors.** Among the four GGT fractions, b-GGT and f-GGT showed the highest positive Pearson's correlation coefficient with serum levels of triglycerides, insulin, glucose, ALT and BMI (Table 3), and inverse correlation with HDL cholesterol. The highest correlations presented by s-GGT fraction were with BMI and ALT.

Multiple regression analysis identified ALT as the common predictor of fractional GGT levels; triglycerides were the main predictor of b-GGT and f-GGT fraction and they were the sole predictor of the b-/s-GGT ratio. Moreover, insulin was independently associated only with b-GGT fraction (Table 4).

In a first attempt to investigate the relationship between fractional GGT and MetS, patients were divided into two subgroups, *i.e.* those presenting with one or two MetS criteria (Control group, C) and those with three or more (MetS group). We found that b-GGT [C vs. MetS, median (25<sup>th</sup>-75<sup>th</sup>): 1.75 (0.95–2.85) U/L vs. 3.19 (1.50–6.59) U/L;  $p < 0.001$ ], m-GGT [0.33 (0.19–0.60) U/L vs. 0.55 (0.26–0.81) U/L;  $p < 0.05$ ], and f-GGT fraction activities [8.8 (7.0–10.6) U/L vs. 10.3 (9.1–13.6) U/L;  $p < 0.001$ ] were significantly higher in subjects belonging to

<b>Metabolic syndrome criteria</b>	
Hypertension	95 (100%)
Fasting glucose $\geq 100$ mg/dL	52 (55%)
BMI M $\geq 29.5$ kg/m <sup>2</sup> ; F $\geq 27.2$ kg/m <sup>2</sup>	33 (35%)
TG $\geq 150$ mg/dL	14 (15%)
HDL M < 40 mg/dL; F < 50 mg/dL	13 (14%)
<i>Drugs:</i>	
Anti-hypertensives	85 (89%)
Calcium channel blockers	48 (50%)
Angiotensin receptor blockers	60 (63%)
Beta-blockers	20 (21%)
Angiotensin-converting enzyme (ACE) inhibitors	13 (14%)
Diuretics	35 (37%)
<i>Lipid-lowering</i>	
Statin	26 (27%)
Bezafibrate	4 (4.2%)
<i>Glucose-lowering</i>	
Metformin	8 (8.4%)
Vildagliptin	1 (1.1%)

**Table 2.** Data represent the number of patients (% of the total). BMI, body mass index; TG, triglycerides.

	<b>Variables</b>					
	<b>BMI</b>	<b>Glucose*</b>	<b>HDL</b>	<b>TG*</b>	<b>ALT*</b>	<b>Insulin*</b>
b-GGT*	0.382 <sup>‡</sup>	0.369 <sup>†</sup>	-0.360 <sup>†</sup>	0.472 <sup>‡</sup>	0.353 <sup>†</sup>	0.434 <sup>‡</sup>
m-GGT*	0.266 <sup>§</sup>	0.199 <sup>n.s.</sup>	-0.281 <sup>§</sup>	0.218 <sup>‡</sup>	0.333 <sup>§</sup>	0.224 <sup>‡</sup>
s-GGT*	0.312 <sup>§</sup>	0.281 <sup>§</sup>	-0.242 <sup>‡</sup>	0.203 <sup>‡</sup>	0.365 <sup>†</sup>	0.282 <sup>§</sup>
f-GGT*	0.452 <sup>‡</sup>	0.438 <sup>‡</sup>	-0.393 <sup>‡</sup>	0.408 <sup>‡</sup>	0.423 <sup>‡</sup>	0.412 <sup>‡</sup>
b-/s-GGT*	0.192 <sup>n.s.</sup>	0.199 <sup>n.s.</sup>	-0.251 <sup>#</sup>	0.478 <sup>‡</sup>	0.079 <sup>n.s.</sup>	0.315 <sup>§</sup>

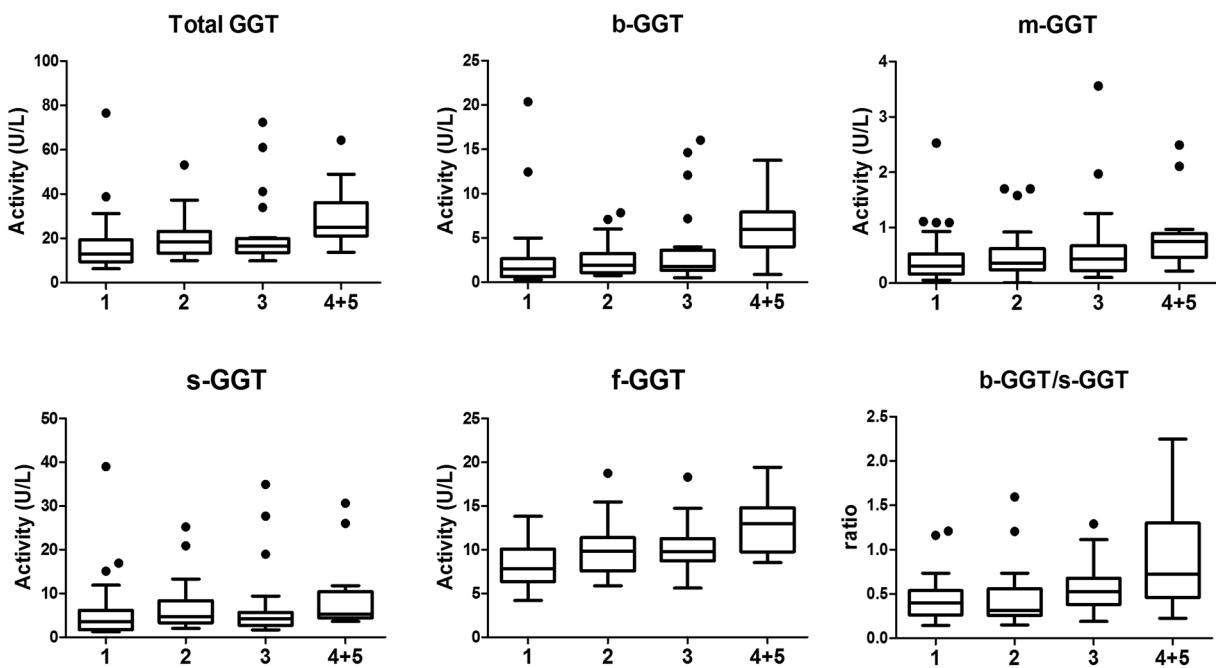
**Table 3.** Pearson's correlation analysis. Data are Pearson correlation coefficients. BMI, body mass index; TG, triglycerides; ALT, alanine aminotransferase. \*Statistical analysis was performed on ln-transformed data. Statistical significance level <sup>‡</sup>P < 0.05; <sup>§</sup>P < 0.01; <sup>#</sup>P < 0.001; <sup>‡</sup>P < 0.0001; n.s. not significant.

	<b>Variables</b>								
	<b>R<sup>2</sup> adj</b>	<b>Gender M = 0 F = 1</b>	<b>Age</b>	<b>BMI</b>	<b>Glucose*</b>	<b>HDL</b>	<b>TG*</b>	<b>ALT*</b>	<b>Insulin*</b>
b-GGT*	0.342	n.s.	n.s.	n.s.	n.s.	n.s.	0.419 <sup>‡</sup>	0.295 <sup>§</sup>	0.212 <sup>‡</sup>
m-GGT*	0.171	-0.234 <sup>‡</sup>	n.s.	n.s.	n.s.	n.s.	0.217 <sup>‡</sup>	0.297 <sup>§</sup>	n.s.
s-GGT*	0.194	-0.232 <sup>‡</sup>	n.s.	0.221 <sup>‡</sup>	n.s.	n.s.	n.s.	0.265 <sup>‡</sup>	n.s.
f-GGT*	0.384	n.s.	n.s.	n.s.	0.239 <sup>‡</sup>	n.s.	0.324 <sup>§</sup>	0.309 <sup>§</sup>	n.s.
b-/s-GGT*	0.219	n.s.	n.s.	n.s.	n.s.	n.s.	0.477 <sup>‡</sup>	n.s.	n.s.

**Table 4.** Multivariable linear regression analysis. Data are correlation coefficients adjusted for the effect of the other variables included in the model (partial correlation coefficient). BMI, body mass index; TG, triglycerides; ALT, alanine aminotransferase. \*Statistical analysis was performed on ln-transformed data. Statistical significance level <sup>‡</sup>P < 0.05; <sup>§</sup>P < 0.01; <sup>#</sup>P < 0.001; <sup>‡</sup>P < 0.0001; n.s. not significant.

MetS group. Also the b-/s-GGT ratio was significantly higher [0.32 (0.26–0.54) U/L vs. 0.58 (0.41–0.78; p < 0.001] in MetS patients.

On this background, patients were then divided into four subgroups, according to the number of co-existing MetS criteria. Due to the low number of patients, those with four or five criteria were grouped together ("4 + 5" subgroup). As shown in Fig. 1, the fractions b-GGT and f-GGT showed a significant increase along with the increase of the number of co-existing MetS criteria (P < 0.0001 for linear trend) and a similar trend was also observed for the b-/s-GGT ratio (P < 0.001). A further analysis revealed that the increase of values from subgroup "1" to subgroup "4 + 5" was more pronounced for b-GGT, as judged by the slope for the linear trend (b-GGT slope = 0.201 vs f-GGT slope = 0.068). The increase was more prominent between the subgroups "3" and "4 + 5".



**Figure 1.** Distribution of fractional GGT activity in patients according to the number of the co-existing MetS criteria; patients with four or five criteria were grouped (“4+5”). The box represents the 25<sup>th</sup> and 75<sup>th</sup> percentiles and the line the median value. Whiskers correspond to the 25<sup>th</sup> percentile minus 1.5 times IQR (interquartile range) and to the 75<sup>th</sup> percentile plus 1.5 IQR.

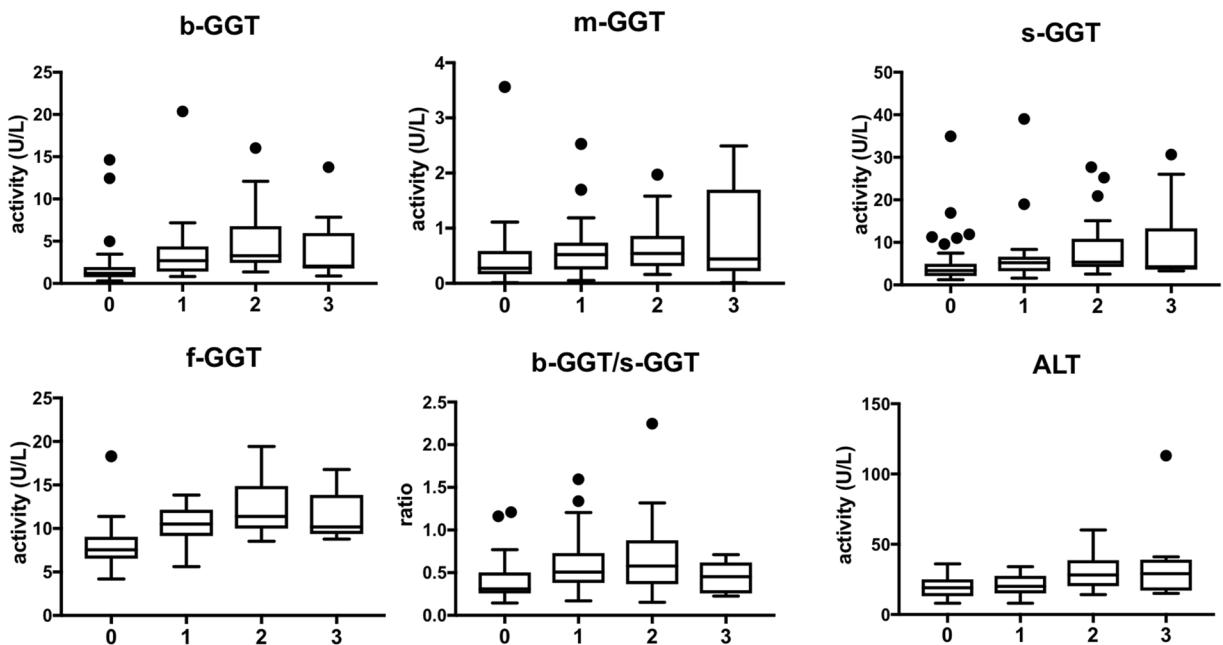
Since bezafibrate and metformin medications have been reported to lower serum total GGT activity<sup>22–24</sup>, we repeated all the analyses excluding the patients treated with these drugs (see Table 2). However, statistical analyses confirmed the results obtained in the whole group (data not shown).

**Fractional GGT analysis and liver steatosis.** Patients were also grouped depending on the steatosis grade as evaluated by liver ultrasound (Fig. 2). The fractions b-GGT, s-GGT and f-GGT showed a progressive increase in patients with low (grade 1) or moderate liver steatosis (grade 2), in comparison with those without liver steatosis (grade 0), while no further increase was seen in patients with grade 3 steatosis ( $P < 0.0001$ ,  $P < 0.01$ ,  $P < 0.0001$  for linear trend from grade 0 to grade 2). The b-/s-GGT ratio showed the same behavior ( $P < 0.001$ ), while variation in m-GGT fraction levels were not statistically significant. The levels of ALT increased all over the subgroups according to the grade of steatosis ( $P < 0.001$  for linear trend from grade 0 to grade 3). A further analysis revealed that the increase of values from grade 0 (g0) to grade 2 (g2) of steatosis was more pronounced for b-GGT median values ( $g0 = 1.19 \text{ U/L}$ ;  $g2 = 3.29 \text{ U/L}$ ;  $\text{ratio } g2/g0 = 2.75$ ), as judged also by the slope for the linear trend (slope = 0.589). Indeed, lower increases were observed for s-GGT ( $g0 = 3.39 \text{ U/L}$ ;  $g2 = 5.37 \text{ U/L}$ ;  $\text{ratio } g2/g0 = 1.58$ ; slope = 0.310), f-GGT ( $g0 = 7.54 \text{ U/L}$ ;  $g2 = 11.38 \text{ U/L}$ ;  $\text{ratio } g2/g0 = 1.50$ ; slope = 0.231), b/s-GGT ratio ( $g0 = 0.31$ ;  $g2 = 0.58$ ;  $\text{ratio } g2/g0 = 1.87$ ; slope = 0.278) and ALT ( $g0 = 19 \text{ U/L}$ ;  $g2 = 28 \text{ U/L}$ ;  $\text{ratio } g2/g0 = 1.47$ ; slope = 0.173). Again, when patients treated with bezafibrate and metformin were excluded, statistical analyses confirmed the results obtained in the whole group (data not shown).

Finally, a two-way ANOVA was used to compare the effect of MetS presence and the grade of steatosis on fractional GGT values. The two-way ANOVA showed no significant interactions between the two factors but, interestingly, revealed only a statistically significant effect of the steatosis grade on b-GGT and f-GGT values ( $p < 0.01$  and  $p < 0.001$ , respectively).

## Discussion

Among the risk factors associated to MetS, in Italy hypertension shows the highest prevalence<sup>16,17</sup>, and thus the identification of early, minimally invasive blood-based biomarkers for MetS diagnosis in hypertensive patients would be of great interest for their early risk stratification. In this perspective, the main findings of this study are that (i) hypertensive patients with three or more criteria for MetS display higher levels of b-GGT, m-GGT and f-GGT as compared to subjects with only one or two risk factors, but (ii) only b-GGT and f-GGT fractions significantly increase along with the number of MetS criteria presented. Accordingly, (iii) b-GGT and f-GGT fractions show the highest and significant correlations with all the others criteria associated to MetS, *i.e.* levels of triglycerides, glucose, HDL and BMI. These results are in good agreement with data from literature suggesting a positive correlation between total serum GGT activity and the onset of MetS (*e.g.*<sup>4,7,25</sup>). Interestingly our data show that b- and f-GGT are the main fractions that contribute to such correlation and that they are the most influenced fractions by liver steatosis, a condition known to be associated with MetS. Overall, our data confirm the higher diagnostic accuracy of fractional GGT analysis as compared to total GGT.



**Figure 2.** Distribution of fractional GGT activity, b-/s-GGT ratio and ALT according to the grade of liver steatosis. Tukey's box and whiskers plot: the box extends from the 25<sup>th</sup> to 75<sup>th</sup> percentiles, the line in the middle is plotted at the median. Whiskers correspond to the 25<sup>th</sup> percentile minus 1.5 times IQR (interquartile range) and to the 75<sup>th</sup> percentile plus 1.5 IQR.

The plasma b-GGT fraction activity increases in NAFLD<sup>13</sup>, a condition frequently associated with insulin resistance<sup>26</sup> and MetS<sup>27</sup>, and is positively associated with several cardiovascular risk factors including atherogenic dyslipidemia<sup>15</sup>. In this perspective our data also show a stronger increase of b-GGT median values - as compared to the other GGT fractions - along with the g0-g2 increase of steatosis grade.

The basis for such correlation might lie in the biogenesis of GGT fractions<sup>28</sup>. We found that the b-GGT corresponds to membrane microvesicles carrying the lipophilic GGT<sup>29</sup> and that it is released *in vitro* by several cell types including the human hepatocyte HepG2 cell line<sup>28</sup>, and inflammatory cells upon activation<sup>30,31</sup>. Since all cell types found in the liver, *i.e.* hepatocytes, cholangiocytes, Kupffer, stellate and endothelial cells are able to release microvesicles<sup>32,33</sup>, and thus potentially b-GGT, further studies are required to establish the origin of b-GGT released during MetS and the contributions brought by the activated/damaged parenchymal liver cells and by the other cell types involved in liver inflammation.

The f-GGT is the most abundant fraction in healthy subjects<sup>12</sup> and corresponds to a soluble and catalytically active form of GGT. We showed that f-GGT is obtained by proteolytic digestion of the other GGT fractions<sup>28,29</sup>. The f-GGT fraction is the simplest form of circulating GGT and its levels are likely to be influenced by the balance between production and catabolism of the other fractions<sup>34</sup>.

As regard the levels of s-GGT, we did not find any significant difference between patients with three or more criteria for MetS when compared to subjects with only one or two factors. We suggested that s-GGT may be constituted of bile-acid micelles carrying lipophilic GGT<sup>29</sup> and we found that an increase in s-GGT levels is associated with hepatocellular damage, as observed in chronic viral hepatitis C and alcoholic-liver disease<sup>13,35</sup>. In confirmation of that, our results show that s-GGT is more significantly correlated with ALT levels rather than MetS factors. The increases of s-GGT and ALT between the second grade (g2) and the third grade (g3) of steatosis may be thus associated with the onset of a hepatocellular damage, and potentially to the onset of steatohepatitis. The ratio b-/s-GGT seems to mainly reflect the b-GGT behavior, thus suggesting the prevalence of metabolic alterations over the hepatocellular damage.

A s-GGT-like trend was observed also for the m-GGT fraction. Indeed, m-GGT levels – even higher in MetS group – did not show any significant increase along with the increase of MetS criteria and, accordingly, they showed a lower correlation with MetS factors when compared to b- and f-GGT. As for s-GGT, we have suggested that also m-GGT may be constituted of bile-acid micelles<sup>29</sup>: the similar pattern of biogenesis and their biochemical properties could thus help to explain the similar behavior of these two fractions. Further studies are however required to elucidate this specific point.

In conclusion our data showed that the investigation of fractional GGT could provide a novel, minimally invasive blood-based tool for a better identification of patients possibly presenting with the typical metabolic alterations of MetS, such as steatosis. Moreover, fractional GGT could be a useful tool for a better comprehension of MetS pathogenesis.

2.10

# SCIENTIFIC REPORTS



OPEN

## Predictive Value of Serum Gamma-glutamyltranspeptidase for Future Cardiometabolic Dysregulation in Adolescents- a 10-year longitudinal study

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Serum gamma-glutamyltransferase ( $\gamma$ -GT) is implicated in the pathogenesis of atherosclerosis and metabolic syndrome (MetS) in adults. The relationships between  $\gamma$ -GT and cardiometabolic dysregulation remains unclear in adolescents. We enrolled 7,072 Taiwanese adolescents and followed them for a median of 6.8 years. The optimal cut-off values (CoVs) of baseline  $\gamma$ -GT to predict future MetS, hypertension (HTN), and type 2 diabetes (T2DM) were determined by receiving operating characteristic (ROC) curve. Using these CoVs, the participants were divided into normal- and high-level groups. Cox proportional hazard analysis was used to calculate hazard ratios (HRs) for the subjects with a high level of  $\gamma$ -GT for the risk of future cardiometabolic dysregulation. Serum  $\gamma$ -GT was significantly higher in the subjects with MetS than in those without MetS at baseline ( $p < 0.001$ ). The optimal CoVs of  $\gamma$ -GT were 12 U/L for boys and 11 U/L for girls. In multivariate Cox regression analysis, a higher serum  $\gamma$ -GT level increased the risk of future MetS (HRs 1.98 and 2.85 for boys and girls, respectively, both  $p < 0.001$ ), but not new onset HTN and T2DM. In conclusion, serum  $\gamma$ -GT levels not only demonstrated an excellent correlation with the presence of MetS and also in predicting future MetS in adolescents.

Adolescents have become increasingly obese worldwide during the last three decades<sup>1,2</sup>. Importantly, obese adolescents are likely to stay obese into adulthood and are more likely to develop non-communicable diseases such as metabolic syndrome (MetS), type 2 diabetes (T2DM) and cardiovascular disease (CVD)<sup>3–7</sup>. Since these diseases are included in the top ten leading causes of death in Taiwan<sup>8</sup>, the early recognition of adolescents at high risk of future cardiometabolic dysregulation and prevention of associated morbidity and mortality are critical public health issues<sup>9,10</sup>.

The pathogenesis of cardiometabolic dysregulation with regards to genetic and social-environmental factors is unclear, however it probably involves an imbalance between pro- and anti-inflammatory adipocytokines<sup>11</sup>. Increased levels of pro-inflammatory cytokines such as leptin, tumor necrosis factor- $\alpha$ , interleukin-6 (IL-6), IL-1 $\beta$  and decreased levels of anti-inflammatory cytokines such as adiponectin have been demonstrated both in children and adults with MetS<sup>11,12</sup>. Even though high molecular weight adiponectin and a high leptin-to-adiponectin ratio have been reported to be useful biomarkers in establishing MetS<sup>13</sup>, the limited testing ability in primary care

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institutes limits their clinical application. With an increasing prevalence of MetS in adolescents<sup>14</sup>, identifying easy and reliable biomarkers to predict cardiometabolic dysregulation and understanding the relationships between these biomarkers and cardiometabolic dysregulation are also important.

Gamma-glutamyltranspeptidase ( $\gamma$ -GT) is a liver enzyme that participates in the synthesis and degradation of glutathione as well as xenobiotic detoxification<sup>15,16</sup>. Serum  $\gamma$ -GT is a widely used biomarker for alcoholic liver injury and nonalcoholic fatty liver disease (NAFLD). Previous studies have also reported the diagnostic role of serum  $\gamma$ -GT in MetS, T2DM, and CVD, and its predictive role of mortality and morbidity associated with cardiometabolic dysregulation<sup>17–20</sup>. However, these studies only enrolled middle-aged patients, and thus cannot be extrapolated to adolescents<sup>17–20</sup>. A recent cohort study recruiting 1,874 adolescents demonstrated that the subjects with NAFLD had higher  $\gamma$ -GT levels and greater liver shear velocity (an indicator of liver fibrosis) than those without NAFLD, even after adjustment for fat mass<sup>21</sup>. Although the association between serum  $\gamma$ -GT and ultrasound scan-determined liver damage was identified<sup>21</sup>, the cross-sectional study cannot determine the causality. In addition, the role of  $\gamma$ -GT in future cardiometabolic dysregulation is also uncertain in adolescents. This longitudinal study aimed to evaluate the relationships between baseline  $\gamma$ -GT levels and MetS and its component, and to assess whether optimal cut-off values (CoVs) of  $\gamma$ -GT can predict future MetS, hypertension (HTN) and T2DM in adolescents.

## Methods

This study was approved by the Ethical Committee of the Cardinal Tien Hospital and the Ethical Committee of MJ Health Screening Centers. Each participant provided written informed consent. The described methods were carried out in accordance with the guidelines of the Declaration of Helsinki.

**Study Participants.** We enrolled subjects from MJ Health Screening Centers, a privately-owned chain of clinics throughout Taiwan which provide regular health examinations to their members. Parental informed consent and assent form the young adolescents were obtained. Data from the participants were collected anonymously and provided for research purposes only. In total, 11,370 subjects aged from 10 to 15 years were enrolled during a 10-year sample period (1999 to 2008) (Fig. 1). The exclusion criteria were those with only one visit ( $n = 3,545$ ), those with missing data of MetS components or  $\gamma$ -GT ( $n = 512$ ) and those with a history of alcohol consumption, HTN, type 1 diabetes and those taking medications known to affect MetS components or serum  $\gamma$ -GT levels including antihypertensive agents, corticosteroid, glycemic control agent, antilipid agent, antipsychotics, antidepressants, antiepileptics and immunosuppressants ( $n = 241$ ). The remaining 7,072 subjects (3,954 boys and 3,118 girls) were enrolled as the study cohort.

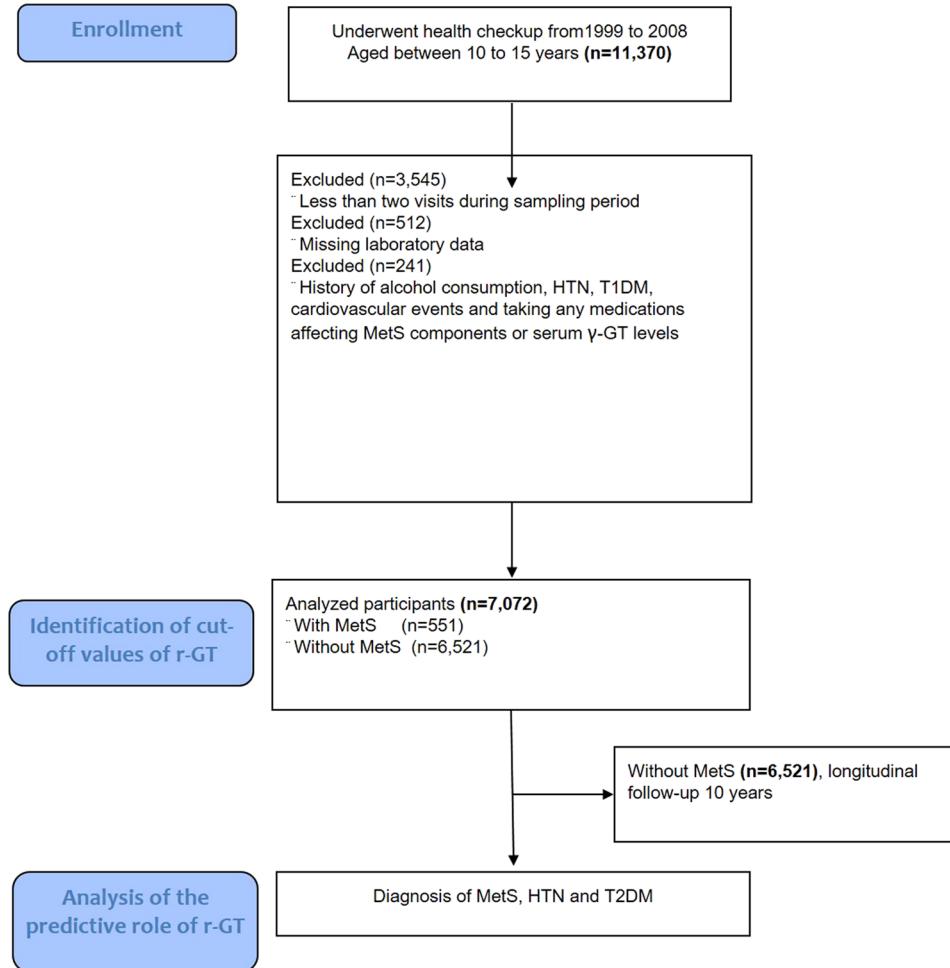
**Study Design.** There are two parts to this study. The first was a cross-sectional observation on the relationships between baseline  $\gamma$ -GT levels and MetS and its components. In addition, the optimal CoVs of baseline  $\gamma$ -GT to differentiate the subjects with and without MetS were identified. The second stage of this study was longitudinal. The primary aim of this stage was to validate the CoVs determined in stage 1. Thus, 551 subjects who had MetS at baseline were excluded, and the remaining 6,521 subjects without MetS were followed up annually with the range of 2 to 10 years (median 6.8 years). Based on the  $\gamma$ -GT CoVs, we grouped the subjects without MetS into those with normal- and high-levels of  $\gamma$ -GT. The incidence rates of developing future MetS, HTN and T2DM were then calculated in the two groups.

**General Data and Anthropometric Measurements.** The senior nursing staff used a questionnaire to obtain the subjects' drinking habits and medical history. Complete physical examinations were then performed. Anthropometric measurement including waist circumference (WC), body weight, body height, systolic blood pressure, and diastolic blood pressure were measured as we described previously<sup>22–24</sup>. After 10-hour fasting, blood samples were drawn from the antecubital vein for biochemical analysis. Plasma was separated from the blood within 1 hour and stored at  $-30^{\circ}\text{C}$  until fasting plasma glucose (FPG) and lipid profile analysis. The FPG was detected using a glucose oxidase method (YSI 203 glucose analyzer, Scientific Division, Yellow Springs Instruments, Yellow Springs, OH). Total cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) concentrations were measured by an enzymatic colorimetric method with a Roche Cobas C501 Chemistry Analyzer (Diamond Diagnostics, USA). Serum levels of high-density lipoprotein cholesterol (HDL-C) were determined using an enzymatic colorimetric assay after dextran sulfate precipitation. Serum  $\gamma$ -GT levels were measured using a CX7 biochemistry analyzer (Beckman, Fullerton, CA)<sup>22–24</sup>.

**Definition of Metabolic Syndrome.** We used the International Diabetes Federation (IDF) consensus definition of MetS in children and adolescents to define MetS<sup>22,25</sup>. Subjects having three or more of the following abnormalities were diagnosed with MetS: abdominal obesity ( $WC \geq 90^{\text{th}} \text{ percentile}$ )<sup>26</sup>, TG  $\geq 150 \text{ mg/dL}$ , HDL-C  $< 40 \text{ mg/dL}$ , HTN (systolic blood pressure  $\geq 130$  or diastolic blood pressure  $\geq 85 \text{ mmHg}$ ), and FPG concentration  $\geq 100 \text{ mg/dL}$ <sup>22</sup>.

**Statistical Analysis.** Anthropometric and biochemical data were expressed as mean  $\pm$  standard deviation. All data were tested for normal distribution using the Kolmogorov-Smirnov test and homogeneity of variance with Levene's test. The *t*-test was used to evaluate differences in demographic data between the subjects with and without MetS. Univariate and multivariate regression analyses were used to assess correlations between  $\gamma$ -GT and MetS components. The optimal CoVs of  $\gamma$ -GT for a higher likelihood of developing cardiometabolic dysregulation was calculated using receiver operating characteristic (ROC) curve analysis (MedCalc Software, Broekstraat, Mariakerke, Belgium).

In stage 2, hazard ratios (HRs) of having MetS, HTN and T2DM were calculated using Cox regression analysis. In addition, Kaplan-Meier plots and the log rank test were performed to evaluate the time effect on the incidence



**Figure 1.** Enrollment flow diagram. A total of 11,370 participants aged from 10 to 15 years who underwent regular health examinations from 1999 to 2008 at MJ Health Screening Centers were enrolled. Among them, the subjects with only one visit ( $n = 3,545$ ), missing data of MetS components or  $\gamma$ -GT ( $n = 512$ ), and a history of alcohol consumption, HTN, type 1 diabetes and those taking medications known to affect MetS components or serum  $\gamma$ -GT levels ( $n = 241$ ) were excluded. The remaining 7,072 subjects were enrolled as the study cohort. In stage 1, the optimal CoVs of baseline  $\gamma$ -GT to differentiate the subjects with and without MetS were identified by ROC curve. Using these CoVs, the aim of second stage was to validate its predictive role on future MetS, HTN and T2DM.

of having MetS, HTN and T2DM between the two groups. All data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL). A  $p$ -value (two-sided)  $< 0.05$  was considered to be statistically significant.

## Results

**Baseline Characteristics and Association between  $\gamma$ -GT and MetS.** The baseline demographic data of the participants with and without MetS are shown in Table 1. Of the 3,954 male subjects, 332 (8.4%) with a mean age of  $13.31 \pm 1.97$  years and 219 (7.0%) of 3,118 females with a mean age of  $13.47 \pm 1.96$  years fulfilled the diagnostic criteria of MetS. There were significant differences in all five components of MetS (WC, blood pressure, FPG, HDL-C, and TG) between the subjects with and without MetS in both genders. Notably, the level of serum  $\gamma$ -GT was significantly higher in the subjects with MetS than in those without ( $p < 0.001$ ).

Univariate regression analysis showed a significant correlation between  $\gamma$ -GT and all five components of MetS in the males, however, only WC, blood pressure and TG were associated with  $\gamma$ -GT in the females (Table 2). In multivariate regression analysis, WC, HDL-C and TG in the males and WC and TG in the females remained significantly associated with  $\gamma$ -GT levels.

ROC curve analysis showed that the optimal CoVs of  $\gamma$ -GT were 12 U/L in males and 11 U/L in females (Fig. 2). The areas under the ROC curve were 0.68 for the males (sensitivity 74.1%, specificity 52.0%) and 0.64 for the females (sensitivity 60.3%, specificity 60.2%) (both  $p < 0.001$ ).

**$\gamma$ -GT in Predicting Future MetS, HTN, and T2DM.** In univariate Cox regression analysis, the subjects with higher baseline levels of  $\gamma$ -GT ( $> 12$  U/L in males,  $> 11$  U/L in females) had a higher risk of developing MetS and HTN in both genders, and T2DM in males during the follow-up period (median 6.8 years) (Table 3). In

	Male				Female					
	MetS (-)		MetS (+)		P value	MetS (-)		MetS (+)	P value	
	n	3622	332			2899	219			
Age (years)	13.2	±2.0	14.0	±1.7	<0.001	13.5	±2.0	13.2	±1.9	0.031
Waist circumference (cm)	68.6	±10.1	82.9	±10.7	<0.001	63.8	±7.4	72.8	±9.2	<0.001
Systolic blood pressure (mmHg)	110.7	±12.7	127.3	±13.1	<0.001	105.1	±11.5	115.3	±14.8	<0.001
Diastolic blood pressure (mmHg)	60.5	±8.6	67.5	±10.1	<0.001	59.1	±7.7	62.2	±8.7	<0.001
Fasting plasma glucose (mg/dl)	94.7	±8.2	99.9	±8.0	<0.001	91.9	±9.4	98.6	±16.4	<0.001
Total cholesterol (mg/dl)	163.5	±29.0	167.6	±34.4	<0.036	166.8	±27.6	170.9	±33.3	0.079
High density lipoprotein (mg/dl)	56.2	±12.9	42.6	±10.3	<0.001	58.1	±12.8	44.1	±8.8	<0.001
Low density lipoprotein (mg/dl)	92.0	±25.4	98.2	±27.7	<0.001	93.6	±24.5	97.8	±29.1	0.038
Triglyceride (mg/dl)	76.3	±35.2	133.6	±65.9	<0.001	75.7	±30.2	145.4	±70.3	<0.001
γ-GT (U/L)	14.1	±7.7	20.3	±13.5	<0.001	10.5	±4.7	13.8	±8.7	<0.001

**Table 1.** Demographic data of the study subjects with and without metabolic syndrome at baseline. Data are shown as mean ± SD. Abbreviations: MetS, metabolic syndrome; MetS(-), without metabolic syndrome; MetS(+), with metabolic syndrome; γ-GT, gamma-glutamyl transferase.

	Univariate		Multivariate			
	γ	p	Model 1		Model 2	
			β	p	β	p
<b>Male</b>						
Waist circumference	0.455	<0.001	0.418	<0.001	0.397	<0.001
Systolic blood pressure	0.221	<0.001	0.026	0.141	0.019	0.285
Diastolic blood pressure	0.140	<0.001	0.014	0.380	0.013	0.406
Fasting Plasma Glucose	0.060	<0.001	0.024	0.082	0.026	0.066
High density lipoprotein	-0.112	<0.001	0.110	<0.001	0.118	<0.001
Triglyceride	0.301	<0.001	0.172	<0.001	0.167	<0.001
<b>Female</b>						
Waist circumference	0.220	<0.001	0.170	<0.001	0.163	<0.001
Systolic blood pressure	0.124	<0.001	0.054	0.009	0.051	0.014
Diastolic blood pressure	0.059	0.001	-0.001	0.941	-0.001	0.946
Fasting Plasma Glucose	0.010	0.568	—	—	—	—
High density lipoprotein	-0.012	0.491	—	—	—	—
Triglyceride	0.195	<0.001	0.154	<0.001	0.152	<0.001

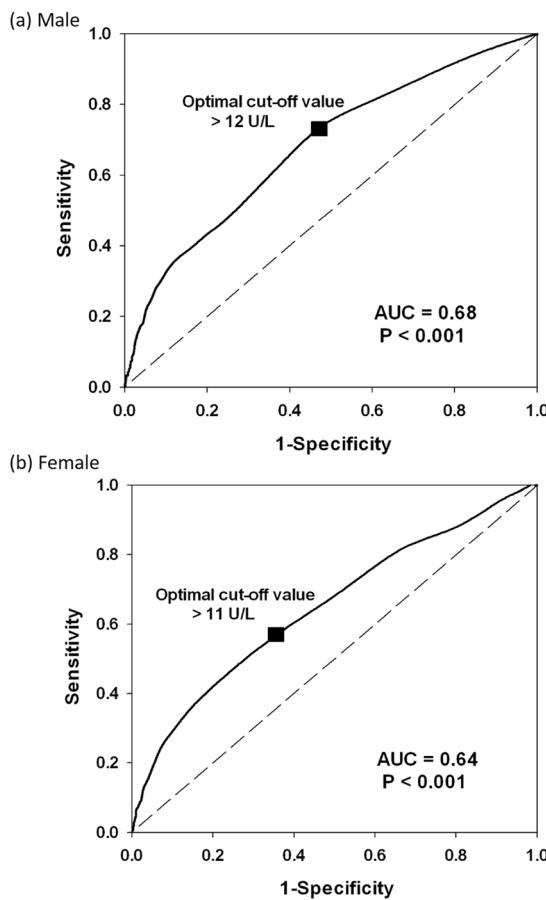
**Table 2.** Univariate and multivariate regression analysis of the γ-GT and components of the metabolic syndrome Model 1: Adjusted for components of metabolic syndrome. Model 2: Adjusted for components of metabolic syndrome as well as age and low-density lipoprotein.

addition, multivariate Cox regression analysis showed that a higher serum γ-GT level remained a significant risk factor for future MetS (HR 1.98, 95% confidence interval (CI) 1.42–2.77 in males; HR 2.85, 95% CI 1.60–5.08 in females, both  $p < 0.001$ ), but not in new-onset HTN or T2DM. Kaplan-Meier plots also demonstrated the same findings (Fig. 3).

## Discussion

The results of this study revealed that the adolescents with MetS not only had higher γ-GT levels, but also a significant association between γ-GT and MetS, particularly WC and TG. These findings suggest that γ-GT may be involved in the pathophysiology of MetS in adolescents. In accordance with this hypothesis, our longitudinal results over a median follow-up period of 6.8 years indicated that a high serum γ-GT level was an independent predictor for future MetS in adolescents. To the best of our knowledge, this is the first large-scale longitudinal study focusing on adolescents to investigate the role of γ-GT on future MetS, HTN and diabetes in the same time.

Since it is well-known that central obesity and insulin resistance are at the core of MetS, the role of γ-GT in the pathogenesis of MetS might be linked through NAFLD. In subjects with NAFLD, overproduction of glucose and TG from the fatty liver may precipitate the occurrence of MetS. On the other hand, the NAFLD is considered the hepatic manifestation of MetS and commonly associated with obesity<sup>27</sup>. Therefore, NAFLD was reported to be a useful predictor of MetS<sup>28</sup>. Conversely, patients with MetS have an increased risk of developing NAFLD<sup>29</sup>. The highly increasing prevalence of T2DM, obesity, and lifestyle changes (mainly exercise withdrawal) in the general population also makes NAFLD the most common diagnosis in daily clinical practices<sup>30</sup>. Even though NAFLD as a cause or a consequence of MetS is still being debated, an elevated level of γ-GT secondary to excessive liver fat



**Figure 2.** Receiver operating characteristic curves for serum  $\gamma$ -GT in both genders. Receiver operating characteristic curves and optimal cut-off values for serum  $\gamma$ -GT for differentiating between MetS and non-MetS in (a) male and (b) female adolescents.

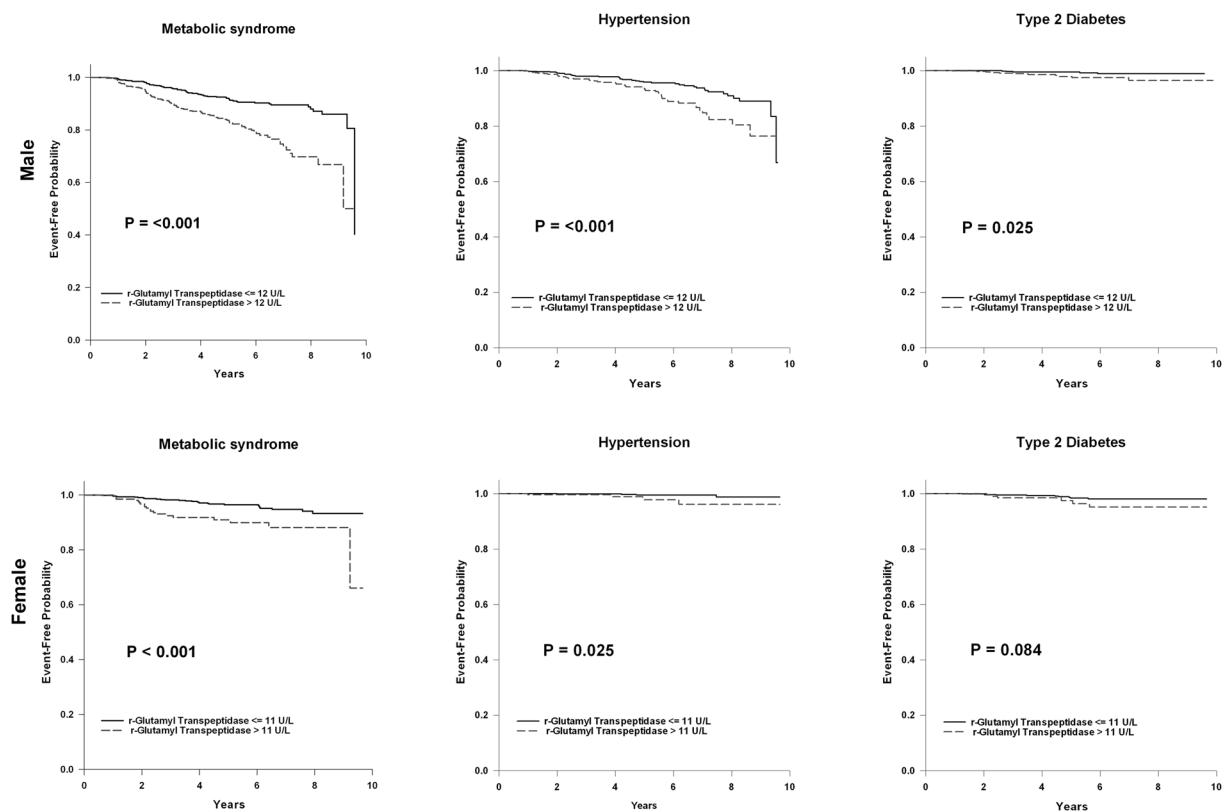
accumulation has been demonstrated in patients with MetS and NAFLD<sup>17</sup>. As expected,  $\gamma$ -GT has been reported to be a surrogate marker of NAFLD, and also a promising biomarker for MetS and its components in adults<sup>19,20</sup>.

However, little is known about the associations of  $\gamma$ -GT concentration with MetS and the role of  $\gamma$ -GT as features of MetS in adolescents. To elucidate this uncertainty, Kong *et al.* enrolled 2,067 healthy Hong Kong participants aged 6–20 years and demonstrated that high  $\gamma$ -GT levels were associated with components of MetS, especially obesity and high blood pressure<sup>31</sup>. Even though these striking findings support the assumption that serum  $\gamma$ -GT might be a potential predictor for MetS in the youth population, the cross-sectional study could not provide information regarding the temporal and causal relationship between  $\gamma$ -GT and MetS<sup>31</sup>. The present study taking advantage of large-scale longitudinal follow-up aimed to assess the predictive value of  $\gamma$ -GT on future MetS in adolescent males and females. Interestingly, our results showed that  $\gamma$ -GT levels were distinctly associated with the WC, HDL-C and TG components of MetS in the males, but only WC and TG in the females. Similarly, previous studies also suggested differences in age and gender in the way MetS is expressed in adults<sup>32</sup> as in adolescents<sup>33</sup>. Even though the phenotype of MetS determined by gender was identifiable, we found that a hyper-triglyceridemic waist (HTGW) was strongly related to  $\gamma$ -GT levels in both genders, suggesting that HTGW is a useful index for metabolic dysregulation<sup>33–35</sup>. In addition to  $\gamma$ -GT, our results also showed that WC in males and HDL-C in females had predictive power for new-onset MetS. These findings reinforce the hypothesis that MetS is a heterogeneous condition, so that the predictive parameters of MetS in affected subjects can be influenced by age, gender, and race/ethnicity<sup>36</sup>. Taken together, our compelling findings not only identify the relationships between  $\gamma$ -GT, current MetS and future MetS, but also validate differences in gender in the variable expression of MetS in adolescents<sup>31,33,35</sup>.

Emerging evidence has revealed the association between NAFLD and increasing odds of MetS<sup>24</sup>. The risk reduction of MetS may be achieved by lowering liver fat. Although pharmacologic therapies for NAFLD remains unavailable<sup>37</sup>, lifestyle interventions such as dieting and exercise have been considered effective<sup>38</sup>. In regard to exercise, Keating *et al.* reported that aerobic exercise training may help to burn fat in liver and viscera regardless of aerobic exercise dose or intensity<sup>39</sup>. Another study on resistance exercise also demonstrated that resistance training lead to a significant reduction in liver fat content and a greater glycemic control in the meanwhile<sup>40</sup>. Even though the existing evidences all supported the role of exercise on improving NAFLD and MetS<sup>38–40</sup>, the precise mechanisms were still unclear. On the other hand, the measurement of serum  $\gamma$ -GT level was a less expensive, widely available and easily interpretable way in primary care institutes to predict MetS, compared to ultrasound

	Univariate Cox Regression			Multivariate Cox Regression	
	Hazard Ratio (95% CI)	p value	Hazard Ratio (95% CI)	p value	
<b>(a) Metabolic syndrome</b>					
Male					
$\gamma$ -GT > 12 U/L	2.526	(1.824–3.500)	<0.001	1.980	(1.417–2.765)
WC > criteria*	4.508	(2.919–6.961)	<0.001	3.881	(2.481–6.069)
BP > criteria*	1.199	(0.740–1.942)	0.461	0.915	(0.562–1.489)
FPG > 100 mg/dl	1.517	(1.068–2.156)	0.020	1.437	(1.008–2.048)
HDL-C<criteria*	1.067	(0.689–1.652)	0.771	0.925	(0.593–1.444)
TG > 150 mg/dl	1.184	(0.641–2.185)	0.590	0.746	(0.401–1.390)
Female					
$\gamma$ -GT > 11 U/L	2.793	(1.589–4.910)	<0.001	2.850	(1.598–5.082)
WC > criteria*	1.859	(1.025–3.373)	0.041	1.547	(0.840–2.850)
BP > criteria*	0.522	(0.072–3.783)	0.520	0.362	(0.049–2.674)
FPG > 100 mg/dl	1.204	(0.477–3.041)	0.694	1.220	(0.483–3.080)
HDL-C<criteria*	2.940	(1.669–5.178)	<0.001	2.880	(1.625–5.104)
TG > 150 mg/dl	0.930	(0.226–3.831)	0.920	0.422	(0.099–1.793)
<b>(b) Hypertension</b>					
Male					
$\gamma$ -GT > 12 U/L	2.068	(1.339–3.194)	0.001	1.551	(0.989–2.433)
WC > criteria*	3.092	(1.816–5.265)	<0.001	2.548	(1.459–4.449)
BP > criteria*	3.281	(2.032–5.299)	<0.001	2.660	(1.624–4.355)
FPG > 100 mg/dl	0.845	(0.484–1.477)	0.554	0.732	(0.416–1.288)
HDL-C<criteria*	1.273	(0.745–2.175)	0.377	0.941	(0.543–1.632)
TG > 150 mg/dl	1.544	(0.773–3.084)	0.218	0.913	(0.447–1.864)
Female					
$\gamma$ -GT > 11 U/L	4.312	(1.069–17.395)	0.040	2.351	(0.517–10.693)
WC > criteria*	5.955	(0.732–48.428)	0.095	3.333	(0.379–29.288)
BP > criteria*	14.494	(3.457–60.769)	<0.001	8.210	(1.830–36.835)
FPG > 100 mg/dl	1.541	(0.189–12.582)	0.686	1.119	(0.130–9.627)
HDL-C<criteria*	1.810	(0.452–7.243)	0.402	1.141	(0.253–5.143)
TG > 150 mg/dl	7.387	(1.477–36.935)	0.015	2.893	(0.448–18.663)
<b>(c) Diabetes</b>					
Male					
$\gamma$ -GT > 12 U/L	3.165	(1.094–9.160)	0.034	2.429	(0.812–7.266)
WC > criteria*	5.224	(1.187–22.985)	0.029	4.023	(0.875–18.496)
BP > criteria*	1.193	(0.271–5.250)	0.815	0.785	(0.175–3.522)
FPG > 100 mg/dl	1.755	(0.610–5.055)	0.297	1.654	(0.568–4.819)
HDL-C<criteria*	1.331	(0.379–4.679)	0.656	1.107	(0.303–4.051)
TG > 150 mg/dl	1.871	(0.425–8.234)	0.407	1.046	(0.224–4.882)
Female					
$\gamma$ -GT > 11 U/L	2.378	(0.862–6.561)	0.094	2.757	(0.980–7.755)
WC > criteria*	0.824	(0.309–2.196)	0.699	0.742	(0.271–2.033)
BP > criteria*	1.667	(0.220–12.619)	0.621	1.555	(0.199–12.164)
FPG > 100 mg/dl	0.672	(0.089–5.091)	0.700	0.680	(0.090–5.161)
HDL-C<criteria*	1.109	(0.403–3.052)	0.841	1.236	(0.444–3.437)
TG > 150 mg/dl	0.046	(0.000–1178.588)	0.553	0.000	(0.000--)

**Table 3.** Hazard ratios of  $\gamma$ -GT and components of the metabolic syndrome in developing future metabolic syndrome, hypertension and type 2 diabetes. BP, blood pressure; CI, confidence interval; FPG, fasting plasma glucose;  $\gamma$ -GT, gamma-glutamyltranspeptidase; HDL-C, high-density lipoprotein cholesterol; WC, waist circumference. \*Criteria for WC were according to the cut-off value by Sung *et al.*<sup>26</sup>; criteria for BP were systolic BP  $\geq$  130 mmHg or diastolic BP  $\geq$  85 mmHg; criteria for HDL-C was  $<$  40 mg/dL. BP, blood pressure; CI, confidence interval; FPG, fasting plasma glucose;  $\gamma$ -GT, gamma-glutamyltranspeptidase; HDL-C, high-density lipoprotein cholesterol; WC, waist circumference. \*Criteria for WC were according to the cut-off value by Sung *et al.*<sup>26</sup>; criteria for BP were systolic BP  $\geq$  130 mmHg or diastolic BP  $\geq$  85 mmHg; criteria for HDL-C was  $<$  40 mg/dL.



**Figure 3.** Kaplan-Meier plot of developing future MetS, HTN and T2DM by different  $\gamma$ -GT levels. Kaplan-Meier curves estimate with log rank test was applied for the event-free probability between the subjects with normal  $\gamma$ -GT levels ( $\leq 12$  U/L) and high  $\gamma$ -GT levels ( $> 12$  U/L).

scan-determined NAFLD<sup>21</sup>. Considering the cost-effectiveness, the CoVs of  $\gamma$ -GT provided in the present study might be a useful tool to evaluate the long-term efficacy of exercise on NAFLD and MetS, and to clarify their relationships, at least in Taiwanese adolescents.

Although the detailed mechanisms that the link  $\gamma$ -GT with HTN and atherosclerotic CVD remain elusive, there are some possible explanations for their relationships<sup>41, 42</sup>. Previous studies showed that  $\gamma$ -GT is significantly related to markers of inflammation such as fibrinogen, C-reactive protein and F2-isoprostanes<sup>42, 43</sup>. Furthermore,  $\gamma$ -GT is thought to be involved in the pathogenesis of atherosclerosis on the basis of expression of  $\gamma$ -GT in human atherosclerotic lesions<sup>43, 44</sup>. Additionally, the activity of ectoenzymatic  $\gamma$ -GT has been reported to play a pivotal role in the generation of free radical species through modulating the redox status of protein thiols at the cell surface<sup>43, 45</sup>. This evidence supports the possibility that serum  $\gamma$ -GT is not only a marker of inflammation and oxidative stress but also a potential predictor for future HTN<sup>42–45</sup>.

However, the results of previous studies have been inconsistent with regards to the relationship between  $\gamma$ -GT and HTN. Kim *et al.* found a meaningful relationship between high  $\gamma$ -GT levels and HTN only in drinkers<sup>46</sup>, but Stranges *et al.* reported that a higher  $\gamma$ -GT level increased the risk of HTN in both subjects who did and did not drink alcohol<sup>43, 47</sup>. Interestingly, our results showed that serum  $\gamma$ -GT levels did not have a predictive power for future HTN, suggesting a possible different pathophysiology in incident HTN in adolescents. The discrepancies between previous studies on adults and our study may be because our subjects were younger, and because they had low CoVs of  $\gamma$ -GT and fewer deleterious lifestyle factors (such as heavy alcohol consumption, cigarette smoking, and physical inactivity)<sup>46, 47</sup>. Further studies including participants with a wide range of age, different genetic background, insulin resistance status, and inflammatory and oxidative condition are needed to elucidate the true role of  $\gamma$ -GT in predicting HTN.

Even within a normal range of concentration, serum  $\gamma$ -GT has been reported to be related to the presence of diabetes<sup>17, 42, 48</sup>. However, our results did not support serum  $\gamma$ -GT activity as a predictor of T2DM in adolescents. Several possible explanations are as follows: First, epidemiological study on prevalence of diabetes in Taiwan reported that adolescents have less than a 1% prevalence of T2DM<sup>49</sup>. Second, the natural time-course of diabetes is a critical confounding factor while assessing the relationship between metabolic predictors and the development of T2DM. Our subjects were relatively young so that normal glucose levels might be observed at a much earlier age in consideration of ‘compensated period’, i.e., higher secretion of plasma insulin to maintain glucose homeostasis<sup>22</sup>. In support of this, Kong *et al.* have shown high  $\gamma$ -GT levels did not pose a significant risk to dysglycaemia because of their young participants<sup>31</sup>. Finally, our participants were around the age of puberty, and higher levels of sex hormones may have inhibited lipogenesis and improved insulin sensitivity<sup>50</sup>. However,

plasma insulin levels parallel to fasting glucose levels were unavailable in this study. Thus we could not evaluate the association between  $\gamma$ -GT and insulin resistance.

The strengths of this study include its longitudinal population-based design and the large number of participants. In addition, this is the first clinical study to identify the optimal CoVs of  $\gamma$ -GT in predicting future MetS in adolescents. Using this simple and widely available biomarker may be helpful in initiating preventive strategies for adolescent MetS. However, there are also several limitations to this study. First, selection bias might exist due to study participants selected from a health screening center rather than from the community. However, the aim of this study was to observe relationships between factors, and thus there should be minimal effects. Second, all subjects of our study were ethnically Chinese, limiting the generalizability of the results to other ethnicities. Finally, data on the levels of serum alanine aminotransferase, insulin, fibrinogen, C-reactive protein, adiponectin and F2-isoprostanes were lacking. Further studies including these parameters and assessing the relationships between  $\gamma$ -GT, systemic oxidative stress, and inflammatory status are needed.

In conclusion, the treatment and prevention of MetS in adolescents has become a public health priority. Our findings suggest that serum  $\gamma$ -GT levels could serve as a clinical predictor for future MetS in adolescents. Using such a low-cost and widely used metabolic biomarker may help pediatricians to screen adolescents at high risk of MetS at an early stage and prevent subsequent deleterious consequences.

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# Alcohol Consumption, Serum gamma-Glutamyltransferase Levels, and Coronary Risk Factors in a Middle-Aged Occupational Population

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**Abstract:** **Alcohol Consumption, Serum gamma-Glutamyltransferase Levels, and Coronary Risk Factors in a Middle-Aged Occupational Population:** **Yuichi YAMADA, et al.** Department of Hygiene, Kanazawa Medical University—The relationships between alcohol consumption, serum gamma-glutamyltransferase (GGT) levels, and the prevalence of major coronary risk factors were analyzed crosssectionally in 2,399 male and 1,402 female middle-aged workers, to clarify the effects of moderate alcohol consumption on the development of the metabolic syndrome. Male moderate drinkers, consuming less than 60 ml of alcohol per day, had a lower prevalence of upper body obesity and low serum HDL-cholesterolemia (LHDLC) in comparison with nondrinkers, but not of hypertension, impaired glucose tolerance or hypertriglyceridemia (HTG). In women, alcohol consumption did not show any significant associations with the coronary risk factors. Men with an elevated serum GGT (EGGT) of 40 U/l or above had a significantly higher odds ratio for all the coronary risk factors as compared with those with normal GGT, even after adjusting for alcohol consumption, together with age, body mass index, cigarette consumption and physical activity. Women with an EGGT of 25 U/l or above had similar findings, although significance was found only in HTG. Nearly 80% and 55% of the appearance of EGGT in men and women were attributable to alcohol consumption, and 20% and 10% of the male and female moderate drinkers had EGGT. These results suggest that even moderate alcohol consumption will increase coronary risk factors characteristic of the metabolic syndrome in drinkers who have an increase in serum GGT. Further studies are required to confirm the causal association between

alcohol consumption, increase in serum GGT and development of the metabolic syndrome.  
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**Key words:** Alcohol consumption, Serum gamma-glutamyltransferase (GGT), Coronary risk factors, The metabolic syndrome, Occupational population

Prevention of the metabolic syndrome<sup>1–3)</sup>, a complex of multiple coronary risk factors, such as hypertension (HYT), impaired glucose tolerance (IGT) and dyslipidemia, should be targeted by health promotion activities for people living in modern society including occupational fields, where coronary heart disease is a leading cause of death and obesity, the most powerful promoter of the metabolic syndrome, is epidemic<sup>4)</sup>.

Meanwhile, moderate alcohol consumption has been shown to protect against the development of coronary heart disease and death<sup>5–7)</sup>, and it may even protect against the development of non-insulin dependent diabetes mellitus (NIDDM)<sup>8,9)</sup> although this is still disputed<sup>10)</sup>. The protective effects of moderate alcohol consumption on coronary heart disease have been attributed to high serum HDL-cholesterol levels, suppressed coagulation capacity of platelets, or the suspected role of anti-oxidant substances contained in alcoholic beverages<sup>11,12)</sup>. More recently, beneficial effects on insulin resistance, the core pathology of the metabolic syndrome, have been proposed as another possible mechanism<sup>13)</sup>, implying that improved insulin resistance in moderate alcohol consumers may suppress the development of the metabolic syndrome, and thus coronary heart disease, but there have also been contradictory studies suggesting increases in insulin resistance after alcohol consumption<sup>14–17)</sup>. In addition, high serum gamma-glutamyltransferase (GGT), a well-known biological indicator of alcohol consumption<sup>18,19)</sup>, has been shown to be associated with the metabolic syndrome<sup>20–22)</sup>. The aim of the present study is to clarify

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if moderate alcohol consumption protects against the development of the metabolic syndrome, and therefore the associations between alcohol consumption, serum GGT levels and major coronary risk factors characteristic of the metabolic syndrome were analyzed crosssectionally in a middle-aged occupational population.

## Subjects and Methods

The study subjects were recruited from 2,656 male and 1,460 female workers aged between 35 and 64 yr in an electronic-parts factory who participated in an annual health check-up and comprised 98.3% of the workers in this age range in the factory. 131 men and 24 women were excluded from consideration since they had diseases that might markedly affect the study results, such as myocardial diseases including signs of old infarction, liver disease mainly due to C-type chronic hepatitis or cirrhosis, renal disease or insufficiency due to glomerulonephritis or diabetes mellitus. In addition, 126 men and 34 women were excluded because of incompleteness of the measurements in the check-ups. Finally, 2,399 men and 1,402 women were selected as the study subjects, and written informed consent was obtained from all of them. Nearly half of the male subjects were engaged in shift and night work, and some hundreds of the subjects have handled toxic chemicals, mainly organic solvents, but no excessive exposure or harmful health effects of the shift work and the chemicals have been detected in the workplaces.

The subjects were measured in the morning after fasting 12 h or longer for height (m) and body weight (kg) in light clothes with the shoes removed, and the body mass index (BMI: kg/m<sup>2</sup>) was calculated. A BMI of 25 or above was defined here as obesity. At the same time, waist circumference (cm) was measured at the umbilicus level. The obese subjects with a waist circumference of 95 cm or more in men and 90 cm or more in women were defined as having upper body obesity (UBO)<sup>4)</sup>. Systolic and diastolic blood pressure (BP: mmHg) was measured with a sphygmomanometer in the sitting position after resting on a chair for five minutes or longer. When BP was higher than 140/90 mmHg in the first measurement, it was measured again 10 min later, and the lower value was recorded. The subjects with a BP of 140/90 mmHg or above in the health check-up, together with those under treatment with medicines for hypertension irrespective of the BP levels, were defined as having HYT.

A fasting serum sample was measured for the concentrations of triglycerides (TG: mg/dl) and HDL-cholesterol (HDLc: mg/dl) with an automatic analyzer (HITACHI 7450, Hitachi, Japan), as well as hepatic enzymes activities including GGT (U/l) and other biochemical parameters. The serum glucose concentration (mg/dl) was measured by an HK-G6PD method with the automatic analyzer. The glycated

hemoglobin concentration (HbA1c: %) was determined using an automatic analyzer, HA8150, Arkray, Japan. The subjects with a fasting serum glucose level of 110 mg/dl or above, and those with an HbA1c of 5.7% or above, were defined as having IGT as well as 23 men and 6 women who were under treatment for NIDDM. The subjects who showed fasting serum TG of 150 mg/dl or above, and 25 men under treatment for high serum TG were defined as having hypertriglyceridemia (HTG), and men who had a serum HDLc below 38 mg/dl and women below 40 mg/dl were defined as having low HDL-cholesterolemia (LHDLC). The male subjects with a serum GGT of 40 U/l or above and females 25 U/l or above, representing the 95% upper limits of serum GGT observed in the male and female non-obese nondrinkers, were defined as having elevated serum GGT (EGGT).

The data on alcohol and cigarette consumption and physical activity at leisure time were obtained by a questionnaire and confirmed by experienced nurses at the health check-up. The average volume of alcohol consumed per day by the subjects was calculated from the data on usual alcohol consumption during the preceding year. The subjects who consumed alcohol less than once a month were classified as nondrinkers as were abstainers and teetotalers. The subjects who consumed alcohol more than once a month were classified into 4 groups according to the average volume of alcohol consumed per day: less than 30 ml, 30–59 ml, 60–89 ml, and 90 ml or more. Smoking habit was classified into 5 groups as nonsmokers, ex-smokers, current smokers consuming less than 1 pack a day, and those consuming more but less than 2 packs, and those consuming 2 packs or more. The subjects were classified into 4 groups of physical activity at leisure: those who performed any kind of exercise lasting 30 min or longer not more often than once a month, those who performed the exercise once a week or less, 2 to 4 times a week, and 5 times or more a week. All the subjects were scored 1–5 or 1–4 for alcohol and cigarette consumption and physical activity according to those groups. The alcohol consumers were further categorized into moderate drinkers consuming less than 60 ml of alcohol per day and excessive drinkers consuming more. The subjects who performed physical exercise only once a week or less were defined as physically inactive subjects.

The associations between alcohol consumption, serum GGT levels and the major components of the metabolic syndrome, such as UBO, HYT, IGT, HTG and LHDLC, in the male and female subjects were analyzed and tested with a  $\chi^2$ -test and a multiple logistic regression (MLR) analysis adjusting for confounders. All the statistical analyses were performed with an SPSS version 11.0 program package for Windows (SPSS Japan, Tokyo), with  $p<0.05$  defined as significance,  $p<0.01$  as high significance, and  $0.05 \leq p < 0.10$  as borderline significance.

**Table 1.** The number (n) and prevalence (%) of upper body obesity (UBO), hypertension (HYT), impaired glucose tolerance (IGT), hypertriglyceridemia (HTG) and low HDL-cholesterolemia (LHDLC), and elevated serum GGT (EGGT) in 2,399 middle-aged men and 1,402 women divided by alcohol consumption categories according to the average volume of alcohol consumed per day

Coronary risk factor <sup>c</sup>	Men						Women					
	Nondrinker		Moderate <sup>a</sup>		Excessive <sup>a</sup>		Nondrinker		Moderate <sup>b</sup>		Results of $\chi^2$ -test <sup>d</sup>	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	*	
UBO	25	(4.2)	26	(2.2)	19	(3.2)	#	58	(4.6)	9	(6.0)	ns
HYT	82	(13.7)	186	(15.5)	145	(24.0)	**	131	(10.5)	16	(10.7)	ns
IGT	55	(9.2)	135	(11.3)	75	(12.4)	ns	58	(4.6)	10	(6.7)	ns
HTG	133	(22.2)	257	(21.5)	145	(24.0)	ns	82	(6.5)	7	(4.7)	ns
LHDLC	79	(13.7)	84	(7.0)	25	(4.1)	**	114	(9.1)	11	(7.4)	ns
EGGT	45	(7.5)	228	(19.0)	256	(42.5)	**	60	(4.8)	14	(9.4)	*

a) For the definitions of the categories of alcohol consumption, refer to text. b) Including 4 women consuming 60 ml or more alcohol per day. c) For the definitions of the risk factors, refer to text. d) ns: not significant ( $p \geq 0.10$ ), #:  $p < 0.10$ , \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

## Results

Of the 2399 male subjects, 599 (25.0%) were nondrinkers, 536 (22.3%) consumed 29 ml of alcohol or less per day, 661 (27.6%) consumed 30–59 ml, 420 (17.5%) consumed 60–89 ml, and 183 (7.6%) consumed 90 ml or more, so that just half of the present male subjects were considered as moderate drinkers, and a quarter of them excessive drinkers. Of the 1,402 female subjects, drinkers were few, and only 126 (9.0%) of them consumed 29 ml of alcohol or less per day, and 23 (1.6%) consumed 30 ml or more. Those who consumed 60 ml or more of alcohol per day were 4 of the 23 women, and therefore the majority of the female drinkers were considered as moderate drinkers. The major coronary risk factors characteristic of the metabolic syndrome: UBO, HYT, IGT, HTG and LHDLC in the male subjects were found in 70 (2.9%), 413 (17.2%), 265 (11.0%), 535 (22.3%) and 188 (7.8%), respectively. In the female subjects, the corresponding figures were 67 (4.8%), 147 (10.5%), 68 (4.9%), 89 (6.3%) and 125 (8.9%), respectively. The prevalence of the coronary risk factors was obtained in the male nondrinkers, moderate drinkers and excessive drinkers, and in the female nondrinkers and drinkers, and the differences were tested by a  $\chi^2$ -test. The results are summarized in Table 1, together with the prevalence of EGGT.

The prevalence of UBO was somewhat lower in the male moderate drinkers in comparison with nondrinkers and excessive drinkers, although the differences in the three categories remained at a borderline significant level ( $p=0.06$ ). The prevalence of HYT in men was increased with increases in alcohol consumption, and the differences

were highly significant. On the other hand, LHDLC was lower in alcohol consumers than in non-consumers, and lower in heavier alcohol consumers. The differences in the three categories were highly significant. The prevalence of IGT and HTG in men was, however, not significantly different in the three categories of alcohol consumption, although both were slightly higher in the excessive drinkers. No significant association of alcohol consumption with the prevalence of the coronary risk factors was found in women, probably because of the low volume of alcohol consumed in the few drinkers.

Although not shown in the table, when adjusting for age and the scores for cigarette consumption and physical activity at leisure by a multiple logistic regression analysis, the odds ratios in the male moderate drinkers and excessive drinkers for UBO as compared with nondrinkers were determined to be 0.54 and 0.78, respectively, and the low odds ratio in the moderate drinkers was significant ( $p=0.03$ ). Multiple logistic regression analyses adjusting for BMI, as well as age and the scores for cigarette consumption and physical activity, showed that the odds ratios in the moderate and excessive drinkers for HYT were 1.2 and 2.0, respectively, and those for LHDLC were 0.53 and 0.25, respectively, as compared with nondrinkers. Those were all significant except for the odds ratio in moderate drinkers for HYT. No significant high or low odds ratios were found for IGT and HTG in the male moderate and excessive drinkers. None of the odds ratios for the coronary risk factors were significant in the female drinkers.

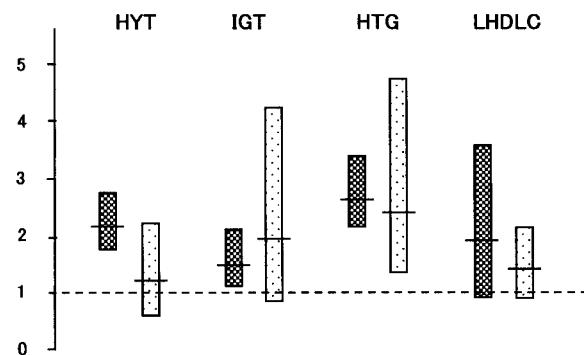
When the threshold was set at 40 U/l in men and 25 U/l in women, EGGT was found in 529 (22.1%) of the male subjects and 74 (5.3%) of the female subjects. As shown

**Table 2.** The results of multiple logistic regression analyses on possible contributors to the appearance of elevated serum GGT in 2,399 middle-aged men and 1,402 women

Contributor	Men			Women		
	Odds ratio	95% C.I. <sup>a</sup>	p <sup>b</sup>	Odds ratio	95% C.I. <sup>a</sup>	p <sup>b</sup>
Age (yr)	1.02	1.01–1.03	*	1.07	1.03–1.11	**
Alcohol consumption	4.92	3.55–6.83	**	2.30	1.21–4.36	*
Obesity	2.72	2.19–3.39	**	2.19	1.32–3.62	**
Current smoking	1.54	1.25–1.91	**	1.70	0.49–5.95	ns
Physical inactivity	1.41	1.02–1.95	*	0.73	0.33–1.59	ns

a) 95% confidence interval. b) ns: not significant ( $p \geq 0.10$ ), \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

#### Odds ratio



**Fig. 1.** Odds ratios in middle-aged men and women with elevated serum GGT ( $\geq 40$  U/l for men and  $\geq 25$  U/l for women) for hypertension (HYT), impaired glucose tolerance (IGT), hypertriglyceridemia (HTG) and low HDL-cholesterolemia (LHDLC) as compared with those with normal GGT, adjusted for age, body mass index, alcohol and cigarette consumption, and physical activity at leisure.

Bars and boxes represent odds ratios and the 95% confidence intervals, respectively. Dark boxes represent men and bright boxes represent women.

in Table 1, the prevalence of EGTT was higher in both male and female drinkers than in nondrinkers, and higher in heavier alcohol consumers. The differences were significant in both men and women. Meanwhile, EGTT was found in less than half (42.5%) of the male excessive drinkers consuming 60 ml or more of alcohol per day, showing a considerable individual difference in the serum GGT elevations even after large volume alcohol consumption.

Table 2 shows the results of multiple logistic regression analyses on possible contributors to the appearance of EGTT in the male and female subjects. The possible contributors defined here were age, obesity (BMI $\geq 25$ ),

alcohol and cigarette consumption, and physical inactivity at leisure time. In men, alcohol consumption was the strongest contributor. Drinkers had an odds ratio of 4.92 in comparison with nondrinkers, with the next strongest being obesity of 2.72. In addition, both current smoking and physical inactivity showed a small but significant contribution to the appearance of EGTT. In women, both alcohol consumption and obesity had strong effects on the appearance of EGTT, and the odds ratios were 2.30 and 2.19, respectively. Smoking and physical inactivity were, however, not significant in women. From these figures, the attributable risk percent of alcohol consumption to the appearance of EGTT was calculated as 79.7% in men and 56.5% in women.

Figure 1 illustrates the odds ratios and the 95% confidence intervals for the appearance of HYT, IGT, HTG and LHDLC in the male and female subjects showing EGTT as compared with those with normal serum GGT, after adjusting for age, BMI and the scores for alcohol and cigarette consumption and physical activity. In men, the subjects with EGTT had the odds ratios (the confidence intervals) of 2.14 (1.65–2.78), 1.43 (1.04–1.96), 2.70 (2.13–3.40) and 1.91 (0.99–3.67), respectively, in HYT, IGT, HTG and LHDLC. Except for a borderline significance in LHDLC ( $p=0.05$ ), all the odds ratios in the risk factors were significant. Women had similar findings, and the odds ratios (the confidence intervals) were 1.13 (0.58–2.19), 1.91 (0.87–4.18), 2.33 (1.18–4.62) and 1.44 (0.98–2.13), respectively, for HYT, IGT, HTG and LHDLC, although statistical significance was detected only for HTG and a borderline significance in LHDLC ( $p=0.07$ ).

It is not shown in the figure, but the odds ratios (the confidence intervals) for UBO in the male and female subjects with EGTT as compared with those with a normal GGT were calculated as 5.25 (3.11–8.85) and 1.63 (0.67–3.96), respectively, when adjustments were done for age, alcohol and cigarette consumption, and physical activity. The odds ratio in men was highly significant.

## Discussion

Upper body obesity (UBO) is a characteristic feature of the metabolic syndrome<sup>2)</sup>. The definitions of UBO adopted in the present study, i.e., men and women having a BMI of 25 or above and a waist circumference of 95 cm or more in men and 90 cm or more in women at the umbilicus level, are not established criteria<sup>4)</sup>. But, the criteria for UBO used in the U.S.<sup>23)</sup>, a waist circumference of 102 cm in men and 88 cm in women, were measured at the top of the iliac bone but not at the umbilicus level, and must be excessive particularly for Japanese men with smaller body height. On the other hand, the criteria proposed by the Japan Society for the Study of Obesity (JSSO)<sup>24)</sup>, 85 cm in men and 90 cm in women measured at the umbilicus level, the unusual lower setting in men than in women, were determined by a principle different from the U.S. criteria, i.e., the waist circumference corresponding to a visceral fat area of 100 cm<sup>2</sup> in the abdominal cavity measured by a CT technique. The validity of JSSO criteria thus remains to be evaluated in further studies with regard to the associations with coronary risk factors. The waist circumferences of 95 cm in men and 90 cm in women adopted here corresponded to the mean waist circumference in men and women having a BMI of 30<sup>4)</sup>, and thus imply a larger waist size relative to the BMI in most of the obese subjects in the present study. The prevalence of UBO was significantly lower in the male moderate drinkers than in the nondrinkers. This lower risk of UBO was not found in female drinkers, and the reasons for the gender difference remain unknown.

The associations of alcohol consumption and coronary risk factors found in the male subjects were in accordance with the previous findings<sup>11, 12)</sup>, i.e., increases in alcohol consumption were significantly related to increases in hypertension (HYT) whereas the prevalence of low HDL-cholesterolemia (LHDLC) was decreased with an increase in alcohol consumption. The odds ratio for HYT in the male moderate drinkers relative to nondrinkers was estimated as 1.2 but was not significant, but that for LHDLC was nearly half and highly significant. The odds ratios in the male moderate drinkers were not significant for impaired glucose tolerance (IGT) and hypertriglyceridemia (HTG). And the odds ratios for all the coronary risk factors in the female drinkers relative to nondrinkers were not significant. These results showed that moderate alcohol consumption, less than 60 ml of alcohol consumed per day, had neither a positive nor negative association with the appearance of coronary risk factors except for the low risk of LHDLC. Moderate alcohol consumption may thus be beneficial for the suppression of the risks of UBO and LHDLC, particularly in men, but not so for the other coronary risk factors of HYT, IGT and HTG, and therefore not so for the

development of the metabolic syndrome.

On the other hand, even after adjusting for alcohol consumption, together with age, BMI, cigarette consumption and physical activity, the odds ratios for HYP, IGT and HTG in the male subjects showing EGTT were significantly higher than in those with normal GGT. Even the high odds ratio for LHDLC in men with EGTT was borderline significant. Women with EGTT had a significantly high odds ratio in HTG and a borderline significantly one in LHDLC, although the odds ratios for HYT and IGT were above 1.0 but not significant in the women. The odds ratio for UBO in men with EGTT was as high as 5.25 after adjusting for age, alcohol and cigarette consumption, and physical activity. These results strongly suggested an association between elevations of serum GGT and the appearance of coronary risk factors characteristic of the metabolic syndrome.

These results, as well as those obtained in previous studies in Japanese and Finnish populations<sup>20-22)</sup>, showed that the association between serum GGT and the coronary risk factors was independent of alcohol consumption. Nevertheless, it should be noted that nearly 80% and 55% of EGTT in the male and female subjects was attributable to alcohol consumption. Therefore, although a considerable individual difference exists in serum GGT elevations after alcohol consumption, and obesity is also a strong contributor to the elevations of serum GGT, and even smoking<sup>25)</sup> and physical inactivity<sup>26)</sup> contributes to it, alcohol consumption is undoubtedly the major cause of serum GGT increase<sup>27, 28)</sup> in this population, particularly in men. EGTT was found in nearly half of the male excessive drinkers consuming 60 ml or more of alcohol per day, but was found even in 20% of the male and 10% of the female moderate drinkers consuming less alcohol.

Summing up these study results, it can be said that moderate alcohol consumption is not beneficial for the suppression of the metabolic syndrome, but that it will increase the risk of the development of the metabolic syndrome in drinkers who show elevations of serum GGT, although considerable limitations exist in interpreting the study results. First, cross-sectional observations cannot provide any evidence of causal associations. Second, the effects of possible alcohol moderation in the subjects who had been detected by physicians or nurses to have coronary risk factors were not evaluated in the present study, and which might have blurred the association between alcohol consumption and the prevalence of coronary risk factors. Third, the appearance of coronary risk factors but not that of the metabolic syndrome itself was analyzed in this study, because of some technical difficulties in confirming the definition of the metabolic syndrome in the present subjects. All those limitations require further studies on the association between alcohol consumption, serum GGT elevations and the development of metabolic syndrome, particularly in follow-up or

interventional designs.

Furthermore, details of the biological link between increase in serum GGT and the development of multiple coronary risk factors also remain unclear. High serum GGT was often found in both alcohol consumers and obese people, and thus it may reflect the progression of hepatic manifestation common in them, probably hepatic steatosis<sup>29,30</sup>. Hepatic steatosis has been suggested to play an important role in the development of insulin resistance<sup>31</sup>, and thus the metabolic syndrome related to upper body obesity<sup>32</sup>. Further studies are also required to clarify the biological link between elevations of serum GGT, the progression of hepatic steatosis, and increase in insulin resistance in alcohol consumers and obese people.

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## Association Between Serum Gamma-Glutamyltransferase Level and Prehypertension Among US Adults

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**Background** Higher serum gamma-glutamyltransferase (GGT) levels, a marker of oxidative stress, are implicated in the development and progression of hypertension; however, data from non-Caucasian ethnicities are limited. Also, currently there is little data available on the association between serum GGT level and clinically relevant blood pressure (BP) categories earlier in the disease continuum, when hypertension prevention efforts may be applicable. The association between serum GGT and prehypertension was examined in a nationally representative sample of US adults.

**Methods and Results** Cross-sectional study among 5,827 National Health and Nutrition Examination Survey 1999–2002 participants aged ≥18 years without cardiovascular disease (CVD) and hypertension. The main outcome-of-interest was the presence of prehypertension (systolic BP 120–139 mmHg or diastolic BP 80–89 mmHg) (n=2,269). Higher serum GGT levels were positively associated with prehypertension, independent of smoking, waist circumference, diabetes, cholesterol levels and other confounders. The multivariable odds ratio (95% confidence intervals) comparing quartile 4 of GGT (>29 U/L) to quartile 1 (<13 U/L) was 1.84 (1.37–2.46), p<0.0001. This association persisted in separate analyses among men and women. The results were consistent in subgroup analyses by race-ethnicity, age, smoking, alcohol intake, body mass index, waist circumference and diabetes. In non-parametric models, the positive association between serum GGT and prehypertension appeared to be present across the full range of GGT, without any threshold effect.

**Conclusions** Higher serum GGT levels are associated with prehypertension in a nationally representative sample of US adults, free of CVD and hypertension. (Circ J 2007; 71: 1567–1572)

**Key Words:** GGT; Hypertension; NHANES; Prehypertension

**G**amma-glutamyltransferase (GGT) is present in serum and the surface of most cell types, and is the enzyme responsible for initiating extracellular catabolism of glutathione, the main antioxidant in mammalian cells.<sup>1</sup> Increased GGT activity may be a response to oxidative stress, which can increase the transport of glutathione precursors into cells.<sup>1,2</sup> Recent reports also indicate a direct role for GGT in the generation of reactive oxygen species.<sup>2–6</sup> In this context, emerging evidence from epidemiological studies indicates that GGT may have a role in the pathogenesis of cardiovascular disease, diabetes mellitus and metabolic syndrome.<sup>7–9</sup> Similarly, recent cross-sectional and longitudinal studies have also noted a relatively independent association between elevated serum GGT levels and hypertension.<sup>9–14</sup> However, for hypertension, with the exception of recent results from the biracial Coronary Artery Risk Development in Young Adults (CARDIA) Study, data from non-Caucasian race-ethnicities in the USA are limited.<sup>9</sup> Also, in light of the overall positive association between serum GGT and clinical hypertension reported in previous epidemiological studies, it is not entirely clear if there is a continuous dose–response relationship

in this association or if this association is evident only beyond a particular threshold level of serum GGT. Further, currently there is little data available on the association between serum GGT level and clinically relevant blood pressure (BP) categories earlier in the disease continuum when hypertension prevention efforts may be applicable. Prehypertension, as defined by the Seventh Joint National Committee (JNC7) on prevention, detection, evaluation and treatment of high blood pressure and including those with systolic BP ranging from 120–139 mmHg or diastolic BP ranging from 80–89 mmHg, is identified as a predictor for developing hypertension and a stage where primary prevention of hypertension is possible.<sup>15–17</sup> In this context, we examined the association between serum GGT levels and prehypertension in a nationally representative sample of US adults, who were free of hypertension, participating in the National Health and Nutrition Examination Survey (NHANES) 1999–2002, after adjusting for several important confounders. We also employed non-parametric analytical techniques to examine the dose–response nature of the association between serum GGT levels and prehypertension graphically.

### Methods

#### Study Participants

The NHANES 1999–2002 was a nationally representative sample of the United States of America's non-institutionalized, civilian population. The procedures involved in NHANES 1999–2002 have been published in detail and are available online.<sup>18,19</sup> In brief, the NHANES study included a

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stratified multistage probability sample based on selection of counties, blocks, households and individuals within households and included the oversampling of non-Hispanic blacks and Mexican Americans to provide stable estimates for these groups. Subjects signed a consent form, and approval was obtained from the Human Subjects Committee in the US Department of Health and Human Service.

Overall, 9,836 adults  $\geq 18$  years of age participated in the interview and examination components of NHANES 1999–2002. Of these participants, systolic and diastolic BP was available for 9,483 participants (96%). We further excluded (not mutually exclusive categories) participants with prevalent hypertension ( $n=3,109$ ), participants with missing covariable data (eg, serum total cholesterol) ( $n=578$ ) and participants with self-reported history of cardiovascular disease ( $n=891$ ), including coronary heart disease, myocardial infarction, angina or stroke. This resulted in 5,827 normotensive participants who were included for all analyses. Out of the 5,827 normotensive participants, 2,269 had prehypertension.

#### *Main Outcome of Interest: Presence of Prehypertension*

Seated systolic and diastolic BPs were measured using a mercury sphygmomanometer according to the American Heart Association and JNC7 recommendations.<sup>15,18</sup> Up to 3 measurements were averaged for systolic and diastolic pressures. Patients were considered hypertensive if they reported current BP-reducing medication use and/or had systolic BPs  $\geq 140$  mmHg and/or diastolic BPs  $\geq 90$  mmHg.<sup>15</sup> In the current analysis, we excluded participants with hypertension. The preferred outcome of interest in the current study was the presence of prehypertension, defined as systolic BP 120–139 mmHg systolic or diastolic BP 80–89 mmHg based on JNC7 criteria.<sup>15</sup>

#### *Exposure Measurements*

Age, gender, race/ethnicity, smoking status, alcohol intake (g/day), level of education, history of diabetes and oral hypoglycemic drug intake or insulin administration, hypertension and antihypertensive medication were assessed using a questionnaire. Individuals who had not smoked  $\geq 100$  cigarettes in their lifetimes were considered never smokers, those who had smoked  $\geq 100$  cigarettes in their lifetimes were considered former smokers if they answered negatively to the question “Do you smoke now?” and current smokers if they answered affirmatively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. After asking the participant to lift up their shirt, waist circumference was measured at the iliac crest to the nearest 0.1 cm.

Detailed descriptions about blood collection and processing are provided in the NHANES Laboratory/Medical Technologists Procedures Manual.<sup>18</sup> Serum GGT concentration was assayed with a Hitachi 737 Analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, IN, USA) at White Sands Research Center, Alamogordo, New Mexico (USA); details of laboratory measurements are available online.<sup>18</sup> Serum total cholesterol was measured enzymatically. Serum glucose was measured at the University of Missouri Diabetes Diagnostic Laboratory using a modified hexokinase enzymatic method. Glycosylated hemoglobin was also measured at the University of Missouri using a boronate affinity high-performance liquid chromatography system. Diabetes was defined using American Diabetes Association criteria as follows: a serum glucose  $\geq 126$  mg/dl after fasting

for a minimum of 8 h, a serum glucose  $\geq 200$  mg/dl for those who fasted  $<8$  h before their NHANES visit, or self-reported current use of oral hypoglycemic medication or insulin.

#### *Statistical Analysis*

Because of their skewed distributions, serum GGT was log-transformed (base 2) when initially analyzed as a continuous variable. We examined serum GGT level as quartiles: <13 U/L, 13–19 U/L, 20–29 U/L and >29 U/L. Initial analyses based on gender-specific GGT quartiles gave similar results as GGT quartiles for the whole cohort; cutoffs for the whole cohort were therefore used to simplify the presented tables. The odds ratio (OR) (95% confidence interval (CI)) of prehypertension was calculated for each GGT level, with the lowest quartile as the reference, using multi-variable logistic regression models. We used 2 models: the age (years), sex-adjusted model; and the multivariable model additionally adjusted for race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (g/day), waist circumference (cm), diabetes (absent, present), glycosylated hemoglobin level (%) and serum cholesterol (mg/dl). Trends in the OR of prehypertension across increasing serum GGT category were determined modeling GGT categories as an ordinal variable. To examine the consistency of the observed association between serum GGT levels and prehypertension, we performed subgroup analyses by gender, race-ethnicity (Non-Hispanic whites, African Americans, Mexican-Americans and others), age ( $<60$ ,  $\geq 60$  years), current smoking (absent, present), current drinking (absent, present), BMI ( $<25$ ,  $\geq 25$  kg/m $^2$ ), waist circumference (low [men  $<102$  cm, women  $<88$  cm], high [men  $\geq 102$  cm, women  $\geq 88$  cm]), and diabetes mellitus (absent, present).<sup>20</sup> In a supplementary analysis, to examine if the observed association between serum GGT and prehypertension was explained by inflammation, we additionally adjusted for C-reactive protein levels (mg/L) in the multivariable model. Sample weights that account for the unequal probabilities of selection, oversampling and non-response were applied for all analyses using SUDAAN (version 8.0; Research Triangle Institute, Research Triangle Park, NC, USA) and SAS (version 9.2.; SAS institute, Cary, NC, USA) softwares; standard errors were estimated using the Taylor series linearization method. To examine the dose–response relationship between the observed association between GGT levels and prehypertension without linearity assumptions, we used flexible non-parametric logistic regression employing the generalized additive modeling approach (R system for statistical computing, available from Comprehensive R Archive Network [<http://www.CRAN.R-project.org>]) to calculate the odds of prehypertension, adjusting for all covariates in the multivariable model. The odds of prehypertension were then plotted against increasing GGT levels (both on the log scale).<sup>21</sup>

## **Results**

Among 5,827 adults  $\geq 18$  years of age, without hypertension and cardiovascular disease included in the current analysis, 2,269 subjects had prehypertension. Table 1 presents the characteristics of NHANES population by serum GGT quartiles. Subjects with higher serum GGT levels were more likely to be: older, Non-Hispanic black, Mexican American, smokers (current and ever), diabetic; to have:

**Table 1 Characteristics of the Study Population by Categories of Serum GGT Levels\***

Characteristics	Serum GGT quartiles				p value <sup>†</sup>
	Quartile 1 (<13 U/L)	Quartile 2 (13–19 U/L)	Quartile 3 (19–29 U/L)	Quartile 4 (>29 U/L)	
No. at risk	1,480	1,610	1,343	1,394	
Age, years	32.7±14.6	36.0±16.9	39.1±16.6	40.9±15.0	<0.0001
Women, %	84.7±0.01	57.1±0.01	40.1±0.01	31.4±0.01	<0.001
Race-ethnicity, %*					
Non-Hispanic whites	50.4±1.2	48.3±1.2	40.8±1.2	38.5±1.2	<0.0001
Non-Hispanic blacks	11.5±0.8	16.0±0.8	21.8±1.0	18.8±0.9	<0.0001
Mexican Americans	27.5±1.1	26.0±1.1	28.2±1.1	34.0±1.2	<0.0001
Others	10.5±0.7	9.6±0.7	9.0±0.7	8.5±0.7	0.2967
Education categories, %*					
Below high school	28.1±1.1	30.0±1.1	34.4±1.2	37.6±1.2	<0.0001
High school	24.5±1.1	25.0±1.1	23.0±1.1	23.8±1.1	0.5976
Above high school	47.2±1.2	44.7±1.2	42.4±1.2	38.3±1.2	<0.0001
Smoking, %*					
Never smoker	71.3±1.1	67.0±1.1	56.2±1.2	49.0±1.2	<0.0001
Ever smoker	28.7±1.1	33.0±1.1	43.8±1.2	51.0±1.2	<0.0001
Current smoker	13.1±0.8	17.3±0.9	23.7±1.0	30.0±1.1	<0.0001
Alcohol intake, g/day	4.4±20.5	7.9±32.1	11.0±33.5	17.0±46.9	<0.0001
Body mass index, kg/m <sup>2</sup>	25.6±5.3	26.1±5.8	27.8±5.8	28.9±6.0	<0.0001
Waist circumference, cm	87.8±13.6	89.3±14.08	94.6±14.3	98.9±14.9	<0.0001
Diabetes, %*	1.7±0.3	2.0±0.3	5.1±0.5	7.4±0.6	<0.0001
Glycosylated hemoglobin, %	5.1±0.5	5.2±0.6	5.4±0.9	5.5±1.1	<0.0001
Total cholesterol, mg/dl	189.5±45.9	185.9±38.3	194.6±40.8	204.2±41.1	<0.0001
C-reactive protein, mg/dl	0.3±0.5	0.3±0.6	0.3±0.5	0.4±0.9	<0.0001
Prehypertension, %*	21.5±1.1	35.1±1.2	46.1±1.2	54.8±1.2	<0.0001
Systolic blood pressure, mmHg	110.0±10.7	114.0±10.6	116.6±10.8	118.5±10.3	<0.0001
Diastolic blood pressure, mmHg	65.0±11.5	67.9±10.6	69.7±10.6	71.2±11.1	<0.0001

\*Data presented are row percentages or mean values and corresponding standard error, based on 5,827 normotensive participants ≥18 years participating in the National Health and Nutrition Examination Survey 1999–2000, USA.

<sup>†</sup>p estimated from linear regression or logistic regression models, as appropriate, with GGT categories as an ordinal variable. GGT, gamma-glutamyltransferase.

**Table 2 Association Between Serum GGT Levels and Prehypertension**

Serum GGT quartiles	No. at risk (n=5,827)	Prehypertension cases (n=2,269)	Age, sex- adjusted OR (95%CI)	Multivariable- adjusted OR (95%CI)*
Quartile 1 (<13 U/L)	1,480	319	1 (referent)	1 (referent)
Quartile 2 (13–19 U/L)	1,610	566	1.60 (1.26, 2.02)	1.28 (1.03, 1.59)
Quartile 3 (19–29 U/L)	1,343	620	2.27 (1.75, 2.95)	1.50 (1.16, 1.95)
Quartile 4 (>29 U/L)	1,394	764	3.28 (2.50, 4.31)	1.84 (1.37, 2.46)
P value			<0.0001	<0.0001
Log-transformed serum GGT, U/L	5,827	2,269	1.57 (1.41, 1.74)	1.39 (1.24, 1.56)

\*Adjusted for age (years), sex (male, female), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (g/day), waist circumference (cm), diabetes (absent, present), glycosylated hemoglobin level (%), and serum cholesterol (mg/dl); based on 5,827 normotensive participants ≥18 years participating in the National Health and Nutrition Examination Survey 1999–2000, USA.

OR, odds ratio; CI, confidence interval. Other abbreviation see in Table 1.

**Table 3 Association Between Increasing Serum GGT Levels and Prehypertension, by Gender**

Serum GGT quartiles	Men (n=2,756)		Women (n=3,071)	
	No. at risk (prehypertension cases)	Multivariable OR (95%CI)	No. at risk (prehypertension cases)	Multivariable OR (95%CI)
Quartile 1 (<13 U/L)	273 (102)	1 (referent)	1,207 (217)	1 (referent)
Quartile 2 (13–19 U/L)	722 (316)	1.24 (0.92, 1.69)	888 (250)	1.33 (0.87, 2.02)
Quartile 3 (19–29 U/L)	805 (424)	1.28 (0.86, 1.91)	538 (196)	1.71 (1.14, 2.57)
Quartile 4 (>29 U/L)	956 (585)	1.92 (1.31, 2.80)	438 (179)	1.70 (1.07, 2.70)
P value		0.0019		0.0293
Log-transformed serum GGT, U/L	2,756 (1,427)	1.42 (1.14, 1.77)	3,071 (842)	1.23 (1.01, 1.49)

\*Adjusted for age (years), sex (male, female), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (g/day), waist circumference (cm), diabetes (absent, present), glycosylated hemoglobin level (%), and serum cholesterol (mg/dl); based on 5,827 normotensive participants ≥18 years participating in the National Health and Nutrition Examination Survey 1999–2000, USA.

Abbreviations see in Tables 1,2.

**Table 4** Association Between Serum GGT Level and Prehypertension, Within Selected Subgroups

Stratified subgroups	No. at risk	Prehypertension cases	Multivariable OR (95%CI) of prehypertension associated with log-transformed GGT, U/L	P-interaction
<i>Race-ethnicity</i>				
Non-Hispanic whites	2,639	1,070	1.37 (1.18, 1.59)	0.42
Non-Hispanic blacks	1,002	420	1.24 (0.98, 1.55)	
Mexican Americans/others	2,186	779	1.59 (1.36, 1.86)	
<i>Age</i>				
<60 years	5,125	1,781	1.43 (1.26, 1.61)	0.31
≥60 years	702	488	1.20 (0.89, 1.61)	
<i>Current smoking</i>				
Absent	4,615	1,753	1.36 (1.20, 1.54)	0.62
Present	1,212	516	1.43 (1.21, 1.71)	
<i>Current drinker</i>				
Absent	4,311	1,593	1.32 (1.17, 1.49)	0.62
Present	1,516	676	1.41 (1.18, 1.69)	
<i>Body mass index</i>				
<25 kg/m <sup>2</sup>	2,407	741	1.21 (0.94, 1.56)	0.27
≥25 kg/m <sup>2</sup>	3,420	1,528	1.61 (1.39, 1.86)	
<i>Waist circumference, cm</i>				
Low (men <102 cm, women <88 cm)	2,407	741	1.24 (1.04, 1.47)	0.31
High (men ≥102 cm, women ≥88 cm)	3,420	1,528	1.50 (1.27, 1.77)	
<i>Diabetes mellitus</i>				
Absent	5,595	2,116	1.39 (1.23, 1.57)	0.67
Present	232	153	1.23 (0.74, 2.05)	

\*OR (95%CI) adjusted for age (years), sex (male, female), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (g/day), waist circumference (cm), diabetes (absent, present), glycosylated hemoglobin level (%), and serum cholesterol (mg/dl); based on 5,827 normotensive participants ≥18 years participating in the National Health and Nutrition Examination Survey 1999–2000, USA.

Abbreviations see in Tables 1,2.

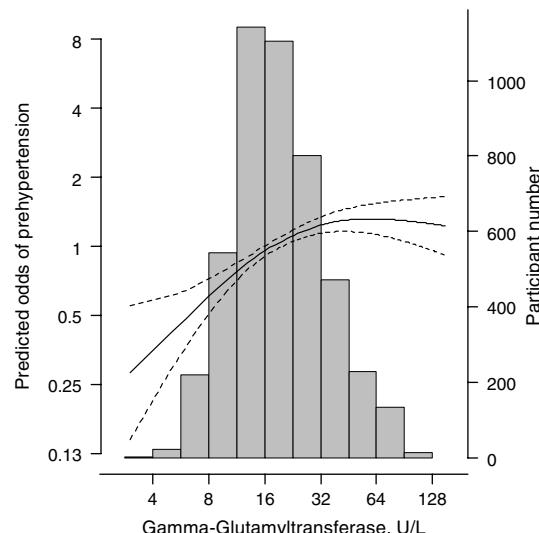


Fig 1. Multivariable-adjusted odds of prehypertension according to serum gamma-glutamyltransferase level (U/L). Solid thick line represents the predicted odds of prehypertension from nonparametric logistic regression; dashed lines, 95% confidence limits for the nonparametric logistic regression estimates. The non-parametric logistic regression was adjusted for age (years), sex (male, female), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (g/day), waist circumference (cm), diabetes (absent, present), glycosylated hemoglobin level (%) and serum cholesterol (mg/dl). Data is based on 5,827 normotensive participants ≥18 years participating in the National Health and Nutrition Examination Survey 1999–2000, USA. X axis: serum gamma-glutamyltransferase level (U/L) plotted in log scale. Y1 axis: predicted odds of prehypertension plotted in log scale. Y2 axis: participant number for each serum gamma-glutamyltransferase level.

consumed more grams of alcohol/day, a higher BMI, a higher waist circumference, higher glycosylated hemoglobin levels, total cholesterol, and C-reactive protein levels; and to be less likely to have a post-high school education than those with lower serum GGT. Mean systolic and diastolic BP and the prevalence of prehypertension increased with increasing GGT categories.

Table 2 presents the ORs of prehypertension with increasing serum GGT quartile. Increasing GGT quartiles were positively associated with prehypertension in both the age, sex-adjusted and multivariable-adjusted models; models evaluating trends in this association were also statistically significant. When serum GGT was analyzed as a continuous variable, the positive association with prehypertension persisted. In Table 3, we present the gender-specific analysis for the association between increasing GGT levels and prehypertension. A clear positive association between GGT and prehypertension was present both among men and women.

In Table 4, we examined the OR of prehypertension associated with increasing levels of log-transformed serum GGT within subgroups of race-ethnicity, age, current smoking, current drinking, BMI, waist circumference and diabetes mellitus. In general, the positive association between higher GGT level and prehypertension was consistently present within these subgroups also and the OR estimates in Table 4 ranged from 1.20 to 1.61.

We then employed non-parametric models to examine if the observed positive association between serum GGT and prehypertension was present across the full range of GGT levels available in the present study (Fig 1). Among the adults without clinical hypertension examined in the present study, overall, there appeared to be a continuous association between serum GGT and prehypertension with

increasing GGT levels; there was no evidence of any threshold effect. On closer examination, the dose-response association between serum GGT and prehypertension appeared to be most evident at serum GGT levels within normal limits (approximately <55 g/L).

In a supplementary analysis, to examine if the observed association between serum GGT and prehypertension was explained by inflammation, we additionally adjusted for C-reactive protein levels (mg/dL) in the multivariable model in Table 2. The results were essentially similar. Compared to serum GGT quartile 1 (referent), the OR (95% CI) of prehypertension was 1.55 (1.22–1.97) in quartile 2, 1.90 (1.44–2.49) in quartile 3 and 2.58 (1.92–3.48) in quartile 4;  $p<0.0001$ . In a second supplementary analysis, we examined the association between quartiles of serum GGT and hypertension. Consistent with the results for prehypertension, we observed a dose-dependent, positive association between increasing serum GGT and hypertension. Compared to subjects in serum GGT quartile 1 (referent), the multivariable OR (95% CI) of hypertension was 1.14 (0.94–1.38) in quartile 2, 1.37 (1.12–1.67) in quartile 3 and 1.78 (1.37–2.30), in quartile 4;  $p<0.0001$ .

## Discussion

Higher serum GGT levels were found to be positively associated with prehypertension in a representative sample of US adults, free of hypertension and cardiovascular disease. This association persisted after adjusting for age, sex, race-ethnicity, waist circumference, smoking, alcohol intake, diabetes mellitus, glycosylated hemoglobin levels and serum cholesterol, and was consistently present in subgroup analysis by gender and important confounders. The OR of prehypertension increased in a dose-dependent manner with increasing quartiles of serum GGT. In a subsequent analysis, employing nonparametric models, the observed positive association between serum GGT quartiles and prehypertension was present continuously across the full range of GGT. Our results are in agreement with the current understanding of the role of GGT in hypertension development, and further contribute to the current literature by: (1) suggesting that GGT levels are related to clinically relevant BP stages even earlier in the disease continuum, including prehypertension when primary prevention is possible; and (2) demonstrating the association between GGT and prehypertension among major race-ethnicities in the USA.<sup>9,13,14,17,22</sup>

Our finding of a positive association between higher serum GGT level and prehypertension shows high internal validity, as shown by the magnitude of this association; independence from related factors such as smoking, alcohol intake, waist circumference and diabetes mellitus; dose-response trend in nonparametric models; and the consistency of this association in subgroup analyses by gender, race and several other factors. In nonparametric models, the dose-response relation between GGT and prehypertension appeared to be most evident at serum GGT levels within normal limits (<55 g/L), a finding consistent with previous reports looking at hypertension.<sup>14</sup> In the current study, the observed association between serum GGT and prehypertension was present among non-drinkers also and persisted after adjusting for grams of alcohol intake among current drinkers. As serum GGT is also a marker of alcohol intake, these findings are consistent with the hypothesis of an association with prehypertension independent of alcohol intake! These results are also in agreement with previous cross-

sectional and longitudinal epidemiologic studies that reported a positive association between higher serum GGT level and clinical hypertension, and extend the evidence to the earlier stage of prehypertension, when primary prevention of hypertension is possible.<sup>9–14,17</sup>

Several lines of recent evidence suggest that an association between serum GGT and hypertension is plausible, including a direct role of GGT in the generation of reactive oxygen species; an indirect role as a marker of increased extracellular catabolism of antioxidant glutathione in response to oxidative stress; its predictive relationship to future elevations in plasma F2-isoprostanes, an oxidative damage product of arachidonic acid; its relationship to markers of inflammation; and its relationship to insulin resistance and components of the metabolic syndrome!<sup>1–6,8,9,23,24</sup> Also, serum GGT has been shown to be associated with reduced kidney function among men without hypertension or diabetes.<sup>25</sup>

As the current study examined a nationally representative sample of US adults, these results are generalizable to US adults. Furthermore, all data were collected following rigorous methodology, including a study protocol with quality control checks as discussed in the NHANES website.<sup>18,19</sup> The main study limitation is the cross-sectional nature of NHANES, which precludes conclusions regarding the temporal nature of the association between serum GGT and prehypertension.

In conclusion, higher serum GGT levels were found to be positively associated with prehypertension in a representative sample of US adults. Approximately 31% of US adults are reported to have prehypertension, a stage with higher risk of converting to clinical hypertension, but when primary prevention is still possible!<sup>16,17,26</sup> In the light of our findings, a corollary observation is that subjects with prehypertension may be a good target group for future hypertension prevention trials that aim to reduce serum GGT levels through interventions based on nutritional or lifestyle factors shown to be inversely associated with serum GGT in observational studies!<sup>1,27</sup>

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## Serum gamma-glutamyl transferase: A novel biomarker for coronary artery disease

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**Background:** Atherosclerosis is a chronic inflammatory process, in which oxidative stress is the key event. Gamma-glutamyl transferase (GGT) is a cellular production of oxidants. We aimed to elucidate the relationship of serum GGT levels and coronary artery disease (CAD) in a Chinese population.

**Material/Methods:** A total of 513 adult subjects who had undergone coronary angiography were enrolled in the study. Clinical characteristics, coronary angiography, and serum samples were collected from all the patients and analyzed for the serum GGT, blood lipids, and cardiovascular risk factors.

**Results:** Subjects with CAD had significantly increased activity of serum GGT ( $p=0.003$ ). Serum GGT levels exhibited positive correlations with alcohol intake ( $\beta=0.177$ ,  $p<0.001$ ), coronary complexity ( $\beta=0.068$ ,  $p<0.001$ ), and triacylglycerol ( $\beta=0.058$ ,  $p<0.001$ ). High-density lipoprotein cholesterol levels ( $\beta=0.157$ ,  $p=0.008$ ) and age ( $\beta=0.004$ ,  $p=0.002$ ) were negatively correlated with serum GGT in the CAD group. The coronary complexity presented a negative correlation with Ig-apolipoprotein AI ( $\beta=-2.517$ ,  $p=0.001$ ) and positive correlations with smoking ( $\beta=0.640$ ,  $p<0.001$ ), Ig-GGT ( $\beta=0.613$ ,  $p=0.004$ ), Ig high sensitivity-C reactive protein ( $\beta=0.320$ ,  $p<0.001$ ), and hypertension ( $\beta=0.286$ ,  $p<0.026$ ).

**Conclusions:** The study showed a positive correlation between serum GGT and CAD in a Chinese population. Serum GGT levels may be a potential biomarker for CAD.

**MeSH Keywords:** **Cardiovascular Diagnostic Technique • Coronary Artery Disease • gamma-Glutamyl Transpeptidase**

**Abbreviations:**

**ALT** – alanine aminotransferase; **ApoAI** – apolipoprotein AI; **ApoB** – apolipoprotein B;  
**CAD** – coronary artery disease; **GGT** – gamma-glutamyl transferase; **HDL-C** – high-density lipoprotein cholesterol; **HsCRP** – high-sensitivity C reactive protein; **LDL-C** – low-density lipoprotein cholesterol; **Lp(a)** – lipoprotein (a); **TC** – total cholesterol; **TG** – triacylglycerol

**Full-text PDF:** <http://www.medscimonit.com/download/index/idArt/890245>

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## Background

Coronary artery disease (CAD) is a worldwide public health problem with high morbidity and mortality, which involves a chronic inflammatory atherosclerosis process with stable CAD and acute coronary syndromes [1,2]. Various mechanisms are associated with the pathogenesis of CAD in which oxidation and inflammation play significant roles [3]. Serum gamma-glutamyl transferase (GGT) has been widely used as a diagnostic index of liver dysfunction [4]. However, in recent years, a number of epidemiological studies showed that serum GGT was positively associated with cardiovascular mortality, myocardial infarction, stroke, high blood pressure, diabetes, and even cancer [5], suggesting that serum GGT may participate in oxidative and inflammatory reactions. In this study, we aimed to investigate the effect of serum GGT activity on oxidation stress and its diagnostic performance for CAD in a Chinese population.

## Material and Methods

### Study population

We retrospectively evaluated adult patients who had undergone coronary angiography between 2004 and 2010 in Shanghai Tongji Hospital, China. We excluded patients with any of the following: 1) severe liver disease or kidney disease; 2) severe infections or heart failure; 3) hyperthyroidism, hypothyroidism, cancer, autoimmune diseases, or chronic connective tissue disease; 4) lipid lowering drugs taken for nearly two months; 5) major surgery, trauma, or burns. A total of 513 subjects were finally enrolled in the study, which consisted of 365 participants with CAD (CAD group) and 148 normal participants (control group). The CAD group was then divided into 3 sub-groups: single-vessel disease ( $n=114$ ), double-vessel disease ( $n=121$ ), and triple-vessel disease ( $n=130$ ). The study protocol was approved by the local ethics committee of Shanghai Tongji Hospital.

All subjects were asked to provide information about cardiovascular risk factors such as hypertension or diabetes mellitus, smoking, and details regarding medication administration received before the admission. Hypertension was defined as a systolic blood pressure  $>140$  mmHg and/or diastolic blood pressure  $>90$  mmHg in resting state, or self-reported use of an antihypertensive drug [6]. Subjects were considered to have diabetes if they had been informed of the diagnosis by a physician, were taking oral anti-hyperglycemic agents or insulin, or were receiving diet therapy, and those who presented with fasting serum glucose of more than 7.0 mmol/L or above on 2 measurements during hospitalization [7]. Subjects were considered as smokers if they smoked at the time of admission or reported cessation  $<6$  months before [8]. Subjects were considered as excessive drinkers if men drank  $>40$  g alcohol per day and women  $>20$  g [9].

### Biochemical analysis

A venous blood specimen (5 mL) was collected at 6:00 a.m. to 7:00 a.m. 1 day before angiography, after an overnight fast. Serum was separated by centrifugation at  $1500 \times g$  for 15 min. All laboratory analysis were performed at the department of Chemical Pathology and standard techniques were used to evaluate triacylglycerol, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein, apolipoprotein AI, apolipoprotein B, lipoprotein (a) and alanine aminotransferase, and high-sensitivity C-reactive protein levels. Serum GGT levels were measured spectrophotometrically with the American Beckman DXC800 automatic biochemical analyzer. Reference range for GGT was 0-40 U/L.

### Coronary angiography

After preoperative preparation, coronary angiography was performed by vascular medicine physicians through the femoral artery or radial artery, using the Judkins method, with 2 projections for the left coronary artery and 4 projections for the right coronary artery, adding extra projections if necessary. Evaluation criteria were according to ACC/AHA Guidelines for Coronary Angiography [10]. Angiographic results were judged by 2 senior physician specialists observing images together according to: the degree of coronary artery stenosis (by comparing the area of narrowing to an adjacent normal segment), percentage reduction of normal vessels, and lesion diameter  $\geq 50\%$  of the normal vessel diameter. When angiographic results involved left anterior descending artery, left circumflex artery, or right coronary artery, branches were divided into single, double, or triple vessel disease. When the left main coronary artery was involved, left anterior descending and left circumflex artery were also calculated.

### Statistical analysis

All data was analyzed by SPSS 14.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables are expressed as mean  $\pm$  standard deviation. The differences between normally distributed numeric variables were evaluated by Student's *t*-test or one-way ANOVA, while non-normally distributed variables were analyzed by Mann-Whitney U test or Kruskal-Wallis variance analysis, as appropriate. Chi-square test was employed for the comparison of categorical variables. Multivariate analysis used multivariate stepwise regression.  $P<0.05$  was considered as statistical significance.

## Results

According to coronary angiography, 114 subjects (22.2%) were diagnosed with single-vessel disease, 121 subjects (23.6%)

**Table 1.** Baseline characteristics of clinic, angiography and laboratory.

	Coronary complexity				P value
	0 (n=148)	1 (n=114)	2 (n=121)	3 (n=130)	
Male	63 (42.6%)	96 (84.2%)	94 (77.7%)	99 (76.2%)	<0.001
Age	56.0±9.14	57.5±12.1	60.6±12.0	64.1±12.1	0.423
Hypertension (%)	22 (24.86%)	50 (20.16%)	70 (28.22%)	128 (51.63%)	<0.001
Diabetes (%)	20 (13.5%)	23 (20.2%)	29 (24.0%)	44 (33.8%)	<0.001
Alcohol intake (%)	22 (14.9%)	29 (25.4%)	20 (16.5%)	19 (14.6%)	0.090
Smoking (%)	35 (23.6%)	61 (53.5%)	71 (58.7%)	76 (58.5%)	<0.001
TC (mmol/L)	4.71±0.89	4.61±0.93	4.73±1.12	4.66±1.24	0.823
TG (mmol/L)	1.66±1.12	1.60±0.95	1.85±1.49	1.79±1.30	0.392
LDL-C (mmol/L)	2.89±0.75	2.94±0.85	3.01±0.99	3.02±0.99	0.549
HDL-C (mmol/L)	1.09±0.30	1.01±0.33	0.97±0.31	0.92±0.26	<0.001
ApoAI (mg/L)	1.30 (1.15~1.44)	1.14 (1.03~1.3)	1.16 (1.0~1.34)	1.14 (1.03~1.33)	<0.001
ApoB (mg/L)	0.82 (0.69~0.99)	0.88 (0.76~1.0)	0.83 (0.69~1.04)	0.83 (0.71~1.02)	0.058
Lp(a) (mg/L)	160 (101~322)	187 (91~332)	187 (65~302)	167 (58~304)	0.548
HsCRP (mg/L)	2.05 (1~8)	8 (1.65~29)	7 (2~25.9)	8.85 (3.7~23.8)	<0.001
ALT (U/L)	18.5 (13.8~27.3)	32.5 (18~57.5)	34.5 (18.3~54.5)	32 (23~50)	<0.001
GGT (U/L)	22 (15~37)	23 (16~49)	24 (17~47)	30 (20~41.5)	0.003

ALT – alanine aminotransferase; ApoAI – apolipoprotein AI – ApoB, apolipoprotein B; GGT – gamma-glutamyl transferase; HDL-C – high-density lipoprotein cholesterol; HsCRP – high sensitivity C reactive protein; LDL-C – low-density lipoprotein cholesterol; Lp(a) – lipoprotein (a); TC – total cholesterol; TG – triacylglycerol.

with double-vessel disease, 130 subjects (25.3%) with triple-vessel disease, and 148 subjects (28.8%) were normal. Clinical, angiography, and laboratory baseline characteristics are summarized in Table 1. Subjects with triple-vessel disease in the tCAD group had statistically significantly higher prevalence ( $p<0.001$ ) of diabetes, hypertension, and smoking compared with double-vessel, single-vessel, and control groups. Lesions associated with the left anterior descending artery were the most common, followed by right coronary and circumflex artery. Left main coronary artery was the rarest, with only 7 cases.

Serum GGT levels had statistical significance ( $p=0.003$ ) in the triple-vessel disease group when compared with the other groups, demonstrating an increase from single-vessel disease as 23 (16~49) to triple-vessel disease as 30 (20~41.5). In addition, serum HDL-C, apolipoprotein AI, high-sensitivity C reactive protein, hypertension, and diabetes were also significantly higher ( $p<0.001$ ) in the triple-vessel group. There was no significant difference ( $p>0.05$ ) in each group concerning serum total cholesterol, triacylglycerol, low-density lipoprotein

cholesterol, apolipoprotein B, lipoprotein (a) level, and alcohol intake (Table 1).

According to the multiple regression analysis, IgGGT was positively correlated with alcohol intake ( $\beta=0.177$ ,  $p<0.001$ ), the coronary complexity ( $\beta=0.068$ ,  $p<0.001$ ), and TG ( $\beta=0.058$ ,  $p<0.001$ ), in which alcohol intake and coronary complexity had high correlation coefficients. However, HDL-C levels ( $\beta=0.157$ ,  $p=0.008$ ) and age ( $\beta=0.004$ ,  $p=0.002$ ) were negatively correlated with IgGGT in the CAD group (Table 2).

With coronary complexity as the dependent variable, multiple-regression analysis revealed that it had a negative correlation with Ig-apolipoprotein AI ( $\beta=-2.517$ ,  $p=0.001$ ), and positive correlations with smoking ( $\beta=0.640$ ,  $p<0.001$ ), IgGGT ( $\beta=0.613$ ,  $p=0.004$ ), Ig high-sensitivity C reactive protein ( $\beta=0.320$ ,  $p<0.001$ ), and hypertension ( $\beta=0.286$ ,  $p<0.026$ ). There was a weak correlation between coronary complexity and age ( $\beta=0.037$ ,  $p<0.001$ ) (Table 3).

**Table 2.** Multivariate analysis between GGT and other factors.

Characters	$\beta$	SE	Standard $\beta$	t	Sig
HDL-C	-0.157	0.059	-0.153	-2.671	0.008
TG	0.058	0.013	0.254	4.571	<0.001
Alcohol intake	0.177	0.046	0.216	3.852	<0.001
Coronary complexity	0.068	0.015	0.269	4.591	<0.001
Age	-0.004	0.001	-0.183	-3.066	0.002

GGT – gamma-glutamyl transferase; HDL-C – high-density lipoprotein cholesterol; TG – triacylglycerol.

**Table 3.** Multivariate analysis between coronary complexity and other factors.

Characters	$\beta$	SE	Standard $\beta$	t	Sig
Age	0.037	0.005	0.387	7.170	<0.001
Smoking	0.640	0.126	0.276	5.087	<0.001
Ig-HsCRP	0.320	0.091	0.185	3.531	<0.001
Ig-ApoA1	-2.517	0.733	-0.179	-3.432	0.001
Ig-GGT	0.613	0.208	0.155	2.940	0.004
Hypertension	0.286	0.128	0.115	2.233	0.026

ApoA1 – apolipoprotein A1; GGT – gamma-glutamyl transferase; HsCRP – high sensitivity C reactive protein.

## Discussion

It is increasingly believed that atherosclerosis is a chronic inflammatory process, the key event of which is oxidative stress resulting from the imbalance between reactive oxygen species and the antioxidant defense system [11]. Reactive oxygen species can inhibit prostacyclin synthetase activity, promote the synthesis of thromboxaneA2, and lead to platelet aggregation and even thrombosis [12]. While increasing lipid peroxidation, reactive oxygen species can cause red blood cells to produce plasma protein cross-linking and increased blood viscosity. However, it results in the injury of endothelial cells and attenuation of lipid infiltration [11]. As a result, the endothelial cells are activated, leading to the release of endothelin and adhesion molecules. In addition, activated monocytes can induce the expression of cytokines, such as tumor necrosis factor and interleukin. Subsequently, macrophages and endothelial cells are induced into the intrarenal arterial wall and oxidize low-density lipoproteins recognized by macrophage scavenger receptors [13]. Eventually, atherosclerosis is induced due to the lipid accumulation and foam cell formation from smooth muscle cells.

GGT is an enzyme on the surface of the cellular membrane, which is responsible for the extracellular catabolism of glutathione of the anti-oxidation mechanism. It has been

always associated with alcohol intake or liver dysfunction [4]. Biologically, GGT divides the gamma-glutamyl part from glutathione. Then, the glutamyl fragment is transferred into amino acid; either a dipeptide or glutathione. Therefore, GGT supplies cellular glutathione resynthesis [14]. Meanwhile, the cysteinyl-glycine moiety on the cellular membrane or in the extracellular space can act as a strongly reduced agent of iron, with the development of the super-oxide ion and hydrogen peroxide. The oxidization of low-density lipoprotein cholesterol particles may occur, which may participate in the formation of inflammatory atheroma within the vascular endothelial wall [15]. Therefore, serum GGT may take part in atherosclerotic plaque progression and rupture. As a result, studies indicated that serum GGT level might be a potential biomarker of atherosomatous plaque in humans [16].

Recent studies showed that serum GGT has a strong association with cardiovascular risk factors [5]. According to a prospective study of 6997 subjects (aged 40–59 years) with no history of CAD or diabetes mellitus in 24 British towns, researchers performed a 24-year followed-up and concluded that the elevated GGT ( $\geq 22$  U/L) was significantly related with the increased risk of fatal CAD events and mortality, which was independent of the established CAD risk factors [17]. In our study, serum GGT levels were related to alcohol intake, coronary lesion count, triacylglycerol, HDL-C, and age in the CAD

group. Alcohol intake and HDL-C levels present high correlation coefficients. However, there is no significant difference in alcohol consumption among the 4 groups in the correlation between coronary complexity and serum GGT levels.

It has been demonstrated that HDL-C possesses significant antioxidant activity, primarily mediated via the inhibition of the ox-low-density lipoprotein with a subsequent reduction of the cellular uptake by the monocyte macrophage system [18]. In addition, HDL-C could prevent atherosclerosis via effects on platelet function, endothelial function, coagulation parameters, inflammation, and interactions with triglyceride-rich lipoprotein. However, the specific mechanisms by which GGT performs the oxidation process of ox-low-density lipoprotein cholesterol need further investigation. In the study, serum GGT levels were higher in triple-vessel disease, double-vessel disease, and diffuse pathology patients.

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The study is limited in that it was a retrospective analysis without histopathology investigation. The relationship between GGT and pathological mechanisms of CAD should be further studied. In addition, the study lacked the follow-up analysis of cardiovascular event and mortality.

## Conclusions

Our study results suggest that serum GGT has a positive correlation with CAD in the Chinese population, which may act as a novel biomarker for CAD.

## Competing interests

None declared.

# Gamma-glutamyl transferase and cardiovascular disease

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**Abstract:** Gamma-glutamyl transferase (GGT) is an enzyme located on the external surface of cellular membranes. GGT contributes in maintaining the physiological concentrations of cytoplasmic glutathione and cellular defense against oxidative stress via cleavage of extracellular glutathione and increased availability of amino acids for its intracellular synthesis. Increased GGT activity is a marker of antioxidant inadequacy and increased oxidative stress. Ample evidence suggests that elevated GGT activity is associated with increased risk of cardiovascular disease (CVD) such as coronary heart disease (CHD), stroke, arterial hypertension, heart failure, cardiac arrhythmias and all-cause and CVD-related mortality. The evidence is weaker for an association between elevated GGT activity and acute ischemic events and myocardial infarction. The risk for CVD or CVD-related mortality mediated by GGT may be explained by the close correlation of GGT with conventional CVD risk factors and various comorbidities, particularly non-alcoholic fatty liver disease, alcohol consumption, oxidative stress, metabolic syndrome, insulin resistance and systemic inflammation. The finding of GGT activity in atherosclerotic plaques and correlation of intra-plaque GGT activity with histological indexes of plaque instability may suggest a participation of GGT in the pathophysiology of CVD, particularly atherosclerosis. However, whether GGT has a direct role in the pathophysiology of CVD or it is an epiphenomenon of coexisting CVD risk factors or comorbidities remains unknown and Hill's criteria of causality relationship between GGT and CVD are not fulfilled. The exploration whether GGT provides prognostic information on top of the information provided by known cardiovascular risk factors regarding the CVD or CVD-related outcome and exploration of molecular mechanisms of GGT involvement in the pathophysiology of CVD and eventual use of interventions to reduce circulating GGT activity remain a duty of future studies.

**Keywords:** Cardiovascular disease (CVD); gamma-glutamyl transferase (GGT); mortality; prognosis

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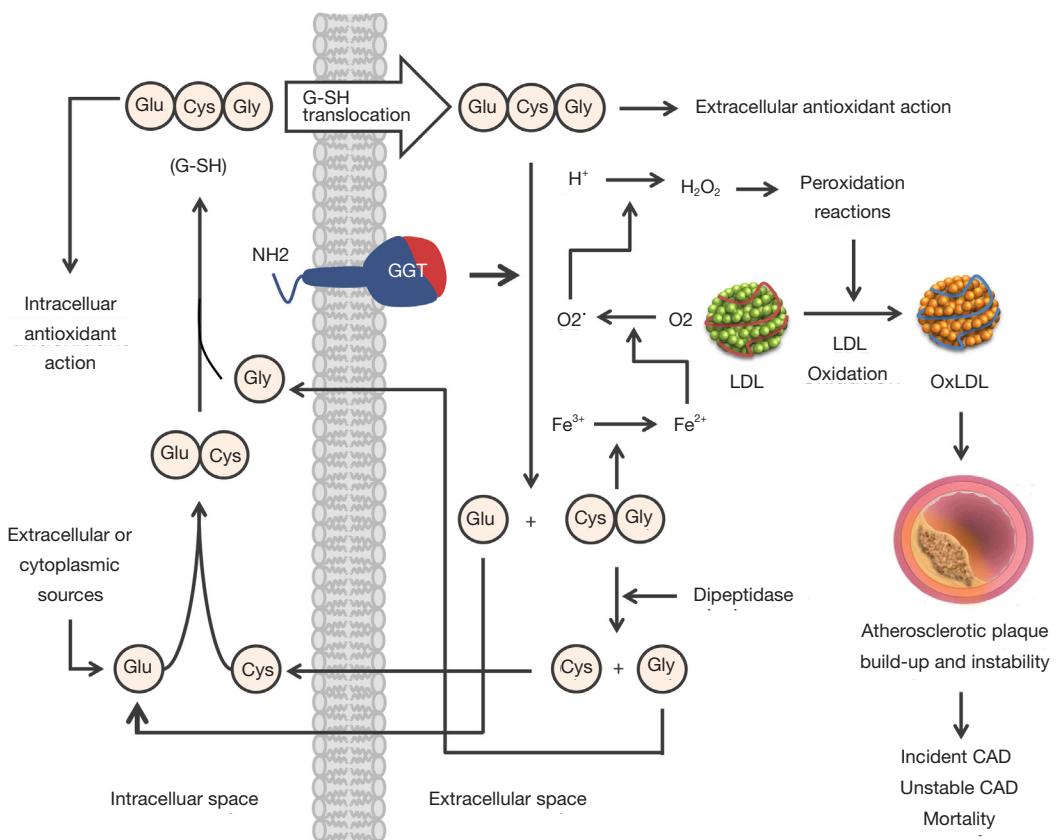
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## Background information

Gamma-glutamyl transferase (GGT; EC2.3.2.2.) is an enzyme located on the external surface of membranes of various cells. Mammalian GGT is a dimeric glycoprotein with a molecular weight of 68 kDa consisting of 2 subunits: a 46 kDa large subunit and a 22 kDa small subunit. However, depending on the degree of glycosylation, the molecular weight has been reported to vary between 38 to 72 kDa for the large and 20 to 66 kDa for the small

GGT subunit (1). The large subunit has an intracellular N-terminal sequence, a transmembrane hydrophobic domain and an extracellular domain and is responsible for GGT anchorage on the cellular membrane surface whereas the small subunit harbors the enzyme active center (1). GGT is present in all cells with the exception of erythrocytes. There is considerable inter-tissue and over the embryonic development variability in GGT activity (2). GGT activity was reported to be particularly high in tissues with secretory and absorptive function



**Figure 1** Gamma-glutamyl transferase (GGT) reaction and the proposed mechanism of related pro-oxidant and atherogenic activity. Cys, cysteine; Glu, glutamic acid; Gly, glycine; GSH, glutathione; LDL, low-density lipoprotein; oxLDL, oxidized LDL.

such as kidney, biliary system, intestine and epididymis and the enzyme activity is greatest in the ductal luminal surface of these tissues (3). Abundant GGT activity has been demonstrated in the proximal tubule of the kidney. GGT activity is particularly intensive in biliary pole of hepatocytes and cholangiocytes (4). GGT is produced as a single polypeptide chain which undergoes autoproteolytic cleavage into the large and small subunits. Human GGT is encoded by a multigene family of at least 7 different genes located in the chromosome 22; nevertheless only 1 of these genes produces complete and functional GGT (5). The gene transcription is controlled by multiple promoters. Related gene sequences that are either nonfunctional or represent pseudogenes are found in chromosomes 18, 19, and 20 (6,7). The multiple promoters and the alternative splicing are responsible for diversity of molecular forms and tissue specificity. Between 50% and 77% of GGT activity is genetically determined (8).

GGT functions are not entirely known. The localization

of GGT in tissues with transport function has led to the suggestion that GGT is involved in the transport of amino acids via the “gamma-glutamyl cycle” (9). However, the enthusiasm for this hypothesis has faded because humans or animals with GGT deficiency have no anomalies in amino acid transport. Cleavage of glutathione—the main thiol antioxidant in humans—is the most important physiological function of GGT. Glutathione has profound cellular functions including protection from oxidative stress, redox signaling, detoxification of xenobiotics, cellular proliferation, fibrogenesis, nitric oxide metabolism, storage and transport of cysteine, sulphur metabolism and apoptosis (10). Glutathione is synthesized in the cytoplasm of cells via a cycle of reactions proposed by Meister (9). After synthesis, glutathione is transported out of the cell and is degraded by GGT into glutamyl moiety (transferred to water or other compounds such as amino acids or peptides) and dipeptide cysteinyl-glycine which is further degraded by dipeptidase into free cysteine and glycine (Figure 1).

Glutathione breakdown in the extracellular space increases the availability of cysteine which is taken up by the cells and used as an essential precursor for the intracellular synthesis of glutathione and proteins. Thus GGT contributes in maintaining the physiological concentrations of glutathione in cytoplasm and cellular defense against oxidative stress. GGT deficiency is an extremely rare autosomal recessive disease characterized by increased glutathione concentration in plasma and urine (due to cellular loss not compensated by GGT action) and central nervous system alterations (11). Other functions of GGT include involvement in the metabolism of leukotrienes, xenobiotics and glutaminase action (cleavage of amide bond of amino acid glutamine to produce glutamate and ammonium) (2).

Circulating GGT is supposed to originate mostly from the liver (12,13) and is influenced by genetic and environmental factors (14). Based on gel filtration chromatography, researchers from the Pisa University have identified 4 GGT fractions with different molecular weight in subjects of both sexes: big (b-GGT), medium (m-GGT), small (s-GGT) and free (f-GGT) (15). Recent studies have suggested that b-GGT consists of membrane microvesicles and it may serve as a precursor for smaller fractions (m-GGT and s-GGT) whereas f-GGT represents free soluble form of the enzyme (4). Emerging evidence suggests an association between elevation of various GGT fractions and specific diseases (16); however, this issue needs further investigation. Although GGT measurement was introduced into clinical laboratories more than 50 years ago and research continuously improved our understanding of clinical utility of GGT, questions still remain as regards the indications for GGT measurement, laboratory methods and reference range. The list of GGT involvement in pathological processes is long. Nevertheless, outside the clinical use as a test for hepatobiliary disease and alcohol abuse, GGT has garnered large interest for its association with cardiovascular disease (CVD), diabetes, metabolic syndrome and cancer. The focus of this review is to summarize the existing knowledge on the association of GGT with CVD. The association of GGT with other morbid conditions is not covered. For detailed information, the reader may refer to recent reviews on these topics (8,17,18).

### **GGT, CVD and mortality**

Several population-based studies have investigated the

association of GGT with CVD or risk of death. The Framingham Offspring Study (FOS) was one of the first epidemiological studies initiated to test the association of GGT with cardiovascular risk and disease. In its second examination cycle, 3,451 patients were recruited between 1978 and 1982 and followed up to a mean of 19 years. GGT was associated with higher body mass index, blood pressure, low-density lipoprotein (LDL)-cholesterol, triglycerides and glucose. On the follow-up, per each standard deviation (SD) higher log GGT, the risk for metabolic syndrome increased by 26%. After adjustment for cardiovascular risk factors, GGT conferred a 13% and 26% increase in the risk for CVD and mortality for each SD higher log GGT. Subjects in the highest GGT quartile showed a 67% increase in the incidence of CVD. The study clearly showed that increased activity of circulating GGT predicted the onset of metabolic syndrome, incident CVD and mortality hinting a role for GGT as a marker of metabolic and cardiovascular risk (19). In the British Regional Heart Study—a prospective study of 7613 middle-aged British men followed for 11.5 years—GGT was associated with an array of cardiovascular risk factors, all-cause mortality and mortality related to coronary heart disease (CHD). Increased GGT [highest quintile (>24 U/L) vs. the rest] was associated with 22% and 42% increase in the relative risk for all-cause and CHD-related mortality. Notably, the risk for CHD-related mortality linked to GGT (a 42% increase in adjusted risk) was higher for subjects with evidence of CHD, particularly prior myocardial infarction, at the time of screening (20). A prospective study by Wannamethee *et al.* (21) included 6,997 men, 40–59 years of age, without a history of CVD, stroke or diabetes. Over a 24-year follow-up, GGT was significantly associated with the increased risk of fatal CHD events (but not non-fatal events), major stroke and overall CVD mortality after adjustment for traditional CVD risk factors. The risk of fatal CHD and CVD-related mortality was elevated in the top quarter ( $\geq 22$  IU/L) only, whereas the risk of stroke showed a tendency to increase with raising GGT level. The adjusted relative risks for GGT quartile 4 versus quartile 1 were 1.43 (1.09–1.84), 1.56 (1.20–2.04) and 1.40 (1.16–1.70) for fatal CHD events, stroke and CVD mortality, respectively. A stronger association of GGT with CVD mortality was seen in the men of younger (<55 years) age (21). The British Women's Heart and Health study assessed the association of GGT with CHD or stroke in 4,286 women (2,961 women with completed data) over a 4.6-year follow-up. For each unit higher log GGT, the hazard ratios (HR) with 95% confidence interval (CI) were

1.15 (0.88–1.48) for incident CHD, 1.45 (0.90–2.34) for incident stroke and 1.17 (0.93–1.48) for CHD or stroke (22). In the meta-analysis of 10 prospective studies incorporated in the same publication, the HR per 1 U/L change of GGT was 1.20 (1.02–1.40) for CHD, 1.54 (1.20–2.00) for stroke and 1.34 (1.22–1.48) for CHD or stroke (22).

Three large Austrian studies have investigated the association of GGT with cardiovascular risk factors, CVD or mortality. The study by Kazemi-Shirazi *et al.* (23) investigated the association of GGT with the risk of mortality in a cohort of 283,438 first attendants (inpatients or outpatients). The median follow-up was 7.6 years. GGT activity was scaled into five categories: normal low (<9 U/L for women, <14 U/L for men), normal high (9–17, 14–27 U/L), moderately elevated (18–26, 28–41 U/L), increased (27–35, 42–55 U/L), and highly elevated ( $\geq 36$ ,  $\geq 56$  U/L). The adjusted HRs for all-cause mortality were 1.2 (1.1–1.2), 1.4 (1.3–1.4), 1.6 (1.5–1.6) and 2.0 (2.0–2.1) for 2nd to 5th categories versus 1st (normal low) GGT category, suggesting a dose-response relationship between GGT and the risk of mortality (23). The study by Ruttman *et al.* (24) assessed the association of GGT with the risk of CVD-related mortality in a cohort of 163,944 Austrian adults, monitored for up to 17 years. High GGT was significantly associated with CVD-related mortality with adjusted HR per log GGT of 1.66 (1.40–1.98) in men and 1.64 (1.36–1.97) in women. Subgroup analyses in men showed a significant association of GGT with incident fatal events of chronic forms of CHD, congestive heart failure, hemorrhagic and ischemic stroke but not myocardial infarction ( $P=0.16$ ). Comparable associations were also observed in women except for the association with hemorrhagic or ischemic stroke which was not significant. The analyses stratified by age showed stronger associations in younger participants (24). In the study by Strasak *et al.* (25), a cohort of 76,113 Austrian men and women with serial measurements of GGT was prospectively followed-up for a median of 10.2 years. Longitudinal changes of GGT were assessed over a period of 6.9 years. A 7-year change of GGT of  $>9.2$  U/L was associated with increased CVD mortality in men [adjusted HR = 1.40 (1.09–1.81)] compared with stable GGT (7-year change of −0.7 to 1.3 U/L). In women, the risk of CVD mortality was also increased with increasing GGT, but the effects were less evident and were significant only for CHD. As in the study by Ruttman *et al.* (24) markedly stronger associations were observed in younger individuals.

Two prospective studies in Finnish population tested the

association of GGT with the risk of stroke or CHD. The prospective cohort study by Jousilahti *et al.* (26) included 14,874 Finnish men and women, 25 to 64 years of age who participated in a cardiovascular risk factor survey in 1982 or 1987. The study showed that serum GGT activity was associated with the risk of total and ischemic stroke in men and women. During a follow-up of 7 or 12 years (depending on the time of survey), the risk ratios in men and women were 1.45 and 1.48 for total stroke and 1.51 and 1.59 for ischemic stroke (calculated per unit increase in log GGT). The prospective study by Lee *et al.* (27) included 28,838 Finnish men and women, 25 to 74 years of age. The risk of CHD was assessed in the 25th, 50th, 75th, and 90th sex-specific percentiles of serum GGT. After adjustment for traditional cardiovascular risk factors, compared with the lowest GGT category (<25th percentile), the HR for the association with CHD were 1.15, 1.25, 1.27, and 1.57 in men and 1.03, 1.22, 1.32, and 1.44 in women in other 4 GGT categories (25th–50th, 50th–75th, 75th–90th and  $\geq 90$ th percentile;  $P$  for trend  $<0.01$ ). Stronger associations were observed in subjects  $<60$  years of age and alcohol drinkers. The strength of association was comparable for non-fatal myocardial infarction and fatal CHD. In subjects with type 2 diabetes, the respective adjusted HRs were 1.29, 1.57, 1.88, and 1.78 ( $P$  trend = 0.03, for combined men and women) (27).

The EUROSTROKE nested case-control study showed a significant increase in the age- and sex-adjusted risk for stroke of 26% per SD (28.7 IU/mL) higher GGT. The odds of hemorrhagic stroke increased linearly with the increase in GGT activity. The association of GGT with stroke was significantly stronger in non-diabetic subjects compared with subjects with diabetes in whom, no association was found (28). The association of GGT activity with the risk for acute ischemic events was investigated in the population-based MONICA (Monitoring trends and determinants on cardiovascular diseases) Augsburg (Southern Germany) survey conducted between October 1984 and June 1985 (29). The study included 1,878 healthy men (25–64 years) who were free of CHD at baseline. Up to 2002, a total of 150 acute ischemic events of new onset occurred. The HR for the association of GGT with incident myocardial infarction across GGT quartiles (<13, 13 to <20, 20 to <35, and  $\geq 35$  U/L) were 1.0, 1.84, 2.02, and 3.08 ( $P$  for trend  $<0.001$ ). After adjustment, the HR for incident myocardial infarction was 2.34 (1.23–4.44) for highest versus lowest GGT quartile. The study strongly suggested that GGT elevation predicts the occurrence of acute ischemic

events in apparently healthy men (29). The population-based Study of Health in Pomerania (SHIP) recruited 4,160 subjects (2,044 men and 2,116 women) without hepatitis B and C or liver cirrhosis at baseline. Over a median of 7.3 years, 307 subjects died. Elevated GGT was associated with the risk of mortality in men [adjusted HR =1.49 (1.08–2.05)] but not in women [adjusted HR =1.30 (0.80–2.12)] with both risk estimates calculated for 5th versus 1st–4th GGT quintiles. The association was particularly strong in men with hepatic steatosis [HR =1.98 (1.21–3.27)]. The authors concluded that in subjects with increased GGT levels, ultrasound examination of liver should be performed for diagnostic and risk stratification reasons (30). Other studies have demonstrated an association of GGT with mortality in elderly subjects (31,32).

Two studies in Asian population (Japan) have suggested a stronger association between GGT and CVD-related mortality and stroke in women than in men. The study by Hozawa *et al.* (33) included 2,724 Japanese men and 4,122 Japanese women without prior CVD or liver dysfunction. Over a follow-up of 9.6 years there were 83 and 82 CVD deaths, in men and women, respectively. In women, the adjusted HR for CVD mortality was 2.88 (1.14–7.28) for the elevated GGT group ( $\text{GGT} \geq 50 \text{ U/L}$ ) versus the reference group ( $\text{GGT}: 1\text{--}12 \text{ U/L}$ ). No significant association was observed in men. The association remained significant in the never-drinker subgroup (33). Similar findings as regards the gender-specific association of GGT with CVD mortality were reported in the prospective study by Fujiyoshi *et al.* (34) which included 7,488 adults (3,089 men). The risk for stroke was not increased with the increase in GGT activity either in women or in men (34). As regards the association between GGT and CVD mortality, a 2009 report of the third National Health and Nutrition Examination Study (NHANES III) 1988–1994 Survey that included 14,950 adult participants without viral hepatitis B and C came to different conclusions. Abnormal GGT was defined as  $>51 \text{ U/L}$  in men and  $>33 \text{ U/L}$  in women. Death certificate-based 12-year mortality was assessed. All-cause mortality [HR =1.5 (1.2–1.8)] and mortality related to liver disease [HR =13.0 (2.4–71.5)], cancer [HR =1.5 (1.01–2.2)] and diabetes [HR =3.3 (1.4–7.6)] but not CVD mortality [HR =1.3 (0.8–2.0)] increased with the increase in GGT activity (35).

Several recent meta-analyses have summarized the results of studies that have assessed the association of GGT with CVD or mortality. A 2013 meta-analysis of 7 studies with 273,141 participants showed an association between

GGT and cardiovascular [relative risk =1.52 (1.36–1.70)] and all-cause [relative risk =1.56 (1.34–1.83)] mortality with both risk estimates calculated for highest versus lowest GGT quartile. Notably the association between GGT and cardiovascular mortality was not significant in the subgroup of Asian participants [relative risk =1.76 (0.76–3.30)] (36). A 2014 meta-analysis by Kunutsor *et al.* (37) of 11 prospective studies has shown a significant association between elevated GGT and mortality [fully adjusted relative risk =1.60 (1.42–1.89) for 3rd versus 1st GGT tertile with substantial heterogeneity between studies]. Another 2014 meta-analysis of 29 cohort studies with 1.23 million participants and 20,406 cardiovascular outcomes showed an association between GGT and CVD with an adjusted relative risk of 1.23 (1.16–1.29) per SD higher log GGT (38). Alkaline phosphatase but not alanine aminotransferase or aspartate aminotransferase showed also an independent association with CVD risk.

In aggregate, evidence from epidemiological studies as regards the association of GGT with the risk for CVD or mortality may be summarized as follows: first, epidemiological evidence strongly supports an association between elevated GGT and incident CVD, stroke or all-cause and CVD-related mortality which appears to be stronger in subjects of younger age. Second, there seems to be a strong correlation between elevated GGT and cardiometabolic risk factors which tend to cluster in subjects with higher GGT levels. Consequently, whether GGT provides prognostic information on top of cardiovascular and metabolic risk factors for prediction of CVD or mortality remains unproven. Third, evidence available is not consistent as regards the association between elevated GGT activity and the risk for acute ischemic events, particularly acute myocardial infarction. Fourth, although a significant association between elevated GGT and CVD or mortality is observed in both sexes, more sex-specific analyses are needed to clarify some existing controversy. Whether the association between GGT and CVD or mortality is weaker in Asian population needs further investigation.

### GGT and coronary events or mortality in patients with CHD

It has been suggested that the presence CHD may strengthen the association between GGT and mortality (20). Emdin *et al.* (39) assessed the association of GGT with mortality or mortality plus nonfatal myocardial infarction over a 6-year follow-up in 469 patients with angiography-

documented CHD. In a subgroup of 262 patients with previous myocardial infarction, cardiac mortality (25.2% vs. 13.9%; P=0.038) or cardiac mortality plus nonfatal myocardial infarction (32.7% vs. 20.4%; P=0.031) were higher in patients with a GGT >40 U/L versus those with GGT <40 U/L. The association between GGT and cardiac events remained significant after adjustment for potential confounders including alcohol consumption. Notably, GGT had no significant prognostic value in patients without previous myocardial infarction (39). In a more recent study, the same authors reconfirmed the independent association of GGT with cardiac mortality in 474 patients with established CHD. At 3 years cardiac mortality was 9% in patients with GGT >25 U/L and 3.5% in patients with GGT <25 U/L (P=0.028). The combination of three biomarkers (higher GGT, C-reactive protein and fasting glucose) identified a group of patients (n=45) with a 3-year risk of cardiac mortality of 26.6% (40). In the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, GGT was measured in 2,556 subjects with and 699 subjects without angiography-proven CHD. There were 754 deaths over a mean follow-up of 7.75 years. All-cause and CVD-related mortality was significantly increased from 1st to 4th GGT quartiles. Compared with subjects with GGT in the lowest quartile, in other 3 quartiles the HR were 1.2 (0.9–1.5), 1.4 (1.1–1.8) and 1.9 (1.5–2.3) for all-cause mortality and 1.4 (1.0–2.0), 1.8 (1.4–2.5) and 2.2 (1.6–2.9) for mortality due to cardiovascular causes. In patients with angiographic CHD, the association between GGT and prognosis was significant and comparable to that of the entire cohort (41). Other studies have shown that GGT predicts prognosis in patients with stable CHD (42) or in-hospital major adverse cardiovascular events in patients presenting with ST-segment elevation myocardial infarction (43).

Our group investigated the association of GGT with cardiovascular events in 5,501 consecutive patients with angiography-proven CHD (44) and in a subgroup of 2,534 consecutive patients with acute coronary syndromes (45). A specific aim of our studies was to test whether GGT provides incremental prognostic information beyond that provided by cardiometabolic risk factors in patients with CHD. In patients with GGT in 1st, 2nd and 3rd GGT tertiles (median GGT activity 21.3, 36.6 and 79.6 U/L, respectively), the Kaplan-Meier estimates of 3-year all-cause and cardiac mortality were, 7.1%, 7.2% and 13.9% (P<0.001) and 4.1%, 3.6% and 7.9% (P<0.001), respectively. After adjustment, GGT was associated with 30% and 21% increase of the risk for all-cause and cardiac mortality, respectively. The

C-statistic of multivariable models was used to test the discriminatory ability of GGT regarding mortality. Of note, GGT improved the discriminatory power of the model(s) of all-cause mortality (P<0.001) and non-cardiac mortality (P<0.001) but not cardiac mortality (P=0.155). This study suggested that GGT provides prognostic information that is incremental to the information provided by CVD risk factors for all-cause and non-cardiac but not cardiac mortality (44). The study in patients with acute coronary syndromes showed comparable results in terms of association of GGT with all-cause mortality (45). Surprisingly, however, adjustment in the multivariable Cox model attenuated the association of GGT with cardiac mortality to the level of statistical insignificance. Discriminatory tests (C-statistic) showed that GGT did not provide prognostic information on top of CVD risk factors regarding prediction of cardiac mortality even in patients with acute coronary syndromes, known to die mostly from cardiac causes (45). In both studies there was no association of GGT with the risk of non-fatal myocardial infarction or stroke (44,45).

Whether GGT correlates with the extent or severity of coronary atherosclerosis remains a matter of controversy. A recent study 442 consecutive patients with stable CHD showed a close correlation between GGT and severity and extent of coronary atherosclerosis assessed by the SYNTAX score (46). Similarly, another recent study that used computed tomography angiography to assess coronary plaque burden and structure in 259 young subjects with coronary atherosclerosis (138 patients with coronary atherosclerosis with a mean age of 41.6 years and 121 controls with a mean age of 41.9 years) showed that patients with plaque formation had significantly higher GGT levels than controls. Furthermore, GGT levels were correlated with the number of plaques and the presence of noncalcified plaques (47). However, other studies came to opposite conclusions. In the study by Saely *et al.* (48) that included 1,000 patients undergoing coronary angiography, GGT was associated with metabolic syndrome but not with angiography-documented coronary atherosclerosis. We also did not find any correlation between GGT level and extent of coronary atherosclerosis or proportion of patients with multi-vessel disease, despite showing a strong association of GGT with all-cause and cardiac mortality (44). One study found that the serum level of GGT may be an independent marker for in-stent restenosis (49). Other studies have shown an association of GGT with coronary

flow reserve in hypertensive patients (50), coronary slow flow (51), no re-flow after primary percutaneous coronary intervention (52), syndrome X (53), carotid intima media thickness (54) or contrast-induced nephropathy in patients with acute myocardial infarction (55).

In aggregate, evidence for an association of GGT with coronary events and mortality in patients with established CHD is weaker than the evidence obtained from epidemiological studies investigating the association of GGT with incident CHD or mortality. Particularly, whether GGT offers prognostic information that is incremental to the information provided by conventional risk factors remains still questionable. Despite using coronary angiography—the gold standard for diagnosis of CHD—studies that assessed GGT in patients with established CHD included smaller numbers of patients and had a short follow-up. Furthermore teasing out the intricate relationship and interdependence between CVD risk factors, GGT and CHD remain difficult. The association of GGT with mortality may be under-adjusted, particularly for the presence and impact of non-alcoholic fatty liver disease—a highly prevalent chronic liver disease and an equivalent of metabolic syndrome with an established role in CVD and mortality (56,57). A large Korean population study showed that GGT predicted an increased risk for all-cause and cancer-related mortality which persisted after adjustment for liver fat. Nevertheless adjustment for the liver fat attenuated the association of GGT with CVD mortality (58). The confounding impact of percutaneous coronary intervention—suggested to abolish the association of GGT with coronary events (39)—should be considered. Finally the vast majority of contemporary patients with CHD are treated with statins and other secondary prevention measures. Statins are known to reduce the risk of fatal and nonfatal coronary events due to their plaque stabilization effects and as shown for other biomarkers may attenuate the association of GGT with cardiovascular events in patients with CHD. Recent evidence shows that non-cardiac deaths have exceeded cardiac deaths in patients with CHD (59), potentially reflecting the efficacy of secondary prevention measures. An experimental study in apolipoprotein E knockout mice showed that statins significantly reduced the GGT expression in aortic atherosclerotic plaques (60). Not surprisingly, all these factors may lead to attenuation of the association of GGT with coronary events or cardiac mortality in patients with CHD.

## GGT and coronary events or mortality in patients with diabetes

Diabetes and elevated GGT activity are associated with increased oxidative stress, poor metabolic profile, high prevalence of non-alcoholic fatty liver disease and heightened inflammatory burden. Hypothetically diabetic status and elevated GGT may have a cumulative or additive action exacerbating the impact of these conditions and strengthening the association with CVD and mortality. It has been suggested that the association of GGT with mortality may be stronger in patients with diabetes (27). An observational cohort study of 1,952 patients with type 2 diabetes has shown an association of GGT with all-cause, cardiovascular and cancer-related mortality, over a mean follow-up of 6.4 years. Adjusted HR for all-cause, cardiovascular and cancer-related mortality was: 1.047 (1.027–1.067), 1.017 (0.973–1.064) and 1.052 (1.017–1.088), each for 10 U/L increase in GGT activity. Notably, GGT but not alanine aminotransferase was associated with mortality (61). In 1280 patients with diabetes, Sluik *et al.* (62) showed that subjects in the 4th GGT quartile had a fully adjusted HR of 3.96 (1.74–9.00] for all-cause mortality over 8.2 years of follow-up. The association was particularly evident in former and current smokers, younger subjects, subjects with a higher waist–height ratio and drinkers. Our group assessed the association of GGT with 3-year mortality in 1,448 diabetic patients and angiography-proven CHD after percutaneous coronary intervention. After adjustment, GGT was associated with the risk for all-cause [HR =1.25 (1.05–1.49)] but not cardiac mortality [HR =1.23 (0.96–1.58)], with both risk estimates calculated per SD higher log GGT. The differences in C-statistics of the models with and without GGT showed that GGT improved the risk for all-cause ( $P=0.02$ ) but not cardiac ( $P=0.135$ ) mortality (63). These studies confirmed an association of GGT with mortality; however, by excluding subjects without diabetes, they offer no information whether the GGT-mortality association is stronger in diabetic patients. A recent study by Kengne *et al.* (64) addressed this issue. The study included a sample of 17,852 subjects (3.3% with diabetes, n=583) who were followed for 10.1 years. The age and sex-adjusted HR was 1.43 (1.13–1.81) in subjects with diabetes and 1.27 (1.18–1.37) in subjects without diabetes for CVD mortality and 1.24 (1.08–1.44) and 1.30 (1.25–1.34), respectively, for all-cause mortality, with all risk estimates calculated per 1 SD increment in log GGT. Notably no evidence was found

that diabetic status *per se* modified the strength of the GGT-mortality association (*P* for interaction =0.16) (64).

These studies showed that elevated GGT activity predicts the risk for all-cause mortality in diabetic patients. The association of GGT with CVD mortality seems to be weaker than the association with all-cause mortality. There is no solid evidence that diabetic status modifies the association of GGT with mortality.

### **GGT and heart failure**

Several studies have assessed the association of GGT with incident congestive heart failure. In 2010, Dhingra *et al.* (65) reported GGT-heart failure relationship in 3,544 participants of the Framingham study cohort who were free of heart failure or myocardial infarction. Over a mean follow-up of 23.6 years, the risk of heart failure was increased by 39% [adjusted HR =1.39 (1.20–1.62)] per each SD higher log GGT. Subjects with a serum GGT level  $\geq$  median had a 1.71-fold risk of heart failure compared with individuals with GGT < median. Of importance was the finding that GGT improved the risk reclassification modestly (net reclassification index of 5.7%; *P*=0.01). The Kuopio Ischaemic Heart Disease (KIHD) study followed 1,780 men free of heart failure for a mean of 22 years (66). Serum GGT was log-linearly associated with the risk of heart failure [adjusted HR =1.25 (1.07–1.45) per 1 SD higher log GGT]. The findings remained consistent in analyses accounting for CHD of new onset and incident impaired renal function. In a meta-analysis of 5 studies (incorporated in the same study) the fully adjusted relative risk for heart failure per each SD higher baseline and long-term GGT values were 1.28 (1.20–1.35) and 1.43 (1.31–1.56), respectively (66). These studies strongly support an association of GGT with the risk for heart failure. Some studies suggest a direct participation of GGT in the pathophysiology of congestive heart failure (67).

### **GGT and arterial hypertension**

Several lines of evidence suggest an association between GGT and arterial hypertension. Cheung *et al.* (68) showed in 235 patients with arterial hypertension and 708 normotensive subjects that GGT but no other liver enzymes predicted new-onset hypertension over a 3-year follow-up. Other studies have shown that GGT correlates with systolic and diastolic blood pressure (69), arterial stiffness (69,70) or impaired aortic elasticity (71). Elevated but still within

normal range GGT correlated with incident hypertension in drinkers and non-overweight subjects (72). Recent meta-analyses summarized the evidence linking elevated GGT level with the risk of arterial hypertension (73,74). A recent meta-analysis by Liu *et al.* (73) summarized 13 prospective studies with 43,314 participants and 5,280 cases of arterial hypertension. The pooled relative risk of hypertension was 1.94 (1.55–2.43) when comparing the highest versus the lowest GGT categories and 1.23 (1.13–1.32) per each SD higher log GGT. No significant association was observed for the subgroup with blood pressure  $\geq$ 160/95 mmHg and nondrinkers. However, heterogeneity across the studies was significant (73). In another recent meta-analysis of 14 cohort studies with 44,582 participants and 5,270 cases of arterial hypertension, the pooled relative risk of association with hypertension was 1.32 (1.23–1.43) comparing 3rd versus 1st GGT tertiles (74). Again, the heterogeneity across the studies was significant. In a pooled dose-response analysis of 10 studies, a linear association between GGT and hypertension risk was found (*P* for nonlinearity =0.37). The pooled relative risk of arterial hypertension per 5U/L increment in GGT levels was 1.08 (1.04–1.13) (74). These two meta-analyses showed that baseline circulating GGT is associated with the risk of arterial hypertension in general population, consistent with a linear dose-response relationship.

### **GGT, cardiac arrhythmias and sudden cardiac death**

The rationale for investigating the association between GGT and cardiac arrhythmias rests on the link of GGT with some well-known factors that predispose for cardiac arrhythmias such as, cardiovascular risk factors, liver disease, alcohol consumption, systemic inflammation and increased oxidative stress. An indirect impact of GGT via other forms of CVD predisposing for cardiac arrhythmias should also be considered. Several studies have addressed the association of GGT with the risk for atrial fibrillation. The Atherosclerosis Risk in Communities (ARIC) study in which 1,021 incident atrial fibrillation events occurred in 9,333 men and women over a 12-year follow-up showed that GGT was linearly associated with the risk for atrial fibrillation after multivariable adjustment. A doubling of GGT activity was associated with 20% increased risk of atrial fibrillation (75). The SHIP study showed that elevated activities of liver enzymes were associated with increased prevalence of atrial fibrillation in general adult

population. The adjusted odds ratio for atrial fibrillation was 2.17 (1.64–2.87) per each SD higher log GGT (76). The KIHD study that included 1,780 Finnish men showed an association between baseline and long-term GGT values and the risk of atrial fibrillation over a 22-year follow-up. However, after full adjustment for conventional risk factors the association was attenuated to below the level of statistical significance (66). A pooled analysis of ARIC and KIHD studies showed that baseline and long-term GGT predicted the risk of atrial fibrillation with fully adjusted HR of 1.09 (1.02–1.16) and 1.14 (1.03–1.25), respectively, per each SD higher baseline and long-term GGT (66). A recent study by Tekin *et al.* (77) that included 81 patients with nonvalvular atrial fibrillation reported higher levels of GGT in patients with nonvalvular atrial fibrillation compared with age and sex-matched controls without atrial fibrillation. Furthermore the study showed an independent association between GGT and chronic nonvalvular atrial fibrillation. In another recent study, elevated GGT levels predicted the occurrence of atrial fibrillation following catheter ablation (78).

An association between GGT and the risk for ventricular arrhythmias or sudden cardiac death has also been shown. In the KIHD study, the HR for an association of GGT with the risk of ventricular arrhythmias was 1.37 (1.04–1.80) (66). A recent publication from the KIHD study showed that serum GGT was log-linearly associated with the risk of sudden cardiac death with a HR =1.30 (10–1.54) per each SD higher log GGT (79). The association of GGT with sudden cardiac death remained significant after adjustment for known risk factors, alcohol consumption, resting heart rate, lipids and C-reactive protein [HR =1.26 (1.05–1.50)].

### Mechanisms of GGT association with CVD

Several putative explanations may be offered to explain the association of elevated GGT with the risk for CVD or CVD-related mortality. GGT is closely associated with established cardiovascular risk factors (which tend to cluster in patients with higher GGT level), metabolic syndrome and insulin resistance (18). Elevated GGT may be a marker of antioxidant inadequacy and of increased oxidative stress (17). Circulating GGT levels are also closely correlated with the markers of inflammation and thus its elevated activity may signify a heightened inflammatory state (80). Ample evidence exists that all these conditions—cardiovascular risk factors, metabolic syndrome, insulin resistance, increased oxidative stress and systemic

inflammation are great promoters of CVD and CVD-related mortality. In this regard, elevated GGT may be considered a marker of increased cardiometabolic stress, in general. The close association of GGT with alcohol consumption may also explain the association of GGT and all-cause mortality (81) and cardiac arrhythmias, particularly atrial fibrillation (82). Nevertheless, light and moderate alcohol consumption are inversely associated with myocardial infarction and CVD mortality (81). Nonalcoholic fatty liver—the most common cause of chronic liver disease, with a prevalence up to 70% in diabetic patients—is associated with the increased activity of GGT and other liver enzymes in addition to more prevalent CVD (83). Nonalcoholic fatty liver disease is associated with an array of cardiometabolic disorders, for which it is considered a metabolic syndrome equivalent. It has been proposed that GGT may indicate a link between fatty liver and early atherosclerotic disease (84). Not surprisingly, the adjustment for fatty liver has attenuated the association between GGT and CVD mortality (58). Nonalcoholic fatty liver disease is associated with the risk of ventricular arrhythmias (85) and atrial fibrillation (86). In particular, elevated GGT is associated with the increased risk for atrial fibrillation either because both conditions share similar risk factors or because of ectopic fat accumulation in atrial myocardium (87) which may modulate the electrophysiological properties and ion currents predisposing for arrhythmogenesis (88). Finally, evidence available suggests a direct participation of GGT in the pathophysiology of CVD, particularly atherosclerosis, on cellular and molecular level. The breakdown of glutathione by GGT in the extracellular space leads to production of cysteinyl-glycine dipeptide—even a stronger reducing agent than glutathione. The cysteinyl-glycine moiety acts as a strong reducing agent of iron from  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  which subsequently catalyzes formation of superoxide and hydrogen peroxide. These species promote peroxidation reactions (including low-density lipoproteins) and exert a local pro-oxidant and proinflammatory action (Figure 1). There is evidence that these reactions occur within atherosclerotic plaques and they present the most accepted putative mechanism of a direct participation of GGT in the pathophysiology of atherosclerosis leading to promotion of atherosclerotic process, plaque instability and coronary ischemic events (89). Catalytically active GGT is found in atherosclerotic plaques (90) and the GGT activity within the atherosclerotic plaques was found to correlate with systemic GGT activity (91) and histological indexes of plaque instability (92).

## Concluding remarks and perspective

Ample evidence from large epidemiological studies strongly suggests the existence of an association of elevated GGT activity with CVD, CHD, arterial hypertension, congestive heart failure, cardiac arrhythmias and CVD-related mortality. However, the evidence linking elevated GGT with acute ischemic events is weaker and inconsistent. Although, it has been suggested that GGT appears to pass the Vasan's criteria (93) as a biomarker of increased cardiovascular risk (89), the intricate association of GGT with cardiovascular risk factors and comorbidities, particularly the liver disease, raises serious questions regarding a direct role of GGT in the pathophysiology of CVD or causality in the GGT-CVD relationship. Recent data suggested that adding GGT to conventional cardiovascular risk factors did not improve the risk prediction for CVD or CHD-related mortality (44,64,94). Thus, at this stage, the crucial question whether GGT has a direct role in the pathophysiology of CVD or it is an epiphomenon of coexisting CVD risk factors or comorbidities remains unanswered. Consequently, the Hill's criteria (95) of causality relationship between GGT and CVD, are not entirely fulfilled. Future studies with the use of specific discriminatory tests to assess whether GGT provides information that is additive and beyond the information provided by known cardiometabolic risk factors as regards CVD or CVD-related outcome are hoped to resolve this dilemma. Exploration of molecular mechanisms of GGT involvement in the pathophysiology of CVD and eventual use of interventions to reduce circulating GGT activity remain a duty of future studies.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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# Gamma-glutamyltransferase, fatty liver index and hepatic insulin resistance are associated with incident hypertension in two longitudinal studies

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**Objective:** We hypothesized that liver markers and the fatty liver index (FLI) are predictive of incident hypertension and that hepatic insulin resistance plays a role.

**Methods:** The association between liver markers and incident hypertension was analysed in two longitudinal studies of normotensive individuals, 2565 from the 9-year data from an epidemiological study on the insulin resistance cohort and the 321 from the 3-year 'Relationship between Insulin Sensitivity and Cardiovascular disease' cohort who had a measure of endogenous glucose production. The FLI is calculated from BMI, waist circumference, triglycerides and gamma-glutamyltransferase (GGT) and the hepatic insulin resistance index from endogenous glucose production and fasting insulin.

**Results:** The incidence of hypertension increased across the quartiles groups of both baseline GGT and alanine aminotransferase. After adjustment for sex, age, waist circumference, fasting glucose, smoking and alcohol intake, only GGT was significantly related with incident hypertension [standardized odds ratio: 1.21; 95% confidence interval (1.10–1.34);  $P=0.0001$ ]. The change in GGT levels over the follow-up was also related with an increased risk of hypertension, independently of changes in body weight. FLI analysed as a continuous value, or FLI at least 60 at baseline were predictive of incident hypertension in the multivariable model. In the RISC cohort, the hepatic insulin resistance index was positively related with the risk of 3-year incident hypertension [standardized odds ratio: 1.54 (1.07–2.22);  $P=0.02$ ].

**Conclusion:** Baseline GGT and FLI, as well as an increase in GGT over time, were associated with the risk of incident hypertension. Enhanced hepatic insulin resistance predicted the onset of hypertension and may be a link between liver markers and hypertension.

**Keywords:** alanine transaminase, fatty liver, gamma glutamyltransferase, humans, hypertension, incidence, insulin resistance, longitudinal studies

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; FLI, fatty liver index; GGT, gamma glutamyl transferase; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has emerged as a growing public health issue worldwide that has reached epidemic proportions, including among young people [1–3]. The fatty liver index (FLI) is a surrogate marker of the presence of a fatty liver, and it is based on BMI, waist circumference, triglycerides and gamma-glutamyltransferase (GGT) [4,5].

The liver enzymes, GGT and alanine aminotransferase (ALT) and the FLI, are known to predict the incidence of type 2 diabetes, independently of the presence of obesity [4–8]. In parallel to the risk for diabetes, epidemiological studies report an association between elevated liver enzymes and cardiovascular disease risk [9–13]. A recent study showed that the development of new fatty liver is predictive of incident hypertension in a Korean population, but the association between liver enzymes and the risk of incident hypertension has not been addressed [14]. Furthermore, the mechanisms underlying the relation between NAFLD and hypertension remain uncertain. Insulin resistance, which is commonly associated with both fatty liver and hypertension, could mediate the relationship between fatty liver elevated liver enzymes and the risk of hypertension [15,16].

We previously showed, in a cohort of nondiabetic individuals, that subtle elevations in liver enzyme activities (both GGT and ALT) are associated with hepatic insulin resistance, independently of abdominal adiposity [17].

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Therefore, we raise the hypothesis that hepatic insulin resistance may be related with the development of hypertension.

The aim of the present study was to investigate whether liver markers and the FLI at inclusion are related with incident hypertension, independently of other metabolic risk factors such as insulin resistance. For this purpose, we used the D.E.S.I.R. (data from an epidemiological study on the insulin resistance syndrome) cohort, a large 9-year prospective cohort recruited in the general population, with measures of fasting insulin and glucose levels at baseline.

In addition, we examined whether hepatic insulin resistance predisposes to incident hypertension, in the nonhypertensive participants from the 3-year prospective Relationship between Insulin Sensitivity and Cardiovascular disease (RISC) (relationship between insulin sensitivity and cardiovascular disease) cohort who had an assessment of endogenous glucose production (EGP) by the infusion of stable isotope tracer [17].

## MATERIALS AND METHODS

### The data from an epidemiological study on the insulin resistance cohort, liver markers and incident hypertension

Men and women, aged 30–65 years, recruited into the 9-year D.E.S.I.R. cohort from volunteers were offered free, periodic health examinations by the French Social Security, in 10 health examinations centres in western France [8,18]. There were four health examinations, carried out every 3 years. Hypertension was defined by treatment for hypertension or resting blood pressure (BP) at least 140 (SBP) mmHg and/or at least 90 (DBP) mmHg.

There were 2565 participants without hypertension at inclusion who had liver markers GGT, ALT and aspartate aminotransferase (AST) activities measured at inclusion and a known hypertension status at year 9; in total, 1021 had an incident hypertension during the follow-up.

All participants signed an informed consent, and the protocol was approved by an ethics committee.

### Clinical assessment

Weight and height were measured in lightly clad participants, and BMI was calculated. Waist circumference was measured at the smallest circumference between the lower ribs and iliac crests using a tape measure. Smoking habits and alcohol intake were recorded by the participants on a questionnaire.

Two measures of BP were taken after 5 min of rest; mean values were used in analyses. The examining physician noted treatment for diabetes and hypertension at each of the four examinations [18]. In a sensitivity analysis, we defined incident hypertension by medication; with this definition, there were 3007 participants without hypertension at baseline and 547 with incident hypertension during the follow-up.

### Biochemical measurements

Fasting plasma glucose, measured by the glucose-oxidase method, was applied to fluoroxydized plasma using a Technicon RA100 analyzer (Bayer Diagnostics, Puteaux,

France) or a Specific or a Delta device (KoneLab, Evry, France). Insulin was quantified by microparticle enzyme immunoassay with an automated analyser (IMX; Abbott, Rungis, France). GGT, ALT and AST were assayed by an enzymatic method (IFCC recommendations, without Pyridoxal Phosphate, 37 °C), using a Technicon DAX24 automated analyser (Bayer Diagnostics) or a Specific or a Delta (KoneLab). HOMA-IR was calculated as (fasting insulin) × (fasting glucose)/22.5 [19].

### The fatty liver index

A surrogate marker of fatty liver, the FLI, based on BMI, waist circumference, triglycerides and GGT was calculated as follows:

$$\text{FLI} = \frac{e^L}{(1 + e^L)} \times 100$$

where  $L = 0.953 \times \log_e (\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e (\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745$

with triglycerides measured in mg/dl, GGT in IU/l and waist circumference in centimetre [4,5]. This index has been validated in the general population and has been shown to be accurate in detecting fatty liver [4]. A FLI at least 60 was used to define those who are likely to have a fatty liver [20].

### The RISC cohort, the hepatic insulin resistance and incident hypertension

The rationale and methodology of the 3-year pan-European RISC cohort study have been published, as well as the characteristics of the individuals recruited [21,22]. Clinically healthy nondiabetic and normotensive individuals, aged 30–60 years, were recruited; 321 had an evaluation of hepatic insulin resistance by the infusion of stable isotope tracer and their hypertensive status at year 3 was known; 63 had incident hypertension. Ethics committee approval was obtained by each recruiting centre. Volunteers were given detailed written information on the study as well as an oral explanation, and they all signed a consent form.

### Clinical assessment

Weight, height and waist circumference (mid-way between the iliac crests and the lower ribs) were measured. Alcohol and tobacco consumption were assessed using a standarized semiquantitative questionnaire [18].

BP was measured in triplicate after 5 min of rest, according to a standardized protocol, by trained study nurses using an OMRON 705CP (Omron Healthcare GmbH, Hamburg, Germany) with participants seated; the median of these readings was used in this analysis for both the baseline and the follow-up examinations. Hypertension was defined by treatment for hypertension or resting BP at least 140 (SBP) mmHg and/or at least 90 (DBP) mmHg [18].

### Hepatic insulin resistance index

Biochemical parameters were centrally assayed in a single centre (glucose and insulin in Odense, triglycerides in Dublin and liver markers in Cambridge). Fasting EGP was assessed using a continuous infusion of (6–6<sup>2</sup>H<sub>2</sub>)

glucose for 2 h in a basal state. After insulin infusion, plasma samples were collected every 15 min, from 0 to 90 min, and every 5–10 min from 90 to 120 min for the determination of plasma glucose and insulin concentrations. Glucose enrichment was measured by gas-chromatography mass spectrometry. Basal EGP was calculated as the ratio of the tracer infusion rate and the tracer-to-tracee ratio. The hepatic insulin resistance index (HIRI) was calculated as the product of fasting insulinaemia and EGP [23].

## Statistical analysis

Data are expressed as mean  $\pm$  SD or as median (interquartile range) for variables with a skewed distribution, and categorical data as percentages. Variables that were not symmetrically distributed were log transformed before statistical analyses: GGT, ALT, AST, insulin, HOMA-IR and HIRI. Baseline characteristics, means and percentages were compared using Student *t* tests and  $\chi^2$  tests, respectively, according to incident hypertension.

## Data from an epidemiological study on the insulin resistance study analyses

There were no significant interactions between sex and GGT, ALT, AST activities or FLI on the risk of hypertension; therefore, we analysed men and women together.

The relations between liver enzymes, as stratified into quartile groups, and incident hypertension were assessed by logistic regression analysis, with a trend test across the four groups. Spearman correlation coefficients ( $r_{SP}$ ) assessed the relation between BP levels and liver enzymes. Furthermore, the relation between GGT and FLI concentrations at baseline, with both SBP and DBP at follow-up, was assessed in a multivariable regression analysis, after adjustment for sex, age, waist circumference (not for FLI), smoking, alcohol intake, glycaemia and the respective BP value at baseline.

Further analyses used the liver markers and FLI as continuous variables with adjustments for sex and for age, waist (not for FLI), glycaemia, smoking and alcohol intake. We assessed in the 1851 participants who had a FLI less than 30 at baseline, whether changes in FLI categories over the follow-up were related to the risk of incident hypertension, after adjustment for sex, age, smoking, alcohol intake, glycaemia and changes in body weight over the follow-up. A sensitivity analysis was performed among those with GGT within the normal range and in those who did not consume alcohol or who consumed little (<5 g/day) at baseline.

## RISC study analyses

In the RISC study, BP levels at year 3 were compared according to the median of the HIRI at baseline, by a *t* test. Multivariable logistic regression analysis assessed the association between both the HIRI at baseline and incident hypertension at year 3, after adjustment for sex, centre and baseline age, waist circumference, smoking and alcohol intake.

Statistical analyses used StatView (version 5.0; SAS Institute Inc., Cary, North Carolina, USA) and SAS version 9.2 (SAS Institute).

**TABLE 1.** Baseline characteristics of individuals in the data from an epidemiological study on the insulin resistance cohort according to incident hypertension over the 9-year follow-up

	Without incident hypertension (n = 1544)	With incident hypertension (n = 1021)	P
Age (years)	43 $\pm$ 9	48 $\pm$ 9	<0.0001
Men (%)	38%	54%	0.0001
BMI (kg/m <sup>2</sup> )	23.2 $\pm$ 3.0	24.8 $\pm$ 3.2	<0.0001
Waist circumference (cm)	78 $\pm$ 9	84 $\pm$ 10	<0.0001
Smoker	22%	29%	0.0003
Alcohol intake (g/day)			<0.0001
<5 g/day (54.3%)	67.2%	32.8%	
5–14 g/day (4.7%)	58.2%	41.8%	
15–29 g/day (25.4%)	53.6%	46.4%	
≥30 g/day (15.6%)	47.0%	53.0%	
Fasting glucose (mmol/l)	5.1 $\pm$ 0.5	5.4 $\pm$ 0.8	<0.0001
Fasting insulin (pmol/l) <sup>a</sup>	39.9 (20.8)	45.3 (23.3)	0.0001
HOMA-IR <sup>a</sup>	1.11 (0.7)	1.35 (0.9)	<0.0001
FLI	16.1 $\pm$ 18.2	29.4 $\pm$ 24.2	<0.0001
GGT (IU/l) <sup>a</sup>	18 (13)	23 (21)	<0.0001
ALT (IU/l) <sup>a</sup>	19 (11)	23 (14)	<0.0001
Aspartate aminotransferase (IU/l)	18 (7)	19 (9)	0.0001

Data shown as mean (SD), median (interquartile range) or %. ALT, alanine aminotransferase; FLI, fatty liver index; GGT, gamma-glutamyltransferase.

<sup>a</sup>Log transformation for statistical analysis.

## RESULTS

### The data from an epidemiological study on the insulin resistance cohort

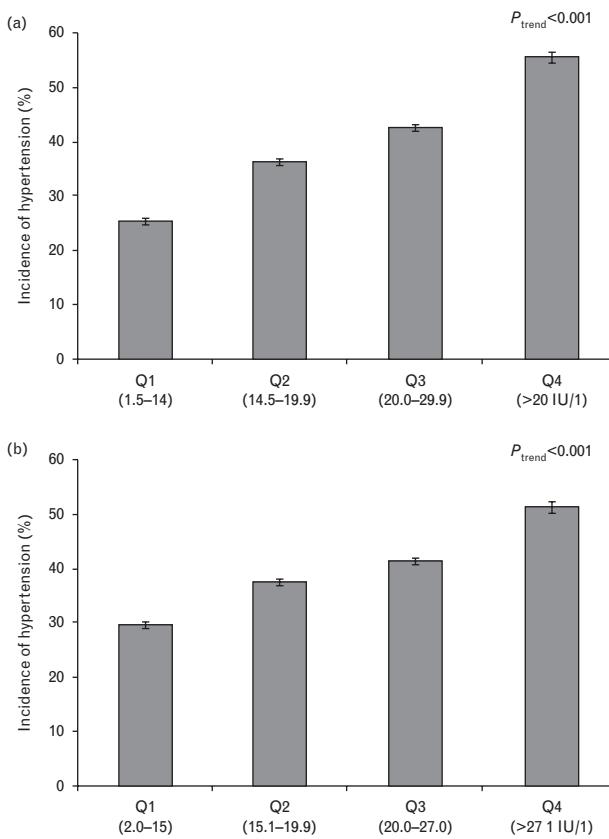
#### Liver enzymes and hypertension

In univariate analysis, those with incident hypertension over the 9-year follow-up had a higher alcohol consumption as well as a higher BMI, waist circumference, fasting glycaemia, HOMA-IR, GGT, ALT and AST activities at baseline as compared with those who remained normotensive (Table 1).

The incidence of hypertension at year 9 increased progressively across the quartile groups of both GGT and ALT at baseline ( $P < 0.0001$ , Fig. 1). When used as continuous variables, baseline GGT and ALT but not AST activity (all log-transformed) were significantly associated with incident hypertension, after adjustment for sex, baseline age, waist circumference and smoking (Table 2). Further adjustment for baseline fasting glycaemia and alcohol intake did not alter the association between GGT and the risk of hypertension (Table 2), but for ALT, the relation was no longer significant. The association between GGT and incident hypertension appeared to be independent of the HOMA-IR index at baseline (Table 2); however, log HOMA-IR was related to the risk of incident hypertension [standardized odds ratio (OR): 1.05; (1.02–1.08);  $P = 0.001$ ].

When both baseline GGT and ALT activities were included in the same model, only GGT was related to incident hypertension [standardized OR: 1.22; 95% confidence interval (1.09–1.37);  $P = 0.007$ ] with a *P* value of 0.89 for ALT.

Plasma GGT levels more than 30 U/l at baseline were also predictive of an increased risk of incident hypertension



**FIGURE 1** Crude 9-year incidence of hypertension (standard error) in the data from an epidemiological study on the insulin resistance cohort, according to quartile groups of (a) gamma-glutamyltransferase and (b) alanine aminotransferase.

in the multivariable model [OR: 1.40 (1.13–1.73);  $P = 0.002$ ]. The change in GGT levels over the follow-up was related with an increased risk of hypertension in the same model, as well as after further adjustment for changes in body weight over the follow-up.

In sensitivity analyses, the OR changed little when we restricted the analysis to those with GGT within the normal range ( $<45 \text{ U/l}$ ) at baseline [standardized OR: 1.23 (1.05–1.44),  $P = 0.009$ ] or if we restricted analyses to those who did not consume alcohol or who consumed little ( $<5 \text{ g/day}$ ) [standardized OR: 1.24 (1.07–1.43),  $P = 0.005$ ]. GGT concentration at baseline was significantly related to both SBP ( $P = 0.0003$ ) and DBP ( $P < 0.0001$ ) at follow-up, after adjustment for sex, age, waist circumference, smoking, alcohol and glycaemia. The results were unchanged when we defined incident hypertension by medication.

### Fatty liver index and hypertension

There was a graded increase in the incidence of hypertension at year 9 across the categories of the FLI, with the highest incidence being observed for those with a FLI at least 60 ( $P < 0.0001$ , Fig. 2, Table 3). FLI at baseline was significantly correlated with both SBP ( $r_{SP} = 0.36$ ,  $P < 0.0001$ ) and DBP ( $r_{SP} = 0.28$ ,  $P < 0.0001$ ) at year 9.

FLI, as a continuous variable, was significantly associated with incident hypertension in a multivariable model with adjustment for sex, baseline age and smoking. Further

**TABLE 2.** Odds ratios for the association between liver markers and the fatty liver index analysed as continuous variables, and 9-year incident hypertension in the data from an epidemiological study on the insulin resistance cohort

	OR (95% CI)	P
GGT (IU/l)		
Unadjusted	1.58 (1.45–1.71)	<0.0001
Adjusted for sex, age, waist circumference and smoking	1.24 (1.13–1.37)	<0.0001
+Adjusted for fasting glucose	1.23 (1.12–1.36)	<0.0001
+Adjusted for alcohol intake	1.21 (1.10–1.34)	0.0001
+Adjusted for HOMA-IR	1.19 (1.09–1.32)	0.0003
ALT (IU/l)		
Unadjusted	1.40 (1.29–1.52)	<0.0001
Adjusted for sex, age, waist circumference and smoking	1.11 (1.00–1.22)	0.04
+Adjusted for fasting glucose	1.10 (0.99–1.21)	0.06
+Adjusted for alcohol intake	1.09 (0.99–1.21)	0.07
+Adjusted for HOMA-IR	1.07 (0.97–1.19)	0.13
Aspartate aminotransferase (IU/l)		
Unadjusted	1.17 (1.08–1.26)	0.0002
Adjusted for sex, age, waist circumference and smoking	0.94 (0.86–1.03)	0.22
+Adjusted for fasting glucose	0.95 (0.87–1.04)	0.28
+Adjusted for alcohol intake	0.95 (0.86–1.04)	0.24
+Adjusted for HOMA-IR	0.94 (0.86–1.03)	0.19
ELI		
Unadjusted	1.89 (1.73–2.06)	<0.0001
Adjusted for sex, age and smoking	1.64 (1.49–1.81)	<0.0001
+Adjusted for fasting glucose	1.60 (1.44–1.76)	<0.0001
+Adjusted for alcohol intake	1.58 (1.43–1.75)	<0.0001
+Adjusted for HOMA-IR	1.44 (1.20–1.74)	0.0001

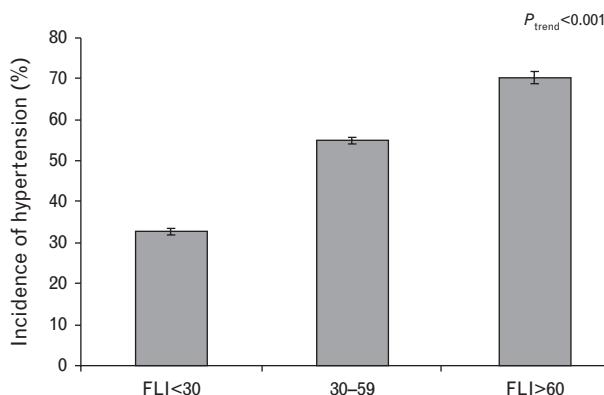
Standardized odds ratio for 1 SD of each variable (after log-transformation). ALT, alanine aminotransferase; CI, confidence interval; FLI, fatty liver index; OR, odds ratio.

adjustment for alcohol intake did not substantially modify the relationship (Table 2). The association between FLI and incident hypertension was independent of the HOMA-IR index at baseline (Table 2).

In the same multivariable model, taking into account alcohol intake, having a FLI at least 30 was associated with a significantly increased risk of incident hypertension after 9 years, with a greater risk for those with a FLI at least 60 at baseline, as compared with those with a FLI less than 30 at baseline (Table 3). Among the 1851 participants who had a FLI less than 30 at baseline, an increase in FLI over the follow-up was associated with an increased risk of incident hypertension as compared with those who retained a FLI less than 30 at follow-up, after adjustment for sex, age, smoking, alcohol intake, fasting glucose and changes in body weight over the follow-up period [for FLI  $> 30$  but  $< 60$  at year 9: OR: 1.76 (1.31–2.36);  $P = 0.0002$ ; for FLI  $\geq 60$  at year 9: OR: 2.74 (1.63–4.62);  $P = 0.0001$ ]. FLI at baseline was significantly related to both SBP ( $P = 0.0002$ ) and DBP ( $P < 0.0001$ ) at follow-up, when analysed as a continuous variables, in multivariable models.

The association between FLI and incident hypertension persisted when we defined incident hypertension by medication.

In addition, when we analysed men and women separately, we observed a significant association between liver markers or FLI and incident hypertension in both sexes, with a stronger effect for men (results not shown).



**FIGURE 2** Crude 9-year incidence of hypertension (standard error) in the data from an epidemiological study on the insulin resistance cohort, according to categories of the fatty liver index.

## The RISC cohort

### Hepatic insulin resistance and hypertension

The HIRI, which is the product of fasting insulinaemia and EGP, was greater in individuals who developed hypertension over the follow-up, as compared with those who remained normotensive (Table 4). A baseline HIRI above the median vs below the median was associated with higher SBP ( $127 \pm 15$  vs  $122 \pm 13$  mmHg,  $P=0.004$ ) and DBP ( $79 \pm 9$  vs  $76 \pm 9$  mmHg,  $P=0.004$ ) at year 3.

The logarithm of HIRI was related to the risk of incident hypertension after controlling for age, recruitment centre, sex, waist circumference, smoking and alcohol intake [standardized OR: 1.54 (1.07–2.22),  $P=0.02$ ]. Similarly, an increased HIRI (above the median) was a risk factor for incident hypertension in the same model [OR: 2.03 (1.03–4.00);  $P=0.04$ ] (Fig. 3).

## DISCUSSION

The main finding of this study is that GGT and the FLI predict incident hypertension in a prospective, general population cohort (the D.E.S.I.R cohort) with a 9-year follow-up. Furthermore, people from the nondiabetic, healthy RISC cohort who had a specific assessment of EGP, provide mechanistic evidence that increased hepatic insulin resistance is associated with incident hypertension.

It has previously been shown that higher GGT activity is associated with an increased risk of cardiovascular disease in the general population [9,12,24,25]. We show that an elevated GGT, even within the normal range, is associated

**TABLE 4. Baseline characteristics in the RISC cohort according to incident hypertension over the 3-year follow-up**

	Without incident hypertension (n = 258)	With incident hypertension (n = 63)	P
Age (years)	$42.6 \pm 7.9$	$47.6 \pm 7.7$	<0.0001
Men (%)	49%	62%	0.07
BMI (kg/m <sup>2</sup> )	$25.5 \pm 3.8$	$27.4 \pm 3.7$	0.0005
Waist circumference (cm)	$87 \pm 13$	$92 \pm 12$	0.005
Smoker (%)	28	23	0.48
Alcohol intake (g/day)			0.89
<5 g/day (38.4%)	81.7%	18.3%	
5–14 g/day (37.2%)	87.5%	12.5%	
15–29 g/day (17.2%)	81.8%	18.2%	
≥30 g/day (7.2%)	84.2%	15.8%	
Fasting glucose (mmol/l)	$5.1 \pm 0.5$	$5.3 \pm 0.7$	<0.0001
Fasting insulin (pmol/l) <sup>a</sup>	32.0 (20.0)	39.0 (28.7)	<0.0001
Hepatic insulin resistance index <sup>a</sup>	0.38 (0.28)	0.48 (0.50)	0.003
FLI	$31.3 \pm 26.9$	$30.9 \pm 25.2$	0.85
GGT (IU/l) <sup>a</sup>	20 (12)	25 (18)	<0.0001
ALT (IU/l) <sup>a</sup>	18 (11)	21 (11)	0.002

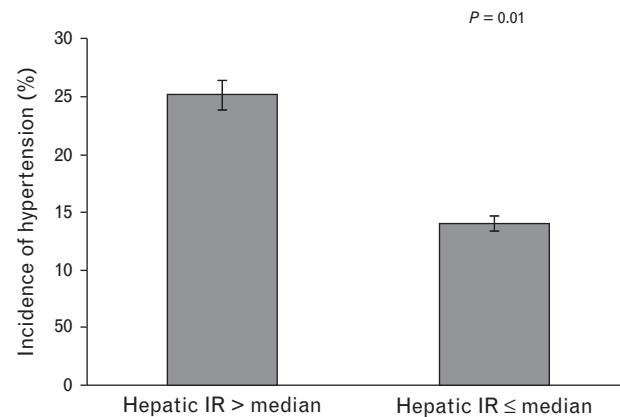
Data shown are as mean  $\pm$  SD, median (interquartile range) or %. ALT, alanine aminotransferase; FLI, fatty liver index; GGT, gamma-glutamyltransferase.

<sup>a</sup>Log-transformed for analysis.

with incident hypertension, independently of conventional risk factors, such as increased waist circumference, but also fasting glucose or HbA1c (data not shown).

The strength of the relation between GGT and incident hypertension was underscored by the fact that the association persisted after exclusion of those with GGT above the normal range and in those who did not consume alcohol or who consumed little (<5 g/day) at baseline in the D.E.S.I.R. cohort, suggesting that the association is not due to individuals with high GGT activity and/or elevated alcohol consumption [17]. Furthermore, the observation that the change in GGT levels over the follow-up was related to the risk of incident hypertension contributes to support the relation between elevated GGT concentration and the development of hypertension.

A potential mechanism underlying the link between GGT and hypertension could be related to oxidative stress and the role of cellular GGT in the metabolism of



**FIGURE 3** Crude 9-year incidence of hypertension (standard error) in the RISC cohort, according to the hepatic insulin resistance index above or below the median value.

Logistic regression analysis with adjustment for age, sex, smoking, fasting glucose and alcohol intake. CI, confidence interval; FLI, fatty liver index; OR, odds ratio.

extracellular reduced glutathione. Indeed, it has been shown that cellular GGT may be involved in the production of reactive oxygen species in the presence of iron or other transition metals [26]. In parallel, oxidative stress is known to be implicated in the pathogenesis of essential hypertension [27] and polymorphisms of antioxidant enzyme genes, including some of the glutathione-S-transferase enzyme genes, have been shown to be associated with the risk of hypertension in the general population [28,29].

On the other hand, a large body of evidence suggests that NAFLD is associated with an increased risk of cardiovascular diseases [11,30]. Cross-sectional studies show a higher prevalence of NAFLD among hypertensive individuals, as compared with those with normal BP [31,32]. A recent study in a Korean population shows that the development of fatty liver was associated with a risk of hypertension at the 5-year follow-up [14]. Furthermore, the degree of NAFLD, as assessed by ultrasonography, has been shown to be related to the risk of incident hypertension in Korean men [33]. We extended these findings by showing that fatty liver, as estimated by an elevated FLI, could also be predictive of incident hypertension, independently of age, sex and alcohol intake in a white cohort. The observation that the increase in FLI over the follow-up was associated with the risk of hypertension supports a possible pathophysiological link between liver fat content and the development of elevated BP. However, confirmation of the link between fatty liver and hypertension risk would require further studies with a more accurate assessment of intrahepatic fat content.

Beyond the presence of increased liver fat content, our study supports a novel role for hepatic insulin resistance in the development of elevated BP, and to our knowledge, is the first to investigate this relationship.

Previous studies have reported a relationship between increased liver enzyme activity and an enhanced risk of type 2 diabetes [6–8,34]. Hepatic insulin resistance could be a pathophysiological link between increased liver enzymes and the risk of both hypertension and type 2 diabetes [15,16].

The first pivotal study that showed insulin resistance was present in essential hypertension reported an increase in muscular insulin resistance without changes in hepatic glucose production among hypertensive individuals, as compared with normotensive volunteers [35]. However, the HIRI is more accurate and takes into account the concomitant concentration of insulin, but was not available in that previous study. Furthermore, it was cross-sectional and did not assess the relation with the subsequent onset of hypertension.

The assessment of fasting EGP by a continuous infusion of a tracer, is complex, restrictive and time consuming. We previously showed in the RISC cohort, that GGT activity is positively correlated with hepatic insulin resistance with a stronger correlation for GGT than for ALT, which suggests that GGT might reflect the degree of hepatic insulin resistance [17].

An experimental study in mice showed that selective and pure hepatic insulin resistance following liver insulin receptor knockout (LIRKO) was associated with increased atherosclerosis, which supports a role for liver insulin

resistance alone, in the development of vascular disease [36]. Indeed, in this model, there was no increase in liver triacylglycerol content [36]. However, BP levels of these LIRKO mice were not reported. Other investigations in mice showed that hepatic insulin resistance was associated with higher plasma levels of harmful metabolites such as malondialdehyde and homocysteine [37,38], which could play a role in vascular dysfunction and the development of hypertension [39].

We cannot exclude the possibility that plasma glucose excursions, which are promoted by enhanced hepatic insulin resistance, may play a role in the association with incident hypertension, as previous studies have shown a link between glucose levels and elevated BP [40,41].

Limitations of the current study include the absence of a direct measure of intrahepatic fat content and a single assessment of liver markers at inclusion. Alcohol consumption was estimated with self-reported questionnaires and we cannot exclude underreporting. However, the results were not altered when restricted to those with very moderate alcohol intakes. Strengths of the study are the analysis of the large D.E.S.I.R. cohort with a long 9-year follow-up. The RISC cohort enables the use of gold standard methodology for measurement of hepatic insulin sensitivity.

In conclusion, our study shows that in the general population, GGT activity and an elevated FLI are independently associated with the risk of incident hypertension after 9 years, even in those with very moderate alcohol consumption. Increases in GGT levels and the FLI over time may help to identify people at higher risk of developing incident hypertension. In addition, enhanced hepatic insulin resistance, as assessed by the estimation of EGP, also predicts the onset of hypertension and may be the link between elevated liver markers and hypertension.

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Author contributions: F.B. researched data and wrote the manuscript. F.B. is the guarantor. A.G. researched data, contributed to the discussion and reviewed/edited the manuscript. F.P. researched data. A.N. researched data and reviewed/edited the manuscript. R.S. contributed to the discussion and reviewed/edited the manuscript. J.P. researched data and contributed to discussion. J.T. researched data. M.M. researched data and reviewed/edited the manuscript. B.F. contributed to the discussion and reviewed/edited the manuscript. B.B. researched data and wrote part of the manuscript.

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# Distribution, Determinants, and Prognostic Value of $\gamma$ -Glutamyltransferase for All-Cause Mortality in a Cohort of Construction Workers from Southern Germany<sup>1</sup>

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**Background.** Serum  $\gamma$ -glutamyltransferase (GGT) is commonly measured as a marker of hepatobiliary disorders in clinical practice, but little is known about its distribution and prognostic value for all-cause mortality.

**Methods.** Distribution and determinants of serum GGT levels were assessed among 8,043 construction workers ages 25–64 who underwent occupational health examinations in six centers in Southern Germany from 1986 to 1988. Study participants were followed for all-cause mortality until 1994.

**Results.** Serum GGT levels were considerably higher in this cohort than among male employees examined in a national survey conducted during the same period. The factors most strongly related to serum GGT were self-reported alcohol consumption, body mass index, diabetes, and hypertension, but relations of GGT levels were also found with nationality, occupation, and smoking. There was a strong dose-response relation between serum GGT levels and all-cause mortality (*P* value for trend <0.001). Compared with men with GGT levels below 15 U/liter (measured at 25°C), relative risks (95% CI) were 1.46 (0.86–2.49), 1.78 (1.08–2.94), 2.09 (1.26–3.45), and 3.44 (2.20–5.38) for men with GGT levels of 15–19, 20–29, 30–49, and ≥50 U/liter, respectively. This relation was reduced but not eliminated by control for body mass index, diabetes, hypertension, alcohol consumption, and other covariates in multi-variable analysis.

**Conclusion.** Serum GGT is a strong risk indicator of all-cause mortality. © 1997 Academic Press

**Key Words:**  $\gamma$ -glutamyltransferase; mortality; risk factors.

## INTRODUCTION

$\gamma$ -Glutamyltransferase (GGT) is a sensitive indicator of hepatobiliary disorders. Elevated levels are often ascribed to excessive alcohol consumption and are commonly used as a marker for excessive alcohol consumption in clinical practice [1,2]. GGT elevations may also be due to other factors, however, such as hepatotoxic drugs, diabetes mellitus, obesity, congestive heart failure, and pancreatic, renal, and pulmonary disorders [3,4].

Several studies have assessed the population distribution of GGT levels. For example, Nilssen et al. assessed GGT levels in more than 20,000 males and females ages 20–59 in the municipality of Tromsø, Norway, in 1986–1987 [5]. Median serum levels (measured at 37°C) were 17 and 12 units/liter for males and females, respectively, and 5.5% of the males and 1.5% of the females had values exceeding 50 units/liter. Somewhat lower levels had been reported in an earlier population survey in Tromsø conducted in 1979–1980 [6]. In the baseline examination of the British Regional Heart Study, the mean (geometric) level among men ages 40–59 was 15.6 units/liter (range 3.0–524.0) [7].

Very few population studies have assessed the prognostic value of GGT levels for mortality [7–9]. In a study from Malmö, Sweden, a significant association was seen between GGT and all-cause mortality, which was mainly due to an excess of alcohol-related deaths [8]. In the British Regional Heart Study, GGT levels were also strongly associated with all-cause mortality, largely due to a significant increase in deaths from ischemic heart disease and noncardiovascular disease causes other than cancer, but the increased mortality was seen only in the top quintile of GGT distributions [7].

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Alcohol consumption is common among construction workers in many countries [10]. Furthermore, construction workers are exposed to a variety of potentially hepatotoxic chemicals, such as organic solvents [11,12]. This occupational group is therefore at increased risk of hepatobiliary disorders. In this study, we analyzed distribution and prognostic value of GGT levels for all-cause mortality in a cohort of construction workers from Southern Germany.

## MATERIAL AND METHODS

### *Design and Study Population*

A retrospective cohort study was conducted in 1992–1995 among all construction workers who had undergone routine occupational health examinations in six occupational health centers of the Workmen's Compensation Board for construction workers in Württemberg (in the South of Germany) between August 1986 and December 1988. In Germany, employees in the construction industry are periodically invited to occupational health examinations by the occupational health service of the Workmen's Compensation Board. In the period of investigation, about 78% of the invited employees in Württemberg participated in the examinations.

The present study includes employees ages 25–64 and belonging to one of the following occupational groups at the time of the baseline examination: plumbers, carpenters, painters or varnishers, plasterers, bricklayers, and unskilled workers and a group of white-collar employees, consisting of office employees, engineers, and architects.

### *Data Collection*

Baseline occupational health examinations included a physician-explored working history (including alcohol and smoking habits), a self-reported occupational and medical history, a physical exam, a lung function test, a test of visual acuity, audiometry, and a blood and serum analysis. In addition, an electrocardiogram or chest X ray was done if necessary. The exams were conducted and recorded by occupational physicians according to a standardized protocol. Serum GGT levels were measured at 25°C with Hitachi 705/717 (reference range 6–28 U/liter). Average daily amount of ethanol uptake was calculated from frequency, type of beverage, and amount of alcohol consumption, assuming that 1 liter of beer, 0.5 liter of wine, or 1 liter of cider corresponds to 50 g ethanol and that alcohol content of 1 unit of liquor (0.02 liters) is approximately 8 g ethanol. These figures reflect typical alcohol concentrations of alcoholic beverages in Southern Germany.

Active follow-up was carried out by the occupational health service between October 1992 and July 1994. Employers, employees, or their relatives (if necessary)

were recontacted, in that order, to ascertain life status. Follow-up information was completed by the system of population registries in Germany for workers who could not otherwise be traced. Vital status could be ascertained for 96.4% of study participants. Completeness of follow-up was somewhat lower for foreign employees (93.6%) than for German employees (97.4%) due to migration.

### *Statistical Analysis*

Distribution of GGT levels was analyzed by age, nationality, occupational group, self-reported alcohol consumption, smoking status, body mass index, and presence or absence of a diagnosis of diabetes, hypertension, or ischemic heart disease at the baseline examination. Alcohol consumption was categorized at cutpoints of 50 and 100 g per day among drinkers. These cutpoints correspond to 2 and 4 customary units of alcohol consumption in Southern Germany (0.5 liters of beer or 0.25 liters of wine), respectively. A minority of study participants who reported occasional alcohol consumption without further quantitative information were included in the category of men consuming 1–49 g per day. Study participants were classified as current, former, or never smokers regardless of the type of smoking (smoking of cigars and pipes was very rare in this study population).

In addition to bivariate analyses, multiple linear regression with the natural logarithm of GGT levels as the dependent variable was performed to quantify the independent effect of the aforementioned factors on GGT levels. Log transformation was performed and geometric rather than arithmetic means were calculated because of the highly skewed distribution of GGT. The age-specific (geometric) mean GGT levels among construction workers of German nationality were compared with the corresponding levels (also measured at 25°C) among male employees of German nationality in a representative national survey carried out in the Federal Republic of Germany in 1987–1988 [13].

All-cause mortality was assessed in relation to GGT levels in both bivariate and multivariable analyses. The following covariates were considered in multivariable analyses: age, nationality, occupation, smoking, body mass index, and presence or absence of a diagnosis of diabetes, hypertension, or ischemic heart disease at the baseline examination. Multivariable analyses were repeated with additional adjustment for self-reported alcohol consumption. The multivariable survival analyses were carried out with the proportional hazards model by Cox [14].

For most study variables, the proportion of missing values was very low (e.g., 0.0% for age and occupation, 0.3% for nationality, and 2.0% for serum GGT). Alcohol consumption and smoking status had been recorded less completely, however, in two of the six occupational health centers (overall completeness for these vari-

ables: 83 and 77%, respectively). Separate categories were created for individuals with unrecorded alcohol consumption or smoking status in multivariable analyses involving the respective variables.

All analyses were carried out on PC with the SAS statistical software package [15].

## RESULTS

### Study Population

Overall, 8,043 men met the inclusion criteria for this analysis (see Table 1). Among these were 850 plumbers (10.6%), 959 carpenters (11.9%), 1,087 painters (13.5%), 880 plasterers (10.9%), 2,703 bricklayers (33.6%), 1,221 unskilled workers (15.2%), and 343 white-collar employees (4.3%). The mean age was 42.8 years. The majority of study participants were of German nationality (74.3%), and 9.4, 8.4, 5.9, and 2.0% of study participants were of Yugoslavian, Italian, Turkish, or other nationality, respectively.

### Distribution and Determinants of GGT

Serum GGT levels were significantly associated with all of the covariates considered in this analysis except for a diagnosis of ischemic heart disease (see Table 1). With regard to age, lowest levels were observed among 25- to 34-year-old employees and highest levels were observed among 45- to 54-year-old employees. GGT levels were considerably lower among Turkish employees than among employees of all other nationalities and among white-collar employees than among all blue-collar occupational groups included in this study. As expected, GGT levels were strongly associated and showed a clear dose-response relation with alcohol consumption. Although GGT levels were highest among former smokers, differences between never, former, and current smokers were of limited magnitude. There was a strong positive association between body mass index and serum GGT levels. Men with a diagnosis of diabetes or hypertension had considerably higher levels of GGT than men without such diagnoses.

The association of GGT levels with self-reported alcohol consumption is illustrated in more detail in Table 2. Overall, abstainers were a small minority in this cohort ( $n = 481$ ), while a considerable proportion of men reported drinking 50–99 ( $n = 1,734$ ) and  $\geq 100$  g ( $n = 852$ ) of alcohol per day. While only a minority of 4.2% of abstainers had serum GGT levels  $\geq 50$  U/liter, this applied to more than one-third of men who consumed  $\geq 100$  g of alcohol per day. Conversely, the majority of abstainers (54.9%) but only 12.2% of the heaviest drinkers had serum GGT levels below 15 U/liter.

The strong association of self-reported alcohol consumption, body mass index, diabetes, and hypertension with GGT levels was confirmed in the multiple regression analysis, with the natural logarithm of GGT

TABLE 1

Levels of GGT by Sociodemographic Variables, Occupation, Alcohol Consumption, Smoking, Body Mass Index, and Pre-existing Diseases

Characteristic	Distribution (n = 8,043)	GGT		
		Geo-metric mean	Proportion $>50$ U/L	P value <sup>a</sup>
Age				
25–34	27.2%	18.5 U/L	11.1%	
35–44	22.7%	23.6 U/L	15.7%	
45–54	36.1%	24.8 U/L	18.7%	
55–64	14.0%	23.1 U/L	13.6%	<0.001
Nationality				
German	74.3%	23.6 U/L	16.9%	
Italian	8.4%	21.3 U/L	10.8%	
Yugoslavian	9.5%	20.7 U/L	13.4%	
Turkish	5.9%	13.6 U/L	2.4%	
Other	1.9%	24.0 U/L	16.8%	<0.001
Occupational group				
White collar	4.3%	17.8 U/L	5.8%	
Plumbers	10.6%	21.3 U/L	13.3%	
Carpenters	11.9%	21.1 U/L	14.8%	
Painters	13.5%	23.6 U/L	17.8%	
Plasterers	10.9%	23.1 U/L	15.3%	
Bricklayers	33.6%	23.6 U/L	16.4%	
Unskilled workers	15.2%	21.3 U/L	14.5%	<0.001
Alcohol consumption				
None	7.4%	14.4 U/L	4.2%	
1–49 g/day	53.3%	18.9 U/L	8.9%	
50–99 g/day	26.4%	28.8 U/L	22.5%	
$\geq 100$ g/day	12.9%	39.6 U/L	36.5%	<0.001
Smoking status				
Never	18.0%	20.7 U/L	12.8%	
Former	20.2%	24.8 U/L	16.6%	
Current	61.7%	23.1 U/L	17.0%	0.003
Body mass index				
$<25.0$ kg/m <sup>2</sup>	35.9%	18.2 U/L	10.9%	
25.0–29.9 kg/m <sup>2</sup>	48.8%	23.6 U/L	16.0%	
$\geq 30.0$ kg/m <sup>2</sup>	15.3%	30.6 U/L	23.1%	<0.001
Diabetes				
No	95.1%	22.0 U/L	14.6%	
Yes	4.9%	32.7 U/L	27.6%	<0.001
Hypertension				
No	77.8%	20.2 U/L	11.9%	
Yes	22.2%	32.3 U/L	26.7%	<0.001
Ischemic heart disease				
No	94.7%	22.3 U/L	15.2%	
Yes	5.3%	24.2 U/L	15.4%	0.924

<sup>a</sup> For  $\chi^2$  test of independence of proportion  $>50$  U/L from group.

levels as the dependent variable and age, nationality, occupation, alcohol consumption, body mass index, and a diagnosis of diabetes, hypertension, or ischemic heart disease as independent variables (see Table 3). Additional analyses did not reveal relevant interactions between alcohol consumption and the other covariates, and therefore no interaction terms were included in the final model. Our analysis also confirmed minor though statistically significant associations of GGT levels with age, occupation, and smoking. All of the adjusted regression coefficients for the blue-collar occupational

**TABLE 2**  
Distribution of Serum GGT by Self-Reported Alcohol Consumption

GGT	Self-reported alcohol consumption			
	None (n = 481)	1–49 g/day (n = 3,490)	50–99 g/day (n = 1,734)	≥100 g/day (n = 852)
<15 U/L	54.9%	39.3%	21.3%	12.2%
15–19 U/L	23.9%	19.0%	15.8%	11.9%
20–29 U/L	11.0%	18.7%	19.3%	18.1%
30–49 U/L	6.0%	13.8%	20.6%	20.7%
≥50 U/L	4.2%	9.3%	23.0%	37.2%

**TABLE 3**

Results of Multiple Linear Regression with the Natural Logarithm of GGT Levels as the Dependent Variable

Predictor variable	b (SE)	t
Age		
25–34	0 <sup>a</sup>	
35–44	0.159 (0.025)	6.41
45–54	0.120 (0.023)	5.25
55–64	0.017 (0.029)	0.57
Nationality		
German	0 <sup>a</sup>	
Italian	-0.117 (0.032)	-3.70
Yugoslavian	-0.194 (0.030)	-6.42
Turkish	-0.368 (0.040)	-9.31
Other	0.077 (0.060)	1.29
Occupational group		
White collar	0 <sup>a</sup>	
Plumbers	0.099 (0.048)	2.05
Carpenters	0.065 (0.048)	1.36
Painters	0.165 (0.047)	3.52
Plasterers	0.100 (0.049)	2.05
Bricklayers	0.104 (0.044)	2.35
Unskilled workers	0.095 (0.048)	1.98
Alcohol consumption		
None	0 <sup>a</sup>	
1–49 g/day	0.148 (0.039)	3.81
50–99 g/day	0.509 (0.041)	12.39
≥100 g/day	0.808 (0.045)	17.93
Smoking		
Never	0 <sup>a</sup>	
Former	0.083 (0.031)	2.66
Current	0.121 (0.026)	4.60
Body mass index		
<25.0 kg/m <sup>2</sup>	0 <sup>a</sup>	
25.0–29.9 kg/m <sup>2</sup>	0.219 (0.019)	11.58
≥30.0 kg/m <sup>2</sup>	0.384 (0.027)	14.29
Diabetes		
No	0 <sup>a</sup>	
Yes	0.231 (0.039)	5.99
Hypertension		
No	0 <sup>a</sup>	
Yes	0.284 (0.021)	13.36
Ischemic heart disease		
No	0 <sup>a</sup>	
Yes	0.013 (0.037)	0.35

Note. b, regression coefficients; SE, standard error; t, t values.

<sup>a</sup> Reference group.

groups were positive, which indicates that the higher GGT levels among these groups than among white-collar employees are not fully explained by self-reported alcohol consumption and the other covariates. Strong differences in GGT levels by nationality with lower levels among employees of Italian, Yugoslavian, and Turkish nationality than among German employees persisted after control for alcohol consumption and the other covariates.

Overall, serum GGT levels were considerably higher among the blue-collar construction workers of German nationality included in this study than among the external comparison group of male employees of German nationality who participated in a representative national survey in 1987–1988 (see Table 4). Differences were large and statistically significant ( $P < 0.001$  in *t* tests for differences between means) for all 5-year age groups up to age 60, and they were most prominent between ages 30 and 59.

#### Prognostic Value of GGT

The survival experience of the cohort is shown in Table 5. A total number of 172 deaths were recorded during the follow-up period. All-cause mortality showed a strong positive association with serum GGT levels in bivariate analyses (*P* value for linear trend  $<0.001$ ). Compared with men with GGT levels below 15 U/liter, relative risk was 1.46 (95% CI 0.86–2.49), 1.78 (95% CI 1.08–2.94), 2.09 (1.26–3.45), and 3.44 (2.20–5.38) for men with GGT levels of 15–19, 20–29, 30–49, and ≥50 U/liter, respectively. Relative risks were reduced to some extent by adjustment for age, nationality, occupation, smoking, body mass index, and a diagnosis of diabetes, hypertension, and ischemic heart disease in multivariable analysis. Relative risks were further reduced by additional adjustment for alcohol consumption, but there still remained a clear dose-response relation between serum GGT levels and all-cause mortality (*P* value for linear trend = 0.01).

**TABLE 4**

Age-Specific (Geometric) Mean GGT Levels among Male Construction Workers of German Nationality in the Cohort and among Male Employees of German Nationality in a National Sample

Age (years)	Construction workers	National sample
25–29	17.6 U/L	14.0 U/L
30–34	22.4 U/L	14.2 U/L
35–39	26.0 U/L	18.4 U/L
40–44	26.8 U/L	17.1 U/L
45–49	27.9 U/L	20.1 U/L
50–54	27.1 U/L	17.5 U/L
55–59	25.0 U/L	17.8 U/L
60–64	22.6 U/L	16.4 U/L

TABLE 5

Numbers of Deaths, Person-Years of Observation, Crude Death Rate, and Relative Risk of Death by Serum GGT

GGT	Deaths	Person-years	Death rate <sup>a</sup>	Relative risk (95% confidence interval)		
				Crude	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
<15 U/L	31	11,904	26.0	1.00 <sup>d</sup>	1.00 <sup>d</sup>	1.00 <sup>d</sup>
15–19 U/L	24	6,296	38.1	1.46 (0.86–2.49)	1.31 (0.76–2.23)	1.26 (0.74–2.16)
20–29 U/L	30	6,473	46.3	1.78 (1.08–2.94)	1.54 (0.92–2.56)	1.52 (0.90–2.56)
30–49 U/L	30	5,516	54.4	2.09 (1.26–3.45)	1.61 (0.95–2.73)	1.49 (0.87–2.57)
≥50 U/L	50	5,585	89.5	3.44 (2.20–5.38)	2.66 (1.65–4.29)	2.24 (1.35–3.72)
				P < 0.001 <sup>e</sup>	P < 0.001 <sup>e</sup>	P = 0.01 <sup>e</sup>

<sup>a</sup> Deaths per 10,000 person-years.<sup>b</sup> Adjusted for age, nationality (categories: German, other), occupational group (categories: white collar, blue collar), smoking (categories: never, former, current, unknown), body mass index (categories: <25.0, 25.0–29.9, and ≥30.0 kg/m<sup>2</sup>), diabetes, hypertension, and ischemic heart disease.<sup>c</sup> Additionally adjusted for alcohol consumption (categories: none, 1–49 g/day, 50–99 g/day, ≥100 g/day, unknown).<sup>d</sup> Reference group.<sup>e</sup> P value for test on linear trend.

## DISCUSSION

This study demonstrated a strong positive dose-response relationship of serum GGT levels with all-cause mortality. This association was partly explained by the impact of prognostic factors associated with GGT elevations, such as diabetes, hypertension, and alcohol consumption. Nevertheless, accounting for these factors, GGT levels were shown to have additional prognostic value. In particular, the monotonic increase of all-cause mortality with GGT levels is unlikely to simply reflect the effects of alcohol consumption, since the relation between alcohol consumption and all-cause mortality was found to be U-shaped in this cohort [16] like in most other pertinent studies [17,18].

Few other prospective population studies have examined the relation between serum GGT levels and all-cause mortality [7–9]. These studies also showed a positive association between GGT and all-cause mortality, which was restricted, though, to the upper end of GGT distributions. Our study differs from previous studies in that it included much higher proportions of individuals with elevated GGT levels, which allowed us to assess dose-response relations with all-cause mortality over a broad range of relevant GGT elevations. Furthermore, unlike the two other large cohort studies that addressed the prognostic value of GGT, this study was not confined to middle-aged men. While we had no information on the cause of death of individuals, the previous studies have suggested that elevated GGT levels mainly lead to an excess of deaths from ischemic heart disease and noncardiovascular disease other than cancer [7,8]. Excess risks for mortality from these diseases might therefore be even higher than the large excess risks observed for all-cause mortality in our cohort.

Our analyses suggest that part of the association between GGT and all-cause mortality is due to diabetes

and hypertension, since control for these factors reduced this relation to some extent. Other chronic diseases, such as pulmonary disorders, congestive heart failure, and pancreatic renal and pulmonary disorders, which have been found to be related to GGT elevations in other studies [3,4], may account for some of the prognostic value of GGT levels that persisted after multivariable adjustment in our study.

Our results are consistent with previous studies that showed a strong association of serum GGT levels with alcohol consumption. The high level of alcohol consumption among construction workers probably also partly explains the large differences in GGT levels between the participants of this study and a sample of male employees from a national survey conducted during the same period. Similarly, alcohol consumption also explains some of the differences in GGT levels between the predominantly muslim Turkish employees and other employees and between blue-collar construction workers and white-collar employees. Nevertheless, part of these differences persisted even after control for alcohol consumption. Although this may reflect residual confounding to some extent (due to the use of broad categories or imperfect reporting of alcohol consumption, to be discussed further below), other factors, such as dietary habits or occupational exposures, may also be relevant and may be worth further study.

Despite the important role of alcohol consumption in GGT elevations, the specificity of GGT as a marker of excessive alcohol consumption is limited. Other reasons for elevated GGT levels, such as those identified in this paper (body weight, diabetes, hypertension), and other potential reasons, such as hepatotoxic drugs, congestive heart failure, or pancreatic, renal, or pulmonary disorders, also require careful consideration. While the higher levels of GGT among painters than among other occupational groups found in our study point to the potential role of hepatotoxic chemicals, our

study had insufficient power to address this question in more detail. Newer, more specific markers, such as carbohydrate-deficient transferrin, may be useful to distinguish GGT elevations due to excessive alcohol consumption from GGT elevations due to other reasons [19].

Like in most other pertinent epidemiologic studies, alcohol consumption is likely to be unprecisely reported with a tendency toward underreporting by part of the study participants in our investigation [20]. Misclassification of alcohol consumption would most likely have led to underestimation of the dose-response relation between this variable and serum GGT levels [21] and to imperfect control for potential confounding by alcohol consumption of the relation between other covariates and GGT levels [22] and of the relation between GGT levels and all-cause mortality.

In contrast to alcohol consumption, serum GGT, the variable of primary interest in this study, is not affected by reporting problems and could be almost completely ascertained in the entire cohort by a simple laboratory test. The results of our study suggest that screening for elevated serum GGT levels could be a powerful tool to identify individuals at increased risk of mortality for whom prevention and treatment of eventual underlying diseases, limitation of alcohol consumption [23], or protection from other relevant exposures could be most beneficial. Preferably, however, pertinent measures should be taken prior to the development of GGT elevations.

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## Association between serum $\gamma$ -glutamyltransferase levels and coronary microvascular function in hypertensive patients

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**Objective** Serum  $\gamma$ -glutamyltransferase (GGT) level is an independent risk factor for cardiovascular (CV) disease, and there is a strong association between serum GGT levels and most CV risk factors, including hypertension; however, the role of serum GGT level as an independent risk factor for target organ damage in hypertension remains controversial. Accordingly, we aimed to determine whether serum GGT level is independently and specifically associated with coronary flow reserve (CFR) impairment in hypertensive patients.

**Methods** We examined 100 never-treated and newly diagnosed hypertensive individuals, and CFR was achieved in 97 (97%) of them. They were divided into two groups based on serum GGT levels.

**Results** Subjects with higher GGT had significantly impaired CFR as compared to those with lower GGT ( $2.10 \pm 0.36$  versus  $2.57 \pm 0.54$ ,  $P < 0.0001$ ). After adjusting for potential confounders, including age, sex, body mass index, blood pressure, lipids and glucose, we found that serum GGT levels were independently associated with CFR impairment ( $\beta = -0.62$ ,  $P < 0.0001$ ). We also found that GGT level was a good predictor of low CFR at the receiver-operating characteristic curve. Area under the

curve was 79% [95% confidence interval: 0.70–0.88], and GGT level was significantly predictive of low CFR ( $P < 0.0001$ ).

**Conclusion** These results support a role for serum GGT level as an independent marker of target organ damage in hypertensive subjects without concomitant risk factors. *J Hypertens* 25:2497–2503 © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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**Keywords:** coronary flow reserve, echocardiography,  $\gamma$ -glutamyltransferase, hypertension, target-organ damage

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### Introduction

Recent epidemiological and clinical studies have shown that  $\gamma$ -glutamyltransferase (GGT) is an independent risk factor for the mortality and morbidity of cardiovascular (CV) disease [1,2]. There is a strong association between serum GGT levels and many CV risk factors, including hypertension [3–5]. In addition, several prospective studies reported that baseline serum GGT concentration was an independent risk factor for the development of coronary artery disease (CAD), diabetes mellitus, stroke and hypertension [5–9].

Patients with hypertension may have symptoms and signs of myocardial ischemia despite angiographically normal coronary arteries, which may be related to impaired coronary microvascular function [10]. In the absence of epicardial coronary artery stenosis, coronary flow reserve (CFR) may be considered a marker of coronary microvascular function, and attenuated CFR is mostly the result of minimal changes in coronary resistance that

are independent of vascular tone [11–13]. Therefore, structural changes in the coronary vasculature are most likely to be the major contributors to altered CFR. Quantitative histological studies performed on septal biopsy tissue showed that reduced coronary dilatory capacity was associated with increased arteriolar media area, and perivascular and interstitial fibrosis in patients with arterial hypertension and angina pectoris in the absence of relevant coronary artery stenosis [12]. These conditions are sensitive indicators of hypertensive target-organ damage [12,13]. Furthermore, it has been suggested that impairment of CFR may occur very early in hypertension before hypertrophy is apparent, and may cause subsequent ischemia and fibrosis [11]. Thus, the search for impaired CFR may be recommended as part of global risk assessment. Accordingly, the present study was performed to evaluate the association between serum GGT concentrations and impaired CFR in a group of middle-aged never-treated and newly diagnosed patients with essential hypertension without concomitant risk factors.

## Methods

### Study population

The overall study population included 100 never-treated and newly diagnosed essential hypertensive subjects. In each subject, blood pressure was measured on three separate days in a week, after 15 min of sitting comfortably, and these three values were averaged. Individuals who had diastolic blood pressure (BP)  $\geq 90$  mmHg and/or systolic BP  $\geq 140$  mmHg in the office setting were diagnosed as hypertensive. A complete physical examination was performed, with particular attention to peripheral arterial pulses and carotid bruits. Each subject was questioned about major CV risk factors, including family history of CAD, current smoking status, alcohol consumption and diabetes mellitus. Family history of CAD was obtained by questioning the subjects about CAD in first-degree male relatives before 55 years and in female relatives before 65 years of age. Age, gender and body mass index (BMI) were recorded. Fasting blood glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglyceride levels, which were measured with original kits using an Abbott-Aeriset autoanalyzer (Chicago, Illinois, USA), were noted. Plasma levels of C-reactive protein were measured by use of a highly sensitive sandwich enzyme-linked immunosorbent assay (ELISA) using the Abbott-Aeriset autoanalyzer. Serum GGT levels were measured at 37°C by enzymatic calorimetric test using a Roche/Hitachi analyzer (Mannheim, Germany) [2]. The normal reference value of the GGT level for a healthy individual was 8–61 U/l in our laboratory. The hypertensive subjects were divided into two groups based on median value of serum GGT levels (21 U/l for women, 30 U/l for men): 49 subjects with lower GGT (group I) and 48 subjects with higher GGT level (group II). Tests for interaction between GGT and gender were significant; therefore, all analyses were conducted with stratifying for gender.

Inclusion criteria included 18–55 years of age, and a regular menstrual cycle for women. Exclusion criteria included the presence of any systemic disease such as hemolytic, hepatic, and renal diseases or any disease that could impair CFR [e.g. diabetes mellitus: fasting plasma glucose level measured on three separate days in a week  $> 126$  mg/dl (7.0 mmol/l); or impaired oral glucose tolerance test: fasting plasma glucose  $< 126$  mg/dl (7.0 mmol/l) but 2-h plasma glucose after a 75-g oral glucose challenge  $> 140$  mg/dl (7.8 mmol/l)], family history of CAD and excessive alcohol consumption ( $> 50$  g/day). Subjects were excluded from the study if they used any vasoactive drug, had undergone previous antihypertensive therapy, were current smokers and had ST-segment or T-wave changes specific for myocardial ischemia (including strain pattern), Q waves and incidental left bundle branch block on ECG. Individuals were also excluded if they had severe dyslipidemia and/or excessive obesity (HDL

cholesterol levels  $< 30$  mg/dl, LDL cholesterol levels  $> 160$  mg/dl, triglyceride levels  $> 400$  mg/dl, BMI greater than 35 kg/m<sup>2</sup>), elevated other liver enzymes, or left ventricular mass index (LVMI)  $> 126$  g/m ( $> 48$  g/m<sup>2.7</sup>) in men and  $> 99$  g/m ( $> 44$  g/m<sup>2.7</sup>) in women [14] (to avoid the confounding effects of LVH on CFR). Written informed consent was obtained from each subject. The institutional ethics committee approved the study protocol.

### Echocardiographic examination

Each subject was examined using an Acuson Sequoia C256 Echocardiography System equipped with 3V2c and 5V2c broadband transducers with second harmonic capability (Acuson Corp., Mountain View, California, USA). Two-dimensional, M-mode and subsequent transthoracic Doppler echocardiographic examinations were performed on each subject.

### Left ventricular mass determination

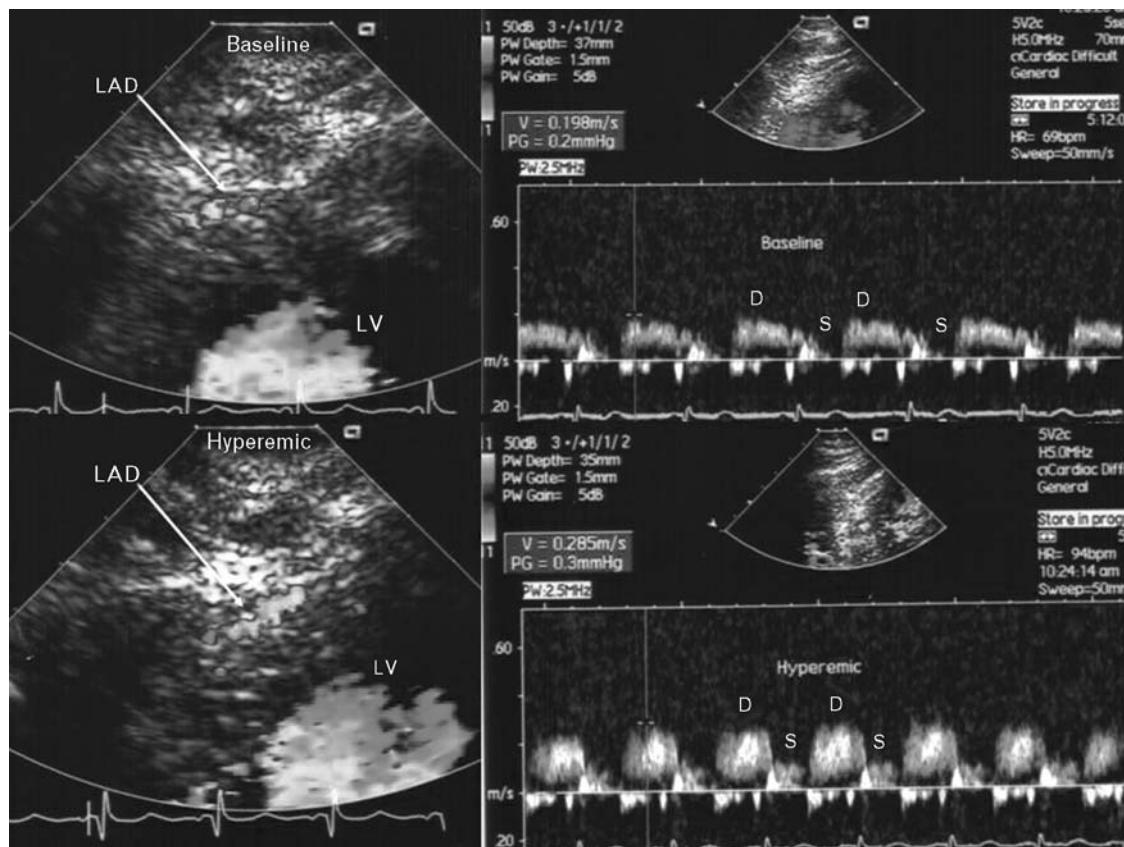
Left ventricular mass (LVM) was calculated from M-mode records taken on parasternal long-axis images according to the formula below (corrected American Society of Echocardiography cube method) [14,15].

$$\text{LVM} = 0.8 \times (1.04[(\text{IVSd} + \text{PWD} + \text{LVDD})^3 - (\text{LVDD})^3]) + 0.6 \text{ g},$$

where IVSd is the interventricular septum thickness at diastole; PWD, the posterior wall thickness at diastole; and LVDD, the left ventricular diastolic diameter. To take into account differences in body size that might influence cardiac size, left ventricular mass was divided by height and height<sup>2.7</sup> to create an LVMI.

### Coronary flow reserve measurement

The visualization of the distal left anterior descending coronary artery (LAD) was performed using a modified, foreshortened, two-chamber view obtained by sliding the transducer on the upper part and medially from an apical two-chamber view, to reach the best alignment to the interventricular sulcus. Coronary flow in the distal LAD was examined by color Doppler flow mapping over the epicardial part of the anterior wall, with the color Doppler velocity set in the range of 8.9–24.0 cm/s [16]. The left ventricle was imaged on the long-axis cross-section, and the ultrasound beam was then inclined laterally. Next, coronary blood flow in the LAD (middle to distal) was searched by color Doppler flow mapping (Fig. 1). All subjects had Doppler recordings of the LAD with dipyridamole infusion at a rate of 0.56 mg/kg over 4 min. By placing the sample volume on the color signal, spectral Doppler of the LAD showed the characteristic biphasic flow pattern with larger diastolic and smaller systolic components (Fig. 1). Coronary diastolic peak velocities were measured at baseline and after dipyridamole by

**Fig. 1**

Mid to distal segment of the left anterior descending coronary artery (LAD) in color-coded transthoracic Doppler echocardiography (arrows): spectral Doppler coronary blood flow by sampling in the mid to distal segment of the LAD. LV, left ventricle; S, systole; D, diastole.

averaging the highest three Doppler signals for each measurement. CFR was defined as the ratio of hyperemic to baseline diastolic peak velocities [16]. CFR  $\geq 2.0$  was considered normal [16–18]. CFR measurement was achieved in 97 of 100 subjects (97%). To test the coefficient of repeatability of the CFR measurement, the measurement was repeated in 10 subjects 2 days later. Intra-observer intra-class correlation coefficients for coronary flow measurements were 0.902 and 0.852 (baseline and hyperemic diastolic peak velocities, respectively), and for CFR value it was 0.886.

#### Statistical analyses

All analyses were conducted using SPSS 9.0 (SPSS Inc., Chicago, Illinois, USA). The groups were compared using the Student *t*-test for continuous variables and chi-squared for categorical variables. Participants were also divided into quartiles of GGT concentration (25th, 50th and 75th percentiles), and the cut-off points were 19, 30 and 49 U/l among men and 15, 21 and 30 U/l among women for the categories of GGT used, respectively. Pearson's correlation analysis was used to test univariate relations. Prediction of variables was obtained by stepwise, forward,

multiple regression including potential confounders (age, sex, BMI, BP, lipids, glucose and LVMI). The receiver-operating characteristic (ROC) curve was determined to evaluate the predictive performance of GGT to detect low CFR. The area under the ROC curve (AUC) and its standard error were calculated. A *P* value of  $< 0.05$  was considered significant.

## Results

#### Clinical characteristics of the study population

The general characteristics and risk factors of the groups are presented in Table 1. The following were similar within the lower and higher GGT groups: age, gender, BMI, systolic and diastolic BP, heart rate, lipid profiles except triglyceride, and hemoglobin. Uric acid and fasting glucose levels were slightly higher, and triglyceride and hsCRP levels were significantly higher in the higher GGT group than in the lower GGT group (Table 1).

#### Analyses of echocardiographic measurements

Interventricular septum thickness, left ventricle posterior wall thickness, left ventricular diastolic diameter, left ventricular systolic diameter, left atrium diameter and

**Table 1 Demographic and biochemical characteristics of the two  $\gamma$ -glutamyltransferase (GGT) groups**

	Patients with lower GGT (n=49)	Patients with higher GGT (n=48)	P
Age (year)	46.6 ± 7.4	45.0 ± 6.9	0.26
Male/female (n/n)	27/22	24/24	0.68
Body mass index (kg/m <sup>2</sup> )	28.3 ± 2.6	27.6 ± 3.0	0.36
Systolic BP (mmHg)	145.4 ± 7.1	146.4 ± 6.9	0.49
Diastolic BP (mmHg)	92.2 ± 5.7	92.2 ± 3.9	0.97
Heart rate (bpm)	73.4 ± 9.1	71.4 ± 8.1	0.27
Total cholesterol (mg/dl)	192.3 ± 30.6	203.5 ± 32.6	0.09
HDL cholesterol (mg/dl)	46.3 ± 8.4	47.4 ± 10.2	0.20
LDL cholesterol (mg/dl)	121.7 ± 24.6	121.3 ± 26.5	0.95
Triglyceride (mg/dl)	127.2 ± 47.0	153.0 ± 65.2	0.03
Hemoglobin (g/dl)	15.1 ± 1.2	14.9 ± 0.8	0.62
hsCRP (mg/l)	2.4 ± 2.2	4.2 ± 2.3	< 0.001
Glucose (mg/dl)	94.4 ± 6.6	96.7 ± 8.0	0.14
Uric acid (mg/dl)	4.8 ± 1.6	5.2 ± 1.6	0.10
GGT (U/l)	17.6 ± 6.6	40.3 ± 12.7	< 0.001

BP, blood pressure; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.

left ventricular ejection fraction were similar between the lower and higher GGT groups, but LVMI was borderline greater in the higher GGT group than in the lower group. Mitral A<sub>max</sub> and mitral E/A ratio were slightly different between the groups (Table 2).

#### Analysis of coronary flow reserve measurements

Baseline and peak heart rate and BPs were similar between the two groups. Baseline diastolic peak flow velocity (DPFV) did not significantly differ between the lower and higher GGT groups. However, hyperemic DPFV and CFR were significantly higher in the lower GGT group than in the higher GGT group (Table 2).

#### Relationship between serum $\gamma$ -glutamyltransferase levels and coronary flow reserve

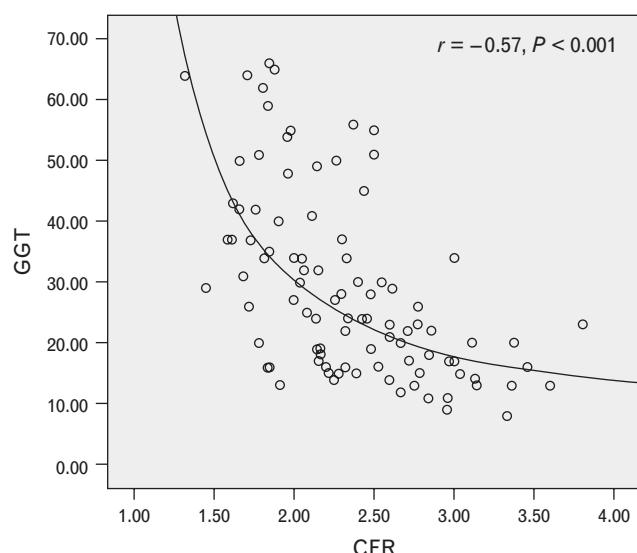
GGT levels were inversely and significantly correlated with CFR (Fig. 2). In addition, after dividing the patients

into quartile-based GGT concentrations (25th, 50th and 75th percentiles), we showed that CFR decreased progressively with higher GGT levels (2.66 ± 0.53, 2.53 ± 0.54, 2.21 ± 0.34 and 1.98 ± 0.37; from quartiles 1 to 4, respectively) (Fig. 3). Furthermore, in stepwise linear regression analysis, when CFR was taken as dependent, and GGT and other study variables including age, sex, systolic and diastolic BP, LVMI, heart rate, hsCRP and lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride) as independent, we found that only GGT level ( $\beta = -0.62$ ,  $P < 0.0001$ ) and mitral E/A ratio ( $\beta = 0.22$ ,  $P < 0.05$ ) were independently correlated with CFR. We also demonstrated that GGT level was an accurate predictor of low CFR on the receiver-operating characteristic (ROC) curve. The area under the curve (AUC) was 79% (95% confidence interval 0.70–0.88), and GGT levels were significantly predictive of low CFR ( $P < 0.0001$ ) (Fig. 4).

**Table 2 Data from echocardiographic examinations of the study subjects**

	Patients with lower GGT (n=49)	Patients with higher GGT (n=48)	P
IVS (cm)	1.07 ± 0.11	1.07 ± 0.12	0.78
PW (cm)	1.01 ± 0.11	1.01 ± 0.11	0.95
LVDD (cm)	4.59 ± 0.36	4.58 ± 0.43	0.82
LVSD (cm)	2.89 ± 0.26	2.89 ± 0.30	0.58
LAD (cm)	3.32 ± 0.30	3.33 ± 0.28	0.76
EF (%)	66.8 ± 2.9	66.6 ± 2.5	0.70
LVMI (g/m)	66.3 ± 13.6	69.5 ± 16.8	0.11
LVMI (g/m) <sup>2.7</sup>	33.9 ± 6.2	35.7 ± 5.0	0.12
Mitral E <sub>max</sub> (cm/s)	71.1 ± 16.2	70.0 ± 15.5	0.74
Mitral A <sub>max</sub> (cm/s)	68.0 ± 13.9	73.3 ± 14.7	0.07
E/A	1.07 ± 0.25	0.98 ± 0.25	0.09
Mitral E deceleration time (s)	207.4 ± 44.2	217.6 ± 34.9	0.21
Baseline heart rate (bpm)	73.2 ± 6.9	72.1 ± 11.2	0.78
Baseline systolic BP (mmHg)	148.9 ± 8.4	150.1 ± 7.3	0.24
Baseline diastolic BP (mmHg)	91.8 ± 4.3	90.1 ± 4.9	0.63
Peak heart rate (bpm)	95.2 ± 12.5	94.9 ± 12.2	0.90
Peak systolic BP (mmHg)	138.1 ± 6.1	142.9 ± 8.8	0.12
Peak diastolic BP (mmHg)	89.7 ± 3.4	90.6 ± 3.8	0.32
Baseline DPFV (cm/s)	24.5 ± 4.3	26.5 ± 6.6	0.08
Hyperemic DPFV (cm/s)	63.1 ± 17.2	55.2 ± 14.7	< 0.01
CFR	2.57 ± 0.54	2.10 ± 0.36	< 0.001

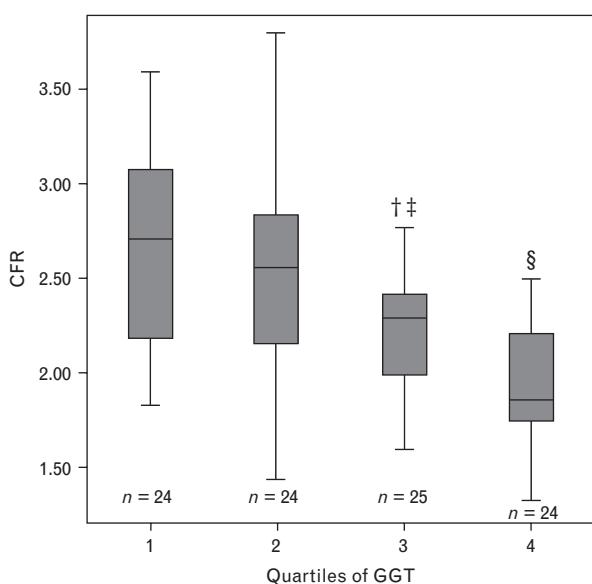
BP, blood pressure; CFR, coronary flow reserve; DPFV, diastolic peak flow velocity of left anterior descending coronary artery; EF, ejection fraction; GGT,  $\gamma$ -glutamyltransferase; IVS, interventricular septum; LAD, left anterior descending coronary artery; LVMI, left ventricular mass index; LVDD, left ventricular diastolic diameter; LVSD, left ventricular systolic diameter; PW, posterior wall.

**Fig. 2**

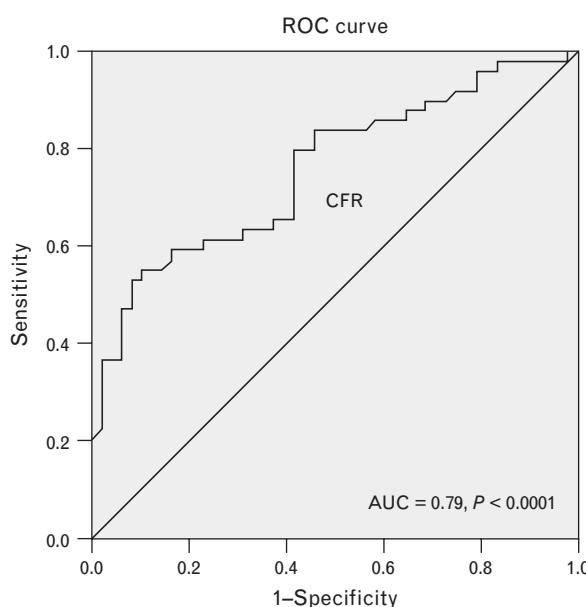
Relationship between serum  $\gamma$ -glutamyltransferase (GGT) levels and coronary flow reserve (CFR).

### Relationships of serum $\gamma$ -glutamyltransferase levels and coronary flow reserve with other study variables

Serum GGT level correlated significantly with glucose level ( $r=0.29$ ,  $P=0.004$ ), triglyceride level ( $r=0.37$ ,  $P<0.001$ ), hsCRP level ( $r=0.34$ ,  $P=0.001$ ), LVMI ( $r=0.23$ ,  $P=0.02$ ), and mitral E/A ratio ( $r=-0.20$ ,

**Fig. 3**

Coronary flow reserve (CFR) decreased progressively with higher  $\gamma$ -glutamyltransferase (GGT) levels.  $^{\dagger}P<0.01$  versus quartile 1;  $^{\ddagger}P<0.05$  versus quartile 2;  $^{\S}P<0.001$  versus quartiles 1 and 2.

**Fig. 4**

Receiver-operating characteristic (ROC) curve analysis of serum  $\gamma$ -glutamyltransferase (GGT) levels for low coronary flow reserve (CFR). Diagonal segments are produced by ties. AUC, area under the curve.

$P=0.04$ ). CFR correlated significantly with glucose level ( $r=-0.19$ ,  $P=0.04$ ), triglyceride level ( $r=-0.25$ ,  $P=0.02$ ), hsCRP level ( $r=-0.37$ ,  $P<0.001$ ), mitral A ( $r=-0.21$ ,  $P=0.02$ ), and mitral E/A ratio ( $r=0.32$ ,  $P=0.002$ ).

### Discussion

The present study used second harmonic transthoracic Doppler echocardiography (TTDE) for CFR determination to evaluate the possible association between serum GGT levels and CFR in arterial hypertension where changes in afterload, renal and LV structure may influence coronary blood flow supply. Hypertensive subjects were divided into two groups based on the median value of GGT, with the cut-off point 30 for men and 21 for women. The main findings of the study were that hypertensive subjects with higher GGT had altered CFR, and that an independent association between serum GGT levels and CFR was evident in essential hypertensive individuals, who were newly diagnosed, had never taken any antihypertensive therapy (including diuretics) and did not have any systemic disease except hypertension. To our knowledge, this is the first study showing an independent association between GGT levels and CFR. Accordingly, CFR was significantly different in our hypertensive subjects with lower and higher GGT.

GGT has a protective function in the antioxidant system with maintaining appropriate hepatic glutathione levels,

which is a crucial antioxidant defense for the cells [19,20]. In addition, it is well known that substantial oxidative stress exists in hypertension [21]. In line with these suggestions, recent studies have reported that there is a positive association between serum GGT levels and BP [22,23]. In addition, it has recently been shown that there is a relationship between serum GGT levels and microalbuminuria, a marker of hypertensive target organ damage [9].

Coronary endothelial dysfunction resulting in an ineffective vasodilator function is common in hypertensive individuals. Reduced CFR is largely the result of minimal changes in coronary resistance that are independent of vascular tone [11–13]. Hypertensive pressure overload of the left ventricle and coronary circulation has several consequences for the coronary circulation: capillaries and myocytes are likely to suffer damage if perfusion pressure in this part of the coronary circulation is increased, and myocyte hypertrophy leads to an increased distance between arterioles oriented in parallel. The thickening of the wall of resistance vessels can be caused by an increase in the number or diameter of the single smooth muscle cells in the media, leading to an increase in the cross-sectional area of the vessel wall (hypertrophy), or by a reorganization of smooth muscle cells without an increase in the vascular wall area (remodeling) [24–26]. A relative or absolute decrease in the number of resistance vessels per myocardial volume in the presence of chronic increased coronary perfusion pressure may also lead to a normalized terminal perfusion pressure. Otherwise, a diminished number of parallel resistance vessels may reduce vasodilator capacity [26,27]. Taken together, these findings imply that structural changes in the coronary vasculature are most likely to be the major contributors to impaired CFR. These structural changes may be qualitatively similar to the well-described effects of hypertension on the peripheral circulation [28]. Accordingly, it has been reported that reduced coronary dilatory capacity was associated with increased arteriolar media area, and with perivascular and interstitial fibrosis in patients with arterial hypertension in the absence of relevant coronary artery stenosis [12]. These findings are surrogate markers of hypertensive target organ damage [12].

Considering the fact that impaired CFR is a surrogate marker of hypertensive target organ damage, and GGT is possibly a marker of oxidative stress and is associated with target organ damage in hypertension, we performed the present study to examine whether GGT is a predictor of impaired CFR among those with newly diagnosed hypertension.

In this study we excluded hypertensive subjects with confounding factors, which are commonly encountered in normal population, such as left ventricular hypertrophy,

diabetes mellitus, obesity, dyslipidemia and CAD, to investigate the independent association between GGT levels and CFR. Therefore, the study does not provide information about the association between serum GGT levels and CFR in the hypertensive population overall.

In the present study, a relatively low dose of dipyridamole was used for hyperemic stimulus. In addition, dipyridamole mildly dilates epicardial coronary vessels. The standardized pharmacologic protocol of dipyridamole to measure hyperemic coronary flow, and thus the CFR, is still controversial, and a 0.56 mg/kg dose has generally been used in most previous studies [16,29,30]. Thus, we used low-dose dipyridamole (0.56 mg/kg over 4 min) for CFR assessment.

In conclusion, the present study demonstrated an independent association between serum GGT levels and CFR impairment in untreated essential hypertensive subjects, even in the absence of known CV risk factors. These results support a role for GGT level as an independent marker of target organ damage in hypertension.

### Acknowledgements

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# GAMMA-GLUTAMYL TRANSPEPTIDASE LEVEL ASSOCIATED WITH METABOLIC SYNDROME AND PROINFLAMMATORY PARAMETERS IN THE YOUNG ROMA POPULATION IN EASTERN SLOVAKIA: A POPULATION-BASED STUDY

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## SUMMARY

**Background:** Elevated gamma-glutamyl transpeptidase (GGT) is present approximately in half of all patients with non-alcoholic fatty liver disease (NAFLD). NAFLD is the liver manifestation of metabolic syndrome (MS). This study aimed to explore the relationship between GGT and MS or proinflammatory parameters.

**Methods:** Data from the cross-sectional HepaMeta study conducted in Slovakia in 2011 among Roma living in rural communities were used. Participants ( $n = 446$ ) were divided into 2 groups: those with elevated GGT and those with normal GGT levels. MS was diagnosed according to the International Diabetes Federation criteria: presence of central obesity and low density lipoproteins (LDL) or high density lipoproteins (HDL), high triglycerides, hypertension, glucose intolerance or type 2 diabetes. Participants were tested for the presence of MS and its components, and biochemical tests for lipid levels (total cholesterol, HDL, LDL, TG) and inflammatory parameters (high sensitivity C-reactive protein – hs-CRP and ferritin) were performed.

**Results:** Of 446 Roma participants, only 29 (6.5%) had GGT levels above the normal value. After exclusion of patients with viral hepatitis and alcohol abuse, patients with elevated GGT suffered from MS more often ( $p < 0.001$ ), and patients with more MS components had a higher risk of elevated GGT. We found a significant association between GGT and the individual MS components, except HDL (waist circumference  $\geq 94\text{cm}$  in men or  $80\text{ cm}$  in women:  $p < 0.01$ ; BMI  $> 30$ :  $p < 0.001$ ; fasting glucose  $\geq 5.6 \text{ mmol/l}$ :  $p < 0.001$ ; arterial hypertension:  $p < 0.05$ , and TAG  $\geq 1.7 \text{ mmol/l}$ :  $p < 0.001$ ). Patients with elevated GGT levels had also significantly higher hs-CRP (hs-CRP  $> 2 \text{ mg/l}$ :  $p < 0.001$ ; hs-CRP  $> 3 \text{ mg/l}$ :  $p < 0.001$ ) and ferritin (ferritin  $> 300 \text{ mg/l}$ :  $p < 0.01$ ) levels.

**Conclusion:** Patients with MS have more significantly elevated levels of GGT. There is a significant association of GGT with individual MS components, except HDL and inflammatory parameters (hs-CRP, ferritin).

**Key words:** gamma-glutamyl transpeptidase, metabolic syndrome, non-alcoholic fatty liver disease, hs-CRP, ferritin

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## INTRODUCTION

Gamma-glutamyl transpeptidase (GGT), a plasma membrane-bound enzyme, is an important catalyst which facilitates glutathione hydrolysis. It is found in many organs, but its presence in the

liver has a significant diagnostic use (1). GGT levels are elevated in most diseases of the liver, especially in alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis (1, 2). GGT, together with obesity and hypertriglyceridemia, are the best markers of NAFLD (3). Approximately 50% of nondiabetic NAFLD patients have elevated GGT levels (4). Elevated GGT level has relatively high sensitivity but low specificity for the diagnosis of NAFLD (5), but we can safely assume that most European patients with elevated GGT, after the exclusion of those

\*HepaMeta Team members are listed in Appendix

with chronic viral hepatitis and alcohol overuse, have NAFLD. The aims of this study were to evaluate the prevalence of elevated GGT in Roma aged 15–45 years in communities in eastern Slovakia; analyse the cause of GGT elevation in this population; analyse the association of elevated GGT with metabolic syndrome (MS) and the individual criteria of metabolic syndrome; analyse the relationship between inflammatory markers (hs-CRP and ferritin) with elevated GGT in patients with NAFLD; analyse the influence of some socioeconomic parameters, diet and physical activity on elevated levels of GGT.

## MATERIALS AND METHODS

Data from the cross-sectional HepaMeta study conducted in Slovakia in 2011 were used. This project aimed to map the prevalence of viral hepatitis B/C and MS in the population living in eastern Slovakia, including Roma settlements. In addition to the general methodology described in detail elsewhere (6), further paper-specific amendments to the methodology follow.

Only Roma participants ( $n=452$ ) were included in this analysis. Participants were considered to have active HBV infection if they were HBsAg positive. Patients with anti-HBc IgG or antiHBsAg positivity were considered to have had encountered HBV in the past or were otherwise vaccinated. GGT levels were considered to be elevated if they were higher than  $0.98 \mu\text{kat/l}$  in men and  $0.66 \mu\text{kat/l}$  in women.

## Social Status and Lifestyle Variables

The questionnaire was developed by a group of experts made up of Roma health mediators and community workers as well as public health experts and academics. It was designed to gather information about socioeconomic background based on attributes of living conditions such as housing, family and per capita income, education and health education, occupation and consumer luxury items e.g., automobiles, television, refrigerator, air conditioning available in the family.

For the majority population, trained assistants were present in the outpatient clinic to assist with questionnaires, if needed. For Roma respondents, questionnaires were administered in community centres by community workers or trained assistants who provided help in case of limited literacy (7).

Physical activity was measured by a questionnaire detailing occupational, household, and leisure time physical activity. Sedentary lifestyle assessment was based on occupational or household activity, along with leisure time activity measures, according to the classification of activities specific to other populations, as previously described (8). The variety of physical activities was measured by asking respondents what physical activity, if any, they had performed during the last week. They could select one or more possibilities from the following list: physical activity at work; physical work around the house or home; brisk walking; dancing; sport; no physical activity. Only walking, dancing or sport activities were analysed. Respondents were also asked how often each week they perform physical activity lasting at least 30 minutes, during which they became breathless or sweaty. Possible responses were: every day; 4–6 times a week; 2–3 times a week; once a week; 2–3 times a month; a few times a year or less. Those

who reported being physically active 2 or more times a week were considered to be sufficiently physically active (8).

## Statistical Analysis

Categorical data is presented in absolute counts and percentages; interval data is presented as a median (interquartile range) because of nonparametric distribution. Measurement of the statistical significance of differences between categorical data was performed using the chi-square test, and for interval data with the Mann-Whitney U test.

## RESULTS

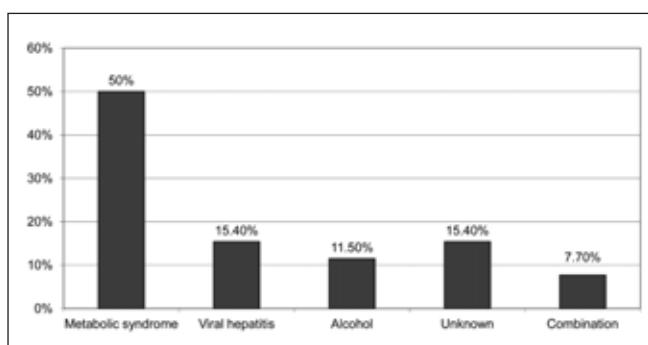
The final sample comprised 452 Roma (mean age = 34.7; SD = 9.14; 35.2% men). The number of participants available for individual analyses is stated separately, as not all results were available for each participant. Baseline parameters of the study cohort are summarised in Table 1.

Out of 446 Roma participants with available GGT test results, only 29 (6.5%) had GGT levels above the normal value. Diseases associated with GGT elevation are depicted in Fig. 1. The most common disease in patients with elevated GGT was metabolic syndrome (in 50% of cases) followed by viral hepatitis (15.4% of cases), 15.4% of participants had elevated GGT with no apparent disease, while 11.5% of participants with higher GGT levels confirmed excessive alcohol use, and 7.7% of participants had a combination of the aforementioned conditions. There was a significantly higher association of metabolic syndrome with elevated GGT ( $p=0.004$ ).

## Laboratory and Clinical Tests in Patients with Elevated GGT

For further analyses all patients with HBs antigen or anti-HCV antibody positivity (56 participants, 12.4%) as well as patients with significant alcohol use (24 participants, 5.3%) together with 12 patients with missing data about viral hepatitis serology or alcohol abuse were excluded. Thus, 354 patients were further analysed. Out of them, 336 participants had a normal GGT serum level and 18 had an elevated GGT serum level.

Table 2 summarizes the clinical data and risk factors for metabolic syndrome. We observed statistically significant difference



**Fig. 1.** Diseases most frequently associated with elevated GGT in the study cohort.

**Table 1.** Baseline parameters of the study cohort by gender

	Men Median (IQR) N=159	Women Median (IQR) N=293	p
Age (years)	34.83 (15.50)	35.54 (15.45)	0.142
BMI ( $\text{kg}/\text{m}^2$ )	26.30 (8.59)	25.45 (8.26)	0.573
Waist (cm)	90 (23.00)	86 (19.00)	<0.001
sBP (mmHg)	126 (19.67)	116 (18.00)	<0.001
dBP (mmHg)	76 (16.00)	72 (14.00)	0.007
TG (mmol/l)	1.14 (0.84)	1.03 (0.77)	0.016
Cholesterol (mmol/l)	4.63 (1.28)	4.74 (1.21)	0.12
LDL (mmol/l)	2.38 (0.96)	2.51 (0.83)	0.397
HDL (mmol/l)	0.96 (0.36)	1.11 (0.35)	<0.001
Fasting glucose (mmol/l)	4.83 (0.73)	4.59 (0.74)	<0.001
hs-CRP (mg/l)	1.88 (3.80)	1.53 (3.18)	0.153
Ferritin (mg/l)	336.45 (305.00)	73.65 (90.25)	<0.001
Metabolic syndrome (%)	29.7% (47 participants)	29.6% (84 participants)	0.97

**Table 2.** Clinical data and risk factors of metabolic syndrome in groups with normal and elevated GGT

	N	GGT normal Median (IQR) or n (%)	GGT elevated Median (IQR) or n (%)	p
Age (years)	441	35.17 (15.04)	42.41 (15.60)	0.006
BMI ( $\text{kg}/\text{m}^2$ )	354	25.4 (8.55)	32.4 (9.76)	<0.001
sBP (mmHg)	341	118.0 (19.00)	127.0 (33.00)	0.005
dBP (mmHg)	341	72.0 (14.00)	82.0 (19.00)	0.001
Waist (cm)	348	87.0 (20.00)	104.5 (29.00)	<0.001
Weight (kg)	343	65.0 (22)	81.0 (12)	0.001
Metabolic syndrome	352	90 (26.9)	13 (76.5)	<0.001
Fulfilled $\geq 3$ MS criteria	336	90 (28.2)	13 (76.5)	<0.001
Fulfilled $\geq 4$ MS criteria	336	36 (11.3)	10 (58.8)	<0.001
Fulfilled 5 MS criteria	336	11 (3.4)	5 (29.4)	<0.001
BMI $> 25 \text{ kg}/\text{m}^2$	341	168 (51.7)	15 (93.8)	0.001
BMI $> 30 \text{ kg}/\text{m}^2$	343	87 (26.6)	11 (68.8)	<0.001
Arterial hypertension	342	94 (29.0)	10 (55.6)	0.017
Waist circumference**	354	184 (54.8)	16 (88.9)	0.004
Specific treatment for hypercholesterolemia	354	8 (2.4)	4 (22.2)	<0.001

\*\*  $\geq 94 \text{ cm}$  (men) or  $80 \text{ cm}$  (women)

in BMI, body weight, waist circumference, and blood pressure. Patients with elevated GGT had also higher risk of having metabolic syndrome and they met significantly more MS criteria.

Biochemical parameters, presented both as interval variables and MS criteria, in patients with elevated and normal GGT levels are summarized in Table 3. Patients with elevated GGT had significantly higher serum levels of cholesterol, TG, glucose, hs-CRP, and ferritin. After exclusion of patients on hypolipidemic treatment, we found significantly higher levels of LDL cholesterol in patients with elevated GGT, although no difference was found in HDL cholesterol. Significantly more patients with elevated GGT met MS criteria for hyperglycemia, hypercholesterolemia, and elevated triglycerides. Interestingly, there was no difference in the incidence of decreased HDL or increased LDL cholesterol

between patients with normal and elevated GGT even after the exclusion of patients on hypolipidemic treatment.

As shown in Fig. 2, participants with elevated GGT met a median of 4 metabolic syndrome criteria compared with participants with normal GGT, who met only a median of 2 criteria. Furthermore, Fig. 3 shows that in patients with normal GGT the largest number (over 30%) met only 1 criterion of metabolic syndrome, and only 3.1% met all five MS criteria. In contrast, patients with the elevated GGT largest numbers met 4 or 5 MS criteria.

### Lifestyle Components in Patients with Elevated GGT

Based on the extensive questioning of almost all of the study participants we have tried to associate some information about

**Table 3.** Biochemical data and risk factors of metabolic syndrome between participants with normal and elevated serum GGT

	N	GGT normal Median (IQR)	GGT elevated Median (IQR)	p
TG (mmol/l)	354	1.2 (0.74)	1.8 (1.72)	<0.001
Cholesterol (mmol/l)	354	4.7 (1.22)	5.1 (1.25)	0.007
LDL (mmol/l)	354	2.5 (0.86)	3.0 (1.02)	0.03
HDL (mmol/l)	354	1.1 (0.36)	0.9 (0.27)	ns
Fasting glucose (mmol/l)	354	4.7 (0.68)	5.2 (1.99)	0.006
hs-CRP (mg/l)	354	1.6 (3.16)	6.3 (8.50)	<0.001
Ferritin (mg/l)	354	104.2 (175.40)	244.5 (451.10)	<0.001
TAG ≥ 1.7 mmol/l	354	70 (20.8)	11 (61.1)	<0.001
Chol ≥ 5.2 mmol/l	354	94 (28.0)	10 (55.6)	0.012
LDL ≥ 3.4 mmol/l	354	32 (9.5)	3 (16.7)	ns
Elevated HDL***	354	234 (69.6)	16 (88.9)	ns
Fasting glucose ≥ 5.6 mmol/l	354	31 (9.2)	7 (38.9)	<0.001
hs-CRP < 1 mg/l*	354	207 (61.6)	16 (88.9)	0.020
hs-CRP 1–3 mg/l*	354	145 (43.2)	16 (88.9)	<0.001
hs-CRP > 3 mg/l*	354	108 (32.1)	16 (88.9)	<0.001
Ferritin > 300 mg/l	354	58 (17.3)	8 (44.4)	0.004

\*hs-CRP < 1 – no CVS risk, \* = 1–3 moderate CVS risk, \*> 3 – high CVS risk; \*\*\*< 1.03 mmol/l (men) or 1.29 mmol/l (women)

eating habits and physical activity with serum levels of GGT. As shown in Table 4, we have not found any statistically significant difference in employment rate, soft drink consumption, diet components, or physical activity in patients with normal and elevated GGT.

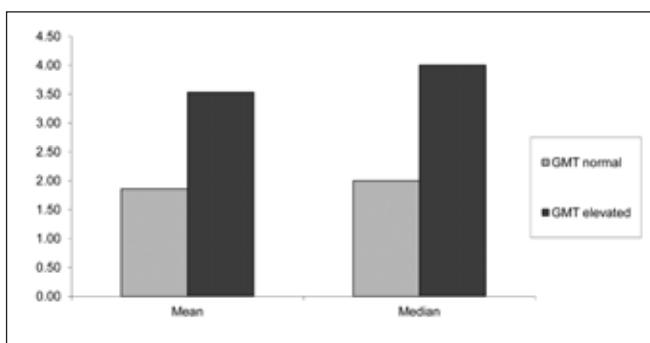
## DISCUSSION

NAFLD is the hepatic manifestation of metabolic syndrome. Insulin resistance plays a pivotal role in NAFLD pathophysiology followed by abnormal accumulation of fat in the hepatocytes (9). NAFLD is present in approximately 16–23% of the general adult population, but it can occur even in childhood (10). In some patients NAFLD progresses through inflammation to liver fibrosis. This process could be divided into 4 stages according to histology: simple steatosis; steatosis with lobular inflammation; hepatocyte ballooning; presence of Mallory hyaline or fibrosis.

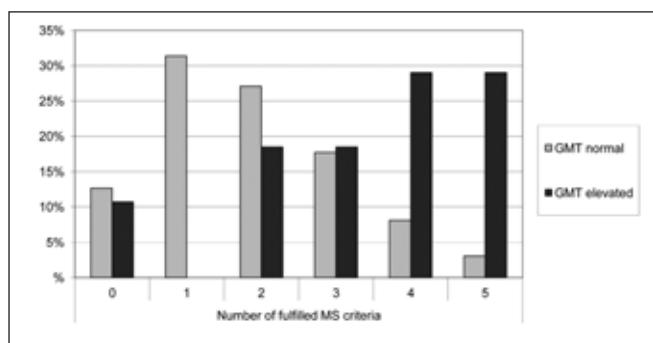
Lobular inflammation with hepatocyte ballooning is the hallmark of nonalcoholic steatohepatitis (NASH) (11, 12). NASH occurs in 2–3% of population and 20–25% patients with NASH progress to liver cirrhosis (13).

We found that 6.5% of Roma had elevated serum levels of GGT. Fifty percent of Roma who had elevated GGT also had metabolic syndrome, 14% had viral hepatitis, 10% confessed to alcohol abuse, and 7% had a combination of at least two of the aforementioned factors. In participants who had negative serology for viral hepatitis B or C, without significant alcohol use and with elevated GGT, metabolic syndrome was present in more than 75% of cases. Metabolic syndrome was the most common disease associated with elevated GGT in the Roma population.

Although elevated GGT has relatively high sensitivity for NAFLD, more tests are required for confirmation of the diagnosis. Foremost, it is necessary to rule out other liver diseases, specifically alcoholic liver disease and viral hepatitis. Insulin resistance or diabetes mellitus and hypercholesterolemia support the diagnosis of NAFLD. Ultrasound of the liver shows remark-



**Fig. 2.** Mean and median of fulfilled MS criteria among participants with normal and elevated GGT.



**Fig. 3.** Percentage of participants with normal and elevated GGT who fulfilled the criteria of MS.

**Table 4.** Eating habits, physical activity and employment rate in patients with normal and elevated levels of GGT

	N	GGT normal n (%)	GGT elevated n (%)	p
Employed	350	33 (9.9)	1 (6.3)	ns
More than 1 family in household	350	143 (43.1)	6 (33.3)	ns
Soft drinks	350	245 (73.8)	10 (55.6)	ns
Fruit in diet	347	174 (52.9)	11 (61.1)	ns
Vegetables in diet	345	166 (50.8)	11 (61.1)	ns
Dairy products in diet	342	188 (58.0)	11 (61.1)	ns
Meat products in diet	350	265 (79.8)	12 (66.7)	ns
Meat in diet	341	214 (66.3)	11 (61.1)	ns
Wheat products in diet	342	225 (69.4)	13 (72.2)	ns
Physical activity – walk	354	63 (18.8)	2 (11.1)	ns
Physical activity – dance	354	56 (16.7)	1 (5.6)	ns
Physical activity – sport	354	36 (10.7)	2 (11.1)	ns
No physical activity	354	36 (10.7)	3 (16.7)	ns
Any physical activity over 30min	350	99 (29.8)	6 (33.3)	ns

ably increased echogenicity of liver tissue (14). The presence of liver fibrosis is important for the staging of NAFLD. It could be assessed via liver biopsy (invasive) or noninvasive methods, such as transient elastography or commercially available fibrosis biomarkers (e.g., Fibrotest®) (15).

After the exclusion of patients with significant use of alcohol and viral hepatitis, approximately 5% of the study population had elevated GGT. Although GGT is one of the more sensitive markers of NAFLD, it is elevated in about half of patients with NAFLD (4). The Dionysos study results show that NAFLD has been confirmed in about 25% of patients with suspected liver disease and 20% of patients without suspected liver disease ( $p=0.203$ ). Patients without suspected liver disease underwent the diagnostic procedure because of metabolic syndrome. Based on the available data we can approximate that the NAFLD prevalence in the study cohort is about 10% in 15–45 years old participants. The overall prevalence of NAFLD in the Roma community is probably much higher, because it increases with age and this study had the upper age limit of 45 years. (16). Further research is needed to estimate the prevalence of NAFLD in this specific community, since significant ethnical differences have been previously reported (17).

Prevalence of obesity ( $BMI > 30 \text{ kg/m}^2$ ) was 26.1%. This number is comparable to other epidemiological studies performed in Slovakia (18), but is very high compared to the prevalence of obesity in India (19), the country Roma people originated in (20).

Metabolic syndrome was present in 76.5% of participants with elevated GGT compared with only 26.9% of participants with normal GGT ( $p<0.001$ ). Patients with a higher number of fulfilled MS criteria had a higher risk of having elevated levels of GGT (Fig. 3). Koller et al. showed similar results in a study on 482 patients. The higher the number of MS criteria met, the higher proportion of patients with elevated GGT or increased echogenicity on the liver ultrasound, significant correlations were found not only with MS in general but with each individual MS criterion, except decreased HDL cholesterol (21).

We also found significant correlations with the individual components of metabolic syndrome. Patients with elevated GGT

had higher mean systolic and diastolic blood pressure and were more frequently diagnosed with arterial hypertension. Similar data have been published by Japanese authors, who report that multiple regression analysis showed that the relationship between GGT and blood pressure was independent of age, obesity and alcohol drinking (22, 23). NAFLD is associated with arterial hypertension even after adjustment for age (24).

Similarly to other studies, significant association of obesity with elevated levels of GGT was observed. In a Norwegian study on 21,782 patients aged 12–59 years, the authors found a significant correlation between BMI and GGT elevation. This correlation was even stronger than the relationship between GGT and alcohol consumption, physical activity, blood pressure, or blood lipids (25). With increasing BMI not only does NAFLD prevalence increase, but also the degree of liver fibrosis (26). The prevalence of NAFLD in the obese is 75% compared with 16% in people with normal body weight (27).

Waist circumference is a relatively strong NAFLD predictor with area under the curve (AUC) of 0.88 (95% CI 0.81–0.94) (28). It also correlates with the degree of fibrosis in children with NASH (29). Participants in this study with a waist circumference of more than 80 cm (women) or 94 cm (men) had NAFLD more frequently.

About one-third of patients with elevated GGT had hyperglycemia compared with only 9.2% of patients with normal GGT ( $p<0.001$ ). This fact was reported immediately after introduction of GGT testing into routine practice (2). Elevated GGT is present not only in patients with overt diabetes mellitus but also in patients with impaired glucose tolerance (30). Hyperglycemia is, together with age, albumin level, BMI, thrombocyte count, and AST/ALT ratio, an independent predictor of fibrosis in patients with NAFLD (31).

Patients with elevated GGT also had higher levels of cholesterol and triglycerides, but not HDL cholesterol. LDL cholesterol was also higher in patients with elevated GGT, but the proportion of patients with LDL of more than 3.4 mmol/l was not significantly higher. Janzon et al. found positive correlations between GGT, cholesterol and triglycerides as well as with HDL (32). In

the Norwegian study mentioned above, the authors also found a significant relationship between GGT, cholesterol, HLD, and TG (25). However, this study did not confirm the relationship between GGT and HDL cholesterol, probably because of the low HDL levels in up to 70% of the total study population. Higher serum TAG ( $p=0.0154$ ) and lower levels of HDL-C ( $p<0.001$ ) are independent predictors of fibrosis in NAFLD patients (33).

Approximately 22% of patients with higher GGT were on hypolipidemic treatment, which is 10-times more than patients with normal GGT ( $p<0.001$ ). Long-term statin treatment leads to decrease of fat accumulation in hepatocytes and has the potential to stop fibrogenesis (34). Addition of fibrate could lead to a small decrease in biochemical activity (ALT), while the addition of ezetimibe in patients with NAFLD and diabetes mellitus has no effect on liver steatosis (35, 36).

Cardiac and hepatic fat are associated with insulin resistance and impaired suppression of lipolysis, ultimately leading to lipotoxicity. In the heart the lipotoxic effect translates into an impairment of energetic and mechanical efficiency, whereas in the liver a fibrogenic response is favoured by the abundance of inflammatory cells. These features precede and likely contribute to left ventricular overload and cardiac hypertrophy through mechanisms similar to those observed in the progression of liver damage in NAFLD. Collectively, these findings suggest the presence of complex and intertwined relationships between NAFLD, myocardial steatosis and coronary artery disease (37).

High sensitivity CRP reflects the level of basal inflammatory activity, which plays an important part in the pathogenesis of atherosclerosis. Elevated hs-CRP confers a significant additive cardiovascular risk. Several authors have identified important hs-CRP cut-offs:

- hs-CRP < 1 mg/l is not associated with any cardiovascular risk;
- hs-CRP = 1–3 mg/l is associated with moderate coronary heart disease risk;
- hs-CRP > 3 mg/l is associated with high risk of cardiovascular disease (38, 39).

Significantly more participants with elevated GGT had elevated hs-CRP (at all three cut-offs) than participants with normal GGT. Patients with NAFLD had also higher hs-CRP compared with patients without NAFLD in an Indian study by Kuppan et al. (40). Almost 90% of patients with elevated GGT and about one-third of patients with normal GGT aged under 45 years had an hs-CRP level of more than 3 mg/l. This fact could at least partially explain the higher cardiovascular morbidity in the Roma population in Slovakia (41, 42). C-reactive protein and other inflammatory markers could also worsen the insulin resistance and obesity. Exact mechanisms for this effect are not known, however, various targets have been proposed, including damage to the appetite controlling region of the hypothalamus (43).

Roma with elevated GGT had higher ferritin levels compared with Roma with normal GGT. On multiple regression analysis performed by Kowdley et al., ferritin  $>1.5 \times$  ULN was independently associated with advanced hepatic fibrosis (OR 1.66; 95% CI 1.05–2.62;  $p=0.028$ ) and an increased NAFLD Activity Score (NAS) (OR 1.99; 95% CI 1.06–3.75;  $p=0.033$ ). A ferritin level  $>1.5 \times$  ULN is also associated with hepatic iron deposition and worsened histological activity (44). Ferritin is also considered to be an inflammatory marker and was found to be positively associated with carotid atherosclerosis (45).

The diet and lifestyle are the main determinants of metabolic syndrome (46). Surprisingly, the analysis of socioeconomic status, diet and physical activity did not reveal any significant difference between patients with elevated and normal levels of GGT. The main reason behind this is probably the low total count of participants with elevated GGT.

Elevated GGT levels probably have some effect on cardiovascular mortality. English authors observed 6,997 men with no prior history of coronary heart disease, stroke or diabetes for 24 years. Patients with elevated GGT (top quarter of the range) compared with patients with GGT in the bottom quarter of the range had a significantly higher risk for fatal CHD events (OR 1.43; 95% CI 1.09–1.84), stroke incidence (OR 1.56; 95% CI 1.20–2.04) and CVD mortality (OR 1.40; 95% CI 1.16–1.70). Stronger associations were found between GGT and CVD mortality in younger men (<55 years) and in patients with low and medium CHD risk based on the Framingham risk score (47). Another study of 4,286 women (aged 60–79 years) reported similar results. Patients with elevated GGT had a higher risk of CHD (HR 1.28; 95% CI 1.01–16.2), stroke (HR 1.56; 95% CI 1.02–2.39) or a combination of endpoints (HR 1.31; 95% CI 1.06–1.62). In the entire meta-analysis cohort, with data pooled from 10 studies, 1 U/l higher GGT (on a log scale) was associated with a 20% increase in the risk of coronary heart disease, a 54% increase in the risk of stroke and a 34% increase in the risk of coronary heart disease and stroke combined (48). Austrian authors followed 2,556 subjects with and 699 subjects without angiographic evidence of CAD in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Compared with subjects in the lowest quartile of GGT, patients in the second, third and fourth quartile had a higher risk for all-cause mortality; unadjusted hazard ratios (95% CI) were 1.2 (0.9–1.5), 1.4 (1.1–1.8) and 1.9 (1.5–2.3), respectively. Hazard ratios (CI) for death from cardiovascular causes were 1.4 (1.0–2.0), 1.8 (1.4–2.5) and 2.2 (1.6–2.9), respectively. The authors concluded that serum GGT is predictive of all-cause and cardiovascular mortality in individuals with CAD independently of other cardiovascular risk factors (49). Elevated GGT in these instances is probably the manifestation of NAFLD/NASH, which on its own significantly increases the risk of fatal and nonfatal cardiovascular events and stroke (50).

## CONCLUSION

Approximately half of patients with NASH have elevated levels of GGT. In this study, which included Roma aged 15–45 years from segregated communities, elevated GGT was associated with MS. Patients with elevated GGT had higher chance of meeting more MS criteria, and elevated GGT was associated with individual MS components, HDL excepted. Inflammatory markers (hs-CRP and ferritin) were associated with elevated GGT as well. More research is needed to assess the influence of elevated GGT on mortality in this community and to assess the influence of pharmacological and nonpharmacological interventions on the prognosis.

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# The Relationship of Gamma-Glutamyltransferase to Aortic Elastic Properties in Young Patients with Prehypertension

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## Abstract

Some cross-sectional studies have demonstrated a positive association between serum gamma-glutamyltransferase (GGT) levels and blood pressure. Accordingly, we aimed to analyze serum GGT levels in patients with prehypertension and examine the relationship with aortic elasticity parameters. The study population consisted of 25 newly diagnosed prehypertensive individuals and 25 healthy control subjects. Aortic strain, distensibility index, and stiffness index beta were calculated from aortic diameters measured by echocardiography and blood pressures simultaneously measured by sphygmomanometry. Prehypertensive patients were detected to have significantly lower aortic distensibility and strain indexes compared to control subjects aortic distensibility. However, aortic stiffness index beta of the prehypertensive group was significantly higher compared to that of the control group ( $3.73 \pm 1.41$  vs.  $2.97 \pm 0.82$ ,  $p = 0.02$ ). The mean GGT levels were found to be higher in patients with prehypertension compared to those of controls ( $47.9 \pm 15.9$  U/L vs.  $36.1 \pm 9.4$  U/L,  $p = 0.003$ ). When multiple linear regression analysis was done to clarify the contributions of GGT to aortic elasticity adjusting for age, body mass index, uric acid, serum glucose, heart rate, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride, we observed that only serum GGT levels were significantly associated with aortic elasticity parameters (for aortic strain beta =  $-0.247$ ,  $p < 0.001$ ; for aortic distensibility beta =  $-0.108$ ,  $p < 0.001$ ; for stiffness index beta =  $0.063$ ,  $p < 0.001$ ). Whatever the mechanism is, young patients with prehypertension have higher serum GGT levels compared to healthy control subjects. More importantly, increased GGT levels are independently associated with impaired aortic elasticity in patients with prehypertension.

**Keywords:** prehypertension, aortic elastic properties, young age, gamma-glutamyltransferase (GGT)

## INTRODUCTION

In the Joint National Committee's (JNCs) seventh report, the prehypertension category has been used for the patients whose systolic and diastolic blood pressure values are 120–139 mmHg and 80–89 mmHg, respectively (1). Patients with prehypertension have been shown to be at increased risk for progression to hypertension; those patients with prehypertension are at twice the risk to develop hypertension as those with lower values (2).

It is well appreciated that the aorta has a fundamental role in the function of the cardiovascular system. One of the most important physiological roles of the aorta is buffering Windkessel function, which is its capability to store part of the stroke volume during systole by aortic distention and to transfer this storage into the peripheral

circulation during diastole (3). It has been demonstrated that arterial stiffness, especially large artery stiffness, is an independent determinant of future cardiovascular disease (4, 5). In addition to its potential etiological role in cardiovascular disease, elevated arterial stiffness may serve as an early marker for the detection of asymptomatic atherosclerotic lesions and/or structural changes resulting from hypertension (6, 7). In particular, it has been proven that large artery stiffness is among the best predictors of cardiovascular mortality and morbidity (8). It has been shown that hypertension is one of the most important predictors of reduced arterial elasticity, independent of age (8–10). More importantly, our group has recently demonstrated that aortic elasticity is impaired in young patients with prehypertension compared to healthy controls (11).

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Gamma-glutamyltransferase (GGT) activity, normally found in serum as well as in the plasma membrane of virtually all cells except erythrocytes, catalyzes the first step in the degradation of extracellular glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis, which is the main thiol antioxidant in mammalian cells (12). Serum GGT determination is widely used as a diagnostic test for hepatobiliary diseases and alcohol abuse (13). However, several clinical studies have shown that slightly elevated serum GGT which is almost within the reference range is significantly associated with all-cause mortality, as well as increased risks of myocardial infarction (MI), and stroke (14–18). Also, serum GGT within a range regarded as physiologically normal is shown to be associated with incident hypertension and diabetes mellitus (19, 20). On the other hand, some cross-sectional studies have demonstrated a positive association between serum GGT levels and blood pressure in spite of the fact that this association had not been specifically examined in young prehypertensive patients (21–23). Accordingly, the current study was designated to evaluate whether GGT could be an additional marker of arterial stiffness by using the echocardiographically derived M-mode indices in young patients with prehypertension.

## MATERIAL AND METHODS

### Patients

The study population consisted of 25 newly diagnosed prehypertensive individuals (18 men, mean age = 34 ± 6 years) and 25 healthy control subjects (16 men, mean age = 33 ± 6 years) eligible for the current retrospectively designed study. The diagnosis of prehypertension was established according to the JNC's seventh report (1). Patients with acute or chronic renal dysfunction, diabetes mellitus, metabolic syndrome, impaired glucose tolerance, heart failure, valvular heart disease, history of coronary artery disease or proven coronary artery disease at coronary angiography or noninvasive tests, familial hyperlipidemia, obesity (body mass index [BMI] > 30 kg/m<sup>2</sup>), asthma or chronic obstructive lung disease, pregnancy or taking oral contraceptives, concurrent therapy with medications that might affect blood pressure, history of smoking, history of alcohol consumption, history of coffee intake, aortic disease (Marfan's syndrome, coarctation of aorta, aortic aneurysm, or aortic surgery etc.), and connective tissue disorders were excluded from the study. Moreover, the patients with a hostile echocardiographic environment were not included in the current study.

### Blood Pressure Measurements

Blood pressure was measured three times for each patient with a standard mercury sphygmomanometer on the right arm in the sitting position following 10 min

resting. Phase I and V Korotkoff sounds were used to determine systolic and diastolic blood pressure measurements. In each patient, measurements were performed by the same investigator, in the same room, and at the same time of day. The average of three measurements was used for the analyses.

### Echocardiographic Examination

Transthoracic echocardiography was performed by using a EASOTE 2,5 MHz probe (ESAOTE, Genova, Italy) at the left lateral decubitus position in a standard manner. Echocardiographic measurements were made on the screen by two cardiologists not aware of the patients' clinical data. M mode tracing of the left ventricle were obtained in the parasternal long axis views at a speed of 50 mm/s. Five consecutive cardiac cycles were averaged for every echocardiographic measurement. Left ventricular systolic and diastolic diameters [LVIDs, LVIDd], left ventricular mass index [LVMI], and left atrial systolic diameter [LAd] were calculated from the parasternal long axis view according to standard criteria. The left ventricular ejection fraction [LVEF] was measured by the software using the Teichholz formula. The left ventricular mass was calculated according to the Penn convention (24) and the LVMI was obtained by the left ventricular mass divided by body surface area. Afterwards, the ascending aorta was visualized in the same views. With the M mode, aortic tracing was recorded at the level of approximately 3 cm above the aortic valve. From the M mode recordings, aortic systolic and diastolic diameters [Aos and Aod] were measured. Aos was determined at the time of the full opening of the aortic valve and Aod was determined at the peak of QRS (25). All parameters were measured in five consecutive cardiac cycles and averaged. Simultaneously, cuff brachial artery systolic blood pressures [SBPs] and diastolic blood pressures [DBPs] were measured with an aneroid sphygmomanometer.

The peak early transmitral filling during early diastole (E), peak transmitral atrial filling velocity during late diastole (A), deceleration time (DT) (time elapsed between peak E velocity and the point where the extrapolated deceleration slope of the E velocity crosses the zero baseline), and isovolumetric deceleration time (IVRT) (time period between the end of mitral diastolic flow Doppler tracing and the starting point of aortic flow Doppler tracing) were used to assess left ventricular diastolic functions. The transmitral diastolic flow Doppler tracing was obtained from the apical four-chamber view by using pulsed Doppler echocardiography with the sample volume size of 1 to 2 mm between the tips of the mitral valve during diastole. To measure isovolumetric relaxation time, a 3-to 4-mm size sample volume was placed in the area of the mitral leaflet tips. Next, the transducer beam was angulated toward the left ventricular outflow tract until the aortic valve closure appeared above and below the baseline.

### The Measurements of Aortic Elasticity Parameters

Aortic elasticity was assessed using the following indexes (26):

$$\text{Aortic strain (\%)} = 100 \times (\text{Aos-Aod})/\text{Aod};$$

$$\text{Aortic distensibility index } (\text{cm}^{-2} \text{ dyn}^{-1} 10^{-6}) = 2 \times \text{Aortic Strain} \times (\text{SBP-DBP});$$

$$\text{Aortic stiffness index beta} = \ln (\text{SBP/DBP})/\text{Aortic Strain}.$$

### Blood Chemistry

Venous blood samples were withdrawn into both the tubes containing K<sub>3</sub> EDTA and the tubes containing no anti-coagulant agent. After all tubes were spun at 5000 rpm for 15 min, plasma and serum samples were stored at -80°C until analyses were made. Total plasma cholesterol, triglyceride, and HDL cholesterol were measured by an enzymatic calorimetric method with the Olympus AU 600 autoanalyzer using reagent from Olympus Diagnostics GmbH (Hamburg, Germany). Low-density lipoprotein cholesterol levels were calculated by Friedwald formula. Serum glucose was measured by the glucose oxidase method. The serum insulin levels were determined with the immunoenzymatic method (Beckman Coulter Inc., Immunotech, IRMA Kit, Prague, Czech Republic). Gamma-glutamyl transferase levels were measured with a spectrophotometric technique by an Olympus AU-2700 autoanalyzer and using commercial kits (Olympus, Hamburg, Germany).

### Assesment of Insulin-Resistance

Insulin-resistance score the homeostatic model assessment [HOMA-IR] was computed with the formula: [HOMA-IR] = [fasting plasma glucose (mg/dl) × immunoreactive insulin (IRI) (IU/ml)]/405 (27). Low

HOMA-IR values represent high-insulin sensitivity, whereas high HOMA-IR values represent low-insulin sensitivity (insulin-resistance) compared to those of the controls.

### Statistical Analysis

Statistical analysis was performed by using the SPSS 11.5 Statistical Package Program for Windows (SPSS Inc., Chicago, IL). Results are expressed as the mean ± SD, median and percentages. The means of groups were compared with each other. The differences between groups were tested by chi-square, an independent samples *t*-test, and a Mann-Whitney *U* test. Intra- and inter-observer variability were calculated as a relative error. A linear regression analysis was performed to evaluate the association between GGT and aortic elasticity parameters. Next, a linear regression analysis was done to evaluate the association between aortic elasticity parameters and other variables. Then, multiple linear regression analysis was done to clarify the contributions of GGT to aortic elasticity adjusting for age, BMI, uric acid, serum glucose, heart rate, LDL-cholesterol, HDL-cholesterol, and triglycerides (TGs). Differences were considered significant at *p* < 0.05.

## RESULTS

When baseline characteristics of patients with prehypertension were compared to those of control subjects, no statistically significant difference was observed between the two groups except for significantly higher GGT and blood pressure levels in patients with prehypertension compared to those in controls as shown in Table 1 (for GGT; 47.9 ± 15.9 U/L vs. 36.1 ± 9.4 U/L, *p* = 0.003).

Table 1. Baseline clinical and biochemical parameters of the study groups

	Prehypertension (n = 25)	Control (n = 25)	<i>p</i>
Age (years)	34 ± 6	33 ± 6	0.35
Gender (M), n (%)	18 (53)	16 (47)	0.54
BMI (kg/m <sup>2</sup> )	25.64 ± 2.04	25.81 ± 1.74	0.74
BSA (m <sup>2</sup> )	1.85 ± 0.13	1.82 ± 0.09	0.36
GGT (U/L)	47.92 ± 15.95	36.12 ± 9.47	0.003
AST (U/L)	38.71 ± 9.50	35.33 ± 7.71	0.18
ALT (U/L)	38.28 ± 9.07	34.44 ± 8.60	0.13
Total cholesterol (mg/dl)	196 ± 41	197 ± 42	0.95
TG (mg/dl) (median)	137 ± 89 (115)	117 ± 50 (96)	0.76
HDL cholesterol (mg/dl)	48 ± 13	45 ± 11	0.39
LDL cholesterol (mg/dl)	121 ± 38	129 ± 40	0.49
Serum glucose (mg/dl)	98 ± 8	94 ± 7	0.07
Serum insulin (μU/mL)	11 ± 4	10 ± 5	0.47
HOMA-IR	2.66 ± 1.06	2.33 ± 1.29	0.33
Heart rate (bpm)	74 ± 9	72 ± 9	0.37

Abbreviations: BMI - body mass index; BSA - body surface area; GGT - gamma-glutamyltransferase; AST - alanine aminotransferase; ALT - aspartate aminotransferase; TG - triglyceride; HDL - high-density lipoprotein; LDL - low-density lipoprotein; HOMA-IR - the homeostatic model assessment of insulin-resistance.

### Echocardiographic Measurements

We noticed no statistically significant difference between two groups regarding LVIDs and LVIDd, LVEF, Lad, and LVMI (Table 2). Although diastolic functions of the left ventricle of the patients with prehypertension were not impaired, the E/A ratio and E velocity values were detected to be lower and deceleration and isovolumetric relaxation times were to be higher in prehypertensives than those in controls (Table 2).

### Aortic Elastic Parameters

Aortic elastic properties of both groups are summarized in Table 3. The mean systolic, diastolic, and pulse pressure measurements were significantly higher in prehypertensive patients than in control subjects. The mean aortic systolic and diastolic diameters of prehypertensive patients were detected to be significantly higher than those of control subjects (aortic systolic diameter:  $31.76 \pm 1.66$  mm vs.  $29.84 \pm 2.28$  mm, respectively,  $p = 0.001$ ; aortic diastolic diameter:  $27.96 \pm 2.03$  mm vs.  $25.44 \pm 2.21$  mm, respectively,  $p < 0.001$ ,

Table 3). However, the mean aortic diameter change of prehypertensive patients was detected to be significantly lower than those of control subjects ( $3.80 \pm 1.04$  mm vs.  $4.40 \pm 0.95$  mm, respectively,  $p = 0.03$ ). The coefficients of variation for aortic systolic diameter, diastolic diameter, SBPs, and DBPs, all of which are the main determinants of the formulas to calculate aortic elasticity, were 5%, 7%, 3%, and 8%, respectively. Intra- and inter-observer variabilities were found to be 2.7% and 4.3% for aortic systolic diameter; 2.8% and 4.9% for aortic diastolic diameter; 2.1% and 3.3% for SBP; 2.4% and 2.9% for DBP, consecutively.

When the mean aortic distensibility and strain indexes were analyzed, aortic distensibility and strain indexes of the patients with prehypertension were found to be significantly lower than those of control subjects (aortic distensibility:  $5.77 \pm 1.91 \text{ cm}^{-2} \text{ dyn}^{-1} 10^{-6}$  vs.  $8.63 \pm 2.67 \text{ cm}^{-2} \text{ dyn}^{-1} 10^{-6}$ , respectively,  $p < 0.001$ ; aortic strain:  $13.81 \pm 4.50\%$  vs.  $17.47 \pm 4.25\%$ , respectively,  $p = 0.005$ , Table 3). In contrast, the mean aortic stiffness index beta [SI] of the prehypertensive

Table 2. Echocardiographic findings of both groups

	Prehypertension (n = 25)	Control (n = 25)	p
LVIDd (mm)	$45.44 \pm 2.00$	$45.20 \pm 1.89$	0.66
LVIDs (mm) (median)	$26.20 \pm 1.63$ (26)	$26.40 \pm 1.68$ (26)	0.67
IVSd (mm)	$9.56 \pm 1.50$	$9.44 \pm 1.08$	0.74
IVSs (mm)	$13.56 \pm 1.50$	$13.44 \pm 1.08$	0.73
LVPWd (mm) (median)	$8.52 \pm 1.68$ (8)	$8.04 \pm 1.27$ (8)	0.24
LVPWs (mm) (median)	$12.88 \pm 1.85$ (13)	$12.24 \pm 1.33$ (12)	0.15
LVEF (%)	$66.70 \pm 2.84$	$65.83 \pm 2.97$	0.29
LVMI ( $\text{gr}/\text{m}^2$ )	$124.53 \pm 17.26$	$117.45 \pm 17.85$	0.16
Left atrial diameter (mm)	$34.00 \pm 3.14$	$32.72 \pm 3.08$	0.14
E velocity( $\text{cm}/\text{s}$ ) (median)	$78.80 \pm 8.91$ (80)	$83.72 \pm 4.60$ (84)	0.07
A velocity( $\text{cm}/\text{s}$ ) (median)	$67.00 \pm 15.80$ (63)	$56.44 \pm 9.25$ (58)	0.02
E/A	$1.23 \pm 0.30$	$1.52 \pm 0.26$	0.001
Deceleration time (ms) (median)	$184.32 \pm 21.42$ (188)	$168.88 \pm 10.17$ (165)	0.004
IVRT (ms) (median)	$78.04 \pm 10.61$ (75)	$72.08 \pm 4.99$ (72)	0.04

Abbreviations: LVIDd - left ventricular internal diameter (diastolic); LVIDs - left ventricular internal diameter (systolic); IVSd - interventricular septum diameter (diastolic); IVSs - interventricular septum diameter (systolic); LVPWd - left ventricular posterior wall diameter (diastolic); LVPWs - left ventricular posterior wall diameter (systolic); LVEF - left ventricular ejection fraction; LVMI - left ventricular mass index; E - early rapid filling wave; A - filling due to atrial contraction; IVRT - isolometric relaxation time.

Table 3. Aortic elastic properties of the study groups

	Prehypertension (n = 25)	Control (n = 25)	p
SBP (mmHg) (median)	$131 \pm 5$ (130)	$107 \pm 9$ (110)	<0.001
DBP (mmHg)	$82 \pm 6$	$65 \pm 6$	<0.001
PP (mmHg) (median)	$49 \pm 7$ (45)	$42 \pm 10$ (40)	0.01
ASD (mm)	$31.76 \pm 1.66$	$29.84 \pm 2.28$	0.001
ADD (mm)	$27.96 \pm 2.03$	$25.44 \pm 2.21$	<0.001
Diameter change (mm)	$3.80 \pm 1.04$	$4.40 \pm 0.95$	0.03
Distensibility ( $\text{cm}^{-2} \text{dyn}^{-1} 10^{-6}$ )	$5.77 \pm 1.91$	$8.63 \pm 2.67$	<0.001
Aortic strain (%)	$13.81 \pm 4.50$	$17.47 \pm 4.25$	0.005
Stiffness index	$3.73 \pm 1.41$	$2.97 \pm 0.82$	0.02

Abbreviations: SBP - systolic blood pressure; DBP - diastolic blood pressure; PP - pulse pressure; ASD - aortic systolic diameter; ADD - aortic diastolic diameter.

group was significantly higher compared to that of the control group ( $3.73 \pm 1.41$  vs.  $2.97 \pm 0.82$ , respectively,  $p = 0.02$ , Table 3).

When the multiple linear regression analysis was done to clarify the contributions of GGT to aortic elasticity adjusting for age, BMI, uric acid, serum glucose, heart rate, LDL cholesterol, HDL cholesterol, and TG, we observed that only serum GGT levels were significantly associated with aortic elasticity parameters as shown in Tables 4–6.

Table 4. Multiple linear regression analysis with aortic strain as the dependent variable in patients with prehypertension

	Beta	95% CI	p
GGT (U/L)	-0.247	(-0.309)–(-0.185)	<0.001
Uric acid (mg/dl)	-0.956	(-2.023)–(0.111)	0.078
Glucose (mg/dl)	-0.120	(-0.293)–(0.054)	0.17
LDL (mg/dl)	-0.011	(-0.035)–(0.013)	0.38
TG (mg/dl)	-0.004	(-0.017)–(0.009)	0.51
HDL (mg/dl)	-0.027	(-0.107)–(0.053)	0.50
Age (years)	-0.076	(-0.230)–(0.077)	0.32
BMI (kg/m <sup>2</sup> )	0.012	(-0.516)–(0.539)	0.97
Heart rate (bpm)	0.015	(-0.097)–(0.127)	0.79

Abbreviations: GGT - gamma-glutamyltransferase; LDL - low-density lipoprotein; TG - triglyceride; HDL - high-density lipoprotein; BMI - body mass index.

Table 5. Multiple linear regression analysis with aortic distensibility as the dependent variable in patients with prehypertension

	Beta	95% CI	p
GGT (U/L)	-0.108	(-0.150)–(-0.066)	<0.001
Uric acid (mg/dl)	-0.361	(-1.087)–(0.365)	0.32
Glucose (mg/dl)	-0.151	(-0.267)–(0.035)	0.05
BMI (kg/m <sup>2</sup> )	-0.214	(-0.537)–(0.110)	0.19
TG (mg/dl)	0.003	(-0.005)–(0.012)	0.42
HDL (mg/dl)	-0.014	(-0.072)–(0.043)	0.62
LDL (mg/dl)	0.004	(-0.013)–(0.021)	0.67
Age (years)	-0.019	(-0.128)–(0.090)	0.73
Heart rate (bpm)	-0.016	(-0.086)–(0.055)	0.67

Abbreviations: GGT - gamma-glutamyltransferase; BMI - body mass index; TG - triglyceride; HDL - high density lipoprotein.

Table 6. Multiple linear regression analysis with stiffness index beta as the dependent variable in patients with prehypertension

	Beta	95% CI	p
GGT (U/L)	0.063	(0.046)–(0.079)	<0.001
Age (years)	0.028	(-0.013)–(0.069)	0.18
Uric acid (mg/dl)	-0.025	(-0.336)–(0.285)	0.87
Glucose (mg/dl)	0.019	(-0.028)–(0.065)	0.42
LDL (mg/dl)	0.000	(-0.007)–(0.007)	0.94
TG (mg/dl)	-0.001	(-0.005)–(0.002)	0.46
HDL (mg/dl)	-0.002	(-0.022)–(0.025)	0.90
BMI (kg/m <sup>2</sup> )	0.025	(-0.111)–(0.162)	0.71
Heart rate (bpm)	0.011	(-0.016)–(0.038)	0.42

Abbreviations: GGT - gamma-glutamyltransferase; LDL - low-density lipoprotein; TG - triglyceride; HDL - high-density lipoprotein; BMI - body mass index.

## DISCUSSION

The current study revealed that not only were serum GGT levels significantly increased in young patients with prehypertension compared to those of controls, but elevated GGT levels were also independently associated with decreased aortic elasticity in young prehypertensive patients.

Hypertension is one of the most common chronic diseases in the world and affects approximately 1 billion people in the world (1). The relationship between blood pressure and risk of cardiovascular events is continuous, consistent, and independent of other known risk factors (1). The classification of prehypertension introduced in the JNC seventh report has delineated these relationships and urged the need for increased education of healthcare professionals to decrease blood pressure levels and prevent the development of hypertension in the general population (28). In addition, patients with prehypertension have also been shown to have an increased risk to develop hypertension (2).

Although serum GGT activity has long been accepted as a marker for hepatobiliary disease and alcohol consumption, there has been some epidemiologic evidence suggesting that GGT might evolve as a potential biochemical risk indicator of cardiovascular mortality and morbidity (29). Serum GGT levels appeared to be an independent risk factor for the development of cardiovascular disease, arterial hypertension, stroke, and type 2 diabetes mellitus, and their complications in several prospective cohort clinical studies after adjusting for alcohol consumption (18, 19, 30, 31). Lee et al. found that even in the young adult population, after adjusting for alcohol consumption, serum GGT concentration was associated with many cardiovascular disease risk factors, including black race, male gender, older age, cigarette smoking, BMI, higher blood pressure, higher fasting blood sugar, higher fasting blood triglycerides, higher blood LDL-cholesterol, and lower blood HDL-cholesterol. In addition, other variables, such as white blood cell count, red blood cell count, hematocrit, and hemoglobin, were positively associated with GGT (19).

Gamma-glutamyltransferase is also a modest risk factor for hypertension (32). Therefore the mechanism underlying these observations is not fully elucidated. Some cross-sectional studies have demonstrated a positive association between serum GGT levels and blood pressure (21–23). Although the positive association between GGT level and blood pressure may simply reflect the well-known relationship between alcohol consumption and hypertension, several investigators reported that the association was independent of the amount of alcohol consumed (22). There have been a few longitudinal studies (33–36), with results that are not entirely consistent. Studies from Japan (33, 35, 36) reported positive associations of serum GGT with blood pressure, but a study from Norway (34) found a

weak positive association, restricted to SBP among women, with no association among men. One Japanese study (33) reported a positive association between baseline GGT and incidence of hypertension only in drinkers, but not in nondrinkers, which could be interpreted as different alcohol-blood pressure relationships in subjects with different serum GGT levels.

Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension. The Coronary Artery Risk Development in Young Adults (CARDIA) study is a large-scale prospective study aiming to examine whether serum GGT predicts diabetes mellitus and hypertension (19). In this landmark study, serum GGT concentrations measured at ages 18–30 years predicted a 15-year incidence of diabetes and/or hypertension and the future concentrations of oxidative stress and inflammation markers such as fibrinogen, uric acid, C-reactive protein (CRP), and F2-isoprostanes, which were measured at various times during the 15 years of follow-up. In that study, the authors concluded that GGT might be an early marker of oxidative or other cellular stress and that it is possibly directly related to the pathogenesis of diabetes and hypertension, perhaps as an oxidative stressor itself. This study suggests that serum GGT is a strong predictor of diabetes and hypertension. Neither alcohol consumption nor liver damage likely explains this association. The authors speculate that it might be involved in the pathogenesis of diabetes and hypertension through a mechanism related to oxidative stress. On the other hand, Lee et al. showed that elevated GGT could be a predictor for hypertension in alcohol drinkers and the relationship between alcohol consumption and hypertension was demonstrated only among those with  $\text{GGT} \geq 30 \text{ U/L}$  at baseline (32). The authors concluded that increased serum GGT levels may reflect individual susceptibility to the blood pressure-raising effect of alcohol. However, in the present study, we found that serum GGT was independently associated with decreased arterial elasticity in young prehypertensives not consuming alcohol in accordance with the data obtained from the CARDIA study.

Although the mechanism underlying the associations of increased serum GGT levels with cardiovascular diseases remains largely unknown, some possible mechanisms exist. Previous experimental studies (37–39) have reported that GGT plays an important role in antioxidant systems with the primary function of maintaining intracellular concentrations of glutathione. Increases in GGT activity can be a response to oxidative stress, marking increased transport of glutathione into cells. In addition, GGT is leaked into the serum, possibly as a result of normal cell turnover and cellular stresses. From that standpoint, increased serum GGT may identify those individuals with low but persistent increases in oxidative and other cellular stresses. On the other hand, recent experimental studies (40–43) indicated that under physiologic conditions, GGT is

involved directly in the generation of reactive oxygen species in the presence of iron or other transition metals. Gamma-glutamyltransferase might alternatively be a specific marker of oxidative stress, e.g., as a result of iron overload, because several experimental and epidemiologic studies have suggested a close relationship between iron overload and cellular or serum GGT activity (44, 45). We speculate that GGT might be an early marker of oxidative or other cellular stress and that it is possibly directly related to the pathogenesis of prehypertension, perhaps as an oxidative stressor itself.

The major limitation of the study is the small sample size. Since coronary angiography was not performed, the probability of subclinical coronary artery disease cannot be fully excluded. But coronary flow is not impaired by insignificant coronary artery stenosis which may result in increased arterial stiffness. On the other hand, our study group consisted of the patients of a younger age in whom the prevalence of coronary artery disease with clinical sequela was relatively low. The current study could not identify a causal role for relationship that was found between serum GGT and aortic elasticity parameters. Self-reported alcohol consumption as a variable was questionable because of its reliability and validity. Lastly, although noninvasive assessment of aortic elasticity is convenient and reproducible, this method itself has some limitation in reflecting arterial stiffness.

In conclusion, increased serum GGT may be an additional marker of decreased aortic elasticity in young patients with prehypertension. Emerging evidence has shown that serum GGT might be an important enzyme in the pathogenesis of cardiovascular diseases, including arterial hypertension. Although arterial stiffness is generally regarded as developing as a consequence of long-standing hypertension, our findings have suggested that it may develop prior to development of overt hypertension, even at the prehypertensive stage, and serum GGT levels may be a marker underlying increased oxidative stress in those patients. Considering that GGT is easily measured and is extensively used, further large-scale prospective studies are needed to clarify the mechanisms responsible for the relationship existing between GGT and aortic elasticity, as well as the clinical implication of that association.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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## Gamma-glutamyl transferase level predicts the development of hypertension in Hong Kong Chinese

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### ABSTRACT

**Background:** Plasma activities of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase, and γ-glutamyl transferase (GGT) are often increased in cardiometabolic diseases. We investigated if hypertension is associated with increased activities of these plasma markers.

**Methods:** We included 235 hypertensive and 708 normotensive subjects (mean age  $47.3 \pm 9.6$  and  $58.0 \pm 10.2$  years respectively) from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2) in 2000–2004 who had drank <1/week. In the follow-up study in 2005–2008 (CRISPS-3), 126 out of the 708 subjects had developed hypertension.

**Results:** Raised plasma ALT (OR = 1.22 per SD of log-transformed level,  $P = 0.045$ ) and GGT (OR = 1.38 per SD of log-transformed level,  $P = 0.001$ ) levels were associated with hypertension at baseline in CRISPS-2 after adjusting for covariates. Among subjects not on anti-hypertensive medications, plasma ALP, ALT and GGT were related to blood pressure ( $P < 0.01$ ). In subjects normotensive at CRISPS-2, plasma GGT, but not ALP, ALT and AST, was an independent predictor of new-onset hypertension at CRISPS-3 (OR = 1.38 per SD of log-transformed level,  $P = 0.020$  and OR = 2.68 for 3rd tertile vs. 1st tertile,  $P = 0.004$ ) after adjusting for covariates.

**Conclusions:** Among the 4 plasma markers, increased GGT activity is the strongest predictor for existing and new-onset hypertension in Hong Kong Chinese.

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### 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is caused by the accumulation of fat in the liver in subjects who do not drink alcohol in excess [1]. It has recently been suggested as the hepatic manifestation of obesity and the metabolic syndrome [1]. In NAFLD, plasma markers of liver injury such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transferase (GGT) are often increased [2].

Apart from liver diseases, these enzymes, especially GGT, have been suggested to be novel markers of cardiovascular diseases [3]. Increased plasma GGT activity has been shown to be associated with hypertension and its development in previous studies in Japanese [4–6], Korean [7], and Caucasians [8,9]. However, all these studies

investigated GGT but not the other enzymes, i.e., ALP, ALT, and AST [4–9]. We previously reported that plasma ALP correlates with the inflammatory marker, C-reactive protein (CRP), in Hong Kong Chinese [10] and Americans [11]. As CRP is known to predict the development of hypertension [12], ALP may also be related to the latter. Moreover, increased plasma ALT is already known to precede the development of the metabolic syndrome [2] and type 2 diabetes [13–17]. Therefore, we hypothesized that plasma markers of liver injury other than GGT may also be increased in hypertension and predict the future risk of hypertension. If this hypothesis is true, the routine liver function test may help to monitor the risk at minimal extra cost. As there is no prospective study on the relationship of hypertension with all the four plasma markers of liver injury, especially in Chinese, we investigated whether plasma ALP, ALT, AST, and GGT were associated with hypertension in a population-based prospective cohort of Hong Kong Chinese. Since alcohol drinking can increase plasma markers of liver injury and may have confounding effect, we limited our analysis to subjects who had alcoholic drinks less often than once a week.

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## 2. Methods

### 2.1. Subjects

The subjects were from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2), details of which have been described previously [18–21]. The study protocol was approved by the Ethics Committee of the University of Hong Kong and all subjects gave written and informed consent. Among the 1944 subjects in the CRISPS-2 study in 2000–2004, plasma activities of all the 4 markers of liver injury were available in 1371 subjects, and only 1197 of whom had alcoholic drinks less often than once a week. Among these subjects, 943 were followed up in 2005–2008 (CRISPS-3) after a median interval of 5.3 years and were included in this analysis.

### 2.2. Variables of interest

Plasma ALP, ALT, AST, and GGT were measured on a Hitachi 912 analyzer. Hypertension was defined as systolic blood pressure (SBP)  $\geq 140$  mm Hg, diastolic blood pressure (DBP)  $\geq 90$  mm Hg, or taking anti-hypertensive medication. Blood pressure was measured 3 times using a mercury sphygmomanometer by a trained nurse. The readings were taken in a seated position after resting in a quiet temperature-controlled room. The Korotkoff V sound was used to determine DBP. The first measurement was to familiarize the subject with the procedure and the sensation of the inflated cuff. The mean of the second and third readings was used for data analysis. Mean arterial pressure (MAP) was calculated as the sum of DBP and one-third of the difference between SBP and DBP. Data on alcohol drinking, smoking, and history of hypertension were obtained by interviewing using a questionnaire. Details of the physical examination and measurement methods of clinical parameters, such as triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, fasting glucose, homeostasis model assessment of insulin resistance index (HOMA-IR), fibrinogen, and plasma high-sensitivity CRP had been described previously [18–23].

### 2.3. Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, IL). Variables with skewed distribution were log-transformed before analysis. Multiple linear or logistic regression models were used to estimate the standardized regression coefficient ( $\beta$ ) or odds ratio (OR) respectively after adjusting for covariates. Variables were used as covariates in the multiple regression analysis if they are recognized determinants of blood pressure or were significantly different between subjects with and without hypertension. For variables that were highly correlated such as body mass index (BMI) and waist circumference, only one was entered into the regression analysis. In a separate analysis, similar results were obtained when BMI was replaced by waist circumference in the adjustment model. The  $P$  values for interaction were estimated by including each multiplicative interaction term in the multivariate regression models in full sample after adjusting for the main effects of all covariates. A 2-tailed  $P < 0.05$  was considered statistically significant.

## 3. Results

**Table 1** shows the clinical characteristics of the subjects in CRISPS-2 according to the hypertension status. Among 708 subjects normotensive in CRISPS-2, 126 subjects had developed hypertension in CRISPS-3. As expected, subjects with prevalent or incident hypertension were older, had higher BMI, waist circumference, SBP, DBP, MAP, triglycerides, fasting blood glucose and HOMA-IR, and had lower HDL cholesterol. Subjects hypertensive at baseline had a higher plasma fibrinogen concentration but less likely to be smokers.

In CRISPS-2, all the 4 plasma markers of liver injury were higher in subjects with prevalent hypertension (**Table 1**). Among 809 subjects not on anti-hypertensive medication in CRISPS-2, the plasma activities of most markers were significantly associated with SBP (ALP:  $\beta = 0.185$ ,  $P < 0.001$ ; ALT:  $\beta = 0.137$ ,  $P = 0.001$ ; and GGT:  $\beta = 0.155$ ,  $P < 0.001$ ), DBP (ALP:  $\beta = 0.184$ ,  $P < 0.001$ ; ALT:  $\beta = 0.149$ ,  $P < 0.001$ ; AST:  $\beta = 0.088$ ,  $P = 0.011$ ; and GGT:  $\beta = 0.171$ ,  $P < 0.001$ ), and MAP (ALP:  $\beta = 0.200$ ,  $P < 0.001$ ; ALT:  $\beta = 0.156$ ,  $P < 0.001$ ; AST:  $\beta = 0.081$ ,  $P = 0.015$ ; and GGT:  $\beta = 0.178$ ,  $P < 0.001$ ) after adjusting for age and sex. In a separate analysis, inclusion of the 134 treated subjects using adjusted blood pressure (by adding 10/5 mm Hg to blood pressure [24]) produced similar results (data not shown). As women had significantly lower plasma activities of all the 4 markers than men ( $P < 0.001$  after adjusting for age and BMI), sex-specific cut-points were used to define the tertiles of the plasma activities in subsequent analysis. As shown in **Table 2**, plasma GGT were significantly associated with prevalent hypertension in the full adjustment model ( $P = 0.003$  for continuous data and  $P$  for trend = 0.008 for tertiles). Plasma ALT was also associated with prevalent hypertension with a borderline significant  $P$  value of 0.045 when the plasma activity was analyzed as continuous data. There was no significant interaction of plasma GGT and ALT with sex ( $P > 0.05$ ). The clinical characteristics of subjects according to the sex-specific tertiles of plasma GGT activities are shown in **Table 3**. In CRISPS-2, plasma GGT activity increased with increasing age, BMI, waist circumference, SBP, DBP, MAP, triglycerides, LDL cholesterol, fasting glucose, HOMA-IR, plasma CRP, plasma ALP, plasma ALT and plasma AST, and decreased with increasing HDL cholesterol (all  $P < 0.05$ , **Table 3**). Plasma fibrinogen and the proportion of smoking did not differ significantly with plasma GGT tertiles.

Among subjects normotensive in CRISPS-2, those who had developed hypertension in CRISPS-3 had significantly higher plasma activities of ALP, ALT, and GGT (**Table 1**). However, only the association of plasma GGT with incident hypertension remained significant in the full adjustment model (**Table 4**). There was no significant interaction of plasma GGT with sex ( $P = 0.207$  for tertiles and 0.072 for continuous activities) and other covariates ( $P > 0.05$  after adjustment for multiple testing). The association of plasma GGT tertiles with incident hypertension was significant in subjects with  $BMI < 25.0 \text{ kg/m}^2$  and  $BMI \geq 25.0 \text{ kg/m}^2$  ( $P$  for trend = 0.038 and 0.042 respectively). Similar results were obtained when baseline SBP in the adjustment model was replaced by baseline DBP or MAP (data not shown).

## 4. Discussion

We report the relationship between all the 4 plasma markers of liver injury and hypertension in a population-based prospective cohort. We demonstrated that only plasma GGT, but not the other markers, was associated with hypertension at baseline and incident hypertension. Our results are consistent with previous findings on the association of plasma GGT with hypertension or pre-hypertension in cross-sectional [25–28] and prospective studies [4–9]. The mechanisms underlying the association of GGT with hypertension have not been fully elucidated. Plasma GGT is usually used as a marker of alcohol intake. In this study, the subjects were not regular alcohol drinkers, but increased GGT activity was still associated with both prevalent and incident hypertension. Previous studies also found a similar association in both drinkers and non-drinkers [8,26]. Therefore, the association cannot be explained by alcohol drinking.

Recently, GGT has been suggested as a novel biomarker of cardiovascular risk [3]. Its increased activity has been shown to be associated with the metabolic syndrome in cross-sectional studies [29,30]. In prospective studies, increased GGT activity can predict incident elevation in plasma ALT [31], and the development of cardiovascular diseases, all-cause mortality, and cardiovascular mortality [30,32–34]. In the Framingham Offspring Study, plasma GGT correlated positively with BMI, blood pressure, LDL cholesterol,

**Table 1**

Clinical characteristics of the subjects in CRISPS-2 (2000–2004).

Characteristics	All subjects		Normotensive subjects in CRISPS-2	
	Normotension (n = 708)	Hypertension (n = 235)	Normotension in CRISPS-3 (n = 582)	Hypertension in CRISPS-3 (n = 126)
Age (years)	47.3 ± 9.7	58.0 ± 10.2‡	46.0 ± 9.0	53.3 ± 10.3‡
Women (%)	60.5	51.1	61.0	57.9
BMI (kg/m <sup>2</sup> )	23.3 ± 3.3	25.4 ± 3.4‡	23.1 ± 3.0	24.5 ± 4.0‡
Waist circumference (cm)	77.3 ± 9.5	84.1 ± 9.4‡	76.7 ± 9.2	80.5 ± 10.4‡
SBP (mm Hg) <sup>§</sup>	113.3 ± 10.9	147.9 ± 15.5‡	111.2 ± 10.0	123.1 ± 9.5‡
DBP (mm Hg) <sup>§</sup>	72.0 ± 8.1	89.0 ± 11.5‡	71.0 ± 8.0	76.6 ± 7.1‡
MAP (mm Hg) <sup>§</sup>	85.8 ± 8.2	108.7 ± 9.5‡	84.4 ± 7.8	92.1 ± 6.8‡
Triglycerides (mmol/l)	1.07 (1.03–1.11)	1.45 (1.35–1.55)‡	1.03 (0.99–1.07)	1.26 (1.15–1.38)†
HDL cholesterol (mmol/l)	1.45 ± 0.39	1.33 ± 0.38‡	1.46 ± 0.38	1.39 ± 0.41†
LDL cholesterol (mmol/l)	3.21 ± 0.81	3.36 ± 0.78	3.20 ± 0.80	3.25 ± 0.85
Fasting glucose (mmol/l)	5.13 (5.06–5.19)	5.55 (5.41–5.69)*	5.05 (4.99–5.11)	5.52 (5.31–5.75)‡
HOMA-IR	1.57 (1.51–1.64)	2.29 (2.10–2.49)‡	1.50 (1.43–1.57)	1.95 (1.77–2.14)‡
Fibrinogen (g/l)	2.90 ± 0.54	3.11 ± 0.62*	2.89 ± 0.56	2.97 ± 0.47
CRP (mg/l)	0.51 (0.47–0.55)	0.85 (0.75–0.96)‡	0.47 (0.43–0.51)	0.74 (0.64–0.86)‡
ALP (U/l)	66.1 (64.7–67.6)	77.2 (74.5–80.0)†	64.8 (63.2–66.3)	72.7 (68.9–76.6)†
ALT (U/l)	20.0 (19.2–20.9)	24.6 (22.9–26.4)‡	19.5 (18.7–20.4)	22.5 (20.1–25.0)†
AST (U/l)	21.6 (21.0–22.2)	24.2 (23.2–25.4)*	21.5 (20.9–22.1)	23.0 (21.5–24.7)
GGT (U/l)	20.5 (19.7–21.4)	28.2 (26.1–30.6)‡	19.8 (18.9–20.8)	24.1 (21.6–26.9)‡
Current smoking (%)	15.1	10.6*	15.3	14.3

Data are expressed as mean ± SD or geometric mean (95% CI) unless otherwise stated.

\*P &lt; 0.05, †P &lt; 0.01, and ‡P &lt; 0.001 for normotensive vs. hypertensive subjects after adjusting for age and sex. For incident elevated blood pressure, P values were further adjusted for follow-up duration.

§ Subjects on anti-hypertensive medication (n = 134) were excluded from analysis.

triglycerides and fasting glucose in cross-sectional analysis, and predicted the development of the metabolic syndrome and cardiovascular diseases over 20 years [35]. It has been suggested that the association of plasma GGT with the metabolic syndrome may be

explained by insulin resistance [36]. Indeed, plasma GGT has also been reported to be predictive of incident diabetes [8,37,38] and the close relationship between raised blood pressure and dysglycemia in our population may also contribute to the association of plasma GGT with

**Table 2**

Association with prevalent hypertension in CRISPS-2 (n = 943).

Marker	Unadjusted model		Model 1		Model 2	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<i>ALP tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.46 (0.99–2.16)	NS	1.33 (0.86–2.04)	NS	1.13 (0.71–1.82)	NS
Tertile 3	2.60 (1.79–3.77)	<0.001	1.66 (1.10–2.52)	0.017	1.15 (0.72–1.84)	NS
P for trend		<0.001		NS		NS
ALP, U/l*	1.76 (1.49–2.08)	<0.001	1.37 (1.15–1.64)	0.001	1.23 (0.99–1.52)	NS
<i>ALT tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.73 (1.18–2.54)	0.005	1.64 (1.06–2.53)	0.025	1.13 (0.71–1.81)	NS
Tertile 3	2.56 (1.75–3.73)	<0.001	3.11 (2.02–4.77)	<0.001	1.59 (0.98–2.57)	NS
P for trend		<0.001		<0.001		NS
ALT, U/l*	1.42 (1.22–1.64)	<0.001	1.56 (1.31–1.87)	<0.001	1.22 (1.01–1.49)	0.045
<i>AST tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.44 (0.99–2.09)	NS	1.20 (0.79–1.82)	NS	1.05 (0.67–1.64)	NS
Tertile 3	2.09 (1.45–3.00)	<0.001	1.74 (1.16–2.61)	0.007	1.43 (0.93–2.21)	NS
P for trend		<0.001		0.022		NS
AST, U/l*	1.36 (1.17–1.57)	<0.001	1.25 (1.06–1.47)	0.008	1.16 (0.97–1.38)	NS
<i>GGT tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.62 (1.08–2.43)	0.020	1.43 (0.91–2.24)	NS	1.12 (0.69–1.81)	NS
Tertile 3	3.71 (2.54–5.41)	<0.001	3.77 (2.46–5.77)	<0.001	1.99 (1.21–3.26)	0.007
P for trend		<0.001		<0.001		0.008
GGT, U/l*	1.65 (1.42–1.91)	<0.001	1.73 (1.45–2.06)	<0.001	1.38 (1.12–1.70)	0.003

For ALP, the cut-off values for tertiles 1, 2, and 3 are ≤67, 68–82 and ≥83 U/l in men, and ≤57, 58–74 and ≥75 U/l in women, respectively.

For ALT, the cut-off values for tertiles 1, 2, and 3 are ≤20, 21–31 and ≥32 U/l in men, and ≤14, 15–21 and ≥22 U/l in women, respectively.

For AST, the cut-off values for tertiles 1, 2, and 3 are ≤21, 22–26 and ≥27 U/l in men, and ≤18, 19–23 and ≥24 U/l in women, respectively.

For GGT, the cut-off values for tertiles 1, 2, and 3 are ≤21, 22–33 and ≥34 U/l in men, and ≤14, 15–21 and ≥22 U/l in women, respectively.

Model 1: Adjusted for age and sex.

Model 2: Further adjusted for BMI, triglycerides, HDL cholesterol, HOMA-IR, CRP, fibrinogen, and current smoking.

\* ORs are expressed in term of per SD of the log-transformed unit.

**Table 3**

Clinical characteristics of the subjects in CRISPS-2 according to tertiles of plasma GGT level.

Characteristics	Tertile 1 (≤21 U/l in men and ≤14 U/l in women)	Tertile 2 (22–33 U/l in men and 15–21 U/l in women)	Tertile 3 (≥34 U/l in men and ≥22 U/l in women)	P for trend
n	338	301	304	
Age (years)	48.3 ± 10.6	50.6 ± 10.6	51.1 ± 11.1	0.004
BMI (kg/m <sup>2</sup> )	22.8 ± 2.9	23.7 ± 3.2	25.2 ± 3.8	<0.001
Waist circumference (cm)	75.7 ± 8.8	79.3 ± 9.4	82.5 ± 10.3	0.003
SBP (mm Hg) <sup>*</sup>	114.9 ± 14.4	117.2 ± 15.2	121.8 ± 18.8	0.013
DBP (mm Hg) <sup>*</sup>	72.6 ± 9.4	73.9 ± 9.3	76.6 ± 11.8	0.039
MAP (mm Hg) <sup>*</sup>	86.7 ± 10.2	88.3 ± 10.4	91.7 ± 12.9	0.014
Triglycerides (mmol/l)	0.93 (0.89–0.97)	1.13 (1.07–1.19)	1.50 (1.42–1.60)	<0.001
HDL cholesterol (mmol/l)	1.43 ± 0.35	1.37 ± 0.37	1.28 ± 0.39	0.009
LDL cholesterol (mmol/l)	3.17 ± 0.80	3.21 ± 0.74	3.37 ± 0.86	0.026
Fasting glucose (mmol/l)	5.03 (4.96–5.10)	5.22 (5.12–5.31)	5.47 (5.34–5.61)	<0.001
HOMA-IR	1.34 (1.27–1.41)	1.71 (1.60–1.83)	2.31 (2.16–2.48)	<0.001
Fibrinogen (g/l)	2.90 ± 0.57	2.92 ± 0.51	3.04 ± 0.62	NS
CRP (mg/l)	0.40 (0.36–0.45)	0.60 (0.54–0.67)	0.82 (0.74–0.91)	<0.001
ALP (U/l)	61.4 (59.6–63.2)	69.2 (67.2–71.3)	77.3 (74.7–80.0)	<0.001
ALT (U/l)	15.9 (15.1–16.6)	19.9 (19.0–20.9)	30.5 (28.5–32.7)	<0.001
AST (U/l)	19.6 (19.0–20.2)	21.5 (20.9–22.2)	26.3 (25.0–27.6)	<0.001
Current smoking (%)	12.4	15.3	14.5	NS
Hypertension (%)	14.8	21.9	39.1	<0.001

Data are expressed as mean ± SD or geometric mean (95% CI) unless otherwise stated.

P for trend was adjusted for age, sex, and BMI, where appropriate.

\* Subjects on anti-hypertensive medication were excluded from analysis.

hypertension [21]. However, in our study, plasma GGT predicted incident hypertension, even after adjusting for covariates including the insulin resistance index, HOMA-IR. Plasma GGT has been suggested as a marker of oxidative stress [3,39], a risk factor of hypertension and cardiovascular diseases. GGT is a key enzyme in the catabolism of glutathione and plays a role in the production of reactive oxygen species through modulating the redox status of cell surface

protein thiols [40]. In a clinical study, serum concentrations of antioxidants can predict GGT level at 10 years, but not vice versa [39]. A prospective study of American adults revealed significant association of plasma GGT with hypertension only among subjects who were overweight or had increased central body fat [9]. This may suggest fatty liver as an underlying mechanism for the association of plasma GGT with hypertension. Our previous work suggested that plasma

**Table 4**

Association with incident hypertension in CRISPS-3 (n = 708).

Marker	Unadjusted model		Model 1		Model 2	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<i>ALP tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.89 (1.11–3.22)	0.019	1.60 (0.88–2.90)	NS	1.48 (0.79–2.77)	NS
Tertile 3	2.98 (1.79–4.95)	<0.001	1.66 (0.94–2.94)	NS	1.48 (0.80–2.74)	NS
P for trend		<0.001		NS		NS
ALP, U/l*	1.49 (1.21–1.83)	<0.001	1.14 (0.90–1.43)	NS	1.08 (0.82–1.42)	NS
<i>ALT tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.44 (0.87–2.36)	NS	1.36 (0.78–2.40)	NS	1.19 (0.65–2.18)	NS
Tertile 3	1.82 (1.11–2.98)	0.017	1.59 (0.91–2.77)	NS	1.30 (0.69–2.43)	NS
P for trend		NS		NS		NS
ALT, U/l*	1.26 (1.05–1.51)	0.012	1.32 (1.05–1.67)	0.018	1.20 (0.92–1.56)	NS
<i>AST tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.11 (0.68–1.81)	NS	1.01 (0.58–1.77)	NS	1.12 (0.63–2.02)	NS
Tertile 3	1.66 (1.03–2.66)	0.037	1.44 (0.82–2.50)	NS	1.47 (0.82–2.64)	NS
P for trend		NS		NS		NS
AST, U/l*	1.22 (1.03–1.46)	0.026	1.20 (0.96–1.49)	NS	1.15 (0.91–1.45)	NS
<i>GGT tertile</i>						
Tertile 1	1.00 (Referent)		1.00 (Referent)		1.00 (Referent)	
Tertile 2	1.54 (0.90–2.63)	NS	1.28 (0.69–2.35)	NS	1.16 (0.60–2.22)	NS
Tertile 3	2.81 (1.69–4.66)	<0.001	2.93 (1.63–5.24)	<0.001	2.68 (1.36–5.26)	0.004
P for trend		<0.001		<0.001		0.004
GGT, U/l*	1.36 (1.13–1.62)	0.001	1.43 (1.14–1.79)	0.002	1.38 (1.05–1.81)	0.020

For ALP, the cut-off values for tertiles 1, 2, and 3 are ≤67, 68–81 and ≥82 U/l in men, and ≤54, 55–70 and ≥71 U/l in women, respectively.

For ALT, the cut-off values for tertiles 1, 2, and 3 are ≤19, 20–31 and ≥32 U/l in men, and ≤13, 14–19 and ≥20 U/l in women, respectively.

For AST, the cut-off values for tertiles 1, 2, and 3 are ≤20, 21–25 and ≥26 U/l in men, and ≤17, 18–22 and ≥23 U/l in women, respectively.

For GGT, the cut-off values for tertiles 1, 2, and 3 are ≤19, 20–30 and ≥31 U/l in men, and ≤13, 14–19 and ≥20 U/l in women, respectively.

Model 1: Adjusted for age, sex, and systolic blood pressure at baseline and follow-up duration.

Model 2: Further adjusted for baseline BMI, triglycerides, HDL cholesterol, HOMA-IR, CRP, fibrinogen, current smoking, and change in BMI.

\* ORs are expressed in term of per SD of the log-transformed unit.

GGT correlates with plasma CRP [10,11], so increased GGT may reflect inflammation that occurs in fatty liver.

Plasma ALP was associated with blood pressure among subjects not on anti-hypertensive medication in CRISPS-2. Although plasma ALP tertiles were not significantly related to hypertension, plasma ALP correlated with SBP and DBP when these were treated as continuous variables. The association of plasma ALP with blood pressure could be explained at least in part by its correlation with plasma GGT activities [10,11,41]. Increased plasma ALT is associated with the development of the metabolic syndrome [2] and type 2 diabetes [13–17], which are closely related to hypertension in our population [19,21]. In our study, plasma ALT was not an independent predictor of prevalent and incident hypertension in stepwise logistic regression analysis.

There are some limitations in this study. The cohort of this study is community-based and so the number of subjects with prevalent and incident hypertension is relatively small. The degree of variations in plasma ALP, ALT, AST, and GGT among subjects and within an individual may influence the degree of significance of their association with hypertension. Elevations of ALP, ALT, AST, and GGT in plasma can be non-specific and found in other diseases such as hepatitis, biliary diseases, musculoskeletal diseases, and myocardial injury. However, these non-specific causes of elevation in plasma markers of liver injury are likely to diminish rather than augment the observed association.

In conclusion, among the four plasma markers of liver injury, GGT is the strongest risk factor for hypertension in Hong Kong Chinese. Therefore, further studies to assess the utility of GGT as a biomarker for hypertension and related diseases are warranted.

#### List of abbreviations

CRISPS	Hong Kong Cardiovascular Risk Factor Prevalence Study
CRP	C-reactive protein
DBP	diastolic blood pressure
HOMA-IR	homeostasis model assessment of insulin resistance index
MAP	mean arterial pressure
OGTT	oral glucose tolerance test
SBP	systolic blood pressure

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ORIGINAL RESEARCH

# Prevalence of cardiometabolic risk factors and metabolic syndrome in obese Kuwaiti adolescents

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**Background:** Childhood and adolescent obesity is associated with insulin resistance, abnormal glucose metabolism, hypertension, dyslipidemia, inflammation, liver disease, and compromised vascular function. The purpose of this pilot study was to determine the prevalence of cardiometabolic risk factor abnormalities and metabolic syndrome (MetS) in a sample of obese Kuwaiti adolescents, as prevalence data might be helpful in improving engagement with obesity treatment in future.

**Methods:** Eighty obese Kuwaiti adolescents (40 males) with a mean (standard deviation) age of 12.3 years (1.1 years) participated in the present study. All participants had a detailed clinical examination and anthropometry, blood pressure taken, and assessment of fasting levels of C-reactive protein, intracellular adhesion molecule, interleukin-6, fasting blood glucose, insulin, liver function tests (alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase), lipid profile (cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides), insulin resistance by homeostasis model assessment, and adiponectin. MetS was assessed using two recognized criteria modified for use in younger individuals.

**Results:** The cardiometabolic risk factors with highest prevalence of abnormal values included aspartate aminotransferase (88.7% of the sample) and insulin resistance by homeostasis model assessment (67.5%), intracellular adhesion molecule (66.5%), fasting insulin (43.5%), C-reactive protein (42.5%), low-density lipoprotein cholesterol (35.0%), total cholesterol (33.5%), and systolic blood pressure (30.0%). Of all participants, 96.3% (77/80) had at least one impaired cardiometabolic risk factor as well as obesity. Prevalence of MetS was 21.3% according to the International Diabetes Federation definition and 30% using the Third Adult Treatment Panel definition.

**Conclusion:** The present study suggests that obese Kuwaiti adolescents have multiple cardiometabolic risk factor abnormalities. Future studies are needed to test the benefits of intervention in this high-risk group. They also suggest that prevention of obesity in children and adults should be a major public health goal in Kuwait.

**Keywords:** obesity, adolescents, prevalence, cardiometabolic risk factors, metabolic syndrome

## Background

Childhood and adolescent obesity is associated with insulin resistance, abnormal glucose metabolism, hypertension, dyslipidemia, inflammation, liver disease, and compromised vascular function.<sup>1–5</sup> As with obesity, these impairments could track into young adulthood, which increases the risk of cardiometabolic diseases and even certain types of cancer independent of adult weight.<sup>6,7</sup>

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The detrimental effects of adolescent obesity on subsequent risk of cardiovascular disease are partly mediated by the presence of cardiometabolic risk factors.<sup>8</sup> Cardiovascular disease is the leading cause of morbidity and mortality worldwide with an estimate of 17.3 million deaths in 2008, and by 2030 this number could reach up to 23.3 million.<sup>9</sup> It is widely believed that atherosclerosis begins in childhood and progresses into adulthood.<sup>10,11</sup> As the number of cardiovascular disease risk factors increases in childhood, so does the severity of both coronary and aortic atherosclerosis in young adulthood.<sup>3</sup> In the Netherlands, two-thirds of obese children and adolescents had more than one cardiovascular disease risk factor in one study.<sup>12</sup> In Germany and Switzerland, around 50% of obese children had at least one cardiometabolic risk factor in one study.<sup>13</sup>

The presence of obesity in childhood and adolescence is also related to the development of fatty liver or steatosis, which is the most common liver abnormality in this age group.<sup>14</sup> Steatosis can be present with or without elevated liver enzymes (aminotransferases).<sup>15</sup> For the long term, the ramifications of having persistently elevated liver enzymes and steatosis are important and could lead eventually to the development of cirrhosis.<sup>14,16</sup>

In two previous studies of obese adolescents in Kuwait, we observed that their health-related quality of life was unimpaired compared with nonobese peers,<sup>17</sup> and that their engagement with therapy to treat obesity was poor.<sup>18</sup> It is possible that knowledge of the presence of cardiometabolic risk factors in obese adolescents may increase the engagement of adolescents and their families with efforts to treat obesity. The aim of the present study was therefore to estimate the prevalence of cardiometabolic risk factors in obese adolescents in order to provide evidence that might be useful to future obesity treatment. In the present study, we carried out assessments of obesity-related cardiometabolic risk factors that could impair vascular health and liver function. These included lipid profile (cholesterol, low-density lipoprotein [LDL], very low-density lipoprotein, high-density lipoprotein [HDL], triglycerides [TG]), interleukin-6 (IL-6), intracellular adhesion molecule (ICAM), C-reactive protein (CRP), adiponectin, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma glutamyltransferase [GGT]), and insulin resistance by homeostasis model assessment (HOMA-IR).

## Materials and methods

### Participants

The study was the baseline element of an intervention to treat adolescent obesity using a randomized controlled

trial, the National Adolescent Treatment Trial for Obesity (NATTO).<sup>18</sup> We recruited 80 obese adolescents participating in the NATTO in Kuwait City<sup>18</sup> at the preintervention stage of the trial. They were all at or above the age- and sex-adjusted 95th body mass index (BMI) percentile, which defines obesity.<sup>19</sup> Age ranged from 10 years to 14 years. All participants underwent physical examination including anthropometric assessment (weight, height, BMI, waist circumference) and had no medical or surgical history. All participants and their parents consented to take part in the study. The study was approved by the Medical Research Committee of the Ministry of Health – Kuwait.

Blood samples were drawn for analysis for fasting blood glucose (FBG), fasting insulin, cholesterol, LDL, HDL, TG, ALT, AST, GGT, CRP, IL-6, ICAM, and adiponectin. Insulin resistance was measured by HOMA-IR (fasting insulin  $\times$  fasting glucose/22.5).<sup>20</sup>

Blood pressure was measured when the participant was sitting quietly in the upright position, with the correct cuff size applied to the right arm. The reading was repeated three times, and the average of the three readings was taken.

### Biochemical assessment

Cholesterol, TG, HDL, sensitive CRP, ALT, AST, and GGT assays were assessed using a C311 Roche analyzer; sensitive CRP immunoturbidimetric assays with cholesterol, TG, HDL, ALT, AST, and GGT being enzymatic colorimetric. Kits were supplied by Roche Diagnosticx GmbH. IL-6, ICAM, adiponectin, and insulin analysis (enzyme-linked immunosorbent assays) was assessed using kits supplied by R&D Systems Europe Ltd (Oxford, UK) and Mercodia AB.

### Cutoff points for defining the cardiometabolic risk factors and metabolic syndrome

There are two commonly used cutoff points for FBG (mmol/L), the World Health Organization (WHO) normal cutoff  $<6.1$  mmol/L<sup>21</sup> and the American Diabetes Association normal cutoff  $<5.6$  mmol/L.<sup>22</sup> However, in Kuwait, the official criterion used for diagnosing and classifying diabetes mellitus is the WHO criterion,<sup>23</sup> and so that was used in the present study.

Ideally, hyperinsulinemia is defined if insulin level exceeds the normal value according to the pubertal stage, due to the impact of physiological insulin resistance of puberty.<sup>24</sup> However, Tanner staging was not assessed during the clinical examination in the present study for social and cultural reasons. Thus, standard values of normal, borderline, and

high fasting insulin levels proposed by the American Heart Association scientific statement were chosen.<sup>25</sup>

HOMA-IR is a proxy for insulin resistance and is widely used in clinical settings and research, with high reliability in determining insulin resistance.<sup>20</sup> There is still a debate about the appropriate cutoff point for HOMA-IR, with proposed values of  $\geq 2.5$ ,<sup>26,27</sup>  $\geq 1.77$ ,<sup>28</sup> and  $> 3.16$ .<sup>20</sup> Keskin et al<sup>20</sup> found that HOMA-IR was the most sensitive and most specific of three proxies for defining insulin resistance, and the cutoff point for insulin resistance diagnosis based on HOMA-IR was 3.16,<sup>20</sup> so that definition was used in the present study.

Assessment of lipid profile for the participants included fasting TG, fasting cholesterol, fasting LDL, and fasting HDL. Jolliffe and Janssen<sup>29</sup> developed age- and sex-specific percentiles for lipoproteins and cholesterol, starting from age 12 years to age 20 years. However, our participants were aged 10–14 years, and it was not possible to use these lipoprotein percentiles for the whole sample. Therefore, the reference values for these parameters were taken from the National Cholesterol Education Program, with fixed cutoff points for normal, borderline, and high values regardless of sex and age.<sup>30</sup>

Liver function tests were obtained in all participants and included ALT, AST, and GGT. The upper limit for ALT and AST in adults differs between populations, and differences exist between males and females.<sup>31</sup> However, in studies examining the prevalence of abnormal ALT, AST, and GGT in adolescents, the most commonly used cutoff points were  $> 40$  U/L,  $> 40$  U/L, and  $> 35$  U/L, respectively.<sup>15,32,33</sup> Therefore, these were the values that we used as cutoff points in our study.

Markers of inflammation were assessed in all participants, including CRP.<sup>34</sup> Generally, normal and abnormal levels of CRP were developed for the adult population,<sup>34,35</sup> and some studies found that the normal range in healthy adults was from 0.08 mg/L to 6.1 mg/L.<sup>36</sup> Our study used the cutoff points set by the American Heart Association and the Centers for Disease Control and Prevention.<sup>34</sup>

The inflammatory cytokine IL-6 has an age-related variability with peak physiological elevation around age 4 years and 15 years in relation to cartilage and bone development.<sup>37</sup> In the literature, precise reference ranges for IL-6 vary greatly depending on the age, weight status, and sex of the participants tested.<sup>37–39</sup> In the present study, we used the reference range of the control group (healthy controls n=37) from a study by Makni et al<sup>40</sup> ( $> 3.9$  pg/mL).

Inflammatory plasma soluble adhesion molecules (ICAM) were also measured in all of the participants.<sup>41</sup> The literature

shows that ICAM values are age related, and when applying the cutoff point for our study we chose a study by Andrys et al<sup>42</sup> to establish reference range for serum soluble adhesion molecules in healthy children and adolescents aged 6–15 years, defined by values between the fifth and 95th percentiles for each inflammatory marker. The normal cutoff range for those aged 6–10 years was 206.8–486.8 ng/mL, and for those aged 11–15 years was 184.1–355.0 ng/mL.<sup>42</sup>

The anti-inflammatory adipokine adiponectin was measured in all participants in the fasting state. It is normally present in plasma concentrations of 2–20 µg/mL.<sup>43</sup> Most studies comparing adiponectin concentration in obese adolescents with its concentration in healthy controls referred to “low levels” when adiponectin concentration was  $< 5$  µg/mL, as compared with its concentration in healthy control subjects at  $> 10$  µg/mL.<sup>44–46</sup> Therefore, in the present study, we used the same cutoff points.

Hypertension was defined as a systolic and/or diastolic blood pressure  $\geq 95$ th percentile for age, sex, and height, measured on three separate occasions.<sup>47</sup> Metabolic syndrome (MetS) was defined according to the International Diabetes Federation (IDF) definition<sup>48</sup> and the Third Adult Treatment Panel (ATP III) definition.<sup>49</sup> Participants were classified as having MetS if they had a waist circumference  $\geq 90$ th percentile plus two or more of the following criteria according to the IDF definition: TG  $\geq 1.7$  mmol/L, HDL  $< 1.03$  mmol/L, blood pressure  $\geq 130/85$  mmHg, and FBG  $\geq 5.6$  mmol/L. Classification of MetS according to the ATP III definition was based on the presence of three or more of the following criteria: waist circumference  $\geq 90$ th percentile, TG  $\geq 1.24$  mmol/L, HDL  $\leq 1.03$  mmol/L, blood pressure  $\geq 90$ th percentile, and FBG  $\geq 6.1$  mmol/L.

## Results

### Characteristics of study participants

Table 1 shows the mean and standard deviation (SD) of all measured parameters for the participants (n=80). The mean age was 12.3 years (SD 1.1 years).

### Prevalence of cardiometabolic risk factors

Twenty-six out of the 80 participants (32.5%) had systolic and/or diastolic blood pressure  $\geq 95$ th percentile for age, sex, and height. Hyperglycemia and hyperinsulinemia were present in 2.5% (two of 80) and 43.8% (35/80) of participants, respectively. Insulin resistance as defined by HOMA-IR value  $> 3.16^{20}$  was found in 67.5% (54/80) of participants. Out of the 80 participants, 27.5% (22/80) had a high TG level, 33.8% (27/80) had a high total cholesterol level, 20% (16/80) had a

**Table 1** Descriptive parameters of the adolescents according to sex, mean (standard deviation)

Variables	All participants (n=80)	Boys (n=40)	Girls (n=40)	Number of participants with abnormality (%)	
				Borderline	High
Age, years	12.3 (1.1)	12.4 (1.2)	12.3 (1.1)	na	na
BMI Z-score	2.2 (0.3)	2.2 (0.3)	2.2 (0.3)	na	na
Waist circumference, cm	93.3 (12.2)	96.6 (12.4)	90.0 (11.2)		
Systolic blood pressure, mmHg	122 (11)	125 (11)	119 (9)	na	24 (30.0%)
Diastolic blood pressure, mmHg	77 (8)	78 (8)	77 (7)	na	14 (17.5%)
Total cholesterol, mmol/L	4.7 (0.9)	4.7 (1.0)	4.7 (0.8)	25 (31.5%)	27 (33.8%)
LDL, mmol/L	3.0 (0.8)	3.0 (0.9)	3.0 (0.7)	20 (25%)	28 (35.0%)
TG, mmol/L	1.3 (0.5)	1.3 (0.5)	1.3 (0.5)	26 (32.5%)	22 (27.5%)
HDL, mmol/L	1.1 (0.2)	1.1 (0.2)	1.1 (0.3)	60 (75%) low	16 (20.0%)
FBG, mmol/L	4.7 (0.8)	4.8 (0.9)	4.5 (0.6)	na	2 (2.5%)
Fasting insulin, $\mu$ U/L	26.7 (23.8)	26.4 (25.8)	27.0 (22.0)	21 (26.5%)	35 (43.8%)
HOMA-IR	6.0 (7.3)	6.4 (9.2)	5.5 (5.0)	na	54 (67.5%)
ALT, U/L	34.2 (23.6)	42.2 (21.3)	26.1 (23.4)	na	21 (26.3%)
AST, U/L	58.1 (19.3)	63.3 (15.6)	52.8 (21.4)	na	71 (88.8%)
GGT, U/L	27.0 (12.6)	31.4 (13.9)	22.7 (9.6)	na	14 (17.5%)
CRP, mg/L	4.2 (5.1)	5.0 (4.6)	3.5 (5.5)	31 (38.5%)	34 (42.5%)
IL-6, pg/mL	2.0 (1.8)	1.9 (1.5)	2.0 (2.1)	na	6 (7.5%)
ICAM, ng/mL	461.3 (158.5)	493.2 (158.0)	429.4 (154.6)	na	53 (66.3%)
Adiponectin, ng/mL	50.7 (25.0)	47.0 (21.5)	54.4 (27.9)	na	

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; FBG, fasting blood glucose; GGT, gamma glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, insulin resistance by homeostasis model assessment; ICAM, intracellular adhesion molecule; IL-6, interleukin-6; LDL, low-density lipoprotein; na, not applicable; TG, triglycerides.

low HDL level, and 35% had a high LDL level. Liver function tests showed high ALT in 26.3% (21/80) of participants, high AST in 88.8% (71/80) of participants, and high GGT level in 17.5% (14/80) of participants. CRP level was high in 42.5% (34/80) of participants, IL-6 level was high in 7.5% (six of 80) of participants, ICAM level was high in 66.3% (53/80) of participants, and adiponectin level was normal in all participants.

Table 2 shows the results of waist circumference, TG, HDL, FBG, systolic blood pressure, and diastolic blood pressure measurements using IDF and ATP III criteria.

**Table 2** Metabolic syndrome prevalence using IDF and ATP III criteria in the participants

Anthropometric and biochemical variables	Mean (standard deviation)	IDF	ATP III
Waist circumference (cm)	93.3 (12.2)	66 (82.5%)	66 (82.5%)
TG (mmol/L)	1.3 (0.5)	12 (15%)	37 (46.5%)
HDL (mmol/L)	1.1 (0.2)	26 (32.5)	26 (32.5%)
FBG (mmol/L)	4.7 (0.8)	4 (5%)	2 (2.5%)
Systolic blood pressure	122 (11)	9 (11.5%)	11 (13.5%)
Diastolic blood pressure	77 (8)		
Metabolic syndrome prevalence		17 (21.3%)	24 (30%)

**Abbreviations:** ATP III, Third Adult Treatment Panel; FBG, fasting blood glucose; HDL, high-density lipoprotein; IDF, International Diabetes Federation; TG, triglycerides.

Seventeen of the 80 participants (21.3%) met the diagnosis of MetS by the IDF definition and 24 of the 80 participants (30%) met the diagnosis of MetS by the ATP III definition.

## Discussion

The current study is the first to estimate the prevalence of cardiometabolic risk factors and MetS in a group of obese Kuwaiti adolescents. The main finding of this study was the high prevalence of multiple cardiometabolic risk factors. Out of the 16 risk factors measured, eight were high in  $\geq 30\%$  of the participants (Table 1). The cardiometabolic risk factors with the highest prevalence of abnormal values included AST (88.7% of the sample), HOMA-IR (67.5% of the sample), ICAM (66.5% of the sample), fasting insulin (43.5% of the sample), CRP (42.5% of the sample), LDL (35.0% of the sample), cholesterol (33.5% of the sample), and systolic blood pressure (30.0% of the sample); 96.3% (77/80) of participants had at least one cardiometabolic risk factor as well as obesity.

As mentioned previously, participants of this study were recruited from the baseline stage of a randomized controlled trial of an office-based treatment trial for adolescent obesity in Kuwait (NATTO). One of the findings of the NATTO was poor engagement with treatment, as evidenced by the poor attendance of families in both the intervention and control arms of the trial.<sup>18</sup> Therefore, findings from the present study might have been useful to demonstrate to the adolescents and

their families that their obesity was a medical problem, and so possibly persuade them to engage more with treatment. Moreover, all of the measured parameters in the present study, except for adiponectin, are readily accessible by physicians working in the Ministry of Health – Kuwait in the clinical setting, so their measurement could be part of any treatment protocol for adolescent obesity in the future.

Risk factors for cardiovascular disease and type 2 diabetes mellitus have extended their roots to reach children and adolescents.<sup>6,7,10,50–54</sup> In a study from Iran<sup>55</sup> on 5,528 adolescents aged 10–18 years assessing the relationship between multiple cardiometabolic risk factors (total cholesterol, TG, LDL, HDL, blood pressure, and FBG) with BMI, low physical activity, and an unhealthy diet, BMI had the greatest direct effect on total cholesterol, LDL, TG, FBG, and blood pressure and an inverse relationship with HDL, more than that contributed by inactivity and an unhealthy diet. Kelishadi et al<sup>55</sup> called for immediate interventions to tackle pediatric obesity and its associated cardiometabolic risk factors in order to prevent future risk of MetS and chronic noncommunicable diseases in Iran.

Kardas et al<sup>56</sup> compared the levels of cholesterol, LDL, TG, HDL, FBG, blood pressure, vitamin D, and adiponectin between obese (n=63) and nonobese (n=51) Turkish adolescents aged 10–16 years. Obesity was defined as BMI >90th percentile for an age- and sex-specific Turkish reference population. Cholesterol, LDL, TG, FBG, and blood pressure were significantly higher in the obese group compared with the nonobese group. Adiponectin, vitamin D, and HDL were significantly lower in the obese group compared with the nonobese group. Mean adiponectin value for the obese group was 3.3 ( $\pm 0.89$ ) ng/mL and in the nonobese group the mean value was 6.0 ( $\pm 1.4$ ) ng/mL.

In the Netherlands, inpatient children and adolescents (n=80, aged between 8 years and 19 years) diagnosed with severe obesity (defined as BMI SDS  $\geq 3$  or BMI SDS  $\geq 2.3$  with comorbidities according to the growth percentiles of the Fourth Dutch Growth Study) were evaluated for the presence of multiple cardiometabolic risk factors, namely blood pressure, fasting insulin, FBG, HOMA-IR, cholesterol, LDL, TG, HDL, and CRP<sup>57</sup> as part of an inpatient treatment trial for their obesity. Data showed that 80% of the participants had at least one impaired cardiometabolic risk factor as well as severe obesity. In comparison with our study, 90% of our participants had at least one impairment with regards to the same cardiometabolic risk factors assessed.

In the present study, almost a third of the participants had MetS according to the ATP III definition.<sup>58</sup> In a study done in Kuwait on apparently healthy female adolescents (n=431, age

10–19 years) to assess the prevalence of MetS using the same definitions that we applied to our study, it was found that MetS was present in 9.1% by the ATP III definition and 14.8% had MetS when the IDF definition was used.<sup>59</sup> In Saudi Arabia, the prevalence of MetS using the IDF definition was 18% among 180 obese 9- to 12-year-olds.<sup>60</sup> Also using the IDF definition in Lebanese adolescents, Nasreddine et al<sup>61</sup> found that 21.2% of the 104 obese adolescents (mean age  $16 \pm 1.3$  years) had MetS, 3.8% of the 78 overweight adolescents (mean age  $16.4 \pm 1.4$  years) had MetS, and 1.2% of the 81 healthy weight adolescents (mean age 16.8 years) had MetS. In Iran, according to the ATP III definition, MetS has been found in 3.3% of Iranian adolescents (n=450, age 15–18 years).<sup>62</sup> In a sample of 321 overweight, obese, and extremely obese adolescents from Brazil (obesity defined using the Centers for Disease Control and Prevention 2000 definition,<sup>19</sup> MetS was found in around 18% of the 10- to 16-year-old adolescents using the IDF definition.<sup>63</sup> Similarly, in the US,<sup>64</sup> it was found that  $>50\%$  of obese children and adolescents (n=439, aged 4–20 years) had MetS according to definitions modified from ATP III and WHO.<sup>24</sup> In summary, global studies suggest that, as in the present study, MetS is relatively common among obese adolescents.

The present study had a number of strengths. Our participants were generally a fairly homogenous group of Kuwaiti adolescents living in Kuwait City and recruited from three State schools who were examined for the presence of cardiometabolic risk factors, including MetS. The use of traditional markers for cardiovascular disease (ie, lipid profile and blood pressure), multiple markers for inflammation (ie, CRP, IL-6, and ICAM), and, for the first time, adiponectin in a sample of Kuwaiti adolescents, assessment of insulin resistance as well as liver function, all add to the novelty of our study.

However, our study had a number of limitations. First, it was not possible to conduct Tanner staging, due to social/cultural and practical reasons. Second, the optimal cutoff to define abnormality for a number of the cardiometabolic risk factors is unclear, but widely used cutoffs were chosen for the present study. Third, no data on changes in cardiometabolic risk factors during obesity treatment were available. Improvements in cardiometabolic risk profile might increase engagement with obesity treatment. Nonetheless, the relatively high prevalence of abnormal values for cardiometabolic risk factors found in the present study could be a useful aid to engage more families into participating in adolescent obesity treatment in future, and might also increase the level of commitment to participation by those who do take part.

## Conclusion

The present study suggests that a number of cardiometabolic risk factors and MetS are prevalent in obese Kuwaiti adolescents. This observation might provide impetus to future strategies to treat pediatric obesity and to prevent or delay the appearance of cardiovascular disease and diabetes mellitus in the future adult generation. The observation might also be used to encourage greater engagement with treatment among families.

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## Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL ARTICLE

## Gamma glutamyltransferase, inflammation and cardiovascular risk factors in isolated coronary artery ectasia

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### KEYWORDS

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### Abstract

**Introduction and Objective:** There are conflicting data on the prevalence of cardiovascular risk factors in coronary artery ectasia (CAE). It is unclear whether CAE is associated with high-sensitivity C-reactive protein (hs-CRP) and gamma glutamyltransferase (GGT). We therefore investigated major cardiovascular risk factors, serum GGT and hs-CRP levels in a large population of patients with CAE.

**Methods:** A total of 167 patients with isolated CAE and 150 controls with normal coronary arteries were selected from 10 505 patients undergoing coronary angiography. Serum GGT and hs-CRP levels were evaluated in addition to cardiovascular risk factors including family history, obesity, smoking, diabetes, hypertension and hyperlipidemia.

**Results:** Hypertension and obesity were slightly more prevalent in CAE patients than in controls, whereas diabetes was slightly less frequent in CAE patients. Other risk factors were similar. Serum GGT (22 [17–42] vs. 16 [13–21] U/l,  $p=0.001$ ) and hs-CRP (2.9 [1.9–3.6] vs. 1.4 [1.1–1.8] mg/l,  $p=0.001$ ) levels were higher in CAE patients than in controls. The presence of CAE was independently associated with diabetes (OR: 0.44, 95% CI: 0.20–0.95,  $p=0.04$ ), obesity (OR: 2.84, 95% CI: 1.07–7.56,  $p=0.04$ ), GGT (OR: 1.08, 95% CI: 1.03–1.12,  $p=0.001$ ) and hs-CRP levels (OR: 3.1, 95% CI: 2.1–4.6,  $p=0.001$ ). In addition, GGT and hs-CRP levels were higher in diffuse and multivessel ectasia subgroups than focal and single-vessel ectasia subgroups (each  $p<0.05$ ).

**Conclusions:** Our findings show that CAE can be independently and positively associated with obesity, GGT and hs-CRP levels, but inversely with diabetes. Moreover, its severity may be related to GGT and hs-CRP levels.

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**PALAVRAS-CHAVE**  
Ectasia coronária;  
Fatores de risco;  
Inflamação;  
Glutamiltransferase

## Gama glutamiltransferase, inflamação e fatores de risco cardíacos na ectasia isolada da artéria coronária

### Resumo

**Introdução e objetivos:** Existem dados contraditórios relativamente à prevalência dos fatores de risco cardiovascular na ectasia da artéria coronária (EAC). Não é claro se a EAC possa estar associada à proteína C reativa de alta-sensibilidade (PCR-as) e à gama glutamiltransferase (gama-GT). Assim examinámos fatores de risco cardiovascular *major*, a gama-GT sérica e os níveis de PCR-as numa população mais alargada de doentes com EAC.

**Métodos:** Foram selecionados um total de 167 doentes com EAC isolada e 150 casos-controlo com artérias coronárias normais dos 10 505 doentes submetidos a angiografia coronária. A gama-GT sérica e os níveis de PCR-as foram avaliados para além dos fatores de risco cardiovascular incluindo a história familiar, obesidade, tabagismo, diabetes, hipertensão e hiperlipidemia.

**Resultados:** A hipertensão e a obesidade foram ligeiramente mais prevalentes nos doentes com EAC do que nos casos-controlo enquanto a diabetes foi menos frequente nos doentes com EAC. Os outros fatores de risco foram semelhantes. Os níveis de gama-GT sérica [22 (17-42) versus 16 (13-21) U/L,  $p = 0,001$ ] e de PCR-as [2,9 (1,9-3,6) versus 1,4 (1,1-1,8) mg/L,  $p = 0,001$ ] foram superiores nos doentes com EAC do que nos casos-controlo. A presença de EAC foi independentemente associada à diabetes (OR: 0,44, IC 95%: 0,20-0,95,  $p = 0,04$ ), obesidade (OR: 2,84, IC 95%: 1,07-7,56,  $p = 0,04$ ), gama-GT (OR: 1,08, IC 95%: 1,03-1,12,  $p = 0,001$ ) e níveis de PCR-as (OR: 3,1, IC 95%: 2,1-4,6,  $p = 0,001$ ). Além disso, os níveis de GGT e de PCR-as foram superiores nos subgrupos de ectasia difusa e multivasos do que nos subgrupos de ectasia focal e de um vaso (cada  $p < 0,05$ ).

**Conclusão:** As nossas conclusões mostram que a EAC pode ser certamente associada à obesidade, aos níveis de gama-GT e de PCR-as, mas de modo inverso à diabetes. Além disso a sua gravidade pode estar associada aos níveis de gama-GT e de PCR-as.

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## Introduction

Coronary artery ectasia (CAE) is characterized by an abnormal dilatation of the coronary arteries.<sup>1-3</sup> More than half of cases of CAE are due to atherosclerosis, and it has thus been considered a variant of atherosclerotic coronary artery disease (CAD).<sup>1-3</sup>

It has been shown that inflammation is one of the causes of atherosclerosis.<sup>4</sup> Similarly, previous studies have shown a link between C-reactive protein (CRP) and CAE.<sup>5,6</sup> However, these studies were relatively small. On the other hand, there is a variety of data on the association of major risk factors for atherosclerosis with CAE.<sup>2,3,7-11</sup>

Gamma glutamyltransferase (GGT) catalyzes glutathione, a major non-protein antioxidant in the cell.<sup>12</sup> It plays a role in oxidation of low-density lipoprotein (LDL) cholesterol and in the pathogenesis of atherosclerosis.<sup>13,14</sup> Epidemiologic studies have reported that serum GGT level has predictive value for cardiovascular disease and mortality in the general population.<sup>15-17</sup>

There have been two studies evaluating GGT levels in CAE patients. They showed that GGT levels were increased in patients with CAE,<sup>18,19</sup> but these studies were small. Therefore, we aimed to investigate serum GGT and CRP levels in addition to major risk factors for atherosclerosis in a larger population of patients with isolated CAE.

## Methods

### Patients

Between January 2007 and December 2012, 427 (4.1%) patients with CAE were selected from 10 505 patients who underwent elective diagnostic coronary angiography in our center. After application of the exclusion criteria, the remaining 167 (1.6%) isolated CAE patients were designated the CAE group. During the same period, 150 age- and gender-matched controls with normal coronary arteries were consecutively selected. The indication for coronary angiography was the presence of typical angina pectoris or significant myocardial ischemia in noninvasive stress tests.

Exclusion criteria were as follows: acute coronary syndromes, history of alcohol consumption, high alanine and/or aspartate transaminase levels, presence of concomitant stenotic lesion (>25% stenosis), significant left ventricular hypertrophy (septal thickness  $\geq 13$  mm), hematologic disorders, acute or chronic infectious disease, hepatitis or previously known inflammatory/autoimmune disorders, renal dysfunction (serum creatinine  $\geq 177$  mmol/l), documented cancer, use of steroids, and significant valvular heart disease (moderate to severe for stenotic lesions or grade  $\geq 2$  for valvular regurgitation).

A detailed medical history and history of cardiovascular risk factors such as diabetes, hypertension and smoking

were obtained from the study population. For each patient, body mass index (BMI) was calculated by the formula of weight (kg) divided by height (m)<sup>2</sup>. BMI was categorized as normal ( $\leq 25 \text{ kg/m}^2$ ), overweight ( $25\text{--}30 \text{ kg/m}^2$ ) or obesity ( $>30 \text{ kg/m}^2$ ). Hypertension was considered to be present if systolic blood pressure was  $\geq 140 \text{ mmHg}$  or diastolic blood pressure was  $\geq 90 \text{ mmHg}$  or both, or if the individual was taking antihypertensive medication. Diabetes was defined as the use of antidiabetic therapy or a fasting plasma glucose level of  $\geq 7 \text{ mmol/l}$  ( $\geq 126 \text{ mg/dl}$ ) in at least two measurements. Family history of CAD was diagnosed if patients had a first-degree male relative  $<55$  years of age or female relative  $<65$  years of age with CAD. Patients who smoked before hospitalization were classified as smokers. Hyperlipidemia was defined as LDL cholesterol  $\geq 3.4 \text{ mmol/l}$  ( $\geq 130 \text{ mg/dl}$ ), triglycerides  $\geq 2.26 \text{ mmol/l}$  ( $\geq 200 \text{ mg/dl}$ ) or use of lipid-lowering drugs.

All patients gave their informed consent for participation in the study, which was approved by the local ethics committee.

### Blood sampling and assays

Blood samples were drawn after a fasting period of 12 hours. Serum glucose, creatinine and lipid profile were determined by standard methods. Whole blood counts were made in a blood sample collected in dipotassium EDTA tubes with an automatic blood counter (Beckman Coulter Inc., CA, USA). Serum GGT levels were measured by the enzymatic calorimetric method using commercially available test kits with an AU640 auto-analyzer (Olympus, Japan). Normal values were defined as 0–50 U/l in our laboratory. In addition, other biochemical tests were performed using original kits with the Olympus autoanalyzer. Serum hs-CRP was measured by nephelometry using commercially available kits in accordance with the manufacturer's instructions (Beckman Coulter Array 360, Brea, CA, USA).

### Angiographic evaluation

Using the Judkins technique, coronary angiography was performed with contrast agents without intracoronary nitroglycerin. Arteriograms were obtained in both right and left anterior oblique projection with caudal and cranial angulation for the left and right coronary system. Additional views were also obtained in patients with inadequate visualization. Images were recorded in digital format and stored for later analysis. Right anterior oblique view was used to evaluate ectasia for the left coronary system and left anterior oblique view for the right coronary artery. Evaluations were performed visually by two experienced angiographers blinded to each other's findings. Vessel diameter was calculated quantitatively in the event of disagreement concerning CAE. Each major coronary artery was subdivided into proximal, mid, and distal segments.

Coronary ectasia was defined as dilation exceeding 1.5 times the normal diameter of normal adjacent segments.<sup>1–3</sup> If no normal adjacent segment could be identified, the mean diameter of the corresponding segment in the control group was taken as the normal value. If the coronary arteries had a normal appearance or no atherosclerotic

plaques with  $\geq 25\%$  stenosis, they were regarded as normal. Patients with concomitant obstructive and ectatic lesions were not included in the study. CAE was defined as focal when involving one segment and as diffuse when involving two or more segments in a major coronary artery, and as severe when diffuse ( $\geq 2$  segments) in  $\geq 2$  vessels.

### Statistical analysis

Continuous variables were presented as mean  $\pm$  standard deviation or median (interquartile range [IQR] of the 25th–75th percentiles of GGT and hs-CRP levels) and categorical variables as number (%). Comparisons between the groups were performed with the Student's t test, the Mann-Whitney U test and chi-square test or Fisher's exact test as appropriate. Gender-specific GGT tertiles were calculated in both groups, with GGT cut points of 18 and 24 U/l in men and 15 and 23 U/l in women delineating low, mid and top tertiles. Similarly, hs-CRP was categorized into low, mid and top tertiles (cut points of 1.53 and 2.56 mg/l). After univariate analyses had been performed for CAE, binary logistic regression analysis was performed to identify independent variables associated with the presence of CAE. Confounders which had significance at the  $p\leq 0.15$  level were entered into the regression analysis. They were hypertension, diabetes, obesity, triglycerides, uric acid, GGT, hs-CRP and platelet count. Odds ratios (OR) and 95% confidence intervals were calculated. The correlation between GGT and CRP levels was evaluated by Pearson correlation analysis. A  $p$  value of  $<0.05$  was considered significant. All analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA).

### Results

The demographic and clinical characteristics of the CAE and control groups are presented in Table 1. Hypertension and obesity were slightly more prevalent in CAE patients compared with controls, whereas the prevalence of diabetes was slightly lower. Other parameters were comparable in the two groups.

Coronary ectasia was located most frequently in the left anterior descending artery (62%), followed by the right coronary artery (59%) and circumflex artery (29%). Diffuse ectasia was seen in 91 patients (55%) and multivessel ectasia in 67 (40%).

Laboratory variables of the study groups are shown in Table 2. Triglyceride level and platelet count were higher in the CAE group than in the control group. Similarly, CAE patients had slightly higher uric acid levels ( $329.2\pm 75.6$  vs.  $311.7\pm 72.7 \mu\text{mol/l}$ ,  $p=0.06$ ). Other parameters were similar in both groups.

Median GGT level was higher in CAE patients than in controls (22 [IQR: 17–42] vs. 16 [IQR: 13–21] U/l,  $p=0.001$ , Table 2, Figure 1A). Similarly, hs-CRP levels were higher in CAE patients than in controls (2.9 [IQR: 1.9–3.6] vs. 1.4 [IQR: 1.1–1.8] mg/l,  $p=0.001$ , Figure 1B). The percentages of patients in the top tertile of GGT and hs-CRP were higher in CAE patients than in controls ( $p$  for each trend= $0.001$ , Figure 2).

**Table 1** Demographic and clinical characteristics of the study population.

Variables	CAE group (n=167)	Control group (n=150)	p
Age, years	58.7±8.2	59.7±7.6	0.38
Male/female	102/65	84/66	0.42
BMI (kg/m <sup>2</sup> )	27.1±2.7	26.9±2.6	0.50
Smoking	64 (38%)	70 (47%)	0.16
Hypertension	124 (74%)	96 (64%)	0.052
Diabetes	31 (19%)	41 (27%)	0.08
Family history of CAD	30 (18%)	22 (15%)	0.45
Dyslipidemia	58 (35%)	46 (31%)	0.47
Obesity	27 (16%)	13 (9%)	0.06
SBP (mmHg)	128±16	127±14	0.55
DBP (mmHg)	77±9	76±8	0.30
<b>Medications</b>			
Aspirin	162 (97%)	148 (99%)	0.45
Beta-blockers	142 (85%)	118 (79%)	0.17
Statins	50 (30%)	39 (26%)	0.51
ACEIs or ARBs	80 (48%)	39 (56%)	0.18
Calcium antagonists	23 (14%)	26 (17%)	0.44

ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; BMI: body mass index; CAD: coronary artery disease; CAE: coronary artery ectasia; DBP: diastolic blood pressure; SBP: systolic blood pressure.

**Table 2** Laboratory parameters of the study population.

	CAE group (n=167)	Control group (n=150)	p
Blood glucose (mmol/l)	6.10±1.51	6.28±2.66	0.45
AST (U/l)	22.6±7.3	23.6±8.7	0.27
ALT (U/l)	20.2±8.8	21.1±9.7	0.39
GGT (U/l) <sup>a</sup>	22 (17–42)	16 (13–21)	0.001
hs-CRP (mg/l) <sup>a</sup>	2.9 (1.9–3.6)	1.4 (1.1–1.8)	0.001
Creatinine (mmol/l)	90.71±17.05	94.18±20.73	0.80
Total cholesterol (mmol/l)	4.78±0.89	4.81±1.02	0.77
Triglycerides (mmol/l) <sup>a</sup>	1.63 (1.34–2.24)	1.51 (1.13–2.25)	0.02
HDL (mmol/l)	1.12±0.17	1.10±0.22	0.36
LDL (mmol/l)	2.68±0.70	2.77±0.67	0.24
Uric acid (μmol/l)	329.2±75.6	311.6±72.7	0.06
Fibrinogen (μmol/l)	10.23±1.79	9.92±1.77	0.13
Hemoglobin (g/dl)	13.4±1.3	13.3±1.4	0.51
WBC ( $\times 10^9$ /l)	7.88±1.9	8.11±2.2	0.32
Platelet count ( $\times 10^9$ /l) <sup>a</sup>	248 (190–286)	194 (170–216)	0.001
Ejection fraction (%)	63±5	62±6	0.34

ALT: alanine transaminase; AST: aspartate transaminase; GGT: gamma glutamyltransferase; hs-CRP: high-sensitivity C-reactive protein; WBC: white blood count.

<sup>a</sup> Median (interquartile range).

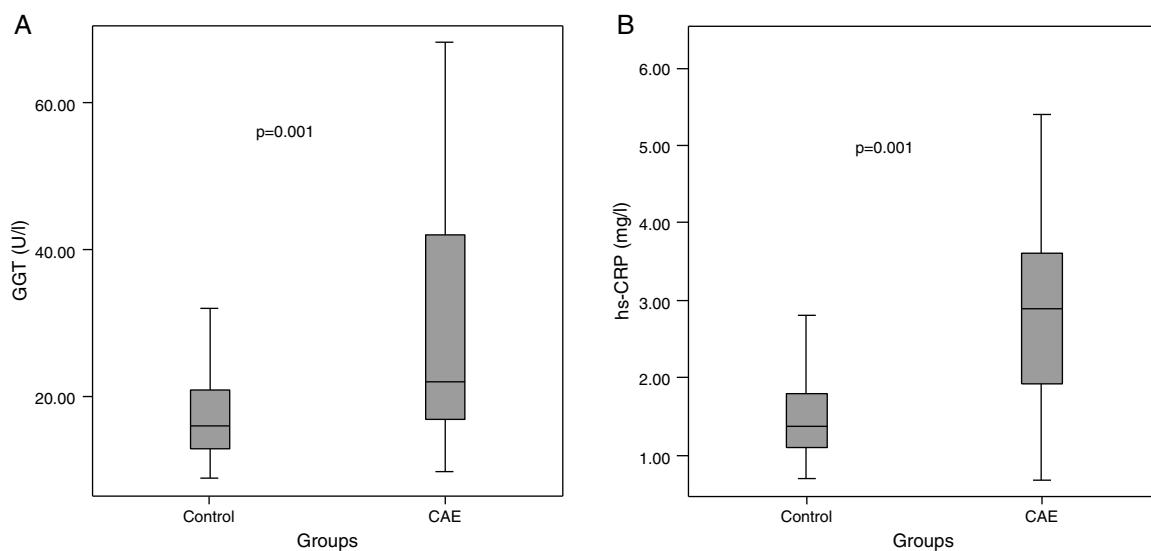
In logistic regression analysis, the presence of CAE was independently and positively associated with GGT levels (OR: 1.08, 95% CI: 1.03–1.12, p=0.001), hs-CRP levels (OR: 3.1, 95% CI: 2.10–4.59, p=0.001) and obesity (OR: 2.84, 95% CI: 1.07–7.56, p=0.038), but negatively with diabetes (OR: 0.44, 95% CI: 0.20–0.95, p=0.036).

In addition, GGT and hs-CRP levels were significantly higher in diffuse and multivessel ectasia subgroups than in focal and single-vessel ectasia subgroups (Table 3). There was a moderate correlation between GGT and hs-CRP levels (r=0.50, p=0.001).

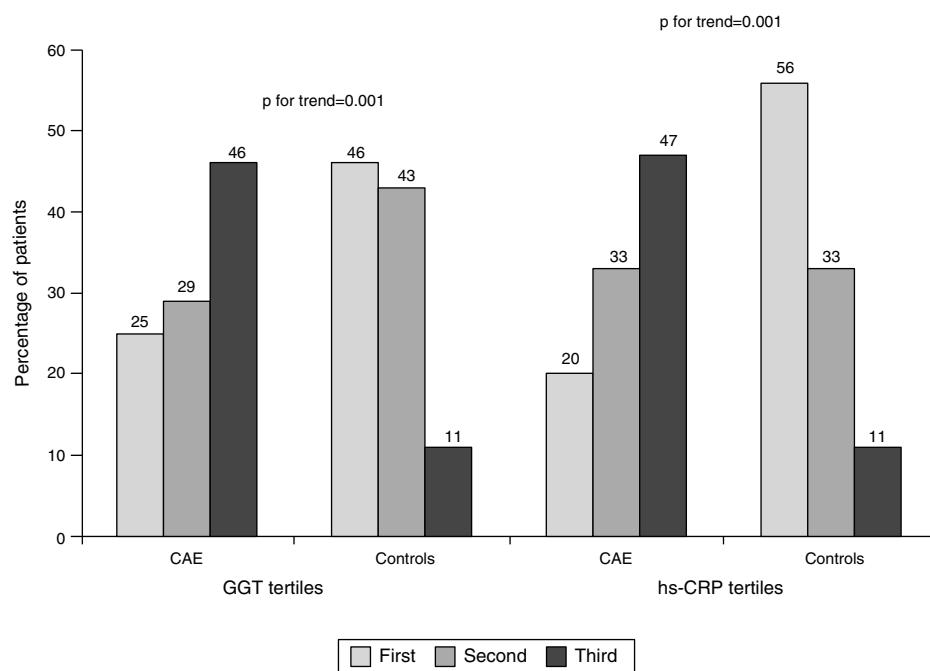
## Discussion

In this study, obesity and hypertension were slightly more prevalent but diabetes less prevalent in CAE patients than in controls. Similarly, serum GGT and hs-CRP levels were higher in patients with CAE. The presence of CAE was positively associated with obesity and GGT and hs-CRP levels but inversely with diabetes. Also, the severity of CAE was linked with GGT and hs-CRP levels.

The prevalence of CAE varies from 1% to 5% in angiographic series.<sup>1–3</sup> Although CAE is largely attributed to



**Figure 1** Box plots showing serum gamma-glutamyltransferase (GGT) (A) and high-sensitivity C-reactive protein (hs-CRP) (B) levels in controls and in patients with isolated coronary artery ectasia (CAE).



**Figure 2** Comparison of GGT and hs-CRP tertiles of patients with coronary artery ectasia (CAE) and of controls with normal coronary arteries. p values were calculated by the chi-square test for independence. GGT: gamma glutamyl transferase; hs-CRP: high-sensitivity C-reactive protein.

**Table 3** Serum gamma glutamyltransferase and high-sensitivity C-reactive protein levels in subgroups of patients with coronary artery ectasia.

	Focal ectasia (n=76)	Diffuse ectasia (n=91)	p	Single-vessel (n=100)	Multivessel (n=67)	p
GGT (U/l)	16 (13–20)	42 (25–47)	0.001	19 (15–22)	45 (40–48)	0.001
hs-CRP (mg/l)	2.4 (1.7–3.5)	3.1 (2.1–3.7)	0.01	2.4 (1.7–3.3)	3.2 (2.7–3.9)	0.001

Values are median (interquartile range). p values were calculated by the Mann-Whitney U test. GGT: gamma glutamyltransferase, hs-CRP: high-sensitivity C-reactive protein.

atherosclerosis, its causative and pathologic mechanisms are not clearly understood.<sup>1–3</sup> It may appear a relatively innocent clinical entity; however, it can cause cardiac events such as stable or unstable angina pectoris, myocardial infarction and cardiac death.<sup>7,9,20</sup>

### **Cardiovascular risk factors and coronary artery ectasia**

Previous small studies have reported different frequencies of major risk factors for atherosclerosis in isolated CAE patients.<sup>2,3,7–11</sup> In our study, there was a predominance of male gender (58%) in CAE patients, as in previous studies.<sup>2,8,11,18,21</sup> The proportion reaches 85% in some studies.<sup>7,9</sup>

Among risk factors, hypertension and obesity were slightly more prevalent in isolated CAE patients in the present study, whereas the rate of diabetes was lower in CAE patients. Other factors were at similar percentages. Only one study has shown a higher frequency of hypertension in CAE patients,<sup>11</sup> in contrast to other studies.<sup>2,3,7–10,18,19</sup> Similarly, hyperlipidemia has been reported to be more common in CAE patients in one study<sup>9</sup> but not in others.<sup>2,3,7,8,10,11,18,19</sup> Both high<sup>8</sup> and low<sup>7,9</sup> prevalences of smoking have also been documented in patients with CAE. We consider that these conflicting results may be mainly due to the selection of the control group from patients undergoing coronary angiography, and the small size of the studies. We constituted the control group from patients with normal coronary arteries, whereas some studies selected patients with CAD as the control group.<sup>7,9</sup>

Interestingly, previous studies<sup>8,10</sup> reported an inverse association between CAE and diabetes, as in our study, in which diabetes independently decreased the likelihood of CAE (OR: 0.44). However, this inverse association was not seen in several other studies.<sup>3,4,7,9,11,18</sup> There are two potential explanations for this association: in diabetic patients, compared with non-diabetic patients, negative arterial remodeling is seen more frequently in the coronary arteries during the progression of atherosclerotic plaques<sup>22</sup>; and consequently obstructive CAD can be found more commonly in diabetic patients.<sup>23</sup>

We found that obesity independently increased the likelihood of CAE (OR: 2.84, p=0.04). Such an association was not present in previous studies.<sup>2,3,7–11,18,21</sup> We think that this finding may be the result of compensatory enlargement due to increased body weight, because we did not routinely measure the diameters of the ectatic vessels and did not index them to body surface area.

### **Gamma glutamyltransferase, inflammation and coronary artery ectasia**

In humans, GGT is responsible for extracellular catabolism of glutathione, an antioxidant.<sup>12</sup> There is evidence that GGT triggers oxidative stress within atherosclerotic plaque and promotes the atherosclerotic process by means of LDL oxidation.<sup>12–14</sup> This finding has been supported by large epidemiologic studies<sup>15,16</sup> in which GGT level is independently associated with cardiovascular disease and mortality.

Furthermore, it is an independent predictor of fatal and non-fatal cardiac events in patients with documented CAD.<sup>22</sup>

Two recent studies have reported that GGT levels are increased in patients with isolated CAE compared to controls with normal coronary arteries, but are not associated with the severity of CAE.<sup>18,19</sup> Their sample size was small, including only 88 and 45 CAE patients. In our larger study, CAE patients had a higher level of GGT compared with controls. Moreover, GGT was independently associated with the presence of CAE as well as with the severity of CAE. We think that GGT may have a prognostic value for CAE, as also reported for atherosclerotic CAD.<sup>24</sup>

Inflammation plays a key role in the atherosclerotic process and CRP can predict future cardiovascular events in men and women at risk.<sup>4</sup> hs-CRP has been documented to be elevated in patients with CAE in some studies<sup>5,6</sup> but not in others.<sup>25,26</sup> However, these studies had small patient populations. In the present study, hs-CRP levels were higher in CAE patients than controls, and independently associated with the presence of CAE.

Previous studies showed an association of GGT with CRP.<sup>13,27,28</sup> GGT can act as a proinflammatory protein in atherogenesis<sup>27</sup> and is associated with atherosclerotic risk factors including obesity, dyslipidemia, metabolic syndrome, hypertension and diabetes.<sup>16,17,28</sup> There was a moderate link between GGT and hs-CRP in our study. This evidence suggests that GGT and CRP may reflect chronic occult inflammation in CAE patients.

In addition, some small studies reported similar platelet counts in CAE and control groups,<sup>29–31</sup> but in contrast, one large study reported significantly lower platelet counts in CAE patients than in controls.<sup>32</sup> Similarly, in our larger study, platelet count was lower in the CAE group. We consider that these conflicting results may be mainly due to the small size of the studies.

### **Limitations**

Our study has some limitations. Firstly, a major limitation is that we did not make prognostic assessments based on GGT levels, because of the small number of major cardiac events: two myocardial infarctions and three presentations with recurrent angina. Secondly, the prevalence of obesity was slightly higher in CAE patients, although mean BMI was similar in the two groups. Hepatic steatosis secondary to obesity may have contributed to higher GGT levels in CAE patients, but we did not evaluate this in each patient. Finally, our findings reflect the situation of patients with chest pain undergoing coronary angiography, but not that of the general population.

### **Conclusion**

Our findings show that the presence of CAE can be independently and positively associated with serum GGT and hs-CRP levels but negatively with diabetes. GGT and hs-CRP may also reflect the severity of CAE. Hence, their measurement may be useful in the evaluation of patients with CAE, as documented in patients with CAD. However, these findings should be supported by further studies.

# Role of gamma-glutamyltransferase in cardiovascular diseases

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Cardiovascular diseases are threatening human health with rising morbidity and mortality rates. Gamma-glutamyltransferase (GGT) has been found to be involved in the pathogenesis of cardiovascular diseases, especially coronary artery disease, and the prognosis of cardiovascular disease may be predicted by increasing GGT levels. GGT levels are related to cardiovascular

Cardiovascular diseases have been attracting increasing attention because of increasing morbidity and mortality rates, and threat to human health. Gamma-glutamyltransferase (GGT), an important enzyme in glutathione (GSH) metabolism, was previously found to be an indicator of liver or biliary tract diseases and alcohol consumption; however, more recently, GGT has shown to be involved in the development of cardiovascular disease. The progress and prognosis of cardiovascular disease may be predicted by increasing GGT levels, a tool preferable to other biochemical indicators such as analysis of blood lipid levels. Serum GGT levels have been shown to be an independent predictor of diabetes, hypertension, the metabolic syndrome and coronary artery disease (CAD) (1). The present review discusses GGT in cardiovascular and related diseases.

## BIOCHARACTERISTICS OF GGT

Human GGT is a multigene family of proteins composed of seven GGT genes and pseudogenes. To date, the exact protein structure, gene-expression patterns and regulatory mechanisms of GGT have not been elucidated. Several GGT complementary DNA segments have been obtained from hepatoma cells, placenta, lung, pancreas and other tissues. These GGT complementary DNA transcripts share the same coding sequence, but their 5'-untranslated regions are different. Therefore, the transcriptional process of GGT was presumed to be controlled by multiple promoters in a linear arrangement similar to the TRE, AP-2 combining site and SP-1 cis elements that exist in the proximal region of the GGT gene.

GGT is a glycosylated protein that is partially embedded in the outer surface of the plasma membrane at the N-terminal transmembrane domain. Franzini et al (2) performed quantitative analysis of serum GGT fractions. In that study, four GGT fractions: big-GGT, medium-GGT, small-GGT and free-GGT fractions of different molecular weight (molecular masses >2000 kDa, 940 kDa, 140 kDa and 70 kDa, respectively) were detected by a procedure based on gel filtration chromatography, followed by postcolumn injection of a fluorescent GGT substrate. Comparatively, GGT activity was decided primarily by the free-GGT and small-GGT fractions. GGT catalyzes the transfer of the gamma-glutamyl moiety from GSH or GSH conjugated to acceptors such as amino acids, dipeptides and molecules with similar traits. GGT can provide cysteine, the rate-limiting amino acid, for GSH de novo synthesis by breaking down extracellular GSH into its constitutive amino acids. It is a vital step in maintaining in vivo homeostasis of GSH and cysteine.

GGT is an enzyme normally present in the serum and on the outer surface of numerous cell types (3). Serum GGT is especially active in the proximal renal tubule, pancreas and intestine, but primarily in the

emergencies of chronic heart failure, and an elevated GGT level has been shown to be an independent predictive marker for cardiac death and cardiac transplantation. Investigation of the role of GGT in the mechanism of cardiac diseases will be helpful in developing preventive strategies and treatment methods.

**Key Words:** Coronary artery disease; Gamma-glutamyltransferase; Heart failure; Hypertension

liver. In most cases, serum GGT levels are examined for the diagnosis of liver, gallbladder and biliary tract diseases (4), especially in alcoholic liver disease (5). GGT is particularly sensitive to alcohol consumption and may be elevated even when other liver function tests remain normal. Its circulating half-life is seven to 10 days, which is increased in alcohol-associated liver injury because of impaired clearance (6). In addition, changes in serum GGT levels can be affected by waist circumference and body mass index (7), hypertension (8), diabetes (9), hyperuricemia (10) and genetic factors (11).

## GGT AND HYPERTENSION

Hypertension is the most common modifiable risk factor for cardiovascular disease, especially in middle-age individuals and the elderly. Recently, GGT has been found to be involved in the pathogenesis of hypertension. In a three-year follow-up study by Cheung et al (8), 235 hypertensive and 708 normotensive Hong Kong Chinese subjects were investigated for plasma alanine aminotransferase, alkaline phosphatase and GGT levels. Statistical analysis showed that plasma GGT, but not alkaline phosphatase, alanine aminotransferase or aspartate aminotransferase levels, was an independent predictor of new-onset hypertension. In another research project involving 10,988 participants (12), GGT showed strong positive correlations with systolic blood pressure and diastolic blood pressure, while demonstrating a positive linear correlation with body mass index, waist circumference, fasting plasma glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, uric acid and high-sensitivity C-reactive protein (CRP) levels. Elevated serum GGT levels within the normal range are considered to be associated with a higher risk of incident hypertension, particularly in drinkers and nonoverweight individuals (13).

Some studies have explored GGT and its role in the development of hypertension. Saijo et al (14) found a connection between GGT and an increased level of arterial stiffness. Celik et al (15) found that regardless of the mechanism, young patients with prehypertension exhibit higher serum GGT levels compared with healthy subjects. More importantly, increased GGT levels are independently associated with impaired aortic elasticity in patients with prehypertension (15). Serum GGT levels can be an alternative indicator of arterial stiffness in hypertension patients. This conclusion was supported by Song et al (16) in a study that involved 1387 participants.

## GGT AND CAD

Although CAD is one of the most common types of heart disease, it is difficult to predict the risk of CAD and intervene at an early stage. GGT has been confirmed to play a role in the occurrence and progression of CAD, especially in prognosis judgment.

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### Elevated levels of serum GGT are involved in the pathogenesis of CAD

GSH is a tripeptide comprised of three amino acids: gamma-glutamic acid, L-cysteine and L-glycine. Its primary biological function is to act as a nonenzymatic reducing agent to help keep cysteine thiol side chains in a reduced state on the surface of proteins. GSH also prevents oxidative stress in most cells and helps trap free radicals that can damage DNA and RNA. The physiological role of GGT is to initiate the hydrolysis of extracellular GSH by cleaving the gamma-glutamyl amide bond of the tripeptide to cysteine and other thiol compounds, which are known to promote LDL oxidation by reducing Fe(III) to redox-active Fe(II) (17). Recently, catalytically active GGT has been found within atherosclerotic coronary plaques from autopsy studies and surgical endarterectomies (18). Some researchers believe that serum GGT is partially adsorbed onto LDL lipoproteins, which can carry GGT activity inside the plaque (in proportion with serum GGT levels), in which free iron has also been described (19). GGT-mediated reactions catalyze the oxidation of LDL lipoproteins, likely contributing to oxidative events influencing plaque evolution and rupture (20). GGT has been considered to play a central role in the formation of the fibrous cap, apoptosis of cellular elements of the lesion, plaque erosion and rupture, enhanced platelet aggregation and thrombosis (19).

Some researchers have focused on the relationship between serum GGT level and coronary blood flow. Caliskan et al (21) confirmed that serum GGT level is independently associated with coronary flow reserve impairment in hypertensive patients. The investigators reported that serum GGT level was an independent marker of target organ damage in hypertensive subjects without concomitant risk factors. Sen et al (22) evaluated the relationship between elevated serum GGT activity and slow coronary flow and found that the mean thrombosis in myocardial infarction frame count showed a positive and moderate correlation with serum GGT activity. Serum GGT activity was the only independent predictor of the mean thrombosis in myocardial infarction frame count (22).

### CAD prognosis can be predicted by measurement of serum GGT levels

It has been confirmed that elevated CRP levels are a predictor of adverse outcomes in patients with acute coronary syndromes and help to identify patients who may be at risk for cardiovascular complications (23). Emiroglu et al (24) performed a comparative analysis of serum GGT and high-sensitivity CRP (hs-CRP) in a trial involving 219 patients presenting with acute coronary syndrome (ACS) and 51 control subjects. Results of the analysis showed that serum GGT and hs-CRP levels were higher in ACS patients and that a moderate but significant correlation was present between GGT and hs-CRP (24). In another prospective study investigating the clinical significance of serum GGT levels during the early postmyocardial infarction period (25), researchers found a significant positive correlation between serum GGT and hs-CRP and homocysteine levels. Left ventricular (LV) end-diastolic diameter remained independently associated with serum GGT activity on day 5 following acute myocardial infarction. Although this study was limited by its small sample size, short-term follow-up period and a noncontrolled study design, the authors reported that serum GGT played a potential role in predicting LV dilation and dysfunction during the early postmyocardial infarction period. The study by Emdin et al (26) agreed with this conclusion. They reported that GGT level – similar to CRP and fasting glucose – was an independent risk factor in patients with established CAD in a study evaluating 474 subjects with angiographically documented CAD. Low serum GGT levels were helpful in identifying patients with the lowest risk of cardiac death (26).

Other studies have focused on the relationship between GGT and types of CAD. Dogan et al (27) found that GGT levels were higher in patients with significant stenosis compared with those without significant stenosis in a study that investigated the association between significant stenosis and major cardiac events (MACE) in 237 non-ST elevation ACS patients. MACE-free survival was slightly poorer in

ACS patients with GGT levels in the upper tertile compared with those with levels in the lower tertile at 12 months (27). In another study that involved 425 patients with ST segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (28), although the TIMI flow percentages were similar in the three GGT tertiles (32%, 45% and 42%), serum GGT activity was associated with in-hospital MACE. Breitling et al (29) reported that serum GGT level was associated with prognosis independent of a variety of established risk markers in patients with stable CAD in a study that included 1152 participants of an in-patient ACS rehabilitation program. The association appeared to be similar to that reported for primary cardiovascular disease, which should prompt additional studies of its clinical utility in cardiovascular patient care (29). In addition, serum GGT activity was found to be associated with higher occlusion rates of venous bypass grafts in a study investigating the relationship between serum GGT levels and saphenous vein bypass graft disease at least one year after coronary artery bypass graft surgery (30). GGT levels have also been confirmed to be an independent predictor of early mortality in STEMI patients without previously known diabetes who underwent mechanical revascularization (31).

### GGT AND CARDIAC SYNDROME X

Cardiac syndrome X (CSX) is a condition in which patients with no physical findings of CAD experience angina. Although it is not clear what causes CSX, some recent studies have investigated the role of GGT. Demir et al (32) compared serum GGT levels between patients with CSX and asymptomatic healthy individuals. In this study, serum GGT activity in patients with CSX was confirmed to be higher than in healthy controls; moreover, GGT activity was further increased in patients with CSX who also had the metabolic syndrome. The relationship between serum GGT activity and carotid intima media thickness in patients with CSX was evaluated by Yagmur et al (33). Serum GGT activity in patients with CSX was shown to be as high as that in patients with CAD. A significant correlation was found between GGT activity and carotid intima media thickness measurements, but serum GGT activity did not correlate with serum CRP levels in patients with CSX. It was suggested that increased GGT levels play a role in the pathogenesis of the microvascular atherosclerotic process of CSX (33).

### GGT AND HEART FAILURE

It has been shown that elevated serum GGT activity exists in the early stages of heart failure (HF), the final and common pathway of all cardiovascular diseases (34). In an evaluation of 1087 ambulatory patients with chronic HF, Ess et al (35) found the prevalence of elevated GGT to be 43% in men and 48% in women. GGT was independently associated with adverse outcomes in these patients. This finding further highlights the clinical importance of GGT in cardiovascular disease (35). In a prospective study involving 3494 men 60 to 79 years of age with no diagnosed HF or myocardial infarction followed-up for a mean period of nine years, in whom there were 168 incident cases of HF (36), elevated GGT was associated with significantly increased risk of incident HF in men <70 years of age but not in men ≥70 years of age. The relevance of serum GGT and disease severity in chronic HF was investigated by Poelzl et al (37), who found that serum GGT was associated with severity of HF as assessed by New York Heart Association class, LV ejection fraction and amino-terminal pro-B-type natriuretic peptide levels. Increased GGT levels are an independent predictor of death or heart transplantation. GGT may provide additional prognostic information, especially in patients with mild HF (37).

Other studies have focused on the mechanism of elevated serum GGT levels in HF patients to explore new methods of intervention in HF progression. Zheng et al (38) identified the role of GGT in reversing pathogenic K<sup>+</sup> channel remodelling in the diseased heart. They found that GSH<sub>o</sub> elicits GGT- and reactive oxygen species-dependent transactivation of tyrosine kinase signalling that upregulates K<sup>+</sup> channel activity or expression via redox-mediated mechanisms. The signalling events stimulated by GGT catalysis of GSH<sub>o</sub> may be a therapeutic

target to reverse pathogenic electrical remodelling of the failing heart (38). Higher central venous pressure has also been found to be related to serum GGT levels in HF patients, and abnormal liver function was attributed to increased serum GGT levels (39). However, further studies should be undertaken to elucidate the mechanism of elevated serum GGT levels in the progression of HF.

### GGT AND OTHER CARDIOVASCULAR DISEASES

The relationship between serum GGT and acute pulmonary embolism (PE) has recently attracted some attention. Serum GGT was confirmed by Zorlu et al (40) to be associated with an increased risk for acute PE-related early mortality in a study evaluating 127 consecutive patients with confirmed PE. The authors reported that a high GGT level was associated with poorer hemodynamic parameters, and it appeared that GGT helped risk stratification in patients with acute PE. Nordenholz (41) confirmed this conclusion, but pointed out that serum GGT would need to be validated in a larger, more heterogeneous population before consideration as a stand-alone risk-stratifying marker of acute PE. Another study investigating the role of serum GGT in elderly patients with nonvalvular atrial fibrillation (AF) (42) indicated that serum GGT activity was significantly higher in patients with AF compared with those without AF. Serum GGT activity was

independently associated with chronic nonvalvular AF. In addition, the significant correlation between GGT and acute glucose dysmetabolism (as indicated by admission glycemia and insulin resistance) can account, at least in part, for the prognostic role of GGT (31). Zhang et al (43) examined the relationship between LV diastolic function and GGT level in diabetic individuals. This study showed that metabolic parameters could affect diastolic function more than systolic functions, and that GGT may be an additional marker of diastolic dysfunction in diabetes patients with cardiovascular disease apart from known cardiovascular disease risk factors (43). Serum GGT may be a biological marker in the formulation of diabetes risk assessments (9).

### SUMMARY

Although frequently used as an indicator of liver or biliary tract diseases, or alcohol consumption in clinical practice, serum GGT has been confirmed to be involved in cardiovascular disease mechanisms. Future studies, including more that examine molecular mechanisms, will help provide insight into the nature of cardiovascular diseases and lead to new preventive and treatment strategies.

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## Relationships of Different Blood Pressure Categories to Indices of Inflammation and Platelet Activity in Sustained Hypertensive Patients with Uncontrolled Office Blood Pressure

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Failure to decrease blood pressure (BP) normally during nighttime (non-dipping) in hypertension is associated with higher cardiovascular morbidity and mortality. In addition, non-dipping BP is associated with increased platelet activity and inflammatory response; however, there has been no study to evaluate the relationship of non-dipping BP to indices of platelet activity and inflammation in uncontrolled hypertensive patients. In the present study, hypertensive subjects with uncontrolled office BP were firstly divided into three groups: 84 subjects with white coat effect and 365 subjects with true uncontrolled hypertension. Then, true uncontrolled hypertensive patients were divided into two groups: 158 patients with dipping and 207 patients with non-dipping. Mean platelet volume (MPV), uric acid (UA),  $\gamma$ -glutamyltransferase (GGT), C-reactive protein (CRP), and high-sensitivity CRP (hs-CRP) levels were studied. The general characteristics and risk factors for coronary artery disease (CAD) of the study population were similar among the groups. MPV, UA, GGT, CRP, and hs-CRP levels were significantly higher in non-dipper group than both dipper and white coat effect groups, and were significantly higher in dipper group than in white coat effect group (MPV:  $9.1 \pm 1.3$ ,  $8.7 \pm 1.1$ , and  $8.0 \pm 0.9$  fL; UA:  $6.9 \pm 1.2$ ,  $5.9 \pm 1.4$ , and  $4.1 \pm 0.8$  mg/dL; GGT:  $38.9 \pm 11.1$ ,  $33.6 \pm 14.9$ , and  $25.2 \pm 9.2$  U/L; CRP:  $7.1 \pm 2.4$ ,  $6.2 \pm 1.9$ , and  $3.9 \pm 0.8$  mg/dL; hs-CRP:  $3.8 \pm 1.5$ ,  $3.3 \pm 1.2$ , and  $2.0 \pm 0.6$ , non-dipper, dipper, and white coat effect groups, respectively, all  $p$  values  $<0.01$ ). All study parameters strongly correlated with each other. In conclusion, in hypertensive patients with uncontrolled office BP, presence of non-dipping BP is associated with increased platelet activity and inflammation, which can be one of the underlying plausible mechanisms of non-dipping BP status.

**Keywords:** Ambulatory blood pressure, atherosclerosis, inflammation, non-dipper, platelet function

### INTRODUCTION

Previous cross-sectional and longitudinal studies have shown that blood pressure (BP) measurements obtained by ambulatory BP monitoring (ABPM) are more prominently correlated with hypertension (HT)-related target organ damage, and have a stronger relationship to cardiovascular (CV) event than office BP measurements in both untreated and treated hypertensives (Mancia et al., 2001, 2007). It has also been suggested that lower achieved ambulatory BPs are associated with a lower rate of CV outcomes (Clement et al., 2003). Furthermore, BP measurements obtained by ABPM is more accurate than office BP in estimating the extent of BP reduction induced by treatment due to higher reproducibility over time (Coats et al., 1992), and ABPM allows for

eliminating “white coat effect” in hypertensive patients who were taking antihypertensive therapy.

Hypertensive individuals are divided into two groups, dippers and non-dippers, according to nighttime reduction in BP  $\geq 10\%$  or  $<10\%$ , respectively. Indeed, antihypertensive treatment similarly changes both daytime and nighttime BPs. However, the prognostic value of nighttime BP is superior to that of daytime BP (Björklund et al., 2004; Kikuya et al., 2005; Segal et al., 2005). Therefore, 24-h ABPM can be more useful at the time of diagnosis and at varying intervals during treatment in hypertensive patients. Accordingly, current guidelines have recommended that effort should be made to extend ABPM to 24 h in order to obtain information on both daytime and nighttime BP profiles, day-night blood pressure difference, morning blood

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pressure rise, and blood pressure variability (Daskalopoulou et al., 2012; Mansia et al., 2007). Normally, it is anticipated that both systolic and diastolic BPs decrease as low as 10% during nighttime. Failure to decrease in systolic BP normally during nighttime is called non-dipping.

Although substantial evidence supports the contention that nighttime BP is more important than daytime BP in predicting outcome and development of target organ damage, particularly in individuals who have nighttime BP fall <10% (non-dipping), to date, there is no study investigating relationship of non-dipping BP to platelet activity and atherosclerosis-related inflammatory markers in uncontrolled hypertensive patients treated by antihypertensive agents regularly. In the present study, we aimed to investigate relationships of BP categories to platelet activity and atherosclerosis-related inflammatory markers in uncontrolled essential hypertensive subjects who were regularly taking antihypertensive drugs.

## METHODS

### Study Population

Between February 2011 and August 2012, a total of 1196 sustained essential hypertensive subjects, who were regularly taking antihypertensive therapy at least 6 mos, were evaluated in the office setting, and 502 (42%) sustained hypertensive subjects with uncontrolled office BP were screened. However, 52 subjects were excluded due to exclusion criteria. Accordingly, the overall study population consisted of 450 subjects. Inclusion criteria were to be 30–75 yrs of age, absence of secondary causes, and regularly taking antihypertensive therapy at least 6 mos. All subjects were asymptomatic and free from a history of CV disease. Exclusion criteria were to have any concomitant systemic disease except diabetes mellitus (DM), such as hemolytic, rheumatic, hepatic, and renal diseases, that could affect studied parameters. The subjects with excessive alcohol consumption (>120 g/d) were also excluded. All patients were on regular antihypertensive treatment for at least 6 mos. Subjects who had not previously taken any antihypertensive therapy were excluded. Written informed consent was obtained from each subject, and the institutional ethics committee approved the study protocol and adhered to the ethical standards outlined in the Helsinki Declaration (Portaluppi et al., 2010).

### Blood Pressure Measurement at the Office Setting

According to current guidelines (Daskalopoulou et al., 2012; Mansia et al., 2007), BP was measured using a mercury sphygmomanometer in office setting; first and fifth phases of Korotkoff sounds were used for systolic and diastolic BPs, respectively. Appropriate cuff sizes were chosen for each subject's arm circumference. BP was measured three times by skilled, trained physicians after 15 min of rest in the sitting position.

The measurements were repeated after 48 h and the average of all measurements was recorded. Physical examination included measurement of height (centimeters) and weight (kilograms), and a resting 12-lead electrocardiogram (ECG) was recorded. The subjects who had a BP above 140/90 mm Hg were considered as uncontrolled hypertensives.

### Ambulatory Blood Pressure Monitoring

Noninvasive 24-h ABPM were performed with a portable compact digital recorder (Tracker NIBP2; Del Mar Reynolds Medical, Hertford, UK), and analyzed using a customized analytical software (CardioNavigator, Spacelabs Healthcare, Issaquah, WA, USA). Appropriate cuff sizes were chosen for each subject. All subjects wore an ABPM device for a single 24-h period. The device was programmed to inflate and record BP at prespecified intervals (every 15 min during daytime hours and every 30 min during nighttime hours), which provided approximately 80 BP recordings during the 24-h period. The display of ABPM was inactivated so that viewing each BP reading did not distract subjects.

Reports generated from a session of ABPM contained BP recordings for the entire 24 h, heart rate, mean arterial pressure, and BP load as well as summary statistics for the overall 24-h, daytime, and nighttime periods. If at least 80% of the total BP readings were valid, the ABPM record was considered satisfactory and used for further analyses. The daytime and nighttime periods were set according to patients' sleeping time.

### Patient Classification

All of the patients used their prescribed antihypertensive medications during ABPM. Based on the definition of current guidelines (Daskalopoulou et al., 2012; Mansia et al., 2007) and according to 24-h ABPM results, the sustained hypertensive subjects with uncontrolled office BP were firstly divided into two groups: false uncontrolled (white coat effect) and true uncontrolled hypertensives. Patients who had a systolic BP  $\geq 140$  mm Hg and/or a diastolic BP  $\geq 90$  mm Hg in office setting, an average 24-h systolic BP  $< 130$  mm Hg and diastolic BP  $< 80$  mm Hg, an average daytime systolic BP  $< 135$  mm Hg and diastolic BP  $< 85$  mm Hg, and an average nighttime systolic BP  $< 120$  mm Hg and diastolic BP  $< 70$  mm Hg in ABPM were considered as false uncontrolled (white coat effect; group I); patients who had a systolic BP  $\geq 140$  mm Hg and/or a diastolic BP  $\geq 90$  mm Hg in office setting, an average 24-h systolic BP  $\geq 130$  mm Hg and/or diastolic BP  $\geq 80$  mm Hg, and an average daytime systolic BP  $> 135$  mm Hg and/or diastolic BP  $\geq 85$  mm Hg or an average nighttime systolic BP  $\geq 120$  mm Hg and/or diastolic BP  $\geq 70$  mm Hg in ABPM were diagnosed as true uncontrolled hypertensives. Then, true uncontrolled hypertensive patients were divided into two groups: dipper (group II) and

non-dipper (group III), according to nighttime reduction in systolic BP  $\geq 10\%$  or  $< 10\%$ , respectively.

### Blood Collection and Laboratory Analysis

Blood samples were drawn from the antecubital vein by careful veinpuncture in a 21-G sterile syringe without stasis at 08:00–10:00 h after a fasting period of 12 h. Hematologic and biochemical measurements including liver enzymes were studied. An automatic blood counter (LH 780 Hematology Analyzer, Beckman Coulter Inc., Miami, FL) was used for whole blood counts. Mean platelet volume (MPV) was measured in a blood sample collected in dipotassium ethylenediaminetetraacetic acid (EDTA) tubes within 30 min after sampling to prevent EDTA-induced platelet swelling. Serum uric acid (UA) levels were measured using Olympus AU2700 autoanalyzer using its own kits (Olympus AU640; Olympus, Tokyo, Japan). Its normal range is 3.5–7.2 mg/dL. Serum  $\gamma$ -glutamyltransferase (GGT) levels were measured by the enzymatic calorimetric method using available kits test with autoanalyzer (Olympus AU 640; Olympus). Its normal ranges is 5–50 U/L. Serum C-reactive protein (CRP) levels were measured using BN2 Nephelometry Analyzer II (Dade Behring, Kalletal, Germany), and serum high-sensitivity CRP (hs-CRP) levels were measured using Siemens Immulite 2000 Immunoassay System Analyzer (Siemens, Los Angeles, CA, USA). The normal values for CRP and hs-CRP are 0–6 and 0–1.1 mg/L, respectively.

### Power Calculation and Statistical Analysis

Based on previous data (Ermis et al., 2012; Kaya et al., 2010), we hypothesized that non-dipper hypertensive patients had plasma MPV, hs-CRP, and GGT levels approximately 0.20–30 of a standard deviation higher as compared with dipper hypertensives. To reach these

numbers at  $p < 0.05$  and  $1 - \beta = 0.85$ , about 180–200 subjects were required for non-dipper group. We recruited in excess of this number to minimize the risk of type II error.

The analyses were performed using SPSS for windows 9.0 (SPSS, Chicago, IL, USA). Categorical variables were defined as percentage and numeric data are expressed as mean  $\pm$  SD. The groups were compared using chi-square test regarding categorical variables. One-way analysis of variance (ANOVA) followed by Tukey's test or Kruskal-Wallis test (comparison of a characteristic across the three study groups if that characteristic did not have a normal distribution, such as GGT, hs-CRP, and triglyceride) was used to compare continuous variables. Univariate correlations were analyzed using Pearson or Spearman (if data were not normally distributed) correlation test, and to provide more statistical power the results were corrected by multiple testing using Bonferroni procedures when the common correlation among the multiple variables exceeded 0.50. Multivariate analysis was used to assess associations of studied markers with potential confounders via multivariate linear regression model. A  $p$  value less than 0.05 was considered significant.

## RESULTS

### Clinical Characteristics of the Study Population

According to ambulatory BP measurements, 450 sustained essential hypertensive patients with uncontrolled office BP were divided into three group: 84 (19%) subjects with white coat effect (group I), 158 (35%) patients with dipper HT (group II), and 207 (46%) patients with non-dipper HT (group III). Their demographic and clinical data are shown in Table 1. The general characteristics and risk factors for CV disease of

TABLE 1. Demographic characteristics and medications of the each study groups.

Characteristic	Group I: White coat effect (n=85)	Group II: Dipper HT (n=158)	Group III: Non-dipper HT (n=207)
Age (years)	50.4 $\pm$ 8.3	51.8 $\pm$ 5.5	51.9 $\pm$ 10.2
Male/female (n/n)	47/38	28/32	119/88
Body mass index (kg/m <sup>2</sup> )	29.1 $\pm$ 2.8	29.4 $\pm$ 3.1	29.1 $\pm$ 3.6
Body mass index $\geq 30$ kg/m <sup>2</sup> (%)	42	44	39
Diabetes mellitus (%)	15	16	15
Dyslipidemia (%)	24	27	32
Current smoker (%)	6	5	12
Duration of HT (months)	62.6 $\pm$ 45.2	65.8 $\pm$ 41.7	66.9 $\pm$ 44.9
Office systolic BP (mm Hg)	159.5 $\pm$ 5.2	159.8 $\pm$ 9.7	161.6 $\pm$ 9.9
Office diastolic BP (mm Hg)	90.2 $\pm$ 4.9	89.9 $\pm$ 7.5	93.8 $\pm$ 8.7
Heart rate (bpm)	77.5 $\pm$ 10.4	76.6 $\pm$ 9.5	79.6 $\pm$ 9.7
ACEI/ARB usage (%)	88	87	92
Beta-blocker usage (%)	28	24	28
Calcium channel blocker usage (%)	14	16	12
Diuretic usage (%)	65	58	59
Oral antidiabetic usage (%)	15	16	15
Statin usage (%)	21	23	24
Aspirin usage (%)	18	24	21

HT = hypertension; BP = blood pressure; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker.

the study population are presented in Table 1. Age, gender, body mass index, duration of HT, office BPs, heart rate, percentage of diabetes mellitus and dyslipidemia, smoking status, and medication status were not significantly different among the groups.

#### Ambulatory Blood Pressure Monitoring Analysis

The results of ambulatory blood pressure monitoring analysis are presented in Table 2. Average heart rates in all periods were significantly higher in non-dipper group than both dipper and white coat effect groups. Average heart rate was different between dipper and white coat effect groups at only nighttime period.

#### Analyses of the Laboratory Findings

The study groups were comparable with respect to hemoglobin, hematocrit, blood urea nitrogen, glucose, lipids, calcium, and liver enzymes (alkaline phosphatase, aspartate transaminase, alanine transaminase). White blood cell count was significantly higher in non-dipper group than both dipper and white coat effect groups. Platelet count was significantly lower in non-dipper group than both dipper and white coat effect groups, and was significantly lower in dipper group than in white coat effect group. Creatinine levels were significantly different between non-dipper and white coat effect groups. MPV, UA, GGT CRP, and hs-CRP levels were significantly higher in non-dipper group than both dipper and white coat effect groups, and were significantly higher in dipper group than in white coat effect group (Table 3, Figure 1). In the white coat effect group, subjects with non-dipping BP ( $n=44$ ) had had significantly increased GGT and UA levels as compared with those with non-dipping BP (GGT:  $27.8 \pm 10.7$  versus  $22.1 \pm 6.1$  U/L,  $p=0.004$ ; UA:  $4.32 \pm 0.87$  versus  $3.94 \pm 0.81$  mg/dL,  $p=0.02$ ). However, the groups were comparable with respect to MPV, CRP, and hs-CRP levels. When the dipper hypertensives were divided into two groups based on dipping ratio, extreme dipper hypertensives had lower MPV, GGT, UA, and hs-CRP levels than dipper hypertensives

(MPV:  $8.3 \pm 1.0$  versus.  $8.8 \pm 1.1$  fL,  $p=0.007$ ; GGT:  $27.1 \pm 13.1$  versus  $34.4 \pm 11.2$  U/L,  $p<0.001$ ; UA:  $5.5 \pm 1.3$  versus  $6.3 \pm 1.2$  mg/dL,  $p<0.001$ ; hs-CRP:  $3.0 \pm 1.1$  versus  $3.6 \pm 1.3$  mg/dL,  $p=0.003$ ). The hypertensive patients with diabetes had significantly increased MPV, GGT, CRP, and hs-CRP levels as compared with those without diabetes (MPV:  $9.3 \pm 1.3$  versus  $8.7 \pm 1.1$  fL,  $p<0.01$ ; GGT:  $41.2 \pm 13.6$  versus  $33.9 \pm 12.4$  U/L,  $p<0.0001$ ; CRP:  $7.0 \pm 3.0$  versus  $6.1 \pm 2.2$  mg/dL,  $p<0.01$ ; hs-CRP:  $4.1 \pm 1.8$  versus  $3.2 \pm 1.4$  mg/dL,  $p<0.0001$ ).

#### Correlation Analyses

Correlations of the study variables with demographic characteristics and risk factors are presented in Table 4. MPV was significantly and positively correlated with glucose levels, presence of DM and non-dipping, and smoking. GGT was significantly and positively correlated with BMI, presence of DM and non-dipping, and smoking. UA was significantly and positively correlated with smoking and presence of non-dipping. Hs-CRP was significantly and positively correlated with creatinine and glucose levels, presence of DM and non-dipping, and smoking. On the other hand, all study variables were strongly correlated with each other (Table 5). Furthermore, in multivariable analysis, GGT, UA, hs-CRP, and MPV were separately taken as dependent, the BP classification status of the subjects (white coat effect, dipper, and non-dipper), age, BMI, lipids, presence of diabetes, smoking, and other confounders including the other hematologic and biochemical parameters were taken as independent, and we found that the presence of non-dipping was a significant predictor of higher GGT, UA, hs-CRP, and MPV ( $\beta=0.36$ ,  $p<0.001$ ;  $\beta=0.57$ ,  $p<0.001$ ;  $\beta=0.37$ ,  $p<0.001$ ;  $\beta=0.30$ ,  $p<0.001$ , respectively).

In multivariate analysis, GGT and MPV were also independently associated with presence of diabetes ( $\beta=0.36$ ,  $p<0.001$ ;  $\beta=0.20$ ,  $p<0.001$ , respectively) and BMI ( $\beta=0.13$ ,  $p<0.01$  for each). In addition, hs-

TABLE 2. Data from ambulatory blood pressure monitoring of the study groups.

Parameter	Group I: White coat effect ( $n=85$ )	Group II: Dipper ( $n=158$ )	Group III: Non-dipper ( $n=207$ )
Average 24-h systolic BP	$121.3 \pm 6.9$	$131.8 \pm 5.8\ddagger$	$135.6 \pm 9.8\ddagger\ddagger$
Average 24-h diastolic BP	$75.7 \pm 6.0$	$78.0 \pm 6.0\ddagger$	$83.0 \pm 8.4\ddagger\ddagger$
Average 24-h mean BP	$88.8 \pm 8.7$	$94.6 \pm 6.5\ddagger$	$99.2 \pm 9.7\ddagger\ddagger$
24-h mean heart rate (bpm)	$71.7 \pm 8.2$	$72.6 \pm 5.2$	$77.3 \pm 8.9\ddagger\ddagger$
Average daytime systolic BP	$126.0 \pm 8.8$	$141.2 \pm 6.2\ddagger$	$138.6 \pm 11.2\ddagger$
Average daytime diastolic BP	$76.7 \pm 6.7$	$82.8 \pm 6.2\ddagger$	$84.9 \pm 9.0\ddagger$
Average daytime mean BP	$96.9 \pm 7.1$	$101.1 \pm 6.5\ddagger$	$101.5 \pm 10.3\ddagger$
Daytime mean heart rate (bpm)	$79.0 \pm 8.4$	$78.6 \pm 5.6$	$81.7 \pm 9.7\ddagger\ddagger$
Average nighttime systolic BP	$103.5 \pm 7.9$	$112.0 \pm 5.6\ddagger$	$129.5 \pm 8.5\ddagger\ddagger$
Average nighttime diastolic BP	$65.9 \pm 5.4$	$70.0 \pm 5.6\ddagger$	$78.3 \pm 8.9\ddagger\ddagger$
Average nighttime mean BP	$80.3 \pm 7.3$	$83.9 \pm 5.0\ddagger$	$93.9 \pm 9.5\ddagger\ddagger$
Nighttime mean heart rate (bpm)	$61.6 \pm 9.7$	$65.3 \pm 5.7^*$	$69.9 \pm 9.1\ddagger\ddagger$

BP=blood pressure.

\* $p<0.01$  versus dipper; † $p<0.0001$  versus white coat effect; ‡ $p<0.0001$  versus dipper; \* $p<0.05$  versus white coat effect.

TABLE 3. Hematologic and biochemical data of the each study groups.

Parameter	Group I: White coat effect (n=85)	Group II: Dipper HT (n=158)	Group III: Non-dipper HT (n=207)
Hemoglobin (g/dL)	14.4 ± 1.6	14.8 ± 1.3	14.5 ± 1.3
Hematocrit (%)	42.1 ± 4.1	42.8 ± 4.8	42.7 ± 3.9
White blood cell count ( $\times 10^3/\text{mm}^3$ )	7.62 ± 1.57	7.84 ± 1.96	9.07 ± 2.24†‡
Platelet count ( $\times 10^3/\text{mm}^3$ )	361.1 ± 89.1	266.2 ± 84.0†	242.8 ± 64.0†¶
PDW (%)	15.8 ± 1.4	15.0 ± 1.1†	14.9 ± 1.4†¶
BUN (mg/dL)	14.9 ± 4.5	15.3 ± 4.4	15.6 ± 4.2
Creatinine (mg/dL)	0.85 ± 0.19	0.89 ± 0.17	0.91 ± 0.18*
Glucose (mg/dL)	98.7 ± 16.1	100.8 ± 10.1	101.4 ± 12.9
Total cholesterol (mg/dL)	189.4 ± 38.7	188.4 ± 32.0	189.7 ± 32.0
HDL cholesterol (mg/dL)	47.8 ± 12.6	48.0 ± 13.1	46.4 ± 10.1
LDL cholesterol (mg/dL)	107.4 ± 30.9	110.0 ± 29.4	112.8 ± 29.3
Triglyceride (mg/dL)	165.4 ± 119.4	153.5 ± 64.6	149.8 ± 76.8
Calcium (mg/dL)	9.3 ± 0.6	9.4 ± 0.5	9.3 ± 0.6
ALP (IU/L)	69.4 ± 18.2	72.6 ± 16.7	70.4 ± 17.3
AST (U/L)	24.0 ± 10.2	23.5 ± 6.9	23.5 ± 10.3
ALT (U/L)	28.9 ± 18.1	28.4 ± 8.3	28.0 ± 13.3
CRP (mg/L)	3.9 ± 0.8	6.2 ± 1.9†	7.1 ± 2.4†¶
Hs-CRP (mg/dL)	2.0 ± 0.6	3.3 ± 1.2†	3.8 ± 1.5†¶
MPV (fL)	8.0 ± 0.9	8.7 ± 1.1**	9.1 ± 1.3†¶
Uric acid (mg/dL)	4.1 ± 0.8	5.9 ± 1.4†	6.9 ± 1.2†‡
GGT (U/L)	25.2 ± 9.2	33.6 ± 14.9†	38.9 ± 11.1†¶

PDW = platelet distribution width; BUN = blood urea nitrogen; HDL = high-density lipoprotein; LDL = low-density lipoprotein;

ALP = alkaline phosphatase; AST = aspartate transaminase; ALT = alanine transaminase; CRP = C-reactive protein.

\* $p < 0.05$  versus white coat effect; \*\* $p < 0.01$  versus white coat effect; † $p < 0.0001$  versus white coat effect; ¶ $p < 0.01$  versus dipper; ‡ $p < 0.0001$  versus dipper.

CRP was also independently associated with presence of diabetes ( $\beta = 0.22$ ,  $p < 0.001$ ).

## DISCUSSION

It is well known that there is a strong association between high BP and CV diseases such as coronary artery disease (CAD) and stroke. In addition, CAD and stroke are the most common forms of target organ damage and most common causes of mortality associated with HT (Sega et al., 2005; Staessen et al., 2001). Although BP measurements in the office setting still remains the cornerstone for decision-making in HT, and most therapeutic trials of HT have used lowering office BP measurements, it has been well documented that BP measurement with ABPM provides a more accurate diagnosis of HT and a better prediction of CV events (Hermida et al., 2013b; Staessen et al., 2001). In addition, BP measurements with ABPM are more closely related to indices of preclinical target organ damage and a better predictor of CV events than BP measurements in the office setting (Staessen et al., 2001; Turnbull, 2003). Furthermore, ABPM can allow white coat effect to be excluded and for detecting masked HT. Indeed, home BP measurements shares similar advantages with ABPM. However, 24-h ABPM gives complete data, especially in the nighttime period, which is considered to be a better marker of CV risk and mortality than daytime period (Fagard et al., 2008; Sega et al., 2005). Normally, both systolic and diastolic BPs decrease about 15–25% in nighttime. A nocturnal systolic BP fall of

<10% is called non-dipping, and failure to decrease in systolic BP is associated with a 2.5 times higher risk of CV events (Verdecchia et al., 1997). Non-dipping BP status is associated with known CV risk factors and is frequent in diabetes. The prevalence of blunted nighttime BP is more than twice in uncontrolled hypertensive patients with type 2 diabetes as compared with those without diabetes (Ayala et al., 2013). There is a significant increase of a blunted nighttime BP decline in treated hypertensive subjects with metabolic syndrome (Hermida et al., 2011b). In the present study, the number of diabetics was relatively small and the percentage of patients with diabetes did not differ among the groups. Although the percentage of diabetics was similar among the groups, there were statistically significant differences between diabetic and nondiabetic hypertensives with respect to GGT, CRP, hs-CRP, and MPV. The prevalence of blunted nighttime BP also elevates with increasing age, and blunted nighttime BP pattern is 4 times more prevalent in patients  $\geq 60$  yrs of age than those  $< 60$  yrs of age (Hermida et al., 2013a). However, administration of one or more antihypertensive medications at bedtime results in an attenuation of the prevalence of blunted nighttime BP decline at all ages as compared with ingestion of all antihypertensive medications at awakening. (Hermida et al., 2013a). Similarly, previous studies have shown that bedtime administration of long-acting antihypertensive agents provides a greater reduction of nighttime BP than morning administration (Hermida et al., 2009, 2010, 2011a). Furthermore, results from the Heart Outcomes

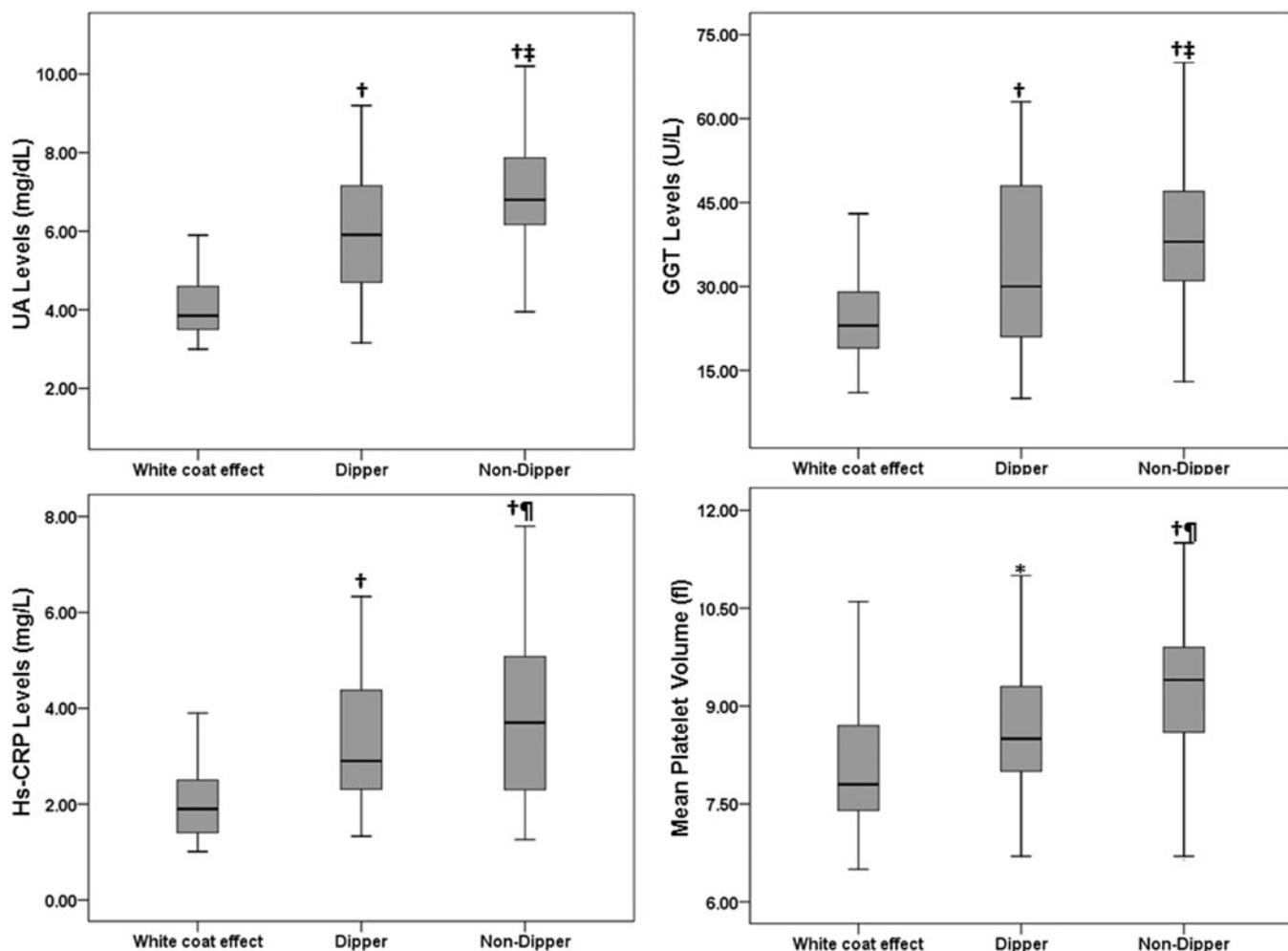


FIGURE 1. Indices of inflammation and platelet activity of each study groups. hs-CRP = high-sensitivity C-reactive protein. † $p < 0.0001$  versus white coat effect; ‡ $p < 0.0001$  versus dipper; ¶ $p < 0.01$  versus dipper; \* $p < 0.01$  versus white coat effect.

TABLE 4. Relationships of the study variables to demographic characteristics and risk factors.

Characteristic	Correlations with			
	GGT	UA	hs-CRP	MPV
Age	0.02	-0.03	0.04	-0.06
Gender (male)	-0.04	-0.01	-0.04	-0.03
Presence of DM	0.20‡	0.04	0.18‡	0.18‡
Smoking	0.21‡	0.17†	0.22‡	0.20‡
Presence of non-dipping	0.38‡	0.60‡	0.37‡	0.35‡
Presence of dipping	0.06	-0.07	0.03	-0.04
Body mass index	0.11*	0.05	0.04	0.08
Creatinine (mg/dL)	0.06	0.09	0.11*	0.03
Glucose (mg/dL)	0.10	0.01	0.14†	0.10*
Total cholesterol (mg/dL)	0.03	0.03	0.03	0.00
HDL cholesterol (mg/dL)	0.04	0.04	0.02	0.01
LDL cholesterol (mg/dL)	0.09	0.11*	0.09	0.06
Triglyceride (mg/dL)	0.08	0.04	-0.08	-0.07

DM = diabetes mellitus.

\*Correlation is significant at the 0.05 level (two-tailed).

†Correlation is significant at the 0.01 level (two-tailed).

‡Correlation is significant at the 0.0001 level (two-tailed).

TABLE 5. Correlations of the study variables.

Variable	GGT	UA	hs-CRP	MPV
GGT	-	0.38‡	0.55‡	0.48‡
UA		-	0.39‡	0.35‡
hs-CRP			-	0.18‡
MPV				-

GGT =  $\gamma$ -glutamyl transpherase; UA = uric acid; hs-CRP = high-sensitivity C-reactive protein; MPV = mean platelet volume.

‡Correlation is significant at the 0.0001 level (two-tailed).

Prevention Evaluation Study (HOPE) substudy where patients were evaluated by ABPM indicated a significant BP reduction mainly during nighttime period if ramipril was administered at bedtime (Svensson et al., 2001). The authors reported that an 8% increase in the diurnal/nocturnal BP ratio was associated with beneficial effects in CV mortality and morbidity. In addition, it has recently been shown that increasing the diurnal/nocturnal ratio of BP was markedly correlated with a

significant decrease in urinary albumin excretion (Hermida et al., 2005).

Although there has been strong evidence that hypertensive patients with non-dipping BP have an increased risk for CV disease and target organ damage, and achievement of nocturnal BP decrease with antihypertensive treatment is associated with beneficial effects in CV morbidity and mortality, there have been limited data evaluating the possible underlying pathophysiological mechanisms of increased CV risk in non-dipper hypertensive patients. In the present study, we showed that sustained hypertensive patients with uncontrolled office BP and non-dipping BP in ABPM had increased serum MPV, GGT, UA, and hs-CRP levels as compared with false uncontrolled (white coat effect) and dipper hypertensives.

Several laboratory techniques have been developed to detect platelet activation. Platelet number and size and the concentration of released substances after platelet activation are surrogate markers of increased platelet activity. Larger MPV is an indicator of increased *in vivo* platelet activation, and MPV correlates well with platelet activity whether measured as aggregation, thromboxane A2 or 3-thromboglobulin release, or adhesion molecule expression (Bath & Butterworth, 1996). Elevated MPV can predict the outcome in vascular thrombotic events such as myocardial and cerebral infarction (D'Erasmo et al., 1990). Previous studies have reported that as compared with normotensive controls, hypertensive patients had increased platelet activity, and there is a relationship between indices of platelet activity and target organ damage in high-risk hypertensive patients (Nadar et al., 2004).

There has been strong evidence that increased serum hs-CRP is a heritable marker of chronic inflammation that is strongly associated with CV disease. Furthermore, as described in the current guidelines, the inflammatory biomarker hs-CRP is now recognized as a major cardiovascular risk factor and as a secondary target for statin therapy (Genest et al., 2009). Previous studies have shown that hypertensive patients with non-dipping BP have increased serum CRP levels compared with those with non-dipping (Kaya et al., 2010; Tsioufis et al., 2008). Serum GGT level is an independent risk factor for CV disease, and there is a strong association between serum GGT levels and most CV risk factors including HT (Lee et al., 2003; Ruttmann et al., 2005). In addition, it has recently been shown that there is a relationship between serum GGT levels and microalbuminuria as well as coronary microvascular function, which are surrogate markers of hypertensive target organ damage (Caliskan et al., 2007; Lee et al., 2005). Patients with elevated serum UA levels had a mean 10-fold increased risk of developing CAD or HT, and that gout incidence was 3-fold higher in hypertensive individuals compared with normotensive subjects (Campion et al., 1987; Fessel, 1980). However, evidence is contradictory regarding whether serum UA level is an independent

risk factor for the development of CAD and hypertension because in two epidemiological studies, hyperuricemia could not be recognized as an independent CV risk factor. An analysis from the National Health and Nutrition Examination Survey (NHANES) III study ensured relevant knowledge to clarify whether serum UA level is an independent risk factor for the development of CAD and HT. This analysis revealed that hypertensive individuals with elevated serum UA levels had a significantly higher relative risk for both heart attack and stroke (Ward, 1998). These results strongly support the hypothesis that elevated serum UA level is an independent risk factor for HT-associated mortality and morbidity. In the present study, we found that MPV, UA, GGT, CRP, and hs-CRP levels were significantly higher in non-dipper hypertensives than both dipper hypertensive and individuals with white coat effect. Furthermore, we also found that there was an independent association between the presence of non-dipping and higher GGT, UA, CRP, hs-CRP, and MPV levels as individually. In addition to the data mentioned above, previous studies have shown that there is a clear relationship among GGT, UA, hs-CRP, and MPV (Bo et al., 2005; Kaya et al., 2010). In line with these findings, we found that GGT, UA, hs-CRP, and MPV were strongly correlated with each other.

In conclusion, the present study showed that non-dipper hypertensives had increased MPV, uric acid, GGT, CRP, and hs-CRP levels as compared with dipper hypertensive patients and individuals with white coat effect. Our results are consistent with the idea that the presence of non-dipping BP is associated with increased platelet activity and inflammation. Accordingly, increased platelet activity and inflammation can be one of the underlying plausible mechanisms of non-dipping BP status, and increased platelet activity and inflammation could contribute to increase the risk of atherosclerotic CV disease and target organ damage in non-dipper hypertensive patients.

## ACKNOWLEDGEMENTS

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## DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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# Serum Gamma-Glutamyl Transferase (GGT) Levels and Inflammatory Activity in Patients With Non-dipper Hypertension

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## Abstract

Non-dipper hypertension is associated with increased cardiovascular morbidity and mortality. We aimed to evaluate serum gamma-glutamyl transferase (GGT) level, which is accepted as a marker for oxidative stress and its relationship with inflammatory activity in patients with non-dipper hypertension. Age and sex matched 43 dipper hypertensive patients, 40 non-dipper patients, and 46 healthy subjects were included into the study. Serum GGT and C-reactive protein (CRP) levels were measured and compared between each of the groups. Serum GGT activity was higher in the non-dipper and the dipper hypertensive groups than in the control group ( $33.5 \pm 11.8$  and  $28.1 \pm 10.1$  U/l, respectively, vs.  $21.2 \pm 6.5$  U/l;  $p < 0.001$ ). There was a statistically significant difference in serum GGT activity between the non-dippers and the dippers ( $p = 0.021$ ). When compared with the control group, serum CRP levels were significantly increased in both the non-dipper and the dipper hypertensive groups ( $6.1 \pm 2.6$  and  $5.4 \pm 2.1$  mg/l, respectively, vs.  $2.8 \pm 1.7$  mg/l;  $p < 0.001$ ). Increased CRP levels were higher in non-dippers than dippers ( $p = 0.046$ ). A significant correlation was found between GGT and CRP measurements ( $r = 0.37$ ,  $p = 0.002$ ). Serum GGT levels, which are markers of the oxidative stress and CRP levels, are both increased in non-dipper hypertension. Increased GGT activity, found to be correlated with CRP levels, may be one of the reasons behind the non-dipper hypertension related cardiovascular complications.

**Keywords:** non-dipper hypertension, Gamma-glutamyl transferase and CRP

## INTRODUCTION

Hypertension is a common chronic condition affecting up to 35% of the adults (1). Most of the hypertensive patients exhibit a blood pressure (BP) fall between 10% and 20% during nighttime hours, who are called dippers. Recent studies implicated that the lack of nocturnal BP fall of less than 10% of the daytime (non-dippers) is associated with increased cardiovascular mortality, silent cerebrovascular disease, and progressive nephropathy, compared to the patients with dipper BP (2–4).

Serum gamma-glutamyl transferase (GGT) activity has been used as a marker for alcohol consumption or hepatobiliary disease (5). Gamma-glutamyl transferase is a plasma membrane enzyme that provides antioxidant glutathione resynthesis (6). Recent reports also indicate a direct role for GGT in the generation of reactive oxygen species (7–9). In this context, evidence from epidemiological studies point out that GGT may have a role in the pathogenesis of cardiovascular disease, diabetes mellitus, and metabolic syndrome (10–12).

Similarly, recent cross-sectional and longitudinal studies have also noted a relatively independent association between elevated serum GGT levels and hypertension (12–14).

Studies evaluating the relationship between inflammatory markers and circadian BP variations showed that non-dipper hypertension is characterized by increased inflammatory activity when compared to the dippers and normotensives (15, 16). However, there is no data indicating the relation between the diurnal BP pattern and serum GGT levels in hypertensive patients. In this study, we aimed to evaluate serum GGT levels together with the inflammatory activity in patients with hypertension in terms of circadian BP patterns.

## METHODS

### Patients

A total of 83 patients with hypertension and 46 healthy control subjects (22 male, 24 female, mean age:  $52.8 \pm 9.6$  years) were included in the study. Hypertensive

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patients were divided into two subgroups: 43 dipper (19 male, 24 female, mean age:  $53.9 \pm 10.5$  years) and 40 non-dippers (18 male, 22 female, mean age  $54.3 \pm 9.6$  years).

Hypertension was defined as systolic blood pressure (SBP)  $\geq 140$  mmHg and/or diastolic blood pressure (DBP)  $\geq 90$  mmHg or presence of the history of taking antihypertensive medication. Non-dipper hypertension was defined as less than 10% decrease in either SBP or DBP during nighttime record over 24-h ambulatory BP monitoring (ABPM). Exclusion criteria were the evidence of coronary artery disease, renal or hepatic dysfunction, active hepatobiliary disease, and alcohol consumption, hematologic disease, cancer, systemic inflammatory conditions, autoimmune disease, anemia, hyperthyroidism, and obstructive sleep apnea. Written informed consent was obtained from each subject and institutional review board approved the study protocol.

### **ABPM Recordings**

Blood pressure was measured using a mercury sphygmomanometer in an office setting. Following a 5-min resting period, SBP and DBP was recorded at Korotkoff phases I and V, respectively. The 24-h ABPM was performed using a portable compact digital recorder (Delmar Reynolds, Tracker NIBP2, Hertford, UK) and analyzer using costumed analytic software. The device was set to obtain BP readings at 15-min intervals during the day (07.00–23.00 h) and at 30-min intervals during the night (23.00–07.00 h). The patients were instructed to attend their usual daily activities but to stay inactive during measurements. Recordings were accepted only if more than 85% of the raw data were valid. The absolute and percentages of the decrease of nighttime SBP vs. daytime SBP were calculated in all subjects.

### **Biochemical Measurements**

Blood samples were drawn following a fasting period of 12 h. Glucose, creatinine, and lipid profiles were determined by standard methods. The activity of GGT was measured by using an Abbott-Architect auto analyzer (Abbott, Chicago, IL, USA) with original kits. C-reactive protein was calculated by the nephelometric method (Behring Nephelometer Analyzer, Marburg, Germany) and expressed as mg/l.

### **Statistical Analysis**

Statistical analysis was performed using the SPSS for Windows (version 11.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics of patients, including frequencies and percentages, were computed. Continuous variables are expressed as mean  $\pm$  standard deviation. Nominal parameters were expressed as percents. Significance of differences between the three groups was assessed by

using one-way ANOVA, followed by the Sheffe post-hoc test for ordinal parameters displaying normal distribution and the Kruskal-Wallis test followed by the Bonferroni corrected Mann-Whitney U post-hoc test for ordinal parameters not displaying normal distribution. Significance of differences between groups for nominal parameters was assessed by using chi-square test. Correlation between GGT activity and CRP levels were evaluated by the Pearson and Spearman rank correlation test. Statistical significance was accepted as p value less than 0.05.

## **RESULTS**

Comparison of baseline characteristics of the non-dippers, dippers, and controls were shown in Table 1. There was no significant difference among the groups with respect to age, gender, resting heart rate, diabetes mellitus, serum creatinine levels, and body mass index (BMI). Triglyceride, total cholesterol, and low-density lipoprotein (LDL) cholesterol levels were higher in the dippers and non-dippers when compared to the controls ( $p < 0.05$ ). Clinical SBPs and DBPs in office settings were similar in both hypertensive groups but were higher than normotensives, as expected ( $p < 0.001$ ). Distribution of the antihypertensive drugs was also illustrated in Table 1. There was no difference between the dippers and non-dippers with respect to the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, and diuretics. The average daytime SBPs, DBPs, and mean BP levels were shown in Table 2.

Serum GGT activity was higher in dipper and non-dipper hypertensive groups than normotensives ( $28.1 \pm 10.1$  and  $33.5 \pm 11.8$  U/l, respectively, vs.  $21.2 \pm 6.5$  U/l;  $p < 0.001$ ) and increased GGT activity was more pronounced in the non-dipper group ( $p = 0.021$ ) (Table 1 and Figure 1). When compared to the control group, serum CRP levels were significantly increased in both dipper and non-dippers ( $5.4 \pm 2.1$  and  $6.1 \pm 2.6$  mg/dl, respectively, vs.  $2.8 \pm 1.7$  mg /dl;  $p < 0.001$ ). Increased CRP levels were higher in non-dippers than in dippers ( $p = 0.046$ ) (Table 1). A significant correlation was found between GGT activity and CRP measurements ( $r = 0.37$ ,  $p = 0.002$ ), (Figure 2).

## **DISCUSSION**

In this study, we found that serum GGT activity and CRP levels were significantly increased in patients with non-dipper hypertension, when compared to the dippers and normotensives. We also found a significant correlation between serum GGT levels and inflammatory activity. To our knowledge, this is the first study comparing GGT and CRP levels together in patients with different circadian BP patterns.

During the last two decades, most of the studies have demonstrated a significant correlation between

Table 1. Laboratory parameters and clinical characteristics of study groups

	Non-dippers (n = 40)	Dippers (n = 43)	Normotensives (n = 46)
Age	54.3 ± 9.6	53.9 ± 10.5	52.8 ± 9.6
Men n/%	18 (45)	19 (44.1)	22 (47.8 %)
BMI(kg/m <sup>2</sup> )	26.8 ± 4.4	27.3 ± 3.6	26.1 ± 3.9
Clinic SBP (mmHg)	149.8 ± 13.4*	148.3 ± 15.2*	110.8 ± 13.1
Clinic DBP (mmHg)	94.2 ± 9.2*	93.6 ± 11.4*	71.7 ± 8.6
Resting heart rate (bpm)	73.5 ± 8.3	72.6 ± 9.2	71.1 ± 6.4
Diabetes, n/%	5 (12.5)	6 (14)	7 (15.2)
Total cholesterol (mg/dl)	198.4 ± 32.7*	202.6 ± 41.2*	186.9 ± 26.9
LDL cholesterol (mg/dl)	130.2 ± 41.5*	132.1 ± 45.5*	119.2 ± 24.5
HDL cholesterol	38.5 ± 11.3	42.4 ± 10.2	41.6 ± 12.5
Triglyceride (mg/dl)	144.6 ± 52.6*	149.6 ± 58.2*	139.6 ± 39.8
Gamma-glutamyl transferase (U/L)	33.5 ± 11.8**#	28.1 ± 10.1*	21.2 ± 6.5
Aspartate aminotransferase (U/L)	24.3 ± 10.1	24.6 ± 8.7	23.4 ± 7.1
Alanine aminotransferase (U/L)	23.9 ± 8.1	23.6 ± 8.5	21.1 ± 7.4
CRP	6.1 ± 2.6**#	5.4 ± 2.1*	2.8 ± 1.7
Creatinine (mg/dl)	0.9 ± 0.13	0.88 ± 0.16	0.86 ± 0.21
ACE inh. n/%	14 (35)	16 (35.6)	—
ARB, n/%	24 (60)	25 (58.1)	—
Ca channel blockers, n/%	9 (22.6)	10 (23.2)	—
Diuretics, n/%	24 (60.0)	26 (60.1)	—

Abbreviations: SBP - systolic blood pressure; DBP - diastolic blood pressure; bpm - beats per minute; BMI - body mass index; ACE inh - angiotensin-converting enzyme inhibitor; ARB - angiotensin receptor blocker.

\*:p < 0.05 non-dippers and dippers vs. normotensives.

#:p < 0.05 non-dippers vs. Dippers.

Table 2. Comparison of ambulatory BP monitoring results of dippers and non-dippers

	Non-Dippers	Dippers	p Value
24-h systolic BP	141.5 ± 9.3	134.5 ± 7.3	<0.001
24-h diastolic BP	90.0 ± 6.8	83.2 ± 5.9	<0.001
24-h mean BP	107.2 ± 7.3	100.2 ± 6.1	<0.001
Daytime systolic BP	145.2 ± 5.8	143.7 ± 6.4	NS
Daytime diastolic BP	90.3 ± 7.1	89.3 ± 7.7	NS
Daytime mean BP	108.5 ± 5.1	107.4 ± 5.7	NS
Nighttime systolic BP	137.8 ± 7.9	126.2 ± 6.3	<0.001
Nighttime diastolic BP	88.5 ± 6.2	78.1 ± 5.0	<0.001
Nighttime mean BP	104.9 ± 5.4	94.1 ± 4.1	<0.001

Abbreviations: BP - blood pressure; NS - nonsignificant.

ABPM recordings and the prevalence and extent of cardiovascular events. The first study showing the relationship between reduced nocturnal BP fall in hypertensive subjects and cardiovascular events was reported by Verdecchia et al. (17). In their study, non-dipper hypertensives were reported to have three-fold increased risk for cardiovascular complications compared to the dippers (15).

Previous studies have reported an association between BP and CRP level, which is used as the standard assay to identify and monitor the inflammatory activity (18, 19). In the Women's Health Study cohort, Blake et al. reported an independent association between CRP and high BP (18). Increasing categories of BP levels were found to be significant predictors of increased CRP levels, after being adjusted for potential confounders. In literature, there are some studies evaluating the relationship between inflammatory activity and circadian BP variations (15, 16). A most recent

study comparing CRP levels between the dippers and non-dippers was carried out by Kaya et al. (16) and CRP levels were reported to be increased in patients showing a non-dipper BP profile. In agreement with this study, we also found increased CRP levels in the non-dippers.

In clinical practice, GGT, which is the enzyme responsible for the extracellular catabolism of glutathione (20), is a commonly used diagnostic test. Gamma-glutamyl transferase has an important role in antioxidant defense systems and as a biomarker would fall under a new classification of "oxidative stress" in view of its role in the degradation of the antioxidant glutathione (8, 9, 21). Oxidative stress is involved in the pathophysiology of some diseases including cardiovascular disease and/or metabolic regulation. Several evidences suggest a plausible relationship between serum GGT level and hypertension, including the following: 1. GGT has a direct role in the generation of

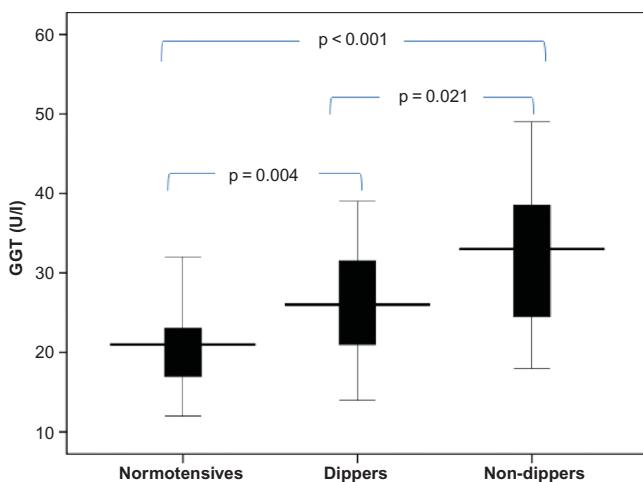


Figure 1. Comparison of serum GGT levels among the three study groups.

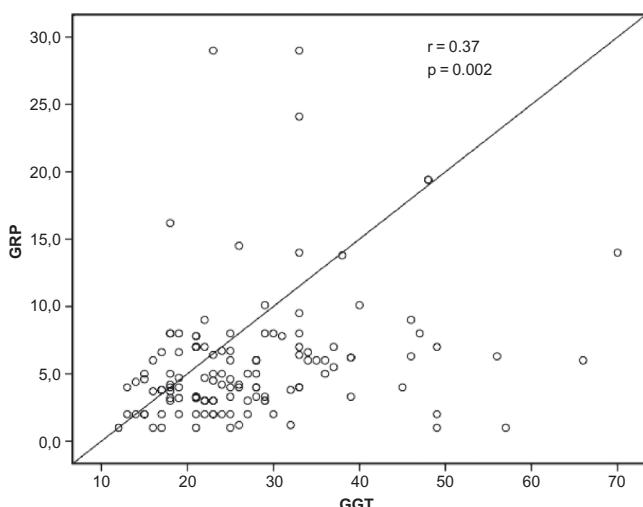


Figure 2. Positive correlation between serum GGT levels and inflammatory activity is shown.

reactive oxygen species; 2. GGT has indirect role as a marker for increased extracellular catabolism of antioxidant glutathione in response to oxidative stress; 3. it has a relationship with inflammatory markers; 4. GGT has also a relationship with insulin-resistance and the other components of the metabolic syndrome (7–9, 21–24).

It is known that oxidative stress is a key component of many reactions associated with chronic inflammation (23, 25). Multiple oxidative processes play a critical role in inflammation and act on various intra- and extracellular pathways through specific mediators in conjunction with free radicals that amplify inflammatory reactions at specific sites (22). In this context, in the Coronary Artery Risk Development in Young Adults (CARDIA) study (12), serum GGT concentrations predicted future serum CRP levels, which was measured 15 years after the earlier GGT measurement and 5 years after the later in dose-response manners. In another study confirming

our results, Lee and Jacobs showed a strong association between serum gamma-glutamyltransferase and C-reactive protein in 12,110 adult participants in the third U.S. National Health and Nutrition Examination Survey (22).

Oxidative stress has been implicated in initiating inflammatory response through chromatin remodeling (histone acetylation/deacetylation), the activation of transcription factors such as nuclear factor-kappa B and activator protein-1 leading to gene expression of proinflammatory mediators (22). Previous studies and our findings suggest that the elevation of serum GGT is involved in the inflammatory response (12, 22, 23). Due to an interrelation between oxidative stress and inflammatory reactivity, it is reasonable to say that one of the triggering pathologies is increased oxidative stress for future cardiovascular events in non-dipper hypertension.

The most important limitations of our study are the small sample size and cross-sectional design of the study which limit our results to generalize. The study was conducted while the patients were taking antihypertensive treatment. However, distribution of drug use was similar in both hypertensive groups. Another limitation is the lack of other markers for defining oxidative stress (i.e., malondialdehyde, superoxide dismutase, and glutathione) and inflammatory markers (i.e., interleukin-6, tumor necrosis factor- $\alpha$ ). Finally, the fact that the diagnosis of dipper vs. non-dippers was based on single BP measurements could be one additional limitation of the study.

From a clinical standpoint, our results showed that both serum GGT levels, which is a marker of the oxidative stress, and CRP levels are correlated with each other and both are increased in non-dippers. Thus, increased oxidative stress may be one of the reasons behind the non-dipper hypertension-related cardiovascular complications.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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## Relationship between serum gamma-glutamyltransferase activity and cardiometabolic risk factors in metabolic syndrome

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### Abstract

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### Objectives:

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The objective of this study was to examine the associations of serum gamma-glutamyltransferase (GGT) levels with the metabolic syndrome (MetS) and its components in Saudi adults.

### Methods:

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The study comprised 400 participants (70 men and 330 women), aged between 40 and 88 years, randomly selected from the medicine clinics at the King Abdulaziz University Hospital in Jeddah, Saudi Arabia, in a cross-sectional study design. A standardized questionnaire was used to determine demographics variables, general health, lifestyle habits, and medical history. Anthropometric and biochemical variables measurements were taken for all study participants. MetS was defined according to the American Heart Association/National Heart, Lung, and Blood Institute report, by the presence of abdominal obesity.

### Results:

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Higher means for triglycerides and insulin resistance indices ( $P < 0.0001$ ) was found among those in the second, third, and fourth GGT quartiles as compared with their counterparts in the first quartile. McAuley index ( $\beta = -0.239$ ,  $P < 0.0001$ , 95% confidence interval:  $-4.1\text{--}1.5$ ) was shown to be a major determinant of circulating GGT in a multivariate analysis.

### Conclusion:

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Elevated serum GGT could be a cardiometabolic risk factor either as a mediator of low-grade systemic inflammation and as a mediator of oxidative stress through mediation of extracellular glutathione transport into cells of organ systems.

**Keywords:** Gamma-glutamyltransferase, metabolic syndrome, Saudi adults, oxidative stress, inflammation

### Introduction

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Metabolic syndrome (MetS) is defined by a clustering of risk factors for cardiovascular disease (CVD), that include abdominal obesity, dyslipidemia, hypertension, and impaired glucose tolerance, all of which increase the risk of CVD and diabetes mellitus.[1] MetS has been acknowledged as one of the major public-health problems globally.[2]

Gamma-glutamyltransferase (GGT) has long been considered an indicator of hepatobiliary dysfunction and alcohol abuse.[3] Recently, several epidemiology studies have shown that GGT participates in common pathophysiological processes, including oxidative stress and lipid peroxidation, which are important to the pathogenesis and development of insulin resistance and the MetS.[4,5,6] Furthermore, when GGT was tested along with other hepatic markers, GGT was the major predictor of type 2 diabetes.[7,8,9] It is clear that the pathways by which biomarkers such as GGT are associated with the causation and/or complications of the MetS represent a rich field for research. It is also possible that GGT is a risk factor and a prognostic indicator of CVD. Further information is needed in regard to the magnitude of risk associated between GGT activity and the individual cardiometabolic disorders. Such a relationship could help to explain the high prevalence of MetS. Nevertheless, the relationship remains uncertain and has not been well researched yet. Therefore, the aim of this study was to examine the associations of serum GGT levels with the MetS and its components in Saudi adults.

## Methods

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The study was approved by the Ethical Committee of King Abdulaziz University Hospital (KAUH) and was carried out in accordance with recommendations from the Declaration of Helsinki. Verbal consent form was provided by all study participants.

A total of 400 Saudi participants (70 men and 330 women), aged between 40 and 88 years, were randomly recruited in a cross-sectional study, between February 2014 and July 2016, from the Department of Internal Medicine Clinics at KAUH, Jeddah, Saudi Arabia, during visits for routine checkups, or for evaluation of cardiovascular risk factors.

Those with a known history of liver disease (e.g., acute and chronic active hepatitis, liver cirrhosis), biliary tract diseases, cardiovascular events (unstable angina, myocardial infarction, and stroke), heart failure, peripheral vascular diseases, cardiovascular surgery, malignant diseases, acute infectious, or inflammatory disorders were all excluded from the study. The demographic, lifestyle, medical history, and use of medications of participants were assessed using an interviewer-based structured questionnaire. The medical history included whether there was a diagnosis and/or treatment of diabetes, hypertension, dyslipidemia, and heart diseases. Lifestyle habits assessed by the questionnaire included supplementation use, smoking history, and physical activity level.

Waist circumference was measured at the plane across the iliac crests, which usually represents the narrowest part of the torso. Systolic and diastolic blood pressures were measured in the sitting position on the right arm three times using a standard zero mercury sphygmomanometer after at least 10–15 min of rest. Then, the average of the three readings was obtained.

MetS was defined according to the American Heart Association/National Heart, Lung, and Blood Institute report, by the presence of abdominal obesity (waist circumference >88 cm in women) with at least two of the following: triglycerides of 150 mg/dl (1.7 mmol/L) or greater, high-density lipoprotein (HDL) cholesterol levels <50 mg/dl (1.29 mmol/L) in women, fasting glucose of 110 mg/dl (6.1 mmol/L) or greater, or blood pressure of 130/85 mmHg or greater.[10]

Venous blood samples were obtained after fasting for at least 12 h. Samples centrifuged and serum, refrigerated at 2–8°C, and analyzed within 24 h. Levels of fasting blood glucose (FBG), plasma insulin, triglyceride, total cholesterol, HDL cholesterol, and liver function test were measured in the routine

biochemistry laboratory of the KAUH. Fasting lipid profile, FBG, and liver enzymes were measured by an enzymatic colorimetric method using an automated chemistry analyzer (Dimension Vista System, Siemens, Germany). Low-density lipoprotein cholesterol was calculated using the Friedewald formula. Fasting plasma insulin concentration was measured with a chemiluminescence method (Modular E170 immunoassay analyzer, Roche, USA). High-sensitivity C-reactive protein (hs-CRP) was measured by immunoturbidimetric assay (Behring Nephelometer-BNA2, Siemens, USA).

Insulin resistance was determined using a number of indices including the homeostatic model assessment of insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICK-I), McAuley's index, and insulin sensitivity index (ISI).[[11](#),[12](#),[13](#),[14](#)]

Continuous variables are presented as mean  $\pm$  standard deviation, while categorical variables are presented as a total number (percentage). If necessary, logarithmic transformation was performed to achieve a normal distribution. Differences of clinical and metabolic features among groups were calculated using ANOVA test and/or Kruskal–Wallis test for parametric and nonparametric variables, respectively. The correlation analysis was performed by calculating the Pearson's or Spearman coefficient correlation for parametric and nonparametric variables, respectively. Multiple linear regression analyses were applied to determine the relationship between GGT and the risk for MetS. Differences were considered statistically significant at two-sided  $P < 0.05$ . Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21 (SPSS, Inc., Chicago, IL, USA).

## Results

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A total of 400 individuals, aged 40–88 years, including 70 men and 330 women, participated in this cross-sectional study. In total, 260 (65%) participants were identified as having MetS.

Sex-specific serum GGT values ( $66.05 \pm 11.2$  U/L for men and  $31.31 \pm 1.94$  U/L for women) are within KAUH laboratory reference ranges.

Clinical characteristics of the study population across GGT quartiles are shown in [Table 1](#). Participants in the third and fourth quartiles had significantly higher means of waist circumference ( $P < 0.05$ ) and serum insulin levels ( $P < 0.05$ ) than those in the first quartile. Higher means for triglycerides, HOMA-IR, QUICK-I, McAuley index, and ISI ( $P < 0.0001$  in all) was found among those in the second, third, and fourth quartiles as compared with their counterparts in the first quartile.

Comparisons of GGT levels were made among groups of participants classified as having 0, 1, 2, 3, 4, or 5 components of MetS [[Figure 1](#)]. Although nonsignificant, the greater the number of clustered risk factors of MetS, the higher the mean levels of GGT.

[Table 2](#) summarizes the correlations between serum GGT and cardiometabolic risk factors in the study population, partially adjusted for age and gender ( $r$  ranging from 0.1 to 0.3). Of all MetS components, blood pressure values failed to show a correlation with GGT levels. Fasting insulin, hs-CRP, and all insulin resistance indices showed a significant correlation with GGT levels ( $P < 0.05$ ).

Stepwise multiple regression analysis for serum GGT was conducted in a model that included all independent variables with  $P$  value up to 0.1 to demonstrate their contribution to GGT level. Only one independent variable that explained 5.7% of the variation in GGT values; McAuley index, exponential ( $2.63 - 0.28$  in insulin [ $\mu\text{U/ml}$ ]  $- 0.31$  in triglycerides [ $\text{mM/ml}$ ]), ( $\beta = -0.239$ ,  $P < 0.0001$ , 95% confidence interval:  $-4.1 - -1.5$ ) was shown to be a major determinant of circulating GGT.

## Discussion

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MetS consists of clustering of atherogenic factors.[[10](#)] In addition, a large number of biochemical and

anthropometric parameters have been reported to be associated with the MetS, including parameters of obesity and products released by adipose tissue, plasma insulin levels, liver enzymes, and CRP.[15,16,17]

Epidemiology studies have indicated that serum GGT concentrations may be related to the development and clinical progression of CVD, even after adjustment for alcohol consumption.[6,18,19,20,21] Although high levels of GGT have been postulated to be directly atherogenic,[22] as have several other biomarkers for the MetS, a direct role in causation of atherosclerosis remains to be determined. As shown in [Figure 1](#), higher GGT levels are accompanied by the additive effect of MetS components and potentially greater risk for subsequent development of type 2 diabetes.

### **Gamma-glutamyl transferase associations with metabolic syndrome components**

There is growing evidence that the liver, the primary source of circulating GGT, is a key target organ for the development of the MetS.[23] A number of studies have also shown that the serum level of GGT directly correlates with an increased risk of MetS.[4,23,24] This was demonstrated by the significant correlations between GGT levels and all MetS components, independent of age and gender, except for blood pressure values [[Table 2](#)]. Although it has been previously proposed that the connection between GGT and MetS could be attributed to an association of higher GGT levels with hypertension.[20,25]

### **Gamma-glutamyl transferase associations with inflammatory markers**

Another important finding was the association between GGT and hs-CRP [[Table 2](#)]. As proposed by Ortega *et al.*[26] a higher GGT production could be secondary to a low-grade hepatic inflammation induced by hepatic steatosis. Alternatively, excess fat in the liver could enhance oxidative stress, leading to overconsumption of glutathione with a compensatory increase in GGT synthesis. The documented predictability of MetS by GGT activity suggests that, as a reflection of oxidative stress, elevated GGT levels are actively involved in the pathogenesis of MetS.[24]

### **Gamma-glutamyl transferase association with insulin resistance indices**

Higher GGT levels were repeatedly reported to be associated with insulin resistance and thus greater risk for type 2 diabetes.[17,20,27] Irrespective of all cardiometabolic risk factors, only the McAuley index showed to be a major determinant of circulating GGT in a stepwise multiple regression models. Such elevations of serum GGT might indicate to be due to ectopic liver fat and/or secondary hepatic inflammation.[22,28]

Strengths and limitations of this study should be acknowledged. The current findings must be interpreted with caution due to the cross-sectional study design, which does not allow us to make inference about the causality for the effects. Nevertheless, the large sample size ensures sufficient evidence in investigating the associations of serum GGT with the MetS and its components.

## **Conclusion**

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Elevated serum GGT could be a cardiometabolic risk factor either as a mediator of low-grade systemic inflammation and as a mediator of oxidative stress through the mediation of extracellular glutathione transport into cells of organ systems. Whether it is implicated as a cause or as a reflection of a metabolic abnormality remains to be discovered. Further longitudinal studies are needed to find out the exact mechanisms underlying the association between GGT and MetS components.

### **Financial support and sponsorship**

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# Gamma Glutamyl Transferase Activity is Associated With Both Paraoxonase Activity and Aortic Stiffness in Hypertensive Patients

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**Background:** We aimed to investigate relationship between gamma glutamyl transferase (GGT) activity with paraoxonase 1 (PON1) activity and aortic stiffness (AS) parameters such as pulse wave velocity (PWV) and augmentation index (Alx). **Methods:** Measurements were obtained from 324 patients with newly diagnosed essential hypertension (mean age:  $55.0 \pm 8.2$  years). The patients were divided into two groups according to their median GGT values. PWV and Alx were calculated using the single-point method via the Mobil-O-Graph® ARCsolver algorithm. **Results:** PWV, Aix, and high-sensitive C-reactive protein (hs-CRP)

values were higher and PON1 activity values were lower in GGT<sub>high</sub> group compared with GGT<sub>low</sub> group ( $P < 0.05$ , for all). Multiple linear regression analysis showed that GGT activity was independently associated with PWV ( $\beta = 0.496$ ,  $P < 0.001$ ) and PON1 activity ( $\beta = -0.343$ ,  $P < 0.001$ ) as well as hs-CRP ( $\beta = 0.334$ ,  $P < 0.001$ ). **Conclusion:** These results may support that increased GGT activity would be associated with both impaired antioxidant system and increased AS in hypertensive patients. *J. Clin. Lab. Anal.* 29:390–396, 2015. © 2014 Wiley Periodicals, Inc.

**Key words:** GGT; aortic stiffness; PWV; paraoxonase; hypertension; CRP

## INTRODUCTION

Gamma glutamyl transferase (GGT) activity is a well-known enzyme marker for increased oxidative stress (1,2). GGT activity is found on the surface of various cells including vascular system and plays a role in the catabolism of glutathione, which is one of the major antioxidants (1, 2). Previous studies have indicated that GGT is involved in the pathophysiologic process of atherosclerosis (3). Also, serum GGT concentrations within the physi-

ologic range have been independently related with most cardiovascular disease risk factors including metabolic

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syndrome and hypertension (4–6). In particular, increased GGT activity has been shown to be associated with hypertension development in previous studies (5, 6).

Increased aortic stiffness (AS) reflects vascular damage and is a measure of the severity of atherosclerosis (7). Also, noninvasive AS is postulated to be a surrogate marker of early atherosclerosis (8). Moreover, AS is independently associated with hypertension (9). Pulse wave velocity (PWV) and augmentation index (AIx), valid, clinically feasible, and reproducible measures of AS, generally allows the comprehensive assessment of patients with cardiovascular diseases (10).

High-density lipoprotein (HDL) cholesterol exerts cardioprotective properties through its antioxidant activity and anti-inflammatory effects, which is largely maintained by paraoxonase 1 (PON1) (11, 12). PON1 protects lipoproteins against oxidative modification and to hydrolyze hydrogen peroxide, a major reactive oxygen species (ROS) produced under conditions of inflammation and atherosclerosis (12).

Our hypothesis is that GGT activity, which reflects increased oxidative stress, is associated with PON1 activity (antioxidant enzyme) and AS, which is related with essential hypertension. Therefore, we aimed to investigate relationship between GGT activity with PON1 activity and AS parameters such as PWV and AIx in patients with newly diagnosed hypertensive patients.

## METHODS

### Study Populations

Of 359 patients having office blood pressure (BP) measurement  $\geq 140/90\text{ mmHg}$ , 35 patients were excluded because of their BP was normal according to ambulatory BP monitoring (ABPM). Measurements were obtained from 324 patients with newly diagnosed essential hypertension (mean age:  $55.0 \pm 8.2$  years, male/female: 125/199). The study population was divided into two subgroups as GGT<sub>low</sub> versus GGT<sub>high</sub> groups with regard to their median GGT levels. Exclusion criteria were secondary hypertension, heart failure, positive history or clinical signs of ischemic heart disease, positive effort test, positive myocardial perfusion scintigraphy, cerebrovascular disease, severe valve disease, atrial fibrillation, usage of any drugs, renal insufficiency (serum creatinine:  $\geq 1.5\text{ mg/dl}$  in men and  $\geq 1.4\text{ mg/dl}$  in women), major noncardiovascular diseases, and known diabetes or fasting glucose  $\geq 126\text{ mg/dl}$ . In addition, patients taking antioxidant vitamin and alcohol were also excluded. The Local Ethics Committee assessed and approved the study and written informed consent for participation in the study was obtained from all individuals.

### BP Measurement and ABPM

BP was measured by using a mercury sphygmomanometer in office setting. Systolic (SBP) and diastolic BPs (DBP) were taken. Noninvasive 24-h ABPM was performed with a portable compact digital recorder (Tracker NIBP2, Delmar Reynolds Ltd., Hertford, UK), and analyzed using a customized analytical software (Delmar Reynolds Medical Inc., Model 2169, Hertford, UK). All subjects wore an ABPM device for a single 24-h period. The device was programmed to inflate and record BP at prespecified intervals (every 15 min during daytime hours and every 30 min during nighttime hours), which provided approximately 80 BP recordings during the 24-h period.

### Diagnosis of Hypertension

In each subject, BP was measured in at least three separate days after 15 min of comfortably sitting and averaged. Then each subject undertook 24-h ABPM. Individuals who had SBP  $\geq 140\text{ mmHg}$  and/or a DBP  $\geq 90\text{ mmHg}$  in office setting, and in ABPM, an average 24-h SBP  $> 130\text{ mmHg}$  and/or DBP  $> 80\text{ mmHg}$ , an average daytime SBP  $> 135\text{ mmHg}$  and/or DBP  $> 85\text{ mmHg}$  or an average nighttime SBP  $> 125\text{ mmHg}$  and/or DBP  $> 75\text{ mmHg}$  were diagnosed as hypertensive (13).

### Blood Samples and Echocardiography

Fasting blood samples were collected after the examination for the evaluation of low-density lipoprotein (LDL) cholesterol, HDL cholesterol, triglyceride, GGT activity, and high-sensitivity C-reactive protein (hs-CRP) levels. Plasma triglyceride, total cholesterol, LDL, HDL concentrations, and fasting glucose were measured using an automated chemistry analyzer (Aerotest; Abbott, Holliston, MN) with commercial kits (Abbott). Hs-CRP was measured using an autoanalyzer (Aerotest; Abbott) with a spectrophotometric commercial kit (Scil Diagnostics GmbH, Vierneheim, Germany). Serum GGT activities were measured by the enzymatic calorimetric test (Roche/Hitachi analyzer, Mannheim, Germany) and the normal range of GGT activity was identified as 7–49 U/l. The coefficient of variation (CV) for measurement of serum GGT activity was 2%.

Measurement of serum PON1 activity was performed in the absence of NaCl (basal activity). The rate of paraoxon hydrolysis (diethyl-*p*-nitrophenylphosphate) was measured by monitoring the increase of absorbency at 412 nm at 37°C. The amount of generated *p*-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was  $17,000\text{ M}^{-1}\text{ cm}^{-1}$  (14). PON1 activity was expressed as U/l serum. The CV for measurement of serum PON activity was 2.3%.

Standard two-dimensional echocardiography was performed using a commercially available echocardiographic machine (Vivid 7R GE Medical System, Horten, Norway) with a 2.0–3.5 MHz transducer. Left Ventricle ejection fraction was determined by the biplane Simpson's method (15).

### The ARC Solver Method

The ARC Solver method is commercially available in the oscillometric Mobil-O-Graph NGW 24-h PWA monitor (IEM; Stolberg, Germany) and aims to be a novel method for the determination of the aortic SBP, aortic BP curves, and AIx based on oscillometric BP measurement. The method (16) has been developed by the Austrian Institute of Technology, Vienna, Austria. The method uses the pulse waves assessed at arteria brachialis. The algorithm for the generation of, using the oscillometric method, have been reported previously (16,17) but are briefly explained. After the conventional oscillometric BP assessment, peripheral pressure waves are recorded, using the appropriately sized brachial cuff and a high fidelity pressure sensor (MPX5050, Freescale Inc., Tempe, AZ), at DBP level for 10 sec. The sensor is connected to a 12 bit A/D converter by means of an active analogue band bass filter (0.425 Hz). Following digitalization, the signal processing is performed using a three-step algorithm. In a first step, the single pressure waves are verified for their plausibility by testing the position of minima and the corresponding wavelengths. Minima are detected by means of an iterative procedure evaluating higher order time derivatives of the pressure signal. The second stage involves comparison of all single pressure waves with one another to recognize artifacts. Aortic pulse waves are then generated via a general transfer function. Modulus and phase characteristics of the ARC Solver transfer function are available. Finally, the coherence of the measured parameters is verified and displayed within the Mobil-O-Graph NG software package, which also allows visual inspection to unveil consistently recorded intrinsic waveform distortion manually.

### Measurements of the Aortic PWV and AIx

All recordings were performed with the ARC Solver method and standard oscillometric BP measurement procedures. After 10 min of rest, an appropriately sized BP cuff was attached to the patient's right arm. Applanation tonometry of the radial artery and oscillometric pulse wave recordings at the brachial artery were performed in the supine position. This was followed by a 10-sec pulsed wave analysis recording with the cuff inflated at the DBP level.

Using the Mobil-O-Graph NG, the aortic BP curves, aortic SBP, and aortic pulse pressure (aPP: aortic SBP –

aortic DBP) were obtained. A characteristic point of the aortic BP curve, the inflection point, is identified within the time domain, indicating the arrival of the reflected wave in the ascending aorta. The BP at this point of time is called "inflection pressure." The difference between aortic SBP and inflection pressure is called "augmentation pressure" (AP). The AIx is then calculated by  $AP/aPP \times 100$ . The Mobil-O-Graph NG software package allows us to automatically calculate aortic PWV and AIx (17).

### Statistical Analysis

Statistical analysis was carried out using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). Data are expressed as mean value  $\pm$  SD. Continuous variables were tested for normality using the Kolmogorov-Smirnov test. An independent simple *t*-test was used in the analysis of continuous variables. Categorical variables were compared using the chi-square test. The correlations between GGT and laboratory, AS parameters, hemodynamic, and echocardiographic parameters were assessed by the Pearson correlation test. A multivariate stepwise linear regression analysis was performed to identify the independent associations of GGT activity. All significant ( $P < 0.05$ ) parameters in the bivariate analysis (Average 24-h SBP, Office SBP, AIx, PWV, hs-CRP, PON1 activity) were selected in the multivariate model. A two-tailed  $P < 0.05$  was considered as statistically significant.

### RESULTS

The patients were divided into two subgroups as GGT<sub>low</sub> (mean age:  $54.7 \pm 6.4$ ; 162 patients) versus GGT<sub>high</sub> (mean age:  $55.3 \pm 9.8$ ; 162 patients) groups with regard to their median GGT levels (GGT<sub>low</sub> < 27.3 U/l and GGT<sub>high</sub>  $\geq 27.3$  groups).

Comparison of baseline and laboratory characteristics was demonstrated in Table 1. Both average 24-h SBP and office SBP values were higher in GGT<sub>high</sub> group compared with GGT<sub>low</sub> group ( $P < 0.05$ , for all). Other baseline characteristics were not different between the groups ( $P > 0.05$ , for all). PON1 activity levels of GGT<sub>high</sub> group were higher than GGT<sub>low</sub> group ( $P < 0.05$ ). Similarly, hs-CRP levels were higher in GGT<sub>high</sub> group compared with GGT<sub>low</sub> group ( $P < 0.05$ ).

PWV and AIx values were higher in GGT<sub>high</sub> group compared with GGT<sub>low</sub> group ( $P < 0.05$ , for all) (Table 1).

GGT activity was significantly associated with office SBP ( $r = 0.112$ ,  $P = 0.043$ ), average 24-h SBP ( $r = 0.176$ ,  $P = 0.002$ ), AIx ( $r = 0.188$ ,  $P = 0.001$ ), PWV ( $r = 0.624$ ,  $P < 0.001$ ), hs-CRP ( $r = 0.509$ ,  $P < 0.001$ ), PON1 activity ( $r = -0.558$ ,  $P < 0.001$ ) in bivariate analysis (Table 2). Relationships between GGT activity with PON1 and PWV were shown in Figures 1 and 2.

**TABLE 1.** Baseline, Laboratory, Echocardiographic, and Aortic Stiffness Characteristics of Groups

Variables	GGT <sub>low</sub> group (n = 162)	GGT <sub>high</sub> group (n = 162)	P value
Baseline characteristics			
Age (years)	54.7 ± 6.4	55.3 ± 9.8	0.541
Gender (male) <sup>a</sup>	56 (34.6%)	69 (42.6%)	0.085
BMI (kg/m <sup>2</sup> )	28.4 ± 5.7	29.1 ± 5.3	0.270
Office SBP (mmHg)	152.3 ± 17.4	157.9 ± 18.4	0.005
Office DBP (mmHg)	92.6 ± 9.5	94.2 ± 10.3	0.148
Average 24-h SBP (mmHg)	132.5 ± 8.8	135.3 ± 11.2	0.013
Average 24-h DBP (mmHg)	83.9 ± 9.6	83.1 ± 9.8	0.439
Heart rate (beat/min)	73.5 ± 8.7	73.9 ± 7.9	0.449
Smoking <sup>a</sup>	46 (28.4%)	41 (25.3%)	0.308
Laboratory findings			
Glucose (mg/dl)	96.7 ± 9.4	95.9 ± 12.5	0.501
Total cholesterol (mg/dl)	207.3 ± 37.2	208.8 ± 41.9	0.748
Triglyceride (mg/dl)	178.2 ± 90.5	175.0 ± 116.8	0.785
HDL-C (mg/dl)	47.4 ± 11.0	47.2 ± 11.5	0.911
LDL-C (mg/dl)	135.7 ± 34.4	137.6 ± 34.8	0.622
Creatinin (mg/dl)	0.77 ± 0.20	0.80 ± 0.23	0.095
hs-CRP (mg/dl)	0.56 ± 0.14	0.76 ± 0.22	<0.001
Uric acid (mg/dl)	5.0 ± 1.14	5.1 ± 1.3	0.404
PON1 activity (UI <sup>-1</sup> )	109.3 ± 44.7	64.5 ± 33.0	<0.001
Echocardiography			
Ejection fraction (%)	61.3 ± 4.6	61.3 ± 5.3	0.954
Aortic stiffness parameters			
PWV (m/sec)	7.4 ± 1.5	9.8 ± 2.5	<0.001
AIx (%)	24.9 ± 11.0	28.0 ± 11.6	0.015

GGT, gamma glutamyl transferase; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; hs-CRP, high-density lipoprotein cholesterol; PON1, paraoxonase1; PWV, pulse wave velocity; AIx, augmentation index.

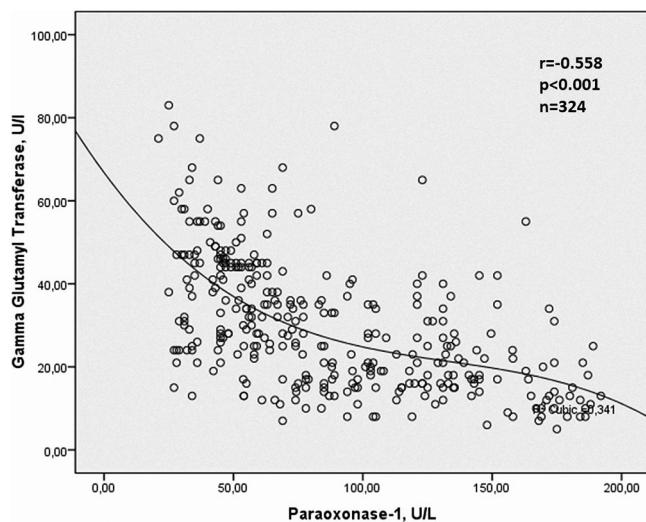
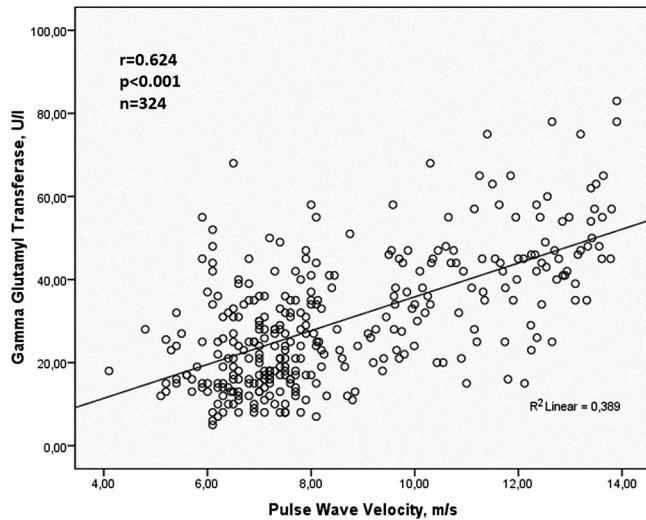
<sup>a</sup>Chi square.

**TABLE 2.** Bivariate and Multivariate Relationships of GGT

Variables	Pearson correlation coefficient	P value	Standardized β-regression coefficients	P value
Average office SBP (mmHg)	0.112	0.043	-0.017	0.703
Average 24-h SBP (mmHg)	0.176	0.002	-0.074	0.110
AIx (%)	0.188	0.001	-0.038	0.366
PWV (m/sec)	0.624	<0.001	0.496	<0.001
hs-CRP (mg/dl)	0.509	<0.001	0.334	<0.001
PON1 activity (UL <sup>-1</sup> )	-0.558	<0.001	-0.343	<0.001

Abbreviations as in Table 1.

Multivariate regression analysis showed that GGT activity was independently associated with PWV ( $\beta = 0.496$ ,  $P < 0.001$ ), hs-CRP ( $\beta = 0.334$ ,  $P < 0.001$ ), and PON1 activity ( $\beta = -0.343$ ,  $P < 0.001$ ) (Table 2).

**Fig. 1.** Relationship between GGT activity and PON1.**Fig. 2.** Relationship between GGT activity and PWV.

## DISCUSSION

This is the first study that investigated the relationship between GGT activity with PON1 activity and AS in newly diagnosed hypertensive patients. The main finding of the present study was that GGT activity was independently associated with PON1 activity and PWV as well as hs-CRP in newly diagnosed hypertensive patients.

It is well known that there is an independent association between GGT activity and hypertension (5, 6). Moreover, GGT activity is positively associated with the development of hypertension (5, 6).

Present study showed that GGT activity within the normal range was independently and negatively associated with PON1 activity. Hypertension is associated with

increased vascular production of ROS (18, 19). Vascular oxidant stress, particularly interactions between nitric oxide and oxygen derived radicals, represents a common pathologic mechanism in many risk factors for atherosclerosis including hypertension (19). GGT has been proposed as a marker of oxidative stress (1,2). Previous experimental studies have reported that GGT plays an important role in antioxidant systems with the primary function of maintaining intracellular concentrations of glutathione, a critical antioxidant defense for the cell (20,21). Also, it was shown that GGT activities within the reference range catalyze oxidative reactions in the presence of iron ions that lead to the production of free radicals and ROS and LDL oxidation (22). Oxidative stress, owing to increased lipid and protein oxidation products and decreased antioxidant enzymes and vitamins, affects PON1 expression and activities (23). There is direct evidence for a mechanistic link between activity of PON1 with systemic oxidative stress and prospective cardiovascular risk, indicating a potential mechanism for the atheroprotective function of PON1 (24). Fallah et al. showed PON1 192 genes potentially play a role in the manifestation of coronary atherosclerosis (25). These findings shows that serum PON1 activity is inhibited by increased oxidative stress (23,24). It has been assumed that antioxidants such as PON1, which physiologically protect against excess of ROS, are consumed, thereby further increasing ROS induced damage (26). Therefore, the inverse relationship between GGT activity and PON1 activity (antioxidant enzyme) may be plausible because GGT activity is associated with oxidative stress.

Present study also showed that GGT activity was independently associated with PWV, with reproducible measures of AS. The relationship between GGT activity and AS was investigated in various patient groups (27, 31). However, this relationship was not investigated in newly diagnosed hypertensive patients. Saijo et al. reported that GGT was independently related to an increased level of arterial stiffness in male patients who were examined for routine checkup (27). Song et al. suggested that serum GGT activity may be an additional marker of arterial stiffness, especially in men, though the relationship with arterial stiffness was weak (28). Jung et al. demonstrated that GGT activity in healthy subjects was independently associated with the increased level of arterial stiffness both in men and women, even it was stronger in men (28). Also, the similar relationship between GGT activity and arterial stiffness was shown in patients with established coronary artery disease and in young patients with prehypertension (30, 31). The pathophysiological mechanisms underlying the association between GGT activity with increased AS are unclear. GGT levels were found to be associated with subclinical aortic atherosclerosis (32). Fur-

thermore, higher serum GGT activity has been reported in atherosclerotic plaques and foam cells (33). Moreover, GGT contributes to oxidative stress pathways in several organ systems, localizes to atheromatous plaques containing oxidized LDL, and is proinflammatory, further implicating this protein in atherogenesis (34). On the other hand, the association between increased AS with oxidative stress, inflammation, and subclinical atherosclerosis has been demonstrated in previous studies (35,36). Therefore, increased GGT activity may reflect to increased oxidative stress, inflammation, and subclinical atherosclerosis in hypertensive patients with increased AS. In present study, the independent relationship between GGT activity with hs-CRP and PON1 activity may support this hypothesis.

Present study showed that GGT activity was also independently associated with hs-CRP levels. This result is consistent with previous studies (27,34,37). Previous studies have reported a significant association between serum GGT and CRP levels after adjustment for age, smoking, alcohol consumption, and BMI (1). Moreover, CRP has been found to be deposited in coronary artery plaque, and it has a pro-oxidative effect on cultured coronary artery smooth muscle cells (37). Therefore, it was suggested that the significant relationship between GGT and CRP may possibly be due to their association with oxidative stress (27).

### Study Limitations

Coronary atherosclerosis may affect GGT and PON1 activities in this patient group. Coronary angiography was not performed in our patients although patients with coronary artery disease have been excluded according to clinical characteristics and patient history, electrocardiography, and treadmill exercise test. Also, smoking may be effective on PON1 activity and AS. However, smoking frequencies of groups were not different and there was no correlation between GGT activity and smoking frequency in our study. Furthermore, in present study, there was an independent relationship between GGT activity with AS and PON1 activity.

Finally, in present study, PON1 phenotype or genotype was not determined. PON1 has two coding region amino acid polymorphisms, one at position 55 and another at position 192 (38). The Q192R polymorphism has such a significant effect on PON1 activity. It is possible, but not advisable, to use paraoxonase activity within a 192 genotype/phenotype (e.g., Q/Q, Q/R, or R/R). Also, it was reported that serum PON1 activity is a better predictor of the risk for cardiovascular diseases than the PON1 genotype (39).

## CONCLUSIONS

In hypertensive patients, GGT activity was independently associated with PON1 activity and PWV as well as hs-CRP. These results may support that increased GGT activity would be associated with both impaired antioxidant system and increased AS in hypertensive patients.

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# Serum $\gamma$ -Glutamyl Transpeptidase Levels and Hypertension in Non-drinkers: A Possible Role of Fatty Liver in the Pathogenesis of Obesity Related Hypertension

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## Abstract

The relationships between increases in body mass index (BMI) and increases in hypertension were compared between non-drinkers with elevated serum  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP) levels ( $\geq 50$  U/l) and those with normal levels, who comprised 10,952 men and 22,107 women aged 40-59 years recruited from an occupational health clinic. Hypertension was found in 16.1% and 13.5% of the men and women, and elevated serum  $\gamma$ -GTP was found in 10.8% and 2.8% of the men and women, respectively. The prevalences of hypertension and elevated serum  $\gamma$ -GTP levels were both increased with increased BMI. Hypertension was, however, shown to be 1.5 times more prevalent in the persons with elevated serum  $\gamma$ -GTP levels than in those with normal levels in both sexes, even after adjusting for BMI by a multiple logistic analysis. It can be concluded that elevations of serum  $\gamma$ -GTP, which are probably a reflection of fatty liver in the non-drinkers, are closely related to the development of hypertension associated with increased obesity.

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## Introduction

Obesity is often accompanied by hypertension. However, the exact mechanisms involved in the development of hypertension in obese persons remain unclear, including the question of why only some obese persons develop hypertension and others do not (3,11). During the last decade, much attention has been paid to

the role of dominant distribution of adipose tissue in the abdominal cavity of some obese persons, i.e., visceral type obesity (1,2,13), in the development of obesity-related complications including hypertension. The development of hypertension in visceral type obesity is attributed to insulin resistance or hyperinsulinemia (5). The details of the associations between visceral type obesity, insulin resistance or hyperinsulinemia, and hypertension, however, remain to be elucidated by further investigations.

On the other hand, we have found a linear association between serum  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP) levels and blood pressure, not only in drinkers (15,17) but also in non-drinkers (16). Since the elevations of serum  $\gamma$ -GTP levels in non-drinkers mainly depend on increased obesity (8,16), these findings suggest that the rise in blood pressure in obese persons is closely related to the elevations of serum  $\gamma$ -GTP levels. The rise in blood pressure may result in hypertension. However, the relationship between serum  $\gamma$ -GTP levels and the development of hypertension could not be determined in our previous study of non-drinkers (16) because of the limited size of the study population. The aim of the present study is, therefore, to elucidate the relationship in a large-scale male and female non-drinker population.

## Subjects and Methods

The study population was recruited from workers who had undergone health check ups conducted during a one-year period at an occupational health check-up service facility. The total number of the participants was around 100,000 (60,000 men and 40,000 women) at the facility. Workers aged between 40-59 years who stated that they had not drunk at all, or had drunk a small volume (< 10 ml) of alcohol not more often than once a month during the one-year period, were regarded as essentially non-drinkers. The present study population, 10,952 men and 22,112 women, comprised about 20%

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**Table 1** Frequencies of hypertension and elevated serum  $\gamma$ -GTP according to body mass index in male and female non-drinkers aged 40-59 years

BMI(kg/m <sup>2</sup> )	Number	Men		Number	Women	
		HT <sup>a)</sup> (%)	$\gamma$ -GTP↑ <sup>b)</sup> (%)		HT (%)	$\gamma$ -GTP↑ (%)
-17.4	135	7 (5.2)	5 (3.7)	294	16 (5.4)	7 (2.4)
17.5-19.9	1,383	99 (7.2)	59 (4.3)	3,077	185 (6.0)	36 (1.2)
20.0-22.4	3,143	348 (11.1)	209 (6.6)	7,538	676 (9.0)	126 (1.7)
22.5-24.9	3,543	577 (16.3)	389 (11.0)	6,455	967 (15.0)	207 (3.2)
25.0-27.4	1,946	472 (24.3)	333 (17.1)	3,109	679 (21.8)	127 (4.1)
27.5-29.9	609	173 (28.4)	122 (20.0)	1,125	260 (23.1)	63 (5.6)
30.0-32.4	131	50 (38.2)	38 (29.0)	367	141 (38.4)	34 (9.3)
32.5	63	32 (50.8)	26 (41.3)	147	72 (49.0)	17 (11.6)
Total	10,952	1,758 (16.1)	1,181 (10.8)	22,107	2,996 (13.5)	612 (2.8)

a) HT:Hypertensives include subjects being treated with anti-hypertensive medicines and those showing blood pressure above 160/95 mmHg.

b)  $\gamma$ -GTP↑: Elevated serum  $\gamma$ -GTP levels were defined as above 50 U/l.

and 80%, respectively, of the total male and female participants aged between 40-59 years.

In the health checkups conducted at the occupational clinic, body weight was measured with only any jacket removed, and the value of body weight was determined as the measured weight minus 1 kg. Blood pressure was determined using automatic equipment, following the recommendations of the Japanese Association for Cerebro-cardiovascular Disease Control (JACD) for the mass screening of hypertension using automatic manometers (9,10). Namely, blood pressure was measured after the subject had rested on a chair for five minutes or longer, using cuffs 13 cm wide and 24 cm long. Serum  $\gamma$ -GTP levels were determined using an automatic analyzer, Hitachi 7250.

The subjects were divided into eight categories of body mass index (BMI) by 2.5 kg/m<sup>2</sup> each, and further divided into two categories of elevated serum  $\gamma$ -GTP levels. Elevated serum  $\gamma$ -GTP levels were defined as above 50 U/l, which was settled as the upper normal limit value at the facility. The prevalences of hypertension in the sixteen categories were calculated. Hypertension was defined as being present in persons showing blood pressure levels above 160/95 mmHg at the health checkups or those being treated with anti-hypertensive medicines.

The relationships between the increases in BMI and the increase in hypertension were then statistically compared between the subjects with or without elevated serum  $\gamma$ -GTP levels by a multiple logistic analysis. The logistic analysis was performed using an SAS program

package distributed by SAS Japan, for a personal computer, PC 98 VX, NEC. Statistical significance was defined as  $p<0.05$ .

## Results

The prevalences of hypertension and those of persons with elevated serum  $\gamma$ -GTP levels according to the levels of BMI in the male and female non-drinkers are shown in Table 1. Both the prevalences of hypertension and persons with elevated serum  $\gamma$ -GTP levels were increased parallel with increases in BMI, in both sexes.

The prevalences of hypertension in the sixteen categories are shown in Table 2a for men and 2b for women. The prevalences of hypertension were increased with increases in BMI in both the subjects with elevated serum  $\gamma$ -GTP levels and those with normal levels. However, hypertension was generally more prevalent in the persons with elevated serum  $\gamma$ -GTP levels at all levels of BMI, which was also common in both sexes.

The differences in the relationships of the increase in hypertensive persons to increases in BMI between the subjects with elevated serum  $\gamma$ -GTP levels and those with normal levels were tested statistically by a multiple logistic analysis. The logistic model was formulated as follows:

$$\text{Log}_e(pX/qX) = \beta_0 + \sum \beta_i X_i; \text{ where } pX \text{ means the probability of hypertension, } qX=1-pX, \text{ and } pX/qX \text{ means the odds of the probability, } X_1 \text{ was BMI (kg/m}^2\text{), } X_2 \text{ was sex: coded 0 (women) or 1 (man), and } X_3 \text{ was serum } \gamma\text{-GTP level: coded 0 (normal) or 1 (elevated).}$$

**Table 2A** Relationships between body mass index and hypertension according to serum  $\gamma$ -GTP levels in male non-drinkers aged 40-59 years

BMI(kg/m <sup>2</sup> )	Normal Number	$\gamma$ -GTP HT <sup>b)</sup>	(%)	Elevated $\gamma$ -GTP <sup>a)</sup>		
				Number	HT	(%)
-17.4	130	7	(5.4)	5	0	(0.0)
17.5-19.9	1,324	92	(6.9)	59	7	(11.9)
20.0-22.4	2,934	313	(10.7)	209	35	(16.7)
22.5-24.9	3,154	487	(15.4)	389	90	(23.1)
25.0-27.4	1,613	377	(23.4)	333	95	(28.5)
27.5-29.9	487	127	(26.1)	122	46	(37.7)
30.0-32.4	93	32	(34.0)	38	18	(47.4)
32.5-	37	17	(45.9)	26	15	(57.7)
Total	9,771	1,452	(14.9)	1,181	306	(25.9)

a) Elevated serum  $\gamma$ -GTP levels were defined as above 50 U/l.

b) HT:Hypertensives include subjects being treated with anti-hypertensive medicines and those showing blood pressure above 160/95 mmHg.

**Table 2B** Relationships between body mass index and hypertension according to serum  $\gamma$ -GTP levels in female non-drinkers aged 40-59 years

BMI(kg/m <sup>2</sup> )	Normal Number	$\gamma$ -GTP HT <sup>b)</sup>	(%)	Elevated $\gamma$ -GTP <sup>a)</sup>		
				Number	HT	(%)
-17.4	287	15	(5.2)	7	1	(14.3)
17.5-19.9	3,041	181	(6.0)	36	4	(11.1)
20.0-22.4	7,412	657	(8.9)	126	19	(15.1)
22.5-24.9	6,248	927	(14.8)	207	40	(19.3)
25.0-27.4	2,982	641	(21.5)	127	38	(29.9)
27.5-29.9-	1,062	244	(23.0)	63	16	(25.4)
30.0-32.4	333	124	(37.2)	34	17	(50.0)
32.5-	130	65	(50.0)	17	7	(41.2)
Total	21,495	2,854	(13.3)	612	142	(23.0)

a) Elevated serum  $\gamma$ -GTP levels were defined as above 50 U/l.

b) HT:Hypertensives include subjects being treated with anti-hypertensive medicines and those showing blood pressure above 160/95 mmHg.

The results are summarized in Table 3. All of the variables, i.e., BMI, sex, and serum  $\gamma$ -GTP levels, were significantly related to the probability of hypertension, and the odds ratios of hypertension were shown to be 1.13 in sex difference (men/women), and 1.51 in different serum  $\gamma$ -GTP levels (elevated/normal). The logistic curves of the increase in hypertension with increased BMI obtained in the statistical results, for the men and women with or without elevated serum  $\gamma$ -GTP levels, are illustrated in Figure 1.

## Discussion

Increases in the prevalence of hypertension with increases in BMI were observed in non-drinkers, both in those with and without elevated serum  $\gamma$ -GTP levels, but were higher at all levels of BMI in the persons with elevated serum  $\gamma$ -GTP levels. These findings were basically common to both men and women, although men showed a slightly higher prevalence of hypertension than women. It can be said from these results that the levels of serum  $\gamma$ -GTP are closely related to the devel-

**Table 3** Results of a multiple logistic analysis for body mass index, sex, and serum  $\gamma$ -GTP levels in relation to hypertension in 10,952 male and 22,107 female non-drinkers aged 40-59 years

Variable	Parameter	Estimate	SE(bi)	$\chi^2$ value <sup>b</sup>	Odds <sup>c</sup> (95% confidence)
Intercept	$\beta_0$	-5.7031	0.1243	2106.6**	
X1:BMI (kg/m <sup>2</sup> )	$\beta_1$	0.1689	0.0051	1113.7**	
X2:Sex (0 or 1) <sup>d</sup>	$\beta_2$	0.1226	0.0340	13.0*	1.13(1.06-1.21)
X3: $\gamma$ -GTP (0 or 1) <sup>e</sup>	$\beta_3$	0.4121	0.0602	46.8**	1.51(1.34-1.70)

a) Logistic model:  $\text{Loge } (\text{pX}/\text{qX}) = \beta_0 + \sum \beta_i \text{Xi}$ ; pX: the probability of hypertension, qX=1-pX, pX/qX: the odds of the probability,  $\beta_i$ : parameter estimate, Xi: variable, SE( $\beta_i$ ): standard error of estimate.

b) \*:  $p < 0.001$ , \*\*:  $p < 0.0001$

c) Odds ratio was calculated only for sex and  $\gamma$ -GTP, which were coded 0 or 1.

d) Sex: coded 0 (women) or 1 (man)

e)  $\gamma$ -GTP: coded 0 (normal; <50 U/l) or 1 (elevated;  $\geq 50$  U/l)

opment of hypertension associated with increased obesity. However, some possible biases involved in the present study should be discussed.

The present non-drinkers included occasional drinkers who consumed a small volume of alcohol, not more often than once a month. In a previous study (14), we found no differences between teetotalers and occasional drinkers who consumed alcohol less than once a month with respect to serum hepatic enzymes and blood pres-

sure levels. On the other hand, we cannot exclude the possibility that our non-drinker group contained some drinkers who consumed more alcohol, because the selections were made by self-reported alcohol consumption data. However, we found a significant correlation between serum  $\gamma$ -GTP and blood pressure in the previous study on a smaller size non-drinker population (16), where the subjects showing higher serum  $\gamma$ -GTP levels were carefully evaluated for alcohol consumption by interviews.

The present subjects may include some persons with elevated serum  $\gamma$ -GTP due to causes other than alcohol consumption, such as hepatobiliary diseases or barbiturate use. However, the presence of these conditions, at least, could not exaggerate the association between elevated serum  $\gamma$ -GTP and hypertension. Some possible artificial effects associated with the manner of measurement of body weight and blood pressure also were not thought to have influenced critically the present results showing a higher prevalence of hypertension in the subjects with higher serum  $\gamma$ -GTP levels at all levels of BMI. Therefore, the present findings were thought to be basically valid.

As shown in Table 2 and Figure 1, the prevalences of hypertension were also elevated with increased BMI even in the subjects with normal serum  $\gamma$ -GTP levels. The difference in the prevalence of hypertension between the two serum  $\gamma$ -GTP levels seemed small, the ratio being around 1.3 in obese subjects with BMI of 30 kg/m<sup>2</sup>. However, it should be noted that the mean value of serum  $\gamma$ -GTP levels in the obese subjects must be also higher even though the levels remained within normal limits. Miura reported in a ten-year follow-up study

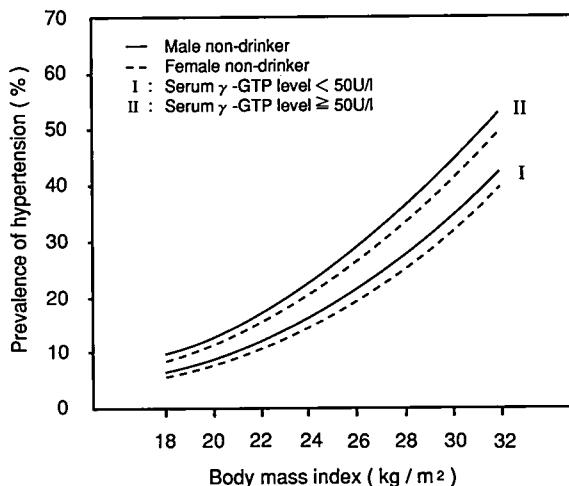


Figure 1: Logistic curves of prevalence of hypertension with increases in body mass index in non-drinkers with elevated serum  $\gamma$ -GTP levels (II:  $\geq 50$  U/l) and those with normal levels (I). Solid lines denote men and dashed lines denote women.

in a Japanese rural population (6), that the subjects with serum  $\gamma$ -GTP levels above 10 U/l showed an incidence of hypertension 2.3 times higher than that of the subjects with lower  $\gamma$ -GTP levels, independent of alcohol consumption. Thus, if the persons with normal serum  $\gamma$ -GTP were divided further into different levels of serum  $\gamma$ -GTP, it can be expected that some differences in the relationship between hypertension and increased BMI would be apparent among the subjects with the different  $\gamma$ -GTP levels.

The significance of the association between the elevation of serum  $\gamma$ -GTP levels and the development of hypertension in non-drinkers, however, remains unclear, i.e., whether it is a biological association or a mere statistical association, and whether it is a causal or non-causal one. The levels of serum  $\gamma$ -GTP in non-drinkers correlated not only with BMI but also with other serum hepatic enzyme levels, such as alanine aminotransferase and aspartate aminotransferase (16). Therefore, the elevations of serum  $\gamma$ -GTP levels in non-drinkers must be closely related to the progress in fatty change in the liver cells, i.e., fatty liver (8,16), and the association between serum  $\gamma$ -GTP levels and hypertension is probably a reflection of an association between fatty liver and hypertension in obese persons.

Although confirming evidence has not yet been provided, fatty liver, as suggested by Kisseebah (5), may have a close association with proliferated adipose tissue in the abdominal cavity, and may play an important role in the development of insulin resistance or hyperinsulinemia, i.e., hepatic insulin resistance, in visceral type obesity. Hypertension is known to be induced by hyperinsulinemia.

Unfortunately, no parameters indicating dominant adipose tissue distribution in the abdominal cavity, such as waist/hip (W/H) ratio, were determined in the present occupational population. A significant correlation between serum  $\gamma$ -GTP levels and W/H ratios was, however, reported in a study of Dutch men (18). Alcohol consumption was not fully evaluated in the Dutch study, and the researchers mentioned that it remained unclear whether or not elevated serum  $\gamma$ -GTP levels should be regarded as an indicator of increased alcohol consumption, or were due to hepatic steatosis in the subjects. Since alcohol consumption is also a major cause of hepatic steatosis, the results of the Dutch study may be interpreted as suggesting an association between fatty liver and visceral type obesity.

It was also noted from the present results that the prevalences of hypertension were different between the two levels of serum  $\gamma$ -GTP even in the persons with BMI of less than  $25 \text{ kg/m}^2$ , who were not obese, even in slender persons. This means that some non-drinkers, even if they are not obese, have higher serum  $\gamma$ -GTP

levels, probably reflecting fatty change in the liver-cells, and tend to have hypertension more frequently. Patients with essential hypertension, even if not obese, often show insulin resistance or hyperinsulinemia (4,7,12). Some relation may exist between the disturbances in insulin metabolism found in non-obese hypertensives and the presence of fatty liver in them.

Although further studies are needed to elucidate the relationships between visceral type obesity, fatty liver and insulin metabolism, especially the question of which one is the primary problem, the present results suggest a possible pathogenetic role of fatty liver in the more frequent development of hypertension in visceral type obese persons. At least, more attention should be paid to the role of fatty liver in further investigations.

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## Serum $\gamma$ -glutamyltransferase is a predictor of incident coronary events in apparently healthy men from the general population

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### Abstract

**Objective:** To investigate whether serum  $\gamma$ -glutamyltransferase (GGT) is an independent predictor for incident coronary events in initially healthy men from the general population.

**Methods and results:** The study was based on 1878 men (aged 25–64 years) who participated in the first MONICA Augsburg survey 1984/1985, and who were free of coronary heart disease at baseline. Up to 2002 a total of 150 incident acute coronary events occurred. Baseline levels of GGT were higher in men who experienced an event than in event-free men ( $28.4 \pm 2.0$  units/l versus  $22.4 \pm 2.1$  units/l,  $p < 0.0002$ ). GGT was highly correlated with other cardiovascular risk factors. In a Cox proportional hazards model after age adjustment hazard ratios (HR) for incident myocardial infarction across GGT quartiles (<13, 13 to <20, 20 to <35, and  $\geq 35$  units/l) were 1.0, 1.84, 2.02, and 3.08 ( $p$  for trend  $0.0001$ ). Further adjustment for hypertension, TC/HDL ratio, diabetes, smoking, physical activity, alcohol intake, education years and BMI attenuated the association; comparing the highest versus lowest quartile of GGT the HR for a first-ever coronary event was then 2.34 (95% CI, 1.23–4.44).

**Conclusions:** Serum GGT is a strong predictor of acute coronary events in apparently healthy men from the general population, independent of other risk factors for cardiovascular disease.

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**Keywords:** Arteriosclerosis; Men; Myocardial infarction; Risk factors;  $\gamma$ -Glutamyltransferase

### 1. Introduction

Usually, an increase in GGT concentration has been regarded as a marker of alcohol consumption or liver disease [1]. Recently, it has been suggested that serum GGT is an independent prognostic marker for cardiac death and reinfarction, both in unselected populations and in patients with coronary artery disease [2–4]. GGT is located on the external surface of most cells and is responsible of glutathione (GSH) catabolism by hydrolysis of its  $\gamma$ -glutamyl bond between glutamate and cysteine. This reaction produces cysteinyl-glycine moieties, which are usually taken

within intracellular milieu by the action of membrane dipeptidases, as precursors for GSH resynthesis [5]. The body of current evidence from studies indicates that GGT may have a role in the pathogenesis of atherosclerosis. GGT activity has been detected in atheromatous plaques of carotid and coronary arteries [6]. Furthermore, cysteinyl-glycine deriving from the hydrolysis of GSH performed by GGT has been found to trigger iron-dependent production of reactive oxygen species (ROS) [7] as well as low-density lipoprotein oxidation in vitro [6]. These facts could provide a pathological basis for the hypothesis of a direct participation of GGT in oxidative processes within the plaque and thus in atherogenesis and coronary artery disease progression. Prior studies have found that serum GGT predicted cardiac mortality or non-fatal myocardial infarction (MI), especially among

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ischaemic patients with established coronary atherosclerosis and previous MI [2,4]. In the present prospective study, we tested the hypothesis whether GGT, possibly as a marker of oxidative stress is an early predictor of incident acute coronary events in initially healthy men aged 25–64 years from the general population in Augsburg, Southern Germany.

## 2. Methods

The presented data were derived from the first population-based MONICA (Monitoring trends and determinants on cardiovascular diseases) Augsburg (Southern Germany) survey conducted between October 1984 and June 1985. The MONICA Augsburg project was part of the multinational WHO MONICA project and the design of the project has been described in detail elsewhere [8]. Briefly, the cross-sectional survey was carried out in the city of Augsburg and the counties Augsburg and Aichach-Friedberg to estimate the prevalence and distribution of cardiovascular risk factors among men and women. Altogether 4022 persons (2023 men, 1999 women, response 79.3%) aged 25–64 years participated in the cross-sectional study. All subjects were prospectively followed within the framework of the Cooperative Health Research in the Region of Augsburg (KORA).

Mortality was ascertained by regularly checking the vital status of all participants through the population registries inside and outside the study area; this procedure guaranteed that the vital status of cohort members who had moved out of the study area could also be assessed. The present study was restricted to men because analyses were not feasible in women due to the small number of events. Follow-up information was available for 2023 men. Up to December 31st 2002 altogether 385 men had died. For the present analyses, we excluded persons with prevalent myocardial infarction or with symptoms or signs of stable chronic angina pectoris at baseline ( $n=106$ ), and all subjects with incomplete data on any of the covariates ( $n=39$ ). Finally, the prospective analyses comprised 1878 men aged 25–64 years at baseline.

Informed consent was obtained from every participant in the study. The study was approved by an institutional review board.

### 2.1. Outcome

The outcome variable for the present analysis was a combination of incident fatal or non-fatal acute MI and sudden cardiac death. They were identified through the MONICA/KORA coronary event registry of the 25–74 year old study population and censored at the 75th year of age [9]. Up to 31 December 2000, the diagnosis of a major non-fatal MI was based on the MONICA algorithm taking into account symptoms, cardiac enzymes, and ECG changes [10]. Since 1 January 2001, all patients with MI diagnosed according to ESC and ACC criteria were included [11,12]. Coronary

deaths were validated by death certificates, autopsy report, chart review, and information from the coroner or the last treating physician.

### 2.2. Data collection

Baseline information on sociodemographic variables, medical history, smoking habits, physical activity level, angina pectoris, and alcohol consumption were gathered by trained and certified medical staff (mainly nurses) through a standardized face-to-face interview. The participants were also asked about the awareness, diagnosis and treatment of hypertension. Information concerning medical drug use was obtained. All participants were asked to bring to the interview all medications taken within the last 7 days preceding the examination. In addition they underwent an extensive standardized medical examination including the collection of a blood sample. All study measurements were conducted by a centrally trained staff according to the World Health Organization MONICA protocol using standard instruments. These procedures have been described elsewhere in detail [8]. A quality assurance program was employed in the study to ensure the quality of the data collection over the entire study period.

Hypertension was defined as a measured blood pressure higher than 140/90 mmHg and/or the use of antihypertensive medication, given that the subjects were aware of being hypertensive. Participants were classified as active during leisure time if they regularly participated in sports in summer and winter for atleast 1 h/week in either season.

### 2.3. Clinical chemical measurements

A non-fasting venous blood sample was obtained from all study participants while sitting. Blood analyses were carried out with an autoanalyser by a clinical laboratory (Central hospital of Augsburg, Germany). Total serum cholesterol analyses were carried out using an enzymatic method (CHOD-PAP; Boehringer Mannheim, Germany). HDL cholesterol was also measured enzymatically after precipitation of the apoprotein B-containing lipoproteins with phosphotungstate/Mg<sup>2+</sup> (Boehringer Mannheim, Germany). Serum  $\gamma$ -glutamyltransferase was determined by a photometric method (smac, Technicon). Internal and external quality control was performed according to the WHO MONICA Manual [8].

### 2.4. Statistical analyses

The duration of the follow-up was calculated as the interval between the baseline examination and the occurrence of an incident fatal or non-fatal acute coronary event, death or the date, when the participants were still alive. Means or proportions for baseline demographic and clinical characteristics were computed for men with and without an incident coronary event. The Chi<sup>2</sup>-test was used to test the differences

in prevalences. The general linear model was used to compare means (*F*-test). The study population was stratified into four groups of GGT concentrations with use of cut-points of 13, 20, and 35 units/l (25th, 50th, and 75th percentiles). Relative risks of incident coronary events were computed for quartiles 2–4, as compared with the lowest quartile in Cox proportional hazards models: The first model included GGT and in addition age (continuous). The second model included the previous factors plus education years (</≥ 12 years), hypertension (yes/no), TC/HDL cholesterol ratio (continuous), physical activity (active/inactive), smoking status (regular smoking, that is a subject who smoked at least one cigarette per day at baseline, yes/no), alcohol intake (0 g/day, 0.1–39.9 g/day, ≥40 g/day), BMI (continuous), and history of diabetes (yes/no and unknown). The TC/HDL cholesterol ratio and GGT were logarithmically transformed before inclusion in analysis as continuous variables since they were not normally distributed. Tests for linear trend across increasing categories of GGT were conducted by assigning the median value within each category to the respective category and by treating the categories as a continuous variable. Furthermore, it was assessed whether GGT would increase the risk of an incident coronary event in men with low and with high alcohol consumption. For this subgroup analyses, the median of the daily alcohol intake (20 g/day) was used as cut-point.

Kaplan–Meier survival plots of GGT quartiles in relation to incident coronary events were examined. Comparisons between survival curves were performed using log-rank test. Results are presented as hazard ratios (HRs) and 95% confidence intervals (CI). Significance tests were two tailed and *p*-values less than 0.05 are stated as statistically significant. All analyses were performed using the Statistical Analysis System (Version 8.2, SAS Institute Inc., Cary, NC).

### 3. Results

In total, 150 incident cases of fatal (*n* = 80) and non-fatal (*n* = 70) coronary events, including sudden cardiac deaths, were registered between 1984 and 2002 (mean follow-up period 15.7 years). Men with an acute coronary event were significantly older, had a higher BMI, higher total cholesterol, and lower HDL cholesterol levels. They had a significantly higher TC/HDL ratio and higher systolic and diastolic blood pressure values. They were more frequently smokers and less frequently physically active. Men with an acute coronary event had also a higher prevalence of diabetes and hypertension. Concentrations of GGT were significantly higher than in those without a coronary event during follow-up. There were no significant differences with regard to education and alcohol consumption between men with an event and men without an event (Table 1).

GGT was positively correlated with age (*p* ≤ 0.0001), BMI (*p* ≤ 0.0001), systolic blood pressure (*p* ≤ 0.0001), total cholesterol (*p* ≤ 0.0001), and the TC/HDL ratio (*p* ≤ 0.0001), but was not correlated with HDL cholesterol. GGT was also significantly related to physical activity (*p* = 0.0005), smoking (*p* ≤ 0.0001), diabetes (*p* = 0.0184), alcohol intake (*p* ≤ 0.0001), and education (*p* = 0.0010) (Table 2).

Serum GGT concentration showed a strong relationship with incident coronary events in men (Table 3). GGT levels in the fourth quartile were significantly associated with incident coronary events independent of age (HR 3.08; 95% CI 1.68–5.63) when compared with the first quartile. Further adjustment for hypertension, TC/HDL ratio, diabetes, smoking, physical activity, alcohol intake, education years, and BMI attenuated the association; comparing the highest versus the lowest quartile of GGT the HR for a first-ever coronary event was then 2.34 (95% CI, 1.23–4.44; *p* for trend

Table 1  
Mean (S.D.) and prevalence of demographic and clinical characteristics of men with and without incident coronary event, age 25–64 years

Characteristics	Men with incident coronary event ( <i>n</i> = 150)	Men without incident coronary event ( <i>n</i> = 1728)	<i>p</i> -value
Age (years) <sup>a</sup>	52.9 (8.3)	44.0 (11.3)	<0.0001
Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	27.9 (3.4)	26.8 (3.5)	0.0003
Systolic blood pressure (mmHg) <sup>a</sup>	139.4 (17.2)	132.8 (16.2)	<0.0001
Diastolic blood pressure (mmHg) <sup>a</sup>	85.5 (10.9)	82.8 (11.3)	0.0044
Hypertension (%) <sup>b</sup>	56.7	37.4	<0.0001
γ-Glutamyltransferase (units/l) <sup>c</sup>	28.4 (2.0)	22.4 (2.1)	0.0002
Total cholesterol (mg/dl) <sup>a</sup>	256.1 (46.1)	234.0 (46.0)	<0.0001
HDL cholesterol (mg/dl) <sup>a</sup>	46.6 (15.5)	51.4 (15.6)	0.0004
Total/HDL cholesterol ratio <sup>c</sup>	5.7 (1.4)	4.7 (1.4)	<0.0001
Physical activity (%)	26.0	45.4	<0.0001
Regular smoker (%)	49.3	33.3	<0.0001
History of diabetes (%)	9.3	2.0	<0.0001
Alcohol intake			
0 g/day (%)	16.7	12.4	0.0693
0.1–39.9 g/day (%)	37.3	46.5	
≥40 g/day (%)	46.0	41.1	
Education <12 years (%)	74.0	68.4	0.1556

<sup>a</sup> Mean (S.D.), *p*-value from *t*-test.

<sup>b</sup> Blood pressure values ≥140/90 mmHg and/or use of antihypertensive medication.

<sup>c</sup> Geometric mean, *p*-value from *t*-test for log-transformed characteristic.

Table 2

Association between  $\gamma$ -glutamyltransferase and other cardiovascular risk factors by Pearson correlation coefficient  $r$  and mean (S.D.) with  $p$ -values

	$\gamma$ -Glutamyltransferase	
	Pearson correlation coefficient	$p$ -value
Age <sup>a</sup>	0.11	<0.0001
BMI <sup>a</sup>	0.27	<0.0001
Total cholesterol <sup>a</sup>	0.30	<0.0001
HDL cholesterol <sup>a</sup>	-0.03	0.1909
Total/HDL cholesterol ratio <sup>a</sup>	0.23	<0.0001
Systolic blood pressure <sup>a</sup>	0.26	<0.0001
	Geometric mean (S.D.)	$p$ -value
Hypertension <sup>b</sup>		
No	19.8 (2.0)	<0.0001
Yes	28.5 (2.1)	
Regular smoker <sup>b</sup>		
No	21.2 (2.1)	<0.0001
Yes	26.1 (2.2)	
Physical activity <sup>b</sup>		
No	24.1 (2.1)	0.0005
Yes	21.3 (2.1)	
History of diabetes <sup>b</sup>		
No	22.6 (2.1)	0.0184
Yes	31.2 (2.5)	
Education <sup>b</sup>		
<12 years	21.0 (2.0)	0.0010
≤12 years	23.7 (2.2)	
Alcohol intake <sup>b</sup>		
0 g/day	17.7 (1.9)	<0.0001
0.1–39.9 g/day	19.2 (1.9)	
≥40 g/day	29.8 (2.2)	

<sup>a</sup> Pearson correlation coefficient.

<sup>b</sup> Geometric mean (S.D.) of  $\gamma$ -glutamyltransferase,  $p$ -value from  $t$ -test and  $F$ -test, respectively.

0.0219). Table 3 further describes the observed crude incidence rates of coronary events by GGT categories. Incidence of coronary events increased with increasing GGT levels from 19.9/10,000 person-years in the first quartile to 77.2/10,000 person-years in the fourth quartile.

To assess whether GGT levels increase the risk of a first-ever coronary event for men with low alcohol intake (<20 g daily) and men with high alcohol intake ( $\geq 20$  g daily), the

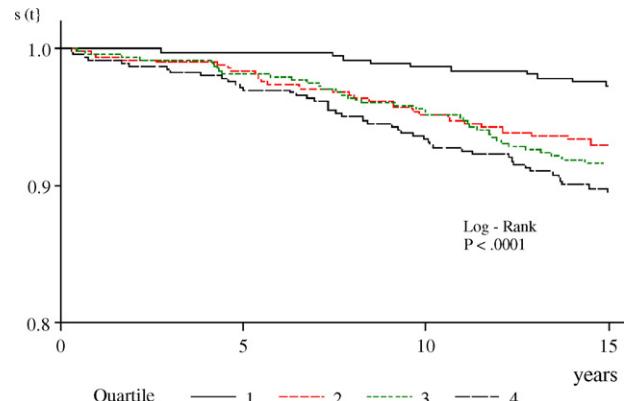


Fig. 1. Association of GGT quartiles with incident coronary events.

impact of GGT on the incidence of coronary events was analyzed separately for the two groups. In the subgroup with low alcohol intake the HR was 2.63 (95% CI, 1.01–6.84) for men in the fourth GGT quartile when compared with men in the first quartile after multivariable adjustment. Comparing the highest versus lowest quartile of GGT the HR for an incident coronary event was 2.12 (95% CI, 0.88–5.13) among men with a high alcohol intake.

Fig. 1 shows the Kaplan–Meier curves for incident coronary events by GGT categories. With increasing GGT concentrations coronary event rates increased significantly over the more than 15 years of follow-up (log-rank test  $p \leq 0.0001$ ). The event curves for the men with GGT concentrations in the second, third and fourth quartile begin to separate from the first quartile very early during follow-up, and separate to a greater extent with increasing follow-up time.

#### 4. Discussion

In this prospective cohort study, the relative risk of a first-ever coronary event associated with serum GGT concentrations in initially healthy men from the general population was investigated. Serum GGT concentrations were elevated in men who subsequently developed an event compared with

Table 3

Relative risks for incident coronary events according to quartiles of GGT among men aged 25–64 years at baseline

Men	GGT				$p$ -value for trend
	<13 units/l	13 to <20 units/l	20 to <35 units/l	≥35 units/l	
Total number $n = 1878$	$n = 395$	$n = 511$	$n = 494$	$n = 478$	
Number of incident cases	13	38	43	56	
Person-years (PY)	6529	8052	7654	7257	
Crude rate per 10000 PY	19.9	47.2	56.2	77.2	
HR (95% CI)					
Model 1 <sup>a</sup>	1.0	1.84 (0.98–3.46)	2.02 (1.08–3.75)	3.08 (1.68–5.63)	0.0001
Model 2 <sup>b</sup>	1.0	1.88 (1.00–3.56)	1.63 (0.86–3.10)	2.34 (1.23–4.44)	0.0219

<sup>a</sup> Model 1: adjusted for age.

<sup>b</sup> Model 2: adjusted for age, education, history of diabetes, hypertension, TC/HDL ratio, regular smoking, physical activity, alcohol intake, and BMI.

those who did not. Furthermore, there was a strong correlation between serum GGT and the known cardiovascular risk factors. Although a part of the association between serum GGT and the risk of an acute coronary event was mediated through these risk factors, GGT was strongly and independently associated with incident coronary events even after multivariable adjustment.

It has been shown that serum GGT is an independent prognostic marker for reinfarction and cardiac death, both in patients with coronary artery disease and in unselected populations [2–4]. Few population studies [3,13–15] have examined the association between serum GGT and all cause mortality, but these studies focused on GGT as an indicator of alcohol consumption. Wannamethee et al. [3] investigated GGT as a prognostic marker for cardiovascular and overall mortality in a large population of middle-aged men. The study found that GGT levels in the top quintile were independently associated with both outcomes. A Swedish study reported that 10-year mortality was twice as high amongst patients in the two highest quartiles as compared with those in the lowest one [2]. Recently, a prospective study showed that GGT is an independent cardiac risk factor in ischaemic patients with established coronary atherosclerosis and previous myocardial infarction [4], using mortality and mortality plus non-fatal myocardial infarction as end-points. GGT showed an independent prognostic value beyond known established risk factors in 262 patients with previous MI, whereas it did not show significant prognostic value in 207 patients without previous MI.

The results of our study have important implications. We showed that serum GGT concentration discriminated men who subsequently developed an acute coronary event from those who remained event-free, even after adjustment for major CHD risk factors and alcohol consumption. A number of epidemiologic studies have examined the association between alcohol consumption and heart disease. Most of these studies have shown a U- or J-shaped relationship of alcohol intake to cardiovascular [16,17] and all-cause mortality [18] suggesting that a mild to moderate alcohol consumption may have a beneficial effect on cardiovascular disease risk [17]. One explanation for this relationship could be that light-to-moderate alcohol consumption is associated with a lower level of coagulatory factors, while higher alcohol intake is associated with impaired fibrinolytic potential [19]. Furthermore, it has been found that moderate alcohol consumption results in dose-dependent increases in plasma concentrations of HDL cholesterol, a well-established major protective factor against CHD [20]. In the present study, there was an increasing trend of incident coronary events with increasing levels of GGT. The dose-response relationship of serum GGT to first-ever coronary events was present in the subgroup with low alcohol intake as well as in the group with high alcohol intake. Thus the hypothesis that GGT may contribute to the clinical manifestation of CHD independently from alcohol consumption is supported. Moreover, the role of GGT in the atherosclerotic process seems to be more com-

plex than it is currently thought. Recent findings suggested a possible role for GGT in the cellular process of LDL oxidation and atherogenesis [6]. Evidence from in vitro studies indicates that atherosclerosis represents a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall. Low-density lipoprotein oxidation is an early event in atherosclerosis and contributes to atherogenesis because oxLDL supports foam cell formation and has a number of potentially proatherogenic activities [21–24]. GGT is found in serum and in the plasma membranes of virtually all cell types. Its physiological role is to initiate the hydrolysis of extracellular GSH, a tripeptide in which cysteine lies between alpha-glycine and gamma-glutamate residues [5]. Cysteine and other thiol components are known to promote LDL oxidation by reducing Fe(III) to redox Fe(II) [6]. Moreover, it was shown that cysteinyl-glycine, a product of GGT/GSH reaction, but not GSH was responsible for reactive oxygen species (ROS) formation initiated by the reductive release of iron from transferring indicating that GGT is directly involved in ROS generation leading to free radical damage of nucleic acids and oxidative modifications of lipids and proteins [25]. Experimental studies have found that active GGT is present within coronary plaques underlining the hypothesis of a direct participation of GGT in LDL oxidation within the plaque and in atherogenesis [26].

The MONICA/KORA Augsburg Study has several limitations that need to be considered. GGT levels were measured only once at baseline, we were therefore unable to account for within-individual variability in the present study. Although we adjusted for a variety of confounders, additional factors that are known to be associated with acute coronary events, such as fasting blood glucose and triglycerides were not available in this study. Thus, confounding by unmeasured variables cannot be entirely excluded. Other reasons for a GGT elevation, for example acute/chronic cholecystitis, pancreatitis and hepatitis were not ascertained and could therefore have biased the present findings. Finally, because the study was limited to men of German nationality between 25 and 64 years of age, caution should be used in generalizing these results to women, other populations and other age groups. The strengths of the MONICA/KORA Augsburg Cohort Study are primarily its prospective design, the representativeness of the cohort, based on a random sample of the general population and the availability of data on lifestyle and multiple cardiovascular risk factors. Because the MONICA/KORA myocardial infarction registry in Augsburg is well established, we should have recovered all incident coronary events that occurred in the cohort.

In conclusion, the present results provide that serum GGT is a strong and independent predictor of acute coronary events in apparently healthy men from the general population. So far, the underlying pathophysiological mechanisms are not entirely clear. It seems that oxidative stress may be involved [27]. Further studies are needed to confirm the present findings and to investigate the biological mechanisms underlying this association.

# Obesity, albuminuria, and gamma-glutamyl transferase predict incidence of hypertension in indigenous Australians in rural and remote communities in northern Australia

Ming Li<sup>a</sup> and Robyn McDermott<sup>a,b</sup>

**Objective:** To describe the incidence of hypertension in a cohort of Australian Aboriginal and Torres Strait Islanders.

**Method:** A follow-up study conducted among 1831 indigenous population aged 15 years and over without hypertension at baseline from 19 communities in North Queensland during 1997–2008. Main measurements included baseline and follow-up weight, waist circumference, blood pressure, fasting glucose, lipids (triglycerides and cholesterol), gamma-glutamyl transferase, urinary albumin creatinine ratio, self-reported tobacco smoking, alcohol intake and physical activity.

**Results:** Hundred cases of hypertension developed over 2633.4 person-years giving a crude incidence of hypertension of 22.6 (16.2–31.4) per 1000 person-years in females and 60.0 (47.1–76.6) per 1000 person-years for males. Age standardized overall incidence was 51.9 per 1000 person-years. Aboriginal participants were twice as likely as Torres Strait Islanders to develop hypertension, which increased with age. Obesity (BMI >30) strongly predicted incident hypertension independently of age or sex (adjusted hazard ratio 2.9, 95% confidence interval 1.9–4.8). Albuminuria and elevated gamma-glutamyl transferase increased the risk of hypertension (adjusted hazard ratio 1.4–1.7) in this population.

**Conclusion:** Incidence of hypertension in indigenous Australian adults is nearly double than that of the general Australian population. High background prevalence of obesity, diabetes and albuminuria contributes to this excess. As well as early detection and management of high blood pressure, albuminuria and diabetes in primary care settings, attention should be equally focused on community-level prevention and management of obesity.

**Keywords:** albuminuria, Australian indigenous population, gamma-glutamyl transferase, incidence of hypertension, overweight and obesity

**Abbreviations:** AusDiab, The Australian Diabetes, Obesity and Lifestyle Study; BP, blood pressure; GGT, gamma-glutamyl transferase; TSIs, Torres Strait Islanders; UACR, urinary albumin creatinine ratio

## INTRODUCTION

High blood pressure (BP) is a major risk factor for death, stroke, and renal and coronary heart disease. The estimated global prevalence of hypertension in 2000 was 26% of adults, and by 2025, it was estimated that this would rise by 24% in developed countries and 80% in developing countries [1]. In Australia, high BP is the most common of all the conditions of the circulatory system. In 2011–2012, it was estimated that 32% Australians aged 18 years and over had high BP (SBP or DBP was  $\geq 140/90$  mmHg or taking medication). Of these, more than two-thirds (68%) had uncontrolled or unmanaged high BP [2]. High BP was the greatest contributor to the burden of cardiovascular disease (CVD), accounting for 42.1% of CVD's total burden in Australian population as reported in 2003 [3]. Most of the studies reporting hypertension incidence and its risk predictors are from the United States [4]. Demographic, anthropometric and dietary factors have been associated with hypertension [4]. Diabetes or fasting insulin concentrations also predict hypertension incidence [5,6]. The Australian indigenous population has higher prevalence of risk factors including poor diet, smoking and features of the metabolic syndrome [7]. A survey of central Australian Aboriginal adults in the 1990s found that the prevalence of hypertension was three times higher than in non-indigenous Australians, and was associated with overweight, higher albumin creatinine ratio (ACR) and diabetes [8]. The incidence of hypertension in Australian population, especially among indigenous populations, has

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not been reported to date. We have previously found that overweight and obesity predict diabetes incidence [9], whereas hyperglycaemia or diabetes and hypertension, in turn, predict coronary heart disease and act conjointly with albuminuria [10]. This study aimed to document hypertension incidence and to find the predictive metabolic and lifestyle factors.

## RESEARCH DESIGN AND METHODS

### Study population

Baseline data were collected from 2152 adults in 19 rural indigenous communities across three health districts in far North Queensland, who participated in the 'Well Person's Health Check' between 1999 and 2000. Methods for this cross-sectional study have been reported in detail elsewhere [11]. Briefly, all indigenous residents of the communities aged 13 years and over were invited to attend a health check through printed media, local radio and word of mouth via local health services, community councils and community groups. On the basis of the local census data, the study achieved a participation rate of 44.5%, with greater participation noted in smaller communities. The follow-up data were collected during 2005–2007. On the basis of the census data, participants overall were not different demographically from the age and sex distribution of the Australian indigenous population as a whole. The study protocols were approved by the Cairns Base Hospital Human Research Ethics Committee with support from the peak Indigenous Health Organizations, Apunipima Cape York Health Council, and the Torres Strait and Northern Peninsula Area Health Council.

### Measurements

Participants were asked to remove foot wear and heavy clothing, and weighed to the nearest 0.1 kg. Height and waist circumference were recorded to the nearest centimetre, with the latter measured by the same technician at the level of the umbilicus. BMI was calculated as weight (kg) divided by the height squared ( $m^2$ ). Fruit and vegetable intake, and alcohol consumption were assessed using a methodology derived from that used in the National Nutrition Survey 1995 [12], and categorized using Australian dietary guideline [13]. Physical activity was measured using a 7-day recall method in which participants were asked to report daily physical activities of at least 30 min duration and moderate intensity performed during the week before their health check. Physical activity was categorized using the WHO criteria in which 'enough' means doing moderate to vigorous physical activity for more than 30 min/day for 5 days in the week before the survey [14]. Current smokers were asked how many cigarettes they smoked daily. The self-reported physical activity, smoking and alcohol intake measures are widely used in other studies [15,16].

Gamma-glutamyl transferase (GGT), fasting total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides and glucose were measured on blood collected in the early morning after at least an 8 h fast by a medical officer, registered nurse or trained phlebotomist, as described in detail elsewhere [11]. GGT was measured using the kinetic photometric procedure with Cobas Integra

800 (Roche Diagnostics, New York, USA). Blood glucose and blood lipids were measured using photometric enzyme endpoint assay with Cobas Integra 700/400 (Roche Diagnostics).

Blood pressure was the average of three measurements taken sitting after 10 min rest. Participants were seated comfortably with their arms outstretched and supported at chest height. An inflatable cuff appropriate to the participants' arm size was applied just above the elbow centred over the radial artery. BP was measured using a Dinamap model 800 automated blood pressure monitor (Critikon; Tampa, Florida, USA). Three separate measurements were recorded over approximately a 10-min period. Baseline hypertension was ascertained either by detection of high BP at examination (measured BP  $> 140/90 \text{ mmHg}$ ) or previous confirmed diagnosis or currently prescribed antihypertensive medication (by medical record review) [11].

Urine specimens provided by participants in sterile 50-ml containers were from the first morning void or a sample at least 2 h from the most recent void. Dipstick urinalysis (Combur-test, Roche) tested the samples for protein, pH, nitrites, leucocytes and blood. ACR was measured by immunoassay in g/mol.

Diabetes was defined as either clinical diagnosis verified by the participants' medical records or a 2-h glucose tolerance test, or fasting blood glucose level at least 7.0 mmol/l [17]. Overweight was defined as BMI 25–30 kg/m<sup>2</sup> and obese as BMI above 30 kg/m<sup>2</sup> using the WHO criteria [14]. Abdominal overweight was defined as waist circumference greater than 80 cm in females and 94 cm in males, and obesity as greater than 88 cm in females and 102 cm in males [13]. Dyslipidaemia was defined as having triglycerides at least 2.0 mmol/l or HDLC below 1.0 mmol/l, as recommended by the National Heart Foundation [18].

### Analysis

This analysis excluded participants who were identified as non-indigenous, who were aged less than 15 years or who had hypertension at baseline.

Incident hypertension was defined as the first study visit, subsequent to baseline, at which the participant had SBP at least 140 mmHg, or DBP at least 90 mmHg, or had initiated treatment with antihypertensive medications. The follow-up period for incident hypertension cases was the time from entering the baseline study to diagnosis date. For those who did not develop hypertension, the follow-up period was the interval between the day of baseline survey and the follow-up. The age–sex-specific cumulative incidence rate stratified by ethnicity was calculated by dividing the number of new cases by the total person follow-up years of the corresponding subgroups. Direct standardization was conducted using the 2007 Australian Bureau of Statistics national data as the reference population.

Baseline characteristics including age, sex and ethnicity, self-reported health behaviours including tobacco smoking, alcohol and fruit and vegetable intake, blood pressure, fasting glucose, blood lipids, urinary ACR (UACR) and GGT levels were compared between incident hypertension cases and non-hypertension cases using log-rank tests. The Cox proportional-hazard model was used to identify the significant baseline factors associated with incident hypertension.

The reported crude hazard ratios show the results of a univariate Cox model. The association for each ethnic group was adjusted for age, sex and further adjusted for ethnicity in the combined hazard ratio estimate. The analysis was carried out using STATA 12 (STATAcorp, College Station, Texas, USA) and significance level was set at two-sided  $P$  value less than 0.05.

## RESULTS

A total of 1831 indigenous population aged 15 years and over without hypertension at baseline from 19 communities in North Queensland during 1997–2008 was included in the analysis. Among them, 401 completed the second survey. Of those lost to follow-up, 83 were dead, 22 were in prison, 344 had moved out of the community and 981 did not attend the follow-up survey. Those lost to follow-up were similar in all baseline characteristics except for a slight excess of younger aboriginal males with a more favourable lipid profile and lower alcohol intake. The baseline characteristics of the 401 participants completing the follow-up were shown in Table 1. Among them, 59% were females, with a mean age of 31.4 years (range 15–78 years). There were 149 aboriginal people (37%) and 190 (47%) Torres Strait Islanders (TSIs). Only 2% met the recommended daily

intake for fruits and vegetables, and 23% met the physical activity guidelines, whereas 43% were self-reported 'heavy' drinkers. One hundred and fifty participants (37%) were obese, 37 (9%) were diagnosed with diabetes and 80 (27.1%) had albuminuria. Compared with the aboriginal participants, the TSIs had substantially higher BMI, lower triglycerides and alcohol intake levels, especially in males.

Hundred hypertension cases developed in a total of 401 participants with 2633.4 person-years of follow-up. The average follow-up period was 6.6 years (ranging from 4.5 to 9 years) among those completing the second survey. Of those, 65 were males with an incidence rate of 60.5/1000 person-years compared to 22.6/1000 person-years in females. The incident rate was 44.4/1000 in aboriginal people compared to 37.3/1000 person-years in TSIs. The overall incidence was 38.3/1000 person-years. The age-standardized hypertension incidence rate in female indigenous participants was 29.8/1000 person-years, and was 74.7/1000 person-years in males, with the total incidence ratio of 51.9/1000 person-years [95% confidence interval (CI) 51.8–52.0]. Males were three times (95% CI 1.9–4.4) more likely to develop hypertension than females. Incidence increased with age. Compared with those aged less than 35 years, those aged 35–54 years had a 2.4 times higher risk of hypertension (95% CI 1.6–3.7), and those aged over

**TABLE 1. Baseline characteristics of participants completing follow-up in rural indigenous communities in North Queensland**

Female	Aboriginal <i>n</i> = 87	TSI <i>n</i> = 110	Joint descendants <i>n</i> = 38	All groups <i>N</i> = 235
Age (years)	33.4 (30.5–36.2)	32.0 (30.0–34.1)	31.1 (27.5–34.6)	32.4 (30.9–33.9)
WC (cm)*	91.2 (87.5–94.9)	101.5 (98.4–104.6)	98.5 (92.4–104.5)	97.2 (94.9–99.5)
BMI (kg/m <sup>2</sup> )*	25.5 (24.0–27.1)	31.4 (30.0–32.8)	30.3 (27.3–33.2)	29.1 (28.0–30.1)
SBP (mmHg)	114.1 (111.6–116.6)	117.9 (116.0–119.80)	116.6 (113.6–119.6)	116.3 (114.9–117.7)
DBP (mmHg)	64.0 (62.0–66.0)	62.9 (61.2–64.7)	64.2 (61.1–67.3)	63.5 (62.3–64.7)
Fasting glucose (g/l)	5.1 (4.7–5.5)	5.4 (5.0–5.7)	5.1 (4.6–5.6)	5.2 (5.0–5.5)
Cholesterol (mmol/l)	4.6 (4.3–4.8)	4.8 (4.6–4.9)	4.3 (4.1–4.6)	4.6 (4.5–4.8)
HDLC (mmol/l)	1.13 (1.06–1.20)	1.08 (1.03–1.12)	1.09 (1.02–1.17)	1.10 (1.07–1.13)
Triglycerides (mmol/l)	1.6 (1.3–1.9)	1.4 (1.2–1.6)	1.1 (1.0–1.3)	1.4 (1.3–1.6)
GGT (IU)	33.3 (28.1–38.6)	24.4 (21.9–27.0)	23.1 (17.7–28.6)	27.5 (25.0–30.0)
UACR (g/mol)	5.7 (2.4–9.0)	7.6 (2.3–12.9)	12.8 (-2.8–28.4)	8.6 (6.3–11.0)
Smokers (%)	54.7 (44.0–65.3)	51.8 (42.4–61.2)	63.2 (47.5–78.8)	54.7 (48.3–61.1)
Drinkers (%)	52.3 (41.7–63.0)	60.7 (51.4–70.1)	66.7 (51.0–82.4)	58.5 (52.1–64.9)
Risky drinkers (%)	30.2 (20.4–40.0)	29.9 (21.1–38.7)	44.4 (27.9–61.0)	32.3 (26.2–38.4)
PA sufficient (%)	21.8 (13.1–30.6)	26.4 (18.0–34.7)	15.8 (4.0–27.6)	23.0 (17.6–28.4)
Male	<i>n</i> = 62	<i>n</i> = 83	<i>n</i> = 24	<i>N</i> = 169
Age (years)	37.5 (34.8–40.3)	35.1 (32.2–38.0)	32.4 (27.4–37.3)	35.6 (33.8–37.5)
WC (cm)*	88.1 (84.5–91.7)	99.3 (95.2–103.5)	100.7 (93.3–108.2)	95.3 (92.6–98.0)
BMI (kg/m <sup>2</sup> )*	23.1 (21.9–24.3)	29.6 (27.9–31.3)	29.2 (26.2–31.3)	27.1 (26.0–28.2)
SBP (mmHg)	123.0 (120.4–125.5)	126.1 (124.4–127.7)	124.2 (121.1–127.2)	124.6 (123.3–125.9)
DBP (mmHg)	69.9 (67.7–72.0)	66.5 (64.5–68.5)	66.9 (63.8–70.0)	67.8 (66.5–69.2)
Fasting glucose (g/l)	5.4 (4.8–6.0)	5.9 (5.3–6.4)	5.3 (4.2–6.3)	5.6 (5.2–6.0)
Cholesterol (mmol/l)	5.2 (5.0–5.5)	5.1 (4.9–5.4)	4.7 (4.3–5.0)	5.1 (4.9–5.3)
HDLC (mmol/l)	1.16 (1.08–1.24)	1.08 (1.02–1.14)	1.13 (1.03–1.24)	1.11 (1.07–1.16)
Triglycerides (mmol/l)*	2.4 (1.9–2.9)	1.9 (1.6–2.2)	1.3 (1.0–1.6)	2.0 (1.7–2.2)
GGT (IU)	86.8 (66.4–107.2)	40.0 (33.6–46.4)	33.8 (26.9–40.7)	56.0 (47.3–64.6)
UACR (g/mol)	13.9 (5.7–22.2)	6.9 (2.4–11.4)	10.4 (-3.4–24.1)	8.1 (5.5–10.6)
Smokers (%)	71.0 (59.5–82.4)	63.3 (52.5–74.1)	54.2 (33.7–74.7)	64.8 (57.5–72.2)
Drinkers (%)	91.7 (84.6–98.8)	79.7 (70.8–88.7)	95.8 (87.6–104.0)	86.5 (81.2–91.8)
Risky drinkers (%)*	73.3 (62.0–84.7)	48.1 (36.9–59.3)	50.0 (29.4–70.6)	57.7 (50.1–65.3)
PA sufficient (%)	21.0 (10.7–31.3)	41.3 (30.3–52.2)	41.7 (21.4–62.0)	33.7 (26.5–41.0)

Figures in the table are means or % (95% CI); \* $P < 0.05$  with analysis of variance (ANOVA) or Chi-square tests. GGT, gamma-glutamyl transferase; HDLC, high-density lipoprotein cholesterol; IU, international unit; PA, physical activity; UACR, urine albumin creatinine ratio; WC, waist circumference. Risk drinker defined as those more than 4 drinks/day for men and more than 2 drinks/day for women [13]; PA sufficiently defined as having moderate to vigorous physical activity for more than 30 min/day for 5 days in the week before the survey [14].

55 years had 3.1 times the rate of hypertension (95% CI 1.6–5.8). Aboriginal adults were twice as likely as TSIs to develop hypertension (adjusted hazard ratio 1.9, 95% CI 1.2–3.0) (Table 2).

Obesity defined either by BMI or waist circumference strongly associated with hypertension incidence regardless of age and sex (hazard ratio ranging between 2.5 and 2.9). Albuminuria increased the risk of hypertension by 70% (hazard ratio 1.7, 95% CI 1.1–2.8) after adjusted for age, sex, ethnicity and BMI. Blood glucose, lipids and behavioural factors such as smoking and drinking, and physical activity did not appear to predict hypertension incidence. Among the aboriginal subgroup, the hazard ratio for at-risk drinkers (>4 drinks/day for males and >2 drinks/day for females) was 2.2 (95% CI 1.03–4.8) and attenuated to null when adjusted for sex. Higher baseline GGT levels increased the risk by 90% (hazard ratio 1.9, 95% CI 1.01–3.5), independent of age, sex and BMI, and drinking (Table 3).

## DISCUSSION

In this cohort of indigenous Australians living in rural and remote communities in North Queensland, we found the standardized hypertension incidence of 51.9 per 1000 person-years with 29.8 in females and 74.7 per 1000 person-years in males. The incidence was approximately twice among a national representative population longitudinal study [The Australian Diabetes (AusDiab) study], which found a 3% annual incidence rate of hypertension using similar methodology and over the same time period [19]. Our findings are similar to those reported in several follow-up studies looking at ethnic differences for African Americans, Hispanics and Asians, which found that incidence varied with age, sex, ethnicity, and year of reporting and the definition of hypertension [4,20]. These make detailed

comparisons difficult. Fewer studies were conducted among ‘aboriginal people’ in other areas of the world, except for the Strong Heart Study, among 4549 American Indians with a 4-year follow-up period [21]. The study shows hypertension incidence increases with age, but does not differ by sex, and is associated with obesity and albuminuria, which is similar to our study [21].

In line with other longitudinal studies among European [22], American [23,24] and Asian [25] populations, we also found that baseline obesity increased the risk of incident hypertension, defined either by BMI and waist circumference. BMI was found to predict the risk of hypertension in a review paper including 15 prospective cohort studies among various populations, although different other factors are added in the risk models in these studies [26]. We found that BMI and waist circumference as measures of obesity were equally predictive of incident hypertension. This is consistent with a recent meta-analysis looking at data from various ethnic groups to evaluate the cross-sectional association between several anthropometric measurements and hypertension [27]. Obesity-related hypertension appears to involve multiple and linked pathways including insulin resistance, inappropriate sympathetic and renal angiotensin system activation, and inflammatory responses leading to endothelial dysfunction, atheroma and arterial wall stiffness. Dipeptidyl peptide-4-mediated incretin signalling can affect vascular function, immune responses and natriuresis in obesity states. Oestrogen-mediated insulin sensitivity in premenopausal women who do not have obesity is compromised when they develop obesity. An alteration in the gut microbiome in obesity is another factor that contributes to insulin resistance and dysfunctional immunity [28].

We also found that albuminuria predicts incident hypertension in this cohort of Australian indigenous adults independent of age, sex and BMI. This is consistent with other prospective cohort studies in population [29–31] or other

**TABLE 2. Hypertension incidence by age, sex and ethnicity among 401 indigenous Australian in North Queensland (cases/1000 person-years)**

Sex and age	Aboriginal (N = 149)		TSIs (N = 193)		Joint descendants (N = 62)		Total incidence (95% CI)
	Case/person year (no.)	Incidence (95% CI)	Case/person year (no.)	Incidence (95% CI)	Case/person year (no.)	Incidence (95% CI)	
<b>Female</b>							
15–24	0/174.9 (25)	0	1/194.6 (29)	5.1 (0.7–36.5)	0/46.3 (9)	0	2.4 (0.3–17.1)
25–34	5/183.0 (26)	27.3 (11.4–65.7)	7/303.0 (46)	23.1 (11.0–48.5)	0/74.4 (14)	0	21.4 (12.2–37.7)
35–44	7/144.1 (20)	48.6 (23.2–101.9)	4/165.5 (24)	24.2 (9.1–64.4)	2/57.7 (11)	34.6 (8.7–138.4)	35.4 (20.5–60.9)
45–54	1/71.4 (9)	14.0 (2.0–99.4)	2/45.7 (8)	43.8 (10.9–175.0)	2/18.4 (4)	108.4 (27.1–433.5)	36.9 (15.4–88.6)
55–64	1/37.6 (5)	26.6 (3.7–188.8)	0/9.0 (1)	0	No observation		21.5 (3.0–152.3)
>=65	1/11.8 (2)	84.7 (11.9–601.2)	2/13.6 (2)	147.1 (36.8–588.0)	No observation		118.1 (38.1–366.0)
<b>Subtotal</b>	<b>15/622.7 (87)</b>	<b>24.1 (14.5–40.0)</b>	<b>16/731.4 (110)</b>	<b>21.9 (13.4–35.7)</b>	<b>4/196.9 (38)</b>	<b>20.3 (7.6–54.1)</b>	<b>22.6 (16.2–31.4)</b>
<b>Male</b>							
15–24	2/54.9 (8)	36.5 (9.1–145.8)	8/158.3 (21)	50.5 (25.3–101.0)	1/42.9 (8)	23.3 (3.3–165.4)	42.9 (23.8–77.5)
25–34	6/115.2 (17)	52.1 (23.4–115.9)	4/138.2 (21)	28.9 (10.9–77.1)	1/45.2 (7)	22.1 (3.1–157.0)	36.8 (20.4–66.5)
35–44	14/144.4 (22)	97.0 (57.4–163.7)	5/99.0 (16)	50.5 (21.0–121.3)	1/30.5 (6)	32.9 (4.6–233.4)	73.0 (47.1–113.2)
45–54	5/73.2 (11)	68.3 (28.4–164.2)	8/89.8 (14)	89.1 (44.6–178.2)	0/5.9 (1)	0	77.0 (44.7–132.5)
55–64	4/30.1 (4)	132.7 (49.8–353.6)	5/38.9 (7)	128.6 (53.5–309.1)	0/11.4 (2)	0	111.9 (58.2–215.0)
≥65	No observation		1/4.6 (1)	217.3 (30.6–1452.3)	No observation		217.3 (30.6–1542.3)
<b>Subtotal</b>	<b>31/417.7 (62)</b>	<b>74.2 (52.2–105.5)</b>	<b>31/528.8 (80)</b>	<b>58.6 (41.2–83.4)</b>	<b>3/135.9 (24)</b>	<b>22.1 (7.1–68.4)</b>	<b>60.0 (47.1–76.6)</b>
<b>Total</b>	<b>46/1040.5 (149)</b>	<b>44.4 (33.1–59.0)</b>	<b>47/1260.2 (193)</b>	<b>37.3 (28.0–49.6)</b>	<b>7/332.8 (62)</b>	<b>21.0 (10.0–44.1)</b>	<b>38.0 (31.2–46.2)</b>

CI, confidence interval; TSIs, Torres Strait Islanders. N, total number of observations of three ethnicity backgrounds; no., number of observations in each age group from the designated ethnicity.

**TABLE 3.** Hazard ratio (95% confidence interval) of risk factors of hypertension incidence by ethnicity among 401 indigenous participants in North Queensland

	Aboriginal (N = 149)		TSIs (N = 193)		Overall (N = 401)	
	Crude HR	Adjusted HR <sup>a</sup>	Crude HR	Adjusted HR <sup>a</sup>	Crude HR	Adjusted HR <sup>a</sup>
Abdominal obesity	Reference = <88 cm in women and = <102 in men					
Yes	0.9 (0.5–1.7)	2.0 (0.9–4.2)	1.1 (0.6–2.0)	2.4 (1.2–4.6)	1.1 (0.7–1.6)	2.5 (1.5–3.9)
BMI category	Reference category, BMI <25					
25–29.9	1.3 (0.7–2.7)	1.3 (0.6–2.7)	0.7 (0.3–1.8)	0.8 (0.3–2.1)	0.9 (0.5–1.6)	0.9 (0.5–1.6)
≥30	1.3 (0.6–2.7)	2.5 (1.03–6.2)	1.7 (0.9–3.5)	2.9 (1.4–6.2)	1.5 (1.0–2.3)	2.9 (1.9–4.8)
Albuminuria	Reference category, UACR <2.5 for males and <3.5 for females					
Yes	1.1 (0.5–2.2)	1.0 (0.5–1.7)	3.5 (1.8–6.7)	2.4 (1.2–4.9)	2.1 (1.4–3.4)	1.7 (1.1–2.8)
GGT categories	Reference category, GGT <50					
≥50	2.8 (1.5–5.0)	1.9 (1.01–3.5)	1.6 (0.7–3.7)	1.1 (0.5–2.5)	2.2 (1.4–3.4)	1.4 (0.9–2.2)
Diabetes	Reference category, No					
Yes	1.0 (0.3–2.7)	0.5 (0.2–1.5)	2.6 (1.2–5.4)	2.1 (0.9–4.7)	2.1 (1.2–3.6)	1.6 (0.9–2.9)
Glucose categories	Reference category, glucose <4.5					
4.5–5.5	1.2 (0.6–2.4)	1.0 (0.5–2.1)	0.9 (0.4–2.0)	0.7 (0.4–1.6)	0.9 (0.6–1.5)	0.8 (0.5–1.3)
≥5.6	1.6 (0.7–3.5)	0.9 (0.4–2.0)	2.0 (0.9–4.6)	1.4 (0.6–3.4)	1.9 (1.1–3.1)	1.3 (0.8–2.4)
Dyslipidaemia	Reference category, No					
Yes	1.3 (0.7–2.3)	1.0 (0.6–1.9)	1.0 (0.5–1.7)	0.9 (0.5–1.7)	1.1 (0.8–1.7)	1.1 (0.7–1.6)
Smoking status	Reference category, No					
Yes	1.6 (0.9–2.9)	1.3 (0.7–2.3)	1.0 (0.5–1.8)	0.8 (0.4–1.4)	0.9 (0.6–1.3)	0.7 (0.5–1.1)
Alcohol drinking	Reference category, No					
Moderate	1.8 (0.7–4.4)	1.8 (0.7–4.5)	0.7 (0.3–1.5)	0.5 (0.2–1.1)	0.9 (0.5–1.6)	0.6 (0.3–1.2)
Risky	2.2 (1.03–4.8)	1.4 (0.6–3.2)	0.8 (0.4–1.5)	0.5 (0.2–1.1)	0.9 (0.6–1.5)	0.6 (0.4–1.0)
PA sufficient	Reference category, No					
Yes	0.7 (0.3–1.5)	1.0 (0.4–2.0)	1.2 (0.7–2.2)	1.1 (0.6–2.1)	0.9 (0.6–1.4)	0.9 (0.6–1.5)

HR, hazard ratio; TSIs, Torres Strait Islanders.

<sup>a</sup>Adjusted for age and sex in each ethnicity subgroup and overall adjusted further for ethnicity; Abdominal obese, and PA sufficient defined by WHO criteria [14]; diabetes defined using WHO criteria [17]; dyslipidaemia defined by National Heart Foundation criteria [18].

ethnic subgroups [32]. Albuminuria is an early marker of endothelial dysfunction and it predicts renal disease progression, cardiovascular and all-cause mortality [33,34]. The mechanisms underlying the link include increased intravascular volume, endothelin secretion, renin–angiotensin and sympathetic nervous system activation, and decreased nitric oxide production and endothelial function [35]. Indeed, the kidney has been proposed as a ‘window’ for early systemic CVD where glomerular albumin leakage signals widespread the disease [36]. Australian indigenous people have higher rates of albuminuria, but lower estimated glomerular filtration rate (eGFR) than the general population, and this may account for much of the excess CVD risk in this group [37]. We also found that increased GGT significantly predicted incident hypertension independent of age, sex, BMI and self-reported alcohol consumption. This is consistent with a recent meta-analysis including 13 prospective cohort studies during 1991–2012 among males and females from Japan, Korea, China, Turkey, France and USA [38]. Elevated GGT can signal liver injury generally, and non-alcoholic fatty liver disease associated with abdominal obesity and other features of the metabolic syndrome [39]. Increased GGT predicts incident hypertension and diabetes via pro-inflammatory pathways and oxidative stress involving increased fibrinogen, C-reactive protein and free radicals [40,41].

Strengths of this study include a representative community-based sample of indigenous adults and objective clinical measurements. Limitations include a relatively short follow-up period, lack of detailed medical and family history, and some potential confounding factors, and a

relatively small follow-up sample. The findings that obesity and other markers of metabolic dysfunction were the strongest predictors of incident hypertension, and that indigenous Australians had excessive risk for both these compared to the general population, were consistent with reports from other ethnic groups in North America and Europe. The challenge going forward is to find effective obesity prevention and treatment measures at a population level which is acceptable and feasible in low-income communities.

## ACKNOWLEDGEMENTS

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## Conflicts of interest

There are no conflicts of interest.

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# Association of Serum $\gamma$ -Glutamyltransferase Level and Incident Prehypertension in Korean Men

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Several lines of evidence indicate that prehypertension is more atherogenic than normal blood pressure. Serum  $\gamma$ -glutamyltransferase (GGT) is known to be positively associated with prehypertensive status and the progression of hypertension. However, there have been no prospective studies of serum GGT level as a predictor of prehypertension. Apparently 13,435 healthy men (mean age  $42.0 \pm 6.6$  yr) with normal blood pressure were included in a prospective cohort study in 2005 and were followed up to 2010 with the endpoint being incident of prehypertension. During the follow up period (median  $2.80 \pm 1.44$  yr; actual follow-up 37,679.1 person-year), prehypertension was developed in 7,867 (58.6%) participants. Risk estimations for incident prehypertension were analyzed based on quartiles of serum GGT levels using multivariate adjusted Cox proportional hazards model. In unadjusted model, the hazard ratio for incident prehypertension for the highest 3 quartiles of baseline serum GGT level was 1.21 (1.13-1.29), 1.29 (1.21-1.38), and 1.57 (1.47-1.67) compared the lowest quartile of serum GGT level, respectively ( $P$  for trend  $< 0.001$ ). These associations still remained statistically significant, even after adjusting for multiple covariates. These findings indicate that increased serum GGT level is independently associated with incident prehypertension in Korean men.

**Key Words:** Gamma-Glutamyltransferase, Prehypertension; Blood Pressure

## INTRODUCTION

Serum  $\gamma$ -glutamyltransferase (GGT), a plasma membrane-bound enzyme, has been used as a biological marker for alcohol intake or liver cell damage (1-3). However, recent studies have shown that serum GGT might be related to oxidative stress and might have a role in the pathogenesis of cardiovascular disease, diabetes mellitus, strokes and metabolic syndrome (4-6).

In the seventh Joint national committee (JNC-7), National Heart, Lung, and Blood Institute (NHLBI) of United States presented a new diagnostic classification for blood pressure (BP) where systolic BP (SBP) is between 120-139 mmHg or diastolic BP (DBP) is between 80-89 mmHg, previously categorized as normotensive and borderline BP in the JNC-6, is now categorized as prehypertension. This is based on the study results that the risk of cerebral infarction and coronary artery disease increases in patients with SBP/DBP above 115/75 mmHg, and the risk is doubled as BP increases by 20/10 mmHg (7). In Strong Heart Study, the incidence rate of hypertension within 4 yr in prehypertensive group was 38%, and in Framingham Heart Study, the risk of hypertension was doubled in prehypertensive

group compared with normotensive group (8, 9). Additionally, the risk of cardiovascular disease in prehypertensive group has 1.8 times higher than that of normotensive group (10).

Recent cross-sectional studies have reported the association between serum GGT and hypertension and prehypertension prevalence (11-15). However, there was no prospective cohort study on the association between serum GGT and incident prehypertension. In this study, we examined the clinical association between baseline serum GGT level and incident prehypertension in Korean men.

## MATERIALS AND METHODS

### Study populations

The present study was a prospective cohort study to examine the association between serum GGT level and incident prehypertension in Korean men participating in a medical health check-up program at the Total Healthcare Center of Kangbuk Samsung Hospital, Sungkyunkwan University, Seoul, Korea. We included in the present study only men who were normotensive at baseline. Initially, participants who had abnormal

blood pressure and participants with prehypertension and hypertension were excluded from this study. In 2005, 46,728 men who had participated in a medical health check-up program were recruited in this study. Among the 46,728 participants, 29,233 men were excluded due to the following reasons: 238 had a history of a malignancy; 323 had a history of cardiovascular disease; 3,241 were receiving antidiabetic medications; 19,793 and 9,591 had a baseline prehypertension and hypertension. Because some participants had more than one exclusion criteria, the total number of men who were eligible for the study was 17,495. We further excluded 4,060 participants who did not attend any follow-up visit between 2006 and 2010. Accordingly, 13,435 participants were included in the final analysis and were observed for incident prehypertension. The total follow-up period was 37,679.1 person-year and average follow-up period was  $2.80 \pm 1.44$  person-years.

### Clinical and Laboratory measurements

Study data included a medical history, a physical examination, information provided by a questionnaire, anthropometric measurements and laboratory measurements. The medical history was assessed by the examining physicians. All the participants were asked about health-related behavior including alcohol intake, cigarette smoking and physical activity. Questions about alcohol intake included the frequency of alcohol intake per week and the usual amount of alcohol intake per day. We considered participants as current smoker who reported to smoke at that time of the study. In addition, the participants were asked about the frequency of physical activity, such as jogging, bicycling, and swimming that lasted long enough to produce perspiration ( $\geq 1$  time/week). Body mass index (BMI) was calculated as the weight (kilograms) divided by height (meters) squared.

After a 5-min rest, BP was measured on the right upper arm in the sitting position using a standardized mercury sphygmomanometer. SBP and DBP were recorded as the first and fifth Korotkoff phases, respectively. According to the JNC-7 guidelines, the measured BP was classified as normal (SBP < 120 mmHg and a DBP < 80 mmHg), prehypertension (SBP 120 to 139 mmHg or DBP 80 to 90 mmHg) and hypertension (SBP  $\geq 140$  mmHg or DBP  $\geq 90$  mmHg, or current use of medication for hypertension). Diabetes mellitus was defined as fasting serum glucose more than 126 mg/dL, or current use of medication for diabetes.

After fasting for at least 12 hr, peripheral venous samples were collected. Total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, creatinine, high-sensitivity C-reactive protein (hs-CRP), fasting blood glucose, insulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and GGT were measured. Serum GGT was measured using Bayer Reagent Packs (Bayer HealthCare, Tarrytown, NY, USA) on an automated chemistry analyzer

(ADVIA 1650 Autoanalyzer; Bayer Diagnostics, Leverkusen, Germany). Insulin levels were measured with immunoradiometric assays (Biosource, Nivelles, Belgium). Insulin resistance was calculated with the homeostasis model assessment of insulin resistance (HOMA-IR): fasting serum insulin ( $\mu$ U/mL)  $\times$  FBG (mg/dL)/22.5 (16). The clinical laboratory has been accredited and participates annually in inspections and surveys by the Korean Association of Quality Assurance for Clinical Laboratories.

### Statistical analysis

Data in the text and tables were expressed as the mean  $\pm$  standard deviation (SD) or medians (interquartile range) for continuous variables and percentages of the number for categorical variables. Participants were divided into quartiles on the basis of serum GGT level as follows: quartile 1, GGT < 17 IU/L; quartile 2, GGT 17 to 25 IU/L; quartile 3, 25 to 40 IU/L GGT; and quartile 4, GGT  $\geq 40$  IU/L. The one-way ANOVA and chi-square-test were used to analyze the statistical differences among the characteristics of the participants at the baseline visit according to the quartile groups of serum GGT level. The distributions of continuous variables were evaluated, and log transformations were used in the analysis as required. For cases with incident prehypertension, the time spent at incident prehypertension was assumed to be the midpoint between the baseline visit and the visit at which prehypertension was first diagnosed. The person years were calculated as the sum of follow-up times from the baseline until the assumed time prehypertension was diagnosed or until the final examination of each individual. Compared to the lowest quartile of serum GGT, the hazards ratios (HRs) of the highest 3 quartiles of serum GGT was calculated by the Cox proportional hazard model after adjustment for baseline characteristics such as age, log (hsCRP), total cholesterol, creatinine, recent smoking status, alcohol intake, regular exercise and diabetes mellitus. For the linear trends of risk, the number of quartiles was used as a continuous variable and tested on each model. To use the Cox proportional hazards models, we checked the validity of the proportional hazards assumption. Two approaches were used to assess the validity of the proportional hazards assumption. First, the assumption was assessed by log-minus-log-survival function and found to graphically hold. Second, to confirm the validity of the proportional hazards assumption, time-dependent covariate analysis was used. The time-dependent covariate was not statistically significant, suggesting that the proportional hazards assumption is not violated ( $P = 0.055$ ).  $P$  values less than 0.05 were considered to be statistically significant. Statistical analyses were performed PASW Statistics 18 (SPSS Inc., Chicago, IL, USA).

### Ethics statement

This cohort study was approved by the institutional review board

**Table 1.** Baseline characteristics of participants according to quartile groups of serum GGT level (n=13,435)

Characteristics	Overall	GGT (IU/L)				<i>P</i> for trend*
		Quartile 1 (< 17)	Quartile 2 ( $\geq 17, < 25$ )	Quartile 3 ( $\geq 25, < 40$ )	Quartile 4 ( $\geq 40$ )	
Person-year (total)	37,679.1	10,005.4	9,773.2	9,093.4	8,807.1	
Person-year (average)	2.80 $\pm$ 1.44	3.00 $\pm$ 1.45	2.85 $\pm$ 1.45	2.77 $\pm$ 1.44	2.59 $\pm$ 1.41	< 0.001
Age (yr)	42.0 $\pm$ 6.6	41.1 $\pm$ 6.9	41.7 $\pm$ 6.5	42.4 $\pm$ 6.6	42.8 $\pm$ 6.3	< 0.001
BMI ( $\text{kg}/\text{m}^2$ )	23.6 $\pm$ 2.6	22.4 $\pm$ 2.3	23.2 $\pm$ 2.4	24.0 $\pm$ 2.5	24.9 $\pm$ 2.5	< 0.001
Systolic BP (mmHg)	103.4 $\pm$ 7.1	102.5 $\pm$ 7.5	103.5 $\pm$ 7.1	103.7 $\pm$ 7.0	104.0 $\pm$ 6.7	< 0.001
Diastolic BP (mmHg)	69.1 $\pm$ 4.4	68.4 $\pm$ 4.7	69.0 $\pm$ 4.3	69.3 $\pm$ 4.2	69.6 $\pm$ 4.2	< 0.001
Total cholesterol (mg/dL)	189.1 $\pm$ 30.9	177.0 $\pm$ 27.3	186.2 $\pm$ 28.9	193.0 $\pm$ 30.2	200.2 $\pm$ 32.2	< 0.001
Triglyceride (mg/dL)	113 (83-158)	88 (68-116)	104 (79-140)	123 (91-167)	150 (111-202)	< 0.001
HDL-cholesterol (mg/dL)	50.3 $\pm$ 10.3	51.6 $\pm$ 10.2	50.4 $\pm$ 10.3	49.7 $\pm$ 10.3	49.6 $\pm$ 10.3	< 0.001
LDL-cholesterol (mg/dL)	111.5 $\pm$ 26.2	103.0 $\pm$ 23.5	110.4 $\pm$ 25.0	114.5 $\pm$ 25.8	117.8 $\pm$ 28.0	< 0.001
Fasting blood glucose (mg/dL)	95.3 $\pm$ 13.7	93.1 $\pm$ 12.5	94.3 $\pm$ 11.8	95.5 $\pm$ 12.2	98.4 $\pm$ 17.2	< 0.001
HOMA-IR	1.81 (1.41-2.38)	1.59 (1.28-2.00)	1.73 (1.36-2.21)	1.87 (1.45-2.46)	2.17 (1.65-2.81)	< 0.001
Insulin ( $\mu\text{U}/\text{dL}$ )	8.4 $\pm$ 3.1	7.4 $\pm$ 2.4	8.0 $\pm$ 2.7	8.6 $\pm$ 3.3	9.6 $\pm$ 3.5	< 0.001
Creatinine (mg/dL)	1.12 $\pm$ 0.12	1.11 $\pm$ 0.10	1.12 $\pm$ 0.15	1.13 $\pm$ 0.10	1.13 $\pm$ 0.11	< 0.001
hsCRP (mg/L)	0.05 (0.02-0.10)	0.03 (0.02-0.07)	0.04 (0.02-0.09)	0.05 (0.03-0.11)	0.07 (0.04-0.14)	< 0.001
AST (U/L)	23 (19-27)	21 (18-24)	22 (18-25)	24 (20-28)	27 (23-33)	< 0.001
ALT (U/L)	24 (18-33)	18 (15-23)	21 (17-27)	26 (20-33)	35 (26-49)	< 0.001
Current smoker (%)	45.9	36.7	42.3	47.4	56.8	< 0.001
Alcohol intake (%)	9.2	4.1	6.1	9.6	16.8	< 0.001
Regular exercise (%)	13.4	15.1	13.6	13.4	11.4	< 0.001
Diabetes mellitus (%)	2.3	1.1	1.5	2.5	3.9	< 0.001

Data are means (standard deviation), medians (interquartile range), or percentages. \**P* value by ANOVA for continuous variables and chi square test for categorical variables.

of Kangbuk Samsung Hospital (Seoul, Korea) (IRB number: KBC12134). Informed consent was obtained from all participants.

## RESULTS

The demographic and biochemical characteristics of the study participants according to the quartile groups of serum GGT level are presented in Table 1. During 37,679.1 person-years of follow-up, 7,867 (58.6%) cases of prehypertension were diagnosed between 2006 and 2010. At the baseline visit, the mean age and BMI of study participants were  $42.0 \pm 6.6$  yr and  $23.6 \pm 2.6$   $\text{kg}/\text{m}^2$ , respectively. There were significant linear trends across the quartiles of serum GGT level with respect to all of the listed variables. Age, BMI, SBP, DBP, total cholesterol, triglyceride, LDL-cholesterol, fasting blood glucose, insulin, HOMA-IR, hsCRP, AST and ALT tended to increase as the serum GGT quartile increased. The percentages of current smoker, alcohol intake participant and diabetes mellitus have a tendency to increase along with the increase of the serum GGT quartile. However, HDL-cholesterol and the percentages of regular exercise participants tended to decrease along with the increase in the serum GGT quartile, respectively. Table 2 shows the incident prehypertension have a tendency to increase according to the quartile groups of serum GGT level.

The baseline characteristics of participants with or without incident prehypertension are shown in Table 3. Participants who were diagnosed prehypertension during the follow-up period

**Table 2.** Incident prehypertension according to quartile groups of serum GGT level

GGT (IU/L)	Incident of prehypertension (%)
Quartile 1 (< 17)	51.0
Quartile 2 ( $\geq 17, < 25$ )	57.8
Quartile 3 ( $\geq 25, < 40$ )	59.6
Quartile 4 ( $\geq 40$ )	65.7
<i>P</i> for trend*	< 0.001

\**P* value by chi square test for categorical variables.

had higher serum GGT level at baseline compared with those who did not develop prehypertension. In contrast to participants without incident prehypertension, those with incident prehypertension were slightly older (42.6 vs 41.2 yr) and more likely to have a less favorable metabolic profiles at baseline. As expected, all clinical variables showed statistically significant differences between two groups except for recent smoking status.

Table 4 presented the results of Cox proportional HRs and 95% confidence interval (CI) for prehypertension according to the quartile groups of serum GGT levels. In unadjusted model, the HRs and 95% CI for incident prehypertension for the highest 3 quartiles of baseline serum GGT level 1.21 (95% CI, 1.13-1.29), 1.29 (95% CI, 1.21-1.38) and 1.57 (95% CI, 1.47-1.67) compared to the lowest quartile of serum GGT, respectively (*P* for trend  $< 0.001$ ). The age adjusted HRs was 1.19 (95% CI, 1.12-1.27), 1.26 (95% CI, 1.18-1.34) and 1.51 (95% CI, 1.42-1.61) compared to the lowest quartile of serum GGT, respectively (*P* for trend  $< 0.001$ ). These associations still maintained statistically significant, even after further adjustments for explanatory vari-

**Table 3.** Comparison between participants with and without incident of prehypertension

Characteristics	Without incident of prehypertension (n = 5,568)	With incident of prehypertension (n = 7,867)	P value*
Age (yr)	41.2 ± 6.6	42.6 ± 6.5	< 0.001
BMI (kg/m <sup>2</sup> )	23.2 ± 2.5	23.9 ± 2.6	< 0.001
Systolic BP (mmHg)	102.2 ± 7.3	104.3 ± 6.8	< 0.001
Diastolic BP (mmHg)	68.3 ± 4.7	69.6 ± 4.1	< 0.001
Total cholesterol (mg/dL)	187.0 ± 30.8	190.7 ± 30.9	< 0.001
Triglyceride (mg/dL)	123.5 ± 69.3	135.3 ± 73.5	< 0.001
HDL-cholesterol (mg/dL)	50.7 ± 10.4	50.0 ± 10.2	< 0.001
LDL-cholesterol (mg/dL)	110.0 ± 26.3	112.6 ± 26.1	< 0.001
Fasting blood glucose (mg/dL)	94.3 ± 12.3	96.1 ± 14.6	< 0.001
HOMA-IR	1.90 ± 0.80	2.04 ± 0.87	< 0.001
Insulin (μU/dL)	8.1 ± 3.0	8.6 ± 3.2	< 0.001
Creatinine (mg/dL)	1.12 ± 0.1	1.13 ± 0.1	< 0.001
hsCRP (mg/L)	0.11 ± 0.3	0.13 ± 0.4	< 0.001
AST (U/L)	24.1 ± 14.6	25.4 ± 15.1	< 0.001
ALT (U/L)	26.9 ± 23.7	29.7 ± 24.3	< 0.001
GGT (IU/L)	31.4 ± 28.2	37.1 ± 34.6	< 0.001
Current smoker (%)	46.0	45.8	0.855
Alcohol intake (%)	8.1	9.9	< 0.001
Regular exercise (%)	12.1	14.3	< 0.001
Diabetes mellitus (%)	1.9	2.5	0.019

Data are expressed as means (standard deviation) or percentages. \*P value by t-test for continuous variables and chi square test for categorical variables.

**Table 4.** Hazard ratios (HRs) and 95% confidence intervals (CI) for incident of prehypertension according to quartile groups of serum GGT level

Variables	Hazard ratios (95% Confidence Interval)			
	Unadjusted	Age-adjusted	Model 1	Model 2
GGT				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	1.21 (1.13-1.29)	1.19 (1.12-1.27)	1.19 (1.10-1.28)	1.17 (1.08-1.26)
Quartile 3	1.29 (1.21-1.38)	1.26 (1.18-1.34)	1.18 (1.09-1.27)	1.16 (1.07-1.26)
Quartile 4	1.57 (1.47-1.67)	1.51 (1.42-1.61)	1.35 (1.24-1.46)	1.32 (1.21-1.43)
P for trend	< 0.001	< 0.001	< 0.001	< 0.001
Age		1.03 (1.02-1.03)	1.03 (1.02-1.03)	1.03 (1.02-1.03)
HOMA-IR			1.12 (1.09-1.15)	1.12 (1.09-1.16)
Triglyceride			1.00 (1.00-1.00)	1.00 (1.00-1.00)
HDL-cholesterol			1.00 (0.99-1.00)	1.00 (0.99-1.00)
Total cholesterol			1.00 (1.00-1.00)	1.00 (1.00-1.00)
Log (hsCRP)			1.06 (1.03-1.09)	1.06 (1.03-1.09)
Creatinine			1.33 (1.04-1.72)	1.29 (1.00-1.67)
Current smoker				1.01 (0.96-1.06)
Alcohol intake				1.12 (1.03-1.23)
Regular exercise				0.90 (0.83-0.97)
Diabetes mellitus				1.10 (0.93-1.31)

Model 1 was adjusted for age, HOMA-IR, triglyceride, HDL-cholesterol, total cholesterol, log (hsCRP) and creatinine. Model 2 was adjusted for model 1 plus recent smoking status, alcohol intake, regular exercise and diabetes mellitus.

ables (age, total cholesterol, log [hsCRP], creatinine, recent smoking status, alcohol intake, regular exercise and diabetes mellitus) in model 1 and 2. In model 1, the adjusted HRs (95% CI) for incident of prehypertension were 1.21 (1.12-1.30), 1.23 (1.13-1.32) and 1.48 (1.37-1.60), respectively (*P* for trend < 0.001). In model 2, the adjusted HRs (95% CI) for incident prehypertension were 1.19 (1.11-1.29), 1.21 (1.12-1.31) and 1.44 (1.33-1.56), respectively (*P* for trend < 0.001).

## DISCUSSION

Serum GGT level was found to be positively associated with the incident prehypertension in Korean men, free of baseline prehypertension, hypertension and many other diseases. The HR of prehypertension was increased with in a dose-dependent manner by increasing quartiles of serum GGT. These association persisted after adjusting for age, total cholesterol, log (hsCRP), creatinine, current smoking status, alcohol intake, regular exer-

cise, and diabetes mellitus.

In our study, 17,495 eligible male participants were selected because of sex differences in serum GGT level. Serum GGT was significantly higher in men compared to women due to the greater consumption of alcohol and higher prevalence of liver disease in men (17). Another reason was the difference in distribution of excess fat in men and women. Serum GGT in women with BMI above 32 kg/m<sup>2</sup> was comparable to the serum GGT in men with BMI in the range of 20-24 kg/m<sup>2</sup> (18, 19).

To the best of our knowledge, our study was the first prospective cohort study demonstrating a positive association between baseline serum GGT level and incident prehypertension; therefore we were unable to compare our results with those of others. Only several previous cross-sectional studies showed that serum GGT is associated with prevalence of prehypertension. Higher serum GGT levels were associated with prehypertension in nationally representative sample of the US adults, with the multivariate odds ratio (OR) (95% CI) of 1.84 (1.37-2.46) comparing quartile 4 of serum GGT (> 29 U/L) to quartile 1 (< 13 U/L). Their results were consistent in subgroup analyses of race-ethnicity, age, smoking, alcohol intake, BMI, waist circumference and diabetes (13). Similar results were found in a community-based cross-sectional study from Japan and Turkey. The multivariate OR (95% CI) for prehypertension was 1.73 (1.06-3.18) for the middle tertile (29-53 IU/L) and 2.37 (1.31-4.31) for the highest tertile (> 53 IU/L), compared to the lowest tertile of serum GGT (< 29 IU/L) in Japanese men (14). And the mean serum GGT level was higher in the prehypertension group than in the control group (24.33 and 18.85 U/L, respectively;  $P < 0.001$ ) in Turkish men and women (15). These findings were consistent with previous reports related to hypertension (16, 20). However, we further suggest that serum GGT level is also related to incident prehypertension, a stage with higher risk of progressing to hypertension. Therefore, if people who have higher serum GGT level change their life-style with the healthy behavior such as a combination of increased physical activity, moderation in alcohol intake, and consumption of a diet that is lower in sodium content and higher in fruit, vegetables, and low-fat dairy products, then risk of progressing to hypertension will be decreased (21, 22). Prehypertension was one of the main contributors to hypertension, and possibly to the future development of cardiovascular disease, although there were differences in the progression rate prehypertension to hypertension (9, 23). Thus, we consistently suggest that higher serum GGT level within physiologically normal range may be useful to predict the risk of prehypertension.

GGT is an enzyme expressed in serum and most cell surfaces; however the function of GGT on these cells is uncertain. Emerging evidence has been shown that serum GGT might be an important enzyme in the pathogenesis of cardiovascular diseases. However, the mechanisms by which the serum GGT

level increases the risk of prehypertension are not fully understood. In our study, the serum GGT predicted the incident prehypertension independently of the alcohol intake. Therefore, the association between serum GGT and prehypertension is not mediated by alcohol intake. Several lines of recent evidence suggest that an association between serum GGT and incident prehypertension is plausible. First, serum GGT has been interpreted as a reliable marker of oxidative stress (3, 6). Serum GGT has a critical role in the maintenance of intracellular defense mechanism by initiating extracellular catabolism of glutathione, the main antioxidant in mammalian cells (1, 2, 24). These associations suggest that GGT may be a marker of oxidative stress. Oxidative stress caused increase of BP by direct vasoconstriction and sodium retention in the vascular smooth muscle and endothelial cells (3). Second, insulin resistance could play a major role in the association between serum GGT and incident prehypertension, because GGT might be interpreted as a marker for hepatic steatosis and hepatic insulin resistance (3, 5). More recent studies suggest that leptin may stimulate sympathetic outflow from the hypothalamus which will increase the heart rate and BP (25). In this study, HOMA-JR increased in dose-response manner with increasing quartiles of serum GGT. Serum GGT might be a reliable predictor of diabetes and hypertension, even within a physiologically normal range in the CARDIA study (6). Third, serum GGT activity probably reflected chronic inflammation associated with low levels of anti-inflammatory hormones or cytokines. Serum GGT was strongly associated with C-reactive protein, the widely recognized marker of chronic inflammation (5, 26). These results strongly suggest that GGT is involved in the pathway of inflammation. However to clarify the mechanisms of prehypertension, further interventional studies that target serum GGT are needed.

Several limitations of this study must be considered. First, participants were self-selected and approximately 23% of participants were excluded because they were unattended any follow up visit. Therefore this study might possess some selection bias. Moreover, a single reading of BP on only 1 visit may have served as a drawback because of large random fluctuations in a casual reading.

In conclusion, serum GGT was an early predictor for progression of prehypertension in healthy Korean men. Therefore increased serum GGT level is implicated in increased BP and the progression of prehypertension. In addition, it is needed to determine whether a more aggressive intervention, such as nutritional or lifestyle modifications that decrease serum GGT level, will decrease the risk of prehypertension.

## DISCLOSURE

The authors have no potential conflicts of interest to disclose.



# Gender Differences in the Association between Serum $\gamma$ -Glutamyltransferase and Blood Pressure Change: A Prospective Community-Based Cohort Study

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## INTRODUCTION

In 2000, more than a quarter of the world's adult population, totaling nearly 1 billion, had hypertension; and this proportion will increase to 29% (1.6 billion) by 2025 (1). High blood pressure (BP) is responsible 7.6 million premature deaths and 92 million disability-adjusted life years (DALYs) in 2001 (2). In addition, BP change is a common condition that elevates the risk of cardiovascular disease (CVD) and mortality in several studies (3-6).

Serum  $\gamma$ -glutamyltransferase (GGT), a commonly used alcohol consumption or liver disease, was an established risk factor for CVD, such as stroke, myocardial infarction, congestive heart failure, metabolic syndrome, and coronary heart disease, suggesting that GGT may be a predictor of CVD (7-9). GGT is also a significant risk factor for increase BP (10-13) and incident hypertension (14-19). It is well reported that several cardiovascular risk factors influences differently according to gender (20). Some studies showed that considerable underlying differences in the distributions of GGT by gender (21, 22). However, the difference of the association between GGT and BP by gender is

We evaluated the gender differences in the relation of baseline serum  $\gamma$ -glutamyltransferase (GGT) levels to blood pressure (BP) change during 4 yr. 4,025 normotensive subjects (1,945 men and 2,080 women) who aged 40-69 yr at baseline participated in the Ansung-Ansan cohort of the Korean Genome Epidemiology Study were included. The associations of GGT with baseline BP or 4-yr change of BP were evaluated. GGT levels were associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) at baseline after adjusting for age, body mass index (BMI), HDL-cholesterol, triglyceride, C-reactive protein (CRP), current smoking status and alcohol intake (SBP,  $\beta = 1.28$ ,  $P < 0.001$ ; DBP,  $\beta = 1.41$ ,  $P < 0.001$ ). GGT levels were also associated with 4-yr change in BP after adjusting for age, BMI, HDL-cholesterol, triglyceride, CRP, current smoking status, alcohol intake and SBP (SBP,  $\beta = 1.08$ ,  $P = 0.001$ ; DBP,  $\beta = 0.64$ ,  $P = 0.003$ ). This association was statistically significant in men (SBP,  $\beta = 1.82$ ,  $P < 0.001$ ; DBP,  $\beta = 1.05$ ,  $P = 0.001$ ), but not in women (SBP,  $\beta = 0.38$ ,  $P = 0.466$ ; DBP,  $\beta = -0.37$ ,  $P = 0.304$ ). Remarkably, this association between GGT and BP was significant in men at 40-49 yr of age. In summary, we found positive associations between GGT levels at baseline and the change of BP. The relation of GGT level and the change of BP was only significant in men, not in women, which warrants further studies to elucidate the biologic mechanisms.

**Keywords:** Blood Pressure;  $\gamma$ -Glutamyltransferase; Gender

unclear. Some previous study has suggested no gender differences in risk of hypertension (18) while other has shown a different risk in women than in men (12). Moreover most previous studies have not been explored gender differences of these associations (10, 11, 13-17, 19).

Therefore, we investigated the association between GGT at baseline and BP and 4-yr change in BP in a large population and assessed whether gender-related difference in this association.

## MATERIALS AND METHODS

### Study subjects

As a part of the Korean Genome Epidemiology Study, the Ansung-Ansan cohort study is an ongoing community-based prospective cohort of 10,038 participants aged 40 to 69 yr. The initial enrollment was carried out in 2001-2002 and follow-up examinations are conducted biennially. The details of the study design and procedures have been previously described (23). The current study was based on an examination of baseline and second follow-up data. A total of 7,260 subjects was enrolled in the current study after the 4-yr follow-up examination; 2,492

subjects declined to participate in the follow-up surveys and 286 died before completing the 2 follow-up visits.

For accurate observation of association between GGT and BP change, 3,067 individuals with hypertension at baseline (BP  $\geq$  140/90 or use of antihypertensive medications) were excluded. In addition, 168 participations with missing information on GGT, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), alcohol intake and smoking status at baseline and SBP and DBP in the 2 follow-up visits were excluded. The remaining 4,025 participants (1,945 men and 2,080 women) are included in this analysis.

### Data collection

Height and weight were measured using standardized techniques and equipment. The BMI was calculated as weight (kg) divided by height squared ( $m^2$ ). BP while the subject was sitting and had rested for at least five minutes was measured on 2 occasions 5 min apart using a standard mercury sphygmomanometer (Baumanometer; W. A. Baum, Copiague, NY, USA) by trained staff; and the mean of value was used for this study. At baseline examination, hypertension was defined as BP  $\geq$  140/90 or use of antihypertensive medications. Blood sample were obtained after a fasting at least 12 hr; serum GGT, triglycerides, high-density lipoprotein (HDL)-cholesterol and C-reactive protein (CRP) were quantified by biochemical assays, performed by a central laboratory (Seoul Clinical Laboratories, Seoul, Korea).

Demographic characteristic was collected at baseline by trained interviewers; age, gender, cigarette smoking status, alcohol consumption (grams/day), menopausal status (yes/no), and medication (yes/no). Non-smokers were defined as those who had reported "never smoking" in the questionnaire. Former smokers were defined as those who had reported "abstain from smoking" in the questionnaire. Current smokers were defined as those who had reported "currently smoking" in the questionnaire. Alcohol consumption determined based on self-report. Subjects were asked how often, on the average, they had consumed the specified amount of each item over the past year.

Then, alcohol consumption in grams per day was calculated as the sum of the average alcohol content per type of alcoholic beverage intake multiplied by the daily number of drinks. Menopausal status was also assessed by the self-report. Subjects who answered "yes" to the following question: "Do you menstruate?" were allotted to premenopausal, and "no" to the question were allotted to postmenopausal.

### Statistical analysis

Variables with skewed distribution were log-transformed before analysis. Descriptive statistics used to characterize the study subjects included means and SDs for continuous variables and proportions for categorical variables. Comparison between men and women were done with *t*-test or with the chi-square test. Correlations were evaluated with Pearson correlation coefficient.

Linear regression models were used to examine the associations of GGT and BP and 4-yr change in BP. We used 3 models. First, we performed unadjusted analysis. Second, we adjusted for baseline levels of age, BMI, HDL-cholesterol, triglyceride, CRP and SBP. Finally, we further adjusted for baseline levels of smoking status and alcohol intake.

Finally we evaluated the association of GGT and BP and 4-yr change in BP for subgroups defined by gender, age, menopausal status. All reported *P* values are two-sided, and *P*  $<$  0.05 was considered statistically significant. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

### Ethics statement

This study was approved by the institutional review board of Seoul National University Hospital (IRB No. H1206-081-414) and all participants provided written informed consent before participation.

## RESULTS

### Participant characteristics in men and women

Table 1 presents study population characteristics at baseline for

**Table 1.** Baseline characteristics of study participants

Parameters	All subjects (n = 4,025)	Men (n = 1,945)	Women (n = 2,080)	P value
Age (yr)	50.0 (8.2)	49.9 (8.2)	50.1 (8.3)	0.436
Body mass index ( $kg/m^2$ )	24.3 (3.0)	24.1 (2.8)	24.4 (3.1)	0.001
HDL cholesterol (mg/dL)	44.8 (9.8)	43.2 (9.6)	46.4 (9.8)	< 0.001
Triglycerides (mg/dL)*	126.0 (82.0)	141.0 (95.0)	115.0 (69.0)	< 0.001
CRP (mg/dL)*	0.1 (0.2)	0.1 (0.2)	0.1 (0.2)	0.001
GGT (IU/L)*	17.0 (20.0)	28.0 (29.0)	13.0 (8.0)	< 0.001
Alcohol intake (grams/day)	9.0 (20.3)	17.3 (26.1)	1.3 (6.1)	< 0.001
Systolic blood pressure (mmHg)	112.5 (11.5)	113.7 (10.7)	111.4 (12.1)	< 0.001
Diastolic blood pressure (mmHg)	75.0 (8.0)	76.7 (7.4)	73.4 (8.2)	< 0.001
Current smoking (No [%])	1,000 (24.8)	935 (48.1)	65 (3.1)	< 0.001

Data are given as mean (SD) unless otherwise indicated. \*Median values (interquartile range). *P* values indicate statistical significance of differences between men and women. HDL, high-density lipoprotein; CRP, C-reactive protein; GGT,  $\gamma$ -glutamyltransferase.

men and women. The mean age of study population was 49.9 yr in men and 50.1 yr in women at baseline. The BP, triglyceride, CRP, GGT levels, and alcohol intake were significantly higher in men than in women. Proportions of current smokers were 48.1% in men and 3.1% in women.

### Associations between baseline GGT and baseline BP

Unadjusted analyses showed that GGT was positively associated with SBP both in men and in women ( $P < 0.001$ , respectively), and regression coefficient is higher in women than in men. Also, this relationship remained significant after adjusting for

age, BMI, HDL-cholesterol, triglyceride, CRP, smoking status, and alcohol intake in all subjects ( $\beta = 1.28$ ,  $P < 0.001$ ). There was no significant gender difference in the association between baseline GGT and baseline SBP ( $P$  for interaction with gender = 0.829). DBP also showed significant association with GGT even after adjusting covariates mentioned above ( $P < 0.05$ ). GGT was not associated with SBP in any age group in men or in women. However, DBP showed significant association with GGT even after adjusting covariates in men younger age group ( $\beta = 0.77$ ,  $P = 0.037$ ). In the menopausal status-stratified association between GGT and BP after adjusted covariates, GGT was not

**Table 2.** Four year change in systolic blood pressure in relation to serum  $\gamma$ -glutamyltransferase (GGT) in men and women

Demographic parameters	Unadjusted model		Model 1		Model 2	
	$\beta$	$P$ value	$\beta$	$P$ value	$\beta$	$P$ value
<b>Total</b>						
40-49 (n = 2,365)	0.84	0.006	1.77	< 0.001	1.11	0.002
50-59 (n = 952)	1.20	0.037	2.15	0.001	1.65	0.013
60-69 (n = 708)	-0.60	0.450	0.16	0.834	0.26	0.767
All age (n = 4,025)	0.67	0.010	1.59	< 0.001	1.08	0.001
<b>Men</b>						
40-49 (n = 1,155)	1.37	0.003	2.13	< 0.001	2.30	< 0.001
50-59 (n = 460)	1.59	0.040	2.29	0.005	1.62	0.060
60-69 (n = 330)	1.75	0.097	2.80	0.011	2.66	0.024
All age (n = 1,945)	1.51	< 0.001	2.30	< 0.001	1.82	< 0.001
<b>Women</b>						
40-49 (n = 1,210)	0.13	0.829	0.44	0.463	0.39	0.515
50-59 (n = 492)	0.78	0.490	2.43	0.038	2.29	0.052
60-69 (n = 378)	-3.10	0.035	-1.55	0.279	-1.44	0.316
All age (n = 2,080)	-0.25	0.626	0.40	0.434	0.38	0.466
<b>Menopausal status</b>						
Premenopause (n = 753)	0.37	0.646	0.45	0.562	0.18	0.818
From premenopause (n = 337) to postmenopause	-0.06	0.965	1.73	0.170	1.52	0.237
Postmenopause (n = 966)	-1.10	0.175	0.06	0.945	0.08	0.920

Model 1 adjusted for age, BMI, HDL- cholesterol, triglyceride (log), CRP (log) and SBP; Model 2 adjusted for model 1 plus current smoking status and alcohol intake.

**Table 3.** Four year change in diastolic blood pressure in relation to serum  $\gamma$ -glutamyltransferase (GGT) in men and women

Demographic parameters	Unadjusted Model		Model 1		Model 2	
	$\beta$	$P$ value	$\beta$	$P$ value	$\beta$	$P$ value
<b>Total</b>						
40-49 (n = 2,365)	0.52	0.024	1.45	< 0.001	0.81	0.003
50-59 (n = 952)	0.40	0.302	1.37	0.001	0.83	0.057
60-69 (n = 708)	-0.80	0.125	-0.20	0.685	-0.39	0.474
All age (n = 4,025)	0.28	0.129	1.19	< 0.001	0.64	0.003
<b>Men</b>						
40-49 (n = 1,155)	0.93	0.010	1.49	0.001	1.09	0.007
50-59 (n = 460)	0.62	0.269	1.29	0.021	0.84	0.155
60-69 (n = 330)	0.60	0.393	1.05	0.135	0.82	0.270
All age (n = 1,945)	0.93	0.001	1.40	< 0.001	1.05	0.001
<b>Women</b>						
40-49 (n = 1,210)	-0.63	0.172	-0.24	0.603	-0.22	0.627
50-59 (n = 492)	-0.27	0.709	0.67	0.360	0.64	0.386
60-69 (n = 378)	-1.89	0.045	-1.79	0.042	-1.75	0.048
All age (n = 2,080)	-0.87	0.016	-0.36	0.315	-0.37	0.304
<b>Menopausal status</b>						
Premenopause (n = 753)	-0.31	0.598	-0.08	0.898	-0.16	0.785
From premenopause to postmenopause (n = 337)	-1.39	0.165	0.24	0.800	0.03	0.979
Postmenopause (n = 966)	-0.96	0.068	-0.67	0.188	-0.66	0.198

Model 1 adjusted for age, BMI, HDL- cholesterol, triglyceride (log), CRP (log) and SBP; Model 2 adjusted for model 1 plus current smoking status and alcohol intake.

**Table 4.** Association between serum  $\gamma$ -glutamyltransferase (GGT) and four year change in blood pressure expect the heavy drinker or diagnosed with hepatitis\*

Demographic parameters	Change of SBP		Change of DBP	
	$\beta$	P value	$\beta$	P value
Total				
40-49 (n = 2,129)	0.90	0.023	0.19	0.550
50-59 (n = 880)	1.67	0.020	0.26	0.599
60-69 (n = 669)	0.12	0.900	-0.99	0.115
All age (n = 3,678)	0.94	0.005	0.02	0.942
Men				
40-49 (n = 966)	1.72	0.003	0.79	0.104
50-59 (n = 401)	1.68	0.088	0.51	0.494
60-69 (n = 298)	2.52	0.046	0.19	0.834
All age (n = 1,665)	1.90	< 0.001	0.70	0.056
Women				
40-49 (n = 1,163)	-0.01	0.991	0.70	0.056
50-59 (n = 479)	2.33	0.051	0.40	0.610
60-69 (n = 371)	-1.47	0.313	-1.56	0.112
All age (n = 2,013)	0.14	0.794	-0.63	0.109

\*Adjusted for age, BMI, HDL-cholesterol, triglyceride(log), CRP(log), SBP, current smoking status and alcohol intake. SBP, systolic blood pressure; DBP, diastolic blood pressure.

associated with BP in pre- and post-menopause, either.

#### Association between baseline GGT and change in BP

Table 2 showed relationship between GGT at baseline and 4-yr change in SBP. Remarkably, after adjusting for age, BMI, HDL-cholesterol, triglyceride, CRP, smoking status, alcohol intake, and SBP at baseline, GGT was still positively associated with change in SBP in men with statistical significance ( $P < 0.001$ ), but not in women. DBP change also showed significant association with GGT even after adjusting covariates mentioned above ( $P = 0.003$ ) (Table 3). There was significant gender difference in this association ( $P$  for interaction with gender = 0.007). Furthermore, the gender difference still remained significant after adjusting body weight change during 4 yr (Supplemental Table 1). Also, these results still remained constant even when heavy drinkers or documented hepatitis patients were excluded (Table 4).

The age-stratified association between GGT and change in SBP is presented Table 2. In unadjusted model, men showed significantly positive association in the age groups 40-49 yr and 50-59 yr, and also positive associating tendency in the age groups 60-69 yr with marginal significance ( $P = 0.097$ ). After adjusting covariates, men still showed significantly positive association in the age groups 40-49 yr and 60-69 yr and also positive associating tendency in the age groups 50-59 yr with marginal significance ( $P = 0.060$ ). DBP change also showed significant association with GGT in age group of 40-49 yr. However, GGT in women was not associated with change in BP in any age group. Women showed significantly inverse association in the age group 60-69 yr ( $\beta = -1.75$ ,  $P = 0.048$ ).

There was significant age difference in this association in women ( $P$  for interaction with age = 0.021), but not in men ( $P$  for interaction with age = 0.476). In the menopausal status-stratified association between GGT and change in BP after adjusted

covariates, GGT was not associated with BP in premenopause, postmenopause or change from premenopause to postmenopause, either.

#### DISCUSSION

This study showed gender differences in the relation of baseline serum GGT levels to BP change in 4 yr. GGT levels were associated with BP at baseline after adjusting for age, BMI, HDL-cholesterol, triglyceride, CRP, current smoking status and alcohol intake. However, baseline GGT levels were significantly associated with BP change only in men not in women. This suggests that the effect of GGT on BP changes may be somewhat different between men and women.

Previous studies have shown that GGT has been used as a simple marker of alcohol consumption (24) or liver dysfunction (25). Recent studies have demonstrated that baseline GGT is known as the major risk factor for development of CVD, diabetes, after adjusting for alcohol consumption (7, 16).

Several previous cross-sectional and longitudinal studies have also reported a positive association of GGT with BP and hypertension (10-19). However, results of these studies on the difference in the association between men and women did not entirely consistent. Nilssen and Forde reported a positive association between GGT and BP only in women, but not in men (12), but Shankar and Li found no significant gender difference in the association between GGT and BP (18). Others had not studied separately in men and women but in only men (11, 13, 17) or pooled together (10, 14-16, 19). In this study, GGT levels were associated with BP at baseline after adjusting for age, BMI, HDL-cholesterol, triglyceride, CRP, current smoking status and alcohol intake in overall population, whereas the statistical significance was lost after dividing overall population into men and women. That might be result from the reduced sample size and overcorrection.

Our study also showed that there was significant age difference in the association of GGT with BP changes in men and women. Namely, in men, there were positive associations between GGT and change in SBP among those with age groups 40-49, 50-59 (borderline significant) and 60-69 yr, but, in women, only age group 50-59 yr (borderline significant). Generally, aging process is associated with a progressive stiffening arterial structure, and this process induces a rise in BP (26). In men, our study showed that the association of GGT with SBP change was stronger in old age groups. Lee et al. showed that clarified the complex interaction between GGT, BP and age in men. The study demonstrated that the relations of GGT with BP altered by age. There were positive associations between GGT and change in BP in at or greater than 35 yr old, but in less than 35 yr old (27). However, the study was made up in men aged 25 to 50 yr, so it seems unreasonable to compare our study. In women, consid-

**Table 5.** Association between serum  $\gamma$ -glutamyltransferase (GGT) and four year change in systolic blood pressure in men and women

Variables	Men						Women					
	$\beta$	P value	$\beta$	P value	$\beta$	P value	$\beta$	P value	$\beta$	P value	$\beta$	P value
Log GGT (IU/L)	1.51	< 0.001	2.3	< 0.001	1.82	< 0.001	-0.25	0.626	0.40	0.434	0.38	0.466
Age (yr)			0.18	< 0.001	0.19	< 0.001			0.33	< 0.001	0.33	< 0.001
Body mass index ( $\text{kg}/\text{m}^2$ )			0.3	0.003	0.33	0.001			0.05	0.562	0.05	0.562
HDL cholesterol (mg/dL)			-0.09	0.003	-0.11	0.001			-0.05	0.088	-0.05	0.085
Log Triglycerides (mg/dL)			-0.63	0.311	-0.77	0.211			-1.17	0.095	-1.17	0.093
Log CRP (mg/L)			-0.15	0.484	-0.17	0.410			0.24	0.269	0.24	0.259
Systolic blood pressure (mmHg)			-0.48	< 0.001	-0.48	< 0.001			-0.42	< 0.001	-0.42	< 0.001
Alcohol intake (grams/day)					0.03	0.001					0.01	0.725
Current smoker (vs. others)					0.88	0.090					0.55	0.711

GGT,  $\gamma$ -glutamyltransferase; HDL, high-density lipoprotein; CRP, C-reactive protein.

ering that on average, natural menopause occurs between the ages of 42 and 50 yr in Asian populations (28), the association of GGT and BP change in women aged 50 to 59 yr may be explained change in menopausal status. So, when subdivide by menopausal status, we showed the strong positive association in change from premenopause to postmenopause, but it was not statistically significant.

In this report, we could not offer the clear mechanism of gender difference in the association between GGT and the BP. However, we evaluated the association between GGT and other risk factors in men and women (Table 5). The association between GGT and BP in men still remained significant after adjusting BMI and HDL-cholesterol, whereas is no more significant in women after adjusting BMI. That finding suggests GGT in men might be influenced thus reflects other stress condition such as oxidative stress.

Although the mechanism of the association between GGT and BP remains not fully understood, substantial evidence supports the biological plausibility of this finding, including an indirect role of GGT in initiating extracellular catabolism of anti-oxidant glutathione in response to oxidative stress (29). So, GGT might be a sensitive marker for oxidative stress (30). An experimental study found that oxidative stress have been found with higher levels in men (31), the result has been described that testosterone increases whereas estrogen inhibits total body oxidative stress (32, 33). In particular, the antioxidant activity of an estrogen depends on scavenging free radicals decrease reactive oxygen species (32, 34). Longitudinal cohort study and cross-sectional study also showed an association between menopause and BP and change of BP (35, 36). Therefore, a positive association of GGT with change in BP may be explained in considering the decreasing estrogen by transition from the pre- to the post-menopausal. Also previous report suggested the association of GGT and prehypertension incidence (37).

Major strengths of this study are the large prospective design, the length of follow-up, and the standardized protocol. Additionally, information on all major risk factors was collected. However, it has also potential limitations. First, GGT was only measured

at baseline. Therefore, we were unable to examine for the effect change in GGT on BP. Second, the age-related increase in BP becomes steeper after the 40 yr old (26), however our study was conducted subjects aged 40-69 yr. And four years' follow up duration might still be too short in confirming gender difference in GGT levels and BP change. Finally, we did not consider the differences in natural and surgical menopause.

In conclusion, the most important findings of this study are the gender differences in the association between GGT and the change of BP. The definitive reasons for this gender difference remain unclear, and require further investigation.

## DISCLOSURE

The authors declare no conflict of interests.

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# Gamma-Glutamyltransferase Level and Risk of Hypertension: A Systematic Review and Meta-Analysis

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## Abstract

**Background:** Several prospective observational studies suggest that gamma-glutamyltransferase(GGT) level is positively associated with risk of hypertension. However, these studies draw inconsistent conclusions. Therefore, we conducted a systematic review and meta-analysis to evaluate the exact association between GGT level and subsequent development of hypertension.

**Methods:** We searched Pubmed, Embase, and Science Citation Index (ISI Web of Science) for prospective cohort studies examining the association between GGT level and hypertension. Then, pooled effect estimates (RRs) for the association between GGT level and hypertension were calculated.

**Results:** A total of 13 prospective cohort studies including 43314 participants and 5280 cases of hypertension were included. The pooled RR of hypertension was 1.94(95%CI: 1.55–2.43; P<0.001) when comparing the risk of hypertension between the highest versus lowest category of GGT levels. Moreover, the risk of hypertension increased by 23% (summary RR: 1.23; 95%CI: 1.13–1.32; P<0.001) per 1 SD logGGT increment. Subgroup analyses showed significant positive associations in each subgroup except in ≥160/95 subgroup (RR: 2.56, 95%CI: 0.87–7.54; P = 0.088) and nondrinkers subgroup (RR: 1.76, 95%CI: 0.88–3.53; P = 0.113). Sensitivity analyses showed no single study significantly affects the pooled RRs. No publication bias was found in our meta-analysis.

**Conclusions:** GGT level is positively associated with the development of hypertension. Further studies are needed to confirm our findings and elucidate the exact mechanisms between GGT level and the incidence of hypertension.

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## Introduction

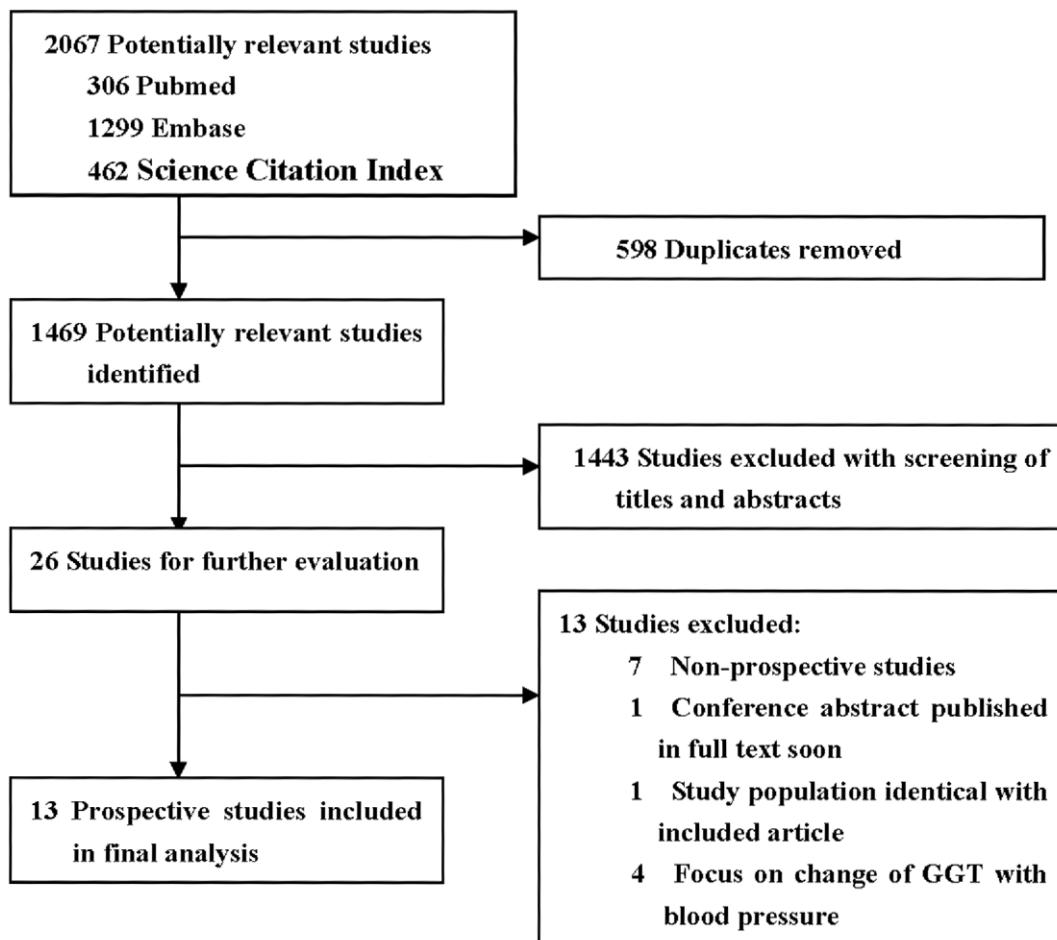
Gamma-glutamyltransferase(GGT) is a common biomarker of liver injury and alcohol consumption [1]. However, recent epidemiologic and clinical studies have also found a close association between GGT level and risk of cardiovascular disease, diabetes, and metabolic syndrome [2,3,4]. The relationships between them were further strengthened by published meta-analyses [5,6,7].

In the past twenty years, the association between GGT and risk of hypertension has been deeply investigated in cross-sectional and longitudinal studies [8–20]. However, these studies drew inconsistent, even opposite conclusions. For example, Kim et al [17] found a significant association between GGT quartiles and hypertension only in the drinkers and non-overweight subgroups, while Stranges et al [15] showed that higher GGT level increased the risk of hypertension in both noncurrent and current drinkers. Because of these inconsistent findings, we therefore conducted a systematic review and meta-analysis to attempt to solve the problem.

## Methods

### Search Strategy

The Pubmed, Embase, and Science Citation Index (ISI Web of Science) databases were searched to collect all publications on the association between GGT and hypertension (last search update: 15th June 2012) without language restriction. The following search terms were used: (Gamma-glutamyltransferase[MeSH] OR Gamma-glutamyltransferase[All Fields] OR gamma-GT[All Fields] OR GGT[All Fields] OR GGTP[All Fields]) AND (Hypertension[MeSH] OR Hypertension [All Fields] OR “Essential Hypertension”[All Fields] OR “Primary Hypertension”[All Fields] OR “High blood pressure”[All Fields]). Similar search terms were applied to Embase and the Science Citation Index. Moreover, reference lists were further retrieved from published articles to avoid missing any relevant studies. Our meta-analysis was performed according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines [21].



**Figure 1. Flow diagram of included studies of meta-analysis.**  
doi:10.1371/journal.pone.0048878.g001

### Study selection

Studies were included if they satisfied the following criteria: 1) the study design should be a prospective design; 2) GGT level in baseline was reported and hypertension as the outcome of interest; 3) the relative risk(RR) or odds ratio(OR) with 95% confidence intervals(CIs) were provided or obtained by calculation.

### Data extraction

Two authors extracted information independently and disagreements were resolved by discussion and consensus. The following data were extracted from each included article: name of the first author, year of publication, country of origin, mean age of the populations, gender component, numbers of participants and cases, durations of follow-up, diagnostic criteria for ascertainment of hypertension, most fully adjusted effect estimates from multivariable for the highest versus the lowest group of GGT level with corresponding 95%CI and study-specific adjusted confounding factors. When the effect estimates of the same population were reported in different follow-up durations, we only included the data with the longest follow-up time.

### Statistical analysis

Relative risk (RR) and 95% CI were chosen as the effect estimate to assess the association between GGT and hypertension. We calculated the combined RR and 95%CI using the most-

adjusted RRs(by comparing the highest versus lowest category of GGT level). Heterogeneity among studies was examined by using chi-square-based Q test and  $I^2$  test. When significant heterogeneity ( $P < 0.05$  and  $I^2 > 50\%$ ) was detected, the pooled RR and 95%CI would be estimated in a random-effect model. Otherwise, a fixed-effect model was chosen. Subgroup analyses were further carried out by race/ethnicity, sex, sample sizes, durations of follow-up, definition of hypertension and number of adjusted confounding factors. In addition, we also analyzed combined RR per 1 SD (standard deviation) logGGT increment in three studies which presented their results (RRs) in per 1 SD logGGT increment.

A sensitivity analysis was performed to evaluate whether the results were markedly affected by a single study. Publication bias was evaluated with Begg's and Egger's test, with a  $P$  value  $> 0.05$  considered as no significant. All statistical analyses were conducted with Stata software, version 11.0 (Stata Corp, College Station, Texas, USA). A  $P$  value  $< 0.05$  was considered statistically significant.

## Results

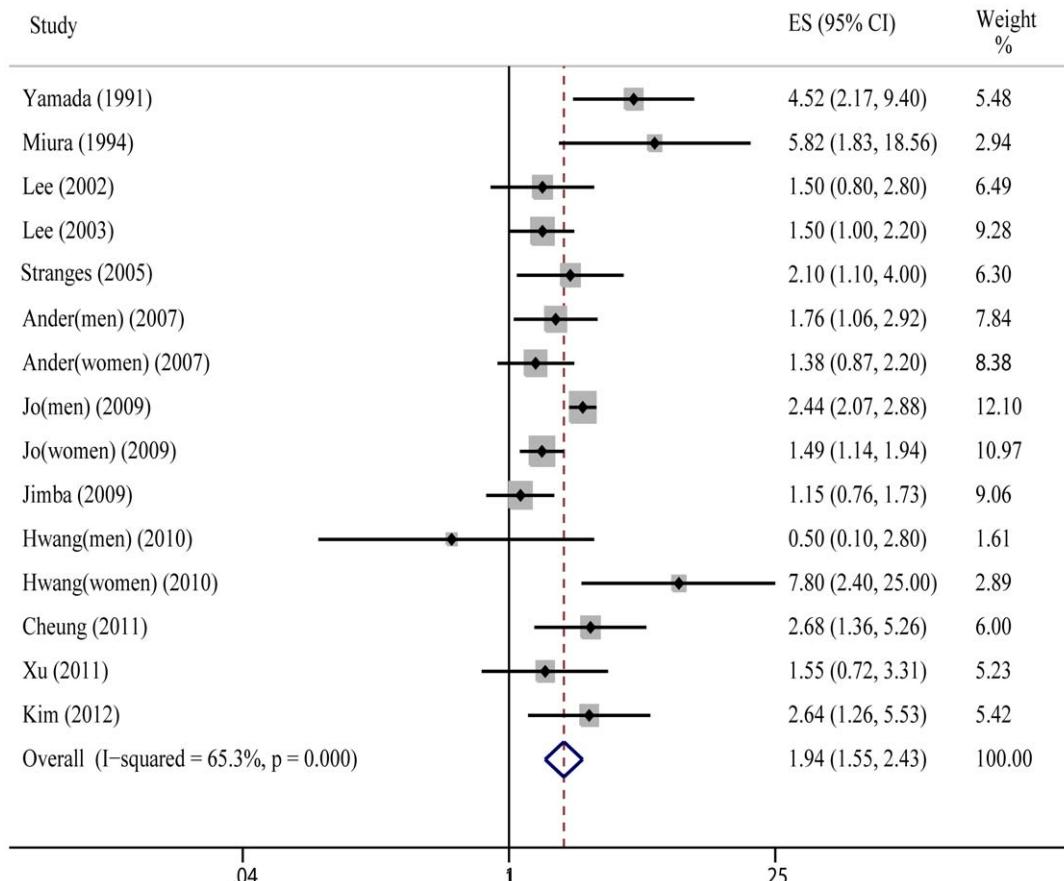
### Literature search

The flow diagram of literature search was shown in Figure 1. 1469 potentially relevant articles were initially identified. Of the 1469 articles identified, 1443 papers were excluded based on titles

**Table 1.** Characteristics of studies included in meta-analysis.

Study	Country	Case/Total [age(y)]	Gender M/F	Definition of Hypertension	Follow-up (years)	Comparison (Highest vs Lowest, U/L)	Adjusted RR(95%CI)	Adjustment for covariates
Yamada [1]: 1991	Japan	29/1393 (35–54)	1393/0	≥160/95	5	≥50 vs <50	4.5(2.17–9.40)	None
Miura [20], 1994	Japan	36/77 (30–69)	77/0	≥140/90, meds	10	≥20 vs <10	5.8(1.83–18.56)	Age, SBP, DBP, alcohol consumption.
Lee [14], 2002	Korea	169/8170 (25–50)	8170/0	≥160/95, meds	4	≥50 vs ≤9	1.4(1.09–1.83)	Age, BMI, smoking, drinking, exercise, family history of hypertension, SBP or DBP, the change of BMI, drinking during four years.
Lee [13], 2003	USA	708/4704 (18–30)	NA	≥140/90, meds	15	>36 vs ≤12	1.5(1.0–2.2)	Age, sex, race, study center, BMI, alcohol consumption, cigarette smoking, physical activity, systolic blood pressure, insulin.
Stranges [15], 2005	USA	195/897 (39–79)	587/310	≥140/90, meds	6	(39–55) vs ≤14	2.1(1.1–4.0)	Age, gender, race, average of alcohol, smoking status, BMI, physical activity, systolic blood pressure.
Ander [10], 2007	France	492/2273 (NA)	1129/1144	≥130/85, meds	3	M: ≥49.4 vs <19.7; F: ≥23 vs <12.6	M:1.76(1.06–2.92); F:1.38(0.87–2.20)	Age
Jo [19], 2009	Korea	2170/17281 (NA)	11659/5622	≥130/85, meds	4	M:>38 vs <19; F:>15 vs <9	M:2.44(2.07–2.88); F:1.49(1.14–1.94)	Age
Jimba [18], 2009	Japan	288/1027 (49±8)	NA	≥130/85, meds	3	≥42 vs ≤24	1.15(0.76–1.73)	Age, sex, alcohol habit, BMI
Hwang [19], 2010	Korea	83/293 (54.1±8.9)	115/176	≥140/90, meds	5	M: >46 vs <17; F: >19 vs <9	M:0.5(0.1–2.8); F:7.8(2.4–25.0)	Age, education, BMI, alcohol intake, cigarette smoking, exercise, salt intake, family history of hypertension, ALT
Chueung [16], 2011	China	126/708 (47.3±9.7)	428/280	≥140/90, meds	5.3	M: ≥31 vs ≤20; F: ≥20 vs ≤13	2.68(1.36–5.26)	Age, sex, systolic blood pressure at baseline, follow-up duration, BMI, triglycerides, HDL cholesterol, HOMA-IR, CRP, fibrinogen, current smoking, change in BMI
Xu [8], 2011	China	119/285 (NA)	NA	≥130/85, meds	3.5	Per 1 SD logGGT increment (41–68) vs <16	1.38(1.05–1.81)	Age, sex
Onat [12], 2012	Turkey	476/1423 (33–84)	735/678	≥140/90, meds	4	Per 1 SD logGGT increment	1.55(0.72–3.31)	Age, sex, menopause, BMI, alcohol usage
Kim [17], 2012	Korea	389/4783 (44±5.8)	3246/1537	≥140/90, meds	3	(29–51) vs ≤12.9	1.20(1.10–1.31)	Age, sex, alcohol amount, smoking status, physical activity, BMI, baseline glucose, uric acid, HDL, LDL, TG, Hs-CRP, baseline systolic blood pressure
							2.638(1.259–5.528)	Age, sex, alcohol amount, smoking status, physical activity, BMI, baseline glucose, uric acid, HDL, LDL, TG, Hs-CRP, baseline systolic blood pressure

Abbreviations: NA: not applicable; M, Man; F, Female; BMI, body mass index; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment index of insulin resistance; CRP, C-reactive protein; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglyceride.  
doi:10.1371/journal.pone.0048878.t001



**Figure 2. Meta-analysis of risk of hypertension between highest vs lowest category of GGT.**  
doi:10.1371/journal.pone.0048878.g002

and abstracts. The remaining 26 articles were then further evaluated by reviewing full text. 13 studies were excluded due to the following reasons: 7 studies not a prospective design, 2 studies repeated with included studies, and 4 studies focused on the change of GGT over time with blood pressure. Finally, 13 articles [8–20] were included in our meta-analysis.

### Study characteristics

Thirteen prospective cohort studies involving 43314 participants and 5280 cases of hypertension were included in our meta-analysis. The characteristics of the 13 articles were summarized in Table 1. Among 13 studies, 10 studies were conducted in Asia, 2 in North-America, and 1 in Europe. The durations of follow-up ranged from 3 to 15 years. As to diagnosis of hypertension, hypertension was defined as blood pressure  $\geq 160/95$  mmHg, 140/90 mmHg, 130/85 mmHg and/or taking antihypertensive medications. Twelve studies including 15 data points (as 3 studies provided their results for men and women separately) offered the RRs and 95%CIs of highest versus lowest category of GGT level. Moreover, three studies [12,16,20] reporting their results in per 1 SD logGGT increment were exclusively analyzed.

### Meta-analysis

As is shown in Figure 2, the pooled RR of hypertension was 1.94(95%CI: 1.55–2.43;  $P < 0.001$ ) for the highest versus lowest category of GGT level; statistical heterogeneity was found in the study results ( $Q = 40.29$ ,  $P = 0.000$ ,  $I^2 = 65.3\%$ ).

As above, three studies [12,16,20] provided their results in per 1 SD logGGT increment. Overall, risk of hypertension increased by 23% (summary RR: 1.23; 95%CI: 1.13–1.32;  $P < 0.001$ ) per 1 SD logGGT increments without evident heterogeneity ( $Q = 1.82$ ,  $P = 0.403$ ,  $I^2 = 0\%$ ).

### Subgroup and sensitivity analyses

The effects of GGT level on risk of hypertension in different subgroups are shown in Table 2. Overall, the positive association between GGT level and risk of hypertension was consistently observed in each subgroup except in  $\geq 160/95$  subgroup (RR: 2.56, 95%CI: 0.87–7.54;  $P = 0.088$ ) and nondrinkers (RR: 1.76, 95%CI: 0.88–3.53;  $P = 0.113$ ).

To evaluate the impact of single studies on the combined results, we conducted sensitivity analyses by omitting one study at a time and reassessed the summary RR for the remaining studies. As is shown in Figure 3, no single study significantly affects the pooled RRs.

### Publication bias

Begg's and Egger's test were performed to test if there was publication bias. As is shown in Figure 4, no publication bias was found (Egger's test:  $P = 0.952$ ).

### Discussion

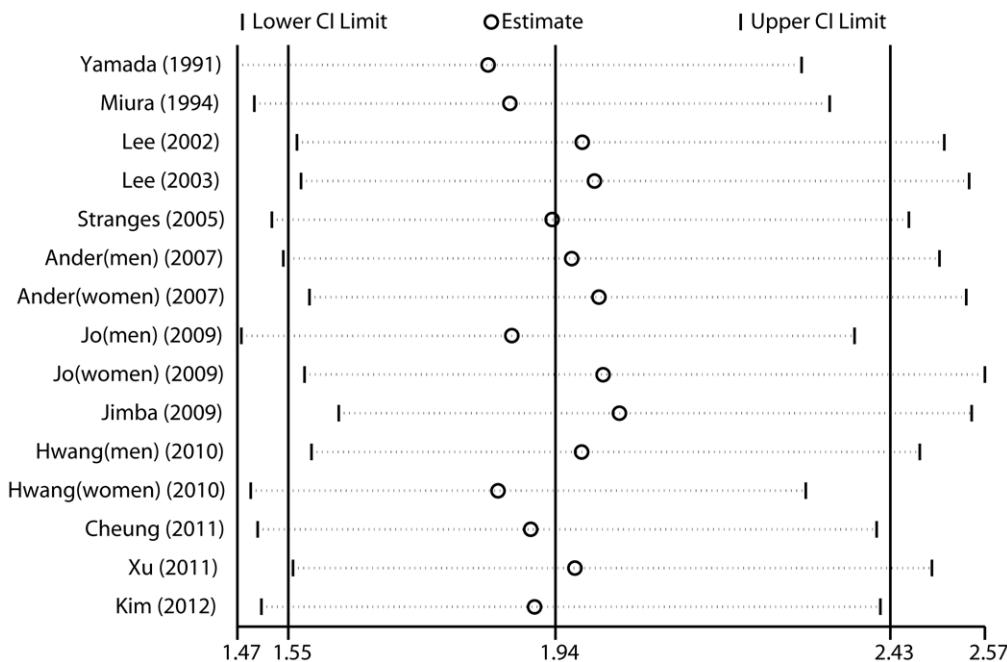
Recent studies suggested that GGT was a novel biomarker of cardiovascular risk. As to the relationship between GGT and

**Table 2.** Subgroup analyses of GGT level and risk of hypertension.

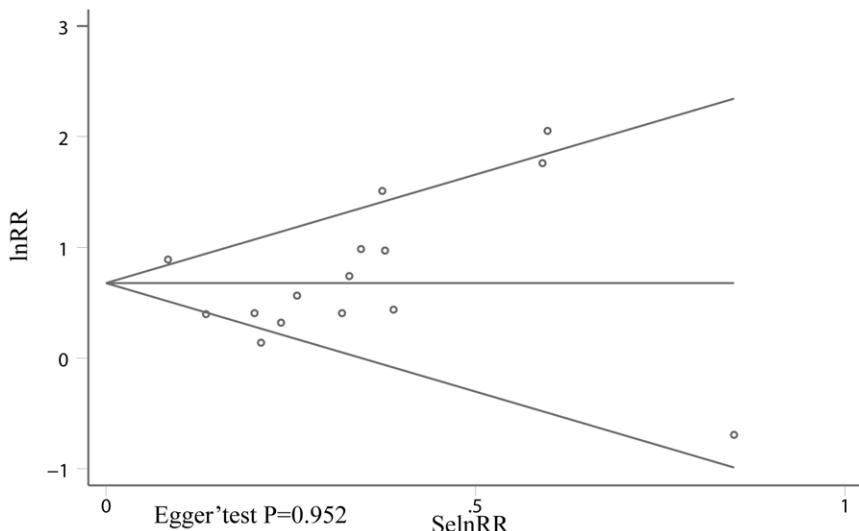
Group	Number of studies	RR(95%CI)	P value for effect estimates	P value for heterogeneity	I <sup>2</sup> (%)
All studies	15a	1.94(1.55–2.44)	<0.001	<0.001	65.3
Race/ethnicity					
Asians	11	2.14(1.58–2.90)	<0.001	<0.001	71.6
Non-Asians	4	1.59(1.25–2.03)	<0.001	0.727	0
Gender					
Men only	6	2.29(1.56–3.37)	<0.001	0.033	58.8
Women only	3	1.91(1.06–3.43)	0.031	0.022	73.8
Both men and women	6	1.70(1.28–2.24)	<0.001	0.203	31.0
Drinking status					
Nondrinkers	5	1.76(0.88–3.53)	0.113	0.136	42.8
Drinkers	4	2.39(1.29–4.44)	0.006	0.051	61.4
Durations, y					
≤5	11	1.87(1.42–2.45)	<0.001	<0.001	70.8
>5	4	2.23(1.40–3.53)	0.001	0.110	50.2
Sample size					
≤1000	5	2.86(1.48–5.54)	0.002	0.053	57.2
>1000	10	1.77(1.39–2.24)	<0.001	0.001	67.8
Definition of HBP					
≥160/95	2	2.56(0.87–7.54)	0.088	0.025	80.1
≥140/90,	7	2.44(1.54–3.86)	<0.001	0.026	58.1
>130/85	6	1.63(1.21–2.18)	0.001	0.001	74.9
Adjustment for covariates					
≤5 factors	8	1.90(1.40–2.57)	<0.001	<0.001	75.3
>5 factors	7	2.05(1.40–3.00)	<0.001	0.066	49.2

a: twelve studies including 15 data points.

doi:10.1371/journal.pone.0048878.t002

**Figure 3. Sensitivity analyses results of given named study omitted.**

doi:10.1371/journal.pone.0048878.g003



**Figure 4. Begg' funnel plot analysis of publication bias.**

doi:10.1371/journal.pone.0048878.g004

hypertension, previous studies have drawn inconsistent conclusions. In this study, we comprehensively evaluated the exact association between GGT level and risk of hypertension. Our present systematic review and meta-analysis of 13 prospective studies showed a significant positive association between GGT level and risk of hypertension (RR: 1.94, 95%CI: 1.55–2.43). Moreover, our results also showed that risk of hypertension increased by 23% (95%CI: 1.13–1.32) per 1 SD logGGT increment. Subgroup analyses showed that the positive association between GGT level and risk of hypertension consistently existed in each subgroup except in ≥160/95 subgroup (RR: 2.56, 95%CI: 0.87–7.54;  $P = 0.088$ ) and nondrinkers subgroup (RR: 1.76, 95%CI: 0.88–3.53;  $P = 0.113$ ).

In our subgroup analyses, an interesting phenomenon was observed: the positive association between GGT level and risk of hypertension was more significant in Asians and male subgroups than that in non-Asians and female subgroups. As is commonly known, Asians have more prevalence of hepatic diseases such as hepatitis B or C, while men are more prone to drink alcoholic beverages. Accordingly, subgroup analysis in nondrinkers showed an increased trend but did not reach a statistical significance (RR: 1.76, 95%CI: 0.88–3.53;  $P = 0.113$ ). Therefore, although most studies in our meta-analysis have adjusted alcohol habits or liver diseases, the possibility that the positive association of GGT and risk of hypertension is explained by alcohol consumption or liver diseases is not completely excluded. However, three studies [15,17,19] performed within normal range of GGT levels showed that relative risk is still much higher in highest category of normal range of GGT levels than that in lowest category (RR: 2.44, 95%CI: 1.15–5.20;  $P = 0.021$ ). Hence, further prospective studies with much more strict inclusion criteria should be conducted to evaluate a more accurate relationship between GGT level and the development of hypertension.

Exact mechanisms that link GGT with hypertension are not fully elucidated, however, several possible explanations are as follows: Longitudinal study showed that GGT is positively associated with inflammation markers such as fibrinogen, C-reactive protein and F2-isoprostanes [13]. In addition, a study found that GGT was expressed in human atherosclerotic lesions

colocalizing with ox-LDL and foam cells, which could contribute to the progression of atherosclerosis [22]. Lastly, GGT plays an important role in the generation of free radical species through its interaction with iron [23]. Base on the above findings, elevated GGT level could be a marker of inflammation condition and oxidative stress, which were important features of hypertension.

Several limitations in our study should be pointed out. First, although most included studies have adjusted for a series of potential confounders, the possibility of residual or unknown confounding still can not be completely excluded. Second, several studies included in our meta-analysis did not specifically exclude subjects with hepatic diseases or alcohol abuse at baseline, this may confuse the true association of GGT with hypertension as liver damage and alcohol intake could affect GGT levels. However, studies that investigated only in nondrinkers [16] or participants without hepatitis [17] and within normal range of GGT levels [15,17,19] also found a positive association between GGT level and risk of hypertension. Third, moderate heterogeneity was observed in our meta-analysis, which is not surprising because of methodological variations, including study population, different ranges of exposure, and number of adjustment factors among studies. To find out source of heterogeneity, we conducted a meta-regression analysis. Regrettably, we did not detect the source of heterogeneity (data not shown). Finally, publication bias may exist for studies with null results as these tend not to be published. However, we did not find evidence of publication bias in our meta-analysis.

In conclusion, our study suggests that GGT level is independently associated with the development of hypertension. However, further studies are needed to confirm our findings and elucidate the exact mechanisms between GGT and the incidence of hypertension.

## Author Contributions

Conceived and designed the experiments: C-FL Y-TG N-YF. Analyzed the data: C-FL Y-TG H-YW. Contributed reagents/materials/analysis tools: C-FL Y-TG H-YW N-YF. Wrote the paper: C-FL N-YF.

# The Association of Oxidative Stress with Hypertensive Retinopathy

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## Abstract

This study was designed to answer the following questions: (i) Do levels of serum gamma-glutamyl transferase (GGT), a marker of oxidative stress, change in hypertensive retinopathy (HR)? (ii) Is there any relation between degree of HR and GGT levels? This study included 80 hypertensive patients with HR. Group 1 comprised 40 patients with grade I HR, and group 2 comprised 40 patients with grade II HR. We selected 40 healthy subjects for the control group. Level of GGT in group 2 was significantly higher than in group 1 ( $P = 0.005$ ) and control group ( $P = 0.001$ ); it was also higher in group 1 than in control group ( $P = 0.025$ ). Our study suggests that oxidative stress, mechanisms known to be involved in vascular lesions, may promote the development of HR.

**Keywords:** gamma-glutamyl transferase, oxidative stress, hypertension, hypertensive retinopathy

## BACKGROUND

Gamma-glutamyl transferase (GGT) is a plasma membrane enzyme with a central role in glutathione homeostasis, which is important in maintaining adequate concentrations of intracellular glutathione to protect cells against oxidants. Elevated serum GGT activity is a sensitive marker of oxidative stress (1). Serum GGT is a clinical marker of several factors: hepatobiliary disease, alcohol consumption, body fat content, plasma lipid/lipoproteins and glucose levels, and medications (2,3). Serum GGT can also reflect other concomitant risk factors such as hypertension (4,5), diabetes mellitus (6,7), obesity (8), dislipidemia, and metabolic syndrome (9). In addition, some studies demonstrated that there is a relationship between diabetic retinopathy and GGT levels (10).

Hypertensive retinopathy (HR) is a condition characterized by a spectrum of retinal vascular signs in people with elevated blood pressure (BP) (11–13). The pathophysiological mechanism of HR is not fully established. Elevated BP alone does not fully account for the extent of retinopathy, other pathogenic mechanisms may be involved, such as increased oxidative stress. Therefore, this study was designed to answer the following questions: (i) Do GGT levels change in HR? (ii) Is there any relation between degree of HR and GGT levels?

## MATERIALS AND METHODS

### Patients

This study was performed at the outpatients' clinic of Department of Internal Medicine of Akdeniz University Hospital. Six hundred and fifty-four adult hypertensive patients were registered in the computer files of our departments. Eighty hypertensive patients without exclusion criteria were invited to participate in this study. None of the patients refused the study. The hypertensive patients were divided into two groups according to the Keith–Wagener classification (14). Group 1 comprised 40 hypertensive patients with grade I HR and group 2 comprised 40 hypertensive patients with grade II HR. Forty normotensive subjects, who were healthy participants and had undergone the checkup program, were used as the control group. The controls had similar body mass index (BMI), age, and sex distribution as the hypertensive group.

The exclusion criteria were as follows: stage 2 hypertension (according to the Eighth Report of the Joint National Committee) with  $BP > 160/100$  mm Hg (15), grades 3 and 4 HR according to the Keith–Wagener Classification (as most of the patients had other complications that could interfere with the GGT results), diabetes mellitus, smoking, alcohol intake more than 30 g/day, hepatitis B or C infection or other known liver diseases, liver enzymes exceeding three times the upper reference range, use of hepatotoxic drugs,

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dyslipidemia, obesity ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ), cardiac, renal, cerebral, and other systemic diseases, recent major surgery or illness.

Dyslipidemia was defined in the presence of at least one of the following conditions: raised plasma triglycerides ( $>200 \text{ mg/dL}$ ), total cholesterol ( $>200 \text{ mg/dL}$ ), low-density lipoprotein (LDL)-cholesterol ( $>130 \text{ mg/dL}$ ), and decreased high-density lipoprotein (HDL)-cholesterol ( $<40 \text{ mg/dL}$  for men and  $<50 \text{ mg/dL}$  for women) (16).

Eligible subjects underwent a comprehensive assessment including documentation of medical history, physical examination, and measurement of laboratory variables. Body weight and height were measured with the subjects in light clothes and without shoes. Body mass index was calculated as the weight ( $\text{kg}/\text{height squared (m}^2\text{)}$ ). The resting electrocardiograms of all the subjects were normal. All patients gave their informed consent to participate in the study.

#### **Measurement of BP**

Arterial BP was measured by a mercury sphygmomanometer after the patient had been in a sitting position for 5 minutes. For each subject, we recorded the average of three readings obtained within 5 minutes. Hypertension was defined as SBP (systolic BP)  $\geq 140 \text{ mm Hg}$  or DBP (diastolic BP)  $\geq 90 \text{ mm Hg}$ , as recommended in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (15).

#### **Fundoscopic Examination**

For the HR evaluation, direct and indirect ophthalmoscopy was performed in all subjects after dilatation of the pupils. A single-blinded observer performed the fundoscopic examinations. The grade of HR was determined according to the Keith-Wagener classification (14).

#### **Biochemical Measurements**

Blood samples were collected from antecubital vein without the use of a tourniquet, between 08.30 and 09.00 hours, after an overnight fast to avoid the differences of diurnal variation. Enzymatic colorimetric assay method (Roche Diagnostics GmbH, Mannheim, Germany) was used to measure triglyceride, cholesterol, and high-density lipoprotein-cholesterol levels. Low-density lipoprotein-cholesterol level was calculated according to the Friedewald formula (17). Fasting glucose level was measured using the enzymatic colorimetric assay method (GLU, Roche Diagnostics GmbH). Serum GGT is measured colorimetrically using nitroanilide method on a Cobas instrument (Roche Diagnostics GmbH).

#### **Statistical Analysis**

Statistical analysis was done using SPSS statistical software (SPSS for Windows 16.0, Chicago, IL, USA). For  $\alpha = 0.05$  (between each group) and a

power of 80%, a sample size per group  $>31$  subjects was needed to detect an actual difference. The normality of the distribution was checked by stem and leaf plots. Gamma-glutamyl transferase values across groups were compared with one-way analysis of variance (ANOVA) followed by the post hoc Bonferroni test. In addition, Pearson's correlation was used to evaluate the association between GGT and degree of HR. Summary data for GGT and other continuous variables are expressed as mean  $\pm$  SD. Statistical significance was defined as  $P < .05$ .

## **RESULTS**

The main characteristics, BPs, and laboratory results of study populations are reported in Table 1. Age, gender distribution, and BMI did not differ among the groups. Similarly, metabolic parameters were not different among the study groups as a result of the selection process.

The level of GGT in group 2 was significantly higher than in group 1 ( $30.57 \pm 6.01 \text{ U/L}$  vs.  $26.57 \pm 6.25 \text{ U/L}$ ,  $P = .004$ ) and normotensive control group ( $30.57 \pm 6.01 \text{ U/L}$  vs.  $23.27 \pm 3.94 \text{ U/L}$ ,  $P = .001$ ); it was also higher in group 1 than in normotensive control group ( $26.57 \pm 6.25 \text{ U/L}$  vs.  $23.27 \pm 3.94 \text{ U/L}$ ,  $P = .025$ ). In addition, GGT showed positive correlation with degree of HR in hypertensive group ( $r = 0.309$ ,  $P = .004$ ).

## **DISCUSSION**

Recent epidemiologic and clinical studies have reported a strong association between GGT, a commonly used biochemical liver test, and hypertension. This association has been shown to be independent of alcohol consumption and to be present among both drinkers and nondrinkers (18,19). Our findings are consistent with previous work. On the contrary, this is the first study, to the best of our knowledge, specifically, to evaluate GGT levels in hypertensive patients with HR. Our study demonstrates that hypertensive patients with retinopathy have increased GGT activity, a marker of oxidative stress. In addition, GGT levels showed positive correlations with a degree of HR in hypertensive group.

Recent studies suggest that excessive production of reactive oxygen species, outstripping endogenous antioxidant defense mechanisms, may be involved in the pathogenesis and complications of hypertension (20–22). Giner et al. (23) reported that oxidative stress is a determinant of microalbuminuria independent BP levels in hypertensive patients. Minuz et al. (24) demonstrated increased oxidative stress and persistent platelet activation in essential hypertension with advanced vascular lesions. Purushothaman et al. (25) reported that oxidative stress promotes left ventricular hypertrophy.

Table 1. The main characteristics and laboratory results of the study groups

Parameters	Group 1	Group 2	Control group
<i>n</i> (men/women)	40 (21/19)	40 (20/20)	40 (20/20)
Age (y)	53 ± 2	52 ± 9	53 ± 1
BMI (kg/m <sup>2</sup> )	25.7 ± 3.1	25.3 ± 3.2	25.4 ± 3.0
SBP (mm Hg)	150 ± 4.6**	149 ± 4.8**	127 ± 4.9
DBP (mm Hg)	98 ± 5.7**	98 ± 5.6**	81 ± 4.7
Fasting glucose (mg/dL)	87.4 ± 9.7	87.9 ± 9.8	87.5 ± 9.6
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
Alanine aminotransferase (U/L)	25.7 ± 3.5	26.6 ± 3.6	25.9 ± 3.5
Total cholesterol (mg/dL)	170.0 ± 22.5	169.4 ± 22.8	169.8 ± 22.9
LDL-cholesterol (mg/dL)	84.3 ± 11.1	83.9 ± 12.2	84.0 ± 12.3
HDL-cholesterol (mg/dL)	50.2 ± 5.2	50.8 ± 5.0	50.4 ± 5.1
Triglyceride (mg/dL)	127.9 ± 15.8	128.0 ± 16.2	127.7 ± 16.7
White blood cell ( $\times 10^9$ /L)	5.8 ± 1.5	5.9 ± 1.6	5.9 ± 1.4
GGT (U/L)	26.57 ± 6.25*	30.57 ± 6.01***†	23.27 ± 3.94

Abbreviations: BMI – body mass index; DBP – diastolic blood pressure; GGT – gamma-glutamyl transferase; HDL – high-density lipoprotein; LDL – low-density lipoprotein.

\* $P < .05$ , group 1 versus control group; \*\* $P < .001$ , groups 1 and 2 versus control group; \*\*\* $P < .001$ , group 2 versus control group; † $P < .005$  group 1 versus group 2.

Hypertensive retinopathy is an important complication and a major site of target organ damage from hypertension. It is known that the autoregulation of the retinal circulation fails as BP increases beyond a critical limit. However, elevated BP alone does not fully account for the extent of HR (26–28). There are cases in which retinopathy was resolved despite the persistence of high BP (29). Although the BP levels in group 1 and group 2 were similar, levels of GGT were higher in group 2 than in group 1 in our study. Thus, the presence of high GGT levels in HR and the correlation of the amount of GGT with the severity of HR imply that oxidative stress may be involved in the mechanism of HR. Moreover, recently we found that there is a relationship between levels of ferritin, a marker of oxidative stress, and HR in essential hypertension (30).

This study has some limitations. Firstly, we accept that our study is a case-control design; it is not easy to predict exactly whether the high GGT levels precede retinopathy or vice versa. Future cohort studies will be helpful in providing an answer. Secondly, the study was conducted while the patients were taking antihypertensive treatment. However, distribution of drug use was similar in both hypertensive groups. Thirdly, grade 1–2 HR is not specific to hypertension.

## CONCLUSION

Our study suggests that there is a relationship between HR and GGT levels in essential hypertension. Oxidative stress, mechanisms known to be involved in vascular lesions, may promote the development of HR. Also upcoming studies will point out whether or not GGT is a predictor of hypertensive vascular outcomes.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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# Serum $\gamma$ -glutamyltransferase within its normal concentration range is related to the presence of diabetes and cardiovascular risk factors

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## Abstract

**Aims** Although many studies have reported an association between serum  $\gamma$ -glutamyltransferase (GGT) and cardiovascular risk factors, the mechanism of this relationship has not been clarified.

**Methods** The medical records of 29 959 subjects (age, median 48, range 14–90 years; 16 706 men, 13 253 women) who visited the Center for Health Promotion at Samsung Medical Center for a medical check-up between January 2001 and December 2003, were investigated. Subjects with hepatic enzyme/GGT concentrations higher than three times the upper limit of the reference range, a positive test for hepatitis C virus antibody, a positive test for hepatitis B virus surface antigen, currently taking anti-diabetic/anti-hypertensive/anti-lipid medication, or a white blood cell (WBC) count higher than 10 000 cells/ml, were excluded. The subjects of each gender were classified into five groups according to their serum GGT concentrations, into quartiles of the normal range of GGT (groups 1, 2, 3 and 4) and into a group with elevated GGT (group 5).

**Results** As the group number increased (group 1 → 5), the frequencies of all of the following increased: (i) diabetes and impaired fasting glucose (IFG); (ii) hypertension, obesity (body mass index  $\geq 27 \text{ kg/m}^2$ ), dyslipidaemia (LDL-cholesterol  $\geq 4.1 \text{ mmol/l}$  and/or triglyceride  $\geq 2.46 \text{ mmol/l}$ , or HDL-cholesterol  $< 1.16 \text{ mmol/l}$ ); (iii) metabolic syndrome. Moreover, these significant relationships between GGT concentrations within its normal range and the presence of diabetes/IFG, hypertension, obesity, dyslipidaemia, and metabolic syndrome persisted after adjusting for several clinical and biochemical variables and for the presence of fatty liver based on ultrasonographic findings. Odds ratios (95% CI) for group 4 (highest quartile of normal range of GGT) vs. group 1 (lowest quartile of normal range of GGT); the referent group, were 3.16 (2.15–4.65) for diabetes, 2.24 (1.73–2.90) for IFG, 1.93 (1.59–2.33) for obesity, 1.38 (1.23–1.55) for dyslipidaemia and 2.88 (2.28–3.65) for metabolic syndrome in men. In women, the odds ratios were 2.72 (1.34–5.52), 3.67 (2.26–5.97), 2.10 (1.61–2.74), 1.80 (1.58–2.04) and 3.57 (2.52–5.07), respectively.

**Conclusions** Our data show that, even within its normal range, serum GGT concentrations are closely associated with the presence of diabetes and cardiovascular risk factors, and that these associations are independent of a fatty liver by ultrasonography.

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**Keywords** diabetes,  $\gamma$ -glutamyltransferase, Korea, metabolic syndrome

**Abbreviations** ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GGT, serum  $\gamma$ -glutamyl transferase; HDL-C, HDL-cholesterol; IFG, impaired fasting glucose; LDL-C, LDL-cholesterol; NFG, normal glucose tolerance; SBP, systolic blood pressure; WBC, white blood cell

## Introduction

Serum  $\gamma$ -glutamyl transferase (GGT) is one of the biliary enzymes and is synthesized in epithelial cells of the intrahepatic duct [1]. In addition to its diagnostic uses as an index of liver dysfunction, and as a biological marker of alcohol intake, serum GGT is of substantial epidemiological significance. Several population studies have shown a strong cross-sectional association between serum GGT concentrations and many cardiovascular risk factors [2–4]. In addition, a number of prospective studies have identified baseline serum GGT concentration as an independent risk marker for the development of both cardiovascular and cerebrovascular disease [5–9]. Excess deposition of fat in the liver, usually termed non-alcoholic fatty liver disease, is strongly associated with elevated serum GGT, obesity, insulin resistance and hyperinsulinaemia [5,10–12]. The Samsung Medical Center is a 1300-bed hospital and one of the largest referral centres in Korea. More than 10 000 subjects visit the Center for Health Promotion at the Samsung Medical Center for an annual medical check-up. Information on lifestyle factors, past medical history, and a family history of diabetes was obtained for all participants by trained nurses. In addition, physical examination, laboratory testing, including serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), GGT, hepatitis C virus antibody and hepatitis B virus surface antigen is conducted routinely. In particular, abdominal ultrasonography is performed in each case according to a standard protocol. Because many factors can affect the relationships between serum GGT concentrations and cardiovascular risk factors, it is essential to adjust for several confounding variables to elucidate the true nature of these associations. In this study, we investigated the relationship between serum GGT concentrations and the frequencies of diabetes, hypertension, dyslipidaemia, obesity and metabolic syndrome, after adjustment for several clinical and biochemical variables and for the presence of fatty liver, based on an ultrasonographic finding.

## Subjects and methods

### Subjects

The medical records of the 29 959 subjects (age, median 48, range 14–90 years; 16 706 men, 13 253 women) who visited our Center for Health Promotion for a medical check-up between 2001 and 2003 were investigated. As routine medical

checks are not covered by the Korean medical insurance system, we suspect that most of our study subjects were members of the upper-middle economic class. Subjects meeting any of the following criteria were excluded: hepatic enzyme/GGT concentrations higher than three times the upper limit of the reference range, a positive test for hepatitis C virus antibody, a positive test for hepatitis B virus surface antigen, those currently taking anti-diabetic/anti-hypertensive/anti-lipid medications, or a white blood cell (WBC) count higher than 10 000 cells/ml. Men and women were classified separately into five groups according to their serum GGT concentrations, into quartiles of the normal range of GGT and into an elevated GGT group (Table 1).

Diabetes was defined as fasting plasma glucose  $\geq 7.0$  mmol/l; hypertension as a systolic blood pressure  $\geq 140$  mmHg and/or a diastolic blood pressure  $\geq 90$  mmHg; dyslipidaemia as a serum LDL-cholesterol (LDL-C)  $\geq 4.2$  mmol/l and/or triglyceride  $\geq 2.46$  mmol/l and/or HDL-cholesterol (HDL-C)  $< 1.16$  mmol/l; obesity as a body mass index  $\geq 27$  kg/m<sup>2</sup>. Metabolic syndrome was defined as three or more of the following abnormalities: body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup>; triglyceride  $\geq 1.7$  mmol/l; HDL-cholesterol  $< 1.04$  mmol/l; fasting plasma glucose (FPG)  $\geq 6.1$  mmol/l; systolic blood pressure (SBP)  $\geq 130$  mmHg and diastolic blood pressure (DBP)  $\geq 85$  mmHg [13]. We used body mass index as a parameter of obesity, because data on waist circumference were not available. This study was approved by the Internal Review Board (IRB) of the Samsung Medical Center.

### Assay methods

Height and weight were measured with subjects wearing light clothing, but no shoes, in the morning; blood pressure was measured with a mercury sphygmomanometer on the right arm with subjects in a sitting position after a 5-min rest. Body mass index was calculated as weight in kilograms divided by the square of the height in metres. Information on lifestyle factors including alcohol consumption, cigarette smoking and a family history of diabetes were obtained by trained nurses. A family history of diabetes was defined as a mother, father, sister or brother with diagnosed diabetes. Questions about alcohol intake included items about frequency of alcohol consumption per week and type of alcoholic beverage. Weekly alcohol intake was calculated and then converted to daily alcohol consumption. Subjects were classified as non-drinkers or current drinkers when they averaged  $< 180$  or 181 to  $< 360$  g/week of alcohol, respectively. Heavy alcohol drinking was defined as  $\geq 360$  g/week of alcohol. Blood samples were obtained in the morning after an overnight fast. Plasma glucose was measured in duplicate by the hexokinase method using an autoanalyser

**Table 1** Clinical characteristics of study subjects according to the level of serum  $\gamma$ -glutamyl transferase

Serum GGT (IU/ml)	Men					Women				
	2–20 (n = 3632)	21–26 (n = 2850)	27–35 (n = 3215)	36–50 (n = 3056)	51–150 (n = 3953)	2–11 (n = 3359)	12–14 (n = 3289)	15–19 (n = 3223)	20–50 (n = 2975)	51–150 (n = 407)
Age (years)	48.8 ± 11.7	49.1 ± 10.5	49.0 ± 9.8	48.3 ± 9.2	47.1 ± 8.5	45.1 ± 9.1	47.3 ± 9.5	48.7 ± 9.5	50.3 ± 9.3	51.7 ± 9.3
Alcohol drinking (%)	63.8	81.3	83.3	86.2	92.8	27.4	26.5	30.8	32.2	30
Heavy alcohol drinking (%)	41.3	50.5	59.8	67.5	79.4	4.8	5.3	8.5	10.3	11.3
Current smoking (%)	28.9	34.7	36.1	42.4	50.0	0.9	3.7	5.2	6.5	7.9
BMI (kg/m <sup>2</sup> )	23.0 ± 2.5	23.9 ± 2.4	24.4 ± 2.4	24.9 ± 2.6	25.3 ± 2.6	21.9 ± 2.4	22.4 ± 2.6	22.9 ± 2.8	23.7 ± 3.1	24.2 ± 3.1
BMI ≥ 27 kg/m <sup>2</sup> (%)	5.2	9.0	13.0	20.0	23.8	2.5	5.0	7.9	14.4	16.8
Body fat percentage	16.5 ± 4.6	21.0 ± 4.9	21.9 ± 4.3	22.8 ± 4.4	23.5 ± 4.6	27.1 ± 4.8	28.2 ± 5.1	29.2 ± 5.3	30.6 ± 5.6	31.7 ± 5.7
FPG (mmol/l)	5.07 ± 0.62	5.23 ± 0.93	5.33 ± 0.99	5.42 ± 1.02	5.53 ± 1.08	4.84 ± 0.50	4.95 ± 0.51	5.04 ± 0.67	5.19 ± 0.83	5.40 ± 1.14
IFG (%) / diabetes (%)	2.7/1.0	4.0/2.4	6.8/3.1	7.7/4.5	10.8/5.7	0.7/0.3	2.1/0.4	3.2/1.0	5.2/2.8	7.9/4.9
SBP (mmHg)	116.7 ± 16.2	117.8 ± 15.9	119.7 ± 15.9	120.3 ± 16.0	122.5 ± 16.5	109.3 ± 15.2	112.9 ± 24.6	113.3 ± 17.2	116.4 ± 18.0	117.8 ± 18.0
DBP (mmHg)	72.9 ± 10.7	74.2 ± 10.5	75.9 ± 10.6	76.2 ± 10.7	77.9 ± 11.1	66.6 ± 10.6	68.2 ± 11.0	68.6 ± 11.3	70.0 ± 11.3	71.1 ± 12.4
Hypertension (%)	9.3	9.3	10.7	12.2	15.0	4.5	7.5	8.6	11.6	14.0
LDL-C (mmol/l)	3.28 ± 0.77	3.45 ± 0.78	3.58 ± 0.80	3.63 ± 0.81	3.63 ± 0.87	3.10 ± 0.77	3.29 ± 0.84	3.45 ± 0.89	3.61 ± 0.92	3.67 ± 0.93
Triglyceride (mmol/l)	1.14 ± 0.56	1.36 ± 0.68	1.55 ± 0.81	1.74 ± 0.93	2.07 ± 1.22	0.92 ± 0.45	1.03 ± 0.54	1.15 ± 0.63	1.34 ± 0.76	1.63 ± 1.04
HDL-C (mmol/l)	1.33 ± 0.32	1.30 ± 0.32	1.28 ± 0.31	1.27 ± 0.29	1.27 ± 0.30	1.55 ± 0.34	1.55 ± 0.36	1.51 ± 0.36	1.47 ± 0.36	1.47 ± 0.36
Dyslipidaemia (%)	43.4	51.8	60.2	65.6	71.7	21.7	30.4	37.6	48.6	53.9
WBC (cell/ml)	5732 ± 1348	5949 ± 1365	6023 ± 1360	6234 ± 1375	6358 ± 1405	5193 ± 1302	5334 ± 1243	5518 ± 1369	5678 ± 1390	5903 ± 1465
AST (IU/ml)	20.2 ± 5.4	21.4 ± 5.6	22.9 ± 6.5	24.9 ± 7.7	28.5 ± 9.4	18.0 ± 4.8	18.8 ± 4.7	20.0 ± 5.3	23.0 ± 8.2	30.6 ± 13.9
ALT (IU/ml)	20.4 ± 8.1	24.1 ± 10.1	28.2 ± 12.6	33.5 ± 16.9	41.1 ± 20.2	14.4 ± 5.2	16.2 ± 6.2	18.8 ± 7.9	25.2 ± 13.7	39.5 ± 21.7
GGT (IU/ml)	16.3 ± 3.0	23.5 ± 1.7	30.7 ± 2.6	42.3 ± 4.3	78.1 ± 24.4	9.6 ± 1.5	13.0 ± 0.8	16.7 ± 1.4	27.6 ± 7.5	74.3 ± 22.3
Family history of diabetes (%)	8.8	9.8	11.3	13.1	13.6	12.9	12.6	12.5	15.4	17.2
College or university graduation (%)	58.3	58.6	57.2	58.8	57.1	60.6	57.9	59.3	57.3	62.3
Household income ≥ 40 000 \$US/year (%)	54.9	54.0	55.4	54.4	53.9	56.2	54.2	54.9	56.7	55.0
Fatty liver in ultrasonography (%)	22.1	35.2	47.7	57.3	60.0	7.9	14.0	23.8	36.5	46.6
Metabolic syndrome (%)	2.8	6.1	10.1	14.9	20.1	1.2	2.9	5.8	11.2	16.9

Data are means ± SD or percentage.

ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG fasting plasma glucose; GGT, serum  $\gamma$ -glutamyl transferase; HDL-C, HDL-cholesterol; IFG, impaired fasting glucose; LDL-C, LDL-cholesterol; SBP, systolic blood pressure; WBC, white blood cell.

Definitions: heavy alcohol drinking as an alcohol intake ≥ 360 g/week; hypertension as an SBP ≥ 140 mmHg and/or a DBP ≥ 90 mmHg; dyslipidaemia as an LDL-C ≥ 4.1 mmol/l and/or triglyceride ≥ 2.46 mmol/l and/or HDL-C &lt; 1.16 mmol/l.

Metabolic syndrome was defined as having three or more of the following abnormalities: BMI ≥ 25 kg/m<sup>2</sup>; triglyceride ≥ 1.7 mmol/l; HDL-C < 1.04 mmol/l; FPG ≥ 6.1 mmol/l; SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg.

(Hitachi, Tokyo, Japan), which had an interassay coefficient of variation of 1.6%. Standard liver testing, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured using an autoanalyser (Hitachi, Tokyo, Japan), as were white blood cell counts (Sysmex, Kobe, Japan). HBsAg was measured using a commercially available immunoradiometric assay (Riakey, Koyang, Korea), and anti-HCV was also tested by immunoradiometric assay (Riakey, Koyang, Korea). Per cent of body fat was measured by bioelectrical impedance analysis (In Body 3.0, Biospace, Seoul, Korea).

Three grades were defined for fatty infiltration of liver: grade 1 (mild), slight diffuse increase in the fine echoes in the hepatic parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders; grade 2 (moderate), moderate diffuse increase in the fine echoes with slightly impaired visualization of the diaphragm and intrahepatic vessels; and grade 3 (severe), marked increase in the fine echoes with poor or no visualization of the diaphragm, intrahepatic vessels, and posterior portion of the right lobe of the liver [14]. In this study, any degree of fatty infiltration including mild, moderate and severe infiltrations were considered as fatty liver. A total of 27 radiologists (medical doctors who were expert on abdominal ultrasonography) performed full standard abdominal ultrasonography. There was no difference in the frequencies of fatty liver according to the radiologist ( $\chi^2 = 22.3$ ).

### Statistics

Data are expressed as means  $\pm$  1 SD. ANOVA or  $\chi^2$  tests were used to compare group variables. Logistic regression analyses were

used to obtain odds ratio for diabetes, impaired fasting glucose, hypertension, dyslipidaemia, obesity and metabolic syndrome after adjusting for several clinical and biochemical variables and the presence of fatty liver. Statistical analyses were performed using the SPSS/PC<sup>+</sup> software program (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at a level of  $P < 0.05$ .

## Results

The clinical characteristics of the study subjects according to serum  $\gamma$ -glutamyl transferase are presented in Table 1. Univariate analyses showed that the following increased in frequency with increasing GGT: heavy alcohol drinking, current smoking, family history of diabetes, obesity, impaired fasting glucose, diabetes, hypertension, dyslipidaemia, fatty liver and metabolic syndrome in both genders. Serum white blood cell count, serum alanine aminotransferase and serum aspartate aminotransferase increased with increasing GGT in both genders. There was no significant difference in income and educational background with increasing serum GGT. In the logistic regression analyses between the lowest quartile of the normal range of GGT (group 1) and the highest quartile (group 4), age, WBC, ALT, AST, the presence of family history of diabetes, smoking history, alcohol consumption, abnormal fasting glucose, dyslipidaemia, obesity and fatty liver independently predicted serum GGT, in both genders (Table 2). Hypertension was a significant predictor of serum GGT in women only.

**Table 2** Odds ratios (95% CI) in the highest quartile of the normal range of GGT with the lowest quartile as the referent group

	Men		Women	
	Odds ratios (95% CI)	P	Odds ratios (95% CI)	P
Age	1.01 (1.00–1.02)	< 0.05	1.03 (1.02–1.04)	< 0.001
WBC	1.16 (1.11–1.22)	< 0.001	1.18 (1.12–1.24)	< 0.001
AST	1.01 (0.99–1.02)	NS	0.96 (0.95–0.98)	< 0.001
ALT	1.11 (1.09–1.12)	< 0.001	1.22 (1.03–1.50)	< 0.001
Family history of diabetes	1.33 (1.08–1.63)	< 0.01	1.24 (1.03–1.50)	< 0.05
Smoking history	P for trend	< 0.001	P for trend	< 0.01
Ex-smoker	1.21 (1.02–1.43)	< 0.05	1.20 (0.90–1.60)	NS
Current smoker	2.14 (1.79–2.55)	< 0.001	1.75 (1.29–2.37)	< 0.001
Alcohol history	P for trend	< 0.001	P for trend	< 0.001
< 180 g/week	1.04 (0.82–1.33)	NS	1.49 (1.25–1.79)	< 0.001
181–360 g/week	1.53 (1.20–1.94)	< 0.01	2.18 (1.67–2.83)	< 0.001
≥ 360 g/week	4.82 (4.01–5.79)	< 0.001	4.28 (3.33–5.51)	< 0.001
Abnormal fasting glucose	P for trend	< 0.001	P for trend	< 0.001
Impaired fasting glucose	2.20 (1.62–2.99)	< 0.001	3.70 (2.19–6.27)	< 0.001
Diabetes	2.96 (1.91–4.60)	< 0.001	2.49 (1.16–5.36)	< 0.05
Hypertension	1.09 (0.89–1.34)	NS	1.37 (1.05–1.77)	< 0.05
LDL-cholesterol ≥ 4.1 mmol/l	1.78 (1.51–2.09)	< 0.001	1.98 (1.66–2.36)	< 0.001
Triglyceride = 2.24 mmol/l	2.68 (2.26–3.18)	< 0.001	1.91 (1.50–2.43)	< 0.001
HDL-cholesterol < 1.16 mmol/l	1.33 (1.15–1.54)	< 0.001	0.92 (0.76–1.120)	NS
BMI ≥ 27 kg/m <sup>2</sup>	1.72 (1.38–2.14)	< 0.001	2.06 (1.53–2.76)	< 0.001
Fatty liver	2.11 (1.83–2.43)	< 0.001	2.00 (1.65–2.41)	< 0.001

Age, WBC, AST and ALT were continuous variables. Diabetes, fasting plasma glucose (FPG)  $\geq$  7.0 mmol/l; impaired fasting glucose (IFG), FPG  $\geq$  6.1 mmol/l. Subjects with GGT (2–20) in men and those with GGT (2–11) in women were the referent group, respectively. Range of GGT in the highest quartile of men was 36–50 and that of women was 20–50 IU/ml, respectively. Hypertension was defined as an systolic blood pressure  $\geq$  140 mmHg and/or a diastolic blood pressure  $\geq$  90 mmHg.

**Table 3** Odds ratios (95% CI) for diabetes and impaired fasting glucose according to serum  $\gamma$ -glutamyl transferase concentrations

Men			Women		
GGT (IU/ml)	Diabetes	IFG	GGT (IU/ml)	Diabetes	IFG
21–26	1.96 (1.31–2.95)*	1.35 (1.02–1.78)**	12–14	0.96 (0.42–2.19)†	2.42 (1.47–3.99)*
27–35	2.30 (1.56–3.40)	2.12 (1.65–2.73)	15–19	1.48 (0.71–3.12)†	3.06 (1.89–4.96)
36–50	3.16 (2.15–4.65)	2.24 (1.73–2.90)	20–50	2.72 (1.34–5.52)*	3.67 (2.26–5.97)
51–150	3.82 (2.58–5.67)	3.16 (2.43–4.09)	51–150	2.72 (1.11–6.69)**	4.31 (2.29–8.12)

Diabetes, fasting plasma glucose (FPG)  $\geq 7.0$  mmol/l; impaired fasting glucose (IFG), FPG  $\geq 6.1$  mmol/l. Subjects with GGT (2–20) in men and those with GGT (2–11) in women were the referent group, respectively. Subjects with impaired fasting glucose were excluded to calculate the odds ratio for diabetes, and those with diabetes were excluded for impaired fasting glucose.

\* $P < 0.01$ , \*\* $P < 0.05$ , †not significant. All other odds ratio were  $P < 0.001$ . All odds ratio were obtained after adjustment for age, family history of diabetes, smoking history, alcohol history, body mass index, body fat per cent, white blood cell counts, aspartate aminotransferase, alanine aminotransferase, systolic and diastolic blood pressure, serum LDL-cholesterol, HDL-cholesterol, triglyceride, the presence of fatty liver based on ultrasonographic finding and radiologist who performed ultrasonogram.

GGT (IU/ml)	Dyslipidaemia	Obesity	Hypertension
Men			
21–26	1.09 (0.98–1.21)†	1.29 (1.05–1.58)**	0.88 (0.74–1.05)†
27–35	1.32 (1.18–1.47)	1.48 (1.22–1.80)	0.95 (0.80–1.13)†
36–50	1.38 (1.23–1.55)	1.93 (1.59–2.33)	1.05 (0.88–1.25)†
51–150	1.65 (1.46–1.86)	1.93 (1.59–2.35)	1.26 (1.05–1.50)**
Women			
12–14	1.27 (1.13–1.43)	1.42 (1.08–1.87)**	1.29 (1.04–1.61)**
15–19	1.45 (1.29–1.63)	1.64 (1.26–2.14)	1.22 (0.98–1.51)†
20–50	1.80 (1.58–2.04)	2.10 (1.61–2.74)	1.33 (1.06–1.68)**
51–150	1.72 (1.34–2.22)	1.62 (1.07–2.43)**	1.35 (0.91–2.00)†

Obesity was defined as body mass index  $\geq 27$  kg/m<sup>2</sup>, dyslipidaemia as an LDL-C  $\geq 4.1$  mmol/l and/or triglyceride  $\geq 2.46$  mmol/l and/or HDL-C  $< 1.16$  mmol/l, and hypertension as a systolic blood pressure  $\geq 140$  mmHg and/or a diastolic blood pressure  $\geq 90$  mmHg. Subjects with GGT (2–20) in men and those with GGT (2–11) in women were the referent group, respectively.

\* $P < 0.01$ , \*\* $P < 0.05$ , †not significant. All other odds ratio were  $P < 0.001$ . Odds ratios for dyslipidaemia; adjusted for age, body mass index, body fat per cent, smoking history, alcohol history, white blood cell counts, aspartate aminotransferase, alanine aminotransferase, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, the presence of fatty liver by ultrasonography and radiologist who performed ultrasonogram. Odds ratios for obesity; adjusted for age, smoking history, alcohol history, white blood cell count, aspartate aminotransferase, alanine aminotransferase, systolic blood pressure, diastolic blood pressure, LDL-C, triglyceride, HDL-C, fasting plasma glucose, the presence of fatty liver by ultrasonography and radiologist who performed ultrasonogram. Odds ratios for hypertension; adjusted for age, smoking history, alcohol history, body mass index, body fat per cent, white blood cell count, aspartate aminotransferase, alanine aminotransferase, LDL-C, triglyceride, HDL-C, fasting plasma glucose, the presence of fatty liver by ultrasonography and radiologist who performed ultrasonogram.

Table 3 shows that a higher concentration of serum GGT, even within its normal range, is a risk factor for diabetes and impaired fasting glucose, and that these relationships are independent of age, gender, a family history of diabetes, smoking history, alcohol history, body mass index, white blood cell count, liver enzyme, blood pressure, lipid profile or the presence of fatty liver. Table 4 presents odds ratios for obesity and dyslipidaemia according to serum GGT concentrations and shows that an elevated serum GGT concentration is an independent risk factor for obesity and dyslipidaemia. However, the relationship between hypertension and serum GGT was not consistent (Table 4). Odds ratios (95% CI) for the meta-

**Table 4** Odds ratios (95% CI) for dyslipidaemia, obesity and hypertension according to serum  $\gamma$ -glutamyl transferase concentrations

bolic syndrome in the highest quartile of normal range of GGT compared with the lowest quartile of normal range of GGT; the referent group were 2.88 (2.28–3.65) in men and 3.57 (2.52–5.07) in women (Table 5).

## Discussion

Our analysis demonstrates that the serum GGT concentration, even within its normal range, is closely related to the presence of components of the metabolic syndrome. Although several studies have already revealed these associations, these have usually involved a relatively small numbers of subjects and the

**Table 5** Odds ratios (95% CI) for metabolic syndrome according to serum  $\gamma$ -glutamyl transferase concentrations

Men			Women		
GGT (IU/ml)	Model 1	Model 2	GGT (IU/ml)	Model 1	Model 2
21–26	1.99 (1.55–2.56)	1.69 (1.31–2.18)	12–14	1.89 (1.30–2.74)*	1.67 (1.15–2.44)*
27–35	3.03 (2.40–3.83)	2.32 (1.83–2.94)	15–19	3.19 (2.25–4.51)	2.53 (1.78–3.60)
36–50	4.01 (3.18–5.05)	2.88 (2.28–3.65)	20–50	4.90 (3.48–6.91)	3.57 (2.52–5.07)
51–150	4.74 (3.76–5.98)	3.57 (2.82–4.51)	51–150	4.87 (3.10–7.65)	3.76 (2.37–5.99)

Metabolic syndrome was defined as having three or more of the following abnormalities: body mass index  $\geq 25 \text{ kg/m}^2$ ; triglyceride  $\geq 1.7 \text{ mmol/l}$ ; HDL-cholesterol  $< 1.04 \text{ mmol/l}$ ; fasting plasma glucose  $\geq 6.1 \text{ mmol/l}$ ; systolic blood pressure  $\geq 130 \text{ mmHg}$  and/or diastolic blood pressure  $\geq 85 \text{ mmHg}$ . Subjects with GGT (2–20) in men and those with GGT (2–11) in women were the referent group, respectively.

\* $P < 0.01$ . All other odds ratios were  $P < 0.001$ .

Model 1, adjusted for age, gender, smoking history, alcohol history, white blood cell counts, aspartate aminotransferase and alanine aminotransferase. Model 2, adjusted for the presence of fatty liver based on ultrasonographic finding, radiologist who performed the ultrasonogram and the variables of model 1.

exclusion criteria used were insufficient. In some studies, subjects with hepatitis C virus antibody and/or hepatitis B virus surface antigen were not excluded. In particular, we found that serum GGT concentration increase, even within its normal range, is a risk factor for diabetes, dyslipidaemia, obesity and metabolic syndrome, independent of the presence of fatty liver by ultrasonography.

The mechanism of the relationship between insulin resistance and GGT elevation has not been clarified, although hepatic steatosis or hepatic insulin resistance, caused by visceral obesity, may be the first possible mechanism [12,15–18]. However, dose-response relations between GGT concentration and the presence of diabetes, or with cardiovascular risk factors, were observed among subjects within a normal ALT range, which usually increases in cases of hepatic steatosis [7]. Also, in the present study, significant associations were found between GGT concentration and the presence of diabetes and with cardiovascular risk factors in subjects with serum ALT within the normal range. These findings suggest that hepatic steatosis and hepatic insulin resistance are not the only mechanisms that explain these relations, although a low normal ALT value does not guarantee freedom from underlying steatohepatitis. Imaging studies, including ultrasonography, cannot be used to determine accurately the severity of liver damage without a liver biopsy. However, the significant association between serum GGT concentration and the presence of the metabolic syndrome, even after adjusting for the presence of fatty liver, suggests that another mechanism governs the relation between serum GGT concentration and metabolic syndrome.

Because oxidative stress can play a role in the pathophysiology of cardiovascular diseases, and GGT has a pivotal role in maintaining intracellular glutathione transport into most types of cells [19–21], oxidative stress provides a second possible mechanism. Increased GGT activity may be a response to oxidative stress, one which can increase the transport of glutathione precursors into cells. In addition, GGT leaks into serum possibly as a result of normal cell turnover and cellular

stress. Subclinical inflammation, which could represent a third underlying mechanism [22,23], may be another possible cause. In the present study, the white blood cell count was a significantly related to serum GGT concentration, after adjusting for age, gender, alcohol history, smoking history, body mass index, blood pressure, lipid profile, serum AST, serum ALT, fasting plasma glucose and the presence of fatty liver ( $\beta = 0.028$ ,  $R^2 = 0.477$ ,  $P < 0.001$ ).

The most important limitation of our study is that this is a cross-sectional study so that causation cannot be inferred. In addition, all variables were only measured once, and therefore prevalence rates of hypertension, impaired fasting glucose/diabetes and the metabolic syndrome may be inflated.

In conclusion, we believe that our data suggest that the serum GGT concentration may be a marker for diabetes and cardiovascular risk factors, independent of its well-known associated variables and of fatty liver by ultrasonography. Further investigation of the mechanisms underlying these associations is warranted.

## Competing interests

None declared.

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## Serum Gamma-Glutamyl Transferase Level Is an Independent Predictor of Incident Hypertension in Korean Adults

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### Abstract

The aim of our study was to assess the relationship between serum gamma-glutamyl transferase (GGT) level within the normal range and incident hypertension according to drinking and obesity status in nonhypertensive individuals. We followed up 4783 normotensive adults (mean age = 44 years) who had serum GGT levels within the normal range at baseline for 3 years. Subjects were divided into four GGT quartile groups according to their serum GGT level at baseline. The overall incidence of hypertension was 8.1%, and the incidence increased with increasing GGT quartile (3.8%, 6.9%, 9.0%, and 12.4% in the lowest, second, third, and highest GGT quartiles, respectively;  $P < .001$ ). In the logistic regression analysis adjusted for age, sex, body mass index, lifestyle factors, glucose, uric acid, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, high-sensitivity C-reactive protein, and baseline systolic blood pressure, the odds ratio (ORs) for incident hypertension increased with increasing GGT quartile ( $P$  for trend = .030). In the above model, the highest quartile group showed increased ORs compared with those in the lowest quartile group (ORs [95% confidence interval], 2.638 [1.259–5.528]). Subgroup analyses revealed a significant association between GGT quartile and the incidence of hypertension in the drinker and non-overweight groups. Our results indicate that elevated serum GGT levels within the normal range are associated with a higher risk of incident hypertension in Korean adults, particularly, in drinkers and non-overweight individuals, suggesting possible different pathophysiologic mechanisms in the incidence of alcohol- and obesity-related hypertension.

**Keywords:** gamma-glutamyl transferase, hypertension, obesity, alcohol consumption, blood pressure

### INTRODUCTION

Gamma-glutamyltransferase (GGT) is a sensitive indicator of hepatic cell inflammation and hepatic triglyceride (TG) accumulation, which are observed in obesity, diabetes mellitus, and excessive alcohol consumption (1,2). GGT has a pro-oxidative effect (3), as it is involved in the degradation of the antioxidant glutathione and has an indirect pro-oxidative effect by causing low-density lipoprotein (LDL) cholesterol oxidation in the presence of iron. GGT is also considered a proinflammatory marker (4), and enzyme activity of serum GGT found within atherosclerotic lesions directly contributes to atherosclerosis progression (5). It has been suggested that serum GGT is independently associated with several pathological conditions including cardiovascular disease (1), diabetes (6), and metabolic syndrome (7).

Several cross-sectional studies have reported a positive association between serum GGT level and prehypertension/hypertension (8–10). Recently, the relationship between serum GGT level and incident hypertension has been investigated in several longitudinal studies. However, the results from these studies are inconsistent (11–16), and most of these studies included subjects with abnormal serum GGT levels and did not exclude subjects with hepatic pathological conditions such as hepatitis B or C that could directly influence serum GGT level. A Western study reported an association between serum GGT level within the normal range and incident hypertension according to drinking status and obesity status (17). Their results showed that increasing GGT quintiles increased the incidence of hypertension in both drinker and nondrinker groups and only in the group with increased central fat distribution.

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However, the study populations were only Western people.

Therefore, our aims in this study were to evaluate whether serum GGT is an independent predictor for the development of hypertension in Korean normotensive adults and to assess the relationship between serum GGT level and incident hypertension according to drinking status and obesity status in this subject group.

## MATERIALS AND METHODS

### Subjects

A total of 7858 Korean adults who were inhabitants of either Seoul or Kyunggi Province visited Kangbuk Samsung Hospital for health examinations in 2002 and 2005. Among them, 3075 subjects were excluded from this study: 438 subjects had positive results for hepatitis B virus surface antigen or hepatitis C virus antibody, 1318 subjects showed GGT concentrations higher than the upper limit of the reference range ( $>51$  U/L), 1244 subjects were hypertensive at baseline (systolic blood pressure [SBP]  $\geq 140$  mm Hg or diastolic blood pressure [DBP]  $\geq 90$  mm Hg, or current use of anti-hypertensive agents), and 75 subjects did not provide information about their alcohol consumption habits. Ultimately, 4783 subjects (mean age =  $44 \pm 5.8$  years, 3246 men) were enrolled in this study and were followed up for an average of 3 years. Nine (0.2%) subjects had taken antidiabetic medication at baseline.

Subjects were classified into one of four GGT quartile groups according to their baseline serum GGT concentrations: quartile 1 consisted of subjects with GGT levels between 0 and 12.9 U/L; quartile 2 included subjects with GGT levels between 13 and 18.9 U/L; quartile 3 was composed of subjects with GGT levels between 19 and 28.9 U/L; and quartile 4 included subjects with GGT levels between 29 and 51 U/L.

This research protocol was approved by the Institutional Review Board of Kangbuk Samsung Hospital (KBC10090). All participants provided written informed consent during health examinations.

### Measurements

Medical and medication histories, smoking status (current smoker or nonsmoker), alcohol drinking frequency ( $\geq 3$  times per week), the amount of alcohol consumed (g/time), and physical activity ( $\geq 3$  times per week) were assessed using the same standard questionnaires in both 2002 and 2005.

The data for alcohol consumption history were collected primarily using self-reported questionnaires. Alcohol consumption history was assessed by asking the frequency of alcoholic beverage consumption and the amount of alcohol consumed per time, using the most popular alcoholic beverage (Soju) in Korea. One bottle of Soju contains 56.5 g of ethanol.

The SBP and DBP were measured by a trained registered nurse by placing a mercury sphygmomanometer on the right arm of the subject, who was in a seated position and had rested for 5 minutes or longer (18). When measured, SBP or DBP was  $\geq 140/90$  mm Hg; blood pressure was measured once more and the average value was used for this analysis.

Height and weight were measured with an automated scale while the participants were wearing a light hospital gown without shoes, and body mass index (BMI) was calculated as weight (kilograms) divided by height squared (meters squared).

Blood samples were obtained in the morning after an overnight fast. Concentrations of high-sensitivity C-reactive protein (hsCRP) were measured using particle-enhanced immunonephelometry, with a lower limit of detection of 0.02 mg/L (Behring Nephelometer II, Dade Behring, Marburg, Germany). Plasma glucose, total cholesterol, TG, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and uric acid levels were measured using an autoanalyzer (Advia 1650 Autoanalyzer, Bayer Diagnostics, Leverkusen, Germany). Serum concentrations of GGT were measured by kinetic spectrophotometric method, using L- $\gamma$ -glutamyl-p-nitroanilide (Advia 1800, Siemens HealthCare Diagnostics, Tokyo, Japan) with a normal range of 0–51 U/L. Serum GGT level was measured using the same reagent on the same autoanalyzer in both 2002 and 2005, and the within-run and total coefficients of variation for GGT determinations were not greater than 10% during the period. Baseline serum GGT levels were categorized by quartile (0–12.9, 13–18.9, 19–28.9, and 29–51 U/L).

Incident hypertension was defined as SBP of 140 mm Hg or greater, DBP of 90 mm Hg or greater, or current use of hypertensive medications at the follow-up visit.

### STATISTICAL ANALYSIS

Data are expressed as mean  $\pm$  standard deviation (SD) or median and interquartile ranges for continuous variables and percentages for categorical variables. Among the variables, TG and hsCRP levels were naturally logarithm-transformed to obtain a normal distribution for statistical analysis.

Differences in baseline characteristics according to GGT quartile were determined using analysis of variance and  $\chi^2$  test. Comparisons of characteristics according to the development of new hypertension were assessed using Student *t* test and  $\chi^2$  test.

Alcohol-adjusted correlation analyses were performed to assess the associations between baseline GGT level, SBP, and DBP, and changes in GGT, SBP, and DBP at baseline and at follow-up.

Multivariate adjusted logistic regression analyses were used to evaluate the association between serum GGT level and incident hypertension: model 1 was

adjusted for age, sex, lifestyle factors (amount of alcohol consumption, smoking status, and physical activity), and BMI; model 2 was adjusted for the variables in model 1 in addition to traditional risk factors (baseline glucose, uric acid, HDL cholesterol, LDL cholesterol, TG, and hsCRP) and baseline SBP.

For subgroup analyses, the study population was divided into two pairs of groups: a nondrinker group versus a drinker group, according to the presence/absence of alcohol consumption, and a nonobese group ( $BMI < 25 \text{ kg/m}^2$ ) versus an overweight group ( $BMI \geq 25 \text{ kg/m}^2$ ), according to the proposed classification of weight by BMI in adults.

Data were analyzed using PASW version 18.0 (SPSS Inc., Chicago, IL, USA), and  $P$  values  $<.05$  were considered statistically significant.

## RESULTS

Among 4783 adults without hypertension at baseline, the overall incidence of new hypertension was 8.1% (389 of 4783). The incidence of new hypertension increased with increasing serum GGT quartile (3.8%, 6.9%, 9.0%, and 12.4% in the lowest, second, third, and highest GGT quartiles, respectively;  $P < .001$ ).

Baseline subject characteristics according to serum GGT quartile are presented in Table 1. As the serum GGT quartile increased, age, the proportion of men, prehypertension at baseline, prevalence of DM at baseline, baseline SBP and DBP, BMI, body weight, current smoking status, and alcohol consumption increased; in other words, subjects in higher serum GGT quartiles had more unfavorable metabolic and lipid profiles than did those in lower serum GGT quartiles (Table 1).

Comparisons of subject characteristics according to the development of new hypertension revealed that subjects in the incident hypertension group were older and more likely to be male. Moreover, subjects in the incident hypertension group consumed alcohol more frequently and had higher baseline SBP and DBP, BMI, and body weight, and more unfavorable metabolic and lipid profiles compared with those in the nonincident hypertension group. Furthermore, the incident hypertension group had higher GGT levels at baseline and higher magnitude changes in GGT level between baseline and follow-up than did the nonincident hypertension group ( $3.4 \pm 13.7$  in nonincident hypertension group vs.  $5.2 \pm 12.8$  in incident hypertension group;  $P = .006$ ; Table 2).

Alcohol-adjusted correlation analyses revealed that baseline GGT level was significantly positively correlated with baseline SBP and DBP ( $r = 0.182$  and  $r = 0.197$ , respectively;  $P < .001$ ). Furthermore, the change in GGT level between baseline and follow-up was positively correlated with changes in SBP and DBP ( $r = 0.05$ ,  $P = .001$  and  $r = 0.039$ ,  $P = .007$ , respectively).

In the multivariate regression analyses of all models, the incidence of new hypertension was significantly

associated with increasing quartile of GGT level (all  $P$  for trend  $<.05$ ). Furthermore, the odds ratios (Ors) for incident hypertension in the highest quartile group in the multivariate regression models were significantly increased compared with that of the lowest quartile group (ORs [95% confidence interval] = 2.408 [1.212–4.785] in model 1; 2.638 [1.259–2.528] in model 2; Table 3). Meanwhile, when substituting GGT quartile with serum GGT (as a continuous variable), the association between serum GGT level and incident hypertension was not significant in any of the models (all  $P$  values  $>.05$ ; data not shown).

Sex-specific analyses showed that baseline serum GGT level was higher in men ( $25.8 \pm 10.7 \text{ U/L}$ ) than in women ( $13.3 \pm 7.1 \text{ U/L}$ ) and that the incidence of hypertension was about twofold higher in men than in women (men vs. women: 9.8% vs. 4.6%). Furthermore, the incidence of hypertension increased according to increasing GGT quartiles in both sexes (8.0%, 7.8%, 11.2%, and 12.1% in the lowest, second, third, and highest GGT quartiles in men,  $P = .003$ ; 2.8%, 2.9%, 4.0%, and 7.8% in the lowest, second, third, and highest GGT quartiles in women,  $P = .002$ ). However, sex-specific regression analyses revealed that both the baseline serum GGT quartiles and the absolute values of serum GGT were not significantly associated with incident hypertension in either sex (data not shown).

In the subgroup analyses in the alcohol/nonalcohol consumption groups, the incidence of new hypertension in the drinker group was higher than that in the nondrinker group (9.3% vs. 6.1%, respectively;  $P < .001$ ). In the multivariate regression analyses, the incidence of new hypertension was significantly associated with increasing GGT quartile in the drinker group, although no significant association was found between incident hypertension and GGT quartile in any of the models in the nondrinker group (Table 4).

The incidence of new hypertension in the nonobese group was lower than that in the overweight group (6.3% vs. 13.5%, respectively;  $P < .001$ ). In the nonobese group, higher serum GGT quartile groups significantly had higher ORs for incident hypertension than did the lowest quartile group in all models. However, the overweight group showed no association between incident hypertension and GGT quartile group in any of the models (Table 5).

When subjects were subdivided into four groups according to drinking status (nondrinker vs. drinker) and BMI status (nonobese vs. overweight), the incidence of hypertension in the drinker and overweight group was highest (4.8% in the nondrinker and nonobese group, 11.5% in the nondrinker and overweight group, 7.3% in the drinker and nonobese group, and 14.3% in the drinker and overweight group). However, the association between serum GGT quartile and incident hypertension was more obvious in the drinker and nonobese group than it was in the remaining three groups (data not shown).

Table 1. Baseline characteristics of the subjects according to GGT quartile

	Serum GGT quartiles				P value
	Q1 (n = 1182)	Q2 (n = 1087)	Q3 (n = 1276)	Q4 (n = 1238)	
Age (y)	43 ± 6.2	44 ± 5.9	45 ± 5.7	45 ± 5.3	<.001
Men, n (%)	252 (21.3)	710 (65.3)	1115 (87.4)	1169 (94.4)	<.001
SBP (mm Hg)	106 ± 11.3	109 ± 10.0	111 ± 9.4	112 ± 8.9	<.001
DBP (mm Hg)	68 ± 8.3	71 ± 8.0	73 ± 7.3	73 ± 7.1	<.001
BMI (kg/m <sup>2</sup> )	21.8 ± 2.3	22.6 ± 2.6	23.7 ± 2.5	24.7 ± 2.5	<.001
Body weight (kg)	57.4 ± 7.9	63.5 ± 9.4	68.2 ± 8.9	71.5 ± 8.8	<.001
Glucose (mmol/L)	4.83 ± 0.49	5.00 ± 0.63	5.05 ± 0.63	5.22 ± 0.94	<.001
TC (mmol/L)	4.87 ± 0.82	5.05 ± 0.82	5.28 ± 0.86	5.44 ± 0.89	<.001
TG <sup>a</sup> (mmol/L)	0.90 [0.71, 1.22]	1.11 [0.82, 1.49]	1.37 [1.01, 1.84]	1.67 [1.23, 2.26]	<.001
LDL-C (mmol/L)	2.80 ± 0.67	2.98 ± 0.71	3.13 ± 0.76	3.21 ± 0.81	<.001
HDL-C (mmol/L)	1.55 ± 0.35	1.48 ± 0.33	1.37 ± 0.31	1.32 ± 0.30	<.001
hsCRP <sup>a</sup> (mg/L)	0.02 [0.02, 0.02]	0.02 [0.02, 0.02]	0.02 [0.02, 0.02]	0.02 [0.02, 0.02]	.005
Uric acid (μmol/L)	255.8 ± 59.5	303.3 ± 77.3	339.0 ± 71.4	362.8 ± 71.4	<.001
Insulin (pmol/L)	47.23 ± 15.97	48.62 ± 16.67	51.39 ± 18.75	56.25 ± 18.06	<.001
GGT (U/L; baseline)	9.6 ± 2.1	15.4 ± 1.7	23.0 ± 2.9	37.8 ± 6.5	<.001
GGT (U/L; follow-up)	10.9 ± 4.9	17.9 ± 8.2	27.1 ± 11.4	43.7 ± 23.4	<.001
Net GGT (U/L)	1.34 ± 4.66	2.50 ± 7.90	4.11 ± 11.08	5.86 ± 22.60	<.001
Alcohol frequency (≥3 times/wk), n (%)	14 (1.2)	46 (4.2)	102 (8.0)	172 (13.9)	<.001
Alcohol amount (g/time)					<.001
<56.5	627 (95.1)	739 (87.7)	917 (81.8)	880 (76.3)	
56.5–113	27 (4.1)	86 (10.2)	157 (14.0)	208 (18.0)	
>113	5 (0.8)	18 (2.1)	47 (4.2)	66 (5.7)	
Smoking (current), n (%)	108 (9.4)	260 (24.3)	469 (37.2)	550 (44.5)	<.001
Physical activity (≥3 times/wk), n (%)	220 (19.3)	227 (21.3)	220 (17.5)	191 (15.6)	.003
Diabetes mellitus, n (%)	4 (0.3)	16 (1.5)	22 (1.7)	32 (2.6)	<.001
BP category, n (%)					<.001
Normal	865 (73.2)	670 (61.6)	667 (52.3)	583 (47.1)	
Prehypertension	317 (26.8)	417 (38.4)	609 (47.7)	655 (52.9)	

Abbreviations: SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; TC – total cholesterol; TG – triglyceride; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; hsCRP – high-sensitivity C-reactive protein; BP – blood pressure; GGT – gamma-glutamyl transferase.

Notes: Data are means ± SD or medians [interquartiles] for continuous variables and percentages for categorical variables. GGT quartile groups were defined as follows: Q1 (0–12.9 U/L), Q2 (13–18.9 U/L), Q3 (19–28.9 U/L), and Q4 (29–51 U/L).

<sup>a</sup>TG and hsCRP are expressed as raw data but were naturally log-transformed prior to statistical analyses.

## DISCUSSION

This longitudinal study showed that the overall incidences of new hypertension in Korean middle- and old-aged individuals with baseline serum GGT level within the normal reference range were 8.1% (9.8% in men and 4.6% in women, respectively) after a 3-year follow-up. Higher GGT levels were significantly associated with the development of new hypertension, irrespective of drinking or obesity status. However, in subgroup analyses, serum GGT level was significantly associated with incident hypertension only in the drinker and nonobese groups.

The mechanisms that lead to increased BP in subjects with increased GGT within the normal range are not completely understood. GGT is present in the serum and on the surfaces of most cell types and plays a direct role in the generation of reactive oxygen species (3,4). GGT has the primary function of maintaining the intracellular concentration of glutathione in response to oxidative stress and is recognized as a marker of

oxidative stress (19). GGT has also been used as a proinflammatory marker because of its indirect involvement in the generation of cysteinyl-glycine, which results in LDL oxidation (4). Recently, a study reported that enzyme activity of serum GGT found within atherosclerotic lesions directly contributes to atherosclerosis progression (5). For the reasons outlined above, it has been hypothesized that serum GGT level may be associated with prehypertension/hypertension, which are pathologic conditions associated with increased production of reactive oxygen species and proinflammatory substances.

Several cross-sectional observational studies have reported that serum GGT level is positively associated with prehypertension/hypertension (8–10). However, these studies included individuals with GGT levels above the normal range; other confounding conditions such as hepatic parenchymal disease that could directly increase serum GGT level were not excluded. Moreover, these studies were not able to

Table 2. Baseline characteristics according to the development of new hypertension

	No development of HTN (n = 4394)	Development of HTN (n = 389)	P value
Age (y)	44 ± 5.7	46 ± 6.6	<.001
Men, n (%)	2927 (66.6)	319 (82.0)	<.001
SBP (mm Hg)	109 ± 10.1	116 ± 9.2	<.001
DBP (mm Hg)	71 ± 8.0	76 ± 6.1	<.001
BMI (kg/m <sup>2</sup> )	23.1 ± 2.7	24.6 ± 2.7	<.001
Body weight (kg)	64.9 ± 10.2	70.1 ± 9.7	<.001
Glucose (mmol/L)	5.05 ± 0.68	5.16 ± 1.00	.027
TC (mmol/L)	5.15 ± 0.87	5.31 ± 0.92	<.001
TG <sup>a</sup> (mmol/L)	1.22 [0.87, 1.73]	1.42 [1.08, 2.02]	<.001
LDL-C (mmol/L)	3.03 ± 0.76	3.13 ± 0.78	.009
HDL-C (mmol/L)	1.42 ± 0.34	1.40 ± 0.33	.014
hsCRP <sup>a</sup> (mg/L)	0.02 [0.02, 0.02]	0.02 [0.02, 0.02]	.084
Uric acid (μmol/L)	315.2 ± 83.3	333.1 ± 77.3	<.001
Insulin (pmol/L)	50.70 ± 17.36	54.17 ± 18.75	.001
GGT (U/L; baseline)	21.4 ± 11.2	25.8 ± 11.3	<.001
GGT (U/L; follow-up)	24.8 ± 18.7	31.0 ± 17.7	<.001
Net GGT (U/L)	3.36 ± 13.75	5.23 ± 12.84	.006
Alcohol frequency (≥3 times/wk), n (%)	293 (6.7)	41 (10.5)	.004
Alcohol amount (g/time)			.557
<56.5	2891 (83.9)	272 (81.7)	
56.5–113	430 (12.5)	48 (14.4)	
>113	123 (3.6)	13 (3.9)	
Smoking (current), n (%)	1272 (29.3)	115 (29.9)	.794
Physical activity (≥3 times/wk), n (%)	770 (17.9)	88 (22.9)	.016
Diabetes mellitus, n (%)	64 (1.5)	10 (2.6)	.088
BP category, n (%)			<.001
Normal	2670 (60.8)	115 (29.6)	
Prehypertension	1724 (39.2)	274 (70.4)	

Abbreviations: SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; TC – total cholesterol; TG – triglyceride; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; hsCRP – high-sensitivity C-reactive protein; GGT – gamma-glutamyl transferase; BP – blood pressure; HTN – hypertension.

Notes: Data are means ± SD or medians [interquartiles] for continuous variables and percentages for categorical variables.

<sup>a</sup>TG and hsCRP are expressed as raw data but were naturally log-transformed prior to statistical analyses.

Table 3. Logistic regression analyses of the association between increased serum GGT quartile and incident hypertension

	Serum GGT quartiles				P for trend
	Quartile 1 (n = 1182)	Quartile 2 (n = 1087)	Quartile 3 (n = 1276)	Quartile 4 (n = 1238)	
Incident HTN, n (%)	45 (3.8)	75 (6.9)	115 (9.0)	154 (12.4)	
Age- and sex-adjusted	1	1.528 [1.019–2.291]	1.885 [1.256–2.830]	2.645 [1.763–3.968]	<.001
Model 1	1	2.189 [1.094–4.379]	1.937 [0.975–3.849]	2.408 [1.212–4.785]	.047
Model 2	1	2.256 [1.079–4.714]	2.000 [0.963–4.154]	2.638 [1.259–5.528]	.030

Abbreviations: HTN – hypertension; GGT – gamma-glutamyl transferase.

Notes: Values are odds ratio [95% confidence interval]. Model 1 was adjusted for age, sex, lifestyle factors (alcohol amount, smoking status, and physical activity), and body mass index. Model 2 was adjusted for model 1 and traditional risk factors (baseline glucose, uric acid, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and high-sensitivity C-reactive protein) and baseline systolic blood pressure.

determine any cause-and-effect relationships between serum GGT level and incident hypertension due to their cross-sectional design.

Recently, several longitudinal studies have investigated the association between serum GGT level and

incident hypertension; however, the results from these studies are not consistent (11–16). One longitudinal study showed a positive association between serum GGT level within the normal range and incident hypertension after a 15-year follow-up (12), consistent with

Table 4. Logistic regression analyses of the association between increased serum GGT quartile and incident hypertension in the presence/absence of alcohol consumption

Nondrinker group	Quartile 1 ( <i>n</i> = 782)	Quartile 2 ( <i>n</i> = 440)	Quartile 3 ( <i>n</i> = 308)	Quartile 4 ( <i>n</i> = 182)
Incident HTN, <i>n</i> (%)	33 (4.2)	22 (5.0)	28 (9.1)	21 (11.5)
Age- and sex-adjusted	1	0.986 [0.552–1.761]	1.648 [0.910–2.985]	2.006 [1.027–3.917]
Model 1	1	0.836 [0.456–1.532]	1.171 [0.619–2.213]	1.297 [0.624–2.695]
Model 2	1	0.727 [0.378–1.396]	0.721 [0.350–1.483]	0.756 [0.317–1.802]
Drinker group	Quartile 1 ( <i>n</i> = 398)	Quartile 2 ( <i>n</i> = 647)	Quartile 3 ( <i>n</i> = 968)	Quartile 4 ( <i>n</i> = 1056)
Incident HTN, <i>n</i> (%)	12 (3.0)	53 (8.2)	87 (9.0)	133 (12.6)
Age- and sex-adjusted	1	2.485 [1.280–4.823]	2.654 [1.380–5.106]	3.841 [2.012–7.332]
Model 1	1	2.289 [1.149–4.559]	2.105 [1.065–4.160]	2.673 [1.355–5.270]
Model 2	1	2.312 [1.112–4.805]	2.124 [1.029–4.386]	2.847 [1.370–5.913]

Abbreviations: HTN – hypertension; GGT – gamma-glutamyl transferase.

Notes: Values are odds ratios [95% confidence interval]. Model 1 was adjusted for age, sex, lifestyle factors (smoking status and physical activity), and body mass index. Model 2 was adjusted for model 1 and traditional risk factors (baseline glucose, uric acid, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and high-sensitivity C-reactive protein) and baseline systolic blood pressure.

Table 5. Logistic regression analyses of the association between increased serum GGT quartile and incident hypertension in the BMI subgroups

Nonobese group	Quartile 1 ( <i>n</i> = 844)	Quartile 2 ( <i>n</i> = 615)	Quartile 3 ( <i>n</i> = 470)	Quartile 4 ( <i>n</i> = 272)
Incident HTN, <i>n</i> (%)	28 (2.7)	58 (6.4)	73 (7.9)	67 (9.5)
Age- and sex-adjusted	1	2.023 [1.236–3.311]	2.323 [1.397–3.862]	2.714 [1.607–4.584]
Model 1	1	3.350 [1.352–8.302]	3.099 [1.246–7.708]	4.037 [1.615–10.089]
Model 2	1	2.956 [1.166–7.496]	2.579 [1.009–6.593]	3.332 [1.280–8.678]
Overweight group	Quartile 1 ( <i>n</i> = 326)	Quartile 2 ( <i>n</i> = 451)	Quartile 3 ( <i>n</i> = 790)	Quartile 4 ( <i>n</i> = 947)
Incident HTN, <i>n</i> (%)	17 (13.1)	17 (9.2)	42 (11.8)	87 (16.3)
Age- and sex-adjusted	1	0.624 [0.293–1.327]	0.797 [0.399–1.594]	1.174 [0.594–2.318]
Model 1	1	1.121 [0.362–3.476]	1.239 [0.431–3.563]	1.676 [0.597–4.704]
Model 2	1	1.323 [0.371–4.712]	1.421 [0.430–4.690]	2.081 [0.635–6.825]

Abbreviation: HTN – hypertension; GGT – gamma-glutamyl transferase; BMI – body mass index.

Notes: Values are odds ratio [95% confidence interval]. Model 1 was adjusted for age, sex, and lifestyle factors (alcohol amount, smoking status, and physical activity). Model 2 was adjusted for model 1 and traditional risk factors (baseline glucose, uric acid, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and high-sensitivity C-reactive protein) and baseline systolic blood pressure.

our results. However, this previous study included only black and white American individuals, whereas we investigated only Asian subjects. Moreover, the previous longitudinal study did not exclude subjects with hepatic diseases that could affect serum GGT level. Another longitudinal study in Korean male workers reported that elevated GGT level was not significantly associated with new hypertension in the total study population after a 4-year follow-up; however, the authors of this study did report that a GGT level  $\geq 30$  U/L was a predictor for new hypertension compared with a GGT level  $< 30$  U/L, in contrast to our results (11). Although the same ethnic group was evaluated in both the previous study and our study, the earlier study did not exclude subjects

with hepatic diseases such as hepatitis B or C, which are prevalent in Korea; furthermore, a different definition of hypertension was used in the earlier study, which may explain the conflicting findings. A study performed in Norway to investigate the association between concurrent changes in GGT level and SBP over a period of 7 years demonstrated a weak association, only in females (13). However, the design of the Norwegian study was very different from those of the other three longitudinal studies, including our study, and may not have had the power to assess the effects of GGT level on the development of new hypertension.

The results of our subgroup analysis according to drinking status showed that GGT quartile and new

hypertension were significantly associated only in the drinker group, and that the drinker group had a higher incidence of hypertension development than did the nondrinker group. Previous results from studies that investigated the relationship between GGT level and drinking status are inconsistent. A cross-sectional Japanese study reported that the association between serum GGT level and the prevalence of hypertension was similar in drinkers and nondrinkers, which differs from our results; moreover, this previous study evaluated a different study population, including individuals with abnormal GGT level (9). In a longitudinal Japanese study with a 5-year follow-up, baseline GGT levels above 50 U/L were significantly associated with incidences of hypertension in drinkers only, which suggest that elevated serum GGT activity may identify drinkers at higher risk of developing alcohol-related hypertension (10). Another Japanese 10-year prospective study including only male drinkers suggested that increased serum GGT level ( $>20$  U/L) may predict the further development of hypertension among drinkers, even though this previous study included a different subject population than our study (14). In addition, the other study reported that abnormal serum GGT values ( $\geq 50$  U/L) were correlated with the development of hypertension in nondrinkers (15,16). Unlike these previous studies, we included only subjects with serum GGT level within the normal range.

Body mass index is known to be an important factor influencing the development of hypertension. Our subgroup analyses showed that the incidence of hypertension in the overweight group was 2.1-fold higher than that in the nonobese group. However, the association between increasing GGT quartile and incident hypertension was only significant in the nonobese group. This finding suggests that obesity itself is more strongly associated than GGT level with the development of hypertension, resulting in the attenuation of the relationship between serum GGT level and incident hypertension in the obese group. The incidence of hypertension in the highest GGT quartile in the nonobese group was lower than that in the lowest GGT quartile in the overweight group (9.5% vs. 13.1%, respectively). A previous study reported that serum GGT level showed a positive association with alcohol consumption and BMI (20), consistent with our results that serum GGT level is correlated with alcohol consumption and BMI. Another 6-year follow-up study investigated the association between GGT and hypertension within normotensive individuals according to drinking and BMI status and suggested that increasing GGT quintiles increased the incidence of hypertension in both drinker and nondrinker groups and only in groups with increased central fat distribution (17). However, this study included only Western subjects, and the results were inconsistent with our study.

Our study had several limitations. First, our definition of hypertension was based on a single estimate of

blood pressure. Although high BPs were measured more than once, misclassification of BP cannot be excluded. Second, although most epidemiological studies have used a single GGT measurement, this single measurement might not be reliable, considering the previous report that 12% of adults in the American general population with initially elevated GGT levels had normal level on their second examination (21). Third, the reliability and the validity of self-reported alcohol consumption are questionable. Generally, individuals tend to conceal their alcohol consumption and report lower than actual consumption. Fourth, certain diet patterns are associated with increased GGT, including consumption of red meat and alcohol, whereas consumption of nonfried or canned vegetables, grains, beans, tree nuts, and coffee seems to be associated with lower levels of GGT (22). Furthermore, high sodium intake is generally associated with the development of hypertension. However, we have no data regarding dietary patterns. Finally, we have no data regarding hepatic ultrasonography to exclude parenchymal liver diseases, such as fatty liver, cirrhosis, and hepatocellular carcinoma. However, our study subjects were apparently healthy at baseline, and we excluded subjects who had viral hepatitis and abnormal baseline serum GGT level.

On the other hand, the strengths of our study are that we excluded individuals who had confounding conditions that could increase serum GGT level and demonstrated an epidemiologic association between serum GGT level and incident hypertension independent of drinking status, BMI status, and various coexisting factors.

In conclusion, we found that increasing serum GGT level within the normal range was independently associated with incident hypertension in Korean adults. This association was significant only in drinkers and nonobese individuals, respectively. Further prospective population-based studies are needed to assess a more accurate relationship between GGT level and the incidence of hypertension.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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# Serum gamma-glutamyl transferase is a predictor of mortality in patients with acute myocardial infarction

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## Abstract

Gamma-glutamyl transferase (GGT) is involved in the pathogenesis of atherosclerosis and has been associated with adverse cardiovascular outcomes in patients with ischemic heart disease. However, the association between GGT and long-term mortality has not been studied in patients with acute myocardial infarction (AMI).

A total of 2239 AMI patients for whom serum GGT values were available and who underwent percutaneous coronary intervention (PCI) were enrolled in the COREA-AMI (CardiOvascular Risk and idEntificAtion of potential high-risk population in Korean patients with AMI) registry. Patients with acute liver injury were excluded. Patients were classified into 2 groups according to normal ( $n=1983$ ) or elevated ( $n=256$ ) levels of serum GGT. The primary clinical outcome was all-cause mortality. The secondary outcome was cardiac death and recurrent non-fatal myocardial infarction (MI).

The median follow-up period was 3.7 years, and both groups had similar characteristics. Patients with elevated GGT had significantly higher all-cause mortality compared to patients with normal GGT (21.9% vs. 14.4%,  $P=.001$ ). The multivariate Cox proportional hazards model showed that elevated serum GGT level was independently correlated with mortality (hazard ratio 2.12 [1.44–3.11];  $P<.001$ ). Although elevated serum GGT was independently associated with long-term mortality after 30 days after PCI, there was no association within 30 days after PCI. Elevated GGT was also associated with death of cardiac causes with statistical significance. In the subgroup analysis, stronger associations were observed in the young and female patients and in patients who had ST-segment elevation MI and preserved left ventricular ejection fraction at the first echocardiography after the indexed PCI.

Elevated serum GGT is an independent predictor of long-term mortality in AMI patients.

**Abbreviations:** 2xULN = two-times of upper limit of normal, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMI = acute myocardial infarction, AST = aspartate aminotransferase, AUC = areas under the curve, BMI = body mass index, CAD = coronary artery disease, CI = confidence interval, ECG = electrocardiogram, GGT = gamma-glutamyl transferase, HbAc1 = glycated hemoglobin, HDL-C = High-density lipoprotein cholesterol, HR = hazard ratio, hsCRP = high-sensitivity C-reactive protein, IQR = interquartile range, LDL-C = low-density lipoprotein cholesterol, LVEF = left ventricular ejection fraction, MI = myocardial infarction, NSTEMI = Non ST-segment elevation myocardial infarction, PCI = percutaneous coronary intervention, ROC = receiver-operating characteristic, STEMI = ST-segment elevation myocardial infarction, TIMI = thrombolysis in myocardial infarction.

**Keywords:** gamma-glutamyl transferase, long-term mortality, myocardial infarction

## 1. Introduction

The enzyme gamma-glutamyl transferase (GGT) is present in the serum and on the surface of various cell membranes. GGT is

considered a marker of liver or biliary tract diseases and alcohol consumption. However, GGT has recently been identified as a novel indicator of the development and prognosis of cardiovascular diseases. Although the exact mechanism has not been elucidated, the abundance of GGT in atheroma and its function in blood vessels may play a role. GGT catalyzes the first step in the extracellular degradation of glutathione. During the process of GGT-mediated glutathione degradation, low-density lipoprotein (LDL) is oxidized and accumulates in the arterial wall; this process is involved in the pathogenesis of atherosclerosis.<sup>[1]</sup> Moreover, degradation of the antioxidant glutathione results in formation of peroxide free radicals and, consequently, oxidative stress. Thus, the combination of abundant oxidized LDL and GGT in atherosclerotic plaques causes oxidative stress in the endothelium, which can affect plaque evolution and rupture.<sup>[2]</sup> Furthermore, many studies have reported an association between serum GGT levels and various established cardiovascular disease risk factors, such as hypertension, diabetes, metabolic syndrome, and coronary artery disease (CAD).<sup>[3–6]</sup> In addition, increased GGT levels in established CAD patients have been associated with an increase in secondary events, including myocardial infarction (MI), stroke, and cardiovascular death.<sup>[7,8]</sup> However, studies of the association between serum GGT levels and long-term clinical outcomes in

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patients with acute MI (AMI) have included only a few patients with ST-segment elevation MI (STEMI) and yielded inconsistent results.<sup>[9,10]</sup> Therefore, we investigated whether higher serum GGT levels can predict short-term and long-term mortality in patients with AMI.

## 2. Methods

This study used data from the CardiOvascular Risk and idEntificAtion of potential high-risk population in Korean patients with AMI (COREA-AMI) registry, which was designed to evaluate real-world outcomes in “all-comers” with AMI. The COREA-AMI, a large, observational registry included clinical, angiographic, short-term and long-term outcome data for AMI patients who underwent percutaneous coronary intervention (PCI) at 9 major cardiac centers in Korea between January 2004 and December 2009.<sup>[11]</sup>

Initially, our study sample included a total of 4748 patients. Among these, 2281 patients who had serum GGT values were available were enrolled. To avoid the confounding effects of unknown underlying active liver disease on the prognosis, patients who had a serum alanine aminotransferase (ALT) level  $>3$  times the upper limit of normal (ULN) and the ALT greater than the level of aspartate aminotransferase (AST) were excluded ( $n=28$ ).<sup>[12]</sup> To avoid unreasonable deviation of GGT level, the data were trimmed with exclusion of extreme values. The value of 5 patients (who were excluded because of lower than measurable range) was recorded near to zero; these cases were deleted for the possibility of data collection errors. We screened the accessible electrical medical records of the patients from the highest GGT level. The number of patients with GGT level over  $2 \times$  ULN,  $3 \times$  ULN, and  $5 \times$  ULN were 47 (2.1%), 14 (0.6%), and 9 (0.4%), respectively. We excluded the only 9 patients of over  $5 \times$  ULN because 3 of them had obvious hepatobiliary problems; one died from GB cancer, another had recurrent cholangitis, and the other had pancreatic disease. We could not find clear reason of GGT elevation among the others. Finally, a total 2239 of patients were included in this analysis (Fig. 1).

AMI was diagnosed based on characteristic clinical symptoms, serial changes on electrocardiograms (ECGs) consistent with infarction, and increased cardiac enzyme values. The diagnosis was confirmed by coronary angiography in all patients. We excluded patients who were not indicated for PCI based on coronary angiography to strengthen the homogeneity of the study

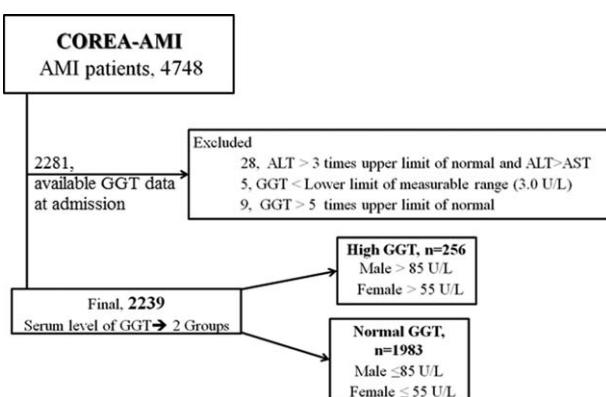
population. All patients received standard medical treatment during PCI and hospitalization. The study protocol was approved by the institutional review board at each participating center and is in accordance with the Declaration of Helsinki. All patients provided written informed consent at the time of admission to enrollment in the registry and the use of their clinical data in future retrospective analyses.

We recorded demographic data, cardiovascular risk factors, and laboratory data for all patients. Cardiovascular risk factors included smoking status, previously diagnosed diabetes mellitus, hypertension, chronic kidney disease, and history of familial CAD. Data on other risk factors were reported by the patients themselves or extracted from medical records. Blood samples were drawn within 24 hours of the initial visit and used for a standard battery of hematological and biochemical tests. Serum GGT levels were measured using the enzymatic colorimetric test at 37°C, and L-g-glutamyl-3-carboxy-4-nitroanilide was used as the substrate at each cardiac center under identical conditions.<sup>[10]</sup> Patients were categorized into 2 groups based on elevated or normal serum GGT levels compared to the upper limit of the clinical reference range. The normal reference range was 9 to 85 U/L for males and 5 to 55 U/L for females.<sup>[13]</sup>

All procedures were performed according to current standard guidelines. The specific drug-eluting stent used in the procedures was chosen by the operator. The operator assessed the type of lesion according to American College of Cardiology/American Heart Association guidelines. After the procedure, aspirin was prescribed indefinitely, and clopidogrel was prescribed for at least 6 months. Immediate post-procedural and in-hospital events were recorded. Patient follow-up was conducted during office visits or through telephone interviews at 1, 6, and 12 months and annually thereafter. Echocardiography was performed within 3 days of the PCI, and a quantitative assessment of the left ventricular systolic function was performed using the modified biplane Simpson method to calculate the left ventricular ejection fraction (LVEF).

The primary objective of this study was to evaluate the association between GGT level and all-cause mortality during clinical follow-up post intervention. The secondary objectives were to evaluate the association between high GGT levels and cardiac death and recurrent non-fatal MI. Cardiac death was defined as death from CAD, heart failure, or arrhythmia, and death was attributed to cardiac events unless non-cardiac death could be clearly identified.<sup>[14]</sup> Recurrent MI was defined as the presence of recurrent symptoms and new ECG changes that considered to be MI or cardiac markers that were at least twice the normal limit. Medical records were thoroughly reviewed by an independent research nurse. Telephone interviews were conducted to collect data on the occurrence of adverse events following PCI. Clinical outcomes of interest were confirmed by source documents and centrally adjudicated by a local events committee at the Cardiovascular Center of Seoul St. Mary's Hospital and an independent group of clinicians who were unaware of patient status. To verify the accuracy of mortality data, we matched our data to official national data collected by the National Statistical Office from death certificates, which previous studies have shown to be reliable.<sup>[11]</sup>

We classified patients into two groups according to normal or high GGT levels and used these 2 categories in the subsequent analyses. Differences between groups of continuous variables were evaluated using an independent t-test or the Mann-Whitney U test. Differences in discrete variables were analyzed using a chi-square or Fisher's exact test and expressed as counts and



**Figure 1.** Study flow chart. Inclusion and exclusion criteria of study population. ALT=alanine aminotransferase, AMI=acute myocardial infarction, AST=aspartate aminotransferase, GGT=gamma-glutamyl transferase.

percentages. Landmark analyses were performed to evaluate the impact of high serum GGT on short-term and long-term mortality. The landmark method of survival analysis uses a fixed time after PCI. In this study, the cut-off for early mortality was the 30th day after PCI. We constructed Kaplan-Meier curves to the end points for patients with normal GGT or high GGT, and differences between the groups were assessed by the log-rank test. Cox proportional hazard models were applied to calculate estimated hazard ratios (HRs) for each end-point. We selected covariates that differed significantly between the groups at baseline and that previous studies have related to GGT level or cardiovascular outcomes after PCI.<sup>[15,16]</sup> The HRs were adjusted for important covariates that had significant effects ( $p < 0.05$ ) on clinical outcomes in the univariate analysis. All analyses were two-tailed, and clinical significance was defined as  $p < 0.05$ . The same process was used for subgroup analyses to evaluate differences according to age, gender, STEMI or Non ST-segment elevation MI (NSTEMI), body mass index (BMI), high or low levels of LDL cholesterol (LDL-C), high or low levels of high-density lipoprotein (HDL) cholesterol (HDL-C), hypertriglyceridemia (triglyceride  $>150$ ), glycated hemoglobin(HbA1c), and LVEF  $\geq 50\%$  at the first echocardiography after the indexed PCI.

To analyze the association between GGT levels and the mortality in our study group of AMI patients, we computed receiver-operating characteristic (ROC) curves, tested for equality of the areas under the curves (AUCs), and calculated 95% confidence intervals (CIs) for GGT. Statistical analyses were performed using the statistical package SPSS V.20.0 (SPSS Inc., Chicago, IL) and MedCalc V.12.7 (MedCalc Software, Mariakerke, Belgium).

### 3. Results

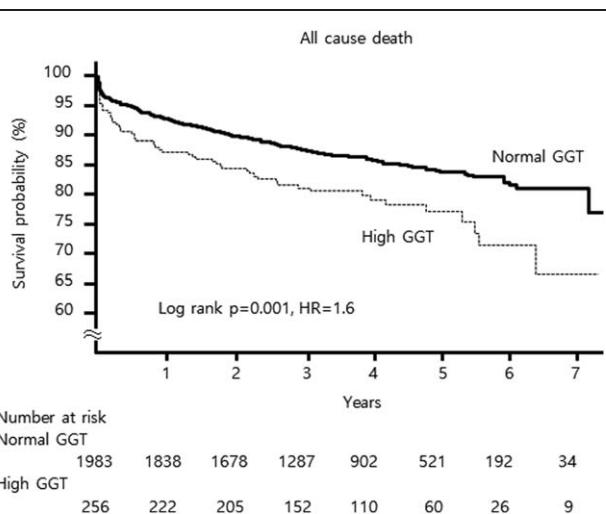
Patient GGT levels were non-normally distributed, and a high GGT level, defined as above the normal range, was observed in 256 patients (11.4%). The median GGT level was 31 (interquartile range [IQR] 20–55; mean  $46 \pm 44.6$ ) U/L in males and 21 (IQR 13–34; mean  $29.3 \pm 26.4$ ) U/L in females, and the percentages of male and female patients with high GGT levels were 11.3% and 11.7%, respectively. The baseline characteristics of the GGT groups are summarized in Table 1. At baseline, patients in the high serum GGT group had more conventional cardiovascular risk factors compared to the normal serum GGT group. Patients in the high GGT group were younger than those

**Table 1**

Baseline characteristics of normal GGT group and high GGT group.

	Overall (n = 2239)	Normal GGT (n = 1983)	High GGT (n = 256)	P
GGT, U/L	28(18–48)	25 (17–39)	113 (92–147)	<.001
Age, y	62.2 $\pm$ 12.7	62.5 $\pm$ 12.6	59.7 $\pm$ 13.6	.002
Women	643 (28.7%)	568 (28.6%)	75 (29.3%)	.828
BMI $\geq 25$ kg/m <sup>2</sup>	828 (37.4%)	719 (36.7%)	109 (43.1%)	.048
Hypertension	1151 (51.4%)	1009 (50.9%)	142 (55.5%)	.167
Diabetes mellitus	796 (35.6%)	698 (35.2%)	98 (38.3%)	.322
Insulin treatment	83 (11.2%)	70 (10.0%)	13 (13.3%)	.277
Familial history of CAD	135 (6.0%)	115 (5.8%)	20 (7.8%)	.203
Current smoking	968 (43.3%)	847 (42.7%)	121 (47.3%)	.166
Chronic kidney disease	152 (6.8%)	130 (6.6%)	22 (8.6%)	.222
LVEF $<50\%$	737 (34.2%)	646 (33.9%)	91 (37.1%)	.308
MDRD-eGFR, mL/min/1.73m <sup>2</sup>	75 (60–75)	75 (60–90)	77 (58–93)	.959
hsCRP, mg/dL	0.62 (0.18–2.41)	0.58 (0.17–2.3)	0.86 (0.27–3.88)	.007
Hemoglobin, g/dL	13.6 $\pm$ 2.1	13.5 $\pm$ 2.1	13.6 $\pm$ 2.3	.386
AST, U/L	33 (23–63)	32 (23–58)	52 (33–97)	<.001
ALT, U/L	27 (18–44)	26 (18–40)	46 (30–69)	<.001
Bilirubin, mg/dL	0.61 (0.49–0.84)	0.61 (0.49–0.83)	0.63 (0.47–0.9)	.117
ALP, U/L	146 (82–205)	144 (81–202)	164 (96–235)	<.001
Uric acid, mg/dL	5.7 $\pm$ 1.9	5.6 $\pm$ 1.8	6.3 $\pm$ 2.2	<.001
Triglyceride, mg/dL	103 (70–150)	102 (69–146)	114 (75–184)	.002
HDL cholesterol, mg/dL	40 (35–47)	40 (35–47)	41 (34–46)	.855
LDL cholesterol, mg/dL	110 (88–133)	110 (89–133)	104 (84–136)	.064
Total cholesterol, mg/dL	175 (150–202)	175 (151–201)	175 (146–206)	.543
STEMI	1373 (61.3%)	1210 (61%)	163 (63.7%)	.412
Killip class $\geq II$	452 (20.0%)	390 (19.7%)	62 (24.2%)	.091
Anterior wall MI	1138 (50.8%)	1014 (51.1%)	124 (48.4%)	.417
Post-PCI TIMI flow $< III$	232 (10.7%)	206 (10.7%)	26 (10.6%)	.956
No reflow	103 (4.6%)	83 (4.2%)	20 (7.8%)	.009
Discharge medication				
Aspirin	2238 (100%)	1982 (99.9%)	256 (100%)	.719
Clopidogrel	2234 (99.8%)	1979 (99.8%)	255 (99.6%)	.547
Statin	2081 (92.9%)	1845 (93%)	236 (92.2%)	.616
ACE-Is or ARBs	1617 (72.2%)	1364 (68.8%)	174 (68%)	.076
Beta-blocker	1538 (68.7%)	1433 (72.3%)	184 (71.9%)	.060

ACE-Is = angiotensin-converting enzyme inhibitors, ALP = alkaline phosphatase, ALT = alanine aminotransferase, ARBs = angiotensin type II receptor blockers, AST = aspartate aminotransferase, BMI = body mass index, CAD = coronary artery disease, eGFR = estimated glomerular filtration rate, GGT = gamma-glutamyl transferase, HDL = high-density lipoprotein, hsCRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, LVEF = left ventricular ejection fraction, MDRD = Modification of Diet in Renal Disease equation, MI = myocardial infarction, STEMI = ST segment elevation myocardial infarction, TIMI = thrombolysis in myocardial infarction.



**Figure 2.** Kaplan-Meier survival curves. All-cause mortality of patients with acute myocardial infarction according to normal or high serum GGT level. GGT=gamma-glutamyl transferase.

in the normal serum GGT group (mean age of  $59.7 \pm 13.6$  vs.  $62.5 \pm 12.6$  years, respectively). Compared with the normal GGT group, more patients in the high GGT group were obese ( $BMI \geq 25 \text{ kg/m}^2$ ; 36.7% vs. 43.1%,  $P=.048$ ). High-sensitive C-reactive protein (hsCRP), uric acid, and serum triglyceride were positively associated with high serum GGT levels, and the difference of them was also significant. No reflow phenomenon after PCI was also more frequently observed in high GGT group (7.8% vs. 4.2%,  $P=.009$ ). Biomarkers associated with liver disease, including AST, ALT, and alkaline phosphatase (ALP), were higher in the high GGT group. No other differences were observed between the two groups.

A total of 341 deaths (15.2%) were recorded during a median follow-up time of 3.7 years (IQR: 2.4–5.0 years). The number of cardiac death and noncardiac death of high GGT versus normal GGT group was 31 (12.1%) vs. 153 (7.7%) and 19 (7.4%) vs. 123 (6.2%), respectively. The proportion of unrevealed cause of death was 2.3% ( $n=6$ ) vs. 0.9% ( $n=18$ ). All-cause mortality

during the entire follow-up period was significantly higher in the high GGT group than the normal GGT group (21.9% vs. 14.4%,  $P=.001$  by the log-rank test). Early mortality at day 30 following PCI (5.9% vs. 3.2%,  $P=.03$ ) and late mortality from day 30 to the end of follow-up (17.1% vs. 11.5%,  $P=.013$ ) were also higher in the high GGT group than the normal GGT group. The Kaplan-Meier curves for all-cause mortality are presented in Figure 2. Compared with the normal GGT group, the high GGT group had an age and sex adjusted HR for death of 1.97 ( $P<.0001$ , Model 1). Additional adjustment for differences at baseline (Model 2:  $BMI \geq 25 \text{ kg/m}^2$ , ALT, AST, ALP, uric acid, hsCRP, hypertriglyceridemia (triglyceride  $>150 \text{ mg/dL}$ ), and presence of no-reflow phenomenon after PCI) and other cardiovascular risk factors (Model 3: hypertension, diabetes, chronic kidney disease, current smoking, high LDL-C ( $LDL \geq 100 \text{ mg/dL}$ ), Killip class  $\geq II$  at admission and final TIMI flow  $<III$  after PCI) could not attenuate this relationship. Elevated GGT remained an independent risk factor for all-cause death (multivariable-adjusted HR 2.12,  $P<.001$ ) (Table 2). In landmark analysis, the effect of high GGT group on the long-term mortality after 30 days after indexed PCI was consistent with the result of overall period death (adjusted HR 1.81,  $P=.009$ ), but there was no difference in early 30 days mortality between 2 groups. When we analyzed cardiac death separately, the association of GGT level was consistent with the former result (multivariable-adjusted HR 1.86  $P=.037$ ) (Fig. 3). The total number of patients who suffered nonfatal MI was 43, the difference between high and normal GGT group was insignificant ( $n=9$  [3.5%] vs.  $n=34$  [1.8%],  $P=.107$ ).

The results of the subgroup analysis are presented in Figure 3. The association between serum GGT level and all-cause mortality differed among the specific groups. After stratifying by sex, an association between serum GGT levels and all-cause death was observed in both female and male patients. However, the association was stronger in female patients than in male patients ( $P$  for interaction,  $<.001$ ). When stratified by the presentation of MI depending on the ST-segment change, the association between serum GGT levels and all-cause mortality was only observed among STEMI patients ( $P$  for interaction,  $<0.001$ ). Also, the association between high serum GGT levels and all-cause mortality was significant in who had preserved LVEF at

**Table 2**

Hazard ratios and 95% confidence intervals for mortality according to the 2 groups of serum GGT.

	Hazard ratio (95% CI)			
	Unadjusted	Model 1*	Model 2†	Model 3‡
High GGT	1.60 (1.14–2.24)	1.97 (1.48–2.63)	1.51 (1.03–2.19)	2.12 (1.44–3.11)
Age, y	1.06 (1.05–1.07)	1.06 (1.06–1.07)	1.06 (1.05–1.07)	1.06 (1.04–1.07)
Female	1.93 (1.56–2.39)	1.13 (0.91–1.41)	1.21 (0.91–1.62)	1.09 (0.80–1.49)
hsCRP			1.06 (1.04–1.08)	1.03 (1.01–1.05)
No-reflow			1.75 (1.05–2.91)	1.02 (0.69–2.19)
Uric acid			1.08 (1.02–1.14)	1.02 (0.96–1.09)
BMI $\geq 25 \text{ kg/m}^2$			0.61 (0.44–0.84)	0.66 (0.48–0.92)
Chronic kidney disease				3.91 (2.76–5.53)
Diabetes				1.58 (1.19–2.11)
Killip Class $\geq II$				1.70 (1.25–2.31)
Post-PCI TIMI flow $<III$				1.52 (1.06–2.18)

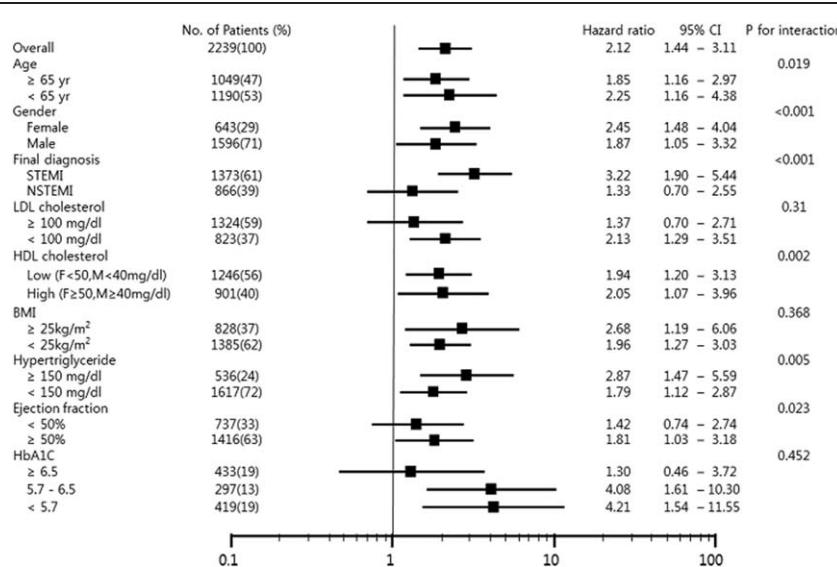
BMI = body mass index, CI = confidence interval, GGT = gamma-glutamyl transferase, hsCRP = high-sensitivity C-reactive protein, PCI = percutaneous coronary intervention, TIMI = thrombolysis in Myocardial Infarction.

The parameters which showed statistical significance after multivariate analysis are demonstrated.

\* Model 1 was adjusted for age and sex; unadjusted hazard ratios of these variables are included for reference.

† Model 2 was adjusted for model 1 plus  $BMI \geq 25 \text{ kg/m}^2$ , AST, ALT, ALP, uric acid, hsCRP, hypertriglyceridemia (triglyceride  $>150 \text{ mg/dL}$ ) and presence of no-reflow phenomenon during PCI.

‡ Model 3 was adjusted for model 2 plus hypertension, diabetes, chronic kidney disease, current smoking,  $LDL \geq 100 \text{ mg/dL}$ , Killip class  $\geq II$  at admission and TIMI flow  $<III$  after PCI.



**Figure 3.** Subgroup analysis. The association between serum GGT level and all-cause mortality differed among the specific groups according to age, sex, final diagnosis, LDL cholesterol, HDL cholesterol, body mass index, hypertriglyceride, ejection fraction and HbA1c. HRs were adjusted for age, sex, hypertension, diabetes, chronic kidney disease, current smoking, BMI  $\geq 25 \text{ kg/m}^2$ , AST, ALT, ALP, uric acid, hsCRP, hypertriglyceridemia (triglyceride  $> 150 \text{ mg/dL}$ ), high LDL cholesterol (LDL  $\geq 100 \text{ mg/dL}$ ), Killip class  $\geq II$  at admission, presence of no-reflow phenomenon during PCI, and TIMI flow  $< III$  after PCI. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMI = acute myocardial infarction, AST = aspartate aminotransferase, BMI = body mass index, GGT = gamma-glutamyl transferase, HDL = high-density lipoprotein, HR = hazard ratio, hsCRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, PCI = percutaneous coronary intervention, TIMI = thrombolysis in myocardial infarction.

admission (LVEF  $\geq 50\%$ ,  $P$  for interaction, .023). The association between high GGT levels and all-cause mortality was stronger in patients under the age of 65 years ( $P$  for interaction, .019) and in those had high HDL-C ( $P$  for interaction, .002), but the HR value of each subgroup showed just a slight difference. Additionally, the lower the HbA1c level of subgroups, the association tended to be stronger, but there were too many missing values ( $n = 1090$ ) to show statistical significance ( $P$  for trend,  $<.452$ ). The difference between obese and nonobese patients was not clear. Whether the value of GGT was more meaningful in low LDL-C ( $< 100 \text{ mg/dL}$ , HR = 2.13,  $P = .003$ ) was not obvious ( $P$  for interaction = .31).

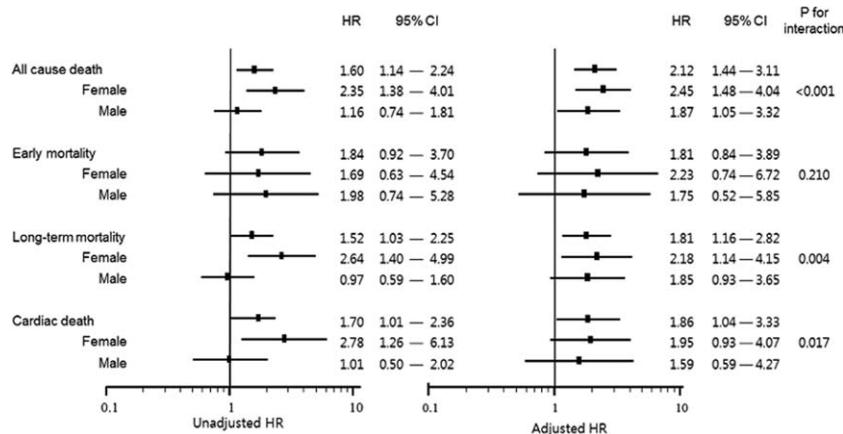
The baseline difference of male and female subgroup is presented in Table 3. Male and female AMI patients have very different cardiovascular risk factors, respectively. Men tend to be obese and have more history of current smoking. Male AMI patients' uric acid level is higher than women's and HDL-C is lower. Female AMI patients are much older and have more hypertension and diabetes. Estimated glomerular filtration rate and hemoglobin are significantly lower in these patients, and total and LDL-C level is higher. Furthermore, more females presented as worse Killip class and NSTEMI. As a result, the prognosis of female AMI patients was shown much worse than male patients (mortality 22.4% vs. 12.3%). On this basis, we repeated the same survival analysis on each sex group. Figure 4 is a summary of the association of high GGT and mortality in according to sex. After stratifying by sex, a stronger association between serum GGT level and all-cause death was observed in female patient. Especially in long-term mortality after 30th day, the association was observed only in females. In the aspect of cardiac death, the association was significant only in females, but the difference between 2 sex groups turned to be uncertain after multivariable adjustment.

ROC curve analyses were done to find out the cutoff value. It demonstrated that cutoff values of the serum GGT for predicting all-cause mortality could not be determined (AUC = 0.501,

**Table 3**  
The baseline characteristics of male and female AMI patients.

	Male (n = 1596)	Female (n = 643)	P
GGT, U/L	31 (20–55)	21 (13–34)	<.001
Age, y	59.1 ± 12.5	70.0 ± 9.8	<.001
BMI $\geq 25 \text{ kg/m}^2$	616 (39%)	212 (33.4%)	.013
Hypertension	721 (45.2%)	430 (66.9%)	<.001
Diabetes mellitus	519 (32.5%)	277 (43.1%)	<.001
Insulin treatment	53 (11.1%)	30 (11.5%)	.874
Familial history of CAD	104 (6.5%)	31 (4.8%)	.127
Current smoking	794 (49.7%)	174 (27.1%)	<.001
Chronic kidney disease	106 (6.6%)	46 (7.2%)	.663
LVEF $< 50\%$	511 (33.2%)	226 (36.7%)	.128
MDRD-eGFR, mL/min/1.73m <sup>2</sup>	78 (64–93)	66 (51–81)	<.001
hsCRP, mg/dL	0.55 (0.17–2.24)	0.81 (0.23–3.26)	.028
Hemoglobin, g/dL	14.1 ± 2.0	12.2 ± 1.9	<.001
AST, U/L	34 (24–64)	32 (23–60)	.678
ALT, U/L	30 (20–46)	22 (15–35)	.143
Bilirubin, mg/dL	0.65 (0.5–0.89)	0.57 (0.4–0.75)	<.001
ALP, U/L	143 (79–199)	158 (89–223)	<.001
Uric acid, mg/dL	5.8 ± 1.8	5.2 ± 2.0	<.001
Triglyceride, mg/dL	103 (71–151)	101 (67–149)	.149
HDL cholesterol, mg/dL	39 (34–46)	42 (35–49)	<.001
LDL cholesterol, mg/dL	109 (88–131)	113 (88–139)	.032
Total cholesterol, mg/dL	174 (150–200)	179 (152–206)	.014
STEMI	1004 (62.9%)	369 (57.4%)	.015
Killip class $\geq II$	278 (17.5%)	174 (27.1%)	<.001
Anterior wall MI (IRA-LAD)	816 (51.1%)	322 (50.1%)	.653
Post-PCI TIMI flow $< III$	160 (10.4%)	72 (11.4%)	.465
No reflow	72 (4.5%)	31 (4.8%)	.751
Discharge medication			
Aspirin	1595 (99.9%)	643 (100%)	.526
Clopidogrel	1593 (99.8%)	641 (99.7%)	.577
Statin	1514 (94.9%)	567 (88.2%)	<.001
ACE inhibitor or ARB	1189 (74.5%)	428 (66.6%)	<.001
Beta-blocker	1133 (71.0%)	405 (63.0%)	<.001

ACE-Is = angiotensin-converting enzyme inhibitors, ALP = alkaline phosphatase, ALT = alanine aminotransferase, ARBs = angiotensin type II receptor blockers, AST = aspartate aminotransferase, BMI = body mass index, CAD = coronary artery disease, eGFR = estimated glomerular filtration rate, GGT = gamma-glutamyl transferase, HDL = high-density lipoprotein, hsCRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, LVEF = left ventricular ejection fraction, MDRD = Modification of Diet in Renal Disease equation, MI = myocardial infarction, STEMI = ST segment elevation myocardial infarction, TIMI = thrombolysis in myocardial infarction.



**Figure 4.** Univariate- and multivariate-adjusted time-to-death curves of high gamma-glutamyl transferase (GGT) on normal GGT group about all-cause mortality (early within 30 days and long term) and cardiac mortality in according to female and male. HRs were adjusted for age, hypertension, diabetes, chronic kidney disease, current smoking, body mass index  $\geq 25 \text{ kg/m}^2$ , aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, uric acid, high-sensitivity C-reactive protein, hypertriglyceridemia (triglyceride  $> 150 \text{ mg/dL}$ ), high low-density lipoprotein (LDL) cholesterol ( $\text{LDL} \geq 100 \text{ mg/dL}$ ), Killip class  $\geq II$  at admission, presence of no-reflow phenomenon during percutaneous coronary intervention (PCI) and thrombolysis in myocardial infarction flow  $< III$  after PCI. CI = confidence interval, HR = hazard ratio.

$P=.94$ ). In female group, we could find statistical significant value ( $AUC=0.58$ ,  $P=.003$ ), but AUC showed very low discrimination accuracy. The cutoff value of GGT in female group was 58 U/L with 21.5% sensitivity and 91.6% specificity. It was very close to the minimum value of in female high GGT group in our study (57 U/L) (Fig. 5).

#### 4. Discussion

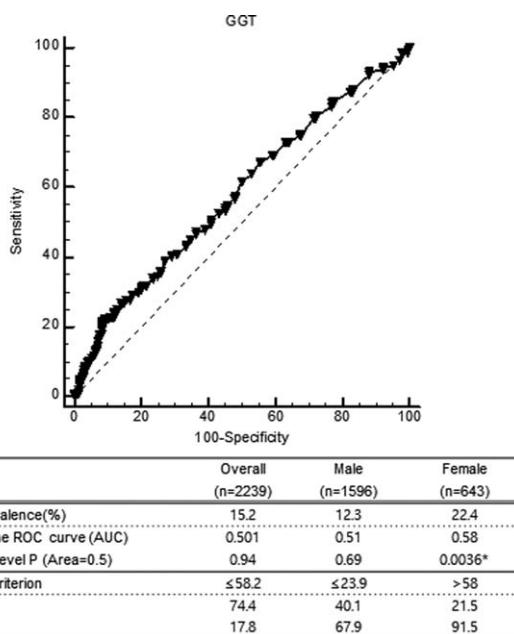
In this study, we observed a significant association of serum GGT levels with all-cause mortality and identified serum GGT as a reliable, independent predictor of long-term mortality and cardiac

mortality following AMI over a median follow-up period of 3.7 years. Interestingly, high GGT exhibited worse clinical outcomes in the female and in patients with STEMI and normal LVEF. These results are consistent with those of previous studies. In addition, this study is stronger than previous studies because it involved a larger AMI sample in which all subjects received PCI with drug eluting stent and had a longer follow-up period.

These findings may be explained by changes in lipid metabolism in the setting of AMI, in association with an acute-phase reaction and inflammatory response.<sup>[17]</sup> The patterns of alteration in the composition of lipid profile were wide in various previous reports, but a consensus about active inflammatory changes after AMI was developed. As previously explained, GGT is an enzyme associated with lipid metabolism and oxidative stress; therefore, the level of serum GGT at admission may reflect the degree of baseline change in lipid metabolism and inflammation after AMI, which may have implications for prognosis. In a small cohort study suggest the alteration of lipid metabolism might be result of secondary liver failure because of extensive and severe MI,<sup>[18]</sup> but the impact of high GGT on mortality was not altered after adjustment of liver enzyme and lipid profiles. It suggests the independent role of GGT on inflammation process apart from the level of lipids and liver enzymes.

The differences in baseline characteristics between the 2 GGT groups observed in this study are also consistent with explanation. The inflammatory markers hsCRP<sup>[19]</sup> and uric acid<sup>[20]</sup> were significantly higher in the high GGT group. Uric acid is considered a metabolic product of inflammation and is elevated in various inflammatory conditions such as CAD and heart failure.<sup>[21,22]</sup>

In general, LDL-C is the most important determinants of atherosclerotic events in the lipid profile.<sup>[17]</sup> It is interesting that there were some trends toward lower LDL-C levels in patients with high GGT. In our multivariable analysis, the high LDL-C tended to be preventive for mortality ( $\geq 100 \text{ mg/dL}$ ; HR = 0.81,  $P=.139$ ) or LDL (as continuous variable, mg/dL; HR 1.0,  $P=.309$ ), but it was not statistically significant. The use of statins could be a confounding factor, but unfortunately there was no



**Figure 5.** ROC curve analyses of serum GGT level and all-cause mortality in female acute myocardial infarction patients. AUC = area under the curve, GGT = gamma-glutamyl transferase, ROC = receiver-operating characteristic.

information about either the usage of statin at the time of blood sampling or the level of LDL-C at the time of discharge in our registry. These might be one of the limitations of retrospective data from real world practice. Other lipid profiles including HDL-C and triglyceride have been previously reported in association with GGT, but the results were conflicting as our study.

Subgroup analyses revealed that the association between serum GGT level and all-cause mortality were more significant in STEMI patients. This association may reflect difference in nature of coronary obstruction in STEMI and NSTEMI. STEMI is primarily attributed to acute plaque rupture owing to inflammation, suggesting a role for GGT. This discrepancy may also be because of the inclusion of an insufficient number of NSTEMI patients.

Adverse outcomes may also be attributed to the association between serum GGT level and metabolic syndrome. Obesity and hypertriglyceridemia were more frequently observed in the high GGT group. In this study, high GGT was more significant in patients who were younger than 65 years or who had normal left ventricular function. Such patients are usually expected to have a favorable prognosis but may be negligent in their long-term health care. In this regard, GGT levels may serve as a guide for these patients because they may receive the greatest benefit from being aware of metabolic disturbances. Although there was no statistical significance, the lower the HbA1c level of subgroups, the association tended to be stronger with GGT that could be an implication in the same vein.

To determine whether male and female patients with AMI differed with baseline risk factors and treatment, we repeated analysis of baseline characteristics after re-grouping. Age at the time of MI showed big difference as previous studies,<sup>[23]</sup> women were much older than men. So, the greater risk of mortality and morbidity owing to age may attenuate the other risk difference of females. But in our study, after adjustment for age the meaning of high GGT was not changed. Our study reported a higher percentage of hypertension, diabetes mellitus and higher Killip classes in women, whereas current smoking, obesity, familial history of CAD, and presentation as STEMI tended to be more in men. The distribution of risk factors was different between them and these findings are consistent with previous reports,<sup>[23]</sup> then we can find out that we should assess differently and separately of each sex group. Interestingly, there was significant difference of discharge medication between 2 groups. In females, statins were less prescribed in spite of higher total and LDL-C, and angiotensin-converting enzyme-inhibitors, angiotensin type II receptor blockers, and beta-blockers were not prescribed either to more females those tended to present lower LVEF. These factors may explain the higher long-term mortality of women with AMI.

A previous STEMI study suggested that the differences in microvascular reperfusion after PCI between men and women may attribute to higher risks in female.<sup>[24]</sup> In the study, TIMI myocardial perfusion grade and incomplete ST segment resolution were used as a parameter of microvascular dysfunction, and females had lower TIMI grade and more incomplete ST segment resolution. In our study, the percentage of lower TIMI flow grade after PCI and presence of no-reflow phenomenon during PCI were compared; no significant difference was observed between both sex groups. But presence of no-reflow was significantly different between high GGT and normal GGT group. Furthermore, no-reflow and TIMI flow after PCI were also important covariates with clinical significance in multivariable analysis of all-cause mortality in association with GGT. This might be an implication of the association of GGT and myocardial

microvascular dysfunction, but more concrete investigation will be needed.

We made 3 interesting observations concerning the association between elevated serum GGT levels and clinical outcome in patients with AMI. First, elevated serum GGT levels are a useful marker for easily and reliably predicting long-term clinical outcomes in patients with AMI. GGT levels are reportedly higher in patients with CAD than in the general population, and GGT levels are higher in patients with NSTEMI and STEMI than in patients with unstable angina.<sup>[19]</sup> In our AMI population, the proportion of patients with high GGT levels who did not have overt liver disease exceeded 11%, and the results were statistically significant after adjusting for liver markers such as AST, ALT, and ALP. Therefore, this method is not only acceptable but also likely easy to apply in clinical practice. Second, elevated GGT levels were more strongly associated with clinical outcome in female patients than in male patients. No previous studies have examined sex differences in the association between GGT level and outcomes in MI.<sup>[23]</sup> Studies of the association of GGT and other cardiovascular risk factors have observed heterogeneity in sex differences. In 2 studies of coronary calcification and hypertension in Korea, GGT level was an independent predictor in men but not women.<sup>[25,26]</sup> However, one study of the association between GGT and vascular events observed a significant positive association between GGT levels and cardiovascular disease in women.<sup>[27]</sup> The sex differences observed in our study may be because of the differences in alcohol consumption between men and women. More Korean men drink alcohol than women.<sup>[26]</sup> As an alternative explanation, BMI differed significantly between the normal and high GGT groups and between men and women. There was a striking 34% to 39% reduction in mortality in patients with  $BMI \geq 25 \text{ kg/m}^2$  at Table 2 and the patients with  $BMI \geq 25 \text{ kg/m}^2$  were more in men than women. It is mostly understood that patients with overweight or mild obesity (BMI of 25–30 kg/m<sup>2</sup>) might have relatively better outcomes, so this might affect the result consequently. Severe obesity (BMI > 30 kg/m<sup>2</sup>) is commonly considered as a predictor of worse outcomes, and the mortality of overt obese (BMI > 30 kg/m<sup>2</sup>; n = 97, 11.3%) group tended to be higher than that of overweight (BMI of 25–30 kg/m<sup>2</sup>; n = 731, 9.7%) group in our AMI patients, but it was not statistically significant (HR = 1.21, Log rank, P = .557).

Third, our study population is unique in that we included both STEMI and NSTEMI patients and all patients underwent PCI with DES. Previous studies have included either STEMI patients or non-ST segment elevation-ACS patients. According to current guidelines on long-term management following ACS, the treatment strategy depends primarily on whether the clinical outcome is MI.<sup>[28–30]</sup> Therefore, the overall outcome following AMI is also crucial. The all-cause mortality rates in our study population and in the high GGT level group were slightly higher and much higher, respectively, than those in the HORIZONS AMI trial in patients with STEMI (approximately 6%–7%).<sup>[31]</sup> When the early mortality rate was assessed, it was similar to the result that obtained using the long-term outcome. This result suggests that the initial GGT level might reflect not only the acute phase of inflammation, but also the chronic systemic metabolic status of an individual, which can affect the long-term outcome.

## 5. Limitations

This study has several limitations. First, this was an observational study and may be subject to bias and confounding. Specifically, information on alcohol consumption, which can influence the

level of GGT, was not included in this study and 52% of patients did not have a GGT serum level recorded. Moreover, the exclusion of liver disease in patients was not confirmed by imaging or serology for viral hepatitis.<sup>[27,32]</sup> Isolated GGT elevation has not been significantly associated with adverse outcomes in liver disease; therefore, the relevance of GGT elevation in MI should not to be attenuated.

Second, there were a limited number of adverse events, and we were unable to specify the various subtypes of adverse cardiovascular events. Third, although many possible hypotheses of the mechanisms of GGT have existed, no clear evidence was proven. Even there is no report on the time course of GGT in the setting of AMI like AST, LDH, or CPK. Recently, various subtypes of GGTs with specific functions have been studied.<sup>[33,34]</sup> These studies might be a key to understand the mechanism of the association between GGT and cardiovascular disease. In addition, a large prospective study with serial GGT values and associated variables will contribute to a detailed understanding of the pathogenesis of cardiovascular disease.

## 6. Conclusion

In summary, an elevated GGT level is an independent predictor of adverse long-term prognosis and increase of cardiac mortality in patients with AMI. Stronger associations were observed in the young and female patients and in patients who had STEMI and initially preserved LVEF after the indexed PCI.

## Author contributions

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**Software:** Jae Gyung Kim.

**Supervision:** Jong-Min Lee, Kiyuk Chang.

**Visualization:** Jae Gyung Kim.

**Writing – original draft:** Jae Gyung Kim.

**Writing – review & editing:** Jae Gyung Kim, Kiyuk Chang.

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## Letter to the Editor

**Changes in serum gamma-glutamyl transferase and blood pressure levels in subjects with normal blood pressure and prehypertension**

Dear editor:

Recently, serum gamma-glutamyltransferase (GGT), a marker of oxidative stress, has been considered to be linked with the development of cardiovascular disorders [1]. In light of the overall positive associations between serum GGT and hypertension (HT) in previous reports [2–7], similarly, a significant and positive association between GGT and prehypertension (PreHT) has more recently reported in the general population [8]. PreHT, defined as systolic blood pressure (SBP) ranging from 120–139 mmHg or diastolic blood pressure (DBP) ranging from 80–89 mmHg (by the Seventh Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure), is identified as a predictor for the occurrence of HT and a stage when primary prevention of HT is still possible [9–12]. The result of the association of GGT with PreHT may contribute to preventive cardiology because the clinical usage of GGT is easy as a biochemical marker; however, a study examining the association of GGT with PreHT was cross-sectional [8], so it is important to confirm the association of GGT changes with SBP/DBP changes in PreHT levels in a longitudinal (over a period of at least 1 y) study.

Moreover, the Seventh Joint National Committee defines <120/80 mmHg of SBP/DBP as normal blood pressure (NBP) [9]. Individuals with NBP are also thought to be in a more possible stage of primary prevention of HT than PreHT; however, no study has examined the association of GGT with NBP.

This background encouraged us to examine whether SBP/DBP changes could be accompanied by GGT changes in subjects with PreHT and NBP. Thus, we conducted a one-year observational study to investigate this association and to observe whether there were any differences between PreHT and NBP subjects.

Overall, 364 asymptomatic Japanese subjects were studied during a 1-y study period: 259 PreHT subjects (79 men and 180 women; mean age:  $49.8 \pm 6.9$  [range: 35–64] y) and 105 NBP subjects (50 men and 55 women; mean:  $47.9 \pm 6.0$  [37–61] y) were included. This study was approved by the Tottori University Ethics Committee and each subject gave informed consent. All subjects were negative for both hepatitis B surface antigen and hepatitis C virus antibody, did not take any continuous medication (untreated during the study period), had no alcohol consumption or smoking habit, and no medical history of cardiovascular, renal, thyroid, malignant or collagen disorders. After a 12-h fast, seated

SBP/DBP, body mass index (BMI), serum lipids (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], triglyceride [TG]), plasma glucose (PG) and GGT were respectively measured. SBP/DBP was measured 3 times with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Japan) and the 3 measurements were averaged. PG was measured with an automatic analyzer (BM2250, JEOL Co. Ltd., Japan). TC, TG, HDL-C and GGT were assayed with an automatic analyzer (TBA-200FR, Toshiba, Japan). In the pre-study data, subjects  $>100$  U/l in serum GGT levels were not included (basically, there is no clear gender difference in the association between GGT and BP [8]). All subjects were of relatively ordinary health, and those with the following pre-study data (mild dyslipidemia and impaired fasting glucose tolerance) were included:  $<6.2$  mmol/l in TC; 0.8–2.6 mmol/l in HDL-C;  $<2.8$  mmol/l in TG;  $<7.0$  mmol/l in PG. In the post-study period, the same variables such as SBP/DBP, BMI and GGT were reexamined.

All values were expressed as the means  $\pm$  SD (geometric means in GGT only). Multiple regression analysis of SBP/DBP changes was used to analyze the correlation with GGT changes after adjusting for measured confounders (age, gender and BMI changes). Because of the skewed distribution, GGT was log-transformed for this analysis. A  $P < 0.05$  was considered significant.

In PreHT, the pre-study mean levels and post-study mean levels (followed by mean change levels [range]) in each variable were as follows:  $126.7 \pm 8.0$  and  $125.3 \pm 12.0$  ( $-1.4 \pm 11.2$  [−30–30]) mmHg in SBP;  $82.1 \pm 6.0$  and  $81.7 \pm 8.3$  ( $-0.4 \pm 8.9$  [−28–27]) mmHg in DBP;  $23.4 \pm 2.8$  and  $23.4 \pm 2.9$  ( $0.0 \pm 0.7$  [−3.1–1.7]) kg/m<sup>2</sup> in BMI;  $40.2 \pm 1.7$  and  $38.8 \pm 1.8$  ( $1.0 \pm 1.5$  [−68–80]) U/l in GGT. Similarly, in NBP, the pre-study mean levels and post-study mean levels (mean change levels [range]) in each variable were as follows:  $104.6 \pm 7.3$  and  $110.2 \pm 10.8$  ( $5.7 \pm 9.5$  [−19–26]) mmHg in SBP;  $67.2 \pm 6.5$  and  $70.6 \pm 8.4$  ( $3.4 \pm 8.1$  [−17–26]) mmHg in DBP;  $21.5 \pm 2.3$  and  $21.6 \pm 2.5$  ( $0.1 \pm 0.8$  [−3.3–2.1]) kg/m<sup>2</sup> in BMI;  $28.1 \pm 1.6$  and  $28.6 \pm 1.7$  ( $1.0 \pm 1.0$  [−68–80]) U/l in GGT.

In PreHT, SBP as well as DBP changes significantly, independently and positively correlated with GGT changes (Table 1). SBP changes also significantly correlated with BMI changes. Age correlated with SBP changes, but did not reach statistical significance. In NBP, only one significant correlation was seen: SBP changes positively correlated with BMI changes.

We found a significant, independent and positive association between GGT and SBP/DBP changes in a population with PreHT, whereas a weak association in NBP. This suggests that GGT may be a predictive biochemical marker for SBP/DBP changes in PreHT, extending the recent cross-sectional data on

Table 1  
Multiple regression analysis of variables correlated to blood pressure changes

Variable	Prehypertension	Normal blood pressure
	$\beta$ -coefficient (P value)	$\beta$ -coefficient (P value)
<i>For Δ systolic blood pressure</i>		
Age	0.118 (0.053)	0.107 (NS)
Gender, male	0.082 (NS)	0.072 (NS)
Δ body mass index	0.128 (0.048*)	0.232 (0.017*)
Δ GGT <sup>a</sup>	0.162 (0.012*)	0.120 (NS)
<i>For Δ diastolic blood pressure</i>		
Age	0.030 (NS)	0.159 (NS)
Gender, male	0.059 (NS)	0.080 (0.420)
Δ body mass index	0.087 (NS)	-0.021 (NS)
Δ GGT <sup>a</sup>	0.150 (0.023*)	0.132 (NS)

GGT: gamma-glutamyl transferase. Δ(change levels) means the values by subtracting pre-study values from post-study values.

<sup>a</sup> GGT was analyzed after log-transformation because of the skewed distribution.

\* Significance: P<0.05.

the association between GGT and PreHT [8]. The association between BMI and SBP changes, observed in PreHT and NBP, is consistent with previous reports [13,14]. Additionally, it is interesting that the effects of GGT on SBP/DBP may be somewhat different between PreHT and NBP. Although the mechanism of the association between GGT and blood pressure remains largely unknown, some plausible explanations exist; e.g., its direct involvement in the generation of reactive oxygen species; on the other hand, its indirect role in maintaining intracellular antioxidant glutathione (in response to oxidative stress, increased transport of glutathione into cells by increased GGT activity); its relation to chronic inflammation and insulin resistance affecting the cardiovascular system [1,2,8]. A significant association of GGT with PreHT, rather than NBP, may be explained in considering the above speculation. Namely, PreHT may be a status with oxidative stress, inflammation or insulin resistance, linked to increased GGT activity, more than NBP. If so, GGT is related to clinically relevant BP stages, at least from the PreHT stage; therefore, GGT could be useful to monitor the disease continuum of HT. In addition, exploring the biological roles of GGT might partly clarify the mechanism of HT development.

Our study had several limitations. BP measured in a single day could be a relatively weak point, although many epidemiological studies have adopted this methodology. Concerning the short-term period and population number, more studies with a longer follow-up period and a larger sample (particularly in NBP subjects) will be needed as a future challenge.

In conclusion, during 1 y, serum GGT level changes were found to be significantly and positively correlated with SBP/DBP level changes, particularly in PreHT. The impact of GGT on SBP/DBP in PreHT was suggested to be greater relative to NBP, maybe reflecting the difference in both pathophysiological conditions.

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# 5.4

## Fatty Liver Index, Gamma-Glutamyltransferase, and Early Carotid Plaques

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An association between fatty liver and carotid atherosclerosis has been established; however, it is not clear whether this relationship is a consequence of shared conventional risk factors or whether it is determined by specific circulating factors originating from liver or adipose tissue. To identify the factors possibly linking fatty liver and atherosclerosis, we assessed, in 1,012 subjects free of confounding diseases (e.g., hypertension, diabetes, cardiovascular diseases, and dyslipidemia) and metabolic syndrome, the relationship between the presence of early plaques at carotid bifurcation and fatty liver index (FLI; a validated surrogate marker of fatty liver), as well as the associations between carotid plaque presence and established atherosclerotic risk factors, family history of cardiovascular disease (FH-CVD) or diabetes, insulin sensitivity, serum liver enzymes, adipokines, fatty free acids, and high-sensitivity C-reactive protein (hsCRP). A total of 55 of 1,012 subjects (5.4%) had small plaque at carotid bifurcation. Subjects with plaque were older and had higher prevalence of FLI  $\geq 60$  and FH-CVD, higher blood pressure, plasma low-density lipoprotein cholesterol, glucose, gamma-glutamyltransferase (GGT), and hsCRP, as compared to subjects without plaques ( $P < 0.05$ ). In a logistic regression model, adjusted for sex, liver transaminase, and alcohol consumption, the independent predictors of plaque presence were age ( $P < 0.0005$ ), FLI  $\geq 60$  ( $P < 0.0005$ ), and current smoking ( $P < 0.05$ ). When FLI in the model was replaced by variables used in its equation (e.g., body mass index, waist circumference, plasma triglycerides, and GGT), the independent determinants of plaque presence were age ( $P < 0.001$ ), GGT ( $P = 0.001$ ), and current smoking ( $P < 0.05$ ). **Conclusions:** Our cross-sectional study suggests that subjects with FLI  $\geq 60$  are at higher risk of atherosclerotic lesions, independently of established risk factors, and that serum GGT may represent a link between fatty liver and the development of early atherosclerosis. (HEPATOLOGY 2012;55:1406-1415)

Published studies have demonstrated an association between fatty liver and carotid atherosclerosis,<sup>1,2</sup> above all, carotid atherosclerotic plaques.<sup>3</sup> However, it is not clear whether the relationship between the fatty liver and atherosclerotic process is a consequence of shared established risk factors (e.g., abdominal obesity, atherogenic dyslipidemia, hypertension, and dysglycemia)<sup>4,5</sup> or whether it is determined by specific circulating factors that originate either from liver or from adipose tissue<sup>6</sup> and are known to parti-

cate in the development and progression of atherosclerosis through their effects on smooth muscle cell (SMC) proliferation and migration, foam cell formation, inflammation, and angiogenesis.<sup>7-11</sup>

To provide insight about the possible links between fatty liver and the development and progression of carotid atherosclerosis, we analyzed data from the Relationship between Insulin Sensitivity and Cardiovascular risk (RISC) Study that included a clinically healthy young-to-middle-aged European Caucasian population

**Abbreviations:** ATP III, National Cholesterol Education Program's Adult Treatment Panel III report; BMI, body mass index; BP, blood pressure; CCA, common carotid artery; CNR, Consiglio Nazionale delle Ricerche; CVDs, cardiovascular diseases; DELFIA, dissociation-enhanced lanthanide fluorescent immunoassay; FH-CVD, family history of cardiovascular disease; FLI, fatty liver index; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HRT, hormone replacement therapy; hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; LDL, low-density lipoprotein; MGL, mean gray level; MII, index of insulin sensitivity; NEFAs, nonesterified fatty acids; OGTT, oral glucose tolerance test; PA, physical activity; RISC, Relationship between Insulin Sensitivity and Cardiovascular risk Study; ROI, region of interest; SD, standard deviation; SE, standard error; SMCs, smooth muscle cells; US, ultrasound.

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that was free of confounding morbidities, such as hypertension, diabetes, dyslipidemia, and cardiovascular diseases (CVDs).<sup>12</sup> In such a selected population, we assessed the relationship between the presence of small plaque at carotid bifurcation and fatty liver, as well as the cross-sectional associations between carotid plaque presence and conventional atherosclerotic risk factors (e.g., sex, age, anthropometric parameters, blood pressure [BP], lipid profile, smoking habit, low physical activity [PA] level, and family history of CVD and diabetes), insulin resistance, and several circulating molecules, possibly proatherogenic and originating from liver or adipose tissue (e.g., liver enzymes, adipokines, fatty free acids, and high-sensitivity C-reactive protein; hsCRP). The presence of fatty liver was estimated by a validated surrogate marker, the fatty liver index (FLI).<sup>13,14</sup>

Small plaques at carotid bifurcation represent a good clinical model of early atherosclerosis, because carotid bifurcation is one of the first, and the most common, sites of atherosclerotic plaque formation resulting from interactions between local hemodynamic factors, local cellular factors, and active circulating molecules.<sup>15,16</sup> Besides the presence of early carotid plaques, we also assessed their acoustic properties by means of videodensitometric analysis, which has been previously shown to provide information on plaque composition, because it can identify plaque with high content of lipid or SMCs.<sup>17,18</sup>

## Patients and Methods

The study population was a subgroup of the RISC study cohort ([www.egir.org](http://www.egir.org)). The details of the study design and protocol have been reported elsewhere.<sup>12</sup> Briefly, RISC recruited apparently healthy Caucasian subjects in 19 centers in 14 European countries between June 2002 and July 2004. They were between 30 and 60 years of age with BP, serum cholesterol, triglycerides, fasting, and 2-hour glucose concentrations within established limits. Exclusion criteria were the presence of overt CVD, chronic diseases (e.g., hypertension, diabetes, dyslipidemia, chronic lung, hepatic and kidney diseases, and neoplastic and inflammatory diseases), class III obesity, the presence of carotid stenosis >40% and calcified carotid plaques, and treatment for hypertension, diabetes, dyslipidemia, obesity,

and steroid treatment (with the exception of hormone replacement therapy [HRT] in menopausal women). A standardized examination protocol included anthropometry, brachial BP measurements, resting electrocardiogram, a fasting blood test, an oral glucose tolerance test (OGTT), a euglycemic hyperinsulinemic clamp, and high-resolution ultrasound (US) of extracranial carotid arteries. Information regarding medical history, drug use, alcohol and cigarette consumption, and family history (i.e., any first-degree family member) of CVD (FH-CVD) (i.e., coronary heart disease and stroke) and diabetes were collected using standardized self-reported questionnaires. For smoking habit, the subjects were categorized as never smoker, current smoker, and ex-smoker (when quitted smoking for 1 year and more before the study). Data on alcohol consumption were not rechecked with family members. Metabolic syndrome was defined according to the criteria of the National Cholesterol Education Program's Adult Treatment Panel III report (ATP III), which have been suggested to provide a practical tool for identification of subjects at increased risk of CVD.<sup>19</sup> The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the local ethics committee in each center. Written consent was obtained from all participants.

**Population of the Present Study.** In the RISC study, 1,566 participants were originally recruited; 356 were excluded for not satisfying inclusion criteria or for incomplete baseline examination (Fig. 1). For the purpose of this study, we also excluded subjects in whom the carotid bifurcation and origin of internal carotid artery could not be adequately visualized as well as subjects with an above-average or a high relative Framingham risk score<sup>20</sup> and with metabolic syndrome according to ATP III criteria.<sup>19</sup> The final study population consisted of 1,012 apparently healthy subjects with complete baseline data, adequate visualization of the entire extracranial carotid tree, at a low-average relative Framingham risk, and free of metabolic syndrome. In a subpopulation of 669 subjects (66.1% of the population of the present study), an objective assessment of habitual PA by means of accelerometer monitoring<sup>21</sup> was also available (Fig. 1).

**Body Composition Assessment and BP Measurement.** Body weight and fat-free mass were measured by electrical bioimpedance using a Body Composition

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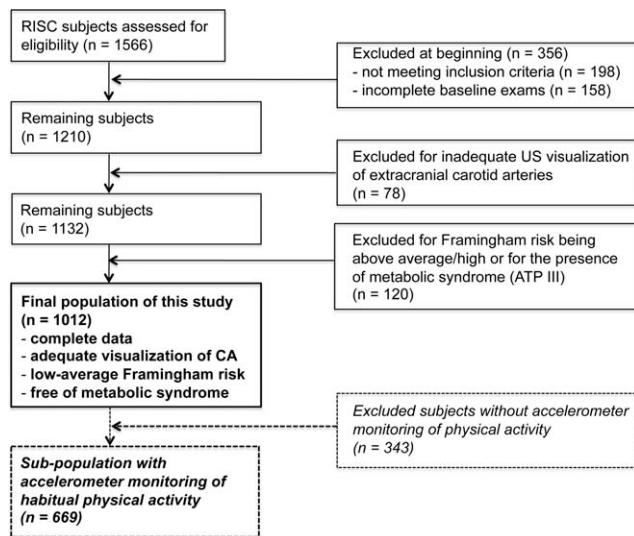


Fig. 1. Selection of the present study population from the RISC population.

Analyzer (model TB-300; Tanita, Tokyo, Japan); fat mass was obtained as the difference between body weight and fat-free mass. Waist circumference was measured as the narrowest circumference between the lower rib margin and anterior superior iliac crest. Brachial BP was measured by a digital electronic tensiometer (model 705cp; Omron, Kyoto, Japan), with regular or large adult cuffs according to the arm circumference, in subjects seated for at least 10 minutes.

**OGTT and Insulin Clamp.** A 75-g OGTT was performed, with blood samples taken before and 30, 60, 90, and 120 minutes into the test. On a separate day within 1 month of the OGTT, a euglycemic hyperinsulinemic clamp was performed. Exogenous insulin was administered as a primed-continuous infusion at a rate of  $240 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$  simultaneously with a variable 20% dextrose infusion adjusted every 5–10 minutes to maintain a plasma glucose level within  $0.8 \text{ mmol/L}$  ( $\pm 15\%$ ) of the target glucose level (4.5–5.5 mmol/L). Additional blood samples were obtained at 20-minute intervals for insulin determination. The clamp procedure was standardized across centers.<sup>12,22</sup> Insulin sensitivity was expressed as the ratio of the M value averaged over the final 40 minutes of the 2-hour clamp and normalized by the fat-free mass, to the mean plasma insulin concentration measured during the same interval (M/I; in units of  $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}_{\text{ffm}}^{-1} \cdot \text{mm}^{-1}$ ).<sup>22</sup>

**Analytical Procedures.** Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (Roche Method for Modular System; Roche Diagnostics, Basel, Switzerland), glucose (Cobas Integra; Roche

Diagnostics), insulin (a specific time-resolved fluoroimmunoassay, AutoDELFIA [dissociation-enhanced lanthanide fluorescent immunoassay] Insulin kit; Wallac Oy, Turku, Finland), liver enzymes (International Federation of Clinical Chemistry method; Dade-Behring Dimension RXL, Newark, DE), leptin (an in-house DELFIA assay on an AutoDELFIA autoanalyzer; Wallac Oy), total plasma adiponectin (in-house time-resolved immunofluometric assay<sup>23</sup>), nonesterified fatty acids (NEFAs; Randox enzymatic kit, Hitachi Modular P unit; Hitachi, Tokyo, Japan), and hsCRP (monoclonal antibodies from R&D Systems; Abingdon, UK) were measured centrally.

**Surrogate Marker of Fatty Liver.** A surrogate marker of fatty liver, the FLI, was calculated.<sup>13,14</sup> An FLI that uses an algorithm based on body mass index (BMI), waist circumference, triglycerides, and gamma-glutamyltransferase (GGT) has been validated against liver US in the general population and has been proven accurate in detecting fatty liver (accuracy, 0.84 [95% confidence interval: 0.81–0.87]).<sup>13</sup> When the index value is greater than or equal to 60 ( $\text{FLI} \geq 60$ ), the probability of having a fatty liver is  $>78\%$ .<sup>14</sup> A validation of FLI against magnetic resonance spectroscopy demonstrated the presence of hepatic fat (range, 8.6%–24.0%) in subjects with  $\text{FLI} \geq 60$  and the absence of hepatic fat in those with  $\text{FLI} < 20$ .<sup>24</sup>

**Physical Activity Assessment.** Habitual PA was estimated by accelerometer monitoring. A single-axis accelerometer (Computer Science Applications Model AM7164; Manufacturing Technology, Inc., South Bend, IN) was used to monitor ambulatory movements.<sup>21</sup> The accelerometer was secured by a belt at the small of the back from waking up until going to sleep. Subjects were asked to wear the monitor for 7 days, if possible, weekend included, and to behave in their usual manner. In the final analysis, only those days when the accelerometer was worn for at least 10 hours were included. Nonwearing periods were identified as 60 minutes or more of continuous zero counts. Accelerometer data were processed with custom software developed for the RISC project and were checked for spurious recording: high counts  $>20,000$  counts/min or repeated recording of the same number of counts<sup>25</sup>; the days with spurious data were excluded. The average intensity of daily PA was expressed as the average number of accelerometer counts per 1 minute of monitoring time.

**Carotid Artery US Imaging and Analysis.** High-resolution B-mode US of extracranial carotid arteries was performed in each recruiting center by trained and certified technicians following a standardized

protocol.<sup>12</sup> Reading of carotid images was centralized at one center (University of Pisa, Pisa, Italy) by a single reader (M.K.) using the Medical Image Processing computer-driven image analysis system (Institute of Clinical Physiology, Consiglio Nazionale delle Ricerche [CNR], Pisa, Italy). Near- and far-wall intima-media thickness (IMT) was measured bilaterally in digitized end-diastolic frames for a single view of the common carotid artery (CCA) and for three different views of the carotid bulb and internal carotid artery. Carotid plaque was defined as an IMT >1.5 mm in any carotid segment.<sup>26</sup> CCA IMT represents a mean value of far-wall IMT measured in the right and left artery. In the RISC study, intraobserver variability of IMT measurements was tested in 140 randomly chosen scans. The correlation between two readings was  $r = 0.95$  ( $P < 0.0001$ ), and the mean difference was  $4.8\% \pm 2.8\%$ .

**Acoustic Videodensitometric Analysis of Carotid Plaques.** The acoustic properties of the carotid plaque were evaluated by means of digital densitometric analysis (Institute of Clinical Physiology, CNR, Pisa, Italy) that was previously validated against histological analysis of intimal lesions.<sup>18</sup> In the long-axis view, a region of interest (ROI) including the entire carotid plaque was selected. Within the ROI, digitized images were analyzed by the first-order analysis that generates a histogram representing the frequency distribution of gray levels of pixels by plotting the gray values on the abscissa and the frequency of the occurrence on the ordinate. The histogram was described in terms of average pixel intensity, that is, mean gray level (MGL). To adjust for different gain settings and different US attenuation in different study subjects, two calibration steps were introduced into the analysis of each subject. The effect-of-gain setting was restrained by calibrating the gray-level amplitude of the ROI against vessel lumen (i.e., blood) taken as the blank (MGL = 0), whereas the effects of imaging depth and attenuation were minimized by calibration against an internal reference represented by the adventitia (MGL = 160).<sup>18</sup> Intraobserver variability of MGL measurement was tested in 30 randomly chosen plaques. The correlation between two readings was  $r = 0.93$  ( $P < 0.0001$ ), and the corresponding mean difference was  $6.7\% \pm 5.0\%$ .

**Statistical Analysis.** Quantitative data are expressed as mean  $\pm$  standard deviation (SD), and categorical data are expressed as percentages. Skewed variables are given as median and interquartile range and were log-transformed for statistical analyses. Analysis of variance was used to compare continuous variables. Relations between the outcome variables and continuous variables were evaluated by univariate Pearson's correlation

**Table 1. Characteristics of Subjects Without and With Early Carotid Plaque**

	Without Plaque	With Plaque	P Value
N	957	55	
FLI	26 $\pm$ 23	40 $\pm$ 28	<0.0001
FLI $\geq$ 60 (%)	11.1	36.3	<0.0001
Sex (men; %)	42.4	50.9	=0.22
Age (years)	43 $\pm$ 8	49 $\pm$ 8	<0.0001
Waist circumference (cm)	85 $\pm$ 12	87 $\pm$ 13	=0.12
BMI (kg/m <sup>2</sup> )	24.9 $\pm$ 3.7	25.6 $\pm$ 3.4	=0.14
Fat mass (kg)	19.8 $\pm$ 8.1	20.7 $\pm$ 7.5	=0.44
Systolic BP (mmHg)	116 $\pm$ 12	121 $\pm$ 12	=0.01
Diastolic BP (mmHg)	74 $\pm$ 8	77 $\pm$ 7	=0.01
Heart rate (bpm)	68 $\pm$ 11	66 $\pm$ 9	=0.32
LDL cholesterol (mmol/L)	2.9 $\pm$ 0.8	3.2 $\pm$ 0.7	<0.001
HDL cholesterol (mmol/L)	1.46 $\pm$ 0.38	1.44 $\pm$ 0.35	=0.61
*Triglycerides (mmol/L)	0.9 [0.5]	1.1 [0.6]	=0.17
Fasting plasma glucose (mmol/L)	5.0 $\pm$ 0.6	5.2 $\pm$ 0.5	=0.01
*Fasting plasma insulin (pmol/L)	29 [20]	35 [23]	=0.32
*M/I ( $\mu$ mol.min <sup>-1</sup> .kg <sub>FFM</sub> <sup>-1</sup> .nmol/L <sup>-1</sup> )	137 [89]	130 [67]	=0.53
*ALAT (IU/L)	17 [10]	17 [17]	=0.12
*ASAT (IU/L)	20 [7]	22 [8]	=0.15
*GGT (IU/L)	20 [12]	27 [21]	<0.0001
*Alcohol consumption (g/week)	42 [82]	42 [76]	=0.52
*Adiponectin (mg/L)	8.3 [4.1]	7.9 [4.5]	=0.98
*Leptin (ng/ml)	8.7 [12.3]	10.3 [9.0]	=0.61
*NEFA (mmol/L)	0.50 [0.27]	0.47 [0.31]	=0.18
*hsCRP (mg/L)	0.4 [0.8]	0.8 [1.2]	<0.05
Smoking habit (never:ex:current; %)	52:24:24	44:25:31	=0.43
FH-CVD (%)	33.1	49.1	=0.01
FH of diabetes mellitus (%)	23.8	27.7	=0.89
†Average daily PA (counts per minute)	377 $\pm$ 177	304 $\pm$ 141	<0.01

Abbreviations: FH, family history; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

\*Skewed variables expressed as median [interquartile range].

†Data on PA available only in 669 subjects.

coefficients. Logistic regression analysis and multiple linear regression analysis adjusted for center were used to test the independence of the associations of outcome variables with their significant correlates in univariate models. Statistical tests were two-sided, and significance was set at a value of  $P < 0.05$ . Statistical analysis was performed by JMP software (version 3.1; SAS Institute, Inc., Cary, NC).

## Results

Of 1,012 apparently healthy young-to-middle-aged subjects, 55 (5.4%) had 1 or 2 small atherosclerotic plaques without calcification at the carotid bulb and/or origin of internal carotid artery (overall, 68 plaques; maximum plaque thickness =  $2.06 \pm 0.38$  mm). Subjects with plaques had higher FLI, higher prevalence of FLI  $\geq$ 60, were older, had higher office BP, plasma level of LDL cholesterol, fasting glucose, GGT, and hsCRP, and a higher prevalence of CVD within first-degree relatives (Table 1), as compared to subjects

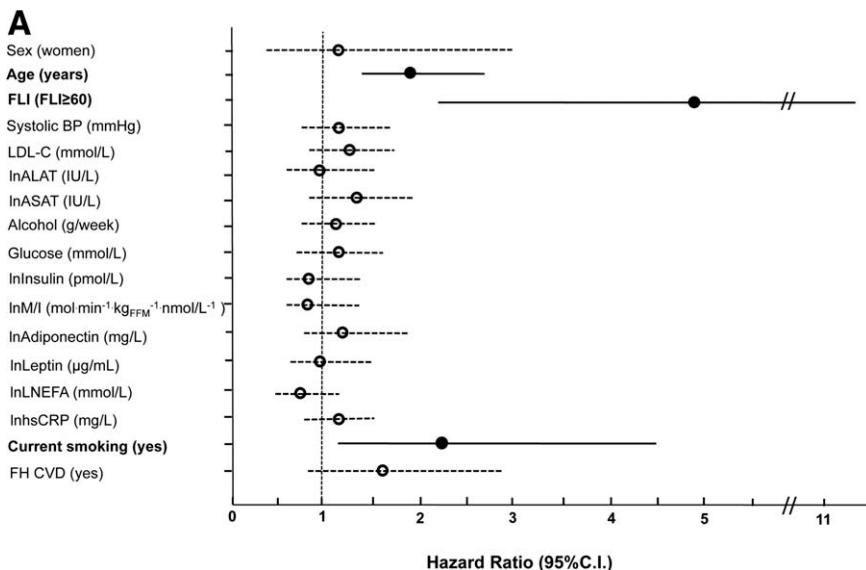


Fig. 2A. Independent predictors (full circles and lines) of the presence of early carotid plaque. Logistic regression model including FLI, used as a dichotomous variable (FLI  $\geq 60$  and FLI  $< 60$ ). Hazard ratios are calculated for 1 SD of the continuous variables. Skewed variables are log-transformed (ln). ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

without plaques. Subjects with and without plaques were comparable for sex distribution, anthropometric parameters, liver transaminase and alcohol consumption, fasting insulin and insulin sensitivity, plasma levels of adiponectin, leptin and NEFA, and prevalence of diabetes within the first-degree relatives. Smoking habit also did not differ significantly between subjects with and without plaque (Table 1). However, within current smokers ( $N = 246$ ; mean duration of smoking,  $22 \pm 9$  years; mean cigarette consumption,  $15 \pm 10$  cigarettes per day), those with plaque ( $N = 17$ ) had smoked for a longer period, as compared to those without plaques ( $29 \pm 10$  versus  $22 \pm 8$  years;  $P < 0.01$ ); average cigarette consumption was comparable ( $15 \pm 7$  versus  $14 \pm 11$  cigarettes per day;  $P = 0.81$ ) between the two groups. One hundred and three women used HRT; the prevalence of FLI  $\geq 60$  was comparable between women with and without HRT (4.9% versus 6.5%;  $P = 0.52$ ).

In a subgroup of 669 subjects (276 men and 393 women) undergoing accelerometer monitoring of PA, the mean monitoring time was  $5.6 \pm 1.5$  days. Subjects with small carotid plaques ( $N = 46$ ) had lower average daily PA, as compared to those without plaques (Table 1).

To assess whether the association between hepatic steatosis and early carotid atherosclerosis was independent of common risk factors, a logistic regression analysis was used, entering as a dependent variable the presence of carotid plaque and as independent variables the index of hepatic steatosis (as a dichotomous variable, FLI  $\geq 60$  and FLI  $< 60$ ), together with possible determinants of atherosclerotic process (e.g., sex,

age, systolic BP, lipid profile, glycemia, insulin and insulin sensitivity, adipokines, NEFA, and hsCRP; normalized for 1 SD) (Fig. 2A). The analysis was adjusted for center, current smoking, alcohol consumption, liver transaminase, and FH-CVD. In such a model, the independent determinants of carotid plaque presence were age ( $P < 0.0005$ ), FLI  $\geq 60$  ( $P < 0.0005$ ), and current smoking ( $P < 0.05$ ). Subsequently, the FLI was replaced in a logistic regression model by variables used in its equation (e.g., BMI, waist circumference, plasma triglycerides, and GGT) to identify the possible pathophysiologic link between fatty liver and early carotid atherosclerosis. In this model (Fig. 2B), the independent determinants of plaque presence were age ( $P < 0.001$ ), GGT ( $P = 0.001$ ), and current smoking ( $P < 0.05$ ). Finally, the same logistic analysis was run only in 967 subjects with plasma GGT within normal limits (up to 40 IU/L in women and up to 68 IU/L in men).<sup>27</sup> Independent determinants of carotid plaque presence in this model were age ( $P < 0.0005$ ) and current smoking ( $P = 0.01$ ), but not GGT ( $P = 0.14$ ).

When average daily PA was added into the logistic regression model in a subpopulation of 669 subjects with accelerometer monitoring, it was entered as an additional independent predictor of plaque presence without affecting the other determinants (Fig. 2C).

CCA IMT was also higher in subjects with FLI  $\geq 60$ , as compared to those with FLI  $< 60$  ( $677 \pm 97$  versus  $590 \pm 79$   $\mu\text{m}$ ;  $P < 0.01$ , after adjustment for age). In the entire study population, CCA IMT correlated with age ( $r = 0.42$ ;  $P < 0.0001$ ), anthropometric parameters ( $r = 0.17$ - $0.28$ ;  $P < 0.0001$  for all),

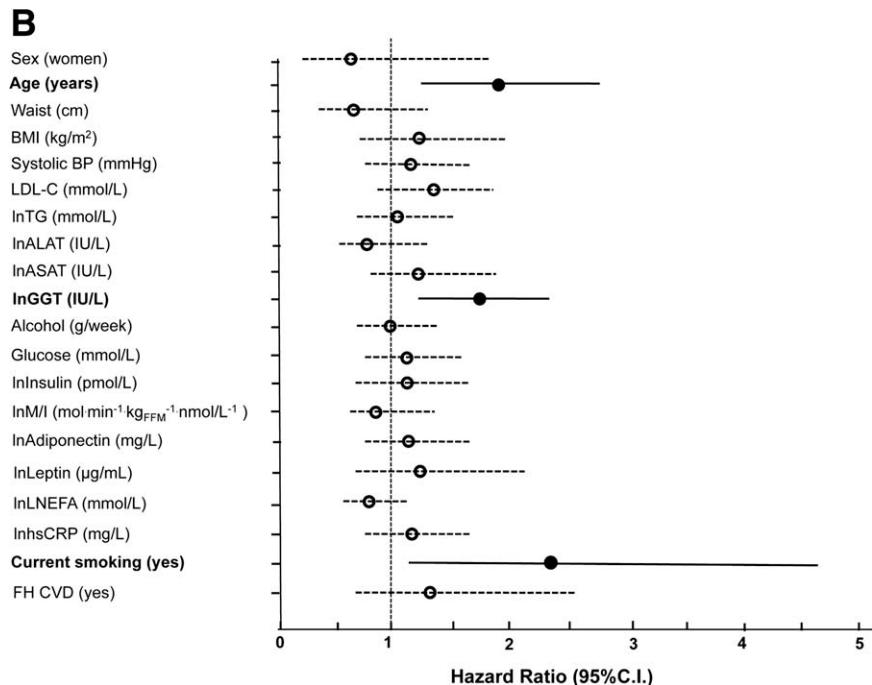


Fig. 2B. Independent predictors (full circles and lines) of the presence of early carotid plaque. Logistic regression model including variables used in FLI equation (BMI, waist circumference, plasma triglycerides, and GGT). Hazard ratios are calculated for 1 SD of the continuous variables. Skewed variables are log-transformed (ln). ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

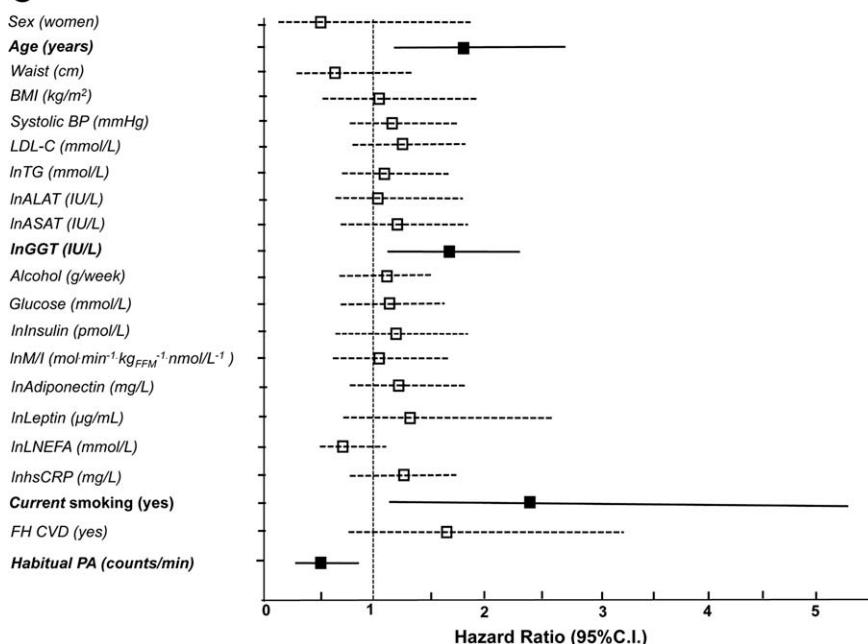
systolic BP ( $r = 0.27$ ;  $P < 0.0001$ ), plasma LDL cholesterol and triglycerides ( $r = 0.26$  and  $0.21$ ;  $P < 0.0001$  for all), fasting plasma glucose ( $r = 0.17$ ;  $P < 0.0001$ ), and plasma GGT ( $r = 0.16$ ;  $P < 0.0001$ ). A multivariate regression model was created, entering as a dependent variable the standardized CCA IMT and as independent variables FLI (as a dichotomous variable) and all the variables that correlated with CCA IMT in a univariate model. The model was adjusted for center, sex, current smoking, alcohol consumption, and family history. Following backward stepwise removal, the independent predictors of CCA IMT were male sex, age, FLI  $\geq 60$ , systolic BP, LDL cholesterol, and FH-CVD (Table 2). When FLI in the model was replaced by the variables used in its calculation, independent predictors of CCA IMT were male sex, age, waist circumference, systolic BP, LDL cholesterol, and FH-CVD.

**Tissue Characterization of Early Carotid Plaques.** Densitometric analysis was performed in 52 plaques (excluding plaques not entirely visualized and, in subjects with 2 plaques, the smaller one). MGL of carotid plaques was higher in subjects with FLI  $\geq 60$ , as compared to those with FLI  $< 60$  (Fig. 3A), and in current smokers, as compared to nonsmokers and ex-smokers ( $81 \pm 26$  versus  $64 \pm 22$ ;  $P < 0.05$ ). Plaque MGL increased with waist circumference, 2-hour plasma glucose, and insulin ( $r = 0.32$ ,  $0.35$ , and  $0.31$ ;  $P < 0.05$  for all) and decreased with plasma adiponectin concentrations (Fig. 3B). In the multivariate regression model (adjusted for center, sex, and smoking habit), plasma adiponectin and current smoking were

the only independent determinants of plaque MGL ( $\beta \pm \text{standard error [SE]} = -0.42 \pm 0.11$ ,  $P < 0.001$ , and  $0.26 \pm 0.11$ ,  $P < 0.05$ ; cumulative  $R^2 = 0.55$ ,  $P < 0.0001$ ). In addition, subjects with FLI  $\geq 60$  had lower plasma levels of adiponectin (Fig. 3C) and plasma adiponectin was inversely related to FLI (Fig. 3D). Also, in the entire study population ( $N = 1,012$ ), plasma adiponectin levels were lower in the subgroup with FLI  $\geq 60$ , as compared to that with FLI  $< 60$  ( $6.8 \pm 2.6$  versus  $9.0 \pm 3.6$ ;  $P < 0.0001$ ) and plasma adiponectin and FLI were inversely correlated ( $r = -0.37$ ;  $P < 0.0001$ ).

## Discussion

This cross-sectional study demonstrates that in apparently healthy young-to-middle-aged subjects without metabolic syndrome and increased cardiovascular risk, the presence of early plaques at the carotid bifurcation is independently associated with FLI  $\geq 60$ , which is used as a surrogate marker of hepatic steatosis. When FLI was replaced by variables used in its equation (e.g., BMI, waist circumference, plasma triglycerides, and GGT), plasma levels of GGT emerged as an independent determinant of early carotid atherosclerosis. The association between GGT and carotid plaques was independent of established atherosclerotic risk factors, insulin sensitivity, adipokines, hsCRP, and PA level, but disappeared when only subjects within the normal GGT range were included into the analysis. These findings suggest that plasma GGT might

**C**

**Fig. 2C.** Independent predictors (full squares and lines) of the presence of early carotid plaque in a subpopulation of 669 subjects with accelerometer monitoring of habitual PA. Hazard ratios are calculated for 1 SD of the continuous variables. Skewed variables are log-transformed (ln). ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

represent a link between hepatic steatosis and the atherosclerotic process, and that GGT above normal limits could be used as a biochemical marker of atherosclerosis.

Our clinical data on GGT are supported by experimental studies, in which a catalytically active GGT has been found within cerebral, carotid, and coronary plaques, when colocalized with oxidized LDLs and cluster of differentiation 68<sup>+</sup> foam cells.<sup>8</sup> Plaque GGT is supposed to derive from the plasma, in the form of complexes with LDL.<sup>7</sup> Once accumulated in the plaque environment, GGT retains its activity and triggers an iron-dependent oxidation of LDL in the extracellular space.<sup>28</sup> It is worth noting that in our population, plasma GGT was an independent determinant of plaque presence in the carotid bulb, but not of the arterial wall thickness in the common carotid artery. Such a difference might indicate that local mechanical forces resulting from flow-separation and flow-profile changes in the carotid bulb are necessary to promote the influx and accumulation of plasma-derived GGT within the arterial wall.<sup>15,16</sup>

The initialization and evolution of atherosclerotic plaque are closely linked with the presence of SMCs; intimal SMCs are the first cells present in adaptive intimal thickening.<sup>16</sup> The presence of SMCs within atherosclerotic plaques has been shown to influence their acoustic properties, which can be quantitatively evaluated by means of acoustic densitometry. We have previously demonstrated, using the same densitometric analysis as in this study,<sup>18</sup> that with increasing SMC

content, MGL (or acoustic reflectivity) of the initial atherosclerotic lesion increases. In the present study, MGL of small carotid plaques was inversely and independently related to plasma adiponectin levels, an observation indicating that increasing circulating adiponectin decreases the SMC content of these early lesions. Furthermore, subjects with FLI  $\geq 60$  had a

**Table 2. Independent Correlates of CCA IMT: Multiple Regression Model**

Model with FLI (Used As a Dichotomic Variable; FLI $\geq 60$ and FLI < 60)		
	$\beta^*$ $\pm$ SE	P Value
Sex (male)	0.11 $\pm$ 0.03	<0.0005
Age (years)	0.37 $\pm$ 0.03	<0.0001
FLI $\geq 60$	0.15 $\pm$ 0.04	<0.0005
Systolic BP (mmHg)	0.14 $\pm$ 0.03	<0.0001
LDL cholesterol (mmol/L)	0.09 $\pm$ 0.03	<0.005
FH-CVD (yes)	0.07 $\pm$ 0.03	<0.05
Cumulative R <sup>2</sup>	0.28	<0.0001

**Model With the Variables Used in FLI Calculation (BMI, Waist Circumference, Triglycerides, and GGT)**

	$\beta \pm$ SE	P value
Sex (male)	0.08 $\pm$ 0.03	=0.01
Age (years)	0.36 $\pm$ 0.03	<0.0001
Waist circumference (cm)	0.11 $\pm$ 0.03	=0.001
Systolic BP (mmHg)	0.13 $\pm$ 0.03	<0.0001
LDL cholesterol (mmol/L)	0.09 $\pm$ 0.03	<0.005
FH-CVD (yes)	0.06 $\pm$ 0.03	=0.05
Cumulative R <sup>2</sup>	0.27	<0.0001

\* $\beta$  = standardized regression coefficient.

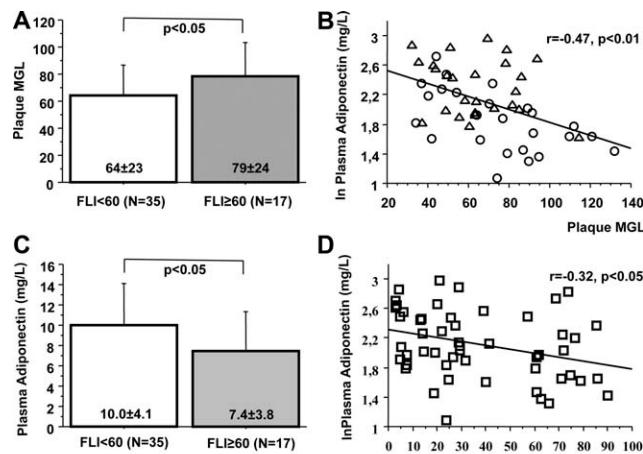


Fig. 3. (A) Plaque MGL in subjects with  $\text{FLI} < 60$  and  $\text{FLI} \geq 60$ . (B) Correlation between plaque MGL and plasma adiponectin level. Circles indicate men, triangles indicate women. (C) Plasma adiponectin levels in subjects with  $\text{FLI} < 60$  and  $\text{FLI} \geq 60$ . (D) Correlation between FLI and plasma adiponectin level in subjects with carotid plaques.

higher plaque MGL and a lower plasma adiponectin level, as compared to those with  $\text{FLI} < 60$ , and plasma adiponectin and FLI were inversely related (Fig. 3). Altogether, these findings imply that the fatty-liver-related decrease in plasma adiponectin level may induce an increase in SMCs in early atherosclerotic plaques. Our observations are in agreement with *in vitro* studies demonstrating that adiponectin suppresses the platelet-derived growth factor-induced proliferation and migration of human aortic SMCs<sup>10,29</sup> and prevents adventitial fibroblasts from proliferating, transforming into myofibroblast, and migrating to the intima,<sup>30</sup> and they also confirm the previous clinical data on decreased plasma adiponectin levels in patients with fatty liver disease.<sup>31</sup> Yet, it must be emphasized that the observed effect of plasma adiponectin on the acoustic properties of carotid plaque might only apply to early atherosclerotic lesions in healthy subjects free of confounding pathologies and not to more advanced atherosclerotic plaques. The Prospective Investigation of the Vasculature in Uppsala Seniors Study, which includes the community-based elderly population with a high prevalence of hypertension, diabetes, and dyslipidemia, has shown a positive association between plaque MGL and plasma adiponectin levels that probably reflects the inhibiting effect of adiponectin on lipid accumulation and foam cell formation.<sup>9,32</sup>

Two additional findings should be briefly discussed. Cigarette smoking is supposed to accelerate the development and/or progression of carotid atherosclerosis,<sup>33</sup> and one of the mechanisms that may participate in early plaque formation in smokers is the effect of nico-

tine on SMCs. Nicotine induces SMC proliferation through the mediation of growth factors and inhibits physiological SMC apoptosis.<sup>34</sup> In line with these findings are our observations on associations between current smoking and smoking duration on one side and the presence and acoustic reflectivity of small carotid plaques on the other. Second, our data confirm the beneficial impact of PA on the atherosclerotic process that have been previously demonstrated in a variety of clinical and experimental studies and that is supposed to reflect the effect of PA on oxidative stress as well as pro- and anti-inflammatory cytokines.<sup>35</sup>

**Strengths and Limitations.** Our study includes a large population free of confounding diseases (e.g., CVD, diabetes, and hypertension), without metabolic syndrome, and at low-average cardiovascular risk. All subjects were well characterized from the metabolic point of view, including M/I by the gold-standard euglycemic hyperinsulinemic clamp. However, there were also some limitations. First, the design of the RISC study did not include liver US for the determination of fatty liver. Therefore, for the purpose of the present study, we used a previously validated surrogate marker, FLI,<sup>13,14</sup> that, in a recent prospective study, has been associated with cardiovascular mortality.<sup>36</sup> We also took into consideration the FLI developed by Kotronnen et al.<sup>37</sup> However, the algorithm was not suitable for this population because it was developed in a group of subjects with diabetes and metabolic syndrome, whereas we studied clinically healthy subjects free of diabetes, hypertension, and metabolic syndrome. Second, because of the large number of centers participating in the RISC study, only standard US scanners were used for carotid imaging; thus, tissue characterization of carotid plaques was performed by densitometric analysis of B-mode images and not by analysis of raw radiofrequency data. Third, after a 3-year period, follow-up carotid US was available only in 35 subjects with carotid plaques, and consequently, follow-up data were not included into this study. Finally, information regarding family history of CVD, stroke, and diabetes as well as data on alcohol consumption and smoking habit were obtained by self-reported questionnaires and were not verified.

The results of this study indicate that in a healthy young-to-middle-aged population without metabolic syndrome and increased cardiovascular risk, plasma GGT may represent a pathophysiologic link between hepatic steatosis and the early atherosclerotic process, and that increased serum GGT might be used as a biomarker of atherosclerosis. Hepatic steatosis seems also to influence the acoustic properties of early carotid lesions, probably through its effect on plasma adiponectin level.

# Gamma-glutamyltransferase and risk of hypertension: a systematic review and dose–response meta-analysis of prospective evidence

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The objective of this review was to obtain a reliable estimate of the magnitude of the prospective association between gamma-glutamyltransferase (GGT) and risk of hypertension, and to characterize the nature of the dose–response relationship. We conducted a systematic review and dose–response meta-analysis of published prospective studies. Relevant studies were identified in a literature search of MEDLINE, EMBASE, and Web of Science databases up to May 2015. Study-specific relative risks (RRs) were meta-analyzed using random effects models. We examined a potential nonlinear relationship using restricted cubic splines. Of the 612 titles reviewed, we included 14 cohort studies with data on 44 582 participants and 5 270 hypertension cases. In a comparison of extreme thirds of baseline levels of GGT, RR for hypertension in pooled analysis of all 14 studies was 1.32 (95% confidence interval: 1.23–1.43). There was heterogeneity among the studies ( $P < 0.001$ ), which was to a large part explained by average age of participants at baseline, average duration of follow-up, and the degree of confounder adjustment. In a pooled dose–response analysis of 10 studies with relevant data, there was evidence of a linear association between GGT and hypertension risk ( $P$  for nonlinearity = 0.37). The pooled RR of hypertension per 5 U/l increment in GGT levels was 1.08 (95% confidence interval: 1.04–1.13). Baseline circulating GGT level is associated with an increased risk of hypertension in the general population, consistent with a linear dose–response relationship. Further investigation of any potential relevance of GGT in hypertension prevention is warranted.

**Keywords:** dose–response, gamma-glutamyltransferase, high blood pressure, hypertension, meta-analysis, prospective studies

**Abbreviations:** BP, blood pressure; CI, confidence interval; CVD, cardiovascular disease; GGT, gamma-glutamyltransferase; NOS, Newcastle–Ottawa Scale; RR, relative risk; SD, standard deviation

this number will reach 1.56 billion by 2025 [1]. In addition to being the leading global risk for mortality in the world [2], hypertension is the most common modifiable and leading risk factor for cardiovascular disease (CVD) [3], which represents a worldwide epidemic and is the leading cause of mortality globally [4]. To date, established risk factors for hypertension include excess body weight, excess dietary sodium intake, reduced physical activity, and excess alcohol intake [5,6]. In line with the 2013 guidelines developed by the European Society of Hypertension and the European Society of Cardiology (ESH/ESC) [7], lifestyle changes have been recommended as the cornerstone for the prevention of hypertension or high BP. These include a combination of population-based and intensive-targeted approaches such as reduction of salt and alcohol consumption, maintaining a healthy body weight, regular exercise, and elimination of smoking [7]. Although established risk factors for hypertension explain a large proportion of its risk, its pathogenesis is still not fully established as multiple factors appear to be involved. There is, therefore, a need to further assess potential risk factors, which may have causal or predictive relevance to hypertension and which will help further tailor preventive and therapeutic interventions.

Gamma-glutamyltransferase (GGT), a sensitive but non-specific index of liver injury and a biological clue of excessive alcohol intake, has been strongly linked to the development of adverse cardiometabolic outcomes [8–10] including hypertension [11]. Elevated serum levels of GGT has been postulated to reflect the development and progression of hepatic steatosis; which may play an important role in the development of insulin resistance and hyperinsulinemia, resulting in high BP or hypertension [12–14].

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## INTRODUCTION

Hypertension or high blood pressure (BP) has risen to pandemic proportions – affecting over 1 billion people worldwide and it has been estimated that

Until recently, there has been uncertainty regarding the magnitude and nature of the prospective association between GGT level and risk of hypertension. Liu *et al.* [15] synthesized available prospective epidemiological data on the association between GGT and hypertension and reported a pooled multivariate adjusted relative risk (RR) [95% confidence interval (CI)] of 1.94 (1.55–2.43) for hypertension in a comparison of top versus bottom category of baseline GGT levels. However, in this review, the authors did not standardize the reported risk estimates (they reported comparisons for the highest versus lowest category of GGT levels irrespective of the risk estimates the eligible studies reported) to a consistent comparison before pooling. In addition, they separately pooled the results of three studies that provided risk estimates per 1 standard deviation (SD) increment in log<sub>e</sub> GGT levels. Given these, the magnitude of the association could not be precisely determined. In addition, although the evidence suggests there is a strong association between elevated baseline circulating GGT and risk of incident hypertension; characterization of the nature and magnitude of the dose–response relationship is, however, still lacking, as this was not addressed by previous studies and the recent review. It is uncertain whether there is a clear continuous dose–response relationship to the association or if this association is evident only beyond a particular threshold level of GGT. It is important to establish this, especially if there exists a threshold that would potentially optimize the detection of individuals at increased risk of hypertension. A dose–response analysis is more efficient than comparing the highest to lowest category approach, as it uses all of the exposure-disease information and provides a detailed description of the risk of the disease throughout the observed range of the exposure [16]. Against this background, our first objective using a meta-analytic approach, was to obtain a reliable estimate of the magnitude of the association between GGT and hypertension, by including all relevant studies and standardizing reported risk estimates from all studies to a consistent comparison (top versus bottom thirds of baseline levels of GGT) before pooling. Our second objective was to quantify and characterize in detail the nature of the dose–response relationship between GGT level and risk of hypertension.

## METHODS

### Data sources and searches

This systematic review and meta-analysis of studies was conducted using a predefined protocol and reported in accordance with Preferred Reporting Items For Systematic Reviews and Meta-Analyses and Meta-analysis Of Observational Studies in Epidemiology guidelines [17,18] (Supplementary Materials 1–2, <http://links.lww.com/HJH/A535>). We searched MEDLINE, EMBASE, and Web of Science for prospective (cohort, case-cohort or ‘nested case control’) population-based studies that measured the level of enzymatic activity of GGT and evaluated associations between baseline circulating level of GGT with risk of hypertension or high BP up to May 2015. The computer-based searches combined free and medical subject heading search terms and combined key words related to GGT (e.g.

‘gamma glutamyltransferase’) and hypertension (e.g. ‘hypertension’, ‘blood pressure’). There were no restrictions on language or the publication date. We scanned the reference lists of retrieved articles for all relevant additional studies and review articles. We restricted the search to studies of humans. Further details on the search strategy are presented in Supplementary Material 3, <http://links.lww.com/HJH/A535>.

### Study selection

Observational cohort studies were included if they had at least 1-year of follow-up, assessed associations of GGT with hypertension in adults (>18 years), measured samples at baseline, recruited participants representative of, approximately, general populations (i.e., did not select participants on the basis of confirmed preexisting medical conditions such as hypertension or high BP, cardiovascular disease, liver disease, or chronic kidney disease at baseline). Retrospective studies were not included.

### Data extraction and quality assessment

Two authors independently abstracted data and performed quality assessments using a standardized predesigned data collection form. Data were abstracted, wherein available, on study, publication date, geographical location, population source, time of baseline survey, sample population, study design, sample source (plasma/serum), nature of sample (fresh or frozen and storage temperature), assay type and source, sample size, number of hypertension cases, hypertension case definition, mean age range at start of study, duration of follow-up, and degree of adjustment for potential confounders (defined as ‘+’ when RRs were adjusted for age and/or sex; ‘++’ further adjustment for potential risk factors for hypertension such as BMI, plasma or serum lipids, smoking status, exercise, or alcohol consumption; and ‘+++’ additional adjustment for other liver enzymes and or inflammatory markers). We extracted RRs reported for the greatest degree of adjustment. In the case of multiple publications involving the same cohort, the most up-to-date study or study with the most comprehensive information was abstracted. We contacted authors of eligible studies wherein the published data were insufficient, to provide relevant missing information.

Study quality was assessed based on the nine-star Newcastle–Ottawa Scale (NOS) [19] using predefined criteria namely: selection (population representativeness), comparability (adjustment for confounders), and ascertainment of outcome. The NOS assigns a maximum of four points for selection, two points for comparability, and three points for outcome. Nine points on the NOS reflects the highest study quality. A score of at least 5 indicated adequate quality for inclusion in the review.

### Data synthesis and analysis

The RR with 95% CIs was used as the common measure of association across studies. To enable a consistent approach to the meta-analysis and enhance interpretation of the findings, reported study-specific risk estimates (per standard deviation change, quintiles, quartiles, and user-defined cutoffs) were transformed to involve comparisons between the top third and bottom third of each study population’s

baseline distribution of GGT levels, using standard statistical methods [20,21], which have been described in detail in Supplementary Material 4, <http://links.lww.com/HJH/A535>. Briefly, log-risk estimates were transformed assuming a normal distribution (or that a transformation of the explanatory variable for which the risk ratio is based was normally distributed), with the comparison between top and bottom thirds being equivalent to 2.18 times the log-risk ratio for a 1 standard deviation increase (or equivalently, as 2.18/2.54 times the log risk ratio for a comparison of extreme quarters and as 2.18/2.80 times the log risk ratio for a comparison of extreme quintiles). Standard errors of the log-risk estimates were calculated using published confidence limits and were standardized in the same way. When studies published more than one estimate of the association according to subgroups (e.g. by sex), we obtained a within-study summary estimate using a fixed effect meta-analysis. Summary RRs were pooled using a random effects model to minimize the effect of between-study heterogeneity [22].

To avoid making an assumption of linearity for an exposure-response (e.g. GGT-hypertension) relation, exposure-response relations are usually reported through RRs corresponding to ranges of exposure levels. Therefore, in a meta-analysis, it is useful to model the relation in a flexible nonlinear manner and assess evidence for or lack of nonlinearity, using graphical and statistical testing procedures [23]. We, therefore, performed a two-stage dose-response meta-analysis using the method proposed by Orsini *et al.* [24], to examine a potential nonlinear relationship between GGT levels and hypertension risk by modeling GGT levels using restricted cubic splines with 3 knots at percentiles 25, 50, and 75% of the distribution [25]. This method requires that the number of cases, person-years of follow-up or non-cases, and the RRs with the variance estimates for at least three quantitative categories of GGT levels are known. The median or mean level of GGT for each category was assigned to each corresponding RR. If data were not available, we estimated the median using the midpoint of each category. When the highest or lowest category was open, we assumed it to be the same amplitude as the adjacent category. In the first stage, as described by Orsini *et al.* [24], a restricted cubic spline model with two spline transformations (3 knots minus 1) was estimated using generalized least-squares regression taking into account the correlation within each set of published RRs. In the second stage, the two regression coefficients and the variance/covariance matrix that had been estimated within each study were combined using the restricted maximum likelihood method in a multivariate random-effects meta-analysis [26]. A *P* value for nonlinearity was calculated by testing that the coefficient of the second spline was equal to zero [27].

Statistical heterogeneity across studies was quantified using Cochran  $\chi^2$  and the  $I^2$  statistics [28,29]. Study-level characteristics including geographical location, sex, average age at baseline, average duration of follow-up, number of cases, case definition for hypertension, degree of adjustment, and study quality were prespecified as characteristics for assessment of heterogeneity, which was conducted using stratified analysis and random effects meta-regression

[30]. We assessed the potential for small study effects such as publication bias through formal tests, namely Begg's funnel plots [31] and Egger's regression symmetry test [32]. Finally, we adjusted for the effect of publication bias by the use of the Duval and Tweedie's nonparametric trim-and-fill method [33]. All analyses were conducted using Stata version 13 (Stata Corp, College Station, Texas, USA).

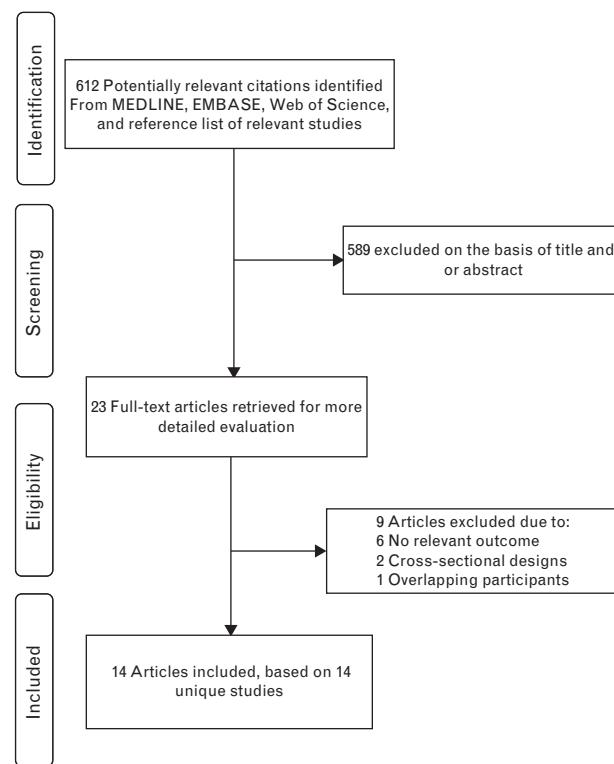
## RESULTS

### Study identification and selection

Our initial search identified 612 potentially relevant citations (Fig. 1). After screening the titles and abstracts, 23 articles remained for further evaluation. We reviewed and assessed these 23 articles, and excluded nine articles because they had no relevant outcome ( $n=6$ ), and they were not prospective ( $n=2$ ) or duplicated a previous publication using the same cohort ( $n=1$ ). In sum, this meta-analysis included 14 articles (Supplementary Material 5, <http://links.lww.com/HJH/A535>) based on 14 unique prospective cohort studies comprising 44 582 participants and 5 270 hypertension cases.

### Study characteristics and quality

Table 1 provides details of the eligible studies. The mean age of participants at baseline ranged from, approximately, 25 to 62 years. One study included participants aged 15 years and over, however, participants who were less than 18 years comprised only 9.3% of the total sample [34]. Two studies included participants from Europe (France and Turkey), two from North America (United States), nine



**FIGURE 1** Selection of studies included in the meta-analysis GGT, gamma-glutamyltransferase.

**TABLE 1. Characteristics of published prospective studies evaluating associations between gamma-glutamyltransferase and incident hypertension**

Lead author, publication year	Name of study or source of participants	Year(s) of baseline survey	Location of study	Baseline mean age (age range), years	% men	Duration of follow-up	Total no. of participants	Number of cases	Hypertension case definition	Covariates adjusted for	Study quality
Yamada, 1991	Metal Products Factory	Japan	1983	43.0 (35–54)	100.0	5.0	1 393	29	SBP ≥160 mmHg, DBP ≥95 mmHg	Unadjusted	6
Miura, 1994	Rural community	Japan	1979–1980	47.8 (30–69)	100.0	10.0	77	36	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Age, SBP, DBP, alcohol consumption	8
Lee, 2002	Steel Manufacturing Company	South Korea	1994; 1998	NS (25–50)	100.0	4	8 170	169	SBP ≥160 mmHg, DBP ≥95 mmHg, and/or taking antihypertensive medication	Age, BMI, smoking (pack years), drinking, exercise, family history of hypertension, SBP or DBP, changes of BMI, drinking during four years	7
Lee, 2003	CARDIA	USA	1985–1986	25.0 (18–30)	NS	15.0	4 704	708	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Study center, race, sex, age, alcohol consumption, BMI, Smoking, PA, fasting serum glucose, insulin for diabetes, SBP, insulin for hypertension	8
Stranges, 2005	WNYS	USA	1986–2001	NS (39–79)	65.4	6.0	897	195	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Age, gender, race, average amount of alcohol, smoking status, BMI, PA, SBP	7
Ande, 2007	DESIR	France	1994–1996	46.0 (30–65)	55.2	3.0	1 776	377	SBP ≥130 mmHg or ≥85 mmHg or treatment of previously diagnosed hypertension	Age	7
Jo, 2009	HPC	South Korea	2002	38.7 (19–86)	70.8	4.0	17 281	2 170	SBP ≥130 mmHg, DBP ≥85 mmHg, or taking antihypertensive medication	Age	6
Jimba, 2009	SSK Hospital	Japan	2002–2003	49.0 (NS)	NS	3.0	1 027	288	SBP ≥130 mmHg, DBP ≥85 mmHg, or taking antihypertensive medication	Age, sex, alcohol habits, BMI at baseline	7
Hwang, 2010	Community	South Korea	2003	54.1 (>30)	39.2	5.0	293	83	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Age, education, BMI, alcohol intake, smoking, exercise, salt intake, family history of hypertension, ALT	7
Cheung, 2011	CRISPS-2	Hong Kong	2005–2008	47.3 (25–75)	39.5	5.3	708	126	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Age, sex, SBP at baseline and follow-up duration, baseline BMI, HDL-C, HOMA-IR, CRP, fibrinogen, current smoking, change in BMI	9

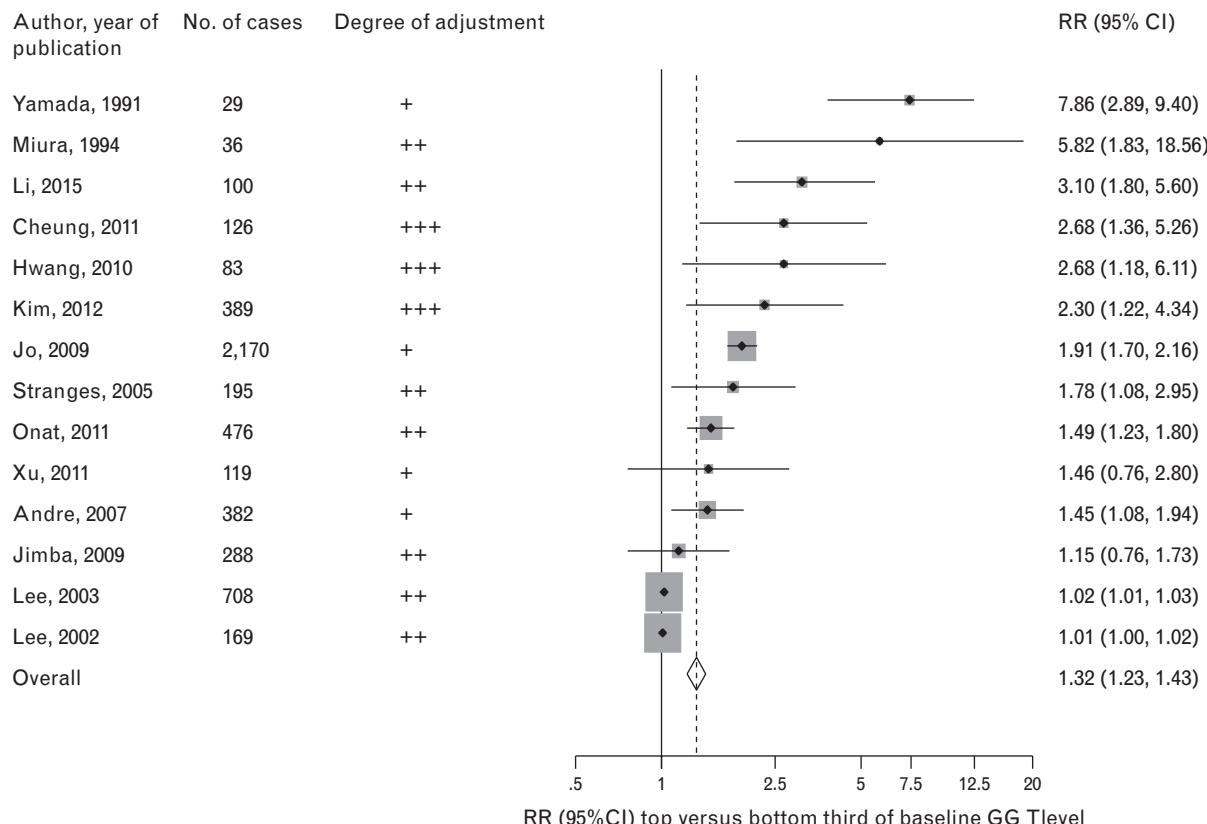
Onat, 2011	TARFS	Turkey	2003–2004	52.0 (33–84)	49.1	4.0	1,422	476	SBP ≥140 mmHg, DBP 90 mmHg, or taking antihypertensive medication	Age, sex, menopause, BMI, alcohol use	8
Xu, 2011	Shanghai	China	2004–2008	NS ( $\geq 40$ )	60.2	3.5	285	119	SBP ≥130 mmHg, DBP ≥85 mmHg, or taking antihypertensive medication	Age and sex	7
Kim, 2012	Kangbuk Samsung Hospital	South Korea	2002–2005	44.0 (NS)	67.9	3.0	4,783	389	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Age, sex, alcohol amount, smoking status, PA, baseline glucose, uric acid, HDL-C, LDL-C, TG, hsCRP, SBP	8
Li, 2015	Rural indigenous community	Australia	1997–2008	31.4 (15–78)	41.0	6.6	1,766	100	SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication	Age, sex, ethnicity, abdominal obese, PA, diabetes, dyslipidemia	7
Total							44,582	5,270			

ALT, alanine aminotransferase; CARDIA, Coronary Artery Risk Development in Young Adults; CRISPS-2, Cardiovascular Risk Factor Prevalence Study; DESIR, Data from Epidemiological Study on the Insulin Resistance Syndrome; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HPC, Health Promotion Centre; hsCRP, high sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol; NS, not stated; PA, physical activity; SSK, Saitama-ken Saiseikai Kurihashi; TARES, Turkish Adult Risk Factor Study; TC, total cholesterol; TG, triglycerides; WNYSH, Western New York Health Study.

from Asia (South Korea, Hong Kong, Japan, and China), and one from Australia. Duration of follow-up to the development of hypertension ranged from 3 to 15 years. Studies ascertained the diagnosis of hypertension (or high blood pressure) using the following definitions: blood pressure at least 130/85 mmHg, 140/90 mmHg, 160/95 mmHg and/or taking antihypertensive medication. All studies evaluated the associations in, approximately, general healthy populations with the exception of one study, which was conducted among prehypertensive adults [35]. The degree of covariate adjustment varied, but majority of studies adjusted for potential risk factors for hypertension such as age, BMI, smoking status, exercise, and alcohol consumption, with three additionally adjusting for another liver enzyme or inflammatory markers. Two studies adjusted for only age. An unadjusted estimate was calculated for one study. Overall, we judged all of the included studies to be of adequate quality (quality score: 6–9). One study scored 9 points, four studies scored 8 points, seven studies scored 7 points, and two studies scored 6 points. Supplementary Material 6, <http://links.lww.com/HJH/A535> provides assay characteristics of measured levels of GGT from studies contributing to the analysis. Apart from seven studies that did not provide specific details of type of assays used for GGT measurements, all other studies employed the enzymatic colorimetric method, which has been shown to be precise for detecting GGT activity [36]. As reported in Supplementary Material 6, <http://links.lww.com/HJH/A535>, the majority of studies assessed the associations within normal reference ranges of GGT.

## Association of gamma-glutamyltransferase and hypertension

The pooled RR (95% CI) of hypertension in a comparison of individuals in the top thirds with those in the bottom thirds of baseline GGT level for all 14 studies was 1.32 (1.23–1.43) (Fig. 2). The combined RR excluding the study, which was conducted among participants with prehypertension was 1.31 (1.22–1.42), which was similar to the main finding. Similarly, the pooled RR was 1.26 (1.18–1.35) on excluding the study with an unadjusted estimate and 1.30 (1.21–1.40) on excluding the study that included participants aged 15 years and over. The pooled RR was minimally attenuated on simultaneously excluding all three studies 1.23 (1.15–1.31). On simultaneous exclusion of the study with an unadjusted estimate and studies that presented only age-adjusted estimates, the pooled RR was attenuated but not significantly altered 1.08 (1.02–1.13). There was substantial heterogeneity between studies ( $I^2 > 70\%$ ), which was partly explained by study level characteristics such as age at baseline ( $P$  for meta-regression = 0.007), average follow-up duration ( $P$  for meta-regression = 0.04), and degree of adjustment ( $P$  for meta-regression < 0.0001) (Supplementary Material 7, <http://links.lww.com/HJH/A535>). A stronger association was observed in studies that included older participants ( $\geq 45$  years) compared to studies with younger participants ( $< 45$  years) and studies with a longer duration of follow-up ( $\geq 5$  years) compared to studies with shorter duration of follow-up ( $< 5$  years). In further subgroup analysis (data not shown), a stronger association was observed in Asian studies 2.16 (1.47–3.19) compared



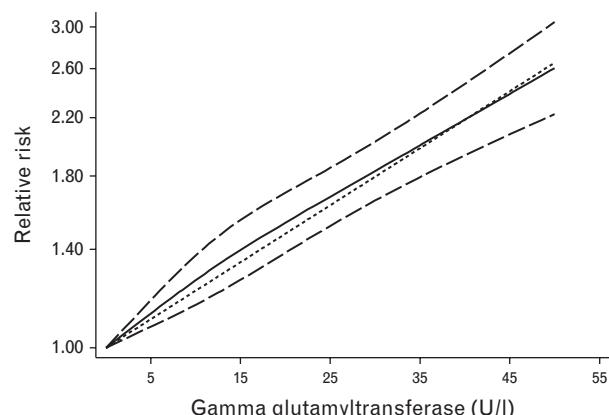
**FIGURE 2** Relative risks for hypertension in individuals in the top compared with the bottom third of baseline levels of gamma-glutamyltransferase in eligible studies. The summary estimate presented was calculated using a random effects model; †, degree of adjustment: +, unadjusted or adjusted for age and/or sex; ++, further adjustment for potential hypertension risk factors; +++, additional adjustment for other liver markers or inflammatory markers; Size of data markers are proportional to the inverse of the variance of the relative ratio; CI, confidence interval (bars); GGT, gamma-glutamyltransferase; RR, relative risk. Risk comparisons originally reported by the eligible studies are as follows: Yamada 1991, reported number of hypertension cases by GGT categories ( $\geq 50$  and  $< 50$  U/L); Miura 1994, userdefined cutoffs; Li 2015, estimates provided by authors; Cheung 2011, tertiles; Hwang 2010, quartiles; Kim 2012, quartiles; Jo 2009, quartiles; Stranges 2005, quintiles; Onat 2011, per standard deviation change; Xu 2011, quartiles; Andre 2007, quartiles; Jimba 2009, tertiles; Lee 2003, user-defined cut-offs; and Lee 2002, user-defined cut-offs. The studies mentioned in this figure are included in the reference list in Supplementary Material 5.

with other populations 1.53 (1.12–2.10) ( $P$  for meta-regression = 0.293). Egger's test was significant ( $P = 0.001$ ), consistent with observed funnel plot asymmetry (Supplementary Material 8, <http://links.lww.com/HJH/A535>), suggesting that studies with less striking results were less likely to have been reported. Despite the concern that small studies with null results often tend not to be published, we found no definitive evidence of such selective reporting when studies were grouped by size in meta-regression analysis (Supplementary Material 7, <http://links.lww.com/HJH/A535>). Duval and Tweedie's trim-and-fill method identified seven missing studies and addition of these hypothetical missing studies did not alter the significant association between GGT and hypertension risk, although substantially weaker (pooled RR comparing top versus bottom third, 1.11: 1.02–1.20).

### Dose-response analysis

In pooled analysis of 10 studies (total of 13 data points because results for men and women were reported separately for some of the studies) providing relevant data, we found no evidence of statistically significant departure from linearity ( $P$  for nonlinearity = 0.37) between GGT levels and risk of hypertension, which was present across the spectrum of GGT values (4.5–54.5 U/L) in our study. Visual

inspection of the plot was also consistent with a linear shape (Fig. 3). The combined RR (95% CI) of hypertension for a 5 U/L increment in GGT level was 1.08 (1.04–1.13).



**FIGURE 3** Dose-response relation between gamma-glutamyltransferase levels and relative risk of hypertension for pooled results of studies providing relevant data. Adjusted relative risks and 95% confidence intervals (CIs; dashed lines) are reported. GGT levels were modeled with restricted cubic splines with 3 knots. Lines with long dashes represent the pointwise 95% CIs for the fitted linear trend (solid line). Lines with short dashes represent the linear trend. The vertical axis is on a log scale; GGT, gamma-glutamyltransferase.

## DISCUSSION

Unlike the previous elegant review by Liu *et al.* [15], who presented a pooled estimate for hypertension comparing the highest versus lowest category of GGT levels irrespective of the risk comparisons reported by the included studies; the present meta-analysis provides a more precise estimate of the magnitude of the association between baseline circulating GGT and incident hypertension. Comparing individuals in the top versus bottom thirds of circulating GGT levels, our results show an, approximately, 30% increased risk of future hypertension in pooled analysis of 14 variably adjusted eligible studies. The risk was attenuated to 8% in pooled results of only studies that adjusted for established risk factors and/or other potential confounders. The observed heterogeneity among the studies seemed to be explained by average age of participants at baseline, average duration of follow-up, and the degree of confounder adjustment. There were more extreme results in studies conducted among older individuals, consistent with established evidence that increasing age is associated with a significant increase in the incidence of hypertension or high BP. As expected, a stronger association with longer follow-up duration was also demonstrated. A stronger association was observed in Asian populations compared with Western populations (though *P*-value for meta-regression >0.05), consistent with findings from the previous review [15] and the fact that liver diseases and metabolic syndrome (strongly associated with hypertension or high BP) are very prevalent in Asians. A stronger association was also observed in men compared with women (although *P*-value for meta-regression >0.05); which is consistent with the significant sex differences in GGT levels, with men having higher levels than women [37]. In addition, men are more likely to develop cardiometabolic diseases at lower average levels of risk markers such as BMI [38], which is also causally associated with GGT levels [39]. However, in the context of the greater proportion of studies featuring more male than female participants in our review, these findings should be interpreted with caution. Our study also provides for the first time, a detailed assessment of the dose-response nature of the association between circulating GGT level and risk of hypertension. The findings were consistent with a linear dose-response relationship, which was characterized by an 8% increase in the risk of hypertension for every 5 U/l increment in circulating GGT level.

### Possible explanations for findings

A large body of evidence has shown that GGT is positively and independently associated with cardiovascular disease (CVD) risk and in a linear fashion [10,40]. Several mechanistic pathways postulated for this association include oxidative stress, increased inflammation, and underlying fatty liver [41]. These same pathways have also been implicated in the relationship between GGT and risk of hypertension. Elevations of serum hepatic enzymes including GGT, have been linked to the development and progression of fatty liver with increasing BMI [42]. Elevated GGT levels are also suggested to signify oxidative stress and a state of chronic inflammation [43]. The states of oxidative stress, increased inflammation, and fatty liver may impair insulin signaling in

the liver, leading to impaired insulin secretion and insulin resistance, which have been implicated in the development of hypertension or high BP [12,14].

### Implications of findings

Our findings are relevant, as they provide further insight concerning the relationship between baseline circulating GGT levels and risk of hypertension and may also have implications for the prevention of hypertension or high BP. Although the cutoff value and reference range for GGT has not been clearly defined, and is essentially arbitrary, being determined ideally by enzyme measuring activity in a healthy population and using the central 95% of values obtained from the population [44]; the recommended cutoff for the upper normal limit of GGT is set at an average of 51 U/l for men and 33 U/l for women [45]. Consistent with the large body of evidence, suggesting an increased risk of adverse cardiometabolic outcomes at GGT levels considered to reflect normal reference ranges [8,9,40], our findings also underscore a potentially deleterious role of increasing GGT levels within the normal range on future risk of hypertension in general population settings. Lifestyle measures such as salt restriction, moderation of alcohol consumption, high consumption of vegetables and fruits and low-fat, maintaining a healthy body weight, regular physical exercise, and elimination of smoking have been recommended as the cornerstone for the prevention of hypertension in nonhypertensive individuals [7]. Given that serum GGT levels can be considerably reduced by most of these lifestyle interventions [46], which also affect levels of established risk factors for hypertension; there remains a possibility that lowering or modification of serum levels of GGT may help in hypertension prediction or prevention. Further evaluation is warranted.

### Strengths and limitations

The strengths and limitations of this meta-analysis merit careful consideration. The notable strengths include our ability to transform reported risk estimates from all contributing studies to a consistent comparison (top versus bottom thirds) to allow a consistent combination of estimates across studies, therefore, obtaining a reliable estimate of the magnitude of the association and enhancing interpretation of the overall findings. We have also provided a detailed assessment of the dose-response relationship between GGT and risk of hypertension, which has not been previously demonstrated. We systematically explored and identified the possible sources of heterogeneity using stratified analyses and meta-regression. Formal tests demonstrated evidence of publication bias, suggesting that studies with less striking results were less likely to have been reported. However, there was no clear evidence of such selective reporting when studies were grouped by size. A detailed quality assessment of eligible studies was performed, with all included studies attaining moderate to high quality scores. Our main weakness was the inability to fully examine the impact of adjustment for potential confounding factors, because the review was based on variably adjusted data reported in the published literature. However, majority of included studies adjusted for major potential confounders (including alcohol consumption which is

known to increase serum levels of GGT) of the GGT-hypertension association and grouping the studies by degree of adjustment did not appreciably alter the direction of the association. In addition, the dose-response analysis was based on data points from 10 out of the 14 eligible studies, as the investigators concerned did not respond to our request for additional data or could not be contacted at all. Finally, it was not possible to correct the estimates for within individual variation in levels of GGT, because the included studies lacked serial assessments of circulating levels of this exposure in the same individuals.

## CONCLUSION

Circulating level of GGT is associated with an increased risk of hypertension in the general population, consistent with a linear dose-response relationship. Further investigation of any potential relevance of GGT in hypertension prevention is warranted.

## ACKNOWLEDGEMENTS

S.K.K. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. We sincerely thank Dr. Ming Li, School of Population Health, Sansom Institute for Health Research, University of South Australia, Adelaide for readily providing additional data on request.

## Conflicts of interest

There are no conflicts of interest.

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## Original Contribution

### Use of A Mendelian Randomization Approach to Assess the Causal Relation of $\gamma$ -Glutamyltransferase with Blood Pressure and Serum Insulin Levels

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Elevated levels of  $\gamma$ -glutamyltransferase (GGT) have been associated with elevated blood pressure (BP) and diabetes. However, the causality of these relations has not been addressed. The authors performed a cross-sectional analysis (2003–2006) among 4,360 participants from the population-based Cohorte Lausannoise (CoLaus) Study (Lausanne, Switzerland). The rs2017869 variant of the  $\gamma$ -glutamyltransferase 1 (*GGT1*) gene, which explained 1.6% of the variance in GGT levels, was used as an instrument for Mendelian randomization (MR). Sex-specific GGT quartiles were strongly associated with both systolic and diastolic BP (all  $P$ 's < 0.0001). After multivariable adjustment, these relations were attenuated but remained significant. Using MR, the authors observed no positive association of GGT with BP (systolic:  $\beta = -5.68$ , 95% confidence interval (CI):  $-11.51, 0.16$  ( $P = 0.06$ ); diastolic:  $\beta = -2.24$ , 95% CI:  $-5.98, 1.49$  ( $P = 0.24$ )). The association of GGT with insulin was also attenuated after multivariable adjustment but persisted in the fully adjusted model ( $\beta = 0.07$ , 95% CI:  $0.04, 0.09$ ;  $P < 0.0001$ ). Using MR, the authors also observed a positive association of GGT with insulin ( $\beta = 0.19$ , 95% CI:  $0.01, 0.37$ ;  $P = 0.04$ ). In conclusion, the authors found evidence for a direct causal relation of GGT with fasting insulin but not with BP.

blood pressure; diabetes mellitus; gamma-glutamyltransferase; insulin; Mendelian randomization analysis; obesity

Abbreviations: CoLaus, Cohorte Lausannoise; GGT,  $\gamma$ -glutamyltransferase; *GGT1*,  $\gamma$ -glutamyltransferase 1; OLS, ordinary least squares; 2SLS, 2-stage least squares; SNP, single nucleotide polymorphism.

Many studies have shown that persons with elevated levels of  $\gamma$ -glutamyltransferase (GGT) have higher levels of blood pressure and insulin and higher prevalences of diabetes and hypertension (1–14). Although these relations have been found to persist after adjustment for a variable number of covariates, significant attenuation usually occurs. Nevertheless, these findings have been interpreted as 1 piece of evidence for the involvement of fatty liver disease and oxidative stress in the pathogenesis of hypertension and type 2 diabetes mellitus (15).

However, we are not aware of any study that has investigated whether these significant relations are truly causal or are due to residual confounding. Evidence for a causal relation would provide important epidemiologic confirmation of prior basic scientific findings about the involvement of oxidative stress in the pathogenesis of insulin resistance,

type 2 diabetes mellitus, and blood pressure elevation (15, 16). A randomized trial showing that selectively lowering GGT levels also lowers blood pressure and insulin levels would support a causal relation, but such a trial is currently unavailable, and other ways to establish causality are needed.

*Mendelian randomization* refers to the random allocation of alleles at the time of gamete formation (17, 18). Such allocation is expected to be independent of any behavioral and environmental factors (known or unknown), usually allowing analysis of largely unconfounded risk associations that are not due to reverse causation (17–19). Therefore, to assess the potential causal role of GGT in the pathogenesis of blood pressure elevation and insulin resistance, we performed a Mendelian randomization analysis of blood pressure and fasting insulin in a population-based cohort study.

## MATERIALS AND METHODS

All subjects were participants in the Cohorte Lausannoise (CoLaus) Study, an ongoing population-based cohort study. Recruitment of the study population has been described in detail previously (20). Briefly, a complete list of the inhabitants of Lausanne, Switzerland, aged 35–75 years ( $n = 56,694$ ) was provided by staff of the city's population registry. Subjects were selected using a simple, nonstratified random selection approach. In total, a random sample of 35% of the overall population was drawn. Recruitment began in June 2003 and ended in May 2006 (20). The study was approved by the ethics committee of the University of Lausanne, and written informed consent was obtained from participants before data collection. Only Caucasian subjects were included. For the present analysis, we excluded all participants with missing values for GGT ( $n = 12$ ), blood pressure ( $n = 5$ ), insulin ( $n = 727$ ), or the genetic polymorphism used as the instrumental variable ( $n = 1,084$ ).

Data were collected by trained field interviewers. Seated blood pressure was measured 3 times on the left arm with an appropriate-sized cuff and after at least 10 minutes of rest, using a validated Omron HEM-907 automated oscillometric sphygmomanometer (Matsusaka Company Ltd., Matsusaka, Japan) (21). The average of the last 2 measurements was used for all blood-pressure-related analyses. Percent fat mass (in percentage of total body weight) was assessed by electrical bioimpedance after a 5-minute rest using the Bodystat 1,500 body mass analyzer (Bodystat Ltd., Isle of Man, United Kingdom), as described in detail previously (22).

Venous blood samples were drawn after an overnight fast. Most clinical chemistry assays were performed by the CHUV Clinical Laboratory (University of Lausanne) on fresh blood samples. Adiponectin and insulin levels were measured by Pathway Diagnostics Corporation (Los Angeles, California). Insulin was assayed using a solid-phase, 2-site chemiluminescent immunometric assay with a maximum intraassay coefficient of variation of 13.7% (Diagnostic Products Corporation, Los Angeles, California). GGT was assayed using an optimized standard method (Roche Diagnostics, Basel, Switzerland) with maximum inter- and intrabatch coefficients of variation of 1.6% and 0.4%, respectively. Details on all measurements performed in the CoLaus study have been published previously (20).

Nuclear DNA was extracted from whole blood. Genotyping was performed using the Affymetrix 500 K single nucleotide polymorphism (SNP) chip, as recommended by the manufacturer (Affymetrix, Inc., Santa Clara, California). Persons with less than 95% genotyping efficiency overall (or <90% efficiency on either array;  $n = 399$ ) and persons with possible gender inconsistencies ( $n = 5$ ) were removed. Monomorphic SNPs with less than 70% genotyping efficiency, with a minor allele frequency less than 1%, and/or not in Hardy-Weinberg proportions were excluded from the analyses. Twenty-seven SNPs were located in and around the  $\gamma$ -glutamyltransferase 1 (*GGT1*) gene ( $\pm 100$  kilobases) and were therefore considered for the present analysis.

Baseline characteristics were compared across sex-specific GGT quartiles using Kruskal-Wallis tests for contin-

uous variables and chi-squared tests for categorical variables. We used multivariable linear regression analyses to compare blood pressure and insulin levels across quartiles of GGT and to adjust for potential confounders. In a first step, crude models were adjusted for age and sex. Subsequently, body mass index (weight (kg)/height (m)<sup>2</sup>), alcohol consumption, and smoking were added to the models. We then performed a stepwise selection procedure, to choose relevant covariates among a wide range of potential confounders. Covariates from prior models were forced into the models. For simplicity, covariates selected for systolic blood pressure models were also used for diastolic blood pressure, with the exception of age squared, which was added to the diastolic blood pressure models. For all analyses, data on insulin and other biomarkers were log-transformed to improve the normality of the residuals, as well as the linearity of their associations.

To explore the potential causal effect of GGT on blood pressure or insulin, we applied a Mendelian randomization approach using instrumental variables. In a first stage, we regressed GGT on our instrument (genotypes at rs2017869), which was the SNP explaining the largest proportion of GGT variance in our sample (1.64%). In a second stage, we regressed the response of interest (e.g., systolic blood pressure, diastolic blood pressure, or insulin) on the fitted values from the first-stage regression, referred to hereafter as “explained” GGT. The regression coefficient associated with explained GGT in this second stage can be interpreted as a causal effect of GGT on the response, provided that the instrument is correlated with GGT and that the instrument has no effect on the response other than its effect through GGT. We ensured that the instrument was sufficiently strong by checking that the *F* value obtained in the first-stage regression was greater than 10 (23). The second assumption cannot be verified from the data, but in our case, it is unlikely that the *GGT1* gene affects either blood pressure or insulin independently of GGT. We analyzed the association of rs2017869 with all potential confounders using 1-way analysis of variance or chi-squared tests and found no significant relations, as shown in Appendix Table 1. For each association of interest, we conducted both ordinary least squares (OLS) regression and 2-stage least squares (2SLS) regression, using the ivregress function in Stata (Stata Corporation, College Station, Texas). We compared OLS and 2SLS estimates using the Durbin-Hausman test (24). Whenever appropriate, we also explored whether the significant interactions found using OLS were confirmed using 2SLS.

We performed tests for linear trend by assigning to each participant the sex-specific median of the GGT quartile to which he or she belonged. Effect modification was assessed using multiplicative interaction terms. All statistical analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina), or Stata, version 10.1. A 2-tailed *P* value less than 0.05 was prespecified to indicate statistical significance.

## RESULTS

In total, 4,360 participants were included in the present analysis. Of those, 2,270 (52.1%) were female. Baseline

characteristics are shown in Table 1 according to sex-specific GGT quartile. With increasing levels of GGT, participants were significantly older, had a higher body mass index, more often had diabetes or hypertension, and had a less favorable lipid profile. They also consumed more alcohol, as evidenced by higher levels of carbohydrate-deficient transferring (Table 1), and had higher levels of C-reactive protein, uric acid, and total protein.

Systolic and diastolic blood pressures increased from median values of 121 mm Hg and 76 mm Hg, respectively, in the lowest GGT quartile to 133 mm Hg and 82 mm Hg, respectively, in the highest GGT quartile (Table 1). After adjustment for age and sex, these relations were significantly attenuated, but a highly significant trend persisted (Table 2). In the fully adjusted model, the increases in systolic and diastolic blood pressure were reduced to 1.0 mm Hg, 1.3 mm Hg, and 2.2 mm Hg ( $P$  for linear trend = 0.005) and 0.9 mm Hg, 1.0 mm Hg, and 1.2 mm Hg ( $P$  for linear trend = 0.08) in the second, third, and fourth GGT quartiles, respectively.

Median insulin levels are also shown in Table 1 according to GGT quartile. Age and sex adjustment did not influence much the highly significant gradient observed across GGT quartiles (Table 3). By contrast, additional adjustment for body mass index, alcohol, and smoking substantially attenuated these relations (Table 3). Sensitivity analyses showed that this attenuation was exclusively due to the effect of body mass index. Additional adjustment further attenuated the gradual increase in insulin levels across GGT quartiles. Nevertheless, a highly significant gradient of insulin levels across GGT quartiles persisted even in the fully adjusted model (Table 3). In the primary analyses, diabetes and antidiabetic treatment were not considered for the multivariable insulin models because of potential overadjustment and the statistical “noise” induced by the effect of treatment on insulin levels. However, forcing these variables into the multivariable models did not significantly change our results (data not shown).

Results of selected subgroup analyses are shown in Appendix Table 2. Associations between blood pressure and GGT were consistent across different strata of sex, body mass index, and alcohol consumption. A significant relation between blood pressure and GGT was evident in persons younger than age 55 years but not among older persons. Accordingly, the multiplicative interaction test with age was highly significant for both systolic ( $P$  = 0.001) and diastolic ( $P$  < 0.0001) blood pressure. With regard to insulin, consistent findings were obtained across different strata of age, sex, and alcohol consumption. By contrast, the highly significant relation between GGT and insulin was seen only among persons with a body mass index of at least 25, not among leaner persons. Accordingly, the GGT × body mass index interaction was highly statistically significant ( $P$  < 0.0001).

The frequency of the rs2017869 variant did not deviate significantly from Hardy-Weinberg proportions ( $P$  = 0.73), and genotype frequencies are shown in Table 1. Median GGT levels were 20 U/L (interquartile range, 14–32), 23 U/L (interquartile range, 15–36), and 25 U/L (interquartile range, 18–42) for the GG, GC, and CC genotypes, respectively.

( $P$  < 0.0001). The rs2017869 variant was an appropriate instrumental variable ( $F$  = 72.87 in the first-stage regression). The strong positive association between GGT and systolic blood pressure in crude OLS analyses ( $\beta$  = 7.75,  $P$  < 0.001) was not confirmed using 2SLS ( $\beta$  = -2.78,  $P$  = 0.389). The OLS coefficient differed significantly from the 2SLS coefficient, regardless of the adjustment procedure used (Table 4). Because of the significant age × log GGT interaction in OLS analyses, we also present results stratified by age group. This age × log GGT interaction was not significant in 2SLS analyses ( $P$  = 0.055). These results provide some evidence against a positive causal relation between systolic blood pressure and GGT. Similar results and conclusions were obtained for diastolic blood pressure (data not shown).

By contrast, the positive association of GGT with fasting insulin in crude OLS analyses ( $\beta$  = 0.25,  $P$  < 0.0001) was confirmed using 2SLS ( $\beta$  = 0.20,  $P$  = 0.034). There was no significant difference between the 2 coefficients ( $P$  = 0.637) (Table 5). Whereas various adjustment procedures strongly attenuated the OLS association of GGT with fasting insulin levels, we observed no such effect with the 2SLS coefficient. Our results are compatible with a direct causal effect of GGT on fasting insulin, used as a proxy for insulin resistance. Results were similar in men and women (data not shown). Unlike what was found for the OLS analysis, there was no GGT × body mass index interaction in the 2SLS analysis ( $P$  = 0.814), which suggests that any causal effect is similar in lean and overweight participants. The rs2017869 variant was a much better instrument in the absence of regular alcohol consumption. In that group, a significant Mendelian randomization coefficient could be established, suggesting that GGT also plays a causal role in persons who abstain from alcohol consumption.

## DISCUSSION

In population-based genetic association studies, random allocation of genetic variants from parents to offspring at conception is expected to be independent of any known or unknown behavioral and environmental factors, usually allowing the analysis of largely unconfounded risk associations, similar to a randomized trial in many ways (17, 18). This random association is usually referred to as Mendelian randomization. Using a Mendelian randomization approach, we found convincing evidence that GGT levels were causally associated with fasting insulin, which represents an indirect measure of insulin resistance. The relation of genetically explained GGT with insulin was similar in men and women and was particularly strong among participants who reported not drinking alcohol regularly.

While we were able to confirm the results of prior studies showing a strong positive association between GGT and insulin levels (25–27), this “nongenetic” association was substantially attenuated by adjustment for potential confounders. By contrast, the coefficient obtained from the instrumental-variable approach was barely affected by various adjustment procedures, consistent with the notion that genetically explained GGT levels should not be confounded. Similarly, the instrumental-variable approach did

**Table 1.** Baseline Characteristics of Participants According to Sex-Specific Quartile of  $\gamma$ -Glutamyltransferase Level, CoLaus Study, Lausanne, Switzerland, 2003–2006

Characteristic	Sex-Specific Quartile of $\gamma$ -Glutamyltransferase								P Value <sup>a</sup>				
	1 (n = 1,068)		2 (n = 1,077)		3 (n = 1,115)		4 (n = 1,100)						
	Median (IQR)	No.	%	Median (IQR)	No.	%	Median (IQR)	No.	%	Median (IQR)	No.	%	
$\gamma$ -Glutamyltransferase, U/L													
Men	16 (14–18)			24 (22–26)			35 (32–40)			67 (53–102)			
Women	11 (9–12)			14 (13–15)			20 (18–22)			35 (29–54)			
Age, years	48 (41–57)			52 (44–61)			56 (46–63)			58 (49–65)		<0.0001	
Body mass index <sup>b</sup>	24.1 (21.9–26.4)			25.2 (22.9–28.0)			26.3 (23.8–29.1)			27.4 (24.3–30.5)		<0.0001	
% body fat	26 (21–33)			28 (23–35)			30 (24–38)			31 (25–39)		<0.0001	
Diabetes mellitus		29	2.7		52	4.8		76	6.8		142	12.9	<0.0001
Antidiabetic treatment		18	1.7		27	2.5		44	4.0		78	7.1	<0.0001
Systolic blood pressure, mm Hg	121 (112–132)			126 (115–138)			130 (119–142)			133 (122–146)		<0.0001	
Diastolic blood pressure, mm Hg	76 (70–83)			79 (73–86)			80 (74–88)			82 (75–90)		<0.0001	
Hypertension		220	20.6		363	33.7		483	43.3		598	54.4	<0.0001
Antihypertensive treatment		98	9.2		163	15.1		250	22.4		335	30.5	<0.0001
History of lipid-lowering treatment		58	5.4		120	11.1		149	13.4		211	19.2	<0.0001
Triglycerides, mmol/L	1.0 (0.7–1.3)			1.1 (0.8–1.6)			1.3 (0.9–1.7)			1.4 (1.0–2.1)		<0.0001	
Total cholesterol, mmol/L	5.3 (4.7–6.0)			5.5 (4.8–6.2)			5.7 (5.0–6.4)			5.9 (5.1–6.6)		<0.0001	
High density lipoprotein cholesterol, mmol/L	1.6 (1.4–1.9)			1.6 (1.3–1.9)			1.6 (1.3–1.9)			1.5 (1.3–1.8)		<0.0001	
Current smoking		254	23.8		278	25.8		283	25.4		316	28.7	0.07
Alcohol consumption, drinks/day	0.4 (0.0–1.0)			0.6 (0.0–1.1)			0.7 (0.0–1.4)			0.9 (0.1–2.0)		<0.0001	
Carbohydrate-deficient transferrin, % of total transferrin	0.8 (0.6–1.0)			0.8 (0.6–1.0)			0.8 (0.6–1.0)			0.9 (0.7–1.1)		<0.0001	
Aspartate aminotransferase, U/L	25 (21–29)			26 (22–31)			27 (23–33)			32 (26–40)		<0.0001	
Alanine aminotransferase, U/L	18 (14–24)			22 (17–29)			24 (19–32)			32 (23–47)		<0.0001	
Insulin, $\mu$ U/L	5.7 (4.0–8.0)			6.7 (4.7–9.9)			7.9 (5.1–11.0)			8.7 (5.8–13.5)		<0.0001	
Glucose, mmol/L	5.3 (5.0–5.6)			5.3 (5.0–5.7)			5.4 (5.1–5.9)			5.6 (5.2–6.1)		<0.0001	
Adiponectin, $\mu$ g/mL	8.56 (5.33–13.11)			8.02 (4.97–12.58)			8.20 (5.14–12.42)			7.68 (4.85–12.12)		0.0037	
Uric acid, $\mu$ mol/L	278 (232–336)			305 (252–360)			319 (265–379)			339 (281–407)		<0.0001	
C-reactive protein, mg/L	0.8 (0.4–1.9)			1.2 (0.6–2.5)			1.5 (0.8–3.1)			2.1 (1.0–4.1)		<0.0001	
Albumin, g/L	44 (42–46)			44 (43–46)			44 (43–46)			45 (43–46)		<0.0001	
Protein, g/L	74 (71–76)			74 (72–77)			75 (72–78)			76 (73–79)		<0.0001	
GGT1 rs2017869 genotype													
GG		518	48.5		454	42.2		411	36.9		348	31.6	<0.0001
GC		458	42.9		491	45.6		529	47.4		554	50.4	
CC		92	8.6		132	12.3		175	15.7		198	18.0	

Abbreviations: CoLaus, Cohorte Lausannoise; GGT1,  $\gamma$ -glutamyltransferase 1; IQR, interquartile range.<sup>a</sup> Kruskal-Wallis test for continuous variables and chi-squared test for categorical variables.<sup>b</sup> Weight (kg)/height (m)<sup>2</sup>.

**Table 2.** Change in Systolic and Diastolic Blood Pressures of Participants (mm Hg) According to Sex-Specific Quartile of  $\gamma$ -Glutamyltransferase, CoLaus Study, Lausanne, Switzerland, 2003–2006

Variable and Regression Model	Sex-Specific Quartile of $\gamma$ -Glutamyltransferase								P for Trend <sup>a</sup>
	1 (n = 1,068) ( $\beta$ = 0)	2 (n = 1,077)		3 (n = 1,115)		4 (n = 1,100)			
	$\beta$	P Value	$\beta$	P Value	$\beta$	P Value			
Systolic blood pressure									
Crude	Referent	4.7	<0.0001	8.1	<0.0001	11.8	<0.0001	<0.0001	
Age- and sex-adjusted	Referent	2.3	0.0008	4.2	<0.0001	6.7	<0.0001	<0.0001	
Multivariable-adjusted 1 <sup>b</sup>	Referent	1.5	0.02	2.6	0.0001	4.4	<0.0001	<0.0001	
Multivariable-adjusted 2 <sup>c</sup>	Referent	1.0	0.15	1.3	0.05	2.2	0.003	0.005	
Diastolic blood pressure									
Crude	Referent	2.5	<0.0001	3.9	<0.0001	5.5	<0.0001	<0.0001	
Age- and sex-adjusted	Referent	2.0	<0.0001	3.2	<0.0001	4.6	<0.0001	<0.0001	
Multivariable-adjusted 1 <sup>b</sup>	Referent	1.3	0.003	1.9	<0.0001	2.6	<0.0001	<0.0001	
Multivariable-adjusted 2 <sup>d</sup>	Referent	0.9	0.03	1.0	0.02	1.2	0.01	0.08	

Abbreviation: CoLaus, Cohorte Lausannoise.

<sup>a</sup> P value for linear trend across quartiles of  $\gamma$ -glutamyltransferase.<sup>b</sup> Adjusted for age, sex, body mass index, alcohol consumption, and smoking.<sup>c</sup> Adjusted for age, sex, body mass index, alcohol consumption, smoking, C-reactive protein, albumin, aspartate aminotransferase, antihypertensive treatment, lipid-lowering treatment, and total cholesterol.<sup>d</sup> Adjusted for age, age squared, sex, body mass index, alcohol consumption, smoking, C-reactive protein, albumin, aspartate aminotransferase, antihypertensive treatment, lipid-lowering treatment, and total cholesterol.

not confirm the significant GGT  $\times$  body mass index interaction of the OLS analysis, which suggests that this interaction is due to confounding effects. Our results are therefore in line with prospective studies showing that GGT levels predict incident type 2 diabetes mellitus (1–9). Remarkably, we also found that the coefficient for insulin obtained from the instrumental-variable approach was larger than the fully adjusted “nongenetic” coefficient (Table 5), potentially reflecting adverse effects of lifelong elevations in GGT levels.

Similar to what was found for insulin, various adjustment procedures led to a dramatic decrease in the strength of the nongenetic association between GGT and blood pressure. Nevertheless, we confirmed results of prior studies showing a “nongenetic” positive association of GGT with blood

pressure and hypertension (1, 10–14). However, in contrast to the insulin results, no evidence for a direct causal role of GGT in blood pressure control emerged in the genetic instrument analysis. These results suggest that the previously reported association of GGT levels with incident hypertension is not causal but most likely due to residual confounding.

Our results are in line with recent experimental findings linking oxidative stress to insulin resistance (28, 29). GGT expression is induced by oxidative stress (30), and GGT deficiency leads to an increase in oxidative stress (31). In animal models, dysregulated glutathione metabolism is associated with impaired insulin action in adipocytes (32). Reactive oxygen species are increased in cellular models of insulin resistance, and a treatment designed to alter levels

**Table 3.** Change in Insulin Level ( $\mu$ U/L) According to Sex-Specific Quartile of  $\gamma$ -Glutamyltransferase, CoLaus Study, Lausanne, Switzerland, 2003–2006

Regression Model	Sex-Specific Quartile of $\gamma$ -Glutamyltransferase								P for Trend <sup>a</sup>
	1 (n = 1,068) ( $\beta$ = 0)	2 (n = 1,077)		3 (n = 1,115)		4 (n = 1,100)			
	$\beta$	P Value	$\beta$	P Value	$\beta$	P Value			
Crude									
Crude	Referent	0.15	<0.0001	0.27	<0.0001	0.39	<0.0001	<0.0001	
Age- and sex-adjusted	Referent	0.13	<0.0001	0.25	<0.0001	0.36	<0.0001	<0.0001	
Multivariable-adjusted 1 <sup>b</sup>	Referent	0.08	0.0005	0.15	<0.0001	0.23	<0.0001	<0.0001	
Multivariable-adjusted 2 <sup>c</sup>	Referent	0.04	0.08	0.08	0.0005	0.09	0.0002	<0.0001	

Abbreviation: CoLaus, Cohorte Lausannoise.

<sup>a</sup> P value for linear trend across quartiles of  $\gamma$ -glutamyltransferase.<sup>b</sup> Adjusted for age, sex, body mass index, alcohol consumption, and smoking.<sup>c</sup> Adjusted for age, sex, body mass index, alcohol consumption, smoking, percent body fat, uric acid, aspartate aminotransferase, alanine aminotransferase, protein, adiponectin, total cholesterol, high density lipoprotein cholesterol, triglycerides, and lipid-lowering treatment.

**Table 4.** Change in Systolic Blood Pressure (mm Hg) According to  $\gamma$ -Glutamyltransferase Level Using an Instrumental-Variable Approach, CoLaus Study, Lausanne, Switzerland, 2003–2006<sup>a</sup>

Variable and Regression Model	No. of Participants	Ordinary Least Squares			2-Stage Least Squares			F Value (First Stage)	P for Difference <sup>b</sup>
		$\beta$	95% CI	P Value	$\beta$	95% CI	P Value		
All participants									
Crude	4,360	7.75	7.00, 8.50	<0.0001	-2.78	-9.13, 3.56	0.389	72.87	0.001
Age- and sex-adjusted	4,360	3.97	3.21, 4.73	<0.0001	-4.53	-10.71, 1.64	0.150		0.007
Multivariable-adjusted 1 <sup>c</sup>	4,359	2.52	1.73, 3.31	<0.0001	-4.82	-10.61, 0.96	0.102		0.012
Multivariable-adjusted 2 <sup>d</sup>	4,359	1.30	0.32, 2.03	0.007	-5.68	-11.51, 0.16	0.056		0.020
Age group									
$\geq 55$ years									
Crude	2,009	4.08	2.90, 5.26	<0.0001	-9.20	-21.0, 2.58	0.126	25.38	0.026
Age- and sex-adjusted	2,009	2.43	1.21, 3.66	<0.0001	-11.7	-24.5, 1.06	0.072		0.029
Multivariable-adjusted 1	2,009	1.32	0.05, 2.60	0.042	-13.0	-25.5, -0.41	0.043		0.025
Multivariable-adjusted 2	2,008	0.13	-1.25, 1.51	0.854	-11.7	-23.5, 0.05	0.051		0.047
<55 years									
Crude	2,351	8.26	7.42, 9.10	<0.0001	2.55	-3.29, 8.39	0.392	53.00	0.053
Age- and sex-adjusted	2,351	5.58	4.65, 6.50	<0.0001	0.43	-5.87, 6.74	0.893		0.106
Multivariable-adjusted 1	2,351	3.69	0.27, 4.65	<0.0001	0.72	-4.96, 6.40	0.804		0.299
Multivariable-adjusted 2	2,351	2.45	1.42, 3.48	<0.0001	-0.75	-6.85, 5.36	0.811		0.298
Alcohol consumption									
<1 drink/day									
Crude	3,279	7.39	6.45, 8.33	<0.0001	-1.02	-7.74, 5.68	0.765	71.60	0.013
Age- and sex-adjusted	3,279	3.31	2.38, 4.24	<0.0001	-1.86	-8.17, 4.46	0.564		0.105
Multivariable-adjusted 1	3,278	2.10	1.16, 3.04	<0.0001	-2.22	-8.34, 3.90	0.476		0.161
Multivariable-adjusted 2	3,278	0.87	-0.11, 1.87	0.082	-3.75	-9.97, 2.82	0.273		0.167
$\geq 1$ drink/day									
Crude	1,081	6.39	4.98, 7.81	<0.0001	-9.93	-26.94, 7.08	0.252	11.11	0.059
Age- and sex-adjusted	1,081	4.98	3.57, 6.39	<0.0001	-14.30	-33.49, 4.88	0.144		0.048
Multivariable-adjusted 1	1,081	3.20	1.69, 4.72	<0.0001	-13.24	-29.05, 2.55	0.100		0.040
Multivariable-adjusted 2	1,081	1.86	0.14, 3.57	0.034	-12.21	-26.11, 1.68	0.085		0.045

Abbreviations: CI, confidence interval; CoLaus, Cohorte Lausannoise.

<sup>a</sup> Systolic blood pressure was the dependent variable, and  $\gamma$ -glutamyltransferase was the independent variable of interest. Data on  $\gamma$ -glutamyltransferase were log-transformed for all analyses. In models including all participants and using multivariable adjustment model 1, the P value for the interaction between age and log-transformed  $\gamma$ -glutamyltransferase was <0.0001 for ordinary least squares and 0.055 for 2-stage least squares.

<sup>b</sup> P value for the difference between ordinary least squares and 2-stage least squares estimates (Durbin-Hausman test).

<sup>c</sup> Adjusted for age, sex, body mass index, smoking, and alcohol consumption.

<sup>d</sup> Adjusted for age, sex, body mass index, alcohol consumption, smoking, C-reactive protein, albumin, aspartate aminotransferase, antihypertensive treatment, lipid-lowering treatment, and total cholesterol.

of reactive oxygen species was found to improve insulin sensitivity and glucose homeostasis in insulin-resistant mice (15). Overall, these recent results strongly support the hypothesis that dysregulation of antioxidant enzymes could lead to dysfunction of adipocytes, including insulin resistance.

In prior studies, researchers have consistently reported an increased incidence of cardiovascular mortality (33, 34) and all-cause mortality (35–37) among persons with elevated GGT levels. For example, in a cohort study of 163,944 Austrian adults with a 17-year follow-up period, GGT levels were associated with a 60% increase in cardiovascular

mortality (33). In the Third National Health and Nutrition Examination Survey, GGT was associated with a 50% increase in all-cause mortality and a 2.4-fold increased risk of diabetes-related mortality (35). Such associations were not observed for other liver enzymes, suggesting that the observed relations for GGT are not just due to diffuse liver pathology. Our study indirectly supports the causality of these associations, although their detailed mechanisms remain largely unclear, and further studies are needed in this regard.

The strengths of this study are its large sample size, the population-based nature of the cohort, the large number of relevant characteristics assessed, and the availability of

**Table 5.** Change in Insulin Level ( $\mu$ U/L) According to  $\gamma$ -Glutamyltransferase Level Using an Instrumental-Variable Approach, Lausanne, Switzerland, 2003–2006<sup>a</sup>

Variable and Regression Model	No. of Participants	Ordinary Least Squares			2-Stage Least Squares				P for Difference <sup>b</sup>
		$\beta$	95% CI	P Value	$\beta$	95% CI	P Value	F Value (First Stage)	
All participants									
Crude	4,360	0.25	0.22, 0.27	<0.0001	0.20	0.02, 0.39	0.034	72.87	0.637
Age- and sex-adjusted	4,359	0.22	0.19, 0.25	<0.0001	0.19	-0.02, 0.39	0.070		0.756
Multivariable-adjusted 1 <sup>c</sup>	4,359	0.14	0.12, 0.17	<0.0001	0.18	-0.01, 0.36	0.055		0.713
Multivariable-adjusted 2 <sup>d</sup>	4,307	0.07	0.04, 0.09	<0.0001	0.19	0.01, 0.37	0.042		0.177
Body mass index <sup>e</sup>									
<25									
Crude	1,918	0.10	0.06, 0.13	<0.0001	0.14	-0.30, 0.57	0.539	14.23	0.860
Age- and sex-adjusted	1,918	0.08	0.04, 0.13	<0.0001	0.13	-0.34, 0.60	0.589		0.853
Multivariable-adjusted 1	1,918	0.09	0.05, 0.13	<0.0001	0.14	-0.32, 0.59	0.558		0.840
Multivariable-adjusted 2	1,889	0.03	-0.02, 0.08	0.235	0.16	-0.20, 0.53	0.373		0.459
$\geq 25$									
Crude	2,442	0.22	0.19, 0.25	<0.0001	0.17	-0.03, 0.36	0.096	61.32	0.621
Age- and sex-adjusted	2,442	0.21	0.18, 0.25	<0.0001	0.16	-0.05, 0.37	0.130		0.646
Multivariable-adjusted 1	2,441	0.18	0.14, 0.21	<0.0001	0.19	0.01, 0.38	0.039		0.847
Multivariable-adjusted 2	2,418	0.08	0.04, 0.11	<0.0001	0.17	-0.03, 0.36	0.097		0.357
Alcohol consumption									
<1 drink/day									
Crude	3,279	0.27	0.24, 0.30	<0.0001	0.22	0.02, 0.43	0.033	71.60	0.637
Age- and sex-adjusted	3,279	0.24	0.21, 0.28	<0.0001	0.21	-0.01, 0.43	0.056		0.792
Multivariable-adjusted 1	3,278	0.15	0.12, 0.18	<0.0001	0.22	0.02, 0.42	0.035		0.541
Multivariable-adjusted 2	3,238	0.07	0.03, 0.10	<0.0001	0.23	0.02, 0.44	0.036		0.139
$\geq 1$ drink/day									
Crude	1,081	0.24	0.20, 0.29	<0.0001	0.12	-0.32, 0.56	0.597	11.11	0.580
Age- and sex-adjusted	1,081	0.21	0.17, 0.26	<0.0001	0.06	-0.43, 0.56	0.803		0.556
Multivariable-adjusted 1	1,081	0.12	0.08, 0.17	<0.0001	0.01	-0.43, 0.46	0.952		0.634
Multivariable-adjusted 2	1,069	0.04	-0.01, 0.10	0.112	0.05	-0.27, 0.38	0.741		0.951

Abbreviation: CoLaus, Cohorte Lausannoise.

<sup>a</sup> Insulin was the dependent variable, and  $\gamma$ -glutamyltransferase was the independent variable of interest. Data on both were log-transformed for all analyses. In models including all participants and using multivariable adjustment model 1, the P value for the interaction between body mass index and log-transformed  $\gamma$ -glutamyltransferase was <0.0001 for ordinary least squares and 0.814 for 2-stage least squares.

<sup>b</sup> P value for the difference between ordinary least squares and 2-stage least squares estimates (Durbin-Hausman test).

<sup>c</sup> Adjusted for age, sex, body mass index, smoking, and alcohol consumption.

<sup>d</sup> Adjusted for age, sex, body mass index, alcohol consumption, smoking, percent body fat, uric acid, aspartate aminotransferase, alanine aminotransferase, protein, adiponectin, total cholesterol, high density lipoprotein cholesterol, triglycerides, and lipid-lowering treatment.

<sup>e</sup> Weight (kg)/height (m)<sup>2</sup>.

a large number of biomarkers and genetic information on all participants. Nevertheless, the following potential limitations should also be taken into account in interpreting the present results. First, only middle-aged Caucasians were included, and generalizability to other populations remains unclear. Second, because our instrument (rs2017869) was rather weak (i.e., 1.6% of GGT variance explained), Mendelian randomization analyses resulted in wide confidence intervals and low precision. In addition, insulin resistance was estimated only indirectly via fasting insulin levels; however, direct measurements such as those made by euglycemic hyperinsulinemic clamp are hardly feasible in large-

scale, population-based studies. Finally, the blood pressure measurements showed high intraindividual variability (38), which may have limited our power to reveal a causal GGT-blood pressure relation.

Using a Mendelian randomization approach, we found evidence for a direct causal association between GGT and fasting insulin levels. These findings suggest that an association of GGT with the risk of type 2 diabetes mellitus might occur via insulin resistance. Given the role of GGT in glutathione homeostasis, our epidemiologic results are in line with experimental data showing the importance of oxidative stress in the pathogenesis of insulin resistance. If confirmed

in other settings, these results underscore the usefulness of the Mendelian randomization approach to infer causality in observational epidemiology and to unravel underlying pathophysiological mechanisms.

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**Appendix Table 1.** Baseline Characteristics of Participants According to *GGT1* rs2017869 Genotype, CoLaus Study, Lausanne, Switzerland, 2003–2006

Characteristic	GGT1 rs2017869 Genotype						P Value	
	GG (n = 1,731)		GC (n = 2,032)		CC (n = 597)			
	Median (IQR)	No.	%	Median (IQR)	No.	%		
γ-Glutamyltransferase, U/L	20 (14–32)			23 (15–36)			<0.0001	
Age, years	53 (45–62)			54 (45–63)			0.29	
Sex		798	46.1		991	48.8		
Body mass index <sup>a</sup>	25.6 (22.9–28.5)			25.7 (23.2–28.6)			0.49	
% body fat	29 (23–37)			29 (23–36)			0.42	
Diabetes mellitus		127	7.3		125	6.2		
Antidiabetic treatment		70	4.0		67	3.3		
Systolic blood pressure, mm Hg	127 (117–140)			128 (117–140)			0.71	
Diastolic blood pressure, mm Hg	79 (73–87)			80 (73–87)			0.89	
Hypertension		659	38.1		783	38.5		
Antihypertensive treatment		321	18.5		406	20.0		
History of lipid-lowering treatment		209	12.1		253	12.5		
Triglycerides, mmol/L	1.2 (0.8–1.7)			1.1 (0.8–1.7)			0.41	
Total cholesterol, mmol/L	5.6 (4.9–6.3)			5.6 (4.9–6.2)			0.69	
High density lipoprotein cholesterol, mmol/L	1.6 (1.3–1.9)			1.6 (1.3–1.9)			0.26	
Current smoking		477	27.6		507	25.0		
Alcohol consumption, drinks/day	0.6 (0.0–1.4)			0.6 (0.0–1.4)			0.54	
Carbohydrate-deficient transferrin, % of total transferrin	0.8 (0.6–1.0)			0.8 (0.6–1.0)			0.66	
Aspartate aminotransferase, U/L	27 (23–33)			27 (23–34)			0.23	
Alanine aminotransferase, U/L	23 (17–32)			23 (18–33)			0.32	
Insulin, μU/L	7.0 (4.9–10.0)			7.0 (5.0–11.0)			0.16	
Glucose, mmol/L	5.4 (5.0–5.9)			5.4 (5.1–5.8)			0.91	
Adiponectin, μg/mL	8.30 (5.05–12.54)			7.93 (5.04–12.43)			0.62	
Uric acid, μmol/L	308 (253–368)			313 (259–373)			0.14	
C-reactive protein, mg/L	1.4 (0.6–2.9)			1.3 (0.7–2.9)			0.80	
Albumin, g/L	44 (43–46)			44 (43–46)			0.29	
Protein, g/L	75 (72–77)			75 (72–78)			0.70	

Abbreviations: CoLaus, Cohorte Lausannoise; *GGT1*, γ-glutamyltransferase 1; IQR, interquartile range.<sup>a</sup> Weight (kg)/height (m)<sup>2</sup>.

**Appendix Table 2.** Change in Blood Pressure (mm Hg) or Insulin Level ( $\mu$ U/L) According to Sex-Specific Quartile of  $\gamma$ -Glutamyltransferase in Subgroup Analyses, CoLaus Study, Lausanne, Switzerland, 2003–2006

Characteristic	Sex-Specific Quartile of $\gamma$ -Glutamyltransferase								<i>P</i> for Interaction <sup>a</sup>
	1 ( $\beta = 0$ )	2	$\beta$	<i>P</i> Value	3	$\beta$	<i>P</i> Value	4	
<b>Age group</b>									
$\geq 55$ years	<i>n</i> = 325	<i>n</i> = 449			<i>n</i> = 600			<i>n</i> = 635	
SBP <sup>b</sup>	Referent	1.0	0.41		-0.2	0.89		0.9	0.47
DBP <sup>b</sup>	Referent	0.2	0.76		0.2	0.76		-0.2	0.80
Insulin level <sup>c</sup>	Referent	0.02	0.54		0.07	0.04		0.07	0.07
$< 55$ years	<i>n</i> = 743	<i>n</i> = 628			<i>n</i> = 515			<i>n</i> = 465	
SBP	Referent	0.7	0.30		2.4	0.001		3.3	0.0001
DBP	Referent	1.1	0.04		1.3	0.02		2.3	0.0003
Insulin level	Referent	0.05	0.07		0.09	0.004		0.12	0.0009
<b>Sex</b>									
Male	<i>n</i> = 481	<i>n</i> = 541			<i>n</i> = 529			<i>n</i> = 539	
SBP	Referent	0.9	0.34		1.3	0.17		2.8	0.008
DBP	Referent	0.8	0.20		1.2	0.08		1.5	0.04
Insulin level	Referent	0.03	0.31		0.04	0.30		0.09	0.02
Female	<i>n</i> = 587	<i>n</i> = 536			<i>n</i> = 586			<i>n</i> = 561	
SBP	Referent	1.0	0.27		1.1	0.26		1.5	0.16
DBP	Referent	1.2	0.04		1.1	0.06		1.1	0.10
Insulin level	Referent	0.03	0.27		0.12	0.0001		0.09	0.007
<b>Body mass index<sup>d</sup></b>									
$\geq 25$	<i>n</i> = 400	<i>n</i> = 559			<i>n</i> = 712			<i>n</i> = 771	
SBP	Referent	0.4	0.67		0.6	0.57		1.3	0.22
DBP	Referent	0.5	0.42		0.6	0.38		0.9	0.18
Insulin level	Referent	0.08	0.01		0.13	<0.0001		0.16	<0.0001
$< 25$	<i>n</i> = 668	<i>n</i> = 517			<i>n</i> = 404			<i>n</i> = 329	
SBP	Referent	1.0	0.22		1.4	0.14		2.6	0.01
DBP	Referent	1.1	0.05		1.2	0.06		1.1	0.13
Insulin level	Referent	0.01	0.71		0.04	0.23		0.01	0.72
<b>Alcohol consumption</b>									
$\geq 1$ drink/day	<i>n</i> = 721	<i>n</i> = 779			<i>n</i> = 831			<i>n</i> = 842	
SBP	Referent	0.5	0.56		1.6	0.04		2.7	0.002
DBP	Referent	1.0	0.04		1.5	0.004		1.7	0.002
Insulin level	Referent	0.06	0.01		0.06	0.03		0.08	0.005
$< 1$ drink/day	<i>n</i> = 347	<i>n</i> = 298			<i>n</i> = 284			<i>n</i> = 258	
SBP	Referent	2.3	0.07		1.9	0.15		3.1	0.03
DBP	Referent	1.1	0.18		0.8	0.33		1.5	0.10
Insulin level	Referent	-0.02	0.59		0.12	0.004		0.10	0.04

Abbreviations: CoLaus, Cohorte Lausannoise; DBP, diastolic blood pressure; SBP, systolic blood pressure.

<sup>a</sup> For every individual trait, multiplicative interaction tests were conducted using nonstratified multivariable regression models.

<sup>b</sup> Adjusted for age, sex, body mass index, alcohol consumption, smoking, C-reactive protein, albumin, aspartate aminotransferase, antihypertensive treatment, lipid-lowering treatment, and total cholesterol.

<sup>c</sup> Adjusted for age, sex, body mass index, alcohol consumption, smoking, percent body fat, uric acid, aspartate aminotransferase, alanine aminotransferase, protein, adiponectin, total cholesterol, high density lipoprotein cholesterol, triglycerides, and lipid-lowering treatment.

<sup>d</sup> Weight (kg)/height (m)<sup>2</sup>.



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## Short communication

# Relationship between $\gamma$ -glutamyltransferase, lipids and lipoprotein(a) in the general population

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## Abstract

**Background:** Population-based epidemiological studies have shown a convincing association between increased  $\gamma$ -glutamyltransferase (GGT) activity and components of the metabolic syndrome, type 2 diabetes, hypertension, ischaemic stroke and myocardial infarction. However, little information is available on the interaction between GGT activity and traditional or emerging markers of cardiovascular risk.

**Methods:** We performed a retrospective analysis to retrieve results of serum GGT, fasting plasma glucose (FPG), creatinine, LDL-cholesterol, HDL-cholesterol, triglycerides and lipoprotein(a) tests performed on outpatients referred by the general practitioners to our laboratory for routine blood testing during the last 5 years.

**Results:** The concentrations of most lipid parameters varied with increasing GGT activities. There were graded, positive, associations of GGT concentrations with LDL-cholesterol, triglycerides, atherogenic index of plasma (AIP) and the total to HDL-cholesterol ratio, whereas a negative association was observed with HDL-cholesterol. Lipoprotein(a) concentrations increased in parallel with GGT activity, though such trend did not reach statistical significance. The frequencies of subjects with undesirable values according to the NCEP-ATP III and AHA/ACC thresholds increased across the spectrum of GGT thresholds for all lipids parameters but lipoprotein(a). These associations remained statistically significant even after adjustment for gender, age, FPG and creatinine concentrations. In multiple linear regression analyses GGT activity predicted plasma concentrations of LDL-cholesterol, triglycerides, HDL-cholesterol (negatively), AIP and the total to HDL-cholesterol ratio independently of age, gender, impaired fasting glucose/diabetes and creatinine levels.

**Conclusions:** The results of this large retrospective study indicate that increased GGT activities are independently associated with a more atherogenic lipid profile in general population.

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**Keywords:** Cholesterol; Lipids; Lipoprotein(a); Triglycerides;  $\gamma$ -glutamyltransferase

## 1. Introduction

Recent population-based epidemiological studies have shown a convincing association between increased  $\gamma$ -glutamyltransferase (GGT) activity, even within the reference range, and components of the metabolic syndrome. It was also prospectively demonstrated

that baseline GGT activity predict development of type 2 diabetes, hypertension, ischaemic stroke and myocardial infarction [1–3]. Moreover, evidence was provided on the interaction between serum GGT levels and individual components of the metabolic syndrome, entailing that people with low-normal serum GGT levels (e.g. <20 U/L) would no longer be considered at high risk of prevalent type 2 diabetes [4]. Nevertheless, little information is available on the interaction between GGT activity, the traditional lipid profile including low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, and emerging markers of cardiovascular risk such as the atherogenic index of plasma (AIP), the total to HDL-cholesterol ratio and lipoprotein(a). Given

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the important clinical implications of an interaction between plasma lipids and GGT in predicting the cardiovascular risk, a careful analysis focused on this relationship is needed.

## 2. Materials and methods

We performed a retrospective analysis on the database of the Laboratory Information System of the Clinical Chemistry Laboratory of the University Hospital of Verona to retrieve cumulative results of serum GGT, fasting plasma glucose (FPG), creatinine, LDL-cholesterol, HDL-cholesterol, triglycerides and lipoprotein(a) tests which have been performed on all outpatients referred by the general practitioners for routine blood testing during the last 5 years (April 2002–April 2007). Venous blood from outpatients is routinely collected in the morning on fasting subjects. Lipoprotein(a) is measured by the reference nephelometric assays on a Behring Nephelometer-II (Dade Behring GmbH, Marburg, Germany). Serum lipids, lipoproteins, FPG, creatinine and GGT are assayed on the Roche/Hitachi Modular System P (Roche Diagnostics GmbH, Mannheim, Germany). LDL-cholesterol is calculated by the Friedewald's equation [5], whereas the AIP is calculated as  $\log(\text{triglycerides}/\text{HDL-cholesterol})$  [6]. The guidelines from the American Heart Association (AHA) and the American College of Cardiology (ACC) [7] and the US-National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATP III) [8] were used to calculate the percentages of study participants with undesirable values of FPG and lipids.

Data are expressed as means  $\pm$  SD or proportions. Skewed variables were logarithmically transformed to improve normality prior to analysis and then back-transformed to their natural units for presentation in table. Statistical analyses included the one-way analysis of variance (ANOVA), the chi-squared test with Yates' correction for continuity (for categorical variables), the analysis of covariance (ANCOVA) and the multivariable linear regression analysis. Nonparametric statistical tests were also used, but because the results were identical to those obtained by parametric procedures, only the latter were presented. In ANCOVA analysis the relationships of plasma lipids with GGT concentrations (categorized into three groups: i.e.,  $\leq 20$ , 20–40 and  $> 40$  U/L) were tested after adjustment for gender, age, fasting glucose and creatinine concentrations. To further assess the independence of the association of GGT concentrations (considered as a continuous measure) with different plasma lipids, we performed multivariable linear regression analyses. In these multivariable regression models

plasma lipids were individually considered as dependent variables (entered as continuous measures), whereas GGT, gender, age, serum creatinine and fasting glucose concentrations were included as covariates. *P* values  $< 0.05$  were considered statistically significant. Statistical analyses were performed using the statistical package SPSS version 12.0 (SPSS, Chicago, IL).

## 3. Results

Cumulative results for all the parameters included in statistical analyses were retrieved for 1227 outpatients  $> 35$  years old (449 females, 37% of total). As shown in Table 1, the concentrations of most lipid parameters remarkably varied with increasing GGT activities. There were graded, positive, associations of GGT concentrations with LDL-cholesterol, triglycerides, AIP and the total to HDL-cholesterol ratio, whereas a negative association was observed with HDL-cholesterol. Similarly, the mean FPG and creatinine concentrations significantly increased across GGT categories. Lipoprotein(a) concentrations increased in parallel with GGT activity, though such trend did not reach a statistical significance. After stratification of patients according to the NCEP-ATP III and AHA/ACC thresholds, the frequencies of those with undesirable values of LDL-cholesterol, triglycerides, HDL-cholesterol, AIP and the total to HDL-cholesterol ratio significantly increased across the spectrum of GGT thresholds. On the contrary, such trend did not achieve a statistical significance for those with undesirable lipoprotein(a) values.

Notably, as also shown in Table 1, the strong associations between serum GGT activity and lipid parameters (except for lipoprotein(a)) remained statistically significant even after adjustment for gender, age, FPG and creatinine concentrations. Almost identical results were observed in multiple linear regression analyses (data not shown). In these analyses, GGT

**Table 1**  
Stratification according to the activity of  $\gamma$ -glutamyltransferase (GGT) of cumulative values for glucose, creatinine and lipids assayed on 1227 consecutive outpatients referred by the general practitioners to our laboratory for routine blood testing over a 5 year-period

	GGT categories			<i>P</i> values <sup>a</sup>	<i>P</i> values <sup>b</sup>
	$\leq 20$ U/L	20–40 U/L	$> 40$ U/L		
<i>n</i>	717	358	202	—	—
Gender (M/F)	199/518	210/148	110/92	<0.0001	NA
Age	55 $\pm$ 17	56 $\pm$ 15	60 $\pm$ 14	<0.005	NA
Creatinine (mmol/L)	64 $\pm$ 17	71 $\pm$ 17	72 $\pm$ 21	<0.0001	NA
Fasting plasma glucose (mmol/L)	5.0 $\pm$ 0.9	5.5 $\pm$ 1.3	5.7 $\pm$ 1.6	<0.0001	NA
%pts. $\geq 6.1$ mmol/L	3%	10%	13%	<0.0001	NA
LDL-cholesterol (mmol/L)	2.8 $\pm$ 0.9	3.0 $\pm$ 0.9	3.1 $\pm$ 1	<0.0001	<0.0001
% pts. $\geq 3.37$ mmol/L	30%	40%	44%	<0.0001	<0.0001
Triglycerides (mmol/L)	1.0 $\pm$ 0.6	1.3 $\pm$ 0.7	1.5 $\pm$ 1.0	<0.0001	<0.0001
%pts. $\geq 1.7$ mmol/L	11%	32%	35%	<0.0001	<0.0001
HDL-cholesterol (mmol/L)	1.6 $\pm$ 0.4	1.4 $\pm$ 0.4	1.5 $\pm$ 0.5	<0.0001	<0.0001
%pts. $\leq 1.04$ mmol/L	4%	16%	13%	<0.0001	<0.0001
Lipoprotein(a) (mg/L)	128 $\pm$ 260	242 $\pm$ 267	268 $\pm$ 285	0.160	0.250
%pts. $\geq 300$ mg/L	24%	27%	31%	0.185	0.210
Atherogenic index of plasma	-0.23 $\pm$ 0.20	-0.02 $\pm$ 0.30	-0.01 $\pm$ 0.30	<0.0001	<0.0001
%pts. > 0	18%	44%	51%	<0.0001	<0.0001
Total to HDL-cholesterol ratio	3.0 $\pm$ 0.9	3.7 $\pm$ 1.3	3.7 $\pm$ 1.2	<0.0001	<0.0001
%pts. $\geq 3.5$	30%	57%	56%	<0.0001	<0.0001

Data are expressed as means  $\pm$  SD or proportions. NA, not applicable.

<sup>a</sup> *P* values for the trend by one-way ANOVA or chi-squared test (for categorical variables).

<sup>b</sup> *P* values for the trend by analysis of covariance (ANCOVA) adjusted for age, gender, plasma glucose and creatinine concentrations.

activity strongly predicted plasma concentrations of LDL-cholesterol, triglycerides, HDL-cholesterol (negatively), AIP and the total to HDL-cholesterol ratio independently of age, gender, FPG (or presence of impaired fasting glucose/diabetes) and creatinine levels (standardized beta coefficients for GGT ranging from 0.219 to 0.368;  $P < 0.0001$ ).

#### 4. Discussion

It has been highlighted that increased GGT concentrations may predict the incidence of type 2 diabetes, metabolic syndrome, cardiovascular disease as well as death [1–3], suggesting that this enzyme may be regarded not only as index of liver damage and alcohol consumption, but also as a reliable marker of cardiovascular risk [9]. Adjusting for traditional risk factors, a 1-SD increase in GGT levels confers a 13% increase in cardiovascular risk and 26% increased risk of death. Accordingly, individuals in the highest GGT quartile experiences a ~70% increase in the incidence of cardiovascular events [10]. Although such association has been attributed to GGT as a marker of metabolic and cardiovascular risk, little information is currently available on the relationship between GGT concentrations and the lipid profile in the general population, since most of previous epidemiological studies have been principally performed in type 2 diabetic population.

It has been observed that GGT activity is significantly associated with total cholesterol, triglyceride, FPG, homocysteine and systolic blood pressure after controlling for possible confounders such as cigarette smoking, daily alcohol consumption and obesity [9]. GGT activities within the normal range have also been positively associated with triglyceride and total cholesterol irrespective of the drinking or obesity status, suggesting that GGT measurement may have important clinical implications as being more than just a marker of alcohol consumption and obesity-related liver disease [11]. After classifying a cohort of Japanese population into five groups according to GGT concentrations, Kim *et al.* observed that the frequencies of undesirable values for LDL-cholesterol and/or triglyceride increased in parallel with the GGT activity after adjustment for a variety of potential confounders [12]. Overall, the main findings of our study – which was performed on a large cohort of general population – essentially agree with this conclusion, since the mean concentration and the frequency of undesirable values according to the AHA and NCEP-ATP III criteria increased steadily across the spectrum of GGT thresholds for LDL-cholesterol, triglycerides, AIP, HDL-cholesterol and the total to HDL-cholesterol ratio. These results remained essentially unchanged even after adjusting for gender, age, FPG and creatinine concentrations, all of which being potentially important correlates of GGT. In addition, in our multivariate analyses, higher concentrations of GGT strongly predicted most of lipid parameters independently of potential confounders. At variance with earlier studies, we have also investigated the potential influence of GGT activity on serum lipoprotein(a). Although both the mean concentration and the prevalence of undesirable values of this lipoprotein tended to be higher across the GGT thresholds, such trend did not reach statistical significance. However, this is not surprising in that the concentration of this atherogenic lipoprotein particle varies over 1000-fold between individuals and it is

determined primarily by quantitative and qualitative polymorphisms at the apolipoprotein(a) locus [13], which globally account for up to 98% of lipoprotein(a) variance in the general population [14].

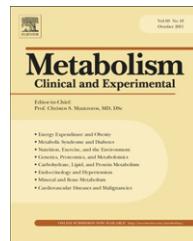
In conclusion, results of this large retrospective study indicate that increased GGT activities are independently associated with a more atherogenic lipid profile in general population. Such an association may be at least partially explained by enhanced oxidative stress, liver steatosis and insulin resistance, which may be important underlying mechanisms by which raised GGT levels might adversely affect lipoprotein metabolism. We are aware of some limitations in this study. First, the cross-sectional design of this study precludes the establishment of causal or temporal relations among lipids and GGT. Prospective studies will be required to sort out the time sequence of events. Another possible limitation is that we cannot definitively exclude that the strong associations between GGT and lipid parameters could be partly explained by some unmeasured anthropometric/demographical variables such as body mass index, concomitant pharmacological treatment and, especially, alcohol consumption, which would also explain the higher prevalence of male subjects in the top tertile of GGT activity. However, it is also to mention that GGT concentrations are associated with lipid abnormalities independently of drinking and obesity status [11]. Thus, irrespective of potential unmeasured effects of lifestyle factors, obesity or other associated comorbidities, we have observed a marked interaction between GGT, even within its normal range, and lipid parameters in the general population, suggesting the possibility that determination of GGT may help in cardiovascular risk prediction with important management implications, provided that laboratory, clinical and other diagnostic data are available. Similarly, if longer term prospective studies will substantiate this findings, the identification of people with raised GGT activity would highlight a subgroup of individuals to be targeted with more intensive lipid-lowering therapy for lowering the risk of cardiovascular events.

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# Metabolism

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## Circulating alanine transaminase (ALT) and $\gamma$ -glutamyl transferase (GGT), but not fetuin-A, are associated with metabolic risk factors, at baseline and at two-year follow-up: The prospective Cyprus Metabolism Study

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### ABSTRACT

**Objective.** To comparatively evaluate traditional liver tests and fetuin A as predictors of cardiometabolic risk, we studied associations between serum alanine transaminase (ALT),  $\gamma$ -glutamyl transferase (GGT), aspartate aminotransferase (AST) and fetuin-A and anthropometric, metabolic, and cardiovascular parameters cross-sectionally at baseline, and prospectively, after 2-years of follow-up.

**Research Design and Methods.** 616 randomly enrolled young healthy participants in the Cyprus Metabolism Study, including all 93 subjects who participated in the follow-up study 2 years after baseline assessment, were included in this study.

**Results.** In the cross-sectional study, serum ALT and GGT were strongly correlated with anthropometric, cardiovascular, and metabolic variables, while serum AST was only correlated with waist circumference and waist-to-hip ratio. Fetuin-A was correlated with anthropometric variables, systolic blood pressure (SBP), insulin, and homeostasis model of

**Abbreviations:** ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; AUC, Area under the curve; BMI, Body mass index; BMR, Basal metabolic rate; BP, Blood pressure; DBP, Diastolic blood pressure; ELISA, Enzyme-linked immunosorbent assay; GGT, Gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, Homeostasis model of assessment of insulin resistance; hsCRP, High sensitivity C-reactive protein; IDF, International Diabetes Federation; LDL, Low-density lipoprotein; LBP, Leptin-binding protein; LFTs, Liver function tests; NAFLD, Non-alcoholic fatty liver disease; NGAL, Neutrophil gelatinase-associated lipocalin; NHANES III, Third National Health and Nutrition Examination Survey; ROC, Receiver operating characteristic; SBP, Systolic blood pressure; SE, Standard error; VAT, Visceral adipose tissue; WHR, Waist-to-hip ratio.

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assessment-insulin resistance (HOMA-IR) in the unadjusted model. In the fully adjusted model, both serum ALT and GGT levels remained positively correlated with total and low-density lipoprotein (LDL) cholesterol. GGT levels also remained correlated with triglycerides. ALT levels remained strongly positively correlated with insulin ( $r = 0.17$ ,  $p < .0001$ ) and HOMA-IR ( $r = 0.16$ ,  $p = 0.0001$ ). Serum fetuin-A levels were no longer significantly correlated with any variables.

Prospectively, ALT and GGT were predictors of anthropometric variables and LDL cholesterol, while baseline levels of AST and fetuin-A were not predictors of any variables at 2-year follow-up.

**Conclusions.** We confirmed associations of ALT and GGT levels but failed to demonstrate an independent association between fetuin-A and cardiometabolic risk factors in young healthy men. Traditional liver tests (LFTs) are thus better than fetuin-A predictors of metabolic risk factors cross-sectionally and prospectively in young healthy adults.

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## 1. Introduction

Obesity is a risk factor for the development of insulin resistance, hypertension and dyslipidemia, which increase an individual's eventual risk of developing type 2 diabetes mellitus and cardiovascular disease [1–3]. The mechanisms underlying these adverse metabolic effects of obesity have been the subject of intense investigation. The clustering of disturbed glucose and insulin metabolism, overweight and abdominal fat distribution, hypertension, and dyslipidemia, i.e. the metabolic syndrome, has also been associated with non-alcoholic fatty liver disease (NAFLD) [4–6]. Similar to NAFLD, abnormal liver function tests are considered markers of the metabolic syndrome and may be stronger predictors of a worse metabolic milieu than other clinical measurements [7–9].

Alanine transaminase (ALT) is a widely used serum marker of liver disease and even a minor elevation of ALT is a good predictor of mortality from liver disease [10,11].  $\gamma$ -glutamyltransferase (GGT) and aspartate aminotransferase (AST) are mainly derived from the liver and hence often considered liver function tests (LFTs), although a significant contribution from other tissues makes them imperfect markers of liver function alone. Recently, a number of observational studies have suggested that abnormal LFTs are associated with obesity, insulin resistance, and metabolic syndromes [4,12–14]. Several prospective studies have also shown that high levels of ALT and GGT are independently associated with increased risk for incident metabolic syndrome and diabetes [12,15–19]. In non-diabetic subjects, some studies suggested that GGT might be a stronger predictor of development of type 2 diabetes than AST or ALT [19–21] while others have suggested that ALT is the only predictor [12]. All prior studies have been focused on middle to old-aged populations and thus data on young adults are lacking.

Fetuin-A, also known as alpha-2-Heremans-Schmid glycoprotein, is another serum protein that is mostly derived from liver, with limited contribution from the tongue and placenta [22]. Fetuin-A has attracted much attention since recent cross-sectional human studies have found that levels of fetuin-A are associated with insulin resistance [23,24], metabolic syndrome [25,26], visceral adipose tissue [27], fatty liver, body mass index (BMI), waist circumference, and an atherogenic

lipid profile. Weight loss induced by bariatric surgery has also been shown to lead to a decrease in fetuin-A levels [28]. In prospective studies, even after adjusting for markers of body composition, increased levels of fetuin-A have been linked to increased amount of visceral adipose tissue at five-years of follow-up in a group of 508 older subjects [29], as well as increased incidence of type 2 diabetes mellitus in both older and middle-aged subjects [27,30]. Prospective studies have also found a higher risk of clinical manifestations of atherosclerotic disease with elevated fetuin-A levels, including myocardial infarction and stroke, potentially through its association with insulin resistance and associated adverse metabolic findings [31,32].

No study to date has studied associations between fetuin-A, anthropometrics and metabolic risk factors in young adults. Moreover, no prior studies have comparatively assessed fetuin-A and traditional LFTs as predictors of cardiovascular and metabolic disease in either young or older individuals.

Thus, we investigated fetuin-A as a marker of cardiovascular and metabolic risk factors in a population of young men, both cross-sectionally and prospectively. We then comparatively evaluated traditional liver enzymes ALT, AST and GGT vs. fetuin-A as predictors of cardiovascular and metabolic diseases.

## 2. Materials and Methods

### 2.1. Subjects

The full design of the Cyprus Metabolism Study has been published elsewhere [33,34]. This research has received institutional approval from both Harvard School of Public Health and the Cyprus National Bioethics Committee.

#### 2.1.1. Cross-sectional study

In brief, 1056 eighteen-year-old candidates for recruitment in the Cypriot Army were enrolled in the study in July 2006 and 2007. For this study, a random subgroup of 616 participants was studied.

#### 2.1.2. Prospective study

All participants who enrolled in the study in July 2006 were contacted for the two-year follow-up study. One hundred and

fifteen out of 417 eligible study participants expressed an interest in participating and could make the appointment to be seen at the Nicosia General Hospital for the follow-up evaluation two years later in July 2008. Twenty-one participants were excluded because of lack of a baseline sample for fetuin-A assays. One other participant was excluded because no follow-up weight was available. This resulted in 93 participants eligible for inclusion in the prospective analyses. No major differences were detected between the initial group of subjects and the 93 participants in the prospective analysis [34].

## 2.2. Measurements

Baseline and follow-up anthropometric and metabolic measures were ascertained using standardized methods [33,34]. Body composition was measured using the TanitaTBF-300A Body Composition Analyzer; basal metabolic rate was calculated by the analyzer using a Tanita proprietary formula. Activity level was assessed using questionnaires and includes both exercise and habitual activities such as walking or farm work. Baseline liver function tests including albumin, ALT, AST, alkaline phosphatase, total bilirubin, and GGT were assessed at Nicosia General Hospital using routine automated laboratory methods (Olympus AU2700™ Chemistry-Immuno Analyzer, Olympus, Center Valley, PA). De-identified frozen samples were shipped to Beth Israel Deaconess Medical Center and serum fetuin-A levels were measured using sandwich enzyme immunoassay (ELISA, BioVendor, Candler, NC). All samples were analyzed in duplicate in the same assay. Assay sensitivities as well as interassay and intraassay coefficients of variation were similar to those reported by the manufacturer.

## 2.3. Statistical analysis

All the variables were tested for normal distribution and logarithmically transformed if not normally distributed. Results were presented as mean values  $\pm$  S.E.M., or for categorical variables, number and percentage, were used for the descriptive statistics. SAS (version 9.1, SAS Institute, Cary, NC) was used for statistical analysis.  $P < 0.0125$  was considered statistically significant based on Bonferroni correction testing four dependent or independent hypotheses at the same time on one set of data.

Baseline characteristics of the follow-up group were compared to those of the entire cohort using t-test for continuous variables and chi-square test for categorical variables. Characteristics of participants at baseline and at follow-up were compared by repeated measures analysis of variance for both continuous and categorical variables.

Pearson correlation coefficients and the probabilities associated with this statistic were obtained between variables in the cross-sectional study. Pearson partial correlation coefficients were obtained after controlling for the effects of age, smoking status, activity, BMI, body fat percentage, and waist-to-hip ratio (WHR) between variables. General linear models and logistic regression analysis were used in the prospective study. The area under the receiver operating

characteristic (ROC) curves was used to compare accuracy of logistic regression models.

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## 3. Results

### 3.1. Cross-sectional study

Descriptive characteristics of the study population are presented in Table 1. As expected from a young healthy population, all mean values were within the normal range. However, 29.5% of the participants had a BMI greater than  $25 \text{ kg/m}^2$ . Dyslipidemia was found in 30.0% of the participants, mainly due to elevated low-density lipoprotein (LDL) or low high-density lipoprotein (HDL) cholesterol.

Findings from the cross-sectional portion of the study are summarized in Table 2. In the unadjusted model, we found that serum ALT and GGT levels were strongly positively correlated with anthropometric, cardiovascular, and metabolic variables, except for height, HDL cholesterol, urea and creatinine. On the other hand, AST levels were only correlated with waist circumference and WHR. Serum fetuin-A levels were significantly positively correlated with most anthropometric variables including body weight ( $r = 0.15$ ,  $p = 0.0002$ ), BMI ( $r = 0.15$ ,  $p = 0.0004$ ), total body fat ( $r = 0.14$ ,  $p = 0.0009$ ), body fat mass ( $r = 0.13$ ,  $p = 0.001$ ), body fat-free mass ( $r = 0.15$ ,  $p = 0.0003$ ), waist ( $r = 0.16$ ,  $p < .0001$ ) and hip circumferences ( $r = 0.16$ ,  $p < .0001$ ), WHR ( $r = 0.11$ ,  $p = 0.010$ ), and BMR ( $r = 0.15$ ,  $p = 0.0003$ ). Serum fetuin-A levels were also significantly correlated with some cardiovascular and metabolic variables, such as systolic blood pressure (SBP,  $r = 0.12$ ,  $p = 0.004$ ), insulin ( $r = 0.15$ ,  $p = 0.0002$ ), homeostasis model of assessment-insulin resistance (HOMA-IR,  $r = 0.14$ ,  $p = 0.0005$ ), and leptin ( $r = 0.10$ ,  $p = 0.012$ ).

After controlling for the effects of age, smoking status, activity, BMI, body fat percentage, and WHR, serum ALT and GGT levels remained strongly positively correlated with total and LDL cholesterol. ALT levels also remained strongly positively correlated with heart rate ( $r = 0.14$ ,  $p = 0.0003$ ), insulin ( $r = 0.17$ ,  $p < .0001$ ) and HOMA-IR ( $r = 0.16$ ,  $p = 0.0001$ ), and serum GGT levels remained positively correlated with triglycerides and leptin. In the fully adjusted model, serum AST and fetuin-A levels were not significantly correlated with any cardiovascular and metabolic variables.

In multi-variable linear regression analysis including ALT, GGT, AST and fetuin-A as dependent variables, ALT has the strongest association with anthropometric variables, cardiovascular risk factors (except for DBP, HDL cholesterol and triglycerides), leptin, uric acid, urea, insulin, and HOMA-IR. GGT has strongest association with triglycerides and LBP. GGT also showed less strong association with anthropometric variables, cardiovascular risk factors, and leptin. AST has much less strong association with anthropometric variables, insulin, HOMA-IR and no association with cardiovascular variables in these models. Fetuin-A does not have any significant association with any variables in these models. After controlling for age, smoking status, activity, BMI, body fat percentage, and WHR, only serum ALT levels are independently strongly associated with insulin, HOMA-IR, and heart rate. Both serum GGT and ALT levels are

**Table 1 – Baseline characteristics of participants in the cross-sectional study.**

Variables	All subjects (n = 616)
Age (year)	18.3 ± 0.02
Activity (h/week)	11.6 ± 0.5
Anthropometric variables	
Height (cm)	175.0 ± 0.2
Weight (kg)	72.6 ± 0.6
BMI (kg/m <sup>2</sup> )	23.7 ± 0.2
Total body fat (%)	14.2 ± 0.3
Fat mass (kg)	11.2 ± 0.3
Fat-free mass (kg)	61.5 ± 0.3
Waist circumference (cm)	82.0 ± 0.4
Hip circumference (cm)	97.3 ± 0.4
Waist-to-hip ratio	0.84 ± 0.002
BMR (kcal/day)	1822 ± 9
Cardiovascular risk factors	
Heart rate (beats/min)	69.8 ± 0.4
SBP (mmHg)	108.1 ± 0.4
DBP (mmHg)	63.8 ± 0.3
Total cholesterol (mg/dL)	154.8 ± 1.3
HDL cholesterol (mg/dL)	48.1 ± 0.4
LDL cholesterol (mg/dL)	103.8 ± 1.1
Triglycerides (mg/dL)	60.4 ± 1.0
Fasting glucose (mg/dL)	81.6 ± 0.3
Insulin (ng/mL)	9.1 ± 0.2
HOMA-IR	1.88 ± 0.05
Leptin (ng/mL)	2.55 ± 0.11
LBP (ng/mL)	23.4 ± 0.2
Fetuin-A (μg/mL)	295.5 ± 2.8
Uric Acid (ng/dL)	6.7 ± 0.1
Urea (ng/dL)	35.6 ± 0.3
Creatinine (ng/dL)	1.0 ± 0.004
Liver Function test	
GGT (U/L)	25.2 ± 0.6
ALT (U/L)	27.7 ± 0.9
AST (U/L)	27.8 ± 1.1
Categorical variables	
Smoking status	
Never	368 (59.7)
Previous	26 (4.2)
Current	222 (36.1)
BMI >25 kg/m <sup>2</sup>	182 (29.5)
Waist circumference >94 cm	96 (15.6)
Total cholesterol >200 mg/dL	48 (7.9)
HDL <40 mg/dL	103 (16.9)
LDL >130 mg/dL	94 (15.4)
Dyslipidemia	183 (30.0)

Results are presented as mean ± SEM, or as n (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, leptin-binding protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; SE, standard error.

independently associated with total, and LDL cholesterol. AST and fetuin-A are no longer associated with any variables in the adjusted model.

### 3.2. Prospective study

Table 3 shows the baseline and follow-up characteristics of the 93 participants who took part in the prospective study.

Interestingly, most of the anthropometric variables and cardiovascular risk factors significantly worsened over the 2-year period between visits.

Findings from the prospective portion of the study are summarized in Table 4. In the unadjusted model, baseline serum ALT and GGT levels were significant predictors of BMI, body weight, total body fat, body fat mass, waist circumference, hip circumference, WHR, body fat-free mass, BMR, and LDL cholesterol at 2-years of follow-up (for ALT, p = 0.0004–0.01, β=0.27–0.37; for GGT, p = <0.0001–0.003, β=0.32–0.52). In the models adjusted for baseline age, smoking status, activity, BMI, body fat percentage, and WHR, the baseline serum GGT level remained a significant predictor of LDL cholesterol (p = 0.003, β=0.32), whereas serum ALT was not predictive of any cardiovascular or metabolic variable. Unlike ALT and GGT, baseline serum AST levels were not predictive of any anthropometric, cardiovascular, and metabolic variables in either unadjusted or adjusted models.

Similarly, the changes of serum ALT and GGT levels from baseline to 2-year follow-up were strong predictors of changes of all anthropometric variables in the unadjusted model. The change of serum ALT levels was also a significant predictor of change of triglycerides (p = 0.005, β=0.28), and the change of serum GGT levels was also a significant predictor of change of total (p < 0.0001, β=0.41), HDL (p = 0.002, β=0.31) and LDL (p = 0.01, β=0.26) cholesterol in unadjusted models. In the model adjusted for baseline age, smoking status, activity, BMI, body fat percentage, and WHR, the change of serum GGT levels remained significantly associated with the change of total (p < 0.0001, β=0.41), HDL (p = 0.002, β=0.33) and LDL (p = 0.01, β=0.26) cholesterol while the change of serum ALT levels were only significantly associated with HDL cholesterol (p = 0.008, β=0.31).

Baseline levels of serum fetuin-A were not a significant predictor of any anthropometrics, cardiovascular, and metabolic variables at two-year follow-up in either unadjusted or adjusted models.

Using logistic regression analysis, serum ALT has fair accuracy to significantly predict a total cholesterol level of >200 mg/dL vs. <200 mg/dL, as measured using the area under the ROC curve method (ROC AUC =0.67, p = 0.02, and/or OR = 0.949 [0.909, 0.992]) indicating that each 1 U/L decrease of ALT conveys an approximately 5% lower probability of having total cholesterol >200 mg/dL. Serum AST and fetuin-A levels were not significant predictors of categorically analyzed cholesterol levels.

### 4. Discussion

In this study, we demonstrated that the liver-derived enzymes ALT and GGT are strongly positively correlated to BMI, adiposity, fat free mass, BMR, blood pressure, total and LDL cholesterol, triglycerides, dyslipidemia, fasting glucose, insulin, and HOMA-IR in young healthy adults. In addition, after adjusting for various confounders, ALT remained associated with heart rate, total and LDL cholesterol, insulin, and HOMA-IR, suggesting that these correlations are independent from any underlying correlation with body composition, age, smoking status, and activity level. Conversely, GGT predicts only LDL cholesterol whereas ALT predicts no parameters at two-year

**Table 2 – Correlations between liver function tests, fetuin-A and baseline values of study variables.**

	ALT				GGT				AST				Fetuin-A			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.01	0.74			-0.02	0.61			0.11	0.01			-0.04	0.31		
Activity	-0.02	0.67			-0.03	0.44			-0.03	0.47			0.02	0.70		
Smoking status	0.06	0.16			0.13	0.001			0.02	0.58			-0.03	0.53		
Anthropometrics																
Height	0.06	0.12			-0.04	0.34			0.02	0.70			0.05	0.22		
Weight	0.44	<.0001			0.39	<.0001			0.09	0.02			0.15	0.0002		
BMI	0.46	<.0001			0.45	<.0001			0.10	0.02			0.15	0.0004		
Total body fat	0.46	<.0001			0.46	<.0001			0.10	0.01			0.14	0.0009		
Fat mass	0.47	<.0001			0.44	<.0001			0.10	0.01			0.13	0.001		
Fat free mass	0.36	<.0001	0.03	0.44	0.30	<.0001	-0.08	0.06	0.08	0.06	0.03	0.51	0.15	0.0003	0.06	0.16
Waist circumference	0.47	<.0001			0.45	<.0001			0.11	0.006			0.16	<.0001		
Hip circumference	0.41	<.0001			0.39	<.0001			0.18	0.06			0.16	<.0001		
WHR	0.39	<.0001			0.40	<.0001			0.13	0.001			0.11	0.010		
BMR	0.42	<.0001	0.05	0.21	0.36	<.0001	-0.07	0.08	0.09	0.03	0.53		0.15	0.0003	0.05	0.23
Cardiovascular risk factors																
Heart rate	0.22	<.0001	0.14	0.0003	0.15	0.0003	0.06	0.13	0.04	0.29	0.01	0.75	0.04	0.39	0.01	0.73
SBP	0.31	<.0001	0.10	0.015	0.29	<.0001	0.08	0.06	0.05	0.21	0.003	0.95	0.12	0.004	0.04	0.30
DBP	0.14	0.0004	-0.001	0.97	0.12	0.002	-0.01	0.76	0.03	0.47	-0.01	0.81	0.07	0.08	0.03	0.54
Total cholesterol	0.32	<.0001	0.19	<.0001	0.35	<.0001	0.24	<.0001	0.08	0.04	0.04	0.32	0.06	0.12	0.03	0.40
HDL	-0.03	0.50	0.08	0.04	-0.07	0.07	0.04	0.33	0.01	0.74	0.04	0.28	-0.02	0.71	0.02	0.61
LDL	0.34	<.0001	0.21	<.0001	0.38	<.0001	0.26	<.0001	0.07	0.06	0.04	0.38	0.10	0.02	0.07	0.11
Triglycerides	0.19	<.0001	-0.002	0.95	0.31	<.0001	0.13	0.001	-0.01	0.76	-0.06	0.13	0.04	0.28	-0.005	0.91
Dyslipidemia	0.18	<.0001	0.05	0.18	0.17	<.0001	0.04	0.33	0.02	0.70	-0.01	0.74	0.01	0.74	-0.02	0.62
Hormonal and metabolic variables																
Fasting glucose	0.13	0.001	0.01	0.77	0.14	0.0003	0.04	0.30	0.02	0.60	-0.003	0.93	0.01	0.74	-0.03	0.53
Insulin	0.37	<.0001	0.17	<.0001	0.30	<.0001	0.09	0.02	0.07	0.09	0.02	0.62	0.15	0.0002	0.08	0.07
HOMA-IR	0.36	<.0001	0.16	0.0001	0.30	<.0001	0.09	0.02	0.07	0.10	0.02	0.63	0.14	0.0005	0.06	0.12
Leptin <sup>a</sup>	0.41	<.0001	0.04	0.31	0.46	<.0001	0.15	0.0002	0.07	0.09	-0.03	0.43	0.10	0.012	-0.02	0.54
LBP	-0.16	<.0001	0.06	0.14	-0.21	<.0001	-0.002	0.97	-0.02	0.56	0.03	0.48	-0.08	0.06	-0.01	0.88
Uric acid	0.13	0.001	0.05	0.26	0.14	0.0003	0.06	0.14	0.06	0.13	0.04	0.27	0.02	0.56	0.001	0.99
Urea	-0.02	0.60	0.02	0.59	-0.04	0.35	0.002	0.95	0.08	0.06	0.09	0.03	0.01	0.87	0.03	0.50
Creatinine	-0.05	0.19	-0.03	0.45	-0.05	0.24	-0.02	0.62	0.02	0.67	0.02	0.55	-0.05	0.26	-0.04	0.35
Liver function tests																
ALT					0.54	<.0001	0.41	<.0001	0.52	<.0001	0.53	<.0001	0.11	0.006	0.06	0.13
GGT					0.54	<.0001	0.42	<.0001			0.11	0.008	0.06	0.13	0.15	0.0002
AST					0.52	<.0001	0.53	<.0001	0.11	0.01	0.06	0.13		-0.005	0.91	-0.02

Model 1: Pearson correlation coefficients and the probabilities associated with this statistics.

Model 2: Pearson partial correlation coefficients were obtained after controlling for the effects of age, smoking status, activity, BMI, body fat percentage, and WHR.

P < 0.0125 was considered statistically significant based on Bonferroni correction testing four dependent hypotheses at same time on one set of data.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, leptin-binding protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; WHR, waist-to-hip ratio.

<sup>a</sup> Values of leptin were logarithmically transformed for analysis.

follow-up in the adjusted models. Similarly, when compared to ALT and GGT, we found that fetuin-A was less strongly associated with anthropometric parameters, SBP, insulin, HOMA-IR, and leptin. In addition, we showed for the first time, that fetuin-A is not an independent predictor of metabolic risk factors in young healthy men since these correlations became nonsignificant in this group of lean insulin-sensitive 18-year-old subjects after adjusting for various confounders. This suggests that fetuin-A is not as closely correlated to these

parameters when compared with traditional LFTs and that to a large extent, its associations with cardiometabolic risk factors may be due to an underlying correlation with body composition.

Our data are in agreement with prior cross-sectional studies of mostly middle-aged participants, linking ALT and GGT to components of the metabolic syndrome, as well as type 2 diabetes mellitus [4,9,12,35]. For example, in a large population-based study analyzing data from the Third National Health and Nutrition Examination Survey (NHANES III), ALT elevation

**Table 3 – Baseline and follow-up characteristics of participants in the prospective study.**

Variables	At baseline (n = 93)	At follow-up (n = 93)	P <sup>a</sup>
Age (year)	18.2 ± 0.01		
Activity (h/week)	11.3 ± 1.4		
Anthropometric variables			
Height (cm)	175.0 ± 0.6	175.2 ± 0.6	<.0001
Weight (kg)	68.9 ± 1.3	72.9 ± 1.3	<.0001
BMI (kg/m <sup>2</sup> )	22.5 ± 0.4	23.7 ± 0.4	<.0001
Total body fat (%)	12.8 ± 0.6	15.6 ± 0.6	<.0001
Fat mass (kg)	9.5 ± 0.7	12.1 ± 0.8	<.0001
Fat-free mass (kg)	59.4 ± 0.7	60.9 ± 0.7	<.0001
Waist circumference (cm)	79.1 ± 1.0	83.8 ± 1.0	<.0001
Hip circumference (cm)	95.7 ± 0.8	97.1 ± 0.8	0.0005
Waist-to-hip ratio	0.82 ± 0.004	0.86 ± 0.004	<.0001
BMR (kcal/day)	1770 ± 20	1814 ± 20	<.0001
Cardiovascular risk factors			
Heart rate (beats/min)	66.1 ± 0.9	71.6 ± 1.0	<.0001
SBP (mmHg)	106.2 ± 1.1	111.7 ± 1.0	<.0001
DBP (mmHg)	61.7 ± 0.8	71.6 ± 0.7	<.0001
Total cholesterol (mg/dL)	144.2 ± 2.7	159.6 ± 2.8	<.0001
HDL cholesterol (mg/dL)	47.1 ± 0.9	46.0 ± 0.9	0.10
LDL cholesterol (mg/dL)	101.9 ± 2.4	98.4 ± 2.4	0.05
Triglycerides (mg/dL)	58.7 ± 2.3	75.8 ± 4.3	<.0001
Fasting glucose (mg/dL)	78.2 ± 0.7	86.9 ± 1.0	<.0001
Insulin (ng/mL)	7.4 ± 0.3		
HOMA-IR	1.44 ± 0.07		
Leptin (ng/mL)	1.76 ± 0.20		
LBP (ng/mL)	25.0 ± 0.5		
Fetuin-A (μg/mL)	333 ± 8.1		
Uric Acid (ng/dL)	6.6 ± 0.1	6.0 ± 0.1	<.0001
Urea (ng/dL)	35.8 ± 0.7	30.4 ± 0.6	<.0001
Creatinine (ng/dL)	1.05 ± 0.01	1.1 ± 0.01	0.002
Liver Function test			
GGT (U/L)	22.6 ± 0.8	22.2 ± 1.0	0.62
ALT (U/L)	24.1 ± 1.4	21.5 ± 1.3	0.07
AST (U/L)	28.1 ± 1.9	20.3 ± 0.5	0.007
Categorical variables			
Smoking status			
Never	60 (64.5)		
Previous	2 (2.2)		
Current	31 (33.3)		
BMI >25 kg/m <sup>2</sup>	17 (18.3)	22 (23.7)	0.35
Waist circumference >94 cm	7 (7.5)	12 (12.9)	0.21
Total cholesterol >200 mg/dL	2 (2.2)	7 (7.5)	0.09
HDL <40 mg/dL	14 (15.1)	22 (23.7)	0.14
LDL >130 mg/dL	12 (12.9)	9 (9.7)	0.49
Dyslipidemia	26 (28.0)	34 (36.6)	0.21

Results are presented as mean ± SEM, or as n (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, leptin-binding protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; SE, standard error.

<sup>a</sup> P for difference of follow-up group between baseline and 2-year follow-up; repeated measure analysis was applied for both continuous and categorical variables.

was associated with a number of risk factors of NAFLD, such as impaired glucose metabolism, insulin resistance, central obesity, high leptin and triglycerides, even when patients with known diabetes (who are at high risk for NAFLD) were excluded [36]. Others have found similar cross-sectional correlations between GGT and components of the metabolic syndrome [37]. Both GGT and ALT have also been shown prospectively to predict the development of insulin resistance and diabetes

mellitus [12,15,17,38,39], which we did not find in this population of healthy 18-year-old men.

In the present study, we found that fetuin-A was significantly correlated with body fat distribution, but not with insulin resistance in young healthy men. Similar to our findings, prior studies in middle-aged or older populations have found that fetuin-A is correlated with truncal obesity [40]. However, many studies have reported a significant

**Table 4 – Univariable and multivariable linear regression models of liver function tests and fetuin-A in predicting anthropometrics, cardiovascular and metabolic risk factors at 2-year follow-up.**

	ALT						GGT						AST						Fetuin-A							
	Model 1			Model 2			Model 1			Model 2			Model 1			Model 2			Model 1			Model 2				
	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>	$\beta$	P	R <sup>2</sup>		
<b>Anthropometrics</b>																										
Height	0.15	0.17	0.02				0.05	0.63	0.00				0.10	0.38	0.009				0.22	0.04	0.050					
Weight	0.37	0.0004	0.14				0.44	<.0001	0.19				0.08	0.46	0.007				0.15	0.17	0.022					
BMI	0.35	0.001	0.13				0.47	<.0001	0.22				0.04	0.70	0.002				0.06	0.56	0.004					
Total body fat	0.34	0.001	0.12				0.49	<.0001	0.24				0.09	0.43	0.008				0.02	0.83	0.001					
Fat mass	0.35	0.001	0.12				0.48	<.0001	0.23				0.08	0.45	0.007				0.05	0.66	0.002					
Fat free Mass	0.34	0.001	0.12	0.02	0.76	0.62	0.33	0.002	0.11	-0.003	0.97	0.62	0.06	0.56	0.004	-0.05	0.48	0.62	0.25	0.02	0.060	0.14	0.05	0.63		
Waist circumference	0.36	0.001	0.13				0.52	<.0001	0.27				0.03	0.81	0.001				0.11	0.33	0.011					
Hip circumference	0.33	0.002	0.11				0.43	<.0001	0.19				0.07	0.53	0.005				0.10	0.36	0.010					
Waist-to-hip ratio	0.30	0.01	0.09				0.51	<.0001	0.26				-0.06	0.60	0.596				0.03	0.43	0.007					
BMR	0.37	0.001	0.13	0.02	0.80	0.74	0.41	<.0001	0.17	0.02	0.78	0.74	0.09	0.42	0.008	-0.03	0.64	0.74	0.17	0.11	0.029	0.07	0.25	0.74		
<b>Cardiovascular risk factors</b>																										
Heart rate	0.23	0.03	0.05	0.21	0.09	0.08	0.25	0.02	0.06	0.24	0.08	0.09	0.06	0.61	0.00	0.05	0.68	0.05	-0.17	0.12	0.03	-0.18	0.11	0.08		
SBP	0.07	0.50	0.01	-0.10	0.38	0.23	0.17	0.114	0.03	0.06	0.63	0.23	-0.02	0.84	0.00	-0.12	0.23	0.24	-0.05	0.63	0.00	-0.09	0.39	0.23		
DBP	0.16	0.15	0.02	0.04	0.72	0.21	0.06	0.55	0.00	-0.04	0.76	0.21	0.04	0.70	0.002	-0.02	0.85	0.20	-0.05	0.62	0.003	-0.13	0.22	0.22		
Total Cholesterol	0.22	0.04	0.05	0.23	0.06	0.10	0.27	0.01	0.07	0.29	0.03	0.12	-0.07	0.55	0.004	-0.07	0.57	0.07	-0.04	0.70	0.002	-0.09	0.42	0.07		
HDL	-0.04	0.71	0.00	-0.04	0.77	0.03	-0.02	0.87	0.00	-0.04	0.78	0.03	-0.06	0.58	0.004	-0.06	0.63	0.04	0.03	0.78	0.001	0.04	0.73	0.03		
LDL	0.27	0.01	0.07	0.29	0.02	0.12	0.32	0.003	0.10	0.39	0.003	0.16	-0.05	0.67	0.00	-0.04	0.75	0.06	-0.05	0.65	0.00	-0.11	0.33	0.07		
Triglycerides	0.02	0.86	0.00	-0.03	0.80	0.11	0.00	0.99	0.00	-0.10	0.43	0.11	-0.02	0.84	0.001	-0.06	0.62	0.11	-0.03	0.80	0.001	-0.03	0.80	0.11		
<b>Hormonal and metabolic variables</b>																										
Fasting glucose	0.06	0.58	0.00	0.00	0.97	0.12	0.06	0.61	0.003	0.04	0.78	0.12	0.17	0.11	0.03	0.11	0.31	0.13	-0.07	0.55	0.00	-0.08	0.47	0.12		
Uric Acid	0.02	0.87	0.00	-0.15	0.18	0.22	0.10	0.36	0.01	-0.05	0.69	0.20	-0.05	0.66	0.00	-0.11	0.30	0.21	0.03	0.81	0.00	-0.04	0.71	0.20		
Urea	0.04	0.69	0.00	0.07	0.55	0.08	-0.08	0.48	0.006	-0.04	0.79	0.07	0.14	0.21	0.02	0.16	0.16	0.09	0.23	0.03	0.05	0.19	0.09	0.10		
Creatinine	-0.09	0.42	0.01	-0.06	0.64	0.08	-0.21	0.05	0.05	-0.16	0.24	0.09	0.08	0.49	0.006	0.08	0.46	0.08	0.06	0.60	0.003	0.05	0.63	0.08		

Model 1: unadjusted model.

Model 2: adjusted for age, smoking status, activity, BMI, body fat percentage, and waist to hip ratio.

 $\beta$  denotes the adjusted regression coefficient. $P < 0.0125$  was considered statistically significant based on Bonferroni correction testing four dependent hypotheses at the same time using one set of data.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure.

correlation between fetuin-A and impaired fasting glucose [41,42], metabolic syndrome [43], insulin resistance [24], type 2 diabetes [42,44–46] and prospective studies have found that fetuin-A has an independent association with type 2 diabetes mellitus [27,30] in middle-aged or old overweight populations. Interestingly, Jenkins et al. found that there was no significant relationship between plasma fetuin-A and insulin or HOMA-IR in the combined groups of older individuals and young participants in their study. However, plasma fetuin-A levels trended to be correlated with insulin and HOMA-IR in older but not in younger participants, suggesting effect modification by age [47]. We also found that fetuin-A is not an independent predictor of metabolic risk factors or dyslipidemia in our younger cohort. It has been shown that higher fetuin-A levels are associated with visceral adipose tissue (VAT) as opposed to overall body fat [29]. Deposition of VAT may play a more important role with advancing age and increasing BMI, explaining the negative result in this young healthy cohort. Jenkins et al. found in their younger participants that plasma fetuin-A was significantly related to blood pressure and blood lipid variables; in our study, fetuin-A was associated with SBP but no other blood pressure or lipid variables at baseline.

In summary, the novel findings of our study are that fetuin-A levels are not independently associated with any metabolic or cardiovascular risk factor at baseline and are not a better than traditional LFTs predictor of these variables cross-sectionally and prospectively in young adults.

The strengths of this study are that it is the first cross-sectional and prospective study comparing associations between serum liver enzymes, serum fetuin-A levels and cardiovascular and metabolic characteristics in young men. We also adjusted for known potential confounders, such as smoking status and activity in our analysis, thus eliminating bias or confounding by these variables. Measurements were performed under code using de-identified specimens and state of the art methodology by technicians who were blinded to the study hypotheses eliminating bias from these sources. Random assay variability could have resulted in misclassification but this random misclassification would have suppressed effect estimates and hence should not have resulted in statistical significance where this does not exist.

The limitations of our study include the relatively short follow-up time of only 2 years; this period of time has been shown to be adequate in terms of evaluation of cardiometabolic predictors of risk in prior studies and in this study in terms of traditional LFTs. Despite the large number of subjects in the cross sectional study, the prospective study included only a relatively small follow-up group (93 subjects) but numbers of subjects were sufficient to demonstrate significant associations between serum liver enzymes levels and outcomes of interest. The results may not be directly generalizable to other populations since we focused on a young and healthy population of Mediterranean descent. Future prospective studies are needed to confirm our data in cohorts of women and/or older subjects in the same and other ethnic groups. Moreover, interventional, mechanistic studies are needed to interpret our findings that fetuin-A may not be a better indicator of the metabolic syndrome, diabetes, and cardiovascular disease compared

to traditional LFTs in younger adults as this study clearly demonstrates.

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## Author contributions

CSM derived the hypothesis and conceived the study design; MP, CAC, SNK, and DCC planned and organized the collection of the data; XL, HG, and JPC performed the laboratory analyses; XL and CSM planned and did the statistical analyses and XL collated the data and run the statistical analyses, XL, OPRH, MP, CAC, SNK, DCC, and CSM contributed to the interpretation and discussion of results; XL, OPRH wrote initial versions of the manuscript and CSM completed the manuscript. This report was critically reviewed and subsequently approved by all authors.

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## Conflict of interest

The authors have no relevant conflict of interests.

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## **$\gamma$ -Glutamyltransferase, but not markers of hepatic fibrosis, is associated with cardiovascular disease in older people with type 2 diabetes mellitus: the Edinburgh Type 2 Diabetes Study**

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### **Abstract**

**Aims/hypothesis** We examined the association of prevalent and incident cardiovascular disease (CVD) with chronic liver disease in a cohort of community-based people with type 2 diabetes, in order to clarify the relationship between these two important conditions.

**Methods** 1,066 participants with type 2 diabetes aged 60–75 years underwent assessment of a range of liver injury markers (non-specific injury, steatosis, steatohepatitis, fibrosis, portal hypertension). Individuals were followed up for incident cardiovascular events.

**Results** At baseline there were 370/1,033 patients with prevalent CVD, including 317/1,033 with coronary artery disease

(CAD). After a mean follow-up of 4.4 years there were 44/663 incident CVD events, including 27/663 CAD events. There were 30/82 CVD-related deaths. Risk of dying from or developing CVD was no higher in participants with steatosis than in those without (HR 0.90; 95% CI 0.40, 2.00;  $p>0.05$ ). The only notable relationship was with  $\gamma$ -glutamyltransferase (GGT) (incident CVD: adjusted HR for doubling GGT 1.24 [95% CI 0.97, 1.59]  $p=0.086$ ; incident CAD: adjusted HR 1.33 [95% CI 1.00, 1.78]  $p=0.053$ ), suggesting that in our study population, chronic liver disease may have little effect on the development of, or mortality from, CVD.

**Conclusions/interpretation** An independent association between GGT and CVD warrants further exploration as a potentially useful addition to current cardiovascular risk prediction models in diabetes. However, overall findings failed to suggest that there is a clinical or pathophysiological association between chronic liver disease and CVD in elderly people with type 2 diabetes.

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### **Abbreviations**

ALT	Alanine aminotransferase
APRI	Aspartate to platelet ratio index
AST	Aspartate aminotransferase
CAD	Coronary artery disease
CK18	Cytokeratin-18
CVD	Cardiovascular disease
dBp	Diastolic blood pressure
eGFR	Estimated glomerular filtration rate

ELF	Enhanced Liver Fibrosis panel
ET2DS	Edinburgh Type 2 Diabetes Study
FIB4	Fibrosis-4 score
GGT	$\gamma$ -Glutamyltransferase
HA	Hyaluronic acid
MI	Myocardial infarction
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NFS	NAFLD fibrosis score
OPCS	Office for Population Censuses and Surveys
P3NP	Aminoterminal peptide of procollagen III
sBP	Systolic blood pressure
TIA	Transient ischaemic attack
TIMP-1	Tissue inhibitor of metalloproteinases-1

## Introduction

Reports of higher cardiovascular mortality rates in people from the general population with non-alcoholic fatty liver disease (NAFLD) [1, 2] raise the possibility that there may be a pathophysiological relationship between NAFLD and the development of cardiovascular disease (CVD). In people with type 2 diabetes, such a relationship could help to explain the higher prevalences of both conditions. However, the association between CVD and NAFLD has not been well researched in diabetic populations, such that the true relationship between these two important conditions remains uncertain. Epidemiological knowledge of the relationship between NAFLD and CVD in diabetes is particularly limited: current studies are restricted to ultrasound scan-detected NAFLD and the secondary care end of the diabetes spectrum [3, 4]. We therefore aimed to determine the association of CVD with a range of biomarkers of chronic liver injury in a large cohort representative of the full spectrum of elderly people with type 2 diabetes.

Biologically, an association between NAFLD and CVD is plausible. Many of the pathogenic factors proposed for NAFLD and atherosclerosis are shared (e.g. insulin resistance, dyslipidaemia, systemic inflammation) and are closely linked to type 2 diabetes. The concept of the liver–vessel axis hypothesis [5] could also explain the biological mechanisms linking the liver directly to the accelerated atherosclerosis proposed in NAFLD. There is evidence indicating that a consequence of advanced NAFLD (non-alcoholic steatohepatitis [NASH]) includes enhanced atherosclerosis via further insulin resistance leading to atherogenic hyperlipidaemia (low HDL-cholesterol, high triacylglycerol and high LDL-cholesterol levels) and systemic inflammation through pro-inflammatory and pro-atherogenic factors (IL-6, TNF- $\alpha$ , nuclear factor kappa-light-chain-enhancer of activated B cells [6, 7]).

One of the challenges in exploring the association between CVD and NAFLD or chronic liver disease in general in

human epidemiological studies is the lack of validated methods to diagnose the various stages of chronic liver disease using non-invasive tests which can be ethically applied to large groups of people who are mostly asymptomatic in terms of liver disease. Attempts to categorise people as ‘diseased’ or ‘not diseased’ based on findings of such non-invasive tests in an epidemiological setting are likely to lead to considerable bias. Therefore, we chose to explore the direct association of a wide range of different liver injury biomarkers with CVD rather than attempt to categorise chronic liver disease based on what would be arbitrary cut-points. We examined the association of prevalent and incident CVD with an array of biomarkers, including those measuring non-specific liver injury (plasma liver enzymes), steatosis (ultrasound), steatohepatitis (cytokeratin-18 [CK18] [8]), surrogate of advanced portal hypertension (platelet count), and liver fibrosis (aspartate to platelet ratio index [APRI] [9], aspartate aminotransferase [AST] to alanine aminotransferase [ALT] ratio, fibrosis-4 score [FIB4] [10], enhanced liver fibrosis panel [ELF] [11] and NAFLD fibrosis score [NFS] [12]).

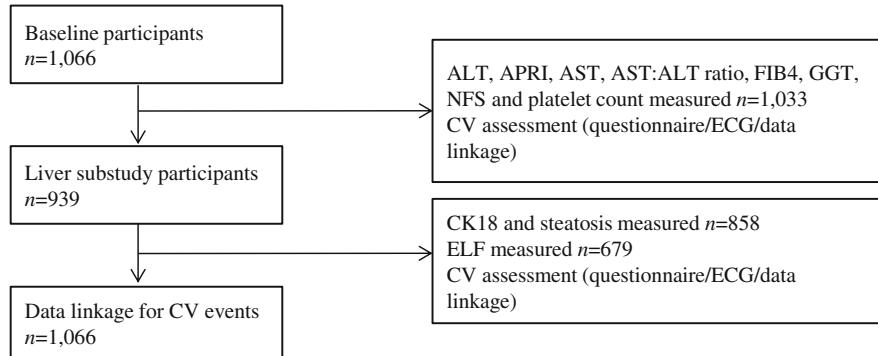
## Methods

### The Edinburgh type 2 Diabetes study

Full methods of the Edinburgh Type 2 Diabetes Study (ET2DS) have been published elsewhere [13]. Patients with type 2 diabetes aged 60–75 years at baseline were selected at random from the Lothian Diabetes Register, a comprehensive register of patients with diabetes living in Lothian, Scotland, UK. Baseline attendees ( $n=1,066$ ) have previously been shown to be representative of all those randomly selected to participate ( $n=5,454$ ), and therefore representative of the target population of older people with type 2 diabetes living in the general population [14]. The liver assessment clinic was attended by 939 participants at year 1 (Fig. 1).

### Clinical examination

Research clinics were held at the Wellcome Trust Clinical Research Facility, Western General Hospital, Edinburgh, UK, at baseline, year 1 and at follow-up and have been described previously [13, 15]. Briefly, attendees underwent fasting venous blood sampling for measurement of plasma liver enzymes (including ALT, AST and  $\gamma$ -glutamyltransferase [GGT]) and platelets; height and weight recording; blood pressure measurement; and a self-administered questionnaire including standard questions on current medications (including diabetes treatment, defined as diet-controlled, oral antihyperglycaemic agent only or insulin±oral antihyperglycaemic agent), alcohol consumption, smoking (categorised as ever or never), history of liver disease and CVD, as well as the Edinburgh Claudication and

**Fig. 1** Participant flowchart

WHO chest pain questionnaires. A 12-lead ECG was also recorded, using recognised standard operating procedures and a MAC 1200 resting ECG analysis system (GE Medical Systems, Milwaukee, Wisconsin, USA), and coded using The Minnesota Code manual [16]. Imaging included abdominal ultrasound scan. Average alcohol intake per week over the previous year and a history of alcohol excess were determined by questionnaire using questions adapted from the Alcohol Use Disorders Identification Test Consumption screening tool. Alcohol excess was defined as >14 units/week in women and >21 units/week in men [17] or self-reported history of an alcohol problem.

NAFLD was defined as the presence of hepatic steatosis on ultrasound scan, without alcohol excess or use of hepatotoxic medication, and a negative liver screen [18].

Alcohol excess was as defined above. Hepatotoxic medication use was defined as the use of non-topical glucocorticoids (isoniazid, methotrexate, amiodarone or tamoxifen) for >2 weeks within the 6 months prior to ultrasound scan. A positive liver screening included any of positive autoantibodies (any of anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody), ferritin >2,247 pmol/l,  $\alpha$ -fetoprotein >6  $\mu$ g/l, or positive hepatitis B or C serology. Clinically significant positive immunology titres were defined as anti-smooth muscle antibody titre >1:160 or anti-mitochondrial antibody titre >1:40 [19].

### Biomarkers of chronic liver injury

Biomarkers of liver injury were categorised and defined as: non-specific liver injury (liver enzyme levels: AST, ALT, GGT), steatosis (ultrasound scan), steatohepatitis (CK18), liver fibrosis (APRI, AST:ALT ratio, ELF, FIB4 and NFS) and advanced portal hypertension (platelet count).

Plasma liver enzymes, APRI, AST:ALT ratio, FIB4, NFS and platelet count were measured at baseline. CK18 and ELF were measured at year 1. All patients underwent a liver ultrasound scan at the 1 year visit. Sonographic grading of hepatic steatosis was performed using standard criteria, as described

previously, following validation against proton magnetic resonance spectroscopy [20].

ALT, AST and GGT were analysed using a Vitros Fusion chemistry system (Ortho Clinical Diagnostics, High Wycombe, UK) at the Western General Hospital, Edinburgh, UK. APRI [9], FIB4 [10] and NFS [12] were calculated as in the original publications. AST:ALT ratio was calculated as AST (U/l)/ALT (U/l). CK18 and ELF tests were undertaken on serum samples taken at the time of the liver ultrasound scan and subsequently stored at -80°C. CK18 was measured using the M30-Apoptosense ELISA (Peviva, Stockholm, Sweden) at the Biomedical Research Unit laboratory, University of Nottingham, UK. ELF scores were derived from the serum hyaluronic acid (HA), aminoterminal peptide of procollagen III (P3NP) and tissue inhibitor of metalloproteinases-1 (TIMP-1) equation as in the original publication [11] and measured using the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, New York, NY, USA) at the iQur laboratory, London, UK.

Given that biomarkers of fibrosis (e.g. ELF) could potentially be influenced by the presence of arthropathies [21] and renal disease, the presence of joint diseases (osteoarthritis, rheumatoid arthritis and others) was actively sought through self-administered questionnaire. Estimated glomerular filtration rate (eGFR) was measured at the time of clinic attendance and analysed using a Vitros Fusion chemistry system (Ortho Clinical Diagnostics) at the Western General Hospital, Edinburgh, UK.

### Identifying CVD

Information on cardiovascular events at baseline and at follow-up clinics was collected from multiple sources including patient- and/or general practitioner-completed questionnaires, 12-lead ECG, and linkage to hospital discharge and death certification data. Data linkage was undertaken, via the National Health Service National Services Scotland, to Scottish Morbidity Record (SMR01) general and acute inpatient discharge records using ICD-10 ([www.who.int/classifications/icd/en/](http://www.who.int/classifications/icd/en/)) (and related ICD-9 [[www.icd9data.com](http://www.icd9data.com)]).

[com/2007/Volume1](http://com/2007/Volume1)) codes and to Office for Population Censuses and Surveys (OPCS) version 4 codes for cardiovascular interventions. A fatal or non-fatal cardiovascular event was recorded if predetermined criteria based on the multiple data sources were met.

**Myocardial infarction** (1) ICD-10 code for myocardial infarction (MI) on discharge/death record, plus either self-report of a doctor diagnosis of MI, positive WHO chest pain questionnaire for MI, report of MI on general practitioner questionnaire or new ECG codes for MI; or (2) clinical criteria for MI met following scrutiny of clinical notes.

**Angina** (1) ICD-10 code for angina as primary diagnosis on discharge record; or (2) at least two of (a) self-report of a doctor diagnosis of angina or of starting angina medication, (b) ECG codes for ischaemia, and (c) positive WHO chest pain questionnaire; or (3) clinical diagnosis of angina on scrutiny of hospital notes.

**Stroke** (1) ICD-10 code for stroke as discharge/death record; or (2) clinical criteria for stroke met on scrutiny of clinical notes in individuals with either self-report of stroke or with non-primary ICD-10 hospital discharge/death code for stroke.

**Transient ischaemic attack** (1) ICD-10 code for transient ischaemic attack (TIA) on discharge record; or (2) clinical criteria for TIA met on scrutiny of clinical notes in individuals with either self-report of stroke or with non-primary ICD-10 hospital discharge code for stroke or TIA.

**Coronary intervention** OPCS-4 code for coronary intervention on discharge record.

**Intermittent claudication** (1) ICD-10 code for intermittent claudication on discharge record; or (2) clinical criteria for intermittent claudication met on scrutiny of clinical notes in individuals with either self-report of intermittent claudication or positive Edinburgh Claudication Questionnaire.

**Peripheral vascular intervention** OPCS-4 code for peripheral vascular intervention on discharge record.

**Carotid endarterectomy** OPCS-4 code for carotid endarterectomy on discharge record.

Prevalent CVD at baseline (for ALT, AST, GGT, AST:ALT ratio, APRI, FIB4, NFS and platelets) or year 1 (for steatosis, CK18, ELF) was defined as any of MI, angina, coronary intervention, intermittent claudication, peripheral vascular intervention, stroke, TIA or carotid endarterectomy at any time prior to this point. Prevalent coronary artery disease (CAD) at baseline/year 1 was defined as any of MI, angina or coronary intervention at any time.

Incident CVD was defined as any of MI, angina, coronary intervention, intermittent claudication, peripheral vascular intervention, stroke, TIA or carotid endarterectomy occurring between baseline/year 1 and end of August 2011, for both non-fatal and fatal events, in those patients without prevalent CVD at baseline. Incident CAD was defined as any of MI, angina or coronary intervention occurring between baseline/year 1 and end of August 2011, for non-fatal and fatal events, in those patients without prevalent CAD at baseline.

## Data analysis

The primary outcome measures were prevalent cardiovascular events and incident cardiovascular events. The secondary outcome measures were prevalent and incident CAD events. Fatal and non-fatal events were combined for analysis.

Data were assessed for normality and where necessary non-normal variables (APRI, CK18 and GGT) were transformed on the log<sub>2</sub> scale.

The follow-up time for each individual for incident disease was from the date of the baseline/liver substudy research clinic attendance until the first of: cardiovascular event, death or end of August 2011.

Analysis was undertaken using a listwise approach for three scenarios—measurements taken at baseline (ALT, APRI, AST, AST:ALT ratio, FIB4, GGT, NFS and platelets), measurements taken at the initial liver substudy clinic (CK18 and steatosis on ultrasound scan) and ELF.

Univariate analysis with normal continuous variables was carried out using Student's *t* test (ALT, AST, AST:ALT ratio, ELF FIB4, NFS and platelets), non-normal continuous variables (APRI, CK18 and GGT) using the Mann–Whitney *U* test, and categorical variables (steatosis) using the  $\chi^2$  test, examining for both the presence of prevalent and incident CVD and CAD.

Logistic regression for the association with prevalent CVD and CAD, and Cox proportional hazards regression for the association with incident CVD and CAD, were undertaken for all markers of liver injury. Both were performed unadjusted, adjusted for age and sex, and additionally adjusted for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation (Scottish Index of Multiple Deprivation quintile), smoking status, excess alcohol consumption, BMI, systolic blood pressure (sBP), diastolic blood pressure (dBp), HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR. Analysis of prevalent disease was undertaken for all participants; analysis of incident disease was undertaken for participants free of CVD at baseline.

Sensitivity analyses of the incident cardiovascular events were undertaken: (1) for participants with NAFLD (defined as the presence of hepatic steatosis on ultrasound scan without alcohol excess or use of hepatotoxic medication and a

negative liver screen); and (2) following inclusion of all participants and adjusted for prevalent CVD at baseline.

Data were analysed using SPSS version 19.0 (SPSS, Chicago, IL, USA).

Ethics approval was obtained from the Lothian Research Ethics Committee and all participants gave written informed consent.

## Results

### Patient characteristics

The baseline research clinic was attended by 1,066 patients, 939 (88%) of whom returned for the liver assessment at 1 year. Figure 1 shows the participant flow. There were no significant differences between attenders at baseline and attenders at the liver assessment (reported previously [22]); participant characteristics are described in Table 1.

Full data from baseline were available for 1,033 participants. From the 1 year liver assessment, steatosis and CK18 data were available for 858 participants. ELF data were available on a random subgroup of 679 participants; there were no significant differences between participants with and without available ELF scores (Table 1).

### Prevalent CVD

At baseline there were 370/1,033 (35.8%) patients with prevalent CVD and 317/1,033 (30.7%) with prevalent CAD. A significantly higher proportion of those with CVD and CAD were male (both 61.8%,  $p<0.001$ ) compared with those free of disease. Those with CVD and CAD were older (mean 68.4 vs 67.6 years,  $p=0.004$ , and 68.6 vs 67.6 years,  $p<0.001$ , respectively) than those without. Results were similar for the 1 year assessment: at baseline there were 303/858 (35.3%) patients with prevalent CVD and 260/858 (30.3%) with prevalent CAD. Again, those with CVD and CAD were significantly more likely to be male and to be older than those without.

There were no significant differences in the distribution of joint disease potentially influencing fibrosis biomarkers between those with and those without CVD (osteoarthritis 22.3% vs 23.8%,  $p=0.785$ ; rheumatoid arthritis 5.3% vs 3.2%,  $p=0.173$ ; other joint disease 15.6% vs 12.5%,  $p=0.440$ , respectively). Mean eGFR was lower in those with prevalent CVD than in those without (62.1 vs 65.7  $\text{mL}^{-1} \text{min}^{-1} 1.73 \text{ m}^{-2}$ ,  $p<0.001$ ).

Participants with prevalent CVD had marginally lower ALT (mean 41.9 vs 43.7 U/l,  $p=0.048$ ) and higher GGT measures (median 20.0 vs 17.0 U/l,  $p<0.001$ ) compared with

**Table 1** Characteristics of all ET2DS participants, those undergoing CK18 and steatosis assessment and subgroups with ELF measurements

Characteristic	All participants (n=1,033)	CK18 and steatosis participants (n=858)	ELF participants (n=679)
Age, years	67.9 (4.2)	67.9 (4.2)	67.8 (4.2)
Sex, % male	51.2 (530)	53.8 (462)	52.6 (357)
Duration of diabetes, years	6.0 (3.0–11.0)	6.0 (3.0–11.0)	6.0 (3.0–10.0)
HbA <sub>1c</sub> , %	7.39 (1.1)	7.38 (1.1)	7.36 (1.1)
HbA <sub>1c</sub> , mmol/mol	57.0 (12.1)	57.2 (12.3)	57.0 (11.9)
Fasting glucose, mmol/l	7.54 (2.1)	7.48 (2.0)	7.49 (2.0)
Diet-controlled, % yes	19.8 (197)	19.3 (161)	19.2 (127)
Oral antihyperglycaemic agent use, % yes	63.0 (628)	64.8 (541)	65.4 (432)
Insulin therapy, % yes	17.3 (172)	15.9 (133)	15.4 (102)
BMI, kg/m <sup>2</sup>	31.3 (5.6)	31.2 (5.7)	31.2 (5.7)
Waist circumference, cm	106.7 (12.7)	106.6 (12.8)	106.5 (12.7)
Serum cholesterol, mmol/l	4.30 (0.9)	4.31 (0.9)	4.33 (0.9)
sBP, mmHg	133.2 (16.4)	133.3 (16.1)	133.5 (16.3)
dBP, mmHg	69.1 (9.0)	69.3 (8.9)	69.4 (8.9)
Alcohol excess <sup>a</sup> , % yes	8.1 (84)	7.6 (65)	8.4 (57)
Ever smoked, % yes	60.7 (527)	59.6 (455)	60.0 (366)

Values are mean (SD), median (interquartile range) or proportion ( $n$ )

All variables were measured concurrently at year 1 examination of the ET2DS, except for BMI and waist circumference, which were measured at baseline

<sup>a</sup> Defined as women >14 units/week, men >21 units/week or patient disclosed history of a current or prior alcohol problem

those without. Patients with prevalent CAD also had significantly higher GGT values than those without (median 20.0 vs 17.0 U/l,  $p<0.001$ ), although all median levels were within the normal range. The proportion of participants with steatosis was lower in those with CVD than in those without (CVD 54.1% vs 57.5%,  $p=0.350$ ; CAD 51.2% vs 58.5%,  $p=0.051$ ). Full data are given in Table 1 of the electronic supplementary material (ESM).

Multivariable analysis of the relationship between liver markers and prevalent cardiovascular events, adjusting for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation, smoking status, excess alcohol consumption, BMI, systolic blood pressure, HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR, is shown in Table 2. GGT was the only liver marker independently associated with prevalent CVD (OR for a doubling of GGT 1.18; 95% CI 1.03, 1.36;  $p=0.021$ ) or CAD (OR 1.21; 95% CI 1.05, 1.40;  $p=0.008$ ).

## Incident CVD

There were 663 participants without CVD. After a mean follow-up of 4.4 years from baseline attendance there were 44/663 (6.6%) patients with incident CVD and 27/663 (4.1%) with incident CAD events. A significantly higher proportion of those with incident CVD were male (59.1% vs 44.3%,  $p=0.061$ ) and they were significantly older (68.9 vs 67.5 years,  $p=0.024$ ), with no differences in those with incident CAD compared with those without incident CAD. Similar results were obtained for those patients followed up from the 1 year assessment (mean follow-up 3.5 years), with 35/561 (6.2%) incident CVD and 19/561 (3.4%) incident CAD events and with a similar age/sex distribution.

There were 82/1,033 (7.9%) deaths in the follow-up period from baseline, with 30/82 (36.6%) attributable to CVD, of which 20 were attributable to CAD.

**Table 2** Multivariable association between liver markers and prevalent cardiovascular events

Liver marker	Model 1	<i>p</i> value	Model 2	<i>p</i> value	Model 3	<i>p</i> value
<b>All CVD</b>						
ALT, U/l	0.99 (0.98, 1.00)	0.079	0.99 (0.98, 1.00)	0.028	0.99 (0.98, 1.00)	0.088
AST, U/l	0.99 (0.98, 1.01)	0.341	0.99 (0.98, 1.01)	0.174	0.99 (0.98, 1.01)	0.385
GGT, log <sub>2</sub> <sup>a</sup>	1.21 (1.07, 1.37)	0.002	1.20 (1.06, 1.35)	0.005	1.18 (1.03, 1.36)	0.021
Steatosis, % yes	0.91 (0.68, 1.22)	0.518	0.96 (0.71, 1.30)	0.774	0.84 (0.60, 1.17)	0.296
CK18, log <sub>2</sub> <sup>a</sup>	1.08 (0.90, 1.30)	0.421	1.09 (0.90, 1.31)	0.405	0.99 (0.81, 1.22)	0.926
APRI, log <sub>2</sub> <sup>a</sup>	0.98 (0.78, 1.23)	0.833	0.85 (0.67, 1.08)	0.189	0.90 (0.70, 1.67)	0.439
AST:ALT ratio	1.34 (0.56, 3.21)	0.509	1.39 (0.56, 3.44)	0.473	1.51 (0.56, 4.07)	0.419
ELF score	1.00 (0.83, 1.21)	0.984	1.01 (0.82, 1.23)	0.964	0.94 (0.74, 1.19)	0.604
FIB4	1.13 (0.90, 1.41)	0.289	1.01 (0.80, 1.28)	0.921	1.03 (0.80, 1.33)	0.801
NFS	1.09 (0.96, 1.24)	0.174	1.07 (0.93, 1.22)	0.350	1.01 (0.85, 1.19)	0.915
Platelets, $\times 10^9/l$	1.00 (1.00, 1.00)	0.569	1.00 (1.00, 1.00)	0.499	1.00 (1.00, 1.00)	0.734
<b>CAD</b>						
ALT, U/l	0.99 (0.98, 1.00)	0.200	0.99 (0.98, 1.00)	0.124	0.99 (0.98, 1.01)	0.390
AST, U/l	0.99 (0.98, 1.01)	0.383	0.99 (0.98, 1.01)	0.230	1.00 (0.98, 1.01)	0.588
GGT, log <sub>2</sub> <sup>a</sup>	1.22 (1.08, 1.39)	0.002	1.22 (1.07, 1.38)	0.002	1.21 (1.05, 1.40)	0.008
Steatosis, % yes	0.75 (0.55, 1.02)	0.064	0.79 (0.58, 1.08)	0.140	0.66 (0.46, 0.94)	0.019
CK18, log <sub>2</sub> <sup>a</sup>	1.05 (0.86, 1.28)	0.650	1.05 (0.86, 1.28)	0.610	0.96 (0.78, 1.18)	0.707
APRI, log <sub>2</sub> <sup>a</sup>	1.00 (0.79, 1.27)	0.987	0.88 (0.67, 1.13)	0.320	0.95 (0.73, 1.24)	0.720
AST:ALT ratio	1.01 (0.40, 2.53)	0.981	0.93 (0.36, 2.42)	0.887	0.94 (0.33, 2.66)	0.912
ELF score	0.98 (0.80, 1.20)	0.848	0.96 (0.77, 1.19)	0.726	0.88 (0.69, 1.13)	0.324
FIB4	1.20 (0.95, 1.50)	0.123	1.07 (0.84, 1.36)	0.599	1.11 (0.85, 1.43)	0.441
NFS	1.11 (0.97, 1.27)	0.138	1.07 (0.93, 1.23)	0.328	1.03 (0.86, 1.22)	0.765
Platelets, $\times 10^9/l$	1.00 (1.00, 1.00)	0.386	1.00 (1.00, 1.00)	0.788	1.00 (1.00, 1.00)	0.967

Values are ORs (95% CI)

<sup>a</sup>APRI, CK18 and GGT analysed on the log<sub>2</sub> scale for linearisation; therefore, ORs relate to a doubling of the marker

Model 1, unadjusted; model 2, adjusted for age and sex; model 3, adjusted for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation (Scottish Index of Multiple Deprivation quintile), smoking status, excess alcohol consumption, BMI, sBP, dBp, HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR at baseline

Mean (or median) liver injury marker levels were largely similar between participants with and without incident CVD (ESM Table 2) and after multivariable adjustment (Table 3). Only GGT appeared to have some independent association with either incident CVD (HR for a doubling of GGT 1.24; 95% CI 0.97, 1.59;  $p=0.086$ ) or incident CAD (HR 1.33; 95% CI 1.00, 1.78;  $p=0.053$ ). None of the individual covariates added to the multivariable model had a major attenuating effect on the HR estimating the GGT–outcome association (ESM Table 3). In further analyses performed on all participants with either a first or subsequent cardiovascular event occurring after baseline (i.e. including those with prevalent CVD at baseline, but with adjustment for prevalent cases), an association between GGT and events was confirmed (ESM Tables 4 and 5). HRs with similar magnitudes were observed with increased statistical significance ( $p<0.05$ ), likely due to the increase in sample size.

When restricted to patients with NAFLD ( $n=319$ ) there were 38 incident cardiovascular events, with 23 attributable

to CAD. Of all the liver injury markers investigated, GGT alone showed an independent association with incident CVD in this subgroup (fully adjusted HR for a doubling of GGT 1.56; 95% CI 1.08, 2.28;  $p=0.019$ ) (ESM Tables 6 and 7).

## Discussion

In this large-scale epidemiological study, we have shown that raised GGT is independently associated with an increase in both prevalent and incident cardiovascular events in older people with type 2 diabetes. Previous studies, predominantly in younger samples of the general population, have found similar results for this plasma liver enzyme; we have now shown that findings are consistent in a high-risk (diabetic) and older subgroup of the population. Despite the availability of a wide range of other liver injury markers, we found no evidence that markers of hepatic steatosis, steatohepatitis, portal hypertension or fibrosis were associated with higher levels

**Table 3** Multivariable association between liver markers and any incident CVD events

Liver marker	Model 1	<i>p</i> value	Model 2	<i>p</i> value	Model 3	<i>p</i> value
<b>All CVD</b>						
ALT, U/l	1.00 (0.97, 1.02)	0.754	1.00 (0.97, 1.02)	0.836	0.99 (0.97, 1.02)	0.669
AST, U/l	1.01 (0.98, 1.04)	0.526	1.01 (0.98, 1.04)	0.544	1.01 (0.97, 1.04)	0.700
GGT, log <sub>2</sub> <sup>a</sup>	1.25 (0.99, 1.59)	0.062	1.26 (0.99, 1.60)	0.059	1.24 (0.97, 1.59)	0.086
Steatosis, % yes	0.78 (0.36, 1.67)	0.525	0.84 (0.39, 1.80)	0.654	0.90 (0.40, 2.00)	0.787
CK18, log <sub>2</sub> <sup>a</sup>	1.05 (0.64, 1.70)	0.857	1.13 (0.68, 1.85)	0.643	1.02 (0.60, 1.75)	0.931
APRI, log <sub>2</sub> <sup>a</sup>	0.88 (0.505, 1.525)	0.644	0.79 (0.43, 1.46)	0.448	0.76 (0.40, 1.45)	0.408
AST:ALT ratio	3.63 (0.61, 21.61)	0.156	2.85 (0.475, 17.06)	0.252	3.58 (0.53, 28.12)	0.183
ELF score	1.220 (0.91, 1.64)	0.185	1.19 (0.85, 1.66)	0.312	1.15 (0.81, 1.64)	0.443
FIB4	1.01 (0.54, 1.91)	0.966	0.82 (0.40, 1.68)	0.586	0.83 (0.39, 1.76)	0.625
NFS	0.81 (0.58, 1.14)	0.226	0.76 (0.54, 1.06)	0.109	0.78 (0.57, 1.09)	0.143
Platelets, $\times 10^9/l$	1.00 (1.00, 1.01)	0.162	1.01 (1.00, 1.01)	0.061	1.00 (1.00, 1.01)	0.110
<b>CAD</b>						
ALT, U/l	1.00 (0.98, 1.03)	0.771	1.01 (0.98, 1.04)	0.497	1.01 (0.98, 1.04)	0.611
AST, U/l	1.02 (0.99, 1.05)	0.213	1.03 (0.99, 1.06)	0.135	1.02 (0.99, 1.06)	0.220
GGT, log <sub>2</sub> <sup>a</sup>	1.27 (0.95, 1.69)	0.103	1.31 (0.88, 1.75)	0.060	1.33 (1.00, 1.78)	0.053
Steatosis, % yes	0.82 (0.32, 2.14)	0.688	0.87 (0.33, 2.27)	0.774	0.91 (0.33, 2.53)	0.858
CK18, log <sub>2</sub> <sup>a</sup>	1.07 (0.58, 1.99)	0.822	1.10 (0.60, 2.01)	0.748	0.96 (0.49, 1.90)	0.908
APRI, log <sub>2</sub> <sup>a</sup>	1.07 (0.56, 2.06)	0.839	1.15 (0.56, 2.34)	0.709	1.10 (0.52, 2.32)	0.804
AST:ALT ratio	4.36 (0.51, 37.18)	0.178	3.40 (0.37, 31.13)	0.278	4.25 (0.39, 46.73)	0.237
ELF score	1.24 (0.85, 1.80)	0.269	1.15 (0.76, 1.74)	0.508	1.12 (0.69, 1.82)	0.642
FIB4	1.28 (0.64, 2.60)	0.486	1.22 (0.57, 2.64)	0.611	1.25 (0.56, 2.79)	0.583
NFS	0.84 (0.55, 1.28)	0.416	0.81 (0.53, 1.23)	0.323	0.76 (0.51, 1.17)	0.225
Platelets, $\times 10^9/l$	1.00 (1.00, 1.01)	0.301	1.00 (1.00, 1.01)	0.286	1.00 (1.00, 1.01)	0.297

Values are HRs (95% CI)

<sup>a</sup>APRI, CK18 and GGT analysed on the log<sub>2</sub> scale for linearisation; therefore, ORs relate to a doubling of the marker

Model 1, unadjusted; model 2, adjusted for age and sex; model 3, adjusted for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation (Scottish Index of Multiple Deprivation quintile), smoking status, excess alcohol consumption, BMI, sBP, dBp, HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR at baseline

of prevalent or incident CVD, suggesting that liver disease may have little effect on the development of vascular complications in our study population.

A major strength of this study is its representation of the full spectrum of people with type 2 diabetes, not just those attending secondary care or receiving advanced treatment modalities. This population is of particular interest as it may show an accelerated progression of liver disease due to the combined effects of age and metabolic risk factors. Community-based populations of people with type 2 diabetes represent the vast majority of all people with type 2 diabetes and, as such, require special attention given the impact of their longer term care on health service provision.

Our findings are consistent with previous findings of a significant association between GGT and both prevalent and incident CVD in the general population [3, 23–30], contributing to the paucity of literature in diabetic populations. In addition, contrary to previous findings, we found that this association persists into older age [26], independently of a wide range of cardiovascular risk factors. There is a biological plausibility for this relationship: GGT degrades glutathione to glutamate, which via cysteinylglycine is involved in iron reduction, allowing lipoprotein oxidation within atherosomatous plaques [31]. What is unclear is whether GGT is a pathogenic factor in atherogenesis or simply a surrogate biomarker of the microinflammatory, plaque-associated inflammatory response. Given that no liver injury markers other than GGT were independently associated with CVD, this strengthens the argument for the GGT association being driven by systemic inflammation as opposed to a direct consequence of chronic liver disease. Whatever the underlying mechanism, our findings indicate that further investigation is warranted into whether or not GGT could add predictive ability to existing vascular risk prediction models in type 2 diabetes [32].

In terms of the association between CVD and other liver injury markers, previous studies are limited and inconclusive. Significant associations between transaminases and both increased and decreased CVD in the general population have been reported [33, 34]. Investigations into the relationship between NAFLD (defined as the presence of hepatic steatosis on ultrasound scan) and cardiovascular events [35, 36], in populations comprised exclusively of patients with type 2 diabetes [3, 4, 37, 38], have reported significant associations between NAFLD and incident CVD (OR 1.53 [3], HR 1.96 [38], after controlling for cardiovascular risk factors), but no association with liver enzymes (including GGT). Although the present study failed to find a similar relationship between sonographic hepatic steatosis and CVD, our cohort differs from diabetic cohorts studied previously, mainly in its broad spectrum of patients with type 2 diabetes. Targher et al used a study population derived exclusively from secondary care diabetes settings (therein limiting generalisability), where the influence of hepatic steatosis may be stronger in the context

of more severe diabetes, consistent with other studies looking at more general populations and cardiovascular mortality [37]. Whilst our findings may also be affected by specific cohort effects, the size and follow-up time are comparable to those of several other similar studies [3, 26].

Our finding of a lower prevalence of CVD in people with steatosis could be explained, at least in part, by regression of hepatic steatosis with advancing liver disease [39]; or it may reflect survival bias, in that those with the most severe NAFLD had already died prior to participation in the ET2DS.

In patients with NAFLD, relative concentrations of serum CK18 can discriminate between steatosis and NASH [8]. However, there are no previous studies examining the relationship between CK18 levels and cardiovascular events in either general or diabetic populations. Several previous studies diagnosing NASH using different methods (such as biopsy or elevated ALT levels) showed mixed results for the association with cardiovascular risk (e.g. risk scores, lipid levels). Both Soderberg et al [40] and Ekstedt et al [2] found associations of all-cause and cardiovascular mortality with the presence of biopsy-proven NASH, but no association with steatosis. Conversely, Lazo et al [41] found no association between NASH and cardiovascular mortality in patients diagnosed by ultrasound scan and elevated hepatic enzymes, suggesting that the criteria for NAFLD and NASH classification may have a significant impact on findings.

Data on the relationship between hepatic fibrosis and CVD are also limited. Kim et al found significant associations between the NFS, APRI and FIB4 with cardiovascular mortality in a general population [42]. Our study used all these, as well as the ELF score, an extracellular matrix-related multi-component panel (HA, P3NP and TIMP-1), validated for use in patients with NAFLD [20], and found no relationship.

It should be noted that the utility of different liver injury biomarkers may be determined by the context in which they are used. For example, there is a body of evidence validating non-invasive liver biomarkers for the cross-sectional stratification of liver disease in secondary care and predicting future liver-related clinical outcomes [43, 44]. Results from this study do not suggest that most of the markers investigated would add prognostic value to existing risk scores used to predict cardiovascular endpoints in diabetes [32]. The exception to this is GGT, which is generally not considered useful for stratifying active liver disease, but which may prove beneficial in predicting CVD. Given the results presented here, further investigation into this question in diabetes is warranted.

The strengths and limitations of this study should be acknowledged. The large size, population-based approach, prospective design with intensive investigation for incident cardiovascular events, and wide range of liver biomarkers investigated are key strengths of the current study. The modest follow-up duration is partially offset by the large sample size, resulting in a significant number of person-years at risk, and

by the high-risk population under study, which resulted in a high number of incident events. Without a liver biopsy it is not currently possible to accurately identify NAFLD. However, we believe that our comprehensive approach of using ultrasound scan, assessment of alcohol consumption and hepatotoxic medication use, and liver screen will identify the vast majority of patients with NAFLD, potentially missing only those with minimal hepatic steatosis due to regression of steatosis in the advanced stages of the disease process.

In conclusion, our study provides evidence that GGT may independently associate with CVD and that its potential prognostic value for CVD in people with type 2 diabetes would be usefully investigated. However, lack of association between CVD and other markers of liver injury (non-specific injury, steatosis, steatohepatitis, significant portal hypertension, fibrosis) suggests that chronic liver disease per se may not have a major influence on the development of CVD, at least in older diabetic populations.

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**Access to research materials** Applications to access the underlying research materials will be considered via the ET2DS standard data sharing procedures. Please contact the corresponding author for details.

**Duality of interest** MWJS has received fees for speaking from Novo Nordisk, Eli Lilly and Pfizer. JRM, JAF, RMW, CMR, SG, ING and JFP report no disclosures.

**Contribution statement** JRM, JAF, RMW, SG, ING, MWJS and JFP are responsible for the conception and design of the study. The data were acquired by JRM, RMW, CMR and SG, analysed by JRM, and interpreted by JRM, JAF, ING, MWJS and JFP. The article was drafted by JRM, JAF, ING and JFP, and critically revised by all the authors. All authors approved the final version. JRM is the guarantor of this work.

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# Association Between Serum Gamma-glutamyl Transferase Level and Hypertension in Indian Adults: A Population Based Cross-Sectional Study

Gamma Glutamyl Transferase (GGT) is an enzyme responsible for initiating extracellular catabolism of glutathione in mammalian cells.<sup>[1]</sup> Recent studies suggest a possible role of GGT in the pathogenesis of hypertension. A literature search was carried out to identify studies conducted with the objective of finding association between hypertension and GGT. It revealed that there is dearth of studies conducted with similar objective.<sup>[2-6]</sup> The present study was conducted in this backdrop to explore any possible association between hypertension and GGT in Indian population. In our study, 194 patients consented of which 96 patients were hypertensive and 98 normotensive. The age and sex of participants in either group did not show any statistical difference. The systolic blood pressure (expressed as Mean  $\pm$  Standard deviation in mm of Hg) in normotensive and hypertensive group was  $116 \pm 8.2$  and  $148 \pm 6.8$ , respectively, while the diastolic blood pressure (in mm of Hg) in normotensive and hypertensive group was found to be  $76 \pm 3.2$  and  $96 \pm 4.8$ , respectively. The GGT level was  $(38.98 \pm 7.53)$  IU/L in the normotensive arm as compared with  $(42.23 \pm 9.06)$  IU/L in the hypertensive arm ( $P < 0.001$ ). The present study indicates that GGT level is elevated in hypertensive patients as compared with their age and sex matched normotensive peers suggesting a positive association between the two.

A population-based cross-sectional study was conducted to address the study objective. The study was approved by the institutional ethics committee. All consecutive patients attending the biochemistry laboratory of the institute were considered eligible for participation using the following inclusion-exclusion criteria.

Patients of either sex with age between 15 and 65 years. Patients were excluded if there is history or clinical evidence of (1) Diabetes Mellitus, (2) Renal Disease,

(3) Liver Disease, (4) Cardiac Disease, (5) Active Infection, or (6) Acute Illness.

On the first day (Study day 0), each patient was explained about the details of the study rationale and confidentiality safeguards. Only those patients who gave their written consent were included in the study. Following informed consent administration, blood pressure (BP) was measured by a physician of the trial team. The BP measurement was repeated for next two consecutive days (Day 1, 2). Patients who were found to have Systolic Blood Pressure (SBP) higher than 140 mmHg and/or Diastolic Blood Pressure (DBP) higher than 90 mmHg on three consecutive days were considered as hypertensive. On the Study day 2, blood sample were collected from all the study participants for estimation of serum GGT. All samples were immediately centrifuged and stored at  $2-8^{\circ}\text{C}$  until analysis for the relevant biochemical parameters. All analyses were performed within 3 hours of sample collection. Serum GGT level was measured by XL600 (Transasia Bio-medicalas Limited)autoanalyzer using Gamma Glutamyl p-Nitroanilidine (GPNA) principle.<sup>[7]</sup>

The statistical software R version 2.11.1 was used to analyze the data. All values were expressed as mean  $\pm$  standard deviation unless otherwise indicated, and differences in mean values between two groups were analyzed using Student's *t*-test. All tests were two tailed and considered statistically significant if  $P <$  level of significance, 0.05.

A total of 194 patients consented into the study of which 96 patients were hypertensive and 98 normotensive. In the present study, the age of the patients in hypertensive group was  $56.71 \pm 8.48$  years as compared with  $57.72 \pm 11.28$  years in the normotensive group ( $P = 0.47$ ). The male:female ratio between the two groups did not show any significant statistical difference ( $P < 0.99$ ). However, the Body Mass Index (BMI) of the hypertensive group was  $32 \pm 4.1$  as compared with  $27 \pm 2.3$  in the normotensive group ( $P < 0.0001$ ). The SBP in normotensive and hypertensive group was  $116 \pm 8.2$  and  $148 \pm 6.8$ , respectively, while the DBP in normotensive and hypertensive group was found to be  $76 \pm 3.2$  and  $96 \pm 4.8$ , respectively. The GGT level was  $(38.98 \pm 7.53)$  IU/L in the normotensive arm as compared with  $(42.23 \pm 9.06)$  IU/L in the hypertensive arm ( $P < 0.001$ ). The details are shown in Table 1.

In the present study, the age of the patients in hypertensive group was  $56.71 \pm 8.48$  years as compared with  $57.72 \pm 11.28$  years in the normotensive group. ( $P = 0.47$ ). The

**Table 1: Baseline demographic and anthropometric data of study participants**

	Normotensive group	Hypertensive group	P value*
Number of patients	98	96	
Male/Female	56/42	54/41	0.99
Age (years)	57.72±11.28	56.71±8.48	0.47
BMI	27±2.3	32±4.1	<0.0001
Systolic blood pressure (in mm of Hg)	116±8.2	148±6.8	
Diastolic blood pressure (in mm of Hg)	76±3.2	96±4.8	
Gamma glutamyltransferase (U/L)	38.98±7.53	42.23±9.06	<0.001

\*A two-tailed P-value <0.05 is considered to be statistically significant

male:female ratio between the two groups did not show any significant statistical difference (*P* value 0.99). Thus, the present study suggests that serum GGT levels are elevated in hypertensive patients as compared with their age and sex matched normotensive peers (*P* < 0.001). Our results are in agreement with previous studies that reported a positive association between higher serum GGT level and clinical hypertension.<sup>[2-6]</sup> Our results are also in concordance with the current understanding of the role of GGT in hypertension development. A mechanism has been put forward to account for the pathological role of GGT in elevation of BP. GGT is known to act as an antioxidant by virtue of its central role in GSH cycle. Hypertension being a state of high oxidative stress elevated GGT level can be explained as a compensatory mechanism.<sup>[8]</sup> Furthermore, recent studies have revealed a pro-oxidant generating role of GGT. GGT generates Reactive Oxygen Species (ROS) in presence of free iron or other transition metal. These authors suggested that the GGT mediated generation of the more reactive thiol cysteinyl glycine could cause the reduction of ferric iron Fe(III) to ferrous Fe(II), thus starting a redox cycling process liable to result in the production of reducted in Pakistan byactive oxygen species (ROS).<sup>[9]</sup>

Another notable finding in our study is BMI of the hypertensive group (32 + 4.1) is statistically higher than normotensive group (27 + 2.3) (*P* < 0.0001). Our finding is in congruence with the study conducted by Iqbal *et al.*<sup>[6]</sup> Raised BMI is a measure of obesity which has many adverse effects on hemodynamics and cardiovascular function. It increases total blood volume and cardiac output thus increasing the cardiac workload. Typically, obese patients have a higher cardiac output but a lower level of total peripheral resistance at any given level of arterial pressure. Most of the increase in cardiac output with obesity is caused by stroke volume, although because of increased sympathetic activation, heart rate is typically mildly increased as well. The Frank-Starling curve is often shifted to the left because of increases in filling pressure and volume, thus increasing cardiovascular work. Obese patients are more likely to be hypertensive than lean patients, and weight gain is typically associated with increases in

arterial pressure.<sup>[10,11]</sup> The study included a sample size of 194 patients attending biochemistry department of a single medical college. Adequately powered multicentric studies involving more study participants are needed to confirm the association between GGT and hypertension.

The present study indicates that GGT level is elevated in hypertensive patients compared with their normotensive peers suggesting a positive association between the two. However, adequately powered multicentric studies are needed to substantiate the association between serum GGT level and hypertension.

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# Serum $\gamma$ -Glutamyltransferase: Independent Predictor of Risk of Diabetes, Hypertension, Metabolic Syndrome, and Coronary Disease

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Serum  $\gamma$ -glutamyltransferase (GGT) is associated with oxidative stress and hepatic steatosis. The extent to which its value in determining incident cardiometabolic risk (coronary heart disease (CHD), metabolic syndrome (MetS), hypertension and type 2 diabetes) is independent of obesity needs to be further explored in ethnicities. After appropriate exclusions, a cohort of 1,667 adults of a general population (age 52 ±11 years) was evaluated prospectively at 4 year's follow-up using partly Cox proportional hazard regressions. GGT activity was measured kinetically, and values were log-transformed for analyses. MetS was identified by Adult Treatment Panel-III criteria modified for male abdominal obesity. Median (interquartile range) GGT activity was 24.9 (17.0; 35.05) U/l in men, 17.0 (12.3; 24.0) U/l in women. In linear regression analysis, while smoking status was not associated, (male) sex, sex-dependent age, alcohol usage, BMI, fasting triglycerides and C-reactive protein (CRP) were significant independent determinants of circulating GGT. Each 1-s.d. increment in ( $= 0.53 \ln \text{GGT}$ ) GGT activity significantly predicted in each sex incident hypertension (hazard ratio (HR) 1.20 (95% confidence interval (CI) 1.10; 1.31)), and similarly MetS, after adjustment for age, alcohol usage, smoking status, BMI and menopause. Strongest independent association existed with diabetes (HR 1.3 (95% CI 1.1; 1.5)) whereas GGT activity tended to marginally predict CHD independent of total bilirubin but not of BMI. Higher serum total bilirubin levels were protective against CHD risk in women. We conclude that elevated serum GGT confers, additively to BMI, risk of hypertension, MetS, and type 2 diabetes but only mediates adiposity against CHD risk.

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## INTRODUCTION

Elevation in serum  $\gamma$ -glutamyltransferase (GGT) activity, previously ascribed to alcohol intake or liver disease, has been shown to predict morbidity and mortality independent of these (1,2). Modest increases within normal range may be an early marker of cellular oxidative stress (3) and explain the strong associations of serum GGT with many cardiovascular risk factors and disease. Oxidative stress, assessed by circulating prostaglandin F2 $\alpha$  levels, is recognized to be related to obesity (4). Indeed, BMI was observed to be a determinant of GGT concentrations in both genders in the Tromsö study (5). Increases in GGT activity have been found to predict hypertension (6,7), as well as incident cases of type 2 diabetes (8–10). GGT activity also predicted all-cause and coronary heart disease (CHD) mortality, independent of alcohol intake or liver disease (1,2). Metabolic syndrome (MetS) also was found to be associated with increased GGT activity in a prospective study of Japanese men (10), in the Framingham Study (2), and cross-sectionally among Turkish adults (11).

Despite the availability of considerable knowledge in the topic, further information is needed in regard to the magnitudes of risk associated between GGT activity and the individual cardiometabolic disorders, the extent of ethnic variation therein and to what extent the observed associations are dependent on excess adiposity.

Serum bilirubin is recognized to act as an antioxidant; and prospective cohort studies have shown that higher serum bilirubin concentrations are associated with decreased risk for CHD (12,13). In dyslipidemic patients with MetS, mean bilirubin levels decreased progressively with the number of MetS components as mean GGT activity increased (14). Whether an interaction exists between the two stated oxidation-related variables with respect to diabetes or CHD is not defined.

Studies examining the relationship of serum GGT levels with each of the risks of hypertension, MetS, diabetes and CHD in the same population-based cohort might provide clues to the preferential oxidative pathways operative in regard to different

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cardiometabolic risks; such information might eventually be used in risk assessment or prevention. Turks have a high prevalence of MetS (15) and enhanced low-grade inflammation (16), hence, are suitable to explore such associations. We, therefore, aimed to examine: (i) the determinants of GGT activity, including sex, age, and inflammatory mediators, (ii) to explore prospectively its associations with the development of individual cardiometabolic disorders independent of adiposity measures, and (iii) to determine whether serum total bilirubin concentrations act as antioxidant against type 2 diabetes or CHD in a population sample representative of middle-aged and elderly Turkish adults.

## METHODS AND PROCEDURES

This study sample is formed by the cohort of the Turkish Adult Risk Factor Study, a prospective survey on the prevalence of cardiac disease and risk factors in a representative sample of adults in Turkey carried out biennially since 1990 in 59 communities throughout all geographical regions of the country (17). Partial logistic support was provided by the Turkish Ministry of Health. Written informed consent was obtained from all participants. Data were obtained by history of the past years via a questionnaire, physical examination of the cardiovascular system, and recording of a resting electrocardiogram. During the 2003–04 screening serum GGT concentrations were determined in 1,875 participants, aged 33–84 years. Exclusions (11%) were made as follows: 56 individuals with GGT values over 100 U/l to minimize confounding by unrecognized hepatic disease and heavy usage of alcohol, hormone replacement therapy or use of lipid lowering drugs (64 subjects), type 2 diabetes with or without renal impairment (97 persons). No subjects existed with recent myocardial infarction or known hepatic disease. This left 1,667 nondiabetic persons (821 men and 846 women) for analysis in this study. Control was made for menopause in 409 women (48.4%), for 245 persons with alcohol intake up to one to four times weekly (moderate) in regression analyses.

### Measurement of risk factors

Blood pressure was measured with an aneroid sphygmomanometer (Erka, Bad Tölz, Germany) in the sitting position on the right arm, and the mean of two recordings 3-min apart was recorded. Weight was measured in light indoor clothes using scales. Waist circumference was measured—with the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest. BMI was computed as weight divided by height squared ( $\text{kg}/\text{m}^2$ ). In regard to cigarette smoking, never smokers, past smokers, and current smokers formed the categories. Anyone who drank alcoholic drinks between once a month and four times per week was considered as moderate user.

Blood samples were collected in a 11-h or longer fasting state in this study except for 15% of individuals. Samples were spun at 1,000 g for 10 min and shipped on cooled gel packs at 2–5°C to Istanbul to be stored in deep-freeze at –75°C, until analyzed at the Yıldız Technical University in the same city. Serum GGT activity was assayed by the kinetic method using Glucana as substrate (Thermo Trace, Noble Park, Australia) with a Hitachi 902 autoanalyzer, normal range being reported as £50 U/l in men, £30 U/l in women. PreciNorm U and PreciPath U universal control sera were used as controls. Inter- and intra-assay coefficient of variation for GGT were 1.2/1.1% and 1.5/1.6%, respectively. Serum concentrations of total cholesterol, fasting triglycerides, glucose, high-density lipoprotein cholesterol (HDL-C plus 2nd generation, directly without precipitation) and total bilirubin were determined by using enzymatic kits from Roche Diagnostics (Mannheim, Germany) with a Hitachi 902 autoanalyzer. Low-density lipoprotein cholesterol values were computed according to the Friedewald formula. Uric acid was determined enzymatically by Infinity kit utilizing modified Trinder method.

Serum concentrations of complement C3, high-sensitivity C-reactive protein (CRP), apolipoprotein A-I, and B were measured by Behring kits and nephelometry (Behring Diagnostics, Westwood, MA). Concentrations of insulin were determined by the chemiluminescent immunoassay method using Roche kits and Elecsys 1010 immunautoanalyzer (Roche Diagnostics).

Diagnosis of CHD was based on the presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the electrocardiogram (18), or on a history of myocardial revascularization (in one-quarter of patients). Among women, age >45 years was prerequisite for a definitive diagnosis added to isolated typical angina. Electrocardiogram changes of “ischemic type” of greater than minor degree (codes 1.1-2, 4.1-2, 5.1-2, 7.1) were considered as myocardial infarct sequelae or myocardial ischemia, respectively.

Hypertension was defined as a blood pressure  $\geq 140$  mm Hg and/or  $\geq 90$  mm Hg, and/or use of antihypertensive medication. Metabolic syndrome was identified when three out of the five criteria of the National Cholesterol Education Program (Adult Treatment Panel-III) (19) were met, modified for prediabetes (fasting glucose  $\geq 100$  mg/dl ( $>5.55$  mmol/l) (20) and further for male abdominal obesity using cutoff of  $\geq 95$  cm, as assessed in the Turkish Adult Risk Factor study (21). Fasting triglyceride values of the previous participation in the survey were taken into account in few instances of missing data. Diabetes was diagnosed with the criteria of the American Diabetes Association (20), namely by self-report or when plasma fasting glucose was  $\geq 7.0$  mmol/l or when 2-h postprandial glucose was  $>11.1$  mmol/l.

### Data analysis

Descriptive parameters were shown as mean  $\pm$  s.d. or in %. Due to skewed distribution, values derived from log-transformed (geometric) means were used for GGT, CRP, and insulin. Pearson correlation was used for continuous variables, and Spearman correlation for log-transformed and categorical variables. GGT was dichotomized by the near-median  $\geq 25$  U/l in men, and  $\geq 17$  U/l in women to evaluate the distribution of baseline characteristics in participants. Quartiles were formed of sex-specific serum total bilirubin, a marker of oxidative stress, using cutoff points of 0.4, 0.585,  $\geq 0.80$  mg/dl in men and 0.3, 0.465,  $\geq 0.60$  mg/dl in women. Multiple linear regression analyses were performed. Since logarithmic values were used for the dependent variable GGT in linear regression models, the  $\beta$ -coefficient of an independent variable was calculated by log-transforming the obtained values and using the exponents corresponding to the standard deviation. The relative risk for GGT by logistic regression or Cox regression analyses was expressed in terms of 1-s.d. increment which corresponded to  $\ln 0.53$ . Statistical analyses were performed using SPSS-10 for Windows (Nr. 9026510; SPSS, Chicago, IL). A value of  $P < 0.05$  on the two-tail test was considered statistically significant.

## RESULTS

In 1,667 nondiabetic subjects at baseline, 101 (6.1%) developed diabetes, and of 1,533 persons free of CHD at baseline, 118 (7.7%) developed CHD over a period of 3.93 ( $\pm 1.0$ , range 2–5) years for either outcome. Median (interquartile range) GGT activity at baseline was 24.9 (17.0; 35.05) U/l in 820 men, 17.0 (12.3; 24.0) U/l in 847 women.

### Correlates of GGT

High compared with low GGT concentrations significantly distinguished practically all risk factors related to MetS and cardiovascular disease including alcohol usage and CRP, except smoking status, HDL-cholesterol and apo A-I (Table 1). Gender was similar in the two groups ( $P = 0.28$ ).

**Table 1 Means (s.d.) of baseline characteristics in the study sample stratified by sex-specific low/high GGT values (*n* = 1,667)**

	Low GGT (<25/17 U/l)			High GGT (≥25/17 U/l)			<i>P</i> value
	<i>n</i>	Mean	s.d.	<i>n</i>	Mean	s.d.	
Age, years	857	51.7	11.7	810	52.4	9.8	0.17
GGT, <sup>a</sup> mIU/l	857	13.75	1.42	810	32.3	1.5	<0.001
Total bilirubin, mg/dl	623	0.59	0.37	588	0.56	0.3	0.067
Waist circumference, cm	842	91.5	12	797	97.7	10.6	<0.001
BMI, kg/m <sup>2</sup>	842	28.1	5.1	797	30.3	5.0	<0.001
Systolic BP, mmHg	843	122.9	20.9	802	127.5	20.2	<0.001
Diastolic BP, mmHg	843	78.6	11.4	801	81.5	10.6	<0.001
Fasting glucose, mmol/l	763	99.3	44.7	733	103	37.7	0.09
Total cholesterol, mmol/l	844	4.84	1.02	806	5.24	1.07	<0.001
Fasting triglycerides, mmol/l	729	1.56	0.87	688	1.99	1.14	<0.001
HDL-cholesterol, mmol/l	844	1.14	0.33	806	1.11	0.31	0.07
Apolipoprotein A-I, g/l	656	1.34	0.29	591	1.37	0.34	0.16
Apolipoprotein B, g/l	648	1.01	0.27	597	1.09	0.30	<0.001
Uric acid, μmol/l	856	296.8	85	809	334.3	88.6	<0.001
CRP <sup>a</sup> mg/l	803	1.72	3.26	757	2.63	2.85	<0.001
Complement C3, g/l	588	1.24	0.27	583	1.38	0.26	<0.001
Fasting insulin, <sup>a</sup> mIU/l	606	6.86	1.99	606	9.43	2.02	<0.001
Fibrinogen, g/l	605	3.14	1.02	562	3.24	1.02	0.085
Alcohol usage, <i>n</i> , %	857	34	4.0	810	85	10.5	<0.001
Current smoking, <i>n</i> , %	857	260	30.3	810	241	29.8	0.39

Some parameters were not measured at baseline in each participant.

BP, blood pressure; CRP, C-reactive protein; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein.

<sup>a</sup>Geometric mean and s.d. values.

Highest correlations of log GGT were observed with fasting triglycerides, waist circumference, alcohol usage, complement C3 and fasting insulin (*r* ranging from 0.2 to 0.3, **Table 2**). **Table 3** depicts a multiple regression analysis for log GGT in a model comprising five independent variables that explained 20% of the variation in GGT values; it showed male sex (values 1.37-fold those in women), usage of moderate alcohol (1.4-fold than in abstainers) and waist circumference (1.16-fold per 1-s.d. increment) to be major determinants of circulating GGT. While smoking was not independently associated, separate analyses in sexes disclosed age to be inversely associated in men but positively in women. Further linear regression analyses for log GGT adjusted for sex, age, alcohol usage, smoking status, and BMI were used with the following additional individual variables that proved to be significantly and independently associated: elevated fasting triglycerides ( $\beta$ -coefficient 1.29 per 1 s.d.), CRP ( $\beta$ -coefficient 1.13) and low-density lipoprotein cholesterol ( $\beta$ -coefficient 1.11).

#### Prediction of incident cardiometabolic disorders

In multivariable Cox models for incident CHD and diabetes, adjusted for sex, age, and menopause, we first examined whether log GGT and serum total bilirubin quartiles were each of independent predictive value. While GGT was modestly

**Table 2 Pearson/Spearman correlations of GGT concentrations (*n* = 1,667)**

	<i>n</i>	<i>r</i>	<i>P</i> value
Waist circumference, cm	1,643	<b>0.28</b>	<0.001
BMI, kg/m <sup>2</sup>	1,639	<b>0.14</b>	<0.001
Systolic BP, mmHg	1,645	<b>0.09</b>	0.001
Diastolic BP, mmHg	1,645	<b>0.12</b>	<0.001
Total cholesterol, mg/dl	1,650	<b>0.16</b>	<0.001
Fasting triglycerides, mmol/l	1,417	<b>0.27</b>	<0.001
HDL-cholesterol, mmol/l	1,650	<b>-0.15</b>	<0.001
Fasting glucose, mmol/l	1,496	<b>0.11</b>	<0.001
Uric acid, μmol/l	1,665	<b>0.06</b>	0.019
Total bilirubin, mg/dl	1,211	0.04	0.15
Complement C3, g/l	1,171	<b>0.21</b>	<0.001
C-reactive protein, mg/l	1,560	<b>0.19</b>	<0.001
Fasting insulin <sup>a</sup> mIU/L	1,212	<b>0.23</b>	<0.001
Apolipoprotein A-I, g/l	1,247	<b>-0.05</b>	0.06
Apolipoprotein B, g/l	1,245	<b>0.13</b>	<0.001
Smoking status	1,667	<b>0.15</b>	<0.001
Alcohol usage	1,667	<b>0.21</b>	<0.001

Some parameters were not measured at baseline in each participant. Significant values are provided in boldface and borderline significant values are italicized.

BP, blood pressure; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein.

<sup>a</sup>Log-transformed values.

**Table 3** Linear regression analyses for GGT

	$\beta$ -coefficient	s.e.	P value	$\beta$ -coefficient	s.e.	P value	$\beta$ -coefficient	s.e.	P value
	Total (n = 1,641)			Men (n = 807)			Women (n = 834)		
Constant	10.0	1.15	<0.001	8.65	1.20	<0.001	4.6	1.17	<0.001
Sex, male	<b>1.37</b>	1.03	<0.001						
Alcohol intake, yes/no	<b>1.40</b>	1.05	<0.001	<b>1.36</b>	1.05	<0.001	<b>1.48</b>	1.05	0.046
Waist circumference, 11/13 cm <sup>a</sup>	<b>1.16</b>	1.00	<0.001	<b>1.17</b>	1.03	<0.001	<b>1.14</b>	1.00	<0.001
Age, 11 years	1.00	1.002	0.80	<b>0.94</b>	1.03	<0.001	<b>1.08</b>	1.002	<0.001
Smokers, current vs. never	1.001	1.016	0.42	1.00	1.02	0.84	1.04	1.016	0.15
Variance r <sup>2</sup>	0.20; P < 0.001			0.15; P < 0.001			0.08; P < 0.001		

Log-transformed values. Significant values are provided in boldface.

GGT,  $\gamma$ -glutamyltransferase.

<sup>a</sup>Denotes 1-s.d. increment in men/women.

**Table 4** Cox regression for prediction of incident CHD and diabetes by serum GGT, adjusted for sex, age and total bilirubin quartiles

	HR	95% CI	HR	95% CI	HR	95% CI
<i>For CHD</i>	Total (n = 103/1,125) <sup>a</sup>		Men (n = 45/542) <sup>a</sup>		Women (n = 58/583) <sup>a</sup>	
Sex, female	0.94	0.42; 2.12				
Age, 11 years <sup>b</sup>	<b>1.94</b>	1.59; 2.36	<b>2.00</b>	1.49; 2.66	<b>2.02</b>	1.27; 3.18
GGT, 1.7-fold <sup>c</sup>	<b>1.15</b>	1.00; 1.33	<b>1.21</b>	0.97; 1.51	<b>1.12</b>	0.91; 1.36
Bilirubin quartile 2 (0.4–0.58/0.3–0.46) <sup>d</sup>	0.78	0.47; 1.29	0.85	0.38; 1.88	0.72	0.37; 1.41
Bilirubin quartile 3 (0.59–0.79/0.47–0.59) <sup>d</sup>	0.63	0.36; 1.09	0.96	0.44; 2.10	<b>0.42</b>	0.19; 0.92
Bilirubin quartile 4 (>0.8/0.6) <sup>d</sup>	0.64	0.37; 1.11	0.65	0.27; 1.57	0.62	0.31; 1.23
Menopause					0.88	0.33; 2.32
<i>For type 2 diabetes</i>	n = 85/1,211 <sup>a</sup>		n = 53/587 <sup>a</sup>		n = 32/624 <sup>a</sup>	
GGT, 1.7-fold <sup>c</sup>	<b>1.35</b>	1.16; 1.58	<b>1.47</b>	1.20; 1.80	1.25	0.96; 1.64
Bilirubin quartile 2 (0.4–0.58/0.3–0.46) <sup>d</sup>	1.05	0.58; 1.90	0.72	0.33; 1.58	1.82	0.68; 4.87
Bilirubin quartile 3 (0.59–0.79/0.47–0.59) <sup>d</sup>	0.91	0.49; 1.68	1.08	0.54; 2.16	0.49	0.12; 1.97
Bilirubin quartile 4 (>0.8/0.6) <sup>d</sup>	1.13	0.63; 2.05	0.82	0.38; 1.77	1.80	0.66; 4.87
Menopause					0.85	0.23; 3.12

Overall samples vary depending on exclusion of the prevalent cases for each disorder at baseline. Age adjusted in both models. Individuals taking hormone replacement therapy or lipid lowering drugs (64 subjects) had been excluded. Significant values are provided in boldface.

CHD, coronary heart disease; CI, confidence interval; GGT,  $\gamma$ -glutamyltransferase; HR, hazard ratio.

<sup>a</sup>Number of cases/number at risk. <sup>b</sup>Denotes 1-s.d. increment. <sup>c</sup>Log-transformed values  $\geq 25/17$  vs.  $< 25/17$  U/l expressed in terms of 1 s.d. = 70% higher geometric mean values. <sup>d</sup>Referent  $< 0.4/0.3$  mg/dl.

predictive of CHD and fairly strongly of diabetes (**Table 4**), high bilirubin quartiles did not prove to be inversely and significantly associated with these diseases. Though GGT was not associated with CHD in females, women with total bilirubin  $\geq 0.47$  mg/dl (in the two highest quartiles) were associated with protection against CHD alone: relative risk (RR) 0.52 (95% confidence interval (CI) 0.28; 0.95) compared with the lowest quartile. While in men RR was not significant with 0.81, in the total study sample, the two highest quartiles disclosed an RR of 0.64 (95% CI 0.40; 1.006).

When BMI was substituted for bilirubin to evaluate the degree of mediation of the GGT associations by adiposity (**Table 5**), the introduced BMI attenuated the associations of GGT for CHD in women, contrasted to a modest attenuation in men. The converse was true in regard to diabetes, namely,

the associations of GGT were modestly attenuated in men but became significant and somewhat stronger in women.

#### GGT and incidence of hypertension and metabolic syndrome

**Table 6** demonstrates prediction of incident hypertension by log GGT in a logistic regression model adjusted for sex, age, menopause and BMI, all powerful determinants of elevated blood pressure. GGT contributed to the associations with an RR of 1.20 (95% CI 1.10; 1.31) per 1-s.d. increment.

After exclusion of 40% of participants who had MetS at baseline, 332 among 975 persons developed MetS during the follow-up. Serum GGT significantly predicted incident MetS at a modest RR in each sex, after adjustment for age (and menopause in women). Further adjustment for alcohol usage and

**Table 5 Cox regression for prediction of incident CHD and diabetes by serum GGT, adjusted for sex, age, BMI and menopause**

	HR	95% CI	HR	95% CI	HR	95% CI
For CHD		Total (n = 116/1,334) <sup>a</sup>		Men (n = 52/647) <sup>a</sup>		Women (n = 64/687) <sup>a</sup>
Sex, female	1.15	0.77; 1.70				
Age, 11 years <sup>b</sup>	<b>1.88</b>	1.56; 2.26	<b>1.94</b>	1.48; 2.58	<b>1.78</b>	1.17; 2.74
GGT, 1.7-fold <sup>c</sup>	1.08	0.94; 1.23	1.16	0.94; 1.42	1.02	0.84; 1.23
BMI, kg/m <sup>2</sup> , 4.5/5.5 <sup>b</sup>	<b>1.21</b>	1.04; 1.42	1.20	0.98; 1.47	1.23	0.98; 1.55
Menopause					1.13	0.47; 2.76
For type 2 diabetes		n = 99/1,439 <sup>a</sup>		n = 59/700 <sup>a</sup>		n = 40/739 <sup>a</sup>
Sex, female	0.66	0.43; 1.02				
Age, 11 years <sup>b</sup>	1.12	0.90; 1.38	<b>1.33</b>	1.00; 1.76	1.02	0.56; 1.86
GGT, 1.7-fold <sup>c</sup>	<b>1.28</b>	1.11; 1.48	<b>1.30</b>	1.07; 1.57	<b>1.35</b>	1.07; 1.71
BMI, kg/m <sup>2</sup> , 4.5/5.5 <sup>b</sup>	<b>1.54</b>	1.34; 1.76	<b>1.44</b>	1.24; 1.68	<b>1.66</b>	1.26; 2.18
Menopause					0.68	0.23; 2.04

Overall samples vary depending on exclusion of the prevalent cases for each disorder at baseline. Significant values are provided in boldface.

CHD, coronary heart disease; CI, confidence interval; GGT,  $\gamma$ -glutamyltransferase; HR, hazard ratio.

<sup>a</sup>Number of cases/number at risk. <sup>b</sup>Denotes 1-s.d. increment in men/women. <sup>c</sup>Log-transformed values  $\geq 25/17$  vs.  $< 25/17$  U/l expressed in terms of 1 s.d. =70% higher geometric mean values.

**Table 6 Logistic regression for prediction of incident hypertension (HT) and metabolic syndrome (MetS) by serum GGT, adjusted for sex, age, menopause and BMI**

	RR	95% CI	RR	95% CI	RR	95% CI
For HT		Total (n = 476/1,422) <sup>a</sup>		Men (n = 224/735) <sup>a</sup>		Women (n = 252/678) <sup>a</sup>
Sex, female	<b>1.40</b>	1.07; 1.84				
Age, 11 years <sup>b</sup>	<b>2.02</b>	1.78; 2.31	<b>1.82</b>	1.52; 2.17	<b>1.80</b>	1.30; 2.48
GGT, 1.7-fold <sup>c</sup>	<b>1.20</b>	1.10; 1.31	<b>1.17</b>	1.03; 1.34	<b>1.15</b>	1.01; 1.31
BMI, kg/m <sup>2</sup> , 4.5/5.5 <sup>b</sup>	<b>1.75</b>	1.53; 1.99	<b>1.98</b>	1.63; 2.42	<b>1.60</b>	1.32; 1.92
Menopause					<b>1.85</b>	1.03; 3.32
For MetS		n = 338/987 <sup>a</sup>		n = 157/486 <sup>a</sup>		n = 181/501 <sup>a</sup>
Sex, female	1.59	1.19; 2.12				
Age, 11 years <sup>b</sup>	<b>1.20</b>	1.05; 1.37	1.01	0.83; 1.23	<b>1.56</b>	1.09; 2.22
GGT, 1.7-fold <sup>c</sup>	<b>1.31</b>	1.18; 1.45	<b>1.14</b>	1.07; 1.22	<b>1.20</b>	1.04; 1.39
Menopause					0.86	0.45; 1.66
		n = 332/975 <sup>a</sup>		n = 153/479 <sup>a</sup>		n = 179/496 <sup>a</sup>
Sex, female	0.93	0.67; 1.30				
Age, 11 years <sup>b</sup>	<b>1.24</b>	1.07; 1.44	1.09	0.88; 1.37	<b>1.64</b>	1.14; 2.38
GGT, 1.7-fold <sup>c</sup>	<b>1.17</b>	1.04; 1.30	1.13	0.95; 1.33	1.14	0.98; 1.32
BMI, kg/m <sup>2</sup> , 4.5/5.5 <sup>b</sup>	<b>2.57</b>	2.12; 3.11	<b>3.89</b>	2.78; 5.42	<b>2.08</b>	1.63; 2.65
Alcohol usage, yes/no	0.89	0.50; 1.58	0.85	0.45; 1.61	0.01	NS
Menopause					0.77	0.38; 1.53

Overall samples vary depending on exclusion of the prevalent cases for each disorder at baseline. Significant values are provided in boldface.

CI, confidence interval; GGT,  $\gamma$ -glutamyltransferase; NS, not significant; RR, relative risk.

<sup>a</sup>Number of cases/number at risk. <sup>b</sup>Denotes 1-s.d. increment in men/women. <sup>c</sup>Log-transformed values  $\geq 25/17$  vs.  $< 25/17$  U/l expressed in terms of 1 s.d. =70% higher geometric mean values.

BMI led to reduction of significance to an RR 1.17 (95% CI 1.04; 1.30) per 1-s.d. increment.

## DISCUSSION

In a middle-aged population-based sample prone to MetS, we found that GGT activity was associated with age inversely in

men but positively in women and furthermore with fasting triglycerides, CRP and low-density lipoprotein cholesterol, all independent of BMI and lifestyle habits. High bilirubin quartiles were not inversely associated with incident diabetes and only in women with CHD, independent of circulating GGT. In logistic regression models adjusted for sex, age, menopause

and BMI, GGT contributed to the association with incident hypertension with an RR of 1.20, significantly predicted incident MetS at a similar RR which was in part BMI-mediated. In similarly adjusted Cox regression models, GGT activity was most strongly predictive of diabetes independently in each sex (hazard ratio (HR) 1.30 per 1-s.d. increment). Yet introduction of BMI in the models attenuated the modest predictive ability of GGT for incident CHD in women, contrasted to a minor attenuation in men. Thus GGT activity, likely a modest independent CHD risk mediator in males, is part of the proinflammatory state/oxidative stress in Turkish females, for which other evidences had been reported (16,22).

#### Elevated general levels and independent covariates

General levels of GGT activity in the study population was substantially higher than those reported for other populations. In four large community-based studies (2,8,23,24) median values ranged between 15–20 U/l in men and 9–11 U/l in women, while current values were higher by 1.4-fold and 1.7-fold, respectively. This reflects, in our opinion, the true difference in GGT levels (rather than variation in measurement methodology) given the high prevalence of MetS and diabetes among Turks, particularly women.

Apart from the well-known GGT determinants of male sex and alcohol intake, fasting triglycerides, CRP and sex-dependent age were the major covariates of GGT activity in the present study. A rise concomitant with age was not unexpected in women, yet the significant moderate independent decline of GGT (by 6%) per 1-s.d. increment in age among men might be ascribed to the concomitant decrease in Turkish men (but not women) of both SHBG and total testosterone with age (25). Serum triglycerides and CRP, recognized mediators of low-grade inflammation and oxidative stress, were documented to be significantly associated with GGT independent of BMI and other covariates. The lack of significant association with high or low GGT activity of serum HDL-C, apo A-I and current smoking is not surprising in this population sample, in view of previously documented dysfunction of HDL and apo A-I and lack of current smoking to confer cardiometabolic risk, especially among Turkish women (16).

#### Serum bilirubin in prediction of CHD risk

In Cox regression analyses jointly with GGT activity, total bilirubin quartiles were not of independent predictive value for the risk of incident diabetes. Regarding incident CHD risk, quartiles 3 and 4 (values >0.46 mg/dl) proved to confer protection in women, but not in men, and marginally in the whole sample. Turkish women rather than men were demonstrated to be more influenced by low-grade inflammation (16) which might explain the observed inverse association of higher bilirubin with decreased CHD risk being confined to females. Latter findings in women are in essential agreement with those reported (12,13).

#### Independent influence of serum GGT on metabolic risk but BMI-mediation in CHD risk

The magnitude of the association between GGT activity and outcome has previously been expressed variably, either in

quantiles (8,24), per doubling of levels or per log unit increase in GGT (26). In agreement with standard expression and in line with the Framingham study (2), we expressed HRs in terms of 1-s.d. increment in GGT. It is to be noted that such expression makes HRs appear lower than previously reported.

We confirmed findings by Lee and associates (2) regarding incident MetS that GGT predicted this development additively to BMI and other confounders. The magnitude of the HR (1.17) was slightly lower than the 1.26 of the Framingham study (2) in which a longer follow-up was available, but relatively high GGT levels in the lowest quartile in this study sample may also be implicated.

The HR in the independent prediction of hypertension, a component of MetS, was of similar magnitude as that for MetS in the current study suggesting that the contribution of GGT to the latter risk was mainly via the component of hypertension. In contrast, type 2 diabetes was linked to GGT more strongly, namely at an HR of 1.3, roughly paralleling the risk magnitude in women of the KORA study (24). This supports our previously expressed view that enhanced proinflammatory state/oxidative stress has an especially prominent role in diabetes among Turks (16,27).

Results on the prediction of CHD by GGT activity have been divergent. Though most studies have reported significant associations, these were of small magnitude, and heterogeneity existed. A meta-analysis of eight studies with respect to incident CHD (26) yielded a “fully-adjusted” pooled HR for 1-s.d. of GGT that corresponded to 1.10 (1.01; 1.20). Greater heterogeneity was notable among women. Our findings showing no independent predictive value of GGT activity for CHD in women and a marginal value in men are closely in line with results of the pooled meta-analysis. Oxidative processes in atherogenesis presumably merely mediate and are not additive to the adiposity-related factors in women; this seems to be distinct from CRP that has additive features and from GGT with respect to metabolic outcomes of diabetes and hypertension in which it contributes additively to adiposity. In the KORA study, the enhanced development of type 2 diabetes among obese subjects required the interaction of high GGT activity in women but not in men (24) suggesting a sex-dependent independent diabetogenic action.

Elevated serum GGT could be involved in cardiometabolic risk either as a marker of hepatic steatosis (28), with or without hepatic insulin resistance (5), and/or as a mediator of oxidative stress via mediation of extracellular glutathione transport into cells of organ systems (9), or as a mediator of low-grade systemic inflammation (29). The documented predictability of all three metabolic disorders of MetS, hypertension, and diabetes by GGT activity suggests that, as a reflection of oxidative stress, elevated GGT levels are actively involved in the pathogenesis of these disorders.

#### Strengths and limitations

Availability of a cohort of both genders representative of the general population, exclusion of potentially confounding conditions and, especially, the simultaneous assessment of

the diverse cardiometabolic disorders form the strengths of the present study. A study sample prone to MetS and having high GGT levels, while constituting strength in view of lack of similar samples, may limit somewhat the applicability of conclusions to populations having lower prevalence of MetS. Our use of multiple analyses may be a limitation in interpreting the findings, as may be the relatively limited number of events for diabetes and CHD. Although the identification of CHD included soft endpoints beyond myocardial infarction, this may reflect the natural distribution of CHD, especially in women. The relatively short mean follow-up of 4 years may have precluded the obtaining of higher HRs for GGT activity regarding cardiometabolic risk. Nonetheless, findings were in close agreement with mainstream results in prior studies.

### Conclusions

In middle-aged adults with a high prevalence of MetS, serum GGT activity is associated not with smoking status but with male sex, alcohol usage, sex-dependent age, fasting triglycerides, CRP and low-density lipoprotein cholesterol, independent of BMI. High bilirubin quartiles did not contribute to the prediction of type 2 diabetes but higher levels were protective of CHD risk in women, independent of circulating GGT. GGT activity modestly predicted hypertension, MetS, and diabetes in each sex, independent of multiple confounders including BMI, whereby the strongest HR existed regarding diabetes risk. GGT is involved in CHD risk mainly by mediating adiposity. Maintenance of normal triglycerides and CRP, regardless of a coexisting obesity, may be relevant in reducing oxidative stress and risk of diabetes.

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### DISCLOSURE

The authors declared no conflict of interest.

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# Persistent elevation of liver function enzymes within the reference range is associated with increased cardiovascular risk in young adults: the Bogalusa Heart Study

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## Abstract

Elevations in alanine aminotransferase (ALT) and  $\gamma$ -glutamyl transferase (GGT), markers of liver dysfunction and nonalcoholic fatty liver, are considered as part of the metabolic syndrome and related diseases. However, information is limited regarding the persistence (tracking) in levels of these enzymes over time and their influence on cardiovascular (CV) risk in young adults. The study sample consisted of white and black subjects ( $N = 489$ , 40% male, 73% white; baseline age, 18–32 years) followed over a period of 12 years as part of the Bogalusa Heart Study, with repeat measurements of CV risk factor variables and liver enzymes. Both at baseline and follow-up, males vs females had higher ALT ( $P < .01$  to  $.0001$ ) and GGT ( $P < .0001$ ); blacks vs whites had higher GGT ( $P < .0001$ ). With respect to persistence in enzyme levels over time, of those individuals who had ALT and GGT at the top quintile specific for age, race, and sex at baseline, about 50% of them continued to remain so with high values after 12 years. Individuals with levels persistently in the highest quintile vs those in the lowest quintile showed higher ( $P < .0001$ ) body mass index, waist circumference, triglycerides, low-density lipoprotein cholesterol, glucose, insulin, insulin resistance index, and systolic and diastolic blood pressures; lower ( $P < .0001$ ) high-density lipoprotein cholesterol; and higher ( $P < .05$  to  $.001$ ) prevalence of obesity, hypertension, dyslipidemia, metabolic syndrome as defined by the National Cholesterol Education Program Adult Treatment Panel III, positive parental history of type 2 diabetes, and coronary heart disease. In addition, based on a multivariate analysis using 2 separate models for ALT and GGT, baseline levels of both enzymes were independent predictors of follow-up; insulin resistance index and baseline GGT were also predictive of follow-up systolic blood pressure. Elevations in liver enzymes ALT and GGT, within “reference” range, persist over time and relate to clinically relevant adverse CV risk profile in young adults.

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## 1. Introduction

Nonalcoholic fatty liver (NAFL), a metabolic consequence of obesity, is increasingly being considered as a hepatic expression of metabolic syndrome [1–5]. Nonalcoholic fatty liver is commonly associated with long-term elevations in liver enzymes such as alanine aminotransferase (ALT) and  $\gamma$ -glutamyl transferase (GGT) [3,6,7]. These enzymes are suggested to have substantial clinical and epidemiological significance as useful noninvasive surrogate markers of NAFL and related liver dysfunction [6,7].

Recent epidemiological and clinical studies have reported a strong association of ALT and GGT with metabolic syndrome and related clinical manifestations including cardiovascular (CV) disease and type 2 diabetes

mellitus [8–13]. However, information is limited regarding the persistence (tracking) of increased levels of these enzymes over time and their effect on CV risk in young adults. As part of the Bogalusa Heart Study, a biracial (black-white) community-based investigation of the early natural history of CV disease [14], the present study examines the tracking of ALT and GGT within “reference” range over time and their association with CV risk in terms of metabolic syndrome and parental histories of coronary heart disease and type 2 diabetes mellitus in apparently healthy young adults.

## 2. Methods

### 2.1. Study population

Three cross-sectional surveys were performed on young adults during 1985–1986, 1988–1991, and 2000–2001 in the

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community (65% whites, 35% blacks) of Bogalusa, LA. The study cohort ( $N = 489$ ; 73% white, 40% male) was selected from those who were fasting and had data on ALT and GGT along with other risk factor variables, and participated in the baseline survey of 1985–1986 or 1988–1991 (baseline age: 25.6 year) as well as the follow-up survey of 2000–2001 (follow-up age, 37.9 years), with a follow-up period of at least 12 years or more. Individuals with liver enzyme values above the reference range (ALT, 0–55 IU/L; GGT, 0–65 IU/L) were excluded. The baseline characteristics of the study cohort were similar to the rest of the participants examined at the baseline survey with respect to liver enzyme levels and other risk factor variables (data not shown). This study was approved by the institutional review board of the Tulane University Health Sciences Center (New Orleans, LA). All participants gave their informed consent.

## 2.2. General examination

Standardized protocols were used by trained observers in all examinations. Subjects were instructed to fast for 12 hours before the screening, with compliance ascertained by an interview on the day of examination. Anthropometric and blood pressure measurements were made in replicate, and mean values were used in all analyses. Height and weight were measured to calculate body mass index (BMI; weight in kilograms divided by the square of height in meters) as a measure of overall adiposity. Waist circumference was measured midway between lower rib cage and iliac crest, as an indicator of visceral fatness. Replicate blood pressure measurements were obtained on the right arm of the subjects in a relaxed, sitting position. Systolic and diastolic blood pressures were recorded at the first and fifth Korotkoff phases, respectively, using a mercury sphygmomanometer. Study subjects were asked through a questionnaire whether either or both biological parents had histories of coronary heart disease (myocardial infarction, bypass surgery, balloon angioplasty, and angina) and type 2 diabetes mellitus, surrogate measures of CV risk. Individuals were considered smoker if they reported current use of cigarette or having stopped smoking only within the past year. Similarly, individuals were considered alcohol drinker if they reported current consumption of alcohol or having stopped alcohol drinking only within the past year.

## 2.3. Laboratory analyses

Cholesterol and triglyceride levels in the serum were assayed using enzymatic procedures on the Hitachi 902 Automatic Analyzer (Roche Diagnostics, Indianapolis, IN). Serum lipoprotein cholesterol levels were analyzed by a combination of heparin-calcium precipitation and agar-agarose gel electrophoresis procedures [15]. The laboratory is being monitored for precision and accuracy of lipid measurements by the Lipid Standardization and Surveillance Program of the Centers for Disease Control and Prevention (Atlanta, GA). A commercial radioimmunoassay kit was used for measuring plasma immunoreactive insulin levels (Phadebas; Pharmacia Diagnostics, Piscataway, NJ). Glucose, ALT, and GGT levels were measured as part of a multiple chemistry profile (SMA20) by enzymatic procedures with the multichannel Olympus Au-5000 analyzer (Olympus, Lake success, NY). Insulin resistance status was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) according to the formula described previously [16]: insulin ( $\mu\text{U}/\text{mL}$ )  $\times$  glucose ( $\text{mmol}/\text{L}$ )  $\div 22.5$ .

## 2.4. Statistical analysis

All statistical analyses were performed with SAS version 9.1 (SAS institute, Cary, NC). Values of ALT, GGT, triglycerides, insulin, and HOMA-IR were log transformed in the analyses to improve normality. General linear models were used to examine race and sex differences in liver enzymes at baseline and follow-up. All  $P$  values were 2-tailed and adjusted for covariates where appropriate. To determine the persistence of ALT and GGT levels over time, the study subjects were ranked according to age-, race-, and sex-specific quintiles of ALT and GGT at baseline and follow-up. Subjects whose liver enzyme levels were persistently in the highest or lowest quintile during both baseline and follow-up surveys were categorized as having persistently high or low ALT or GGT levels. Follow-up risk factor characteristics (measures of obesity [BMI and waist circumference], systolic and diastolic blood pressures, lipids and lipoprotein variables [low-density lipoprotein cholesterol—LDL-C, high-density lipoprotein cholesterol—HDL-C, and triglycerides], and measures of glucose homeostasis

Table 1

Mean  $\pm$  SD of the liver enzymes by race and sex at baseline and follow-up in young adults: the Bogalusa Heart Study

Variable	Male		Female		Comparison ( $P$ )	
	White (n = 150)	Black (n = 45)	White (n = 207)	Black (n = 87)	Sex	Race
Baseline						
Age (y)	25.7 $\pm$ 3.1	26.0 $\pm$ 3.03	25.6 $\pm$ 3.1	25.3 $\pm$ 3.0	NS	NS
ALT (IU/L)	27.2 $\pm$ 20.1	29.3 $\pm$ 25.3	14.2 $\pm$ 7.7	14.1 $\pm$ 8.7	<.0001	NS
GGT (IU/L)	20.9 $\pm$ 19.1	34.3 $\pm$ 44.9	10.8 $\pm$ 8.3	18.4 $\pm$ 18.9	<.0001	<.0001
Follow-up						
Age (y)	38.0 $\pm$ 3.1	38.3 $\pm$ 3.1	37.8 $\pm$ 3.1	37.6 $\pm$ 3.0	NS	NS
ALT (IU/L)	37.2 $\pm$ 24.7	30.5 $\pm$ 14.6	19.2 $\pm$ 13.0	16.9 $\pm$ 9.5	<.01	NS
GGT (IU/L)	46.7 $\pm$ 52.2	55.8 $\pm$ 72.5	21.6 $\pm$ 18.2	35.5 $\pm$ 46.6	<.0001	<.01

NS indicates not significant.

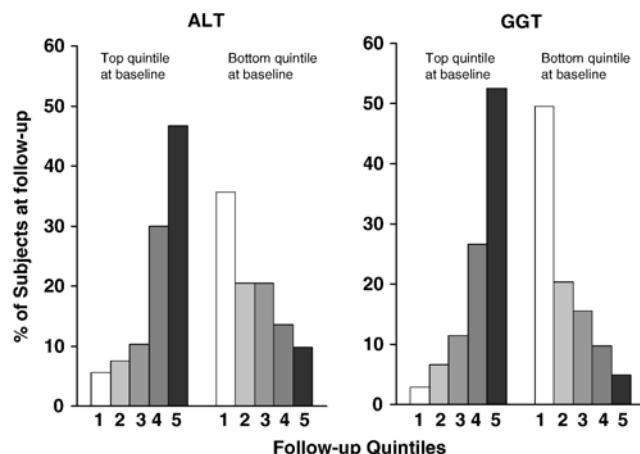


Fig. 1. Tracking of ALT and GGT over a 12-year period in young adults: the Bogalusa Heart Study. The degree of tracking was evaluated in terms of distribution by quintiles at follow-up of subjects who were in the extreme top or the bottom quintile specific for age, race, and sex at baseline. The percentage on the vertical axis denotes the proportion of subjects remaining in each quintile at follow-up.

[insulin, glucose, and HOMA-IR]) associated with persistently high vs persistently low ALT and GGT levels were compared after adjusting for age, race, and sex. Parental histories of type 2 diabetes mellitus and coronary heart disease were also compared between subjects with persistently high vs persistently low ALT and GGT levels.

The prevalence of obesity ( $BMI > 30 \text{ kg/m}^2$ ), hypertension (systolic blood pressure  $> 140 \text{ mm Hg}$  or diastolic blood pressure  $> 90 \text{ mm Hg}$  or being treated for the condition), dyslipidemia (total cholesterol  $> 240 \text{ mg/dL}$  or LDL-C  $> 160 \text{ mg/dL}$  or HDL-C  $< 40 \text{ mg/dL}$  or triglycerides  $> 150 \text{ mg/dL}$  or being treated for the condition), and metabolic syndrome ( $\geq 3$  risk factors defined by National Cholesterol Education Program Adult Treatment Panel III [17]) were compared with the use of  $\chi^2$  analysis between groups with persistently high and low levels of ALT and

GGT to examine the status of abnormalities (high-risk) at follow-up.

In 2 separate multiple regression analyses, levels of ALT (model 1) and GGT (model 2) at baseline were evaluated as an independent predictor of follow-up level of risk factor variables. The model adjusted for age, race, sex, BMI, alcohol consumption status, and cigarette smoking status included either baseline ALT or GGT as the main predictor along with follow-up HOMA-IR, systolic blood pressure, triglyceride, HDL-C, and LDL-C as applicable. For example, when HOMA-IR was a dependent variable, other follow-up risk variables were used as covariates.

### 3. Results

Mean levels of ALT and GGT at baseline and follow-up are shown in Table 1 by race and sex. At baseline and follow-up, GGT levels were higher in blacks vs whites ( $P < .0001$  to  $.01$ ) and males vs females ( $P < .0001$ ), whereas ALT levels were higher only in males vs females ( $P < .0001$  to  $.01$ ).

Subjects with relatively high/low liver enzyme levels at baseline tended to have retained such levels 12 years later. As shown in Fig. 1, when subjects were grouped into quintiles according to age-, race-, and sex-specific rankings of ALT and GGT levels, a higher-than-expected number of individuals who ranked high ( $> 80\text{th percentile}$ ) or low ( $< 20\text{th percentile}$ ) in ALT and GGT levels at baseline maintained their respective ranks at follow-up. Of those individuals who had high levels of liver enzymes at baseline, about 50% continued to have elevated levels after 12 years. If there were no tracking of levels, one would expect only 20% in the highest or lowest quintile at follow-up by chance alone.

Follow-up risk factor characteristics of subjects with persistently high vs low levels of ALT and GGT are shown in Table 2. Subjects with persistently high vs low levels of

Table 2

Follow-up characteristics related to CV risk in young adults with persistently high vs low levels of liver enzymes over a 12-year period: the Bogalusa Heart Study

Risk factor variables (mean $\pm$ SD)	ALT		GGT	
	Persistently low <sup>a</sup> (n = 47)	Persistently high <sup>b</sup> (n = 50)	Persistently low (n = 51)	Persistently high (n = 55)
BMI ( $\text{kg/m}^2$ )	26.8 $\pm$ 6.6	30.7 $\pm$ 5.4**	27.6 $\pm$ 5.9	30.0 $\pm$ 5.9**
Waist circumference (cm)	83.3 $\pm$ 13.3	101.9 $\pm$ 15.1**	86.7 $\pm$ 15.9	100.3 $\pm$ 16.0**
Systolic BP (mm Hg)	113.0 $\pm$ 13.2	123.4 $\pm$ 12.5**	111.2 $\pm$ 10.8	125.6 $\pm$ 11.7**
Diastolic BP (mm Hg)	75.8 $\pm$ 8.8	84.6 $\pm$ 9.9**	74.5 $\pm$ 7.8	86.0 $\pm$ 9.7**
Triglyceride (mg/dL)	99.7 $\pm$ 45.4	190.3 $\pm$ 143.6**	107.3 $\pm$ 59.9	229.4 $\pm$ 192.4**
HDL-C (mg/dL)	53.8 $\pm$ 13.6	41.9 $\pm$ 14.5**	54.8 $\pm$ 11.5	47.5 $\pm$ 19.1*
LDL-C (mg/dL)	113.3 $\pm$ 33.1	128.2 $\pm$ 39.8**	115.6 $\pm$ 32.1	132.1 $\pm$ 40.5**
Insulin ( $\mu\text{U/mL}$ )	9.3 $\pm$ 5.8	18.1 $\pm$ 12.0**	9.0 $\pm$ 4.0	16.8 $\pm$ 10.5**
Glucose (mg/dL)	82.0 $\pm$ 10.4	90.0 $\pm$ 24.7*	80.6 $\pm$ 7.3	92.1 $\pm$ 26.5**
HOMA-IR	2.0 $\pm$ 1.5	4.2 $\pm$ 3.1**	1.8 $\pm$ 0.9	3.9 $\pm$ 2.7**

Systolic BP indicates systolic blood pressure; diastolic BP, diastolic blood pressure.

<sup>a</sup> Less than age-, race-, and sex-specific 20th percentile.

<sup>b</sup> Greater than age-, race-, and sex-specific 80th percentile.

\*  $P < .001$ , persistently high vs low (adjusted for covariates where appropriate).

\*\*  $P < .0001$ , persistently high vs low (adjusted for covariates where appropriate).

Table 3

Prevalence of CV risk factors at follow-up according to liver enzyme status over a 12-year period in young adults: the Bogalusa Heart Study

Prevalence (%)	ALT		GGT	
	Persistently Low <sup>a</sup> (n = 47)	Persistently High <sup>b</sup> (n = 50)	Persistently Low (n = 51)	Persistently High (n = 55)
Obesity	21.3	58.0**	29.4	45.5*
Hypertension	8.5	30.0**	2.0	38.2**
Dyslipidemia	4.3	14.0**	21.7	61.8**
Metabolic Syndrome	8.5	40.0**	7.8	38.2**

Obesity indicates BMI of >30; hypertension, systolic blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg or being treated for the condition; dyslipidemia, total cholesterol of ≥ 240 mg/dL or LDL-C of ≥ 160 mg/dL or HDL-C of <40 mg/dL or triglycerides of ≥ 150 mg/dL or being treated for the condition; metabolic syndrome, ≥ 3 risk factors defined by the National Cholesterol Education Program Adult Treatment Panel III.

<sup>a</sup> Less than age-, race-, and sex-specific 20th percentile.

<sup>b</sup> Greater than age-, race-, and sex-specific 80th percentile.

\* P < .05.

\*\* P < .001.

these enzymes had significantly higher BMI, waist circumference, systolic and diastolic blood pressures, triglycerides, LDL-C, insulin, glucose, and HOMA-IR ( $P < .001$  to  $.0001$ ), and lower HDL-C ( $P < .001$ ). In addition, as shown in Table 3, individuals with persistently high vs low enzyme levels had increased prevalence of high-risk conditions of obesity, hypertension, dyslipidemia, and metabolic syndrome ( $P < .05$  to  $.0001$ ). With respect to metabolic syndrome, as shown in Fig. 2, significant ( $P < .05$ ) clustering of 2 or more metabolic syndrome components occurred in subjects with persistent elevations in either ALT or GGT levels. On the other hand, the prevalence of individuals with no clustering was 2.4-fold ( $P < .05$ ) higher among those with persistently low vs high levels of ALT or GGT.

Baseline levels of liver enzymes as predictors of adverse levels of CV risk factor variables at follow-up were

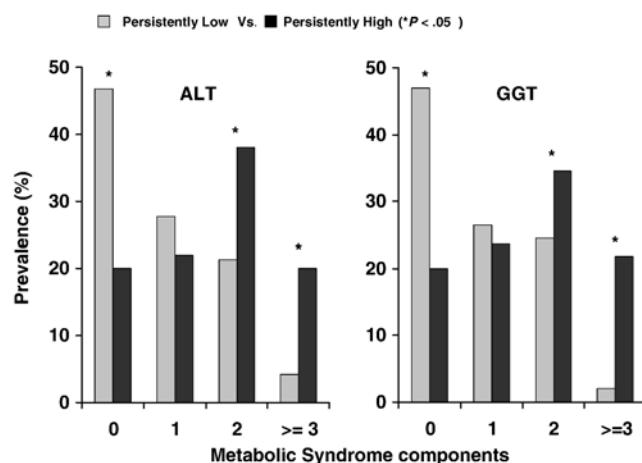


Fig. 2. Clustering of metabolic syndrome components at follow-up in young adults by liver enzyme status over a 12-year period: the Bogalusa Heart Study.

Table 4

Baseline liver enzymes as predictors of follow-up risk factor variables in young adults: the Bogalusa Heart Study

Follow-up risk variables	Baseline ALT (regression coefficient $\beta^a$ )	Baseline GGT (regression coefficient $\beta^a$ )
HOMA-IR	.006*	.004**
Systolic blood pressure	.043	.062**
Triglyceride	.001	.002
HDL-C	.033	.052
LDL-C	-.154	-.030

<sup>a</sup> The model adjusted for age, race, sex, BMI, alcohol consumption status, and cigarette smoking status included either baseline ALT or GGT as the main predictor along with follow-up HOMA-IR, systolic blood pressure, triglyceride, HDL-C, and LDL-C as applicable.

\* P < .05.

\*\* P < .001.

examined in separate multivariate models. Baseline ALT as well as GGT was predictive of follow-up insulin resistance index (HOMA-IR) independent of other risk factors, whereas baseline GGT also predicted follow-up systolic blood pressure (Table 4).

The prevalence of parental histories of type 2 diabetes mellitus and coronary heart disease is shown in Fig. 3 by the status of ALT and GGT. Among those with persistently high vs low ALT levels, parental type 2 diabetes mellitus and parental CHD occurred 2.0-fold ( $P < .0001$ ) and 1.5-fold ( $P < .001$ ), respectively. Likewise, parental type 2 diabetes mellitus and parental CHD occurred 1.7-fold ( $P < .001$ ) and 1.8-fold ( $P < .001$ ), respectively, among those with persistently high vs low levels of GGT.

#### 4. Discussion

The present community-based study demonstrates that elevations in enzymes ALT and GGT, biomarkers of liver dysfunction and NAFL, persist over time and relate adversely to metabolic syndrome and its components as well as to parental histories of coronary artery disease and

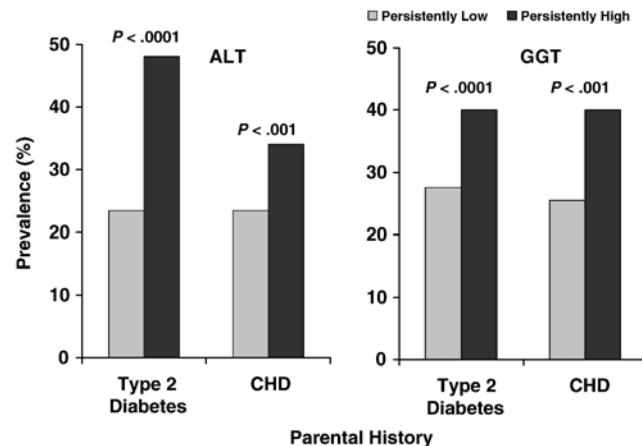


Fig. 3. Prevalence of parental history of type 2 diabetes mellitus and coronary heart disease at follow-up in young adults by liver enzyme status over a 12-year period: the Bogalusa Heart Study.

type 2 diabetes mellitus in asymptomatic, healthy young adults. These longitudinal observations in a relatively younger adult cohort are noteworthy in that they strongly support the notion of pathophysiologic link between NAFL, metabolic syndrome, and related CV risks, as part of the natural history of CV disease.

In this study, about 50% of the cohort maintained their baseline levels (high or low) after 12 years of follow-up. Although earlier studies have shown tracking of CV risk factor variables, individually or in combination, over time [18–21], no such data on ALT and GGT are available for comparison.

The adverse associations between NAFL disease (or related abnormal elevations in liver function enzymes) and pathophysiologically interrelated conditions of metabolic syndrome and its components have been found in previous studies [1–5,8–11]. In the present study, even within the reference range of ALT/GGT enzymes, excess prevalence of clinically high-risk conditions of obesity, dyslipidemia, hypertension, and metabolic syndrome was noted among those who were persistently in the highest vs lowest quintile distribution of either of these enzymes. Of note, in a multivariate analysis adjusted for adiposity and other CV risk factor variables, baseline ALT as well as GGT predicted follow-up measure of insulin resistance, the underlying feature of metabolic syndrome [22,23], in an adverse manner. In addition, GGT predicted systolic blood pressure. Although the observational nature of this study cannot address the issue of causality or the underlying mechanisms involved in the previously mentioned relationships, current knowledge on this subject provides some explanation.

The adverse relationship of liver enzymes to risk variables of metabolic syndrome may be the consequence of link between excess central (visceral) adiposity, NAFL, and hepatic insulin resistance mediated by increased hepatic free fatty acid flux from visceral fat leading to increased hepatic lipogenesis and triglyceride-rich lipoprotein secretion [2,3,24,25], which in part is due to induction of sterol-regulating binding protein and fatty acid synthase [26,27]. Furthermore, excess central adiposity (and by inference NAFL) enhances the expression of proinflammatory adipocytokines including tumor necrosis factor  $\alpha$  and decreases the expression of insulin-sensitizing and anti-inflammatory adiponectin, resulting in increase in insulin resistance [28–30]. In turn, insulin resistance increases reactive oxygen species and oxidative stress by attenuating the inhibitory effect of insulin on lipid oxidation and by activating CYP2E1, a component of the cytochrome P-450 system [31].

The observed independent association of baseline GGT, but not ALT, with follow-up systolic blood pressure is consistent with the results of earlier reports [13,32,33] and may reflect the role of GGT in the dynamics of free radical generation, a factor involved in the pathogenesis of hypertension [34–36].  $\gamma$ -Glutamyl transferase is considered to help maintain adequate levels of hepatic glutathione, an antioxidant [34,35]. Generation of excess free radicals

associated with NAFL and central obesity may deplete glutathione levels thereby causing induction of GGT to counteract the adverse effect. However, the production of the GGT reaction in the presence of iron may itself lead to excess generation of free radicals [34,35].

A positive parental history is recognized as a surrogate indicator of future risk in the offspring, given the familial nature of CV disease and type 2 diabetes mellitus [37,38]. In addition, parental histories of CV disease and type 2 diabetes mellitus are shown to be associated with unfavorable CV risk factor profile in the offspring [39,40]. This study shows a significantly higher prevalence of parental histories of coronary heart disease and type 2 diabetes mellitus in the study cohort with persistently high vs low levels of ALT or GGT.

The observed higher ALT in blacks vs whites and males vs females are consistent with previous findings [6,11]. However, no such data are available for GGT for comparison. The race-sex differences in ALT and GGT may reflect the differences in the prevalence of NAFL noted previously [6].

This study has certain limitations in that it lacks direct assessment of body fat mass and distribution, liver fat content, and in vivo insulin action used in clinical and etiological studies. Instead, we used well-established measures that are appropriate to population studies. Furthermore, parental histories of coronary heart disease and type 2 diabetes mellitus were not verified in this study. Previous studies, including our own, noted a concordance of 78% to 83% between reported and verified cases [41,42]. It should be mentioned that nonsystemic misclassification of self-reported parental histories would most likely result in an underestimation of the strength of association.

In summary, elevations in levels of liver enzymes ALT and GGT persist over time and relate to higher prevalence of obesity, dyslipidemia, hypertension, and metabolic syndrome as well as parental histories of coronary heart disease and type 2 diabetes mellitus in apparently healthy young adults. When viewed in the context of upward secular trends in the prevalence of obesity [43,44] and ALT [45], and higher prevalence of metabolic syndrome [46] and NAFL [6] in the US population, these results underscore the potential utility of ALT and GGT as biomarkers in the evaluation of CV risk in asymptomatic young people.

## Acknowledgments

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 Open Access Full Text Article

ORIGINAL RESEARCH

# Lack of a relationship between circulating gamma-glutamyltransferase levels and carotid intima media thickness in hypertensive and diabetic patients

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**Background:** By increasing the intracellular prooxidant burden, gamma-glutamyltransferase (GGT) may accelerate atherosclerotic vascular disease. That noxious influence may be reflected by circulating enzyme levels, a correlate of cardiovascular risk factors, and a predictor of incident events. To evaluate this hypothesis, we tested the association between circulating GGT and common carotid intima-media thickness (CIMT), a surrogate index of systemic atherosclerotic involvement, in a large and well-characterized group of patients at risk of cardiovascular disease (CVD).

**Patients:** This study analyzed 548 patients with hypertension and/or diabetes and a widely prevalent history of CVD. Subjects with known hepatic disease and abnormal GGT values were excluded.

**Methods:** CIMT (B-mode ultrasonography) values were the mean of four far-wall measurements at both common carotids. Metabolic syndrome (MetS) was diagnosed according to National Cholesterol Education Program-Adult Treatment Panel III criteria. Due to inherent sex-related differences in GGT levels, the data were analyzed separately in males and females in samples dichotomized by the median.

**Results:** The age-adjusted CIMT values did not differ by GGT levels in males or females. In contrast, the carotid wall was consistently thicker in patients with a history of CVD and MetS independent of age and concurrent GGT values. In both sexes, GGT was associated with key components of the MetS such as triglycerides, fasting plasma glucose, and body mass index.

**Conclusion:** The data collected in this mixed group of hypertensive and/or diabetic patients with widely prevalent history of CVD do not support the concept of a direct pathophysiological link between GGT levels within reference limits and atherosclerotic involvement.

**Keywords:** gamma-glutamyltransferase, carotid intima-media thickness, atherosclerosis, metabolic syndrome

## Introduction

Consistent evidence associates increased circulating serum gamma-glutamyltransferase (GGT), a parameter conventionally used for diagnosing hepatobiliary diseases and alcohol abuse,<sup>1</sup> with incident cardiovascular disease (CVD)<sup>2</sup> and major proatherogenic risk factors.<sup>3</sup> For this reason, GGT determination has been added to the array of biomarkers useful for stratifying cardiometabolic risk.<sup>4</sup> Furthermore, the enzyme's active involvement in the atherogenic process was hypothesized on the basis of its potential to increase the intracellular prooxidant burden through the iron-reducing properties of cysteinylglycine moieties generated during GGT-catalyzed glutathione hydrolysis.<sup>5</sup> The identification of prooxidant GGT activity in atherosomatous plaques of carotid and coronary arteries<sup>6</sup> adds to the need for further clinical evaluation.

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Since the first demonstration of its close correlation with directly measured arterial wall thickness, carotid intima-media thickness (CIMT) determination<sup>7</sup> has become an easily obtained and noninvasive standard surrogate measure of systemic atherosclerosis and a prognostic and therapeutic end-point in epidemiological and pharmacological trials.<sup>8</sup> Therefore, ultrasound-derived CIMT offers a way to assess the relationship between GGT levels and atherosclerotic vascular disease. This rarely addressed issue was evaluated in this retrospective cross-sectional analysis of a large and well-characterized sample of patients screened at our institution.

## Materials and methods

### Patients

This study examined 548 consecutive Caucasian subjects who were referred to our department between January 2006 and June 2010 for hypertension and/or diabetes. Table 1 provides clinical and demographic characteristics of the sample. Subjects with history of liver disease, self-reported alcohol abuse, history of hepatitis B or C, anticonvulsants, and microsomal enzyme-inducing drugs active on hepatic GGT release<sup>1</sup> were excluded. Only patients with GGT levels within the reference values of our laboratory (<60 and 40 U/L for males and females, respectively) were included in the

analysis. Statin and antihypertensive (mostly angiotensin-converting enzyme inhibitors (ACEIs), angiotensin-receptor blockers (ARBs), and calcium channel blockers) treatment at the time of the visit was retrieved from the records. Table 1 presents the relative percentages.

### Carotid-scanning protocol

The patients were screened while in supine position with the head and neck gently rotated 45 degrees from the side where the scanning was performed. The examination started by visualizing the longitudinal image of the midportion of the common carotid arteries in the supraclavicular region by moving and rotating the transducer until the sonographer demonstrated and marked the bifurcation with the cursor. Next, the sonographer focused on the interfaces required for the measurements of the arterial wall thickness of the common carotid artery (CCA) segment within 40 mm proximal to the carotid bulb. Patients with arteries in which the references were unidentifiable, tortuous, or calcified were excluded. All measurements were made with the image at the maximum depth of focus. The operator set up the gains and image pre- and post-processing options for every patient and for each artery to obtain the best possible image. Measurements of the distance from the leading edge of the first echogenic luminal, bright line to the leading edge of the second echogenic line were taken manually from frozen images as indicated by Pignoli<sup>7</sup> in order to express the distances in mm. Scanning and measurements were obtained by a Philips ie33® instrument (Philips, Eindhoven, The Netherlands) equipped with a linear 7.5 MHz probe (axial resolution: 0.1 mm) and by the same observer (MN, within-observer variability: 5.3%, the average variation coefficient of 20 triplicate measurements in control subjects).

### Biochemistry

GGT was measured colorimetrically by the nitroanilide method on a Cobas Mira Plus (Roche, Mannheim, Germany) chemistry instrument. Alanine aminotransferase (ALT), fasting plasma glucose (FPG), total cholesterol (CHOL), low-density lipoprotein-cholesterol (LDL-CHOL), high-density lipoprotein-cholesterol (HDL-CHOL), and triglycerides were assessed by automated standard enzymatic and colorimetric methods. All the samples were processed in the same laboratory, and quality control was ensured by the regional branch of the National Health System (Regione Toscana, Controllo di Qualità in Medicina di Laboratorio<sup>9</sup>).

The systolic (S) and diastolic (phase V Korotkoff) blood pressure (BP) values refer to sphygmomanometric measurements taken in sitting position at the time of CIMT determination.

**Table 1** Demographic and clinical characteristics by sex

Variables	Females n = 217	Males n = 331	P-value
CIMT (mm)	0.80 ± 0.19	0.86 ± 0.20	<0.001
GGT (U/L)	15 (9)	27 (20)	<0.001
ALT (U/L)	17 (7)	22 (13)	<0.001
Triglycerides (mg/dL)	93 (57)	118 (77)	<0.001
FPG (mg/dL)	90 (16)	95 (18)	<0.001
BMI (Kg/m <sup>2</sup> )	25.6 ± 4.7	26.8 ± 3.5	<0.001
Diabetes	10%	22%	<0.001
Active smokers	12%	25%	<0.001
History of CVD	19%	40%	<0.001
Statins	54%	67%	<0.01
Antihypertensive Rx	51%	70%	<0.001
Total-CHOL (mg/dL)	216 ± 38	195 ± 38	<0.001
LDL-CHOL (mg/dL)	140 ± 36	131 ± 37	<0.01
HDL-CHOL (mg/dL)	67 ± 15	52 ± 14	<0.001
Age (years)	59 ± 14	60 ± 13	NS
SBP (mmHg)	133 ± 18	133 ± 16	NS
Hypertension	75%	80%	NS

**Note:** Means ± SD, medians (interquartile range) and percentages.

**Abbreviations:** ALT, alanine transferase; BMI, body mass index; CVD, cardiovascular disease; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL-CHOL, high-density lipoprotein-cholesterol; LDL-CHOL, low-density lipoprotein-cholesterol; NS, nonsignificant; SBP, systolic blood pressure; SD, standard deviation.

Body weight was measured to the nearest 0.1 kg on a scale with an attached height measurement device.

## Definitions

Cardiovascular disease (CVD) includes coronary heart disease (previous myocardial infarction, unstable and stable angina, coronary artery bypass graft, or angioplasty), peripheral arterial disease (previous lower limb surgery, angioplasty, or current claudication confirmed by echo-Doppler studies, angiograms, or others), and carotid disease (previous endarterectomy or carotid stenosis  $>50\%$  at echo-Doppler imaging) (Table 2). Diabetes and hypertension were either diagnosed based on ongoing treatment or by the presence of fasting plasma glucose  $>125$  mg/dL and BP  $>130/85$  respectively. Metabolic syndrome (MetS) was defined according to National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) criteria<sup>10</sup> in the presence of at least three of the following criteria: antihypertensive treatment or BP  $>130/85$  mmHg, triglycerides  $\geq 150$  mg/dL, HDL-C  $<40$  mg/dL in males and  $<50$  mg/dL in females, glucose-lowering treatment, or FPG  $>110$  mg/dL, abdominal obesity. The thresholds for abdominal obesity were BMI  $\geq 29.5$  kg/m<sup>2</sup> and  $\geq 27.2$  kg/m<sup>2</sup> in men and women, respectively since those values corresponded to 102 cm and 88 cm of waist circumference in men and women, respectively, in a regression of BMI on the waist as validated previously.<sup>11</sup> Smokers were either categorized as current smokers independent of the number of cigarettes they had per day or as never/previous smokers, meaning tobacco-free for at least 6 months.

## Data processing

The data were analyzed by sex-specific median GGT values (cutoffs: 15 U/L and 27 U/L for females and males, respectively). CIMT was the average of the four values measured bilaterally approximately 1 cm from the other at the far wall of the CCA, provided that these points were free of plaque. Plaques (a local thickening exceeding 1.4 mm and protruding into the lumen) were excluded from the measurements. BMI was calculated as weight/height<sup>2</sup> (Kg/m<sup>2</sup>). For the sake

**Table 2** Distribution (absolute numbers and percentages) by type of vascular disease ( $n = 174$ ) in descending order of frequency

Type of vascular disease	n = 174	%
Multivessel disease	71	40
Coronary heart disease	50	29
Carotid artery disease	43	25
Peripheral vascular disease	10	6

**Note:** Multivessel disease indicates the coexistence in the same patient of two or more of the listed vascular diseases.

of clarity, only the SBP values were reported since diastolic BP did not vary across comparisons.

## Statistics

Differences in continuous and categorical variables were assessed by one-way analysis of variance and chi-square statistics, respectively, and were adjusted for age by analysis of covariance and logistic regression. Unless otherwise specified, descriptive statistics were mean  $\pm$  standard deviation or median (interquartile range) for skewed data and percentages for categorical variables. The limit for statistical significance was  $P < 0.05$ .

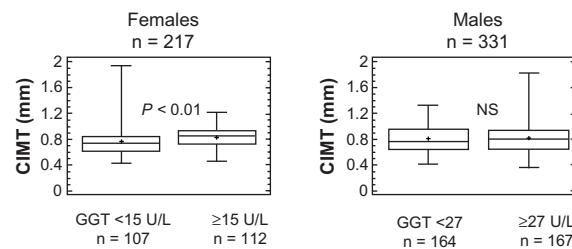
## Results

### Clinical and demographic characteristics by gender

CIMT, GGT ALT, triglycerides, FPG, BMI were higher and diabetes, active smoking, history of CVD, and pharmacological treatment more frequent in males than females whereas total and fractionated CHOL showed opposite trends. Age, SBP, and history of hypertension did not differ by gender (Table 1).

### Clinical and demographic characteristics by GGT status in males and females

In contrast to the homogeneous distribution of such parameters in men, thicker carotid walls (Figure 1), higher SBP, and more frequent hypertension and statin treatment distinguished women with above from those below median GGT levels (Table 3). However, differences in CIMT (Age-corrected means [95% confidence interval (CI)]: 0.80 [0.77–0.83] versus 0.79 [0.76–0.81] mm) and in other parameters (data not shown) were abolished by accounting for the older age of the female subgroup (Table 3). In both sexes, above-median GGT levels were associated with higher ALT, triglycerides, FPG, and BMI (Table 3).



**Figure 1** Box-and-whisker plots of carotid intima-media thickness (CIMT) by above- and below-median gamma-glutamyltransferase (GGT) levels in females (left panel) and males (right panel).

**Notes:** The statistical difference in women was abolished when age difference was taken into account. The central box encloses the middle 50% of the data; the horizontal line inside the box represents the median, and the mean is plotted as a cross. Vertical lines (whiskers) extend from each end of the box and cover four interquartile ranges.

**Table 3** Demographic and clinical characteristics by above- and below-median sex-specific GGT levels

Variables	Females n = 217			Males n = 331			
	<15 U/L n = 107		≥15 U/L n = 112	P-level	<27 U/L n = 164		≥27 U/L n = 167
	<15 U/L n = 107	≥15 U/L n = 112			<27 U/L n = 164	≥27 U/L n = 167	
GGT (U/L)	11 (3)	20 (8)	–	19 (9)	39 (14)	–	
Age (years)	55 ± 14	64 ± 12	<0.001	61 ± 15	59 ± 12	NS	
SBP (mmHg)	128 ± 18	138 ± 17	<0.001	132 ± 15	135 ± 15	NS	
Hypertension	64%	87%	<0.001	79%	87%	NS	
Statins	40%	68%	<0.001	63%	71%	NS	
Antihypertensive Rx	40%	63%	<0.01	65%	74%	NS	
ALT (U/L)	15 (6)	18 (7)	<0.001	19 (9)	25 (15)	<0.001	
Triglycerides (mg/dL)	81 (42)	108 (67)	<0.001	102 (71)	126 (74)	<0.01	
FPG (mg/dL)	86 (13)	95 (16)	<0.001	94 (17)	97 (21)	<0.01	
BMI (Kg/m <sup>2</sup> )	24.8 ± 4.5	26.3 ± 4.4	<0.05	26.0 ± 2.9	27.5 ± 3.6	<0.001	
Total-CHOL (mg/dL)	209 ± 38	223 ± 37	<0.01	190 ± 39	200 ± 35	<0.05	
LDL-CHOL (mg/dL)	133 ± 38	146 ± 35	<0.01	127 ± 37	136 ± 35	<0.05	
HDL-CHOL (mg/dL)	68 ± 15	66 ± 15	NS	54 ± 15	51 ± 13	NS	
Active smokers	12%	12%	NS	22%	25%	NS	
History of CVD	16%	22%	NS	41%	39%	NS	
Diabetes	7%	13%	NS	19%	25%	NS	

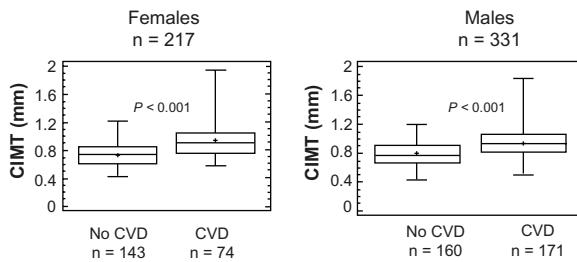
Note: Means ± SD, medians (interquartile range) and percentages.

Abbreviations: ALT, alanine transferase; BMI, body mass index; CVD, cardiovascular disease; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL-CHOL, high-density lipoprotein-cholesterol; LDL-CHOL, low-density lipoprotein-cholesterol; NS, nonsignificant; SBP, systolic blood pressure; SD, standard deviation.

The flat behavior of CIMT by GGT levels diverged quite sharply from the carotid thickening shown by patients with a history of CVD as compared with those without, a statistically significant ( $P < 0.001$ ) pattern unaffected by the adjustment for age (Figure 2). Circulating GGT levels were comparable in patients with a history of CVD and not, either females (17 [9] versus 14 [8] U/L, n = 41 versus 176 respectively, NS) or males (26 [20] versus 28 [19] U/L, n = 133 versus 198 respectively, NS).

## Clinical and demographic characteristics by MetS status

Besides the expected liver enzyme elevations<sup>4</sup> and definition-related<sup>10</sup> modifications of the metabolic and pressor profile (Table 4), CIMT was higher in patients with MetS (Figure 3)



**Figure 2** Box-and-whisker plots of CIMT by history of cardiovascular disease (CVD) in females (left panel) and males (right panel).

Notes: The central box encloses the middle 50% of the data; the horizontal line inside the box represents the median, and the mean is plotted as a cross. Vertical lines (whiskers) extend from each end of the box and cover four interquartile ranges.

and was unchanged after adjusting for GGT levels (GGT-corrected means [95% CI]: 0.82 [0.80–0.84] versus 0.88 [0.86–0.92] mm,  $P < 0.01$ ).

## Discussion

### The lack of relationship between GGT and CIMT

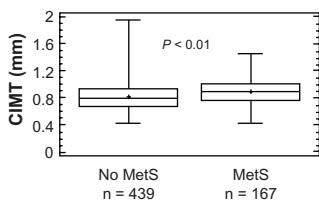
Our cross-sectional evaluation of a large and rather heterogeneous group of hypertensive and/or diabetic patients widely affected by CVD shows a lack of association

**Table 4** Demographic and clinical characteristics by MetS status

Variables	No MetS n = 439	MetS n = 109	P-level
GGT (U/L)	19 (16)	27 (15)	<0.001
ALT (U/L)	19 (9)	24 (18)	<0.001
Triglycerides (mg/dL)	96 (51)	186 (68)	<0.001
FPG (mg/dL)	94 (15)	115 (29)	<0.001
HDL-CHOL (mg/dL)	61 ± 15	45 ± 15	<0.001
BMI (kg/m <sup>2</sup> )	25.4 ± 3.6	29.6 ± 4.1	<0.001
SBP (mmHg)	132 ± 17	139 ± 16	<0.001
Diabetes	10%	46%	<0.001
Hypertension	65%	90%	<0.001
M/F	42%/58%	32%/68%	NS
Age (years)	60 ± 14	61 ± 12	NS

Note: Means ± SD, medians (interquartile range) and percentage.

Abbreviations: ALT, alanine transferase; BMI, body mass index; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL-CHOL, high-density lipoprotein-cholesterol; MetS, metabolic syndrome; NS, nonsignificant; SBP, systolic blood pressure; SD, standard deviation.



**Figure 3** Box-and-whisker plot of carotid intima-media thickness (CIMT) by National Cholesterol Education Program-Adult Treatment Panel III defined metabolic syndrome (MetS) status.<sup>10</sup>

**Notes:** The central box encloses the middle 50% of the data; the horizontal line inside the box represents the median, and the mean is plotted as a cross. Vertical lines (whiskers) extend from each end of the box and cover four interquartile ranges.

between circulating GGT levels and CIMT, a surrogate measure of systemic atherosclerosis.<sup>8</sup> This negative result was immediately evident in men and emerged quite clearly in women after accounting for the older age of those with higher GGT. That demographic influence was consistent with previous reports<sup>1</sup> of trends toward declining values in elderly males whose large representation in our sample may explain why age and GGT showed a different association profile between sexes. One might also ask whether the effect of statins on liver enzymes<sup>12</sup> might have contributed to the divergent pattern, but this is unlikely since statin treatment did not differ by GGT levels in males, and was appropriately more frequent in older females with higher GGT levels. The strength of these findings is augmented when contrasted with the carotid thickening that characterized patients with a history of CVD, a reassuring piece of evidence in agreement with the concept of carotid imaging as an indicator of the atherosclerotic burden across different vascular beds.<sup>8</sup>

Our results are inconsistent with the active contribution of GGT in the initiation and progression of atherosclerotic vascular disease, at least to the extent reflected by carotid imaging. This issue has been addressed by a few studies biased by low statistical strength of the reported correlations, limited sample size, unbalanced male-to-female ratios and, most importantly, missing adjustment for sex and age.<sup>13–15</sup> This latter limitation is of particular relevance when considering the confounding effect of demographic variables on CIMT in our study. This is in agreement with Volzke et al's study on a large sample of patients with nonalcoholic fatty liver disease (NAFLD)<sup>16</sup> in which anatomic alterations ranging from mere liver steatosis to steatohepatitis coexisted with elevated GGT and related metabolic abnormalities.<sup>17,18</sup> Our conclusions are further supported by negative results reported in several series of patients with NAFLD,<sup>19–22</sup> a condition that affected also an undefined but large portion of our patients, particularly those with higher ALT, a measure of

hepatic fat accumulation.<sup>23</sup> It must be recognized, however, that the issue of the NAFLD as a marker of more advanced carotid atherosclerosis is controversial<sup>24</sup> and our data cannot provide any evidence in favor or against that possibility since we have no information about the liver status of our patients.

## GGT, CIMT, and MetS

The clustering of elevated GGT and ALT levels with higher BMI, FPG, triglycerides, and BP by the NCEP-ATP III definition of MetS<sup>10</sup> agrees with the findings of previous epidemiological observations<sup>25</sup> linking the liver, the primary source of those enzymes,<sup>17,18</sup> to a biological phenotype at high risk of CVD and diabetes.<sup>26</sup> In concordance with previous studies<sup>27,28</sup> based on similar diagnostic criteria,<sup>10</sup> we found evidence of more advanced subclinical atherosclerosis in patients with MetS. More importantly, in light of our specific aims, the persistence of that difference after accounting for GGT implies an overcoming influence of metabolic abnormalities on atherosclerotic progression, which is fully concordant with other studies.<sup>29,30</sup>

## Limitations of the study

The conclusions of our study must be considered in the context of some important limitations. First and more importantly, cross-sectional studies such as this one may establish associations or lack thereof, but are weak tools for assessing mechanistic links. Second, the common carotid arteries might be less prone to atherosclerosis than the bulb or the internal carotid arteries<sup>31</sup> and the impact of cardiovascular risk factors may differ across carotid segments.<sup>32</sup> Moreover, carotid plaques, which were not considered in our study, could relate to GGT more tightly than CIMT<sup>16</sup> as a reflection of different biological and genetic determinants of the atherosclerotic process.<sup>33</sup> Third, the pervasive use of statins as well as ACEIs and ARBs – a group of drugs endowed with pleiotropic anti-inflammatory and antioxidant properties<sup>34,35</sup> – may have obscured associations discernible in untreated conditions. That source of confounding is impossible, however, to be circumvented in retrospective studies as ours. Fourth, circulating GGT includes several heterogeneous molecular fractions that are undifferentiated by routine assays of which only the b-fraction may be associated with cardiovascular risk factors and may penetrate the atherosclerotic plaque.<sup>36</sup> Fifth, the impact of GGT on CIMT may only be evident in patients with pathological GGT elevations that were excluded from our series to avoid confounding from liver disease other than NAFLD, given the absence of ultrasound

or biopsy verification. However, this possibility applies, by definition, to a minority of subjects.

In conclusion, higher GGT values bore no relationship to common carotid IMT, a surrogate measure of atherosclerotic vascular disease, in this large group of high-risk subjects. The data do not support the concept of a pathophysiological link between GGT levels within reference limits and atherosclerotic involvement although further studies are needed to evaluate this possibility.

## Author contributions

MN measured CIMT, PS and CG retrieved data from clinical records, GDO supervised clinical processing, AB provided input to result interpretation, RP wrote the paper and acted as senior author.

## Disclosure

The authors have no actual or potential conflict of interests including any financial, personal, or other relationships with people or organizations within 3 years of beginning the work submitted that could inappropriately influence their work.

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## Aging and hypertension decrease endothelial NO-related dilating function and gamma-glutamyltransferase activity but not S-nitrosoglutathione-induced aortic vasodilation

**Running title:** GGT and GSNO in aged SHR

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### ABSTRACT

S-nitrosoglutathione (GSNO), which is involved in the transport and the storage of NO, induces vasorelaxation. It requires gamma-glutamyl-transferase (GGT), an enzyme present on the endothelium, to transfer NO into the cell. We evaluated whether aging and hypertension, which induce NO-related dilating dysfunction, are associated with decreased vascular GGT activity and modify the vasorelaxant effect of GSNO.

Thoracic aortic rings isolated from male Spontaneous Hypertensive Rats (SHR) and Wistar Kyoto Rats (WKY) aged 20-22 (adult) or 57-60 weeks (mature) were preconstricted with phenylephrine, then submitted to concentration-vasorelaxant response curves (maximal response:  $E_{max}$ ;  $pD_2$ ) to GSNO and carbachol (the latter to measure NO-related dilating function). GGT activity was measured using chromogenic substrate.

Both aging and hypertension lowered  $E_{max}$  values for carbachol ( $E_{max}$  -8% in adult SHR, -42% in mature SHR *versus* age-matched WKY,  $p_{age}$  and  $p_{hypertension} < 0.05$ ) demonstrating NO-related dilating dysfunction. Aortic GGT activity also decreased with aging and hypertension (-22% in adult and -75%, reaching 3 nmol/min/g of tissue, in mature SHR *versus* 12 in age-matched WKY and 23 in adult WKY,

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*p<sub>age</sub>* and *p<sub>hypertension</sub>* < 0.05). The pD<sub>2</sub> values of GSNO were similar in mature SHR and WKY but higher in adult SHR (*p<sub>interaction</sub>* < 0.05).

Aging in hypertensive rats decreased NO-related vasorelaxant function and vascular GGT activity, but did not lower the vasorelaxant response to GSNO. This opens perspectives for GSNO-based therapeutics restoring nitric oxide bioavailability and vascular protection in a context of endothelial dysfunction.

**Keywords:** NO-dependent vasorelaxation, Spontaneous Hypertensive Rat, S-nitrosoglutathione, gamma-glutamyltransferase

## INTRODUCTION

S-nitrosoglutathione (GSNO), the nitrosated form of glutathione, is an endogenous low molecular weight S-nitrosothiol involved in the storage and transport of NO. Several studies reported the vasorelaxant properties [1,2] and/or hypotensive effects of GSNO [3], as its protective effects against platelet aggregation [4]. GSNO is an interesting candidate for therapeutics as it mimics endogenous GSNO-related functions. It is currently investigated as NO-donor to restore the decreased NO homeostasis occurring in many cardiovascular diseases such as hypertension or atherosclerosis, the latter mainly concerning large conductance arteries [5]. In the present study, we used aortic rings as model for studying integrative functions of GSNO.

Uptake of NO from GSNO into the cells requires the action of membrane enzymes [6]. Gamma-glutamyl transferase (GGT) is one of the enzyme implicated in the release of NO from GSNO and its uptake into the cell [7, 8]. GGT specifically catalyzes endogenous as exogenous GSNO breakdown producing cysteinylglycine and NO in endothelial cells. There, either NO diffuses into smooth muscle cells to activate the soluble guanylyl cyclase/cyclic guanosine monophosphate pathway and induce vasorelaxation, or it reacts with endothelial glutathione or proteins cysteine residue to form S-nitrosothiols [1, 2, 9].

We have previously documented that endothelial GGT is critical for GSNO-dependent NO-delivery and vasorelaxation in aortic rings isolated from normotensive rats [10]. More recently, we showed that hypertension in adult rats is associated with a slight endothelial NO-dependent dysfunction and a moderate decrease in aortic GGT activity. However, at this age, this does not impair GSNO-induced vasodilation and aortic rings of hypertensive rats even show enhanced sensitivity to GSNO [11].

In the present study, we evaluated GSNO-induced vasorelaxation in older animals, the mature spontaneous hypertensive rat (SHR), which represents a suitable model for aging and hypertension associated with NO-related dilating dysfunction. Our hypothesis was that endothelial dysfunction

may heavily impair GGT activity of the vessel wall, and thus, the bioactivity of exogenous treatment with GSNO would be modified. To this purpose we analyzed: (i) NO-related dilating function of SHR; (ii) the corresponding vascular GGT activities; (iii) the corresponding exogenous GSNO-inducible vasorelaxation.

## MATERIAL AND METHODS

### Chemicals

All reagents were of analytical grade and obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). Ultrapure deionized water ( $18.2\text{ M}\Omega\text{.cm}$ ) was used to prepare solutions. Standard solutions of GSNO were prepared by nitrosation of glutathione after mixing glutathione with sodium nitrite (ratio 1:1) in acidic medium according to the method previously described [12].

The purity of GSNO was assessed by ultraviolet spectrophotometry using its molar absorbance at 334 nm ( $\epsilon = 922\text{ M}^{-1}\text{.cm}^{-1}$ ).

### Rats and ethical statements

All experiments were performed in accordance with the European Parliament guidelines (2010/63/EU) for the use of experimental animals and the respect of the 3 Rs' requirements for Animal Welfare. The protocols and procedures were approved by the advisory regional ethical committee on animal experiments: Comité d’Ethique Lorrain en Matière d’Expérimentation Animale, CELMEA (project “NitroVivo”, APAFIS#1614-2015090216575422 v2).

Male young adult normotensive Wistar-Kyoto rats (WKY) or SHR (11 weeks-old, 300-325 g) were purchased from Janvier Laboratories (Le Genest St Isle, France), kept under standard conditions (temperature:  $21 \pm 1^\circ\text{C}$ , hygrometry  $60 \pm 10\%$ , light on 6 am to 6 pm). They ate standard diet (A04, Safe, Villemoisson-sur-Orge, France) and drank water (reverse osmosis system, Culligan, Brussels, Belgium) *ad libitum*, until 20-22 weeks of age (adult rats; mean body weight  $436 \pm 3$  and  $430 \pm 12$  g; mean systolic blood pressure, tail-cuff method,  $141 \pm 10$  and  $211 \pm 23$  mmHg in WKY and SHR, respectively) and until 57-60 weeks of age (mature rats; mean body weight  $598 \pm 9$  and  $411 \pm 20$  g; mean systolic blood pressure, tail-cuff method,  $146 \pm 7$  and  $207 \pm 8$  mmHg in WKY and SHR, respectively).

Rats were anesthetized with sodium pentobarbitone ( $60\text{ mg}\cdot\text{kg}^{-1}$ , intraperitoneal injection, Sanofi Santé Nutrition Animale, Libourne, France) and the adequacy of anesthesia was checked by testing the loss of the corneal and pinch paw withdrawal reflexes. If a change in the reflexes occurred, a bolus of sodium pentobarbitone was immediately administered. After administration of heparin

(1000 IU.kg<sup>-1</sup> heparine Choay, penis vein), rats were sacrificed by exsanguination and segments (3 cm) of the descending thoracic aorta were removed. Vessels were cleaned from surrounding connective tissues, cut into 2-mm long rings (8 rings per rat) and immediately used for vasoactivity. Some samples of aortic rings were frozen in liquid nitrogen and kept at -80°C until biochemical studies were analyzed.

### Vasorelaxation studies

Vasorelaxation was evaluated on endothelium-intact aortic rings [10]. Aortic vasoactivity was measured using an isometric tension recording system in 10 mL organ chambers (EMKABATH, Emka Technology, France). All manipulations and assays involving GSNO were performed under conditions of subdued light, in order to minimize light-induced degradation. The bath was filled with Krebs' solution containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 1.6 mM CaCl<sub>2</sub>, 24 mM NaHCO<sub>3</sub>, 5.5 mM glucose, adjusted to pH 7.4 (10 mL, 37°C) and continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Following 60-min equilibration with a basal resting tension determined at 2 g, rings were exposed 2 times to KCl (60 mM, 5 min). Aortic rings ( $n = 5 - 13$  per group, from 3 - 8 different rats in each group) were then preconstricted with 10<sup>-6</sup> M phenylephrine. At the plateau of contraction, concentration-relaxation response curves to increasing concentrations of GSNO (10<sup>-10</sup> to 3.10<sup>-5</sup> M) were performed. The role of GGT was assessed in adult rats by stimulating GGT activity with the exogenous  $\gamma$ -glutamyl acceptor glycylglycine (20 mM), as well as by inhibition with the serine-borate complex (SBC, 20 mM), a competitive reversible inhibitor [10].

NO-related dilating dysfunction was evaluated by measuring the ability of carbachol, a muscarinic acetylcholine receptors agonist, to relax preconstricted aortic rings. Decreases in maximal response ( $E_{max}$ ) to carbachol (10<sup>-10</sup> to 10<sup>-5</sup> M response curves) witness NO-related dilating dysfunction [13, 14].

### GGT activity in aorta

GGT activity was measured spectrophotometrically after hydrolysis of the synthetic GGT substrate *L*- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide as previously described [15]. Briefly, aortic rings were homogenized and incubated for 2 h at 37°C in Tris buffer (100 mM, pH 7.4) containing 1 mM *L*- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide, 20 mM glycylglycine and 10 mM MgCl<sub>2</sub>. After centrifugation at 42,000  $\times g$  for 10 min at 4°C, supernatant absorbance was read at 405 nm to monitor the release of 5-amino-2-nitrobenzoate ( $\epsilon = 9500 \text{ M}^{-1}.\text{cm}^{-1}$ ) from *L*- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide. Enzyme activities are expressed in nmol of 5-amino-2-nitrobenzoate per min per g of tissue.

## Data analysis and statistical tests

Relaxant responses to GSNO or carbachol were given as the percentage of  $10^{-6}$  M phenylephrine precontraction and calculated as:

$$\% \text{ of relaxation} = [\text{Tension (PHE } 10^{-6} \text{ M, g)} - \text{Tension (GSNO or carbachol, g)}] / \text{Tension (PHE } 10^{-6} \text{ M, g)} \\ - \text{Tension (BASELINE, g)}] \times 100.$$

The half maximal effective concentration ( $EC_{50}$ ) and maximal response ( $E_{max}$ ) were calculated by fitting each individual concentration response curve using the Hill logistic equation (Graph Pad prism® software version 5.0):

$$\% \text{ relaxation} = E_{min} + ((E_{max} - E_{min}) / (1 + 10^{((\log EC_{50} - \text{concentration}) \times \text{Hill slope}))})$$

where  $E_{min}$  and  $E_{max}$  = minimal and maximal response reached in each concentration-response curve.

The  $pD_2$  was calculated as  $-\log EC_{50}$ .

After modelling individual concentration response curve, means  $\pm$  S.E.M. of  $E_{max}$  and  $pD_2$  were analyzed by a 2-ways (age, hypertension) ANOVA followed by a post-hoc Bonferroni test. The null hypothesis was rejected at  $p < 0.05$ . A 2-ways (GGT modulators, hypertension) ANOVA was also performed to analyze GSNO  $pD_2$  values in 20-22 weeks SHR and WKY rats.

All data are shown as means  $\pm$  S.E.M

## RESULTS

All the concentration response curves to carbachol fitted the Hill model and gave similar  $pD_2$  values, around 7.1-7.4 in all groups (Figure 1). Both aging and hypertension lowered  $E_{max}$  values for carbachol ( $E_{max}$  -8% in adult SHR, -42% in mature SHR versus age-matched WKY,  $p_{age}$  and  $p_{hypertension} < 0.05$ ) demonstrating NO-related dilating dysfunction.

Thoracic aortic GGT activity followed similar trends ( $p_{age}$  and  $p_{hypertension} < 0.05$ ) with a -22% decrease in adult and -75% in mature SHR, versus age-matched WKY (Figure 2).

Concentration-response curves to GSNO reached similar  $E_{max}$  in all groups (Figure 3). The values for  $pD_2$  were similar in mature SHR and WKY and higher in adult SHR ( $p_{interaction} < 0.05$ ).

The influence of GGT modulators to GSNO-induced vasorelaxation in adult SHR was similar to that of age-matched WKY, with higher responses to GSNO in adult SHR in all circumstances (Figure 4,  $p_{GGT \text{ modulators}}$  and  $p_{hypertension} < 0.05$ ). Indeed, inhibition of GGT activity with SBC was associated with a decrease in the vasorelaxant effect of GSNO (decrease in its  $pD_2$  value) both in adult WKY and SHR group. On the opposite, the  $pD_2$  value for GSNO increased after activation of the enzyme with glycylglycine.

## DISCUSSION

Our previous work on adult SHR, submitted (or not) to a high salt diet showed that hypertension is accompanied by a decreased activity of GGT, the main enzyme catalyzing the release of bioactive NO from GSNO [11]. Nevertheless, vasorelaxation induced by GSNO was slightly improved. In the present study, we evaluated GSNO-induced vasorelaxation in older animals, the mature SHR, with a marked NO-related dilating dysfunction, and showed strong decline in aortic wall GGT activity. Despite, bioactivity of an exogenous treatment with GSNO remained stable (while, again, it increased in adult SHR).

The degree of severity of endothelial dysfunction is commonly evaluated on the basis of a decrease in either  $pD_2$  and/or  $E_{max}$  of the concentration-response curves to carbachol or acetylcholine (endothelial muscarinic receptor agonists). This mainly reflects NO-related dilating dysfunction. We and Isabelle et al. [16] showed strong NO-dependent dysfunction in mature SHR ( $E_{max}$  -60% in Isabelle et al. 2012; -42% present Figure 1). It has however to be noticed that global endothelial dysfunction in SHR involves not only decreased NO bioavailability but also decreased production of endothelium-hyperpolarizing factor and prostacyclin [17, 18], higher production of endothelium-dependent vasoconstrictive agents such as endothelin-1 [19, 20]. However, endothelial-dependent hyperpolarisation is a mechanism of relaxation more important in resistance vessels than in larger calibre vessels as aorta. Furthermore, there are structural changes (e.g., vascular wall thickening, smooth muscle hypertrophy, vascular inflammation inducing alteration of the extracellular matrix, vascular infiltration and activation of immune cells leading to vascular remodeling), especially with aging, that can additionally impede the endothelium's ability to relax vessels [19, 21].

Several studies showed that serum GGT levels was increased in cardiovascular diseases [22]. Besides the soluble enzyme found in serum, GGT is also present at cellular level in vascular tissues and is highly expressed in arterial endothelium [23]. We have previously shown that such GGT activity is involved in the utilization of GSNO, in that it promotes the local release of NO from GSNO thus mediating its vasorelaxant effect [10]. Thus, damages of the endothelial cells occurring during aging in SHR affect endothelial GGT enzymatic function. Contrary to our hypothesis, this decline in aortic GGT activity did not induce any decrease in the vasorelaxant effect of GSNO. In adult SHR, GSNO-induced vasodilation still depends on GGT, as shown by our experiments performed in the presence of either an activator or an inhibitor of the enzyme. At this age, GSNO is even more potent in SHR than in WKY rats (present higher  $pD_2$  values, and [11]). In the latter paper, we suggested that (i) even if it decreases, the GGT activity remains sufficient to maintain GSNO vasorelaxant effect and/or (ii) other pathways involved in the denitrosation process of GSNO are substituting for GGT. Membrane

PDI, a membrane enzyme from the redoxins family, which is involved in the release of NO from GSNO and in GSNO vasorelaxant effect [9], increased its expression under oxidative stress [24, 25]. Moreover, hypertension and ageing present oxidative stress, *e.g.* through upregulation of NOX [11, 26, 27, 28]. Moreover, we previously showed [11] that inhibition of PDI with bacitracin did decrease the pD<sub>2</sub> values of GSNO in adult SHR. Therefore, the decreased GGT activity occurring during hypertension and/or endothelial dysfunction may be balanced by an oxidative stress-related increase in PDI expression and/or activity, maintaining the vasorelaxant effect of GSNO in mature SHR, and even slightly improving it in adult SHR.

## CONCLUSION

In conclusion, NO-related dilating dysfunction in mature SHR is accompanied by a 75% decline of GGT aortic wall bioactivity, one of the main enzymes catalyzing the release of bioactive NO from GSNO. Nevertheless, vasorelaxation induced by GSNO is unaffected. As many cardiovascular diseases are associated with a decreased bioavailability of NO, leading to impaired vasodilation, pro-inflammatory/oxidative, pro-proliferative and pro-thrombotic status [29, 30], our results open new perspectives to further development of GSNO-based therapeutics for restoring nitric oxide bioavailability and vascular protection in a context of endothelial dysfunction. Atherosclerosis, for example, which mainly concerns large conductance arteries where vasoactive functions depend specifically on the bioavailability of nitric oxide NO [5, 31] leads to the use of several NO donors (organic nitrates) for therapeutics. However, these treatments are known to provide fast NO release concomitant with induction of oxidative stress and tolerance [32, 33] and new NO-donors, such as S-nitrosothiols are interesting therapeutic alternatives as they do not present the drawbacks of organic nitrates.

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Gender differences in the association of hypertension with gamma-glutamyltransferase and alanine aminotransferase levels in Chinese adults in Qingdao, China

### **Abstract**

### **Objective**

To study the associations of hypertension with gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) levels.

### **Methods**

Data of 3575 men and 5504 women were analyzed. Multivariable logistic regression analysis was performed to estimate the odds ratio (OR) for hypertension with GGT and ALT.

### **Results**

Compared with the lowest quartile, the multivariate adjusted ORs for hypertension were 0.97(0.79, 1.19) in men and 0.88(0.74, 1.04) in women for ALT and 2.29(1.68, 3.14) and 1.52(1.27, 1.83) for GGT in the highest quartile group. The ORs for hypertension in the low waist circumference (WC) category were 2.61(1.56, 4.36) in men and 1.41(0.94, 2.12) in women, and in the high WC category 4.01(2.21, 7.29) and 2.26 (1.54, 3.32) for GGT.

### **Conclusions**

The elevated GGT, but not ALT, was associated with the presence of the hypertension in men and women. The association is stronger in obese men and women than in their lean counterparts.

## Introduction

Serum gamma-glutamyltransferase (GGT) is commonly used as an indicator of alcohol consumption and oxidative stress [1, 2]. Another liver enzyme, alanine aminotransferase (ALT), is the most specific marker of liver pathology, and a strong bio-marker for liver fat accumulation and hepatic insulin sensitivity [3, 4]. Recently, emerging evidence suggests GGT and ALT are associated with the presence of hypertension [5-16].

However, the levels of ALT and GGT differ between men and women, with higher values observed in men [17, 18], only a few studies have compared these liver enzymes for their associations with hypertension separately by men and women [15, 18-20], and it is not entirely clear to clarify if there is a difference in this association between men and women. Moreover, both ALT and GGT are associated with obesity, it is, thus, important to check whether the associations of elevated blood pressure levels with ALT and GGT are not confounded by obesity.

In this study, first, the association of hypertension with serum GGT and ALT levels is examined separately by men and women in a Chinese adult living in Qingdao, China. Second, a stratified analysis by the waist circumference (WC) levels was, thus, performed to check whether the association between the serum GGT and ALT with hypertension depend on obesity in men and women.

## Research design and methods

### Study population

Population-based cross-sectional surveys were conducted separately in 2006 and 2009 in Qingdao, China. A stratified, random cluster sampling method was used to recruit a representative sample of the general population aged 35-74 years old for all surveys. Both surveys were conducted in the same three urban districts (Shinan, Shibei and Sifang) and three rural counties (Jiaonan, Huangdao and Jimo). Five residential communities from each area with 200-250 individuals from each community were randomly selected, and a total of 6100 individuals were invited to the survey in 2006 and 6000 individuals in 2009, respectively. All participants were invited to a survey site near their resident communities. Similar approaches were applied in two surveys. The number of participants in each survey was 5355 (giving a response rate of 87.8%) in 2006 and 5165 (giving a response rate of 86.1%) in 2009.

Each survey participant completed a questionnaire and underwent a detailed medical examination by a trained doctor or nurse. Waist circumference (WC) was calculated at the umbilical level. Height and weight was measured with participants wearing light clothes and without shoes. Body mass index (BMI) was then measured by dividing weight (kg) by height (m) squared ( $\text{kg}/\text{m}^2$ ). Blood pressure was measured with mercury sphygmomanometer (Yuyue, China). Three consecutive blood pressure readings, apart by at least 30 seconds, were taken from the right arm of seated subjects in a quiet room, and the average of the three readings was used in the data analysis. The alcohol-drinking was classified as heavy drinkers (with an alcohol intake of  $\geq 40$  gram per day), moderate drinkers (with an alcohol intake of  $<40$  gram per day) and

non-drinkers (including ex-drinking or not drinking at all) [21]. The smoking status was defined as current smokers (smoking every day) and non-smokers (including ex-smoking, smoking now and then and not smoking at all). A family history of hypertension was classified as having at least one of parents, siblings or offsprings with diagnosed hypertension. Education levels was divided into two levels ( $\leq 9$  or  $>9$  school years). Blood samples were collected locally and all participants were informed to be fast at least 10 hours before blood samples were collected. The lab assays were performed in the central laboratory of Qingdao Hiser Medical Center using Olympus AU analyzers in 2006 and in Qingdao Endocrinology and Diabetes Hospital using Hitachi AU analyzers in 2009. Fasting serum triglycerides (TG) and total cholesterol (TC) were determined by enzymatic method while fasting serum high-density lipoprotein cholesterol (HDL-C) by direct method. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Fasting plasma glucose (FPG) were determined by the glucose oxidize method. ALT and GGT were measured by using an International Federation of Clinical Chemistry (IFCC) method. The concentration of fasting insulin was measured using the chemiluminescence immunoassay method (Abbott AxSym). The index of the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula [HOMA-IR =fasting insulin (mU/L)  $\times$  FPG (mmol/L)/22.5].

The inclusion criteria for the current study were participants who had no data missing for age, BMI, WC, alcohol status, smoking status, lipids, FPG and blood pressure.

Finally, a total of 9079 (40% men) subjects were included in the analysis. The two surveys were approved by the Ethic Committee of Qingdao Municipal Hospital and Qingdao Municipal Center for Disease Control and Prevention, respectively. Verbal and written consent was obtained from each participant before the data collection.

### **Classification of hypertension**

Newly diagnosed hypertension was defined as systolic blood pressure (SBP)  $\geq 140$  mm Hg and /or diastolic blood pressure (DBP)  $\geq 90$  mm Hg. Subjects who reported a history of hypertension and/or who were under treatment with oral anti-hypertensive medication were considered as previously diagnosed hypertension, regardless of their blood pressure levels. There was no difference in the mean levels of ALT and GGT between the subjects with a history of hypertension and the subjects with newly diagnosed hypertension, therefore newly and previously diagnosed hypertension were included in the data analysis.

### **Statistical analysis**

Data were summarized as mean ( $\pm$  standard error) for continuous variables and proportions for categorical variables. Due to skewed distribution, values derived from logarithmically transformed means were used for GGT and ALT in data analysis. The general linear model approach for continuous variables and a chi-square test for categorical variables were used to compare differences in age-adjusted means and prevalence between hypertension and normotension in both surveys. The linear association of ALT and GGT with SBP and DBP was tested using multivariable linear

regression model, adjusting for age, school years, family history of hypertension, *current smoking, alcohol-drinking, BMI, HDL and TG*, and the standard  $\beta$  coefficients and 95% confidence interval (CI) were calculated. The *multivariable logistic regression was performed to investigate the association of hypertension with serum ALT and GGT levels in both genders, adjusting for age, current smoking, alcohol-drinking, school year, family history of hypertension, BMI, HDL and TG*. We divided the serum values of ALT and GGT into four groups based on gender-specific quartiles (25th, 50th and 75th percentiles). Odds ratios (ORs) (95% CI) of hypertension were tested for each ALT and GGT quartiles, with the lowest quartiles as the reference.

The participants between two surveys were not different in mean values of age, BMI, WC, blood pressure, FPG and lipids, and there might be a few variables in which the two surveys seemed to differ, such as the proportions of heavy drinkers, so we pool data from both surveys for data analysis. The distribution of BMI is normal, so a linear measure of the BMI was fitted in the model as a co-variable. To examine the quadratic association, a squared term of the BMI was added into a model together with the linear BMI, and the result showed the linear associations was significant. Moreover, the linear associations between other continuous variables with the presence of hypertension were validated also, and the results remained no changes. Thus a linear measure of the BMI and other continuous variables were fitted into the *multivariable logistic regression model*. All analyses were performed using SPSS (Version15.0; SPSS Inc, Chicago, IL, USA) or SAS 9.3 (SAS Institute, Cary, NC). A p-value less than 0.05 (two tailed) was

considered statistically significant.

## Results

The baseline characteristics of the study population in two surveys were shown in table 1. The subjects with hypertension were older, more obese, had greater levels of FPG and lipids in men and women in comparison with individuals with normotension. They also had significantly higher levels of GGT, but the mean levels of ALT were higher only in men with hypertension. The proportions of family history of hypertension, current smoker and heavy drinker were not statistically significant between hypertensive and non-hypertensive groups of either gender.

The standard beta coefficients and 95% CI were summarized in table 2. The correlation coefficients for GGT-SBP, GGT-DBP were statistically significant in men and women, whereas those for ALT were significant with DBP in men. There is no major differences in the contributions of GGT with SBP between the two surveys but slight discrepancies are identified in the standard beta coefficients of GGT with SBP between two surveys.

Compared with the lowest quartile, the multivariable adjusted ORs for hypertension was significantly higher within the top three quartiles of the GGT in men and women. The relationship of hypertension with ALT was positive merely in men, but faded significantly when BMI was adjusted into the model (table 3).

In addition, a positive interaction of the GGT with the WC in men ( $P<0.001$ ) and women ( $P<0.001$ ) was discovered, a stratified analysis according to the quartiles of WC

was performed to further check the association between GGT and hypertension in low and high WC categories. The ORs for hypertension in the low waist circumference (WC) category were 2.61(1.56, 4.36) in men and 1.41(0.94, 2.12) in women, and in the high WC category 4.01(2.21, 7.29) and 2.26 (1.54, 3.32) for GGT. The association between GGT and hypertension is stronger in high WC categories than in low one in both genders (table 4).

## Discussions

In these population-based cross-sectional studies, we demonstrated that increased serum GGT levels were positively associated with the presence of hypertension in men and women. Such an association was, however, not observed for the ALT. The effect of elevated GGT on hypertension was significant only in obese women, but GGT was positively associated with hypertension in both lean and obese men. Specifically, the association between and hypertension appeared to be stronger in obese men and women than in their lean counterparts.

To the best of our knowledge, many cross-sectional and longitudinal studies have investigated the association between GGT and hypertension [5-10, 13-16]. However, only a few previous studies that examined sex-specific association between concentrations of GGT with hypertension demonstrated inconsistent results and none of these studies have been carried out in Chinese [15, 17, 19, 20]. A population-based prospective study including 1556 men and 1889 women aged 35-69 years old showed the positive association only in men [19]. But another prospective population-based

study including 1171 men and 1267 women aged 20-54 years old performed in Norway showed a weak association between GGT levels and blood pressure only in women but not in men [17]. In the prospective population-based study including 1167 adults aged 33-84 years old performed in Turkey, the positive association between GGT levels and the presence of hypertension was significant in men and women [15], which consisted with our results.

The mechanisms underlying the association of the development of hypertension with the elevated levels of GGT have not been fully addressed and several possible mechanisms may explain this. First, GGT might be an early sensitive enzyme related to oxidative stress [2], and oxidative stress may play an important role in the initiation and progression of hypertension in the long run [8], which suggested that the significant association of hypertension with the elevated levels of GGT might be explained by oxidative stress. Second, prospective study indicated that increased GGT activity is significantly associated with inflammation markers, such as white blood cell (WBC) count, C-reactive protein, fibrinogen and F2-isoprostanes [8, 22, 23], so high GGT levels could be a marker of subclinical inflammation, which was a causal factor of hypertension. Finally, GGT was shown to be associated with insulin resistance [24, 25], and insulin resistance existed in subjects with hypertension [26, 27].

In our study, the effect of elevated GGT on hypertension was significant only in obese women, but GGT was positively associated with hypertension in both lean and obese men. Specifically, the association between GGT and hypertension appeared to be

stronger in obese men and women than in their lean counterparts. This indicated that obesity enhances the effect of GGT on blood pressure. This is partly due to the fact that obesity increases the GGT concentrations [9], and all these may explain the weak association between GGT and hypertension in women who had low waist circumference. The other reason might explain our finding that GGT was associated with hypertension only in obese women might be sex hormone levels, such as the sex hormones binding globulin (SHBG), which are lower in obese women than in lean women. So the decrease in SHBG contributes to the high GGT concentration [28]. This may largely explain the association of GGT with hypertension in obese women observed in our current study.

Our results lent support to the previous studies showing that there is not an independent association between the presence of hypertension with the elevated serum levels of ALT in men and women in this Chinese population [9, 14]. It is possible that higher serum levels of ALT is an early reflection of hepatic steatosis and considered a more liver-specific marker than GGT [29, 30], but GGT is the enzyme for the extracellular catabolism of antioxidant glutathione and commonly used as an indicator of oxidative stress [1, 2, 31]. The lack of relationship between the presence of hypertension with the elevated serum levels of ALT further showed that the association of GGT with hypertension may largely depend on oxidative stress rather than merely liver injury.

The main strengths of our study are: (1) this is a population-based study with a random

sampling approach and represents the general population in China; (2) the collaborative analysis of two surveys can increase the statistical power, and the sample size of the study is large enough to examine sex-specific associations between the presence of hypertension with the liver enzymes; However, the cross-sectional nature of present study limits it from going further to investigate the direct causation between elevated GGT levels and hypertension. In addition, self-reported alcohol consumption was questionable because of its validity and reliability.

### **Conclusions**

In summary, the elevated serum GGT levels, but not ALT, were independently associated with the presence of the hypertension in men and women in this Chinese population. The association is stronger in obese men and women than in their lean counterparts. Further study on the natural relationship between GGT concentration and hypertension as well as the effect of obesity on the relationship is warranted.

### **Acknowledgement**

We are grateful to the participants, primary care doctors and nurses who took part in the survey. We would also like to thank the World Diabetes Foundation (WDF05-108&07-308) for supporting a large community-based diabetes prevention project (QD-DPP) which provides with a platform for carrying out the survey; and for the Bayer Healthcare in China and Lifescan of the Johnson & Johnson Company in China for providing support in the field surveys.

**Declaration of competing interests:** Nothing to declare.

Table1. Baseline characteristics of participants in two surveys.

	Men		Women	
	Normotension	Hypertension	Normotension	Hypertension
Number (%)	1668(46.6%)	1907(53.4%)	2918(53.0%)	2586(47.0%)
Age (years)	49.5(49.3,49.7)	53.4(53.1,53.7)*	47.8(47.6,48.0)	55.0(54.8,55.2)*
School years> 9 (yes, %)	23.6	19.9*	26.6	8.5*
Current smoking (yes, %)	21.3	19.6	1.0	0.5
Current drinking (%)				
non-drinkers	72.7	72.5	99.1	99.3
Moderate-drinkers	12.1	14.0	0.9	0.7
heavy-drinkers	15.2	13.5	0	0
Family history of hypertension (yes, %)	12.1	12.9	11.6	13.1
Body mass index (kg/m <sup>2</sup> )	24.2(23.5,24.9)	25.9(25.1,26.7)*	24.5(23.9,25.1)	26.7(26.1,27.2)*
Waist circumference (cm)	83.5(83.2,83.8)	87.2(86.9,87.5)*	79.6(79.7,79.8)	85.2(85.0,85.4)*
Fasting plasma glucose(mmol/L)	5.77(5.72,5.62)	6.17(6.11,6.23)*	5.65(5.62,5.68)	6.25(6.14,6.36)*
Haemoglobin A1c	5.50(5.47,5.53)	5.65(5.62,5.67)*	5.44(5.37,5.51)	5.76(5.68,5.84)*
HOMA-IR	10.9(9.7,12.1)	12.4(10.5,14.3)	12.1(11.2,13.0)	16.7(14.8,18.6)*
Low density lipoprotein cholesterol (mmol/L)	2.84(2.77,2.91)	3.02(2.94,3.10)*	2.89(2.85,2.93)	3.13(3.06,3.20)*
High density lipoprotein cholesterol (mmol/L)	1.61(1.58,1.64)	1.62(1.59,1.65)	1.67(1.62,1.72)	1.61(1.57,1.65)
Total cholesterol (mmol/L)	5.15(5.11,5.19)	5.35(5.30,5.40)*	5.09(5.07,5.11)	5.49(5.42,5.56)*
Triglyceride (mmol/L)	1.40(1.36,1.44)	1.59(1.56,1.62)*	1.21(1.15,1.27)	1.56(1.47,1.65)*
Alanine aminotransferase (U/L) §	20.4(19.7,21.1)	22.1(21.2,23.0)*	17.5(16.3,18.7)	18.7(17.4,19.1)
Gamma-glutamyltransferase (U/L) §	23.9(22.6,25.2)	29.5(27.8,31.2)*	14.6(13.2,16.0)	17.9(16.4,19.4)*

Data are age-adjusted mean (95% confidence interval) or number (percentages) indicated. \*P<0.05, hypertension vs. normotension

by the same survey within the same gender. §Geometric mean (95% CI). || With missing data. HOMA-IR, homeostasis model

assessment of insulin resistance.

Table2. Multiple linear regression analysis for alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) in association with diastolic blood pressure and systolic blood pressure.

	Diastolic blood pressure		Systolic blood pressure	
	Standard β coefficients	95%CI	Standard β coefficients	95%CI
<b>Men</b>				
ALT(U/L)	0.09*	(0.08, 0.11)	0.03	(0.01, 0.05)
GGT(U/L)	0.07*	(0.05, 0.08)	0.11*	(0.10, 0.13)
<b>Women</b>				
ALT(U/L)	0.04	(0.03, 0.05)	0.05	(0.04, 0.06)
GGT(U/L)	0.07*	(0.06, 0.08)	0.09*	(0.07, 0.10)

Adjusted for age, school years, family history of hypertension, *current smoking*, *alcohol-drinking*, body mass index, triglycerides and high density lipoprotein cholesterol. ALT and GGT are logarithmic transformed. \* P<0.01.

Table3. Odds ratio (95% confidence interval) for hypertension in relation to quartiles of alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) concentrations.

		Model1	Model2	Model3	Model4	Model5	Model6
<b>Men</b>	<b>Number</b>						
<b>ALT</b>							
Q1 (<=10 U/l)	820	1	1	1	1	1	1
Q2 (10-15 U/l)	871	0.97(0.79, 1.20)	1.01(0.83,1.27)	1.06(0.85,1.32)	1.04(0.83,1.29)	1.04(0.83,1.30)	1.02(0.82,1.27)
Q3 (15-22 U/l)	962	0.90(0.74,1.12)	0.99(0.80,1.22)	0.97(0.78,1.22)	0.94(0.75,1.17)	0.94(0.75,1.17)	0.89(0.71,1.11)
Q4 (>22 U/l)	922	1.36(1.15, 1.64)	1.31(1.08,1.57)	1.17(0.98,1.52)	1.10(0.90,1.34)	1.10(0.91,1.35)	0.97(0.79,1.19)
P for trend		P=0.003	P=0.005	P=0.243	P=0.442	P=0.436	P=0.667
<b>GGT</b>							
Q1 (<=16U/l)	844	1	1	1	1	1	1
Q2 (16-24 U/l)	883	1.47(1.07, 2.10)	1.50(1.07,2.10)	1.55(1.09,2.10)	1.55(1.09,2.20)	1.50(1.05,2.13)	1.50(1.05,2.13)
Q3 (24-38 U/l)	938	2.13(1.55, 2.93)	2.13(1.55,2.93)	2.04(1.47,2.84)	1.99(1.43,2.47)	1.95(1.40,2.71)	1.95(1.40,2.71)
Q4 (>38 U/l)	910	3.59(2.68, 4.83)	3.41(2.54,4.60)	2.48(1.82,3.38)	2.33(1.70, 3.181)	2.29(1.68,3.14)	2.29(1.68,3.14)
P for trend		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
<b>Women</b>							
<b>ALT</b>							
Q1 (<=8 U/l)	1322	1	1	1	1	1	1
Q2 (8-12 U/l)	1376	0.86(0.74, 1.01)	0.87(0.74,1.02)	0.87(0.75,1.03)	0.87(0.73,1.02)	0.87(0.74,1.02)	0.87(0.74,1.02)
Q3 (12-18 U/l)	1455	0.82(0.69, 1.02)	0.83(0.71,1.03)	0.88(0.74,1.04)	0.86(0.72,1.02)	0.86(0.73,1.02)	0.86(0.73,1.02)
Q4 (>18 U/l)	1351	1.02(0.87, 1.18)	1.02(0.87,1.19)	0.98(0.83,1.16)	0.93(0.79,1.10)	0.93(0.79,1.10)	0.88(0.74,1.04)
P for trend		P=0.066	P=0.034	P=0.121	P=0.234	P=0.242	P=0.235
<b>GGT</b>							
Q1 (<=11U/l)	1343	1	1	1	1	1	1
Q2 (11-14 U/l)	1458	1.35(1.15, 1.59)	1.34(1.14,1.58)	1.20(1.02,1.41)	1.17(0.99,1.40)	1.17(0.98,1.38)	1.16(0.98,1.38)
Q3 (14-20 U/l)	1376	2.06(1.75, 2.45)	2.05(1.72,2.44)	1.56(1.30,1.88)	1.50(1.25,1.80)	1.47(1.22,1.77)	1.47(1.22,1.77)
Q4 (>20 U/l)	1327	2.42(2.04, 2.85)	2.39(2.02,2.83)	1.67(1.40,2.00)	1.57(1.32,1.88)	1.52(1.27,1.83)	1.52(1.27,1.83)
P for trend		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Model1: Adjusted for age.

Model2: Adjusted for age, school years, alcohol-drinking, current smoking, family history of hypertension and survey years.

Model3: Model2+ body mass index.

Model4: Model3+ triglycerides + high-density lipoprotein cholesterol.

Model5: Model4+ fasting plasma glucose.

Model6: GGT and ALT fitted simultaneously into the Model5.

Table 4. Odds ratio (95% confidence interval) for hypertension in relation to quartiles of gamma-glutamyltransferase (GGT) concentrations stratified by waist circumference categories.

	Odds ratio (95% CI)	P value
<b>Men</b>		
<b>Waist circumference (&lt;79cm) (n=892)</b>		
GGT		
Q1 (<15U/l)	1	
Q2 (15-19 U/l)	1.33(0.76,2.35)	0.314
Q3 (19-26 U/l)	1.75(1.03,2.99)	0.037
Q4 ( $\geq 26$ U/l)	2.61(1.56,4.36)	<0.001
<b>Waist circumference (79-87cm) (n=893)</b>		
GGT		
Q1 (<16U/l)	1	
Q2 (16-22 U/l)	1.55(0.84, 2.87)	0.109
Q3 (22-34 U/l)	2.72(1.25,4.10)	0.009
Q4 ( $\geq 34$ U/l)	2.68(1.54,4.65)	0.001
<b>Waist circumference (87-94cm) (n=930)</b>		
GGT		
Q1 (<19U/l)	1	
Q2 (19-28U/l)	1.86(0.87, 3.98)	0.020
Q3 (28-45 U/l)	2.58(1.26, 5.28)	0.001
Q4 ( $\geq 45$ U/l)	3.10(1.62, 5.96)	<0.001
<b>Waist circumference (<math>\geq 94</math>cm) (n=860)</b>		
GGT		
Q1 (<23U/l)	1	
Q2 (23-33 U/l)	2.25(1.14, 4.46)	0.001
Q3 (33-53 U/l)	3.12(1.64, 5.94)	<0.001
Q4 ( $\geq 53$ U/l)	4.01(2.21, 7.29)	<0.001
<b>Women</b>		
<b>Waist circumference (&lt;76cm) (n=1363)</b>		
GGT		
Q1 (<10U/l)	1	
Q2 (10-14 U/l)	1.20(0.88,1.65)	0.243
Q3 (14-17U/l)	1.27(0.92,1.89)	0.177
Q4 ( $\geq 17$ U/l)	1.41(0.94,2.12)	0.096
<b>Waist circumference (76-83cm) (n=1414)</b>		
GGT		
Q1 (<12U/l)	1	
Q2 (12-15 U/l)	0.96(0.69, 1.32)	0.800

Q3 (15-21U/l)	1.25(0.88, 1.78)	0.199
Q4 ( $\geq 21\text{U/l}$ )	1.16(0.82, 1.64)	0.409

**Waist circumference (83-90cm) (n=1332)**

GGT

Q1 (<12U/l)	1	
Q2 (12-17U/l)	1.32(0.94, 1.85)	0.107
Q3 (17-22 U/l)	1.87(1.32, 2.64)	<0.001
Q4 ( $\geq 22\text{U/l}$ )	2.07(1.48, 2.91)	<0.001

**Waist circumference ( $\geq 90\text{cm}$ ) (n=1395)**

GGT

Q1 (<14U/l)	1	
Q2 (14-19U/l)	1.57(1.05, 2.35)	0.028
Q3 (20-26U/l)	1.89(1.27, 2.83)	<0.001
Q4 ( $\geq 26\text{U/l}$ )	2.26(1.54, 3.32)	<0.001

Adjusted for age, school years, family history of hypertension, *current smoking*, *alcohol-drinking*, body mass index, triglycerides, high density lipoprotein cholesterol and alanine aminotransferase.

Table1. Baseline characteristics of participants in two surveys.

Data are age-adjusted mean (95% confidence interval) or number (percentages) indicated. \*P&lt;0.05, hypertension vs.

	Men		Women	
	Normotension	Hypertension	Normotension	Hypertension
Number (%)	1668(46.6%)	1907(53.4%)	2918(53.0%)	2586(47.0%)
Age (years)	49.5(49.3,49.7)	53.4(53.1,53.7)*	47.8(47.6,48.0)	55.0(54.8,55.2)*
School years> 9 (yes, %)	23.6	19.9*	26.6	8.5*
Current smoking (yes, %)	21.3	19.6	1.0	0.5
Current drinking (%)				
non-drinkers	72.7	72.5	99.1	99.3
Moderate-drinkers	12.1	14.0	0.9	0.7
heavy-drinkers	15.2	13.5	0	0
Family history of hypertension (yes, %)	12.1	12.9	11.6	13.1
Body mass index (kg/m <sup>2</sup> )	24.2(23.5,24.9)	25.9(25.1,26.7)*	24.5(23.9,25.1)	26.7(26.1,27.2)*
Waist circumference (cm)	83.5(83.2,83.8)	87.2(86.9,87.5)*	79.6(79.7,79.8)	85.2(85.0,85.4)*
Fasting plasma glucose(mmol/L)	5.77(5.72,5.62)	6.17(6.11,6.23)*	5.65(5.62,5.68)	6.25(6.14,6.36)*
Haemoglobin A1c	5.50(5.47,5.53)	5.65(5.62,5.67)*	5.44(5.37,5.51)	5.76(5.68,5.84)*
HOMA-IR	10.9(9.7,12.1)	12.4(10.5,14.3)	12.1(11.2,13.0)	16.7(14.8,18.6)*
Low density lipoprotein cholesterol (mmol/L)	2.84(2.77,2.91)	3.02(2.94,3.10)*	2.89(2.85,2.93)	3.13(3.06,3.20)*
High density lipoprotein cholesterol (mmol/L)	1.61(1.58,1.64)	1.62(1.59,1.65)	1.67(1.62,1.72)	1.61(1.57,1.65)
Total cholesterol (mmol/L)	5.15(5.11,5.19)	5.35(5.30,5.40)*	5.09(5.07,5.11)	5.49(5.42,5.56)*
Triglyceride (mmol/L)	1.40(1.36,1.44)	1.59(1.56,1.62)*	1.21(1.15,1.27)	1.56(1.47,1.65)*
Alanine aminotransferase (U/L) §	20.4(19.7,21.1)	22.1(21.2,23.0)*	17.5(16.3,18.7)	18.7(17.4,19.1)
Gamma-glutamyltransferase (U/L) §	23.9(22.6,25.2)	29.5(27.8,31.2)*	14.6(13.2,16.0)	17.9(16.4,19.4)*

normotension by the same survey within the same gender. §Geometric mean (95% CI). || With missing data. HOMA-IR, homeostasis model assessment of insulin resistance.

Table2. Multiple linear regression analysis for alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) in association with diastolic blood pressure and systolic blood pressure.

	Diastolic blood pressure		Systolic blood pressure	
	Standard β coefficients	95%CI	Standard β coefficients	95%CI
<b>Men</b>				
ALT(U/L)	0.09*	(0.08, 0.11)	0.03	(0.01, 0.05)
GGT(U/L)	0.07*	(0.05, 0.08)	0.11*	(0.10, 0.13)
<b>Women</b>				
ALT(U/L)	0.04	(0.03, 0.05)	0.05	(0.04, 0.06)
GGT(U/L)	0.07*	(0.06, 0.08)	0.09*	(0.07, 0.10)

Adjusted for age, school years, family history of hypertension, *current smoking*, *alcohol-drinking*, body mass index, triglycerides and high density lipoprotein cholesterol. ALT and GGT are logarithmic transformed. \* P<0.01.

Table3. Odds ratio (95% confidence interval) for hypertension in relation to quartiles of alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) concentrations.

Model1: Adjusted for age.

	Model1	Model2	Model3	Model4	Model5	Model6
<b>Men</b>		<b>Number</b>				
<b>ALT</b>						
Q1 (<=10 U/l)	820	1	1	1	1	1
Q2 (10-15 U/l)	871	0.97(0.79, 1.20)	1.01(0.83,1.27)	1.06(0.85,1.32)	1.04(0.83,1.29)	1.04(0.83,1.30)
Q3 (15-22 U/l)	962	0.90(0.74,1.12)	0.99(0.80,1.22)	0.97(0.78,1.22)	0.94(0.75,1.17)	0.94(0.75,1.17)
Q4 (>22 U/l)	922	1.36(1.15, 1.64)	1.31(1.08,1.57)	1.17(0.98,1.52)	1.10(0.90,1.34)	1.10(0.91,1.35)
P for trend		P=0.003	P=0.005	P=0.243	P=0.442	P=0.436
						P=0.667
<b>GGT</b>						
Q1 (<=16U/l)	844	1	1	1	1	1
Q2 (16-24 U/l)	883	1.47(1.07, 2.10)	1.50(1.07,2.10)	1.55(1.09,2.20)	1.55(1.09,2.20)	1.50(1.05,2.13)
Q3 (24-38 U/l)	938	2.13(1.55, 2.93)	2.13(1.55,2.93)	2.04(1.47,2.84)	1.99(1.43,2.47)	1.95(1.40,2.71)
Q4 (>38 U/l)	910	3.59(2.68, 4.83)	3.41(2.54,4.60)	2.48(1.82,3.38)	2.33(1.70, 3.181)	2.29(1.68,3.14)
P for trend		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
<b>Women</b>						
<b>ALT</b>						
Q1 (<=8 U/l)	1322		1	1	1	1
Q2 (8-12 U/l)	1376	0.86(0.74, 1.01)	0.87(0.74,1.02)	0.87(0.75,1.03)	0.87(0.73,1.02)	0.87(0.74,1.02)
Q3 (12-18 U/l)	1455	0.82(0.69, 1.02)	0.83(0.71,1.03)	0.88(0.74,1.04)	0.86(0.72,1.02)	0.86(0.73,1.02)
Q4 (>18 U/l)	1351	1.02(0.87, 1.18)	1.02(0.87,1.19)	0.98(0.83,1.16)	0.93(0.79,1.10)	0.93(0.79,1.10)
P for trend		P=0.066	P=0.034	P=0.121	P=0.234	P=0.242
						P=0.235
<b>GGT</b>						
Q1 (<=11U/l)	1343		1	1	1	1
Q2 (11-14 U/l)	1458	1.35(1.15, 1.59)	1.34(1.14,1.58)	1.20(1.02,1.41)	1.17(0.99,1.40)	1.17(0.98,1.38)
Q3 (14-20 U/l)	1376	2.06(1.75, 2.45)	2.05(1.72,2.44)	1.56(1.30,1.88)	1.50(1.25,1.80)	1.47(1.22,1.77)
Q4 (>20 U/l)	1327	2.42(2.04, 2.85)	2.39(2.02,2.83)	1.67(1.40,2.00)	1.57(1.32,1.88)	1.52(1.27,1.83)
P for trend		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Model2: Adjusted for age, school years, alcohol-drinking, current smoking, family history of hypertension and survey years.

Model3: Model2+ body mass index.

Model4: Model3+ triglycerides + high-density lipoprotein cholesterol.

Model5: Model4+ fasting plasma glucose.

Model6: GGT and ALT fitted simultaneously into the Model5.

Table 4. Odds ratio (95% confidence interval) for hypertension in relation to quartiles of gamma-glutamyltransferase (GGT) concentrations stratified by waist circumference categories.

	Odds ratio (95% CI)	P value
<b>Men</b>		
<b>Waist circumference (&lt;79cm) (n=892)</b>		
GGT		
Q1 (<15U/l)	1	
Q2 (15-19 U/l)	1.33(0.76,2.35)	0.314
Q3 (19-26 U/l)	1.75(1.03,2.99)	0.037
Q4 ( $\geq 26$ U/l)	2.61(1.56,4.36)	<0.001
<b>Waist circumference (79-87cm) (n=893)</b>		
GGT		
Q1 (<16U/l)	1	
Q2 (16-22 U/l)	1.55(0.84, 2.87)	0.109
Q3 (22-34 U/l)	2.72(1.25,4.10)	0.009
Q4 ( $\geq 34$ U/l)	2.68(1.54,4.65)	0.001
<b>Waist circumference (87-94cm) (n=930)</b>		
GGT		
Q1 (<19U/l)	1	
Q2 (19-28U/l)	1.86(0.87, 3.98)	0.020
Q3 (28-45 U/l)	2.58(1.26, 5.28)	0.001
Q4 ( $\geq 45$ U/l)	3.10(1.62, 5.96)	<0.001
<b>Waist circumference (<math>\geq 94</math>cm) (n=860)</b>		
GGT		
Q1 (<23U/l)	1	
Q2 (23-33 U/l)	2.25(1.14, 4.46)	0.001
Q3 (33-53 U/l)	3.12(1.64, 5.94)	<0.001
Q4 ( $\geq 53$ U/l)	4.01(2.21, 7.29)	<0.001
<b>Women</b>		
<b>Waist circumference (&lt;76cm) (n=1363)</b>		
GGT		
Q1 (<10U/l)	1	
Q2 (10-14 U/l)	1.20(0.88,1.65)	0.243
Q3 (14-17U/l)	1.27(0.92,1.89)	0.177
Q4 ( $\geq 17$ U/l)	1.41(0.94,2.12)	0.096
<b>Waist circumference (76-83cm) (n=1414)</b>		
GGT		
Q1 (<12U/l)	1	
Q2 (12-15 U/l)	0.96(0.69, 1.32)	0.800

Q3 (15-21U/l)	1.25(0.88, 1.78)	0.199
Q4 ( $\geq 21\text{U/l}$ )	1.16(0.82, 1.64)	0.409

**Waist circumference (83-90cm) (n=1332)**

GGT

Q1 (<12U/l)	1	
Q2 (12-17U/l)	1.32(0.94, 1.85)	0.107
Q3 (17-22 U/l)	1.87(1.32, 2.64)	<0.001
Q4 ( $\geq 22\text{U/l}$ )	2.07(1.48, 2.91)	<0.001

**Waist circumference ( $\geq 90\text{cm}$ ) (n=1395)**

GGT

Q1 (<14U/l)	1	
Q2 (14-19U/l)	1.57(1.05, 2.35)	0.028
Q3 (20-26U/l)	1.89(1.27, 2.83)	<0.001
Q4 ( $\geq 26\text{U/l}$ )	2.26(1.54, 3.32)	<0.001

Adjusted for age, school years, family history of hypertension, *current smoking*, *alcohol-drinking*, body mass index, triglycerides, high density lipoprotein cholesterol and alanine aminotransferase.

# Serum Gamma-Glutamyltransferase Levels are Associated With Concomitant Cardiovascular Risk Factors in Korean Hypertensive Patients

## A Nationwide Population-Based Study

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**Abstract:** Previous studies suggested that serum gamma-glutamyltransferase (GGT) levels were associated with the prevalence of cardiovascular disease (CVD) risk factors including hypertension, diabetes mellitus (DM), and metabolic syndrome (MetS) in the general population. We aimed to investigate the relationship between serum GGT levels and CVD risk factors in Korean hypertensive patients.

This cross-sectional study was based on data from the Korea National Health and Nutrition Examination Survey (KNHANES) 2011 to 2012. The analysis included 1541 hypertensive participants. Study participants were divided into groups according to tertiles of serum GGT with cutoff points of 20 and 35 U/L.

Serum GGT levels were positively associated with the components of MetS ( $P$  value  $< 0.05$ , except for systolic blood pressure and high-density lipoprotein cholesterol). After adjusting for possible confounders, serum GGT levels were associated with an increased risk of MetS, high waist circumference, high triglyceride level, fasting plasma glucose, DM, and the urinary albumin-to-creatinine ratio ( $P = 0.001$ ).

In hypertensive patients, serum GGT levels are positively associated with major cardiovascular risk factors such as MetS, DM, and urinary albumin excretion.

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**Abbreviations:** BMI = body mass index, CHD = coronary heart disease, CHF = congestive heart failure, CVD = cardiovascular disease, DBP = diastolic blood pressure, DM = diabetes mellitus, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, GGT = serum gamma-glutamyltransferase, HDL-C = high-density lipoprotein cholesterol, MetS = metabolic syndrome, SBP = systolic blood pressure, TC = total cholesterol, TG = triglycerides, UACR = urinary albumin-to-creatinine ratio, WBC = white blood cell, WC = waist circumference.

## INTRODUCTION

Hypertensive patients have a 2-fold risk of cardiovascular disease (CVD) including coronary heart disease (CHD), congestive heart failure (CHF), ischemic and hemorrhagic stroke, renal failure, and peripheral arterial disease. Appropriate antihypertensive therapy reduces cardiovascular and renal disease risk, but significant portions of the hypertensive population are either untreated or inadequately treated.<sup>1</sup>

Serum gamma-glutamyltransferase (GGT) levels may be elevated in hepatobiliary disease with or without the elevation of other liver enzymes. Thus, GGT may be an indicator of alcohol abuse or alcoholic liver disease. Serum GGT levels can also elevate due to the ingestion of certain medications such as barbiturates or phenytoin. Recently, several studies demonstrated associations between serum GGT levels and CVD including hypertension, diabetes, and metabolic syndrome (MetS).<sup>2–7</sup> In addition, previous studies of Korean adults showed that serum GGT levels were independently associated with incident hypertension.<sup>2</sup> This is potentially explained by the oxidative stress mechanism demonstrated in the series of Coronary Artery Risk Development in Young Adults (CARDIA) studies.<sup>3,4,8</sup> Although the relationship between serum GGT and cellular GGT is unknown, cellular GGT has been known to play an important role in antioxidant defense systems.<sup>9</sup> Moreover, serum GGT was revealed as an early and sensitive marker of oxidative stress in a recent study.<sup>10</sup> However, previous investigations of the association between serum GGT levels and cardiovascular risk factors were based on the general population. We therefore aimed to evaluate this relationship in Korean hypertensive patients. We considered diabetes mellitus (DM), MetS, and the urinary albumin-to-creatinine ratio (UACR) as additional cardiovascular risk factors in hypertensive individuals.

## METHODS

### Survey and Subject

This cross-sectional study was based on data from the Korea National Health and Nutrition Examination Survey

(KNHANES) 2011 to 2012. The KNHANES was designed to evaluate national health and nutritional status and was conducted by the Division of Chronic Disease Surveillance under the Korea Centers for Disease Control and Prevention (KCDC). The survey consists of an interview on health status, a nutritional assessment, and a health examination.

Of the 8518 KNHANES 2011 to 2012 participants, 6977 were excluded from the present analysis. Reasons for exclusion were an age <19 years ( $n=1952$ ), absence of hypertension ( $n=4634$ ), cancer ( $n=5$ ), pulmonary tuberculosis ( $n=103$ ), chronic hepatitis B or C ( $n=7$ ), missing data ( $n=212$ ), and not fasting for at least 8 h ( $n=29$ ). Hypertension was defined as a systolic blood pressure (SBP)  $\geq 140$  mm Hg, diastolic blood pressure (DBP)  $\geq 90$  mm Hg, or antihypertensive medication use. Finally, a total of 1541 subjects were included in the analysis. All participants provided written informed consent and the Institutional Review Board of the KCDC approved the study protocol.

## Sociodemographic and Lifestyle Characteristics

Monthly household income and educational level, physical activity, and antihypertensive medication use were assessed via individual interviews by trained staff. Alcohol consumption and smoking status were assessed using a self-reported questionnaire. A lower income level was defined as the lowest 25th percentile of the total participants. A higher educational level was defined as high school graduate or more. Alcohol consumption was classified into 3 categories based on the mean amount of alcohol consumed per day up to 1 month before the interview: subjects who consumed  $\geq 30$  g/day of alcohol were classified as heavy drinkers and those who consumed  $\leq 30$  g/day were classified as mild to moderate drinkers.<sup>11</sup> Smoking status was classified as the current smoker or the nonsmoker at the time of the interview. As for physical activity, subjects who exercised moderately for >30 min per session >5 times per week or vigorously for >20 min per session >3 times per week were defined as regular physical exercisers.<sup>12</sup>

## Anthropometric and Biochemical Measurements

Height and body weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. The body mass index (BMI) was calculated using the formula: body weight (kg)/height<sup>2</sup> (m<sup>2</sup>). Waist circumference (WC) was measured at the midpoint between the lower costal margin and the iliac crest during expiration. Blood pressure was measured from the right arm in the sitting position using a standard mercury sphygmomanometer (Baumanometer, WA Baum Co.) after 5 min of rest. Systolic blood pressure and DBP were measured 3 times in 5-min intervals and the average of the second and third measurements was used in the analysis.

Blood samples were obtained after a fasting period of at least 8 h and single spot midstream urine samples were collected from the first morning voiding. Serum GGT levels were measured enzymatically using a Hitachi Automatic Analyzer 7600. Levels of fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) were measured enzymatically with the same equipment. White blood cell (WBC) count was measured with using an XE-2100D (Sysmex) via laserflow cytometry. HbA1c levels were measured using an HLC-723G7 (Tosoh) via high-performance liquid chromatography. To calculate the UACR, serum and urine creatinine levels were measured by kinetic colorimetry using a Hitachi Automatic Analyzer 7600. Urine

albumin levels were measured with the same equipment by means of a turbidimetric assay. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease study Equation.<sup>13</sup>

## Definitions of MetS

We used guidelines from the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA) to define MetS. And it is defined as any 3 or more of the followings: (1) FPG  $\geq 100$  mg/dL (or receiving drug therapy for hyperglycemia); (2) blood pressure  $\geq 130/85$  mm Hg (or receiving drug therapy for hypertension); (3) triglycerides  $\geq 150$  mg/dL (or receiving drug therapy for hypertriglyceridemia); (4) HDL-C  $< 40$  mg/dL in men or  $< 50$  mg/dL in women (or receiving drug therapy for reduced HDL-C); (5) WC  $\geq 90$  cm (35 in) in Asian men or  $\geq 80$  cm (32 in) in Asian women.<sup>14</sup>

Type 2 DM was defined according to the updated American Diabetes Association criteria. The diagnosis could be made when any of the 4 following criteria was satisfied: (1) HbA1c  $\geq 6.5\%$ ; (2) FPG  $\geq 126$  mg/dL; (3) 2-h plasma glucose  $\geq 200$  mg/dL during a 75 g oral glucose tolerance test; (4) classic symptoms of hyperglycemia or hyperglycemic crisis with a random plasma glucose measurement of  $\geq 200$  mg/dL. Impaired fasting glucose was defined as an FPG of 100 to 125 mg/dL. In this study, participants were defined as having DM when they were previously diagnosed with DM by physicians or were taking DM medications. Participants in the present study were defined as having newly diagnosed DM if they had an HbA1c  $\geq 6.5\%$  or an FPG  $\geq 126$  mg/dL.

High WC ( $\geq 90$  cm in men or  $\geq 80$  cm in women), high TG ( $\geq 150$  mg/dL), high FPG ( $\geq 100$  mg/dL), and low HDL-C ( $< 40$  mg/dL in men or  $< 50$  mg/dL in women) levels were defined using the same criteria used to define the MetS components. A high UACR was defined as the highest tertile of UACR.

## Statistical Analysis

SAS 9.2 software (SAS Institute) was used for all statistical analyses. A *P* value of  $<0.05$  was regarded as statistically significant.

Participants were classified into 3 groups according to tertiles of serum GGT and the cutoff values were 20 and 35 U/L. Baseline clinical and metabolic characteristics are expressed as the mean  $\pm$  standard error or percentage (standard error). Differences in clinical characteristics according to GGT categories were assessed using an analysis of variance (ANOVA) or chi square test. Logistic regression models were used to calculate multivariable adjusted odds ratios (ORs) and 95% confidence intervals (CIs). Covariates in the minimally adjusted model (Model 1) were age and gender. The second model (Model 2) was additionally adjusted for BMI. The third model (Model 3) was adjusted for alcohol consumption, smoking status, physical activity, and antihypertensive medication use in addition to the Model 2 variables. The fourth model (Model 4) was adjusted for total energy intake and fat intake percentage per day in addition to the Model 3 variables.

## RESULTS

### Baseline Characteristics According to the GGT Level

Table 1 presents participants' baseline clinical and metabolic characteristics according to serum GGT tertile. Age and

**TABLE 1.** Subject Baseline Characteristics by GGT Category

Characteristics	T1 (GGT < 20 U/L)	T2 (20 ≤ GGT < 35 U/L)	T3 (GGT ≥ 35 U/L)	P Value <sup>†</sup>
N	497	534	510	
Sex (female), %	76.2(2.7)	45.6(2.5)	17.7(1.8)	<0.001
Age, years	62.2 ± 1	57.4 ± 0.8	50.7 ± 0.9	<0.001
BMI, kg/m <sup>2</sup>	24.2 ± 0.2	25.3 ± 0.2	25.7 ± 0.2	<0.001
WC, cm	82.9 ± 0.7	86.7 ± 0.6	89.3 ± 0.5	<0.001
SBP, mm Hg	134.8 ± 1.1	134.4 ± 0.9	135.5 ± 0.9	0.733
DBP, mm Hg	80.1 ± 0.7	83.6 ± 0.7	88.5 ± 0.6	<0.001
FPG, mg/dL	99 ± 1.2	105.2 ± 1.3	106.7 ± 1.4	<0.001
HbA1c, %	5.9 ± 0	6 ± 0	5.9 ± 0	0.036
TG, mg/dL*	109.1(102.9,115.6)	133.9(125.4,143)	167.1(156,179)	<0.001
TC, mg/dL	194.1 ± 1.8	194.1 ± 2.1	196.8 ± 2.1	0.604
WBC count*	5.7(5.5,5.9)	6.3(6.1,6.5)	6.6(6.4,6.8)	<0.001
eGFR, mL/min/1.73m <sup>2</sup>	86.1 ± 1.2	86.7 ± 1.0	89.8 ± 0.9	0.012
UACR*	7.5(6.3,8.8)	7.1(6.1,8.3)	7(6.8,3)	0.847
Monthly income (lowest quartile), %	36.4(2.5)	22.7(2)	19.2(2.3)	<0.001
Education (high school), %	31.4(3.2)	44(2.7)	65.1(3.1)	<0.001
Alcohol consumption				<0.001
Nondrinker, %	48.3(3.1)	31.2(2.2)	11.7(1.7)	
Mild to moderate drinker, %	49(2.9)	60.1(2.4)	53.1(2.6)	
Heavy drinker, %	2.7(1.6)	8.7(1.6)	35.3(2.8)	
Current smoker, %	7.1(1.6)	19.8(2.4)	43.1(2.8)	<0.001
Regular exercise, %	18.3(2.7)	21.1(2.3)	19.4(2.1)	0.739
Use of antihypertensive medications, %	65.9(3.1)	62.9(2.8)	41.3(2.9)	<0.001
Energy intake, kcal	1686 ± 48.8	2050.5 ± 67.6	2344.8 ± 67.3	<0.001
Fat intake, %	13.3 ± 0.5	15.4 ± 0.5	18.4 ± 0.7	<0.001
Protein intake, %	13.4 ± 0.3	13.9 ± 0.2	15.5 ± 0.3	<0.001
Carbohydrate intake, %	73.3 ± 0.6	70.8 ± 0.6	66 ± 0.8	<0.001

Data are presented as the mean ± standard error (SE) or as percentages (SE). BMI = body mass index, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, GGT = gamma-glutamyltransferase, SBP = systolic blood pressure, TC = total cholesterol, TG = triglycerides, UACR = urinary albumin-to-creatinine ratio, WBC = white blood cell, WC = waist circumference.

\* TG, WBC count, and UACR were tested after logarithmic transformation.

† P-values were obtained by an ANOVA or chi square test.

the proportion of women differed significantly among the 3 GGT groups. Metabolic variables including BMI, WC, DBP, FPG, HbA1c, TG, WBC, and eGFR showed significant differences across GGT groups. With the exception of HbA1c, these values were significantly higher in the higher GGT groups. Meanwhile, the rate of antihypertensive medication use was highest in the lowest GGT group. Sociodemographic and lifestyle variables including household income and educational level, alcohol consumption, and smoking status also differed significantly among the 3 GGT groups.

### Relationship Between Serum GGT level and CVD Risk Factors

Table 2 shows correlations between the serum GGT level and variables related to CVD risk. The serum GGT level correlated negatively with age and positively with BMI, WC, DBP, FPG, TG, WBC, and eGFR.

Figure 1 shows the associations between the serum GGT level and the number of satisfied MetS components (a), tertiles of UACR (T1 ≤ 2.42 mg/g, 2.42 < T2 < 9.43 mg/g, T3 ≥ 9.43 mg/g) (b), and glucose metabolism status (c). Serum GGT levels showed increasing trends with higher numbers of MetS components (*P* for trend < 0.001), higher UACRs (*P* for trend = 0.003), and higher glucose levels (*P* for trend < 0.001).

Subjects with newly diagnosed DM had higher serum GGT levels than those with previously diagnosed DM.

### ORs and 95% CIs for Major CVD Risk Factors and Their Associated Traits According to Serum GGT

Table 3 presents ORs (95% CIs) for MetS, high WC, high TG, high FPG, low HDL-C, high UACR, and DM according to serum GGT. The ORs for MetS, high WC, high TG, high FPG, high UACR, and DM tended to increase in the higher GGT groups in all adjusted analyses. However, the ORs for low HDL-C were not significantly associated with serum GGT levels.

### DISCUSSION

A number of previous studies suggested that serum GGT levels may play an important role in various CVDs including hypertension and DM. The present study showed a significant association between serum GGT levels and various CVD risk factors even among Korean hypertensive patients.

The pro-oxidant and pro-inflammatory activities<sup>15</sup> of GGT and its direct involvement in atherosomatous plaque formation have been suggested<sup>16</sup> as possible mechanisms underlying the association between increased cardiovascular risk and elevated serum GGT levels. Gamma-glutamyltransferase was recently reported to be composed of 4 fractions with distinct molecular

**TABLE 2.** Pearson's Correlations Between GGT and Cardiovascular Risk Factors

	<i>γ</i>	<i>P</i> Value <sup>†</sup>
Age, years	-0.3	<0.001
BMI, kg/m <sup>2</sup>	0.17	<0.001
WC, cm	0.28	<0.001
SBP, mm Hg	0.05	0.162
DBP, mm Hg	0.3	<0.001
FPG, mg/dL	0.16	<0.001
HbA1c, %	0.001	0.969
TG, mg/dL*	0.37	<0.001
TC, mg/dL	0.03	0.475
WBC count*	0.18	<0.001
eGFR, mL/min/1.73m <sup>2</sup>	0.12	<0.001
UACR*	-0.01	0.684

BMI = body mass index, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, GGT = gamma-glutamyltransferase, SBP = systolic blood pressure, TC = total cholesterol, TG = triglycerides, UACR = urinary albumin-to-creatinine ratio, WBC = white blood cell, WC = waist circumference.

\* TG, WBC count, and UACR were tested after logarithmic transformation.

<sup>†</sup>  $\gamma$  (Pearson correlation coefficient) and *P* values were obtained via Pearson's correlation analysis.

weights and physiochemical properties, namely big-GGT (b-GGT), medium-GGT (m-GGT), small-GGT (s-GGT), and free-GGT (f-GGT).<sup>17</sup> Of these fractions, the b-GGT fraction is known to correlate strongly with conventional cardiovascular and metabolic risk factors and is the only fraction found in atherosclerotic plaque.<sup>16</sup>

Several previous studies showed that the GGT level is positively associated with blood pressure change and hypertension.<sup>2,3,18–20</sup> In our study, DBP tended to increase as the serum GGT level increased. However, the rate of antihypertensive medication use was the highest in the lowest GGT group. This may indicate an association between strict blood pressure control and low serum GGT levels.

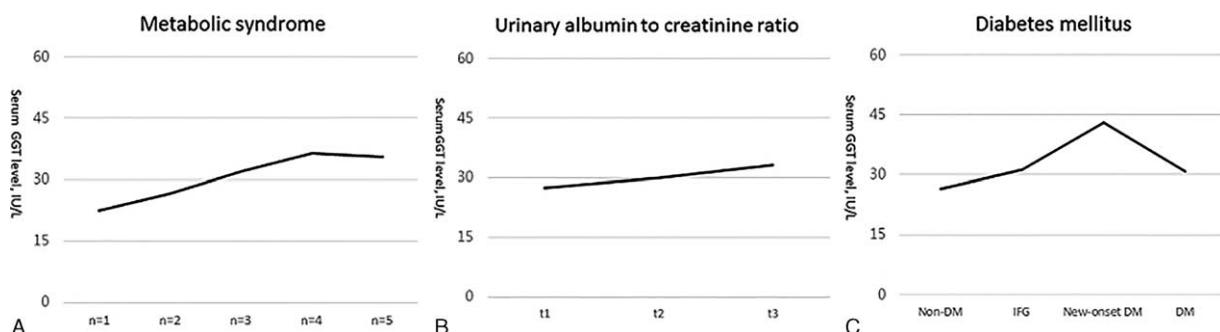
Insulin resistance appears to be involved in the relationship between serum GGT levels and CVD risk factors. Although the mechanism underlying the relationship between elevated serum

GGT levels and insulin resistance is not well understood, several studies found that an elevated serum GGT level is a predictor of type 2 DM and MetS, both of which are closely related to insulin resistance.<sup>21–24</sup> In our study, the FPG level was positively associated with serum GGT level both in newly diagnosed diabetic patients ( $\gamma = 0.28022$ , *P* = 0.0080) and in previously diagnosed diabetic patients ( $\gamma = 0.28828$ , *P* = 0.0257). Additionally, serum GGT level was slightly higher in newly diagnosed DM patients than in previously diagnosed patients, although there was no significant difference between these 2 subgroups. Newly diagnosed DM is thought to be a earlier stage of diabetes with shorter disease duration or less complication than previously diagnosed DM; therefore, the higher level of serum GGT in newly diagnosed diabetic patients than previously diagnosed diabetic patients might possibly be in accordance with the predictable role of GGT in type 2 DM that was shown in the previous studies. Whereas the no relationship was observed between HDL-C and serum GGT levels, higher serum GGT levels were observed in subjects with more MetS components.

It was reported that a significant proportion of Korean hypertensive patients has albuminuria and that those with albuminuria tend to be older, have a longer duration of hypertension, and have a higher prevalence of obesity, and an SBP  $\geq 130$  mm Hg and/or DBP  $\geq 80$  mm Hg compared with normoalbuminuric patients.<sup>25</sup> Our study showed that the UACR elevated as the serum GGT level increased. Taken together, these findings may explain the possible association between serum GGT levels and albuminuria in hypertensive patients.

Notably, the serum GGT level showed a significantly negative association with age in the present study. Most previous studies reported positive associations between the serum GGT level and age. Because our study focused on hypertensive patients, this could be explained by the greater likelihood of uncontrolled hypertension in younger participants, which may be associated with elevated serum GGT levels. Younger subjects may also have greater exposure to alcohol consumption and cigarette smoking than older subjects.

The limitations of this study are as follows. First, this was a cross-sectional study, and therefore, causal relationships could not be demonstrated. Second, subjects with fatty liver were not excluded either ultrasonographically or using invasive diagnostic tools. Third, the GGT level is a sensitive indicator of alcohol intake, but chronic heavy alcohol drinkers were not excluded. Fourth, serum GGT levels are known to increase during an



**FIGURE 1.** Figure shows the distribution of serum GGT levels for each component after adjustment for age and sex: (A) association between the serum GGT level and the total number of metabolic syndrome components (*P* < 0.0001), (B) association between the serum GGT level and the UACR level (T1 < 2.42 mg/g, 2.42 mg/g  $\leq$  T2 < 9.43 mg/g, T3  $\geq$  9.43 mg/g) (*P* = 0.0025), (C) association between the serum GGT level and the diabetic status (*P* < 0.0001). GGT = gamma-glutamyltransferase, UACR = urinary albumin-to-creatinine ratio.

**TABLE 3.** ORs and 95% CIs for CVD Risk Factors and Their Associated Traits According to the Serum GGT Group

	<b>Mets</b>	<b>High WC</b>	<b>High TG</b>	<b>High FPG</b>	<b>Low HDL-C</b>	<b>High UACR</b>	<b>DM</b>
T1 T2 T3 <i>P</i> for trend	1	1	1	1	1	1	1
	2.31(1.60,3.33)	1.70(1.19,2.44)	2.05(1.54,2.74)	1.95(1.38,2.75)	1.06(0.75,1.50)	1.24(0.80,1.92)	2.30(1.57,3.35)
	3.23(2.04,5.09)	2.54(1.69,3.81)	3.23(2.17,4.79)	2.42(1.59,3.66)	0.82(0.55,1.21)	2.00(1.24,3.23)	2.23(1.43,3.49)
	<0.001	<0.001	<0.001	<0.001	0.283	0.004	0.001
T1 T2 T3 <i>P</i> for trend	1	1	1	1	1	1	1
	1.91(1.26,2.88)	1.03(0.66,1.61)	1.92(1.42,2.59)	1.83(1.28,2.60)	0.92(0.64,1.32)	1.21(0.77,1.91)	2.21(1.50,3.26)
	2.64(1.51,4.63)	1.80(1.10,2.93)	2.99(1.96,4.55)	2.20(1.42,3.39)	0.67(0.43,1.03)	1.92(1.15,3.21)	2.08(1.31,3.29)
	0.001	0.013	<0.001	0.001	0.062	0.012	0.004
T1 T2 T3 <i>P</i> for trend	1	1	1	1	1	1	1
	1.83(1.22,2.76)	0.98(0.63,1.54)	1.88(1.40,2.53)	1.76(1.24,2.50)	0.89(0.61,1.29)	1.21(0.77,1.90)	2.16(1.49,3.14)
	2.67(1.53,4.65)	1.72(1.03,2.87)	2.97(1.95,4.54)	2.25(1.44,3.51)	0.73(0.45,1.18)	2.00(1.17,3.41)	2.38(1.44,3.94)
	0.001	0.029	<0.001	0.001	0.201	0.011	0.001
T1 T2 T3 <i>P</i> for trend	1	1	1	1	1	1	1
	1.84(1.24,2.74)	1.27(0.79,2.06)	1.77(1.29,2.43)	2.00(1.40,2.84)	0.88(0.61,1.27)	1.68(1.00,2.84)	2.39(1.60,3.57)
	2.66(1.58,4.49)	2.15(1.29,3.58)	3.11(2.01,4.80)	2.44(1.61,3.68)	0.74(0.45,1.22)	2.54(1.44,4.50)	2.53(1.48,4.30)
	<0.001	0.003	<0.001	<0.001	0.244	0.001	0.001

Data are presented as ORs (95% CIs).

Data were analyzed using multiple logistic regression analysis after adjusting for age, sex, BMI, alcohol consumption, smoking status, physical activity, antihypertensive medication use, and nutritional status. (Model 1: adjusted for age and sex, Model 2: adjusted for BMI in addition to Model 1 variables, Model 3: adjusted for alcohol consumption, smoking status, physical activity, and antihypertensive medication use in addition to Model 2 variables, Model 4: adjusted for energy and fat intake in addition to Model 3 variables.)

BMI = body mass index, CI = confidence interval, CVD = cardiovascular disease, DM = diabetes mellitus, FPG = fasting plasma glucose, GGT = gamma-glutamyltransferase, HDL-C = high-density lipoprotein cholesterol, MetS = metabolic syndrome, OR = odds ratio, TG = odds ratio, WC = waist circumference.

inflammatory response.<sup>26</sup> However, subjects with inflammatory diseases or other such conditions were not excluded in advance, and the analysis was not corrected for this factor. Fifth, the potential influence of specific types of antihypertensive medications on urinary albumin excretion or renal function was not considered. Despite these limitations, to our knowledge, this study is the first to investigate the relationship between GGT levels and CVD risk factors specifically in hypertensive patients, whereas most previous studies investigated this relationship in the general population.

In conclusion, this study found that the level of serum GGT is associated with major CVD risk factor such as DM, MetS, and an increased UACR in Korean hypertensive patients. Although the mechanism remains to be fully clarified, the serum GGT level can be presumed to be associated with CVD risk factors.

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## Relationship between elevated serum gamma-glutamyltransferase activity and slow coronary flow

Yüksek serum gama-glutamiltransferaz aktivitesi ile yavaş koroner akım arasındaki ilişki

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**Objectives:** We evaluated the relationship between coronary blood flow and serum gamma-glutamyltransferase (GGT) activity in patients with slow coronary flow (SCF).

**Study design:** The study included 90 patients (47 men, 43 women; mean age 50.8±9.4 years) with SCF and 88 patients (45 men, 43 women; mean age 51.4±8.8 years) with coronary artery disease (CAD), whose diagnoses were made by coronary angiography. Patients with CAD had normal coronary flow. Coronary flow was quantified using the corrected TIMI frame count (TFC) method and serum levels of gamma-glutamyltransferase were measured. The results were compared with those of a control group consisting of 86 age- and sex-matched patients who had normal coronary arteries and normal coronary flow.

**Results:** The three groups were similar with respect to body mass index, presence of hypertension and diabetes mellitus, lipid profiles, and fasting glucose. The use of medications was significantly more common in the CAD group ( $p<0.01$ ). Compared to the control group, serum GGT activity was significantly increased in both SCF and CAD groups ( $p<0.01$ ), but these two groups did not differ significantly in this respect ( $p=0.71$ ). The TFCs for all the epicardial coronary arteries and the mean TFC were significantly higher in the SCF group ( $p<0.01$ ). Patients with CAD and the controls had similar TFC parameters. The mean TFC showed a positive and moderate correlation with serum GGT activity ( $r=0.326$ ;  $p<0.001$ ). In regression analysis, serum GGT activity was found as the only independent predictor of the mean TFC ( $\beta=0.309$ ;  $p<0.001$ ).

**Conclusion:** We have shown for the first time an association between increased serum GGT activity and SCF. Further clinical studies are needed to clarify the physiopathologic role of serum GGT activity in SCF.

**Key words:** Blood flow velocity; coronary angiography; coronary circulation; coronary disease; gamma-glutamyltransferase; heart catheterization.

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**Amaç:** Çalışmamızda yavaş koroner kan akımı (YKA) olan hastalarda serum gama-glutamiltransferaz (GGT) aktivitesi ile koroner kan akımı arasındaki ilişki araştırıldı.

**Çalışma planı:** Çalışmaya YKA saptanan 90 hasta (47 erkek, 43 kadın; ort. yaşı 50.8±9.4) ve koroner arter hastalığı (KAH) olan 88 hasta (45 erkek, 43 kadın; ort. yaşı 50.8±9.4) alındı. Yavaş koroner kan akımı ve KAH tanıları koroner anjiyografi ile kondu. Koroner arter hastalığı olan grupta normal koroner akım vardı. Tüm hastalarda koroner akım düzeltilmiş TIMI kare sayısı ile değerlendirildi ve serum GGT düzeyleri ölçüldü. Sonuçlar, yaş ve cinsiyet uyumlu ve koroner arterleri ve koroner akımı normal bulunan 86 hastadan oluşan kontrol grubuya karşılaştırıldı.

**Bulgular:** Gruplar arasında beden kütle indeksi, hipertansiyon ve diyabet varlığı, lipit profili ve açlık kan şekeri açısından fark yoktu. Koroner arter hastalığı olan grupta ilaç kullanımı anlamlı derecede fazlaydı ( $p<0.01$ ). Kontrol grubuyla karşılaştırıldığında, serum GGT aktivitesi YKA'lı ve KAH'lı gruplarda anlamlı derecede yüksek bulundu ( $p<0.01$ ); ancak, iki grup arasında bu açıdan fark yoktu ( $p=0.71$ ). Epikardiyal koroner arterlerde ölçülen TIMI kare sayıları ve ortalama TIMI kare sayısı YKA grubunda anlamlı derecede yüksek bulundu ( $p<0.01$ ). TIMI kare sayıları açısından KAH grubu ile kontrol grubu arasında fark yoktu. Ortalama TIMI kare sayısı serum GGT düzeyi ile orta düzeyde pozitif ilişki gösterdi ( $r=0.326$ ;  $p<0.001$ ). Regresyon analizinde, serum GGT aktivitesi ortalama TIMI kare sayısını öngörmekte tek bağımsız değişken idi ( $\beta=0.309$ ;  $p<0.001$ ).

**Sonuç:** Çalışmamızda artmış serum GGT aktivitesi ile YKA arasındaki ilişki ilk kez gösterilmiş olmaktadır. Serum GGT aktivitesinin YKA'da tam patofizyolojik rolünü ortaya koymak için ileri çalışmalarla ihtiyaç vardır.

**Anahtar sözcükler:** Kan akım hızı; koroner anjiyografi; koroner dolaşım; koroner hastalık; gama-glutamiltransferaz; kalp kateterizasyonu.

The slow coronary flow (SCF) phenomenon is an angiographic finding characterized by delayed passage of angiographic contrast along the coronary arteries, in the absence of stenosis in the epicardial vessels. This phenomenon was first described in 1972 by Tambe et al.<sup>[1]</sup> Many etiological factors such as microvascular and endothelial dysfunction, small-vessel disease, and diffuse atherosclerosis are included among the causes of SCF,<sup>[2-4]</sup> but its etiopathogenesis is still unclear. Occlusive disease of small coronary arteries, which may be a form of early-phase atherosclerosis, has also been suggested as a cause.<sup>[5]</sup>

In the CARDIA study (Coronary Artery Risk Development in Young Adults), serum gamma-glutamyltransferase (GGT) values were demonstrated to be strongly and positively correlated with determinants of oxidative stress such as C-reactive protein (CRP), uric acid, and fibrinogen.<sup>[6]</sup> In addition, in patients with a history of myocardial infarction and documented coronary artery disease (CAD), it has been found that the level of GGT has an independent predictive value for mortality and non-fatal myocardial infarction.<sup>[7]</sup>

We hypothesized that serum GGT activity may be associated with coronary blood flow since it was also shown to be associated with atherosclerosis and oxidative stress. Therefore, we aimed to evaluate the relationship between coronary blood flow (expressed by means of thrombolysis in myocardial infarction -TIMI- frame count) and serum GGT activity in patients with SCF.

## PATIENTS AND METHODS

**Patient selection.** The study included 90 patients (47 men, 43 women; mean age  $50.8 \pm 9.4$  years) with SCF and 88 patients (45 men, 43 women; mean age  $51.4 \pm 8.8$  years) with CAD whose diagnoses were made by coronary angiography. Diagnosis of SCF was based on TIMI frame count (TFC) and the presence of normal coronary arteries without luminal irregularities. All the patients in the CAD group had stenotic lesions of greater than 20% and normal coronary flow. The control group consisted of age- and gender-matched 86 patients who had normal coronary arteries and normal coronary flow on coronary angiography. In all the groups, the indication for coronary angiography was either the presence of typical angina or positive or equivocal results of noninvasive screening tests for myocardial ischemia.

Exclusion criteria were prior myocardial infarction, valvular heart disease, cardiac rhythm other than

sinus, heart failure, peripheral vascular disease, severe systemic disease, active hepatobiliary disease, and alcohol consumption. The study was approved by the institutional ethics committee, and informed consent was obtained from all patients.

**Coronary angiography and documentation of TIMI frame count.** Patients underwent selective coronary angiography using the standard Judkins technique. Coronary arteries were visualized in left and right oblique planes, and cranial and caudal angles. Left ventriculography was performed in left and right anterior oblique views. Injection of contrast medium (Iopromide, Ultravist-370; Schering AG, Berlin, Germany) was carried out by an automatic injector at a speed of 3-4 ml/sec for the left coronary artery and 2-3 ml/sec for the right coronary artery. Arteriographies were recorded at a speed of 25 frames/sec. Coronary flow was quantified objectively by two independent observers who were blinded to the clinical details of the individual participants, using the corrected TFC method. The first frame was defined by a column of contrast extending across more than 70% of the arterial lumen in an anterograde motion.<sup>[8]</sup> Since the normal frame counts for the left anterior descending (LAD) coronary artery are 1.7 times greater than the mean for the left circumflex coronary artery and the right coronary artery,<sup>[9]</sup> the TFCs for the LAD were divided by 1.7 to derive the corrected TFC as described earlier.<sup>[10]</sup>

**Definition of slow coronary flow.** All participants with a corrected TFC greater than two standard deviations from the normal range reported for the particular vessel were accepted as having SCF while those whose corrected TFC fell within two standard deviations were considered to have normal coronary flow.<sup>[8]</sup> After assessment of coronary flow using the corrected TFC method,<sup>[8]</sup> the mean corrected TFC was derived by averaging the sum of the corrected TFCs for the LAD, left circumflex coronary artery, and right coronary artery. Intra- and interobserver variabilities for TFC were 0.961 and 0.933, respectively.

**Biochemical measurements.** Blood samples were drawn without stasis at 7-8 AM after 20 minutes of supine rest following a fasting period of 12 hours. Glucose, creatinine, and lipid profiles were determined by standard methods. The activity of GGT was measured by using a Roche Modular P-800 autoanalyzer with original kits.

**Statistical analysis.** Continuous variables were given as mean  $\pm$  standard deviation (SD) and cat-

**Table 1. Baseline clinical and laboratory characteristics of the three groups**

	SCF group (n=90)			CAD group (n=88)			Control group (n=86)			<i>p</i>
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	
Age (years)	50.8±9.4			51.4±8.8			51.6±10.1			0.39
Sex										0.40
Males	47	52.2		45	51.1		44	51.2		
Female	43	47.8		43	48.9		42	48.8		
Systemic hypertension	21	23.3		21	23.9		20	23.3		0.32
Heart rate (beat/min)			75.3±8.8			77.6±9.1			74.1±7.9	0.27
Diabetes mellitus	12	13.3		11	12.5		11	12.8		0.58
Smoking	35	38.9		31	35.2		27	31.4		0.26
Body mass index (kg/m <sup>2</sup> )	25.9±5.5			26.1±5.8			26.2±6.0			0.32
Laboratory findings										
Fasting glucose (mg/dl)			99.0±12.0			105.0±15.0			102.0±11.0	0.51
Gamma-glutamyltransferase (U/l)			30.5±7.2			30.0±7.4			22.1±5.2	<0.01
Aspartate aminotransferase (U/l)			22.7±6.7			21.9±6.5			21.9±6.2	0.84
Alanine aminotransferase (U/l)			22.9±5.7			22.9±5.8			22.6±5.8	0.81
Alkaline phosphatase (U/L)			155.4±55.8			155.6±52.9			154.2±51.2	0.77
Total bilirubin (mg/dl)			0.70±0.22			0.74±0.24			0.68±0.19	0.82
Direct bilirubin (mg/dl)			0.23±0.13			0.25±0.13			0.22±0.14	0.85
Hemoglobin (g/dl)			13.8±1.6			13.4±1.3			13.5±1.2	0.28
Total cholesterol (mg/dl)			195.8±50.9			196.5±43.7			198.0±39.5	0.82
LDL-cholesterol (mg/dl)			120.0±26.2			122.0±24.6			117.0±28.7	0.80
HDL-cholesterol (mg/dl)			44.2±11.4			44.8±10.6			45.7±9.8	0.82
Triglycerides (mg/dl)			147.8±51.3			149.4±45.8			152.3±44.7	0.93
Creatinine (mg/dl)			0.94±0.18			0.92±0.13			0.93±0.15	0.80
Medications										
Beta-blocker	10	11.1		44	50.0		7	8.1		<0.01
ACE inhibitor	9	10.0		40	45.5		10	11.6		<0.01
Aspirin	25	27.8		80	90.9		6	7.0		<0.01
Statin	8	8.9		62	70.5		11	12.8		<0.01
Calcium channel blockers	4	4.4		4	4.6		5	5.8		0.34
TIMI frame count (TFC)										
Left anterior descending (LAD)			44.1±6.4			28.6±5.5			27.1±4.8	<0.01
Corrected TFC of LAD			26.0±3.8			17.2±3.2			16.6±3.1	<0.01
Left circumflex artery			22.2±3.7			16.7±3.1			16.3±3.3	<0.01
Right coronary artery			21.1±6.1			15.9±2.9			15.5±2.8	<0.01
Mean TFC			23.1±2.5			16.6±2.6			16.1±2.3	<0.01

SCF: Slow coronary flow; CAD: Coronary artery disease; ACE: Angiotensin-converting enzyme.

ategorical variables were expressed as percentages. Data were tested for normal distribution using the Kolmogorov-Smirnov test. Groups were compared with the Kruskal-Wallis test for multiple comparisons. When a significant difference between three groups was observed by using Kruskal-Wallis test, Mann-Whitney U-test was used for determination of difference between couples. Correlations between the mean TFC and clinical-laboratory parameters were assessed by the Pearson correlation test. Multiple linear regression analysis was performed to identify the independent predictors of the mean TFC. Statistical significance was defined as  $p<0.05$ . The SPSS statistical software (SPSS for windows 10.0) was used for all statistical calculations.

## RESULTS

The clinical characteristics, laboratory parameters and TFC values of the SCF, CAD, and control groups are presented in Table 1. Age, body mass index, presence of hypertension and diabetes mellitus, lipid profiles, and fasting glucose levels were not different between the three groups. Laboratory characteristics of the groups were not statistically different (Table 1). The use of medications including angiotensin-converting enzyme (ACE) inhibitor, beta-blocker, statin, and aspirin was significantly higher in the CAD group ( $p<0.01$ ). Compared to the control group, serum GGT activity was significantly increased in both SCF and CAD groups ( $p<0.01$ ), but the two groups did not differ significantly in this respect ( $p=0.71$ ).

**Table 2. Relationship between the mean TIMI frame count and clinical and laboratory parameters**

	Pearson analysis		Regression analysis	
	r	p	$\beta$	p
Age	0.122	0.542		
Sex	0.237	0.168		
Body mass index	0.156	0.215		
Hypertension	0.044	0.875		
Diabetes mellitus	0.245	0.124		
Smoking	0.219	0.466		
Heart rate	-0.231	<b>0.017</b>	-0.098	0.174
Total cholesterol	-0.194	0.365		
LDL-cholesterol	-0.064	0.411		
HDL-cholesterol	-0.094	0.251		
Triglyceride	0.107	0.687		
Fasting glucose	0.156	0.569		
Creatinine	0.105	0.765		
Hemoglobin	0.187	0.654		
Serum GGT activity	0.326	<b>&lt;0.001</b>	0.309	<b>&lt;0.001</b>
Coronary artery disease	0.198	0.123		

Of the three groups, the TFCs for all the epicardial coronary arteries and the mean TFC were significantly higher in the SCF group ( $p<0.01$ ; Table 1). Patients with CAD had similar TFC parameters compared to the controls.

Relationships between the mean TFC and clinical and laboratory data are presented in Table 2. The mean TFC showed a positive and moderate correlation with serum GGT activity. In linear regression analysis, serum GGT activity was found as the only independent predictor of the mean TFC ( $\beta=0.309$ ;  $p<0.001$ ).

## DISCUSSION

In the present study, we found that (i) GGT activity was significantly increased in patients with SCF and in patients with CAD compared to subjects with angiographically normal coronary arteries and normal coronary flow, (ii) serum GGT activity was significantly and moderately correlated with the mean TFC, and (iii) GGT was an independent predictor of the mean TFC and the presence of SCF.

The precise pathophysiological mechanism of the SCF phenomenon still remains uncertain. Small vessel abnormality and dysfunction have been implicated in its pathogenesis.<sup>[1]</sup> Mangieri et al.<sup>[9]</sup> reported histopathological findings of left ventricular endomyocardial biopsy specimens in a group of 10 patients with SCF without any other cardiac or systemic diseases. They showed evidence for small vessel abnormality as endothelial thickening due to cell edema, capil-

lary damage, and reduced luminal diameter of the small vessels. Additionally, inflammation,<sup>[11,12]</sup> platelet function disorder,<sup>[13,14]</sup> and imbalance of vasoactive substances<sup>[15,16]</sup> have also been implicated in the pathogenesis of the SCF phenomenon.

Serum paraoxonase (PON) is a high-density lipoprotein-bound antioxidant enzyme that inhibits atherosclerosis and endothelial dysfunction. Yıldız et al.<sup>[17]</sup> reported that serum PON activity was independently associated with the mean TFC, suggesting that reduced serum PON activity might represent a biochemical marker of SCF. Enli et al.<sup>[18]</sup> showed that patients with SCF had significantly increased serum malondialdehyde and erythrocyte superoxide dismutase levels and decreased erythrocyte-reduced glutathione levels compared to patients with normal coronary flow. These findings indicate that free radical damage may play a role in the pathogenesis of SCF.

Serum GGT activity has been used as a marker for alcohol consumption or hepatobiliary disease.<sup>[6]</sup> However, it has been shown in *in vitro* experiments that GGT activity is directly related to oxidative events, playing a role in the evolution of atheromatous plaque and induces LDL oxidation in the presence of iron ions.<sup>[19-22]</sup> Gamma-glutamyltransferase activity has been identified in human atheromatous plaques.<sup>[22]</sup> The prognostic significance of GGT has been studied extensively. A prospective research among CAD patients revealed that the prognostic significance of serum GGT activity was particularly evident in a subset of ischemic patients with multi-vessel CAD and previous myocardial infarction.<sup>[23]</sup> This finding suggests that the significance of serum GGT activity is more pronounced in patients having vulnerable plaques. *In vitro* studies showed that, in the presence of an iron source, such as transferrin, LDL oxidation catalyzed by GGT played an important role in plaque development.<sup>[19-22]</sup> All these findings point out to the possible role of GGT in the development of SCF.

Coronary microvasculature, with small-diameter and well-developed media, is the major vascular determinant of coronary vascular resistance<sup>[24]</sup> and atherosclerosis and dysfunction of coronary microvasculature are well-known pathophysiologic mechanisms of SCF.<sup>[5]</sup> In a recent article, Erdogan et al.<sup>[25]</sup> reported that the coronary flow reserve, which reflects coronary microvascular function, was impaired in patients with SCF and corrected TFC was correlated with coronary flow reserve.

In our study, the TFCs for all the epicardial coronary arteries and the mean TFC were significantly

higher in the SCF group compared to patients with CAD and controls. On the other hand, serum GGT activity was significantly increased in both SCF and CAD groups. These findings were consistent with our expectations for the SCF group, but were unexpected for the CAD group with normal coronary flow. This may suggest a relationship between increased GGT levels and the pathogenesis of atherosclerosis. Furthermore, several studies have demonstrated that medications such as dipyridamol, ACE inhibitors, calcium channel blockers, and statins have positive effects on microvascular dysfunction and SCF.<sup>[9,26-30]</sup> The use of these medications was significantly more common in the CAD group, which may account for the presence of normal coronary flow and TFCs.

There are some limitations of our study that should be taken into account. Diagnosis of normal coronary arteries depends on contrast angiograms of the vessel lumen, which may underestimate the presence of atherosclerotic plaque.<sup>[31]</sup> Intravascular ultrasound (IVUS) provides a more precise assessment of the presence and distribution of atherosclerosis in vessel lumen and throughout the wall.<sup>[32]</sup> We did not have the opportunity to perform IVUS in this study. On the other hand, heart rate, nitrate use, and coronary catheter size may influence the frame count.<sup>[33]</sup> In this study, patients using nitrates were excluded and the catheters used in all the participants were of the same size. Heart rate was similar in the three groups and it was not an independent factor to affect the mean TFC in regression analysis. Thus, increased GGT levels in patients with CAD in the absence of SCF may have different mechanisms other than drug use. This can be better evaluated with the inclusion of another group of patients having both CAD and SCF. The absence of such a group is another limitation of our study.

To our knowledge, this is the first study to report an association between increased serum GGT activity and SCF. Further studies are needed to clarify the physiopathologic role of serum GGT activity in patients with SCF.

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## Increased serum gamma-glutamyltransferase activity in patients with metabolic syndrome

### Metabolik sendromu olan hastalarda artmış serum gama-glutamiltransferaz düzeyi

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#### ABSTRACT

**Objectives:** Accumulating data indicate that serum gamma-glutamyltransferase (GGT) activity represents a true marker of atherosclerotic cardiovascular disease and has prognostic importance. In this study, we sought to evaluate serum GGT activity in patients with metabolic syndrome (MetS).

**Study design:** We enrolled 232 patients (mean age 60.4 years) from our outpatient cardiology clinic, 117 with and 115 without MetS (control group) as defined by the ATP-III criteria. The results of serum liver function tests including serum GGT and C-reactive protein (CRP) levels were compared between the two groups.

**Results:** The two groups were similar with regard to age, sex, smoking, and family history of coronary artery disease ( $p>0.05$ ). The prevalences of hypertension and dyslipidemia were significantly higher in patients with MetS. Compared with controls, patients with MetS had significantly higher serum GGT [(median 21, interquartile range (16-33) vs. 19 (14-26) U/l;  $p=0.008$ ] and C-reactive protein levels [6.2 (3.6-9.4) vs. 5.0 (3.1-7.0) U/l;  $p=0.044$ ]. A high GGT activity (>40 U/l) was determined in 14.5% of the patients with MetS and in 4.4% of the control subjects ( $p=0.012$ ). Serum GGT level showed significant correlations with MetS ( $r=0.24$ ,  $p=0.001$ ), CRP ( $r=0.20$ ,  $p=0.003$ ), triglyceride ( $r=0.18$ ,  $p=0.006$ ), HDL cholesterol ( $r=-0.19$ ,  $p=0.004$ ), aspartate aminotransferase ( $r=0.15$ ,  $p=0.02$ ), alanine aminotransferase ( $r=0.32$ ,  $p=0.001$ ), and alkaline phosphatase ( $r=0.16$ ,  $p=0.01$ ). This significant association continued only for MetS ( $\beta=-0.25$ ,  $p=0.03$ ), HDL cholesterol ( $\beta=-0.18$ ,  $p=0.03$ ), and alkaline phosphatase ( $\beta=0.17$ ,  $p=0.01$ ) in multivariate regression analysis.

**Conclusion:** Our findings suggest that patients with MetS have higher serum GGT and CRP levels compared with controls. This increased GGT level might be a marker of increased oxidative stress and premature atherosclerosis.

#### ÖZET

**Amaç:** Giderek artan veriler serum gama-glutamiltransferaz (GGT) düzeyinin aterosklerotik kardiyovasküler hastalık için gerçek bir belirteç olduğunu ve прогнозik değer taşıdığını göstermektedir. Bu çalışmada metabolik sendromu (MetS) olan hastalarda GGT düzeyinin incelenmesi amaçlandı.

**Çalışma planı:** Kardiyoloji polikliniğine başvuran 232 hasta (117 MetS, 115 kontrol; ort. yaşı 60.4) çalışmaya alındı. Metabolik sendrom tanısı ATP III ölçütlerine göre kondu. Hasta ve kontrol grubunun GGT dahil karaciğer fonksiyon testleri sonuçları ve C-reaktif protein (CRP) düzeyleri karşılaştırıldı.

**Bulgular:** İki grup yaş, cinsiyet, sigara içme ve ailede koroner arter hastalığı öyküsü açısından benzerdi ( $p>0.05$ ). Hipertansiyon ve hiperlipidemi sıklığı MetS grubunda daha yüksek idi. Kontrol grubuyla karşılaştırıldığında, MetS olan hastalarda serum GGT [medyan 21, çeyreklerarası aralık (16-33) ve 19 (14-26) U/l;  $p=0.008$ ] ve C-reaktif protein [6.2 (3.6-9.4) ve 5.0 (3.1-7.0) U/l;  $p=0.044$ ] düzeyleri anlamlı derecede yüksek saptandı. Yüksek GGT aktivitesi (>40 U/l) MetS grubunda %14.5 oranında, kontrol grubunda %4.4 oranında görüldü ( $p=0.012$ ). Serum GGT düzeyi şu parametrelerle anlamlı ilişki gösterdi: MetS ( $r=0.24$ ,  $p=0.001$ ), CRP ( $r=0.20$ ,  $p=0.003$ ), triglisirit ( $r=0.18$ ,  $p=0.006$ ), HDL-kolesterol ( $r=-0.19$ ,  $p=0.004$ ), aspartat aminotransferaz ( $r=0.15$ ,  $p=0.02$ ), alanin aminotransferaz ( $r=0.32$ ,  $p=0.001$ ) ve alkalin fosfataz ( $r=0.16$ ,  $p=0.01$ ). Çokdeğişkenli regresyon analizinde bu anlamlılık sadece MetS ( $\beta=-0.25$ ,  $p=0.03$ ), HDL-kolesterol ( $\beta=-0.18$ ,  $p=0.03$ ) ve alkalin fosfataz ( $\beta=0.17$ ,  $p=0.01$ ) için vardı.

**Sonuç:** Bulgularımız MetS olan hastalarda serum GGT ve CRP düzeylerinin yüksek olduğunu göstermektedir. Artmış GGT düzeyi MetS'li olgularda artmış oksidatif stresin ve erken aterosklerozun bir belirteci olabilir.

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Serum gamma-glutamyltransferase is a marker of hepatobiliary disease and alcohol consumption. It is a plasma membrane enzyme with a central role in glutathione homeostasis which is important in maintaining adequate concentrations of intracellular glutathione to protect cells against oxidants.

It has been shown in rat lung epithelial cells that GGT expression becomes more apparent by oxidants, suggesting that increased GGT activity may be a marker for oxidative stress.<sup>[1]</sup> These findings have been supported by other research, demonstrating that serum concentrations of GGT could be used as a marker for increased oxidative stress in humans.<sup>[2]</sup> Accumulating data indicate that there is an association between serum GGT levels (within the normal range) and cardiovascular diseases.<sup>[3,4]</sup> An association has been shown between elevated GGT and obesity.<sup>[5]</sup> Nonalcoholic fatty liver disease, a manifestation of obesity, has been reported to be associated with GGT elevation.<sup>[6]</sup> Several studies have revealed that elevated serum GGT is a predictor for the development of diabetes mellitus.<sup>[3,7,8]</sup> A population-based study demonstrated a significant association between serum GGT levels and type 2 DM.<sup>[9]</sup>

Factors responsible for elevated liver enzymes, especially GGT, have been shown to include increasing age, obesity, DM, physical inactivity, insulin resistance, hypertension, and dyslipidemia.<sup>[4]</sup> Metabolic syndrome is a constellation of atherosclerotic risk factors and identifies patients who are at high risk for DM and cardiovascular disease.

Considering these associations between GGT and cardiovascular disease, we evaluated the possible relationship between serum GGT activity and MetS. We also investigated potential associations between serum GGT levels and cardiac risk factors, and the levels of other liver enzymes and C-reactive protein.

## PATIENTS AND METHODS

We enrolled 232 patients from our outpatient cardiology clinic, 117 with and 115 without MetS (control group). The diagnosis of MetS was based on the National Cholesterol Education Program, ATP III criteria.<sup>[10]</sup> Patients having at least three of the following five criteria were considered to have MetS: (i) fasting blood glucose  $\geq 110$  mg/dl; (ii) serum triglyceride  $\geq 150$  mg/dl or being on lipid lowering therapy; (iii) serum HDL  $< 40$  mg/dl in men and  $< 50$  mg/dl in women or being on antilipidemic therapy; (iv) blood pressure  $\geq 130$

mmHg systolic and/or  $\geq 85$  mmHg diastolic or being on antihypertensive therapy; and (v) waist circumference  $>102$  cm in men and  $>88$  cm in women.

### Abbreviations:

<i>ALT</i>	Alanine aminotransferase
<i>AP</i>	Alkaline phosphatase
<i>AST</i>	Aspartate aminotransferase
<i>CRP</i>	C-reactive protein
<i>DM</i>	Diabetes mellitus
<i>GGT</i>	Gamma-glutamyltransferase
<i>MetS</i>	Metabolic syndrome
<i>NAFLD</i>	Nonalcoholic fatty liver disease

Exclusion criteria involved the presence of the following: alcohol intake more than 30 g/day, hepatitis B or C infection or other known liver diseases, liver enzymes exceeding three times the upper reference range, use of hepatotoxic drugs, acute infectious/inflammatory conditions, familial hyperlipidemia, or New York Heart Association class 3-4 heart failure.

Dyslipidemia was defined as a total cholesterol level  $>200$  mg/dl, LDL cholesterol level  $>130$  mg/dl, HDL cholesterol level  $<40$  mg/dl, or a triglyceride level  $>150$  mg/dl or being on lipid lowering treatment (ATP III). Body mass index was calculated as weight (kg)/[height (m)]<sup>2</sup>. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest with the patient in the standing position and at the end of a normal expiration. A measuring tape was placed around the abdomen parallel to the floor, taking care not to compress the skin while reading. Hypertension was defined as blood pressure  $\geq 140/90$  mmHg on two or more measurements or being on antihypertensive medication. Smoking was defined as current cigarette smoking or abstinence  $\leq 2$  years.

Venous blood samples were obtained after overnight fasting. Serum liver enzymes, CRP levels, and other hematochemical variables were determined and compared between the groups. Serum GGT levels were measured by the enzymatic calorimetric test at 37 °C on a Roche/Hitachi analyzer (Mannheim, Germany), using L-gamma-glutamyl-3-carboxy-4-nitroanilide as a substrate. Using this method, the normal reference range of the GGT level was 8 to 61 U/l. Serum CRP levels were determined by the immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany) with a normal reference value of  $< 10$  mg/l. Enzymatic measurements of total cholesterol, triglyceride, and HDL levels were performed on a Hitachi 912 auto-analyzer using commercial kits. LDL was calculated using the Friedewald formula.

The study protocol was approved by the local ethics committee and informed consent was obtained from each subject.

**Table 1.** Demographic and clinical characteristics of the study and control groups

	Metabolic syndrome (n=117)			Control (n=115)			<i>p</i>
	n	%	Mean±SD	n	%	Mean±SD	
Age (years)			60.8±9.7			60.1±9.7	0.5
Gender							0.4
Female	86	73.5		79	68.7		
Male	31	26.5		36	31.3		
History of myocardial infarction	15	12.8		8	7.0		0.1
Atrial fibrillation	3	2.6		5	4.4		0.7
Body mass index (kg/m <sup>2</sup> )			30.2±4.6			27.6±4.9	<0.001
Waist circumference (cm)			101.1±9.9			94.5±11.3	<0.001
Risk factors							
Hypertension	104	88.9		67	58.3		<0.001
Dyslipidemia	93	79.5		69	60.0		0.001
Smoking	30	25.6		27	23.5		0.07
Menopause (females)	68	79.1		60	76.0		0.7
Family history of early CAD	51	43.6		50	43.5		1.0
Medications							
ACE inhibitor/angiotensin receptor blocker	53	45.3		34	29.6		0.001
Calcium channel blocker	28	23.9		18	15.7		0.009
Beta-blocker	28	23.9		26	22.6		0.1
Diuretics	38	32.5		30	26.1		0.02
Statin	43	36.8		17	14.8		<0.0001
Fibrates	6	5.1		2	1.7		0.2

Statistical analyses were performed using the SPSS software (ver. 9.0). Data were expressed as means ± standard deviation (SD) or median and interquartile ranges, or as frequencies and group percentages, where appropriate. Distribution of continuous variables for normality was tested with the one-sample Kolmogorov-Smirnov test. Differences between patients with MetS and controls for variables with or without normal distribution were evaluated using the unpaired t-test and Mann-Whitney U-test, respectively. Categorical variables were analyzed with the chi-square test. Correlations were sought by the Pearson correlation analysis. Multivariate linear regression analysis was used to assess the independent associations with GGT. All *p* values were two-sided, and a *p* value of <0.05 was considered significant.

## RESULTS

The mean age of the study population was 60.4±9.7 years, and 165 (71.1%) were females. Table 1 shows demographic and clinical characteristics and labora-

tory results for both groups. The two groups with and without MetS were homogenous with regard to age and sex (*p*>0.05).

As expected, the prevalences of hypertension and dyslipidemia were significantly higher in patients with MetS, whereas the two groups were similar with respect to smoking and family history of coronary artery disease. The mean values for body mass index and waist circumference were significantly higher in the MetS group. Concerning the medications, the use of an ACE inhibitor/angiotensin receptor blocker, calcium channel blocker, and diuretics was higher in patients with MetS (*p*<0.05), while beta blocker use was similar in both groups (*p*>0.05). As expected, statin use was significantly higher in the MetS group (*p*<0.05). Eight patients were on fibrate therapy, six in the MetS group and two in the control group.

Compared with the controls, patients with MetS had a significantly higher median serum GGT level (*p*=0.008, Table 2). This association was also observed after exclusion of patients with a history of

**Table 2.** Laboratory results of the study and control groups

	Metabolic syndrome (n=117)		Control (n=115)		<i>p</i>
	Mean±SD	Median (Range)	Mean±SD	Median (Range)	
Fasting blood glucose (mg/dl)	105±19		94±14		<0.001
Total cholesterol (mg/dl)	211±38		204±53		0.2
HDL cholesterol (mg/dl)	45±10		55±13		<0.001
LDL cholesterol (mg/dl)	132±32		119±32		0.003
Triglyceride (mg/dl)		193 (153-259)		115 (84-158)	<0.001
Uric acid (mg/dl)	5.6±1.3		5.6±1.3		0.1
C-reactive protein (mg/l)		6.2 (3.6-9.4)		5.0 (3.1-7.0)	0.044
Gamma-glutamyltransferase (U/l)		21 (16-33)		19 (14-26)	0.008
Aspartate aminotransferase (U/l)	22.3±6.2		24.0±9.8		0.1
Alanine aminotransferase (U/l)	22.8±11.5		22.6±13.6		0.6
Alkaline phosphatase (U/l)	190.4±51.6		198.1±56.8		0.3
Total bilirubin (mg/dl)	0.61±0.32		0.69±0.30		0.1
Direct bilirubin (mg/dl)	0.16±0.13		0.17±0.10		0.7
Hemoglobin (g/dl)	13.8±1.2		13.8±1.2		0.9
Leukocyte (K/mm <sup>3</sup> )	6.9±1.6		6.8±2.3		0.8
Platelets (K/mm <sup>3</sup> )	256±74		259±70		0.7

alcohol consumption of less than 30 g/day (*p*<0.001). When the patients were divided into two groups as in previous studies based on serum GGT levels of ≤40 U/l (low GGT activity) and >40 U/l (high GGT activity), a high GGT activity was identified in 14.5% of the patients with MetS and in 4.4% of the control subjects (*p*=0.012). Further evaluation of the two groups based on the median GGT values showed that 53.9% of MetS patients had a GGT concentration above the median value, compared to 41.7% in the control group (*p*=0.043).

The median serum CRP level was significantly higher in patients with MetS than in controls (*p*=0.044). Patients with MetS had significantly higher LDL cholesterol (*p*=0.003) and triglyceride (*p*<0.001) concentrations, and lower HDL cholesterol levels (*p*<0.001), whereas total cholesterol levels were similar in the two groups (*p*>0.05). Serum levels of other liver enzymes including alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, and total and direct bilirubin concentrations did not differ between the two groups (*p*>0.05). The two groups were also comparable with regard to complete blood count results (*p*>0.05).

In correlation analysis, serum GGT level showed significant correlations with MetS (*r*=0.24, *p*=0.001),

and CRP (*r*=0.20, *p*=0.003), triglyceride (*r*=0.18, *p*=0.006), and HDL cholesterol (*r*=-0.19, *p*=0.004) levels, and with liver enzymes AST (*r*=0.15, *p*=0.02), ALT (*r*=0.32, *p*=0.001), and AP (*r*=0.16, *p*=0.01). Leukocyte count was not correlated with serum GGT (*r*=0.07, *p*=0.28). This significant association continued only for MetS ( $\beta$ =-0.25, *p*=0.03), HDL cholesterol ( $\beta$ =-0.18, *p*=0.03), and alkaline phosphatase ( $\beta$ =0.17, *p*=0.01) in multivariate regression analysis. (Table 3).

## DISCUSSION

The present study demonstrates that patients with MetS have increased GGT activity, a marker of oxidative stress, and serum CRP, a marker of systemic inflammation.

It has been clearly demonstrated that serum GGT levels even within normal range are associated with some atherosclerotic risk factors and are predictors of future heart disease, hypertension, stroke, and type 2 DM.<sup>[4,7,11]</sup> Although the exact mechanism responsible for this association is unknown, several possible mechanisms have been proposed for the role of serum GGT in increasing cardiovascular risk. The most widely accepted mechanism is oxidative stress, followed by hepatic insulin resistance and subclinical inflammation.

**Table 3. Multivariate predictors of serum GGT activity adjusted for other liver enzymes**

	Coefficient	95% CI	p
Metabolic syndrome (MetS)	-0.259	-16.0, -0.84	<b>0.03</b>
Number of MetS components	-0.20	-6.92, 1.27	0.17
Age	0.013	-0.23, 0.28	0.86
Fasting glucose	0.049	-0.11, 0.20	0.55
HDL cholesterol	-0.182	-0.42, -0.02	<b>0.03</b>
Triglyceride	-0.003	-0.02, 0.01	0.96
Waist circumference	0.08	-0.11, 0.36	0.30
Systolic blood pressure	0.06	-0.12, 0.20	0.61
Diastolic blood pressure	-0.14	-0.49, 0.12	0.24
Alkaline phosphatase	0.17	0.01, 0.09	<b>0.01</b>

Through these mechanisms, elevated GGT is thought to play a role in the initiation and progression of atherosclerosis.

Second, elevated serum GGT might be a marker of NAFLD, which is thought to cause hepatic insulin resistance. Although serum ALT and GGT levels have also been found to be associated with fatty liver, only GGT activity has been reported to be related to oxidative stress. An association between GGT and systemic inflammation has also been demonstrated.<sup>[3,7,12,13]</sup> Studies have shown that NAFLD, in which liver enzymes (including GGT) are usually elevated, is associated with insulin resistance, and patients with this condition are at high risk for cardiovascular diseases.<sup>[5,14]</sup> Conversely, a study in Pima Indians found that ALT concentration but not serum GGT or AST was related to hepatic insulin action.<sup>[15]</sup>

The third possible mechanism implicated is subclinical chronic inflammation. Evidence indicates that serum GGT elevation might be due to inflammation, an important mechanism in all stages of atherosclerotic cardiovascular disease.<sup>[16]</sup> C-reactive protein synthesized by the liver as a marker of systemic inflammation has been shown to be associated with MetS, DM, and cardiovascular disease.<sup>[17]</sup> Indeed, oxidative processes are components of chronic inflammation acting on different pathways and stimulating the inflammatory response. It has been shown that an association exists between serum GGT and CRP levels, while no such association has been reported between ALT and CRP.<sup>[18]</sup> This finding is important in that increased GGT activity is associated with an inflammation marker, CRP. Recent data have shown that, in the presence of Fe<sup>3+</sup> and Cu<sup>2+</sup>, GGT is involved in generating free oxygen radicals, which in turn, induce

oxidative stress to cells.<sup>[19]</sup> Thus, subclinical inflammation and oxidative stress are implicated as important mechanisms in the development of atherosclerosis and MetS. In the CARDIA study (Coronary Artery Risk Development in Young Adults), serum GGT levels within normal limits were found to predict CRP levels, a marker of inflammation, and F<sub>2</sub>-isoprostanes, a marker of oxidative activity.<sup>[20]</sup>

Nakanishi et al.<sup>[8]</sup> reported that GGT activity was related to the development of impaired fasting glucose or type 2 DM. These authors also found an association between serum GGT and white blood cell count and stated that this finding could provide evidence for subclinical inflammation as an underlying mechanism. In our study, we found a significant association between serum CRP levels and GGT activity, suggesting that subclinical inflammation might act as an underlying mechanism.

Data on serum GGT levels and MetS are limited. In a cross-sectional study, Onat et al.<sup>[20]</sup> reported that waist circumference was a major determinant of serum GGT activity. Analysis of the Mexico City Diabetes Study revealed that all four liver enzymes –serum ALT, AST, GGT, and AP– were associated with multiple features of MetS, with GGT being associated with the largest number of features.<sup>[21]</sup> Bo et al.<sup>[18]</sup> reported that serum levels of GGT in healthy adult subjects with no measurable metabolic abnormalities were associated with fasting glucose levels of normal range, providing evidence for oxidative stress (increased nitrotyrosine levels) and inflammation (elevated CRP levels).

In sum, it may be said that GGT levels seem to be elevated in patients with MetS, a condition that

poses a high risk for atherosclerotic cardiovascular disease. Our findings suggest that GGT might act as an intervening factor in the association between obesity, MetS, and DM. We speculate that the association between GGT levels and MetS might be due to the adverse oxidative pattern of this patient population.

### Limitations

Patients in the MetS group had, by definition, 3, 4, or 5 components of the syndrome, while some subjects in the control group had 1 or 2 components. A control group having none of these components would yield better results. The probability of NAFLD is expected to be higher in patients with MetS, a factor that should be taken into account when interpreting our findings. If high-sensitivity CRP, instead of conventional CRP, had been studied, it could have provided us more valuable data.

In conclusion, patients with MetS have a higher serum GGT activity than those without this syndrome. Since GGT can be determined easily, this inexpensive and eligible marker might have important use in clinical practice. Details regarding the underlying link between elevated GGT and multiple coronary risk factors remain unclear. Further research is required to elucidate the exact role of GGT and how the activity of this enzyme is related to MetS or its components.

**Conflict-of-interest issues regarding the authorship or article:** None declared

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## Usefulness of admission gamma-glutamyltransferase level for predicting new-onset heart failure in patients with acute coronary syndrome with left ventricular systolic dysfunction

### Gama-glutamil transferaz enziminin akut koroner sendroma bağlı sol venrikül sistolik fonksiyon bozukluğu gelişen hastalarda yeni başlangıçlı kalp yetersizliğini öngörmede yararı

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#### ABSTRACT

**Objectives:** Our aim was to determine whether there is a relationship between admission gamma-glutamyltransferase (GGT) and subsequent heart failure hospitalizations in patients with acute coronary syndrome.

**Study design:** We selected 123 patients with newly diagnosed acute coronary syndrome of ejection fraction (EF) <45%. Patients were followed 15±10 months, and the relationship between admission GGT level and hospitalization because of heart failure during the follow-up was examined.

**Results:** Twenty-three (18.7%) patients were hospitalized during the follow-up of 15±10 months. Receiver operating characteristic (ROC) curve analysis showed that the cut-off point of admission GGT related to predict hospitalization was 49 IU/L, with a sensitivity of 81.7% and specificity of 65.2%. Increased GGT >49 IU/L on admission, presence of hypertension and hyperlipidemia, left ventricular ejection fraction (LVEF), right ventricular dysfunction, moderate-to-severe mitral regurgitation, alanine aminotransferase level, and antiplatelet agent usage were found to have prognostic significance in univariate Cox proportional hazards analysis. In multivariate Cox proportional-hazards model, increased GGT >49 IU/L on admission (hazard ratio [HR] 2.663, p=0.047), presence of hypertension (HR 4.107, p=0.007), and LVEF (HR 0.911, p=0.002) were found to be independent factors to predict new-onset heart failure requiring hospitalization.

**Conclusion:** Hospitalization in heart failure was associated with increased admission GGT levels. Increased admission GGT level in acute coronary syndrome with heart failure should be monitored closely and treated aggressively.

#### ÖZET

**Amaç:** Çalışmamızın amacı akut koroner sendrom nedeniyle hastaneye kabul sırasında gama-glutamil transferaz (GGT) düzeyleri ile kalp yetersizliği nedeniyle hastaneye yatışlar arasında ilişki olup olmadığını araştırmaktır.

**Çalışma planı:** Çalışmaya akut koroner sendrom ile başvurup ejeksiyon fraksiyonu (EF) %45'in altında olan 123 hasta alındı. Hastalar 15±10 ay takip edildi. Hastaların kabul sırasında GGT düzeyleri ile izleme süresinde kalp yetersizliği nedeniyle hastaneye yatışları arasındaki ilişki incelendi.

**Bulgular:** İzleme süresi olan 15±10 ay içinde 23 hasta (%18.7) kalp yetersizliğine bağlı olarak hastaneye yatırıldı. ROC (receiver operating characteristics) eğrisi analizi yöntemi ile hastaneye yışı öngördüren GGT kesim değeri 49 IU/L olarak saptandı (%81.7 duyarlılık ve %65.2 özgüllük). Tek değişkenli Cox orantısal risk analizinde, GGT düzeyinin >49 IU/L olması, hipertansiyon ve hiperlipidemi, sol venrikül ejeksiyon fraksiyonu (SVEF), orta-ciddi mitral yetersizliği varlığı, alanin aminotransferaz seviyesi ve antitrombosit ilaç kullanımı anlamlı bulundu. Çok değişkenli Cox orantısal risk modelinde, kalp yetersizliği nedeniyle hastaneye yışı ile ilişkili bağımsız risk faktörleri olarak ilk kabul sırasında GGT >49 olması (risk oranı [RO] 2.663, p=0.047), hipertansiyon varlığı (RO 4.107, p=0.007) ve SVEF (RO 0.911, p=0.002) parametreleri saptandı.

**Sonuç:** Akut koroner sendromlu hastalarda kabul GGT düzeyleri takipte kalp yetersizliğine bağlı hastaneye yatışlar ile ilişkilidir. Bu hastalar daha yakın takip edilmeli ve tedavileri optimal düzeyde ayarlanmalıdır.

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Heart failure (HF) is an important problem of public health worldwide and is associated with significant morbidity and mortality. In addition to the traditional ones, different types of biomarkers, such as systemic inflammation and metabolism, were shown to be associated with disease severity and disease progression.<sup>[1]</sup>

Gamma-glutamyltransferase (GGT) is a second-generation enzymatic liver function test, and it was used initially as a sensitive indicator of alcohol ingestion and hepatobiliary dysfunction.<sup>[2]</sup> It is found not only in the liver but also in the kidney and vascular epithelium, as well as in the extracellular fluid, attached to albumin carrier molecules and lipoproteins.<sup>[3-5]</sup> It may also play a role in oxidative stress in accompaniment with glutathione metabolism, and has a possible role as a proatherogenic marker because of its indirect relationship in the biochemical steps to low-density lipoprotein cholesterol oxidation.<sup>[2]</sup>

Recent studies have reported that serum GGT, which is an inexpensive and easily accessible laboratory test, is a predictor for incident cardiovascular diseases, and is associated with prognosis in cardiopulmonary disorders such as coronary artery disease, acute myocardial infarction, HF, and acute pulmonary embolism.<sup>[6-21]</sup> However, the prognostic significance of GGT in patients presenting with acute coronary syndrome (ACS) with left ventricular systolic dysfunction (LVSD) has not been searched yet. We hypothesized that increased admission serum GGT activity may be associated with future acute HF in ACS patients with LVSD.

## PATIENTS AND METHODS

### Study population

A total of 247 consecutive patients who presented with their first ACS were considered for enrollment. Patients who had no previous history of myocardial infarction or other cardiac diseases and who were not on any medications were selected. Patients with alcohol usage, malignancy, hepatobiliary pathology, and acute inflammatory diseases were excluded. Of these patients, echocardiographic examination at the index admission, which yielded low ejection fraction (EF <45%), was available in 123 patients with ACS. Finally, 123 ACS patients who presented with an initial reduced left ventricular systolic function (EF <45%)

were enrolled into the study. Of these 123 patients, 75 presented with ST-segment elevation myocardial infarction (STEMI), 33 with non-ST-segment elevation myocardial infarction (NSTEMI), and 15 with

### Abbreviations:

ACS	Acute coronary syndrome
AUC	Area under the curve
EF	Ejection fraction
GGT	Gamma-glutamyltransferase
HF	Heart failure
LVSD	Left ventricular systolic dysfunction
NSTEMI	Non-ST-segment elevation myocardial infarction
ROC	Receiver operating characteristic
STEMI	ST-segment elevation myocardial infarction
USAP	Unstable angina pectoris

unstable angina pectoris (USAP). These patients were followed-up for 15±10 months after discharge, based on the endpoints of rehospitalization with acute HF with regard to the initial admission serum GGT activity. The diagnosis of acute HF was based on the existence of novel symptoms or characteristic clinical signs and the evidence of left ventricular dysfunction, determined by echocardiography. The study was approved by the local ethics committee. Informed consent was obtained from all patients.

The optimal cut-off point of GGT (at which sensitivity and specificity would be maximal) for the prediction of HF-related rehospitalization was defined with receiver operating characteristic (ROC) curve analysis. Patients were categorized according to this GGT cut-off value. Group I consisted of patients with GGT ≤49 IU/L (n: 89) and Group II consisted of patients with GGT >49 IU/L (n: 34). A detailed history was obtained from patients, including history of hypertension and diabetes mellitus, cardiac rhythm, echocardiographic parameters such as right ventricular dilatation/hypokinesia, presence of pulmonary hypertension, mitral, aortic and tricuspid regurgitation, laboratory findings, and medication at discharge. Hypertension was defined as blood pressure ≥140/90 mmHg on more than two occasions during office measurements or receipt of antihypertensive treatment. Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL or receipt of antidiabetic treatment.

### Measurements

Blood samples were drawn without stasis on admission. GGT activity was measured using a Beckman Coulter Synchron LX20 autoanalyzer with original kits. The laboratory reference limit differs significantly by sex and was set at 9-35 U/L for women and 9-40 U/L for men according to the test kit specification. All

other data including echocardiographic data, demographics and laboratory tests were obtained from the patients' files.

### Echocardiographic evaluation

Echocardiographic examinations were performed in all patients within 24 hours of admission. All examinations were evaluated via Vivid 7 system (GE Healthcare; Wauwatosa, WI) in all participating centers using a 2.5–5-MHz probe. The modified Simpson method was used in EF calculations. Chamber sizes were defined according to recent guidelines.<sup>[22]</sup> Right ventricular dysfunction was defined as dilatation of the right ventricle (right ventricle dimension >3.4 cm at basal plane or >3.8 cm at midplane), combined with the presence of McConnell sign.<sup>[22,23]</sup> Valvular regurgitations were graded into two categories (moderate-to-severe versus non-moderate-to-severe) via a combination of color flow jet Doppler signal intensity and vena contracta width according to guideline recommendations.<sup>[24]</sup> Systolic pulmonary artery pressure was calculated as previously shown.<sup>[25]</sup>

### Statistical analysis

Parametric data were expressed as mean ± standard deviation, nonparametric data as median (interquartile range) and categorical data as percentages. The Statistical Package for the Social Sciences (SPSS) 17.0 (SPSS, Inc.; Chicago, IL) was used to perform statistical procedures. Comparisons between groups of patients were made by use of chi-square or Fisher's exact test for categorical variables, independent samples t test for normally distributed continuous variables, and Mann-Whitney U test when the distribution was skewed. ROC curve analysis was performed to identify the optimal cut-off point of GGT (at which sensitivity and specificity would be maximal) for the prediction of new-onset (acute) HF-related rehospitalization. Area under the curve (AUC) was calculated as a measure of the accuracy of the tests. We compared the AUC with use of the Z test. Patients were categorized as having unchanged (Group I) or increased (Group II) GGT based on a cut-off value. Kaplan-Meier curves were used to display HF-related rehospitalization in two patient groups, defined as having unchanged (Group I) or increased (Group II) GGT, based on a cut-off value. A p value <0.05 was accepted as significant.

We used univariate Cox proportional-hazards analysis

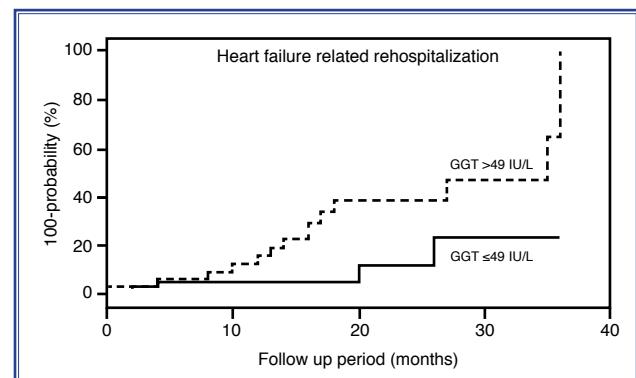
to quantify the association of variables with HF-related hospitalization. Variables found to be statistically significant ( $p<0.25$ ) in univariate analysis were used in a multivariate Cox proportional-hazards model with backward stepwise method in order to determine the independent prognostic factors of HF-related rehospitalization in patients with ACS with reduced LVEF.

## RESULTS

The mean age of the patients was  $65\pm11$  years (24% females, 76% males). Twenty-three (18.7%) patients were admitted to the hospital with acute decompensated HF during the follow-up.

Receiver operating characteristic (ROC) curve analysis of GGT is shown in Figure 1. According to the ROC curve analysis, the optimal cut-off value of GGT to predict HF-related rehospitalization was found as 49 IU/L, with 81.7% specificity, 65.2% sensitivity, 44.1% positive predictive value, and 91% negative predictive value (AUC 0.793, 95% confidence interval (CI): 0.693 to 0.893).

Clinical characteristics and laboratory parameters with regard to the GGT cut-off value are presented in Table 1. Alanine aminotransferase, GGT and creatinine levels and acute HF-related rehospitalization rates were significantly different between the two groups ( $p<0.05$ ), whereas, age, gender, presence of hypertension, diabetes mellitus and atrial fibrillation, medications, and other laboratory findings were not different between the two groups ( $p>0.05$ ). Initial echocardiographic findings with regard to the GGT cut-off value are also presented in Table 1. Right ven-



**Figure 1.** ROC curve for heart failure-related rehospitalization.

**Table 1.** Baseline characteristics of the study patients

	All patients (n=123)	Gamma-glutamyltransferase		p
		≤49 IU/L (n=89)	>49 IU/L (n=34)	
Mean age (year)	65±11	64±10	67±10	0.234
Females	29 (24)	20 (21)	9 (27)	0.818
Presence of hypertension, n (%)	48 (39)	34 (38)	14 (41)	0.924
Presence of diabetes mellitus, n (%)	25 (20)	19 (21)	6 (18)	0.837
Atrial fibrillation, n (%)	11 (9)	6 (7)	5 (15)	0.166
STEMI, n (%)	75 (61)	55 (62)	20 (59)	0.924
Echocardiography at admission				
Left ventricular ejection fraction (%)	33±7	34±6	32±8	0.404
Right ventricular dysfunction, n (%)	14 (11)	4 (5)	10 (29)	<b>&lt;0.001</b>
Moderate-to-severe tricuspid regurgitation, n (%)	12 (10)	5 (6)	7 (21)	0.019
Moderate-to-severe mitral regurgitation, n (%)	23 (29)	12 (14)	11 (32)	0.032
Moderate-to-severe aortic regurgitation, n (%)	3 (2)	3 (3)	0 (0)	0.560
Laboratory findings				
Gamma-glutamyltransferase (IU/L)	31 (18-53)	23 (17-35)	82.5 (58-107)	<b>&lt;0.001</b>
Hemoglobin (g/dL)	14±2.1	14.1±2.0	13.9±2.6	0.330
Presence of anemia, n (%)	28 (24)	21 (24)	7 (22)	0.964
Creatinine (mg/dL)	1.1 (0.9-1.4)	1.0 (0.9-1.2)	1.25 (1.0-1.7)	0.008
Alanine aminotransferase (IU/L)	30 (18-52)	24 (17-43)	53.5 (34-107)	<b>&lt;0.001</b>
Troponin I (ng/mL)	2.0 (0.25-11.5)	1.1 (0.20-13.4)	2.6 (0.25-9.0)	0.766
Medication at discharge				
Antiplatelet agents, n (%)	118 (96)	86 (97)	32 (94)	0.616
Beta-blockers, n (%)	105 (85)	76 (85)	29 (85)	<b>1.000</b>
ACE inhibitors/ARB, n (%)	97 (79)	73 (82)	24 (71)	0.253
Statins, n (%)	112 (91)	82 (92)	30 (88)	0.494
Aldosterone antagonist, n (%)	82 (67)	62 (70)	20 (59)	0.354
Primary endpoint				
Heart failure-related rehospitalization, n (%)	23 (18.7)	8 (9)	15 (44)	<b>&lt;0.001</b>

ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; STEMI: ST-segment elevation myocardial infarction.

tricular dysfunction and moderate-to-severe tricuspid and mitral regurgitation were more frequent in the group with increased serum GGT activity.

Increased GGT >49 IU/L on admission, presence of hypertension and hyperlipidemia, LVEF, right ventricular dysfunction, moderate-to-severe mitral regurgitation, alanine aminotransferase level, and antiplatelet agent usage were found to have prognostic significance in univariate Cox proportional-hazards analysis (Table 2). In multivariate Cox proportional-hazards

model with backward stepwise method, increased GGT >49 IU/L on admission ( $p=0.047$ , hazard ratio [HR] 2.663, 95% CI 1.012-7.007), presence of hypertension ( $p=0.007$ , HR 4.107, 95% CI 1.464-11.521), and LVEF ( $p=0.002$ , HR 0.911, 95% CI 0.858-0.966) were found to be independent factors to determine future acute HF requiring hospitalization (Table 3).

In Figure 2, we also demonstrated the probability of future hospitalization acute HF in a patient with ACS over time, based on the GGT cut-off value.

**Table 2.** Univariate analysis of heart failure-related rehospitalization in patients with acute coronary syndrome with left ventricular systolic dysfunction

	Univariate		
	p	HR	(95% CI)
Gamma-glutamyl transferase >49 IU/L	<b>0.009</b>	3.222	1341-7.744
Baseline characteristics			
Age (year)	0.504	1.015	0.971-1.058
Gender	0.395	1.565	0.558-4.393
Presence of hypertension	0.015	3.038	1.237-7.459
Presence of diabetes mellitus	0.834	0.899	0.331-2.444
Presence of hyperlipidemia	0.104	3.366	0.778-14.559
Atrial fibrillation	0.499	1.592	0.413-6.140
STEMI	0.779	1.135	0.470-2.740
Echocardiography at admission			
Left ventricular ejection fraction (%)	<b>&lt;0.001</b>	0.895	0.844-0.949
Right ventricular dysfunction	0.058	0.399	0.154-1.033
Moderate-to-severe tricuspid regurgitation	0.649	0.775	0.258-2.326
Moderate-to-severe mitral regurgitation	0.022	0.337	0.133-0.855
Moderate-to-severe aortic regurgitation	0.400	0.420	0.056-3.172
Laboratory findings			
Creatinine (mg/dL)	0.402	1.054	0.932-1.191
Alanine aminotransferase (IU/L)	<b>0.004</b>	1.007	1.002-1.011
Troponin I (ng/mL)	0.279	1.010	0.992-1.029
Presence of anemia	0.310	0.608	0.233-1.587
Medication at discharge			
Antiplatelet agents	0.142	0.196	0.022-1.730
Beta-blockers	0.378	1.581	0.571-4.377
ACE inhibitors/ARB	0.581	0.745	0.263-2.117
Statins	0.334	1.831	0.536-6.256
Aldosterone antagonist	0.824	0.905	0.375-2.181

ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; CI: Confidence interval; HR: Hazard ratio; STEMI: ST-segment elevation myocardial infarction.

## DISCUSSION

The present study demonstrated that elevated serum GGT level on admission was significantly and independently associated with the risk of acute HF development in ACS patients with LVSD.

Wannamethee et al.<sup>[26]</sup> found that increased serum GGT levels were associated with cardiac mortality. This finding triggered the studies focusing on the usefulness of GGT as a predictor of cardiovascular diseases.<sup>[6-21]</sup> For the time being, this laboratory test

has been searched by many groups regarding its suitability as a significant predictor for cardiometabolic diseases. Serum GGT was found to be a risk factor for cardiovascular mortality by the Vorarlberg Health Monitoring and Promotion Program Study Group.<sup>[7]</sup> Lee et al.<sup>[11]</sup> demonstrated that serum GGT also predicted cardiovascular mortality in those aged less than 70 years. In addition, it was found to predict non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women.<sup>[6]</sup> Fraser et al.,<sup>[12]</sup> in a recent meta-analysis of fully adjusted results of 10 prospective studies, described that

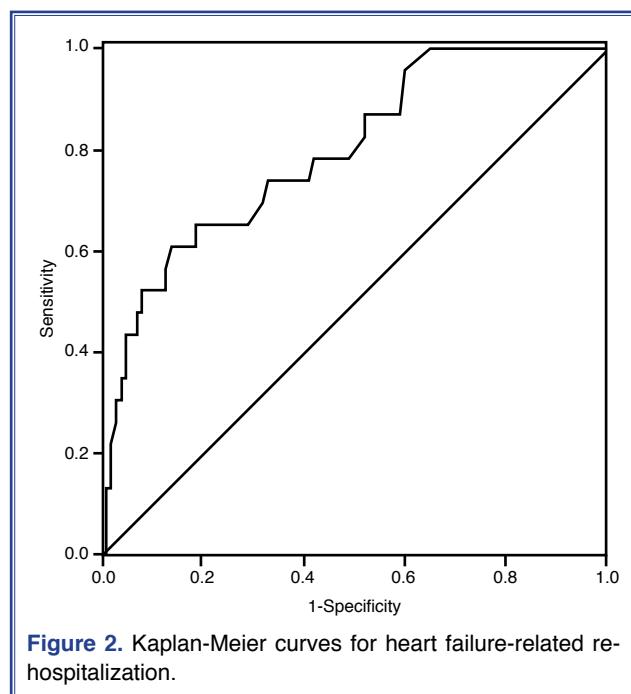
**Table 3.** Multivariate Cox proportional-hazards analysis of heart failure-related rehospitalization in patients with acute coronary syndrome with left ventricular dysfunction

		p	HR	95.0% CI	
				Lower	Upper
Step 1	Gamma-glutamyltransferase >49 IU/L	0.096	2.613	0.844	8.090
	Presence of hyperlipidemia	0.961	1.042	0.202	5.376
	Presence of hypertension	0.008	4.473	1.490	13.431
	Left ventricular ejection fraction (%)	0.012	0.916	0.856	0.981
	Right ventricular dysfunction	0.220	2.362	0.599	9.317
	Moderate-to-severe mitral regurgitation	0.109	0.382	0.118	1.239
	Antiplatelet agents	0.220	0.247	0.027	2.304
	Alanine aminotransferase (IU/L)	0.573	1.002	0.995	1.009
Step 2	Gamma-glutamyltransferase >49 IU/L	0.087	2.629	0.870	7.944
	Presence of hypertension	0.008	4.477	1.492	13.434
	Left ventricular ejection fraction (%)	0.007	0.916	0.859	0.976
	Right ventricular dysfunction	0.219	2.365	0.600	9.327
	Moderate-to-severe mitral regurgitation	0.108	0.382	0.118	1.237
	Antiplatelet agents	0.220	0.247	0.027	2.302
	Alanine aminotransferase (IU/L)	0.571	1.002	0.995	1.009
Step 3	Gamma-glutamyltransferase >49 IU/L	0.066	2.759	0.935	8.140
	Presence of hypertension	0.008	4.236	1.454	12.343
	Left ventricular ejection fraction (%)	0.004	0.912	0.857	0.971
	Right ventricular dysfunction	0.225	2.366	0.588	9.514
	Moderate-to-severe mitral regurgitation	0.114	0.378	0.113	1.263
Step 4	Antiplatelet agents	0.220	0.250	0.027	2.286
	Gamma-glutamyltransferase >49 IU/L	0.145	2.141	0.769	5.958
	Presence of hypertension	0.011	3.829	1.360	10.786
	Left ventricular ejection fraction (%)	0.006	0.919	0.865	0.976
Step 5	Moderate-to-severe mitral regurgitation	0.234	0.499	0.159	1.568
	Antiplatelet agents	0.250	0.271	0.029	2.510
	Gamma-glutamyltransferase >49 IU/L	0.128	2.203	0.798	6.085
Step 6	Presence of hypertension	0.012	3.787	1.340	10.704
	Left ventricular ejection fraction (%)	0.005	0.918	0.864	0.975
	Moderate-to-severe mitral regurgitation	0.213	0.483	0.153	1.521
	Gamma-glutamyltransferase >49 IU/L	0.047	2.663	1.012	7.007
	Presence of hypertension	0.007	4.107	1.464	11.521
	Left ventricular ejection fraction (%)	0.002	0.911	0.858	0.966

Gamma-glutamyltransferase >49 IU/L, presence of hypertension and hyperlipidemia, left ventricular ejection fraction, right ventricular dysfunction, moderate-to-severe mitral regurgitation, alanine aminotransferase, and antiplatelet agent usage were entered into the multivariate Cox proportional-hazards model with backward stepwise method. CI: Confidence interval; HR: Hazard ratio.

a change in GGT of 1 U/L was associated with a 20% increase in the risk of coronary heart disease and a 54% increase in the risk of stroke. Serum GGT is also

associated with coronary artery disease, acute myocardial infarction, diabetes mellitus, hypertension, and metabolic syndrome.<sup>[13-17]</sup> In addition, higher se-



**Figure 2.** Kaplan-Meier curves for heart failure-related re-hospitalization.

rum GGT concentrations were shown to be associated with greater risk of HF.<sup>[18,19]</sup> Wang et al.<sup>[19]</sup> showed that moderate-to-high levels of serum GGT were significantly associated with incident HF in males and females. Poelzl et al.<sup>[20]</sup> described the increased prevalence of elevated GGT in patients with chronic HF. They found the GGT levels to be associated with disease severity and increased GGT to be an independent predictor of death or heart transplantation. Finally, in addition to these studies, Zorlu et al.<sup>[21]</sup> showed that GGT was associated with impaired hemodynamics and can be used for risk stratification of patients with acute pulmonary embolism.

Risk stratification is extremely crucial for ACS patients. The GRACE risk scoring system, an international registry including STEMI, NSTEMI and USAP, which was derived from clinical parameters at the time of hospitalization, was found to accurately predict mortality at six months. Parameters in this GRACE scoring system are age, history of congestive heart failure, history of myocardial infarction, elevated resting heart rate, low systolic blood pressure on arrival, ST-segment depression, elevated initial serum creatinine, and elevated cardiac enzymes in-hospital.<sup>[27]</sup> Among patients hospitalized for ACS, the subsequent development of HF portends a poor prognosis. The CARE trial found age and LVEF as

the most important predictors of HF. Other predictors included diabetes, history of hypertension, previous myocardial infarction, and baseline heart rate. Furthermore, moderate exercise three or more times per week was independently associated with a 30% lower risk of HF.<sup>[28]</sup> In VALIANT, the most important predictors of HF were older age, antecedent diabetes, prior infarct before index myocardial infarction, and reduced renal function.<sup>[29]</sup>

Serum GGT also takes part in the cellular glutathione synthesis and thus the antioxidant defense system.<sup>[30,31]</sup> Increased serum GGT may reflect increased oxidative stress in humans.<sup>[32]</sup> It is also strongly related to systemic inflammation.<sup>[33]</sup> Oxidative stress and systemic inflammation are involved in ventricular remodelling and endothelial dysfunction, both of which contribute to progression of the HF syndrome.<sup>[1,34,35]</sup>

To identify patients who develop acute HF after ACS and to determine the specific therapy are of clinical importance. Hence, throughout this time, biomarkers that might predict future HF were a topic of focus. C-reactive protein was found to be an independent predictor of death and development of HF in patients with ACS in previous studies.<sup>[36,37]</sup> Furthermore, B-type natriuretic peptide (BNP) was also found to be a significant predictor.<sup>[38,39]</sup> Ess et al.<sup>[40]</sup> showed that GGT and total bilirubin were associated with disease severity in CHF. However, only GGT was independently associated with adverse outcome. New biomarkers that predict future cardiovascular events are still needed.

In our study, we found that the subsequent acute HF risk was increased with the increase in serum GGT. Patients with higher GGT also had more frequent pulmonary hypertension, right ventricular dilation/hypokinesia, moderate-to-severe tricuspid and mitral regurgitation, and higher alanine aminotransferase and creatinine levels at the first admission with ACS. Only lower LVEF, presence of hypertension and higher GGT levels were determined as independent predictors for subsequent acute HF in ACS patients by univariate and multivariate regression analyses. Previous studies pointed out the same findings about LVEF being a predictor for subsequent cardiac deterioration; however, the relation between increased GGT level at admission with ACS with subsequent hospitalization for acute HF seems to be a novel finding. Serum GGT may act as a simple and inexpensive biomarker for a

physician to predict the subsequent risk of HF in ACS patients. This would provide a more careful approach for critical patients during their follow-up.

### Study limitations

Even though medications were generally similar after discharge, patients were not monitored for changes in medication and doses during the follow-up. Hence, this may constitute a potential confounder for the study results. In addition, though most of the documented risk factors were included in the analysis, the possibility of residual confounding factors that were not accounted for cannot be entirely excluded. We could not assess which patients underwent percutaneous coronary intervention or cardiac surgery or those on medical therapy. All procedures may affect EF and patient outcomes. Finally, data were derived from a moderate-sized population from a single center, and hence, larger and multi-centered studies are needed to draw certain conclusions.

In conclusion, to our knowledge, this is the first study to report an association between increased serum GGT activity in ACS patients and subsequent risk for acute HF. Further studies are necessary to evaluate the physiopathological role of serum GGT activity in these patients and GGT's overall importance and independence from previously accepted biomarkers and traditional risk factors used for predicting the development of acute HF. However, because of its widespread availability and inexpensive cost for screening, identifying higher than expected GGT levels in ACS patients should alert the physician to give particular attention to caring for these patients and assessing the appropriate aggressiveness of the therapy, with the hopeful outcome of preventing subsequent acute HF development.

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## A comparative research on obesity hypertension by the comparisons and associations between waist circumference, body mass index with systolic and diastolic blood pressure, and the clinical laboratory data between four special Chinese adult groups

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### ABSTRACT

**Background:** The obesity-hypertension pathogenesis is complex. From the phenotype to molecular mechanism, there is a long way to clarify the mechanism. To explore the association between obesity and hypertension, we correlate the phenotypes such as the waist circumference (WC), body mass index (BMI), systolic blood pressure (SB), and diastolic blood pressure (DB) with the clinical laboratory data between four specific Chinese adult physical examination groups (newly diagnosed untreated just-obesity group, newly diagnosed untreated obesity-hypertension group, newly diagnosed untreated just-hypertension group, and normal healthy group), and the results may show something. **Objective:** To explore the mechanisms from obesity to hypertension by analyzing the correlations and differences between WC, BMI, SB, DB, and other clinical laboratory data indices in four specific Chinese adult physical examination groups. **Methods:** This cross-sectional study was conducted from September 2012 to July 2014, and 153 adult subjects, 34 women and 119 men, from 21 to 69 years, were taken from four characteristic Chinese adult physical examination groups (newly diagnosed untreated just-obesity group, newly diagnosed untreated obesity-hypertension group, newly diagnosed untreated just-hypertension group, and normal healthy group). The study was approved by the ethics committee of Hangzhou Center for Disease Control and Prevention. WC, BMI, SB, DB, and other clinical laboratory data were collected and analyzed by SPSS. **Results:** Serum levels of albumin (ALB)alanine aminotransferase (ALT), low density lipoprotein cholesterol (LDLC), triglyceride (TG), high density lipoprotein cholesterol (HDLC), alkaline phosphatase (ALP), uric acid (Ua), and TC/HDLC (odds ratio) were statistically significantly different between the four groups. WC statistically significantly positively correlated with BMI, ALT, Ua, and serum levels of glucose (GLU), and TC/HDLC, and negatively with ALB, HDLC, and serum levels of conjugated bilirubin (CB). BMI was statistically significantly positively related to ALT, Ua, LDLC, WC, and TC/HDLC, and negatively to ALB, HDLC, and CB. DB statistically significantly positively correlated with ALP, BMI, and WC. SB was statistically significantly positively related to LDLC, GLU, serum levels of fructosamine (FA), serum levels of the total protein (TC), BMI, and WC. **Conclusion:** The negative body effects of obesity are comprehensive. Obesity may lead to hypertension through multiple ways by different percents.

GGT, serum levels of gamma glutamyltransferase; ALB, serum levels of albumin; ALT, serum levels of alanine aminotransferase; LDLC, serum levels of low density lipoprotein cholesterol; TG, serum levels of triglyceride; HDLC, serum levels of high density lipoprotein cholesterol; FA, serum levels of fructosamine; S.C. R, serum levels of creatinine; IB, serum levels of indirect bilirubin; ALP, serum levels of alkaline phosphatase; CB, serum levels of conjugated bilirubin; UREA, Urea; Ua, serum levels of uric acid; GLU, serum levels of glucose; TC, serum levels of the total cholesterol; TB, serum levels of the total bilirubin; TP, serum levels of the total protein; TC/HDLC, TC/HDLC ratio.

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## Introduction

Hypertension and obesity have become increasingly serious public health problems worldwide (1). The principle of obesity leading to hypertension, including function of adipose tissue derivatives (adipokines and cytokines), neurohumoral pathways, metabolic

functions and modulation of pressor/depressor mechanisms, has been elaborated in many studies (2–5). However, the direct mechanisms of how obesity causes hypertension are still unknown (5). It is reported that central adiposity, measured as waist circumference (WC), is closely associated with cardiovascular disease (CVD), hypertension, diabetes, dyslipidemia, and the body

mass index (BMI) represents general overweight/obesity (2,6). The associations between obesity and hypertension may vary with the diagnosed criteria changes of center or general obesity according to WC or BMI (7–9). Anthropometric indices vary among different ethnic groups (10,11). The criteria of overweight and obesity for Chinese people have been developed based on BMI and WC values (12), instead of the World Health Organization (WHO) criteria which are more fit for European descents (13). The data of the correlations of obesity and hypertension from Chinese populations have continued to be found.

In this study, the associations between WC, BMI, SB, DB, and other related clinical laboratory data among four Chinese adult physical examination groups were investigated, and the different expressions of these clinical laboratory indices between the four groups were also detected. And the four investigated groups were newly diagnosed untreated just-obesity (shorten for JO) group, newly diagnosed untreated obesity-hypertension (shorten for OH) group, newly diagnosed untreated just-hypertension (shorten for JH) group, and normal healthy group (shorten for NH).

## Data and methods

### *Subjects and methods*

The cross-sectional study was performed, including 153 adults (34 women and 119 men) from 21 to 69 years. The data were collected from September 2012 to July 2014. All the participants took annual routine physical examination in hospital during that time. They were divided into four characteristic groups as (1) newly diagnosed untreated just-obesity (55 subjects) group, (2) newly diagnosed untreated obesity-hypertension (41 subjects) group, (3) newly diagnosed untreated just-hypertension (31 subjects) group, and (4) normal healthy group (26 subjects) group. The study was approved by the ethics committee of Hangzhou Center for Disease Control and Prevention.

Firstly, the inclusion criteria of the subjects were set. The JO group definition is that the group people haven't been diagnosed for obesity and haven't been treated with any anti-obesity drugs till this annual physical examination. The JH group definition is that the group people haven't been diagnosed for hypertension and haven't been treated with any anti-hypertension drugs till this annual physical examination. The OH group definition is that the group people haven't been diagnosed for obesity or hypertension and haven't been treated with any anti-obesity or anti-hypertension drugs till this annual physical examination. The NH group definition is that the group people haven't been diagnosed for any clinical serious disease such as obesity, hypertension, etc. To minimize the possible influence by other diseases, some exclusion criteria were defined. Patients with diabetes mellitus, secondary hypertension, heart diseases, and acute inflammatory diseases (liver and kidney diseases) were excluded. Secondly, for the people referred to the physical examination center, after informed consent, they were selected according to inclusion criteria and assigned to the four groups. And the approximate matching of age and gender between the four groups and the suitable subject number for statistics were considered. And in the end, 55 subjects were selected for the JO group, 41 subjects for the OH group, 31 subjects for the JH group, and 26 subjects for the NH group.

And then subjects underwent the following procedure. The blood pressure (BP) (both systolic and diastolic) of all the participants was measured in the right arm by trained nurses according to a standard protocol (14). The average readings of the three BP measurements, with an interval of from 5 to 15 minutes between measurements, were regarded as the BP value for each subject. And the anthropometric measurements were taken after the subjects taking off their shoes and any heavy clothing or belts. The body weight and height were measured using an electronic scale. The WC was measured at the level midway between the lower rib margin and the iliac crest while the participants breathed out gently (11,15). And other routine clinical laboratory tests for subjects were taken about the blood sample and urine sample, etc.

### **Definition of obesity and hypertension**

The BMI was defined as weight (kg)/height<sup>2</sup> (m<sup>2</sup>), and the subjects' weights were classified according to the Chinese criteria raised by Chinese Obesity Working Group: BMI < 18.5: underweight; 18.5 ≤ BMI < 24: normal; 24 ≤ BMI < 28: overweight; and BMI ≥ 28: general obesity (12,16). A WC ≥ 85 and ≥80 m for males and females were identified as central obesity, respectively (12). An SBP ≥ 140 mmHg or/and a DBP ≥ 90 mmHg at each three separate appointments was defined as hypertension. All the patients were newly diagnosed. In this study, the general obesity was used to divide the subject groups.

### **Clinical laboratory data collection**

Blood samples were obtained after a 12-hour diet. The baseline demographic and clinical laboratory data were obtained from hospital records and reviewed by experienced physicians. These data include the information of age, gender, serum levels of gamma glutamyltransferase (GGT), albumin (ALB), alanine aminotransferase (ALT), low density lipoprotein cholesterol (LDLC), triglyceride (TG), high density lipoprotein cholesterol (HDLC), fructosamine (FA), creatinine (S.C.R), indirect bilirubin (IB), alkaline phosphatase (ALP), conjugated bilirubin (CB), glucose (GLU), total cholesterol (TC), total bilirubin (TB), total protein (TP), uric acid (Ua), Urea (UREA), etc.

### **Statistical analysis**

Descriptive analysis has been performed firstly. When the data were normally distributed and the equal variance test was satisfied, Pearson test was performed to analyze the statistical significance of the correlations between groups, respectively. And if the data were not normally distributed, the analyses were conducted using Mann-Whitney rank sum test and Spearman's correlation test. And partial correlations were also conducted. Clinical index difference between 4 group were analyzed by the least significant difference (LSD) method using the general linear models (GLM) procedure implemented in Statistical Package for the Social Sciences software (SPSS 16.0). p-Values less than 0.05 were considered statistically significant. All statistics were performed using SPSS (version 16.0 for Windows, SPSS Inc., Chicago, IL, USA).

**Table 1.** Demographic and clinical laboratory data of subjects.

Items	Mean (min-max)/number
Age, mean $\pm$ SD years	40.78 $\pm$ 11.77
Sex, male/female	119/34
SB	135.92(98–203)
DB	82.89(55–131)
BMI	27.25(18.9–36.6)
AC	94.21(69–125)
GGT (U/L)	34.72(6–202)
ALB (g/L)	46.69(40.9–52.3)
ALT (U/L)	33.13(4–341)
LDLC(mmol/L)	103.51(45–170)
TG (mg/dl)	168.58(42–998)
HDLC(mg/dl)	50.59(29–90)
FA(mmol/L)	1.57(1–3.2)
SCR ( $\mu$ mol/L)	83.8(55–106)
IB( $\mu$ mol/L)	8.09(1.6–25.8)
ALP (U/L)	64.98(29–113)
CB( $\mu$ mol/L)	3.84(1–11.5)
UREA(mmol/L)	5.05(2.88–9.36)
Ua( $\mu$ mol/L)	344.53(161–585)
GLU (mmol/L)	5.28(4.02–14.13)
TC(mg/dl)	200.82(113–419)
TB( $\mu$ mol/L)	11.93(3.6–37.3)
TP (g/L)	76.55(68.2–84)

## Results

### Demographic and clinical laboratory data of subjects

Demographic information and clinical laboratory data of the participants are shown in Table 1, including age, gender, serum levels of Gamma glutamyltransferase (GGT), Albumin (ALB), Alanine aminotransferase (ALT), Low density lipoprotein cholesterol (LDLC), Triglyceride (TG), High density lipoprotein cholesterol (HDLC), Fructosamine (FA), Creatinine (S.C.R) Indirect bilirubin(IB), Alkaline phosphatase(ALP), Conjugated bilirubin (CB), Glucose(GLU), Total cholesterol(TC), Total bilirubin(TB), Total protein(TP), Uric acid (Ua), Urea(UREA), etc.

### Correlations between WC, BMI, and the clinical laboratory data

Correlation analysis (Table 2) and partial correlation analysis (Table 3) were both conducted in our study. A correlation between WC and BMI is statistically significantly positive ( $r = 0.80, p < 0.001$ ). And DB and SB both statistically significantly positively related with BMI ( $r = 0.249, p = 0.002, r = 0.303, p = 0.000$ , respectively) and WC ( $r = 0.279, p = 0.000, r = 0.307, p = 0.000$ , respectively).

And BMI is statistically significantly positively related to seven clinical laboratory indices, i.e., LDLC ( $r = 0.224, p = 0.005$ ), TG ( $r = 0.169, p = 0.036$ ), S.C.R ( $r = 0.190, p = 0.018$ ), Ua ( $r = 0.234, p = 0.004$ ), Glucose ( $r = 0.162, p = 0.045$ ), ALT ( $r = 0.252, p = 0.002$ ), TC/HDL odds ( $r = 0.272, p = 0.001$ ), statistically significantly negatively correlating to HDLC ( $r = -0.256, p = 0.001$ ), and ALB ( $r = -0.166, p = 0.041$ ). And BMI also nearly statistically significantly positively correlates to three clinical laboratory indices, i.e., FA ( $r = 0.143, p = 0.078$ ), TC ( $r = 0.143, p = 0.078$ ), nearly statistically significantly negatively correlating with CB ( $r = -0.148, p = 0.068$ ).

WC is statistically significantly positively correlated with ten clinical laboratory indices, i.e., GGT ( $r = 0.179, p = 0.027$ ), FA ( $r = 0.169, p = 0.037$ ), ALT ( $r = 0.252, p = 0.002$ ), LDLC ( $r = 0.182, p = 0.024$ ), S.C.R ( $r = 0.303, p = 0.000$ ), ALP ( $r = 0.179, p = 0.027$ ), UREA ( $r = 0.169, p = 0.037$ ), Ua ( $r = 0.337, p = 0.000$ ), GLU

**Table 2.** Bivariate analysis on the relationship of WC and BMI to the clinical laboratory data (only indices with the analysis results of  $p$ -value less than or nearly less than 0.05 being listed below).

Correlates	WC		BMI	
	<i>r</i>	<i>p</i> -Value	<i>r</i>	<i>p</i> -Value
HDLC(mg/dl)	-0.277	0.001	-0.256	0.001
ALB (g/L)	-0.137	0.090	-0.166	0.041
CB( $\mu$ mol/L)	-0.118	0.147	-0.148	0.068
TC(mg/dl)	0.099	0.221	0.143	0.078
TG (mg/dl)	0.155	0.056	0.169	0.036
FA(mmol/L)	0.169	0.037	0.143	0.078
UREA(mmol/L)	0.169	0.037	0.055	0.503
GGT (U/L)	0.179	0.027	0.129	0.113
ALP (U/L)	0.179	0.027	0.119	0.143
LDLC(mmol/L)	0.182	0.024	0.224	0.005
TC/HDLC	0.238	0.003	0.272	0.001
GLU (mmol/L)	0.239	0.003	0.162	0.045
ALT (U/L)	0.252	0.002	0.252	0.002
DB	0.303	0.000	0.249	0.002
SCR ( $\mu$ mol/L)	0.303	0.000	0.190	0.018
SB	0.307	0.000	0.279	0.000
Ua( $\mu$ mol/L)	0.337	0.000	0.234	0.004
BMI	0.800	0.000	1.000	0.800
AC	1.000	0.000	0.800	0.000

**Table 3.** Statistically significantly Partial correlations between WC, BMI and the clinical laboratory data controlled by age and gender.

Correlates	WC		BMI	
	<i>r</i>	<i>p</i> -Value	<i>r</i>	<i>p</i> -Value
ALB (g/L)	-0.217	0.007	-0.212	0.009
ALT (U/L)	0.227	0.005	0.242	0.003
LDLC(mmol/L)	0.103	0.21	0.189	0.02
HDLC(mg/dl)	-0.23	0.004	-0.235	0.004
CB( $\mu$ mol/L)	-0.161	0.049	-0.167	0.041
Ua( $\mu$ mol/L)	0.215	0.008	0.182	0.025
GLU (mmol/L)	0.216	0.008	0.149	0.067
BMI	0.806	0	1	0
AC	1	0	0.806	0
TC/HDLC (odds ratio)	0.165	0.043	0.241	0.003

( $r = 0.239, p = 0.003$ ), TC/HDL ( $r = 0.238, p = 0.003$ ), and nearly statistically significantly positively correlating to TG ( $r = 0.155, p = 0.056$ ). And WC is statistically significantly negatively correlated with HDLC ( $r = -0.277, p = 0.001$ ), nearly statistically significantly negatively correlating with Albumin ( $r = -0.137, p = 0.090$ ).

But, when the age and gender conditions were controlled, the associations had some changes. WC was statistically significantly positively correlated to some clinical laboratory indices, i.e., ALT ( $r = 0.227, p = 0.005$ ), Ua ( $r = 0.215, p = 0.008$ ), GLU ( $r = 0.216, p = 0.008$ ), BMI ( $r = 0.806, p = 0.000$ ), and TC/HDLC ( $r = 0.165, p = 0.043$ ), statistically significantly negatively correlating with ALB ( $r = -0.217, p = 0.007$ ), HDLC ( $r = -0.23, p = 0.004$ ), and CB ( $r = -0.161, p = 0.049$ ).

For BMI, the statistically significantly positively related clinical laboratory indices were ALT ( $r = 0.242, p = 0.003$ ), LDLC ( $r = 0.189, p = 0.02$ ), Ua ( $r = 0.182, p = 0.025$ ), WC ( $r = 0.806, p = 0.000$ ), and TC/HDLC ( $r = 0.241, p = 0.003$ ), and the negatively related ones were ALB ( $((r = -0.212, p = 0.009)$ , HDLC ( $r = -0.235, p = 0.004$ ), and CB ( $r = -0.167, p = 0.041$ )).

### Correlations between DB, SB, and the clinical laboratory data

Correlation analysis (Table 4) and partial correlation analysis (Table 5) were both conducted. In our study, correlation relationship

**Table 4.** Bivariate analysis on the relationship of DB and SB to the clinical laboratory data (only indices with the analysis results of  $p$ -value less than or nearly less than 0.05 being listed below).

Correlates	DB		SB	
	r	p-Value	r	p-Value
ALB (g/L)	-0.052	0.527	-0.203	0.012
TC(mg/dl)	0.054	0.504	0.210	0.009
FA(mmol/L)	0.106	0.192	0.315	0.000
LDLC(mmol/L)	0.117	0.148	0.195	0.016
GLU (mmol/L)	0.138	0.088	0.309	0.000
GGT (U/L)	0.146	0.071	0.028	0.728
UREA(mmol/L)	0.176	0.029	0.164	0.043
Ua(μmol/L)	0.196	0.015	0.082	0.311
ALP (U/L)	0.248	0.002	0.185	0.022
BMI	0.249	0.002	0.279	0.000
AC	0.303	0.000	0.307	0.000
SB	0.705	0.000	1.000	
DB	1.000		0.705	0.000

**Table 5.** Statistically significantly partial correlations between DB, SB and the clinical laboratory data controlled by age and gender.

Correlates	DB		SB	
	r	p-Value	r	p-Value
LDLC(mmol/L)	0.066	0.423	0.174	0.033
FA(mmol/L)	0.025	0.762	0.225	0.005
ALP (U/L)	0.205	0.011	0.139	0.089
GLU (mmol/L)	0.079	0.337	0.231	0.004
TC(mg/dl)	0.003	0.974	0.174	0.032
BMI	0.205	0.012	0.248	0.002
AC	0.206	0.011	0.227	0.005

between DB and SB is statistically significantly positive ( $r = 0.705$ ,  $p = 0.00$ ). DB statistically significantly positively correlated to three clinical laboratory indices, i.e., ALP ( $r = 0.248$ ,  $p = 0.002$ ), UREA ( $r = 0.176$ ,  $p = 0.029$ ), GLU ( $r = 0.138$ ,  $p = 0.088$ ), and nearly positively correlated with GGT( $r = 0.146$ ,  $p = 0.071$ ).

For SB, the statistically significantly positively related clinical laboratory indices were LDLC ( $r = 0.195$ ,  $p = 0.016$ ), FA ( $r = 0.315$ ,  $p = 0.000$ ), UREA( $r = 0.164$ ,  $p = 0.043$ ), GLU ( $r = 0.164$ ,  $p = 0.043$ ), and TC( $r = 0.210$ ,  $p = 0.009$ ), and the negatively related one was ALB( $r = -0.203$ ,  $p = 0.012$ ),

But, when the age and gender conditions were controlled, the correlations changed to some extent. DB statistically significantly positively correlated with three clinical laboratory indices, i.e., ALP ( $r = 0.205$ ,  $p = 0.011$ ), BMI ( $r = 0.205$ ,  $p = 0.012$ ), and WC( $r = 0.206$ ,  $p = 0.011$ ).

For SB, the statistically significantly positively related clinical laboratory indices were LDLC ( $r = 0.174$ ,  $p = 0.033$ ), FA ( $r = 0.225$ ,  $p = 0.005$ ), GLU( $r = 0.231$ ,  $p = 0.004$ ), TC ( $r = 0.174$ ,  $p = 0.032$ ), BMI ( $r = 0.248$ ,  $p = 0.002$ ), and WC ( $r = 0.227$ ,  $p = 0.005$ ).

Comparisons of the clinical laboratory data between four groups

When the age and gender conditions were controlled, clinical laboratory indices having statistical differences between the four groups were as the followings (Table 6): ALB of NH was statistically significantly higher than JO, OH, JH groups, ALT of JO was statistically significantly higher than NH and JH groups. LDLC of OH was statistically significantly higher than NH group. TG of JH was statistically significantly higher than NH group. HDLC of JO was statistically significantly lower than JO and NH groups. ALP of NH

**Table 6.** Clinical laboratory indices comparisons between four groups (only indices with the analysis results of  $p$ -value less than 0.05 being listed below).

item	Rank ( $p < 0.05$ )
ALB	NC > JO,OH,JH
ALT	JO > NC
LDLC	OH > NC
TG	JH > NC
HDLC	JO < JH,NC
ALP	NC < JH,OH
Ua	NC < JO,OH
TC/HDLC	JO > JH,NC

was statistically significantly lower than JH and OH groups. Ua of NH was statistically significantly lower than JO and OH groups. TC/HDLC (odds ratio) of JO were statistically significantly higher than JH and NH groups.

## Discussion

Obesity, hypertension, high blood sugar, high blood fat are recognized as the metabolic disorders, whose pathogenesis underlie with Insulin resistance (17). And the disorders infect each other. The mechanisms between the obesity and the hypertension are complicated. The mechanisms, by which obesity can induce the hypertension, include the mediators of abnormal kidney function, enhanced noxious influence of inflammation on the vasculature, sympathetic nervous system activation, increased visceral adiposity, renin-angiotensin-aldosterone system activation, etc. (1,5,18,19). Some research shows that in Chinese people, the prevalence of general and central obesity is strongly related to high blood pressure, and men with obese BMI but normal WC may be at increased risk of high blood pressure (18). Association information between obesity and hypertension among Chinese specific populations has been obtained.

To explore the relationships between the obesity and the hypertension step by step, the 17 lab indices were analyzed by correlating with WC, BMI, SB, and DB among the subjects, and were compared between the 4 groups of NH, JO, OH, and JH.

The results showed that WC and BMI both statistically significantly positively correlated with SB and DB. It is speculated that the obesity correlates with hypertension and may spawn it. This result may back on it at some extent.

When the age and gender conditions were controlled (the following is the same), in the study, ALB, ALT, LDLC, ; TG, HDLC, HDLC, ALP, ;Ua, and TC/HDLC were significantly different between the four groups.

ALP was found in NH statistically significantly to be lower than JH and OH groups. The role of inflammation on the rising risk of hypertension has been speculated. And it also may be considered benign that inflammation and immune activation lead to modest elevations of blood pressure (20–22). ALP was considered as a Marker of Inflammation in CKD Patients or a protector against renal inflammation, paradoxically (23). Also, ALP may play a special role in hypertension and contribute to higher BP. ALP was also positively correlate with WC, and obesity may contribute to more ALP.

ALT in JO group was found to be statistically significantly higher than that in NH group. Elevated ALT is recognized to be associated with a worse cardiac risk profile and metabolic syndrome (24). And ALT in this study statistically significantly

positively correlated with WC and BMI. From the data, it is speculated that obesity may contribute to higher ALT.

And only LDLC in the OH group was statistically significantly higher than that in NH group. LDLC is considered to be bad cholesterol, leading to plaque, a thick, hard deposit and less flexible arteries and clogs. In the study, LDLC were statistically significantly positively associated with WC□BMI, and SB. So, maybe, elevated LDLC have influence with both obesity and hypertension (25).

In our study, HDLC was found in the JO group to be statistically significantly lower than in JH or NH group. And HDLC statistically significantly negatively correlated with WC and BMI, but had no correlations with SB and DB. And GLU had statistically significantly positive correlation with SB and DB. It is said that high HDLC helps to reduce the risk of heart disease. Also, Fats and cholesterol can be dislodged from cells by high density lipoprotein cholesterol particles. But, in this study, HDLC was not directly associated with hypertension. These data indicate there are a lot of factors those can influence BP, and HDLC may just a intermediate linking factor.

ALB was found in the NH group to be statistically significantly higher than in the JH group, in the OH group or in the JO group. And ALB statistically significantly negatively correlated with BMI, WC, and SB. Obesity may lead to more microalbuminuria or frank proteinuria and low serum albumin by the physical compression to the kidneys by fat in and around kidneys (21). This result had no insistence with the study by Cho et al. (26), in which, increased serum levels of albumin were found to be related with higher prevalence of metabolic syndrome. And the difference need more research.

TG in JH group was found to be statistically significantly higher than in NH group. And TG may statistically significantly positively correlate with BMI. But this association may be influenced by age and gender. TG is said to be an independent risk factor for hypertension□and low HDLC and high triglyceride levels were more prevalent with the existence of hypertension and obesity (19,20).

In our study, Ua of NH group was found to be statistically significantly lower than that of JO group or of OH group. And Ua statistically significantly positively correlated with WC and BMI. Also it statistically positively correlated with DB, but this association may be influenced by age and gender. And it is said that enhanced serum uric acid, recognized as a predictor of BP elevation and obesity, correlated with Angiotensinogen in obese patients with untreated hypertension (27). Also, there are associations between Ua and inflammation biomarkers, endothelial dysfunction and carotid atherosclerosis (28). Maybe, the obesity contributes to more uric acid compared with normal population and then the more Angiotensinogen. And in the end, this can do some contribution for the happening of hypertension.

From the results, it can be speculated that obesity's negative body effects are comprehensive. And obesity can lead to hypertension through multiple ways. ALP and ALT may be at the way of "inflammation on the vasculature", and LDLC, HDLC and TG may be at the way of "increased visceral adiposity", and ALB may be at the way of "the mediators of abnormal kidney function," and Ua may be at the way of "renin-angiotensin-aldosterone system activation".

In summary, mechanisms between the obesity and hypertension are complex. In this study, the criteria of obesity developed

for Chinese people were used, and some statistically significant differences of some clinical laboratory indices were found among four special Chinese population, i.e., the newly diagnosed untreated just-obesity group, newly diagnosed untreated obesity-hypertension group, newly diagnosed untreated just-hypertension group, and normal Chinese population group. The data are precious and may clarify some relationships between the obesity and hypertension. Most results in this study were consistent with previous researches' findings. But conflicting results were found about serum levels of ALB between our study and other one research. Maybe, different mechanisms about obesity's leading to hypertension are all working, but for various individuals, the different mechanisms account for different percents.

And the continual studies will be done by our research team to further touch the related mechanisms between obesity and hypertension.

## Declaration of interest

The authors declare no conflict of interest.

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# Gamma-glutamyltransferase and cardiovascular mortality in Korean adults: A cohort study

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## ABSTRACT

**Background and aims:** Insufficient evidence has been reported on the associations between gamma-glutamyltransferase (GGT) and cardiovascular disease (CVD) mortality from studies with an adequate number of participants.

**Methods:** 512,990 Korean adults who participated in routine health examinations during the period 2002–2003 were followed up until 2013. Hazard ratios (HRs) were calculated after adjusting for potential confounders.

**Results:** Each 1-unit higher natural-log-transformed GGT ( $\text{Log}_e \text{GGT}$ ) level was associated with approximately 30–50% higher mortality risk of CVD (HR = 1.31): hypertensive diseases (HR = 1.31), ischemic heart diseases (IHD, HR = 1.29), total stroke (HR = 1.29), acute myocardial infarction (HR = 1.30), chronic IHD (HR = 1.27), heart failure (HR = 1.48), hemorrhagic stroke (HR = 1.42), and ischemic stroke (HR = 1.27). The associations with CVD mortality did not vary by sex, or alcohol use, whereas they were stronger in younger (<60 years), non-hypertensive (systolic blood pressure [SBP] <140 mmHg), physically more active, normal-weight (body mass index<25 kg/m<sup>2</sup>), and normocholesterolemic (total cholesterol <200 mg/dL) adults than in their respective counterparts. Adding  $\text{Log}_e \text{GGT}$  to prediction models for CVD mortality increased AUC value (0.0020,  $p < 0.001$ ), especially in persons aged <60 years (0.0055), with SBP <140 mmHg (0.0030), and with both age <60 years and SBP <140 mmHg (0.0086).

**Conclusions:** Higher GGT significantly increased the risk of mortality due to CVD and its subtypes. The relative risks were greater in subjects with younger age, no hypertension, more physical activity, normal weight, and normocholesterolemia than in their respective counterparts. In the general population, adding GGT to conventional CVD risk factors may improve the prediction of CVD mortality, especially in subjects younger than 60 years and in those without hypertension.

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## 1. Introduction

Cardiovascular disease (CVD), including ischemic heart disease (IHD) and stroke, is currently the major cause of premature mortality and disability worldwide [1,2]. Several studies have shown that elevated gamma-glutamyltransferase (GGT) activity is associated with higher risk of CVD [3–5], although blood GGT levels have

been mainly used as a liver function test and a marker of alcohol ingestion. Blood GGT is suggested to have the potential to be an indicator, or a risk factor, for cardiovascular risk prediction and evaluation [6–8].

The available evidence, however, is not consistent regarding the association of elevated GGT activity with the risk of the leading cause of premature mortality: IHD, particularly acute myocardial infarction (MI) [5,7,9]. Information from prospective studies on the associations between GGT and subcategories of CVD, such as heart failure and hemorrhagic stroke, is lacking [10,11]. It is unclear whether the associations between GGT and CVD differ by risk factors such as age [5,12], sex [13], alcohol intake [4,12], and metabolic

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risk factors. Furthermore, despite some evidence linking GGT and CVD, it remains unclear whether GGT provides additional information on top of known conventional risk factors for the prediction of CVD, considering the existence of strong correlations between GGT and conventional risk factors [12,14].

Through a prospective cohort study that included approximately 513,000 participants, we aimed to examine whether blood GGT levels were associated with the risk of CVD mortality, and whether any such associations varied by individually specific factors such as sex, age, alcohol use, and blood pressure. Additionally, whether blood GGT provides an incremental benefit on top of known risk factors for the prediction of CVD mortality was examined.

## 2. Materials and methods

### 2.1. Study population and follow-up

The National Health Insurance Service (NHIS) provides compulsory health insurance that covers 97% of the Korean population [15]. The study cohort ( $n = 514,795$ ) comprised a 10% random sample of 5.15 million NHIS beneficiaries aged 40–79 years in 2002 who participated in health examinations during the period 2002–2003. A total of 1805 people were excluded due to missing information ( $n = 1753$ ) on GGT, serum glucose, systolic blood pressure, total cholesterol, and body mass index (BMI) or because they had an extremely high BMI ( $\geq 50 \text{ kg/m}^2$ ,  $n = 52$ ). For the remaining 512,990 people, follow-up on underlying causes of death until December 31, 2013 was carried out using national death records. The International Classification of Diseases-10th Revision (ICD-10) was used to define death from CVD (I00–I99), and instances of CVD mortality were classified into hypertensive diseases (I10–I13), IHD (I20–I25), acute MI (I21), chronic IHD (I25), other heart diseases (I26–I51), heart failure (I50), total stroke (I60–I69), hemorrhagic stroke (I60–I62), and ischemic stroke (I63). For research in accordance with the conditions documented in Korean laws, health examination data can be provided without specific informed consent from the participants [16]. This study was approved by the Institutional Review Board of Catholic Kwandong University, Republic of Korea. Anonymized data were provided to the authors by the NHIS.

### 2.2. Data collection

GGT was measured using the method recommended by the International Federation of Clinical Chemistry (IFCC), or using the Szasz method. Fasting serum glucose and total cholesterol were assayed using enzymatic methods [17]. Blood pressure was measured in a seated position using a standard mercury sphygmomanometer. Weight and height were measured to the nearest kilogram and centimeter, respectively [15]. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters ( $\text{kg}/\text{m}^2$ ). Smoking history, alcohol use, and known CVD were self-reported via a questionnaire. The health examinations and data collection followed a standard protocol officially documented by the Ministry of Health and Welfare. External quality assessments for clinical chemistry, such as GGT measurements, in hospitals was supervised by the Korean Association of Quality Assurance for Clinical Laboratory, and the quality of assays was regularly assessed [18].

### 2.3. Statistical analysis

GGT values were categorized into six groups using the 20th (reference), 40th, 60th, 80th, and 90th percentiles as sex-specific

cut-points (quintiles, with the top quintile split). The cut-points corresponded to 20, 28, 40, 64, and 98 U/L in men, and 11, 15, 19, 26, and 35 U/L in women. The participants were also categorized into four groups based on quartiles for comparison with other research [19,20]. Natural-log transformed GGT ( $\text{Log}_e \text{GGT}$ ) values were also analyzed as a continuous variable.

HRs for CVD mortality were calculated using Cox proportional hazards models stratified by age (years) at baseline (40–44, 45–54, 55–64, 65–74, 75–80) after adjustment for age at baseline (continuous variable within each age group), sex (when applicable), a history of heart disease or stroke (yes or no), smoking status (current smoker, former smoker, never-smoker, or missing information [ $n = 21,660$ ]), alcohol use (frequency; rarely, 2 days/month to 2 days/week, 3–7 days/week, and missing information [ $n = 9657$ ]), physical activity (at least once a week; yes or no), beneficiary income status (deciles; below 4 [low income], 4–7, 8–10 [high income]), systolic blood pressure (SBP; mmHg), serum total cholesterol (mg/dL), fasting serum glucose (mg/dL), and BMI ( $\text{kg}/\text{m}^2$ ). Dose-response analysis using a restricted cubic spline transformation of  $\text{Log}_e \text{GGT}$  with 4 knots (5th, 35th, 65th, and 95th percentiles) with CVD mortality was done to evaluate the non-linearity of association.

The area under the curve (AUC) values were estimated using Proc Logistic (ROC statement). A prediction model with an AUC value of 1.0 or 0.5 represents a perfect or an uninformative model, respectively. When investigating changes in the AUC upon addition of GGT, a CVD mortality prediction model that included all variables in the fully adjusted analysis was used.

Subgroup analyses were done to examine evidence of differences in HRs according to individually specific characteristics, such as age, sex, and alcohol consumption. An inverse-variance weighted average method was used for the interaction test between subgroups [21]. Subgroup analyses were also used as a sensitivity test.

All  $p$ -values were 2-sided. All analyses used SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. General characteristics

During 5.3 million person-years of follow-up of 512,990 people (45.8% women), 4647 men and 3114 women died from CVD. At baseline, the mean (standard deviation) age was 53.1 (9.7) years and the mean  $\text{Log}_e \text{GGT}$  level was 3.26 (0.76) U/L (Table 1), with values of 3.59 (0.75) U/L for men and 2.87 (0.56) U/L for women. Subjects with higher GGT values tended to be more likely to be current smokers and to exhibit more frequent alcohol use, and less likely to be elderly (70 or above), than those with lower GGT levels. Higher GGT levels were generally associated with higher SBP, fasting glucose, total cholesterol, and BMI values (Table 1).

### 3.2. GGT and CVD mortality

Clear dose-response relationships between GGT and CVD mortality were observed in all participants (Fig. 1) and in both sexes (Supplementary Fig. 1 and 2). Compared with the lowest baseline GGT group, the sex-age adjusted HRs for CVD mortality were 1.06, 1.20, 1.32, 1.58, and 1.86 across the other five GGT categories (Fig. 1). After adjustment for age and sex, each 1-unit increase in  $\text{Log}_e \text{GGT}$  was associated with an approximately 35% higher risk of CVD mortality (HR per 1-unit increase in  $\text{Log}_e \text{GGT} = 1.35$  [95% CI = 1.31–1.39]); and elevations of 33%–42% for deaths from hypertensive diseases, IHD, other heart diseases, and stroke (HRs of 1.42 [1.26–1.59], 1.33 [1.26–1.41], 1.35 [1.25–1.47], and 1.34

**Table 1**

Characteristics of participants according to GGT categories.

Characteristics	Total N = 512,990	Sex-specific GGT percentile									
		<20%		20% to <40%		40% to <60%		60% to <80%		80% to <90%	
		n = 89,774	n = 112,011	n = 104,014	n = 100,740	n = 53,164	n = 53,287				
GGT, U/L	37.9 ±54.7	12.6 ±4.0	17.8 ±5.7	25.4 ±8.7	36.9 ±15.0	55.4 ±25.1	131.5 ±127.2				
Log <sub>e</sub> GGT, U/L	3.26 ±0.76	2.47 ±0.36	2.83 ±0.33	3.17 ±0.36	3.52 ±0.43	3.90 ±0.49	4.58 ±0.73				
Age, years	53.1 ±9.7	53.3 ±10.3	52.9 ±9.9	53.0 ±9.6	53.0 ±9.4	53.0 ±9.4	53.4 ±9.2				
SBP, mmHg	127.2 ±18.3	123.4 ±17.4	124.9 ±17.8	126.9 ±17.9	128.9 ±18.1	130.6 ±18.5	132.4 ±19.2				
FSG, mg/dL	98.4 ±34.8	94.0 ±28.5	95.1 ±31.8	97.3 ±33.1	100.1 ±35.4	102.9 ±39	107.6 ±44.5				
Total cholesterol, mg/dL	200.4 ±38.8	188.3 ±34.3	196.1 ±36	201.1 ±37	205.8 ±38.9	209.1 ±40.9	209.8 ±45.3				
BMI, kg/m <sup>2</sup>	24.0 ±3	22.9 ±2.7	23.5 ±2.8	24.0 ±2.8	24.6 ±2.9	24.9 ±3	24.8 ±3.2				
Sex											
Women	234,743 (45.8)	34,661 (38.6)	57,241 (51.1)	47,436 (45.6)	45,728 (45.4)	24,355 (45.8)	25,322 (47.5)				
Men	278,247 (54.2)	55,113 (61.4)	54,770 (48.9)	56,578 (54.4)	55,012 (54.6)	28,809 (54.2)	27,965 (52.5)				
Age group											
< 60 years	370,540 (72.2)	63,171 (70.4)	80,915 (72.2)	75,657 (72.7)	73,478 (72.9)	38,953 (73.3)	38,366 (72.0)				
60–69 years	104,822 (20.4)	18,316 (20.4)	22,392 (20.0)	21,019 (20.2)	20,679 (20.5)	10,858 (20.4)	11,558 (21.7)				
≥ 70 years	37,628 (7.3)	8287 (9.2)	8704 (7.8)	7338 (7.1)	6583 (6.5)	3353 (6.3)	3363 (6.3)				
Self-reported comorbidity											
Heart diseases, stroke	9692 (1.9)	1448 (1.6)	1814 (1.6)	1964 (1.9)	2103 (2.1)	1158 (2.2)	1205 (2.3)				
Smoking status											
Never smoker	329,465 (64.2)	59,542 (66.3)	77,418 (69.1)	67,134 (64.5)	62,423 (62.0)	31,832 (59.9)	31,116 (58.4)				
Past smoker	43,577 (8.5)	8336 (9.3)	8783 (7.8)	9090 (8.7)	8876 (8.8)	4549 (8.6)	3943 (7.4)				
Current smoker	118,288 (23.1)	18,011 (20.1)	21,145 (18.9)	23,300 (22.4)	25,102 (24.9)	14,631 (27.5)	16,099 (30.2)				
Missing	21,660 (4.2)	3885 (4.3)	4665 (4.2)	4490 (4.3)	4339 (4.3)	2152 (4.0)	2129 (4.0)				
Alcohol use, frequency (days)											
Rarely	285,046 (55.6)	57,722 (64.3)	70,415 (62.9)	58,372 (56.1)	50,950 (50.6)	24,521 (46.1)	23,066 (43.3)				
2/month–2/week	160,334 (31.3)	25,419 (28.3)	32,235 (28.8)	33,936 (32.6)	34,600 (34.3)	18,233 (34.3)	15,911 (29.9)				
3–7/week	57,953 (11.3)	4683 (5.2)	7085 (6.3)	9733 (9.4)	13,441 (13.3)	9572 (18.0)	13,439 (25.2)				
Missing	9657 (1.9)	1950 (2.2)	2276 (2.0)	1973 (1.9)	1749 (1.7)	838 (1.6)	871 (1.6)				
Physical activity											
≥1 time/week	210,789 (41.1)	37,432 (41.7)	45,886 (41.0)	43,538 (41.9)	42,012 (41.7)	21,643 (40.7)	20,278 (38.1)				
Income status											
<4 decile (low income)	118,061 (23.0)	19,576 (21.8)	26,062 (23.3)	23,778 (22.9)	22,989 (22.8)	12,448 (23.4)	13,208 (24.8)				
4–7 decile	167,098 (32.6)	29,342 (32.7)	35,628 (31.8)	33,277 (32.0)	32,504 (32.3)	17,463 (32.8)	18,884 (35.4)				
>7 decile (high income)	227,831 (44.4)	40,856 (45.5)	50,321 (44.9)	46,959 (45.1)	45,247 (44.9)	23,253 (43.7)	21,195 (39.8)				

Data are expressed as mean ± standard deviation or n (%). GGT categories: 1–19 (<20%), 20–27 (20% to <40%), 28–39 (40% to <60%), 40–63 (60% to <80%), 64–97 (80% to <90%), and ≥98 (≥90%) U/L in men, 1–10 (<20%), 11–14 (20% to <40%), 15–18 (40% to <60%), 19–25 (60% to <80%), 26–34 (80% to <90%), and ≥35 (≥90%) U/L in women. *p*-values, which were calculated by the Chi-squared test and 1-way analysis of variance across the GGT groups, were <0.001 for each variable including age at baseline. To convert cholesterol from mg/dL to mmol/L, multiply by 0.0259. To convert glucose from mg/dL to mmol/L, multiply by 0.0555.

BMI, body mass index; FSG, fasting serum glucose; GGT, gamma-glutamyltransferase; SBP, systolic blood pressure.

[1.28–1.40], respectively. After further adjustment for person-specific factors including smoking status, frequency of alcohol use, and SBP, the HRs modestly changed (Table 2). The restricted cubic spline analysis showed that the association of Log<sub>e</sub>GGT with CVD mortality was generally linear in both sexes (*p*<sub>non-linearity</sub> = 0.1999 in men, 0.4890 in women) and the pattern of association was similar between men and women (Supplementary Fig. 3).

Mortality was 33%–50% higher for every 1-unit higher Log<sub>e</sub>GGT score for the various subtypes of heart diseases and stroke, including acute MI, chronic IHD, heart failure, hemorrhagic stroke, and ischemic stroke (HRs of 1.34 [95% CI = 1.25–1.43], 1.33 [95% CI, 1.15–1.54], 1.45 [95% CI, 1.24–1.70], 1.50 [95% CI, 1.39–1.69], and 1.33 [95% CI, 1.23–1.44], respectively), after adjusting for age and sex. When fully adjusted for potential confounders, mortality was on average 27%–48% higher for each 1-unit increment in Log<sub>e</sub>GGT for the subtypes of heart disease and stroke (Supplementary Table 1). The magnitude of the HRs per 1-unit increase in Log<sub>e</sub>GGT were similar between acute MI and chronic IHD, while it was greater for hemorrhagic stroke than for ischemic stroke (sex-age adjusted HR, 1.50 vs. 1.33, *p*<sub>heterogeneity</sub> = 0.029; fully adjusted HR, 1.42 vs. 1.27, *p*<sub>heterogeneity</sub> = 0.052), especially in men (fully adjusted HR, 1.58 vs. 1.22, *p*<sub>heterogeneity</sub> < 0.001).

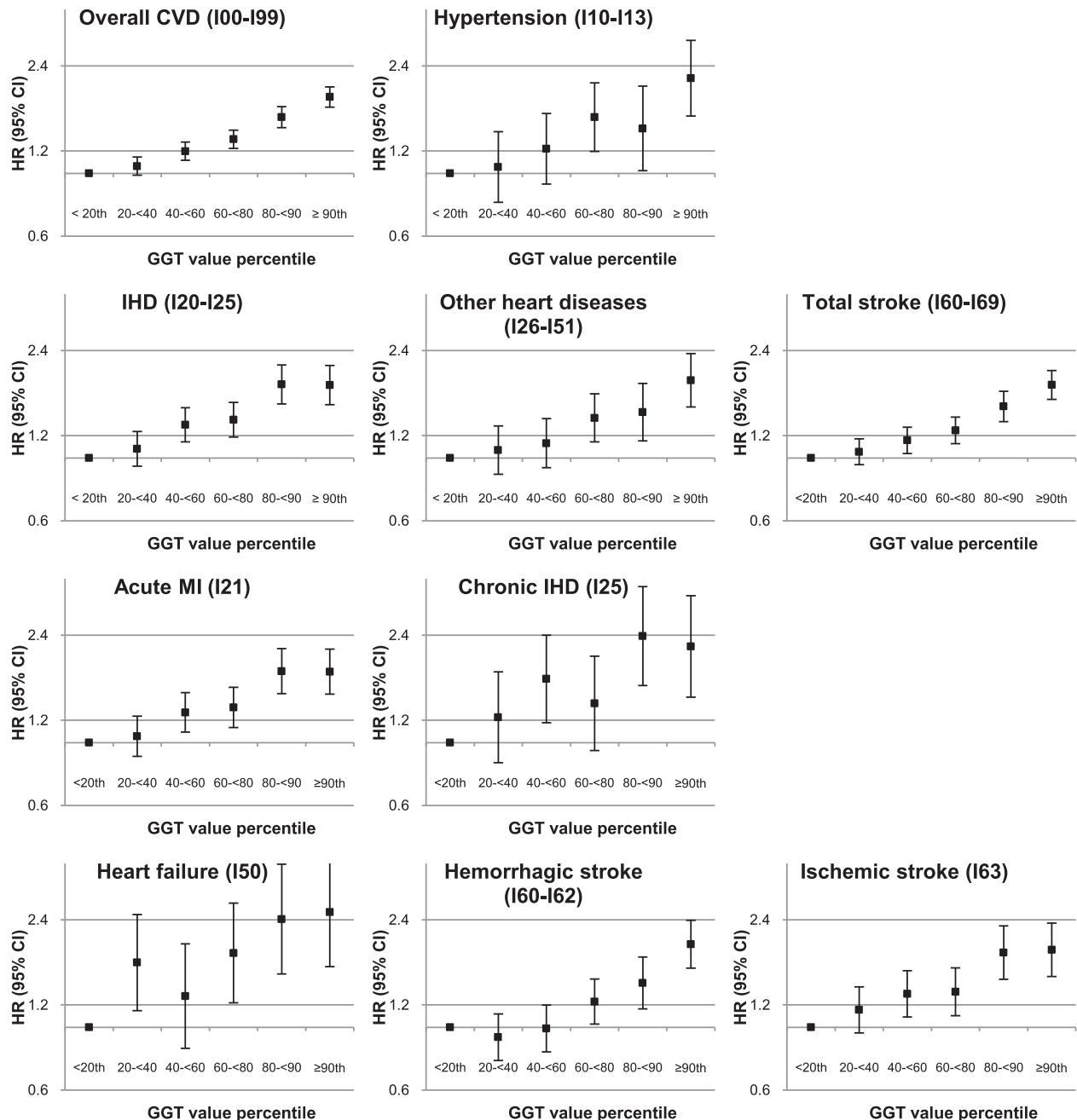
When HRs were analyzed in various subgroups (Fig. 2), the HRs for CVD mortality generally did not vary between men and women, persons with or without self-reported heart diseases or stroke,

never versus ever smokers, rare alcohol users versus regular alcohol users, high versus low income earners, and normoglycemic versus hyperglycemic persons. The association, however, was different between subgroups according to age (*p*<sub>interaction</sub> = 0.001), SBP (*p*<sub>interaction</sub> = 0.001), physical activity (*p*<sub>interaction</sub> = 0.052), BMI (*p*<sub>interaction</sub> = 0.026), and blood cholesterol levels (*p*<sub>interaction</sub> = 0.020). For IHD mortality, the estimated relative risk was greater in persons with lower blood pressure (Supplemental Fig. 4, *p*<sub>interaction</sub> = 0.001), while for total stroke mortality, it was greater in younger adults (*p*<sub>interaction</sub> < 0.001), men (*p*<sub>interaction</sub> = 0.006), and physically active persons (*p*<sub>interaction</sub> = 0.009) than their counterparts (Supplemental Fig. 5).

Additional analysis was performed after excluding persons with a history of liver disease, and further adjustment for alanine transaminase and aspartate transaminase in order to minimize the potential impact of liver disorders and to examine the associations of GGT independent of liver function. After such a restriction and further adjustment, the associations of GGT were not weakened (Supplemental Table 3).

### 3.3. GGT and prediction of CVD mortality

In persons without known heart disease or stroke, without GGT in the model, the fully adjusted prediction model showed a fairly good predictive ability for discriminating CVD mortality (AUC = 0.8442). When Log<sub>e</sub>GGT was added into the model, the AUC



**Fig. 1.** Age and sex adjusted HRs\* across 6 categories of baseline GGT for mortality from CVD and its subtypes. GGT categories (1–19, 20–27, 28–39, 40–63, 64–97, and ≥98 U/L in men, 1–10, 11–14, 15–18, 19–25, 26–34, and ≥35 U/L in women). \*HRs and 95% CIs were calculated using Cox proportional hazards models. CI, confidence interval; CVD, cardiovascular disease; GGT, gamma-glutamyltransferase; HR, hazard ratio; IHD, ischemic heart disease; MI, myocardial infarction.

increased significantly (change in AUC, 0.0020,  $p < 0.001$ , Table 3). The increase in AUC upon the addition of GGT was greater in persons  $<60$  years of age than in persons  $\geq 60$  years of age (0.0055 vs. 0.0021,  $p_{\text{heterogeneity}} = 0.024$ ), in persons with no hypertension (SBP  $<140$  mmHg) than in those with hypertension (0.0030 vs. 0.0013,  $p_{\text{heterogeneity}} = 0.007$ ), and in persons  $<60$  years of age with an SBP  $<140$  mmHg than in those aged  $\geq 60$  years with an SBP  $<140$  mmHg (0.0086 vs. 0.0030,  $p_{\text{heterogeneity}} = 0.031$ ). When a model that only included Framingham risk score variables such as age, sex, smoking status, SBP, total cholesterol, and comorbid diabetes, but not high-density lipoprotein cholesterol due to the unavailability of that

information, was fitted [22], among persons with an SBP  $<140$  mmHg, the increase in the AUC upon the addition of GGT was also greater in persons aged  $<60$  years than in persons aged  $\geq 60$  years (0.0088 vs. 0.0022,  $p_{\text{heterogeneity}} = 0.008$ ).

#### 4. Discussion

In our cohort study of 512,990 Korean adults, we found that higher blood GGT levels were associated with higher mortality from CVD and its subtypes. The presence of clear dose-response associations strengthens the evidence that GGT is a risk factor for

**Table 2**

Hazard ratios (HRs) for CVD mortality across GGT categories.

Causes of death	GGT category	No. of deaths	Model 1			Model 2		
			p	HR	(95% CI)	p	HR	(95% CI)
Total CVD (I00–I99)	<20%	1365		1.00	(Reference)		1.00	(Reference)
	20% to <40%	1493	0.069	1.07	(0.99–1.15)	0.128	1.06	(0.98–1.14)
	40% to <60%	1489	<0.001	1.21	(1.12–1.30)	<0.001	1.18	(1.10–1.27)
	60% to <80%	1499	<0.001	1.34	(1.24–1.45)	<0.001	1.30	(1.20–1.40)
	80% to <90%	884	<0.001	1.59	(1.46–1.74)	<0.001	1.51	(1.38–1.65)
	≥90%	1031	<0.001	1.87	(1.72–2.04)	<0.001	1.72	(1.57–1.87)
	1 unit higher Log <sub>e</sub> GGT	7761	<0.001	1.36	(1.32–1.40)	<0.001	1.31	(1.26–1.35)
Hypertensive diseases (I10–I13)	<20%	89		1.00	(Reference)		1.00	(Reference)
	20% to <40%	101	0.784	1.04	(0.78–1.39)	0.892	1.02	(0.76–1.36)
	40% to <60%	101	0.242	1.19	(0.89–1.59)	0.371	1.14	(0.85–1.53)
	60% to <80%	120	0.003	1.53	(1.15–2.03)	0.012	1.44	(1.08–1.92)
	80% to <90%	54	0.077	1.37	(0.97–1.94)	0.207	1.26	(0.88–1.79)
	≥90%	80	<0.001	2.04	(1.49–2.81)	<0.001	1.81	(1.31–2.50)
	1 unit higher Log <sub>e</sub> GGT	545	<0.001	1.39	(1.23–1.57)	<0.001	1.31	(1.15–1.48)
IHD (I20–I25)	<20%	374		1.00	(Reference)		1.00	(Reference)
	20% to <40%	400	0.206	1.10	(0.95–1.26)	0.420	1.06	(0.92–1.22)
	40% to <60%	435	<0.001	1.34	(1.16–1.54)	0.001	1.27	(1.10–1.46)
	60% to <80%	411	<0.001	1.42	(1.23–1.64)	<0.001	1.31	(1.14–1.52)
	80% to <90%	268	<0.001	1.89	(1.61–2.23)	<0.001	1.70	(1.44–2.00)
	≥90%	265	<0.001	1.89	(1.61–2.24)	<0.001	1.66	(1.40–1.97)
	1 unit higher Log <sub>e</sub> GGT	2153	<0.001	1.37	(1.29–1.45)	<0.001	1.29	(1.21–1.37)
Other heart disease (I26–I51)	<20%	190		1.00	(Reference)		1.00	(Reference)
	20% to <40%	214	0.405	1.09	(0.89–1.32)	0.390	1.09	(0.89–1.33)
	40% to <60%	200	0.158	1.16	(0.95–1.41)	0.148	1.16	(0.95–1.42)
	60% to <80%	226	<0.001	1.44	(1.18–1.76)	<0.001	1.45	(1.19–1.78)
	80% to <90%	117	0.001	1.52	(1.20–1.92)	0.001	1.52	(1.19–1.93)
	≥90%	149	<0.001	2.00	(1.59–2.50)	<0.001	1.97	(1.56–2.48)
	1 unit higher Log <sub>e</sub> GGT	1096	<0.001	1.40	(1.29–1.53)	<0.001	1.39	(1.27–1.51)
Total stroke (I60–I69)	<20%	668		1.00	(Reference)		1.00	(Reference)
	20% to <40%	734	0.313	1.06	(0.95–1.17)	0.317	1.06	(0.95–1.17)
	40% to <60%	710	0.009	1.15	(1.04–1.28)	0.013	1.15	(1.03–1.28)
	60% to <80%	705	<0.001	1.25	(1.12–1.40)	<0.001	1.24	(1.11–1.38)
	80% to <90%	424	<0.001	1.50	(1.33–1.71)	<0.001	1.46	(1.28–1.66)
	≥90%	500	<0.001	1.77	(1.56–1.99)	<0.001	1.64	(1.45–1.86)
	1 unit higher Log <sub>e</sub> GGT	3741	<0.001	1.33	(1.27–1.40)	<0.001	1.29	(1.23–1.35)

GGT categories: 1–19 (<20%), 20–27 (20% to <40%), 28–39 (40% to <60%), 40–63 (60% to <80%), 64–97 (80% to <90%), and ≥98 (≥90%) U/L in men, 1–10 (<20%), 11–14 (20% to <40%), 15–18 (40% to <60%), 19–25 (60% to <80%), 26–34 (80% to <90%), and ≥35 (≥90%) U/L in women.

CI, confidence interval; CVD, cardiovascular diseases; GGT, gamma-glutamyltransferase; IHD, ischemic heart disease.

Model 1: adjustment was done for age at baseline, sex, smoking status, alcohol use, physical activity, income status, and self-reported heart diseases or stroke.

Model 2: adjustment was done for model 1 plus systolic blood pressure, fasting glucose, total cholesterol, and body mass index.

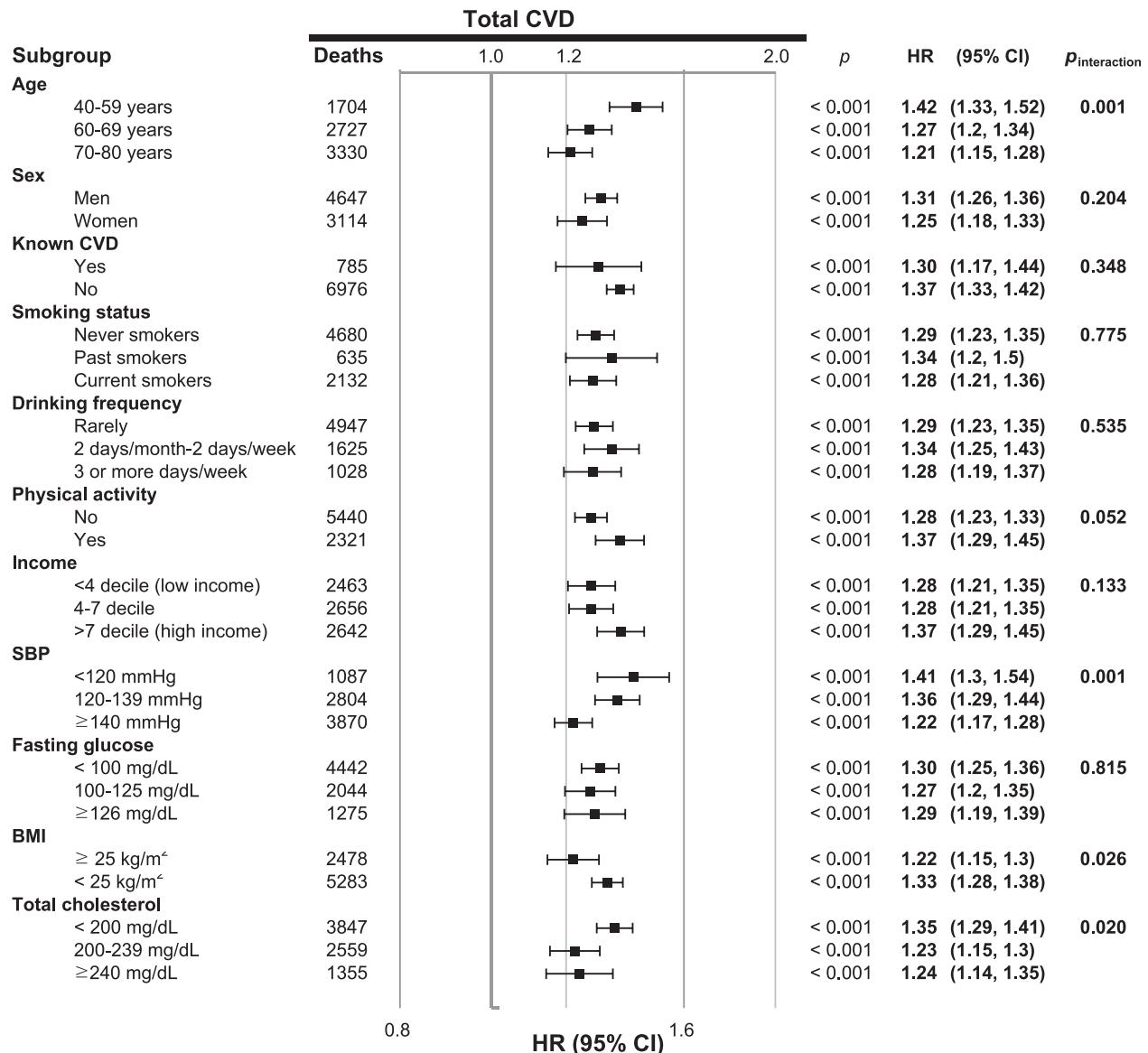
CVD. Each 1-unit increase in the Log<sub>e</sub>GGT value was on average associated with an approximately 30% higher risk of death from CVD (HR = 1.31), and a 30%–50% greater risk of death from the subgroups of CVD such as hypertensive diseases (HR = 1.31), IHD (HR = 1.29), other heart diseases (HR = 1.39), total stroke (HR = 1.29), acute MI (HR = 1.30), chronic IHD (HR = 1.27), heart failure (HR = 1.48), hemorrhagic stroke (HR = 1.42), and ischemic stroke (HR = 1.27), after full adjustment. In the subgroup analysis, the HRs for CVD mortality generally did not vary by sex, self-reported comorbidity of heart diseases or stroke, smoking status, frequency of alcohol use, income status, or blood glucose level. The association, however, was stronger in younger adults (below 60 years), non-hypertensive persons, physically more active persons, persons with normal weight (BMI <25 kg/m<sup>2</sup>), and individuals with total cholesterol <200 mg/dL than in their respective counterparts. Upon the addition of Log<sub>e</sub>GGT in the CVD mortality prediction model, which included age, sex, SBP, fasting glucose, total cholesterol, BMI, smoking status, alcohol use, income status, and physical activity, the AUC value showed a modest increase (0.0020, p < 0.001). The change in AUC values by including GGT was more profound in persons aged <60 years (0.0055), with an SBP <140 mmHg (0.0030), and with both age <60 years and SBP <140 mmHg (0.0086).

Through previous systematic reviews and our own literature search, six prospective cohort studies that reported associations

between GGT and CVD in Asian populations were identified [9,13,20,23–25]. The results in Asian populations have been a source of heterogeneity in several meta-analyses [11,26], and the possibility that the association is weaker in Asians than in European-origin populations has been suggested [7,11]. The current study, however, suggests that the overall association is not substantially different between Asian and European-origin populations, since the magnitude of the associations of GGT with CVD found in this study (HR per 1-SD increase in Log<sub>e</sub>GGT = 1.22 [95% CI = 1.19–1.25]), stroke (1.21 [1.17–1.25]), and heart failure (1.34 [1.19–1.52]) was similar to that of previous meta-analyses (1.23 [95% CI = 1.16–1.29] [3], 1.28, [95% CI = 1.16–1.43] [11], and 1.25 [95% CI = 1.07–1.46] [10], respectively).

IHD, especially its acute form, has been associated with controversial results; in several large studies, higher GGT activity was not associated with a greater risk of IHD, at least in a subgroup analysis according to sex [5,9,20,26]. In the current study, mortality from IHD, including acute MI, was positively associated with GGT levels in both sexes, and the magnitude of the HRs was found to be similar to those for other forms of CVD.

For subtypes of stroke, our study confirmed that the association of GGT was stronger for hemorrhagic stroke than for ischemic stroke. The majority of prospective studies [5,9,26,27], except for a few studies with a small number of stroke cases [13,28], showed similar evidence, but without formal statistical tests. A stronger

**Fig. 2.** HRs\* per 1 unit increment in Log<sub>e</sub>GGT for total CVD mortality by individual-specific characteristics.

\*HRs and 95% CIs were calculated using Cox proportional hazards models stratified by baseline age (years: 40–44, 45–54, 55–64, 65–74, 75–80), after adjustment for age at baseline (continuous variable), sex, smoking status, alcohol use, physical activity, income status, a history of heart diseases or stroke, SBP, fasting glucose, total cholesterol and BMI. BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; Log<sub>e</sub>GGT, natural-log transformed gamma-glutamyltransferase; SBP, systolic blood pressure.

**Table 3**

AUC changes upon addition of Log<sub>e</sub>GGT to the CVD mortality prediction model in participants without self-reported heart disease or stroke at baseline.

Subgroup	No. of participants	No. of deaths	Without Log <sub>e</sub> GGT		With Log <sub>e</sub> GGT		Difference in AUC	p <sub>diff</sub>	p <sub>heterogeneity</sub>
			AUC	(95% CI)	AUC	(95% CI)			
Total	503,298	6976	0.8442	(0.8397–0.8486)	0.8461	(0.8417–0.8506)	0.0020	<0.001	
Age <60 y	366,850	1602	0.7627	(0.7512–0.7742)	0.7682	(0.7568–0.7795)	0.0055	<0.001	0.024
Age ≥60 y	136,448	5374	0.7332	(0.7266–0.7397)	0.7353	(0.7288–0.7418)	0.0021	<0.001	
SBP ≥ 140 mmHg	134,056	3525	0.7914	(0.7840–0.7988)	0.7927	(0.7854–0.8001)	0.0013	<0.001	0.007
SBP < 140 mmHg	369,242	3451	0.8490	(0.8424–0.8556)	0.8519	(0.8455–0.8584)	0.0030	<0.001	
Age <60 y, SBP <140 mmHg	287,456	877	0.7493	(0.7335–0.7651)	0.7579	(0.7422–0.7735)	0.0086	<0.001	0.031
Age ≥60 y, SBP <140 mmHg	81,786	2574	0.7374	(0.728–0.7467)	0.7403	(0.731–0.7496)	0.0030	0.002	

A prediction model with an AUC value of 1.0 or 0.5 represents a perfect or an uninformative model, respectively.

AUC, area under the receiver operating characteristics curve; CI, confidence interval; FRS, Log<sub>e</sub>GGT, natural log-transformed GGT; SBP, systolic blood pressure.

p<sub>diff</sub>: p-value for the difference in the AUC value between models with or without Log<sub>e</sub>GGT.

Heterogeneity: p-value for heterogeneity of the difference in AUC value between models with or without Log<sub>e</sub>GGT between age and SBP subgroups.

The prediction model includes age at baseline, sex, smoking status, alcohol use, physical activity, income status, SBP, fasting glucose, total cholesterol, and body mass index.

association for hemorrhagic than ischemic stroke, however, was found only in men in the current study. An additional analysis with 8 GGT groups (<15 [reference], 15–19, 20–24, 25–29, 30–39, 40–59, 60–79, and ≥80 U/L) revealed that only the highest GGT groups (60–79 and ≥80 U/L in both sexes combined and in men, ≥80 U/L in women) were associated with higher mortality from hemorrhagic stroke with a P-value <0.05, whereas GGT levels ≥25 U/L were generally associated with higher mortality from ischemic stroke in both sexes, compared with persons with a GGT level <15 U/L. These results suggest that the underlying mechanisms of the impact of GGT may be different between ischemic and hemorrhagic stroke to some degree.

GGT has long been used as a marker of alcohol intake, and several studies have found evidence suggesting that the associations of GGT with CVD may be modified by alcohol consumption [12,20,29]. It has not been completely resolved whether GGT is associated with CVD independently of alcohol intake. Our study found that GGT was associated with higher CVD mortality in rare drinkers and that the associations were not modified by alcohol intake frequency. Thus, our study strengthens the evidence that higher GGT activity increases the risk of CVD mortality independently of alcohol intake.

Evidence from several previous studies has suggested that the relative risk of GGT for CVD may be higher at younger ages [5,8,12]. The current study demonstrated that the HRs of GGT for CVD mortality were greater in younger persons (<60 years) and normotensive individuals (SBP <140 mmHg) than in older adults (60 years old or above) and persons with hypertension (SBP ≥140 mmHg). In our study, the association of GGT was also stronger in persons who engaged in physical activity at least once a week, had a normal weight (BMI <25 kg/m<sup>2</sup>), and had normal total cholesterol levels (<200 mg/dL) than in persons who engaged in no physical activity, were overweight or obese (BMI ≥25 kg/m<sup>2</sup>), and had higher cholesterol levels (≥200 mg/dL). Wannamethee et al. similarly reported that the risk associated with elevated GGT was higher in persons with a lower cardiovascular risk score, but without providing detailed information about the specific variables [8]. Kunutsor et al. likewise reported similar findings, stating that subgroups with lower age, BMI, blood lipids, and SBP seemed to show stronger associations, although the p-value for interaction was below 0.05 only between the age subgroups [12].

Although it has been suggested that GGT measurements may be useful for CVD risk assessment [6,8], a few recent studies reported that adding GGT to the conventional CVD risk factors did not improve the prediction of risk for CVD or IHD-related mortality [12,14]. The current study found that upon the addition of Log<sub>e</sub>GGT to the prediction model, the AUC showed a statistically significant increase (change in AUC, 0.0020, *p* < 0.001). The increase of 0.0020 in AUC can be deemed modest; however, the addition of GGT seemed to have the potential to provide an important improvement in risk prediction. For instance, when comparing AUC values with versus without a risk factor in our full model in persons without self-reported heart disease or stroke, only age (change in AUC, 0.1298), SBP (0.0067), and smoking status (0.0033) had more impact than GGT on the prediction of total CVD mortality, while sex, fasting glucose, total cholesterol, BMI, alcohol use, physical activity, and income status had a lower predictive ability than GGT. Furthermore, when we fitted the prediction model in younger adults and in persons with an SBP <140 mmHg, in whom the association of GGT was greatest, the predictive ability (AUC) upon addition of GGT increased from 0.0020 to 0.0055 in persons <60 years, and to 0.0086 in persons aged <60 years with an SBP <140 mmHg. These findings suggest that GGT measurements have the potential to improve the prediction of CVD mortality, especially in persons younger than 60 years old and with an SBP <140 mmHg.

Additionally, since GGT levels increased the AUC more than other cardiometabolic risk factors, except SBP, upon addition into the prediction model, GGT has the potential to replace other conventional risk factors for the prediction of CVD mortality, not to mention that GGT is stable in storage and that measuring GGT is simple, inexpensive, and does not require fasting for measurements to be made.

Several putative mechanisms have been suggested to explain the association of elevated GGT with increased CVD risk [7]. GGT may be a marker of antioxidant inadequacy, oxidative and nitrosative stress, and systemic inflammation [7,30], potentially independent of cardiometabolic risk factors [31]. The direct role of GGT in the formation of atheromatous plaque was also proposed [6].

#### 4.1. Strengths and limitations of the study

A large number of participants, a prospective design, and complete follow-up for death are clear strengths of this study. However, it has several limitations. Non-IFCC methods for measuring GGT, which are associated with lower accuracy [32], were used in some hospitals. However, since lower accuracy was not related to CVD mortality, this may not contribute to an overestimation of the HRs of GGT for CVD mortality. Second, collection of alcohol use information via questionnaire may have some limitations. For example, rare drinkers in the current study were not necessarily lifelong abstainers. However, since the associations were similar across alcohol intake subgroups, this potential misclassification is unlikely to significantly affect the observed associations in rare drinkers. Furthermore, we performed a validation analysis of alcohol measure by examining associations of alcohol with the mortality risk of alcoholic liver disease, which showed that our alcohol measure was fairly reliable and valid. Third, several risk factors, such as C-reactive protein and subgroups of lipids (such as low-density and high-density lipoprotein cholesterol, as well as triglycerides) and several predictors including anti-hypertensive and lipid-lowering medications were not adjusted for in the current study. Although additional adjustment for such factors did not substantially change the associations in other studies [10,33], this remains a limitation. Fourth, relative risk estimation using a single baseline measurement of GGT may underestimate its true association, due to the regression dilution effect [34]. Fifth, several medications use, such as anti-epilepsy drugs [35], that may affect both GGT levels and CVD risk, were not adjusted for. Non-adjustment for such medications use might affect the results. Finally, the fact that the study population was homogeneously Korean may affect its generalizability. Some results, such as the magnitude of the relative risk estimation associated with GGT or the magnitude of the change in AUC upon addition of GGT to the risk prediction model, may need to be assessed in other ethnic and regional populations with varying distributions of environmental and individual risk factors, as well as of subtypes of CVD morbidity.

#### 4.2. Conclusions

Our cohort study of 512,990 Korean adults suggests that higher blood GGT levels increase mortality from CVD and its subtypes. The associations were stronger in younger, non-hypertensive, physically more active, normal-weight, and normocholesterolemic persons than in their respective counterparts. In the general population, GGT measurements have the potential to improve and to replace other conventional risk factors in the prediction of CVD mortality risk, especially in persons aged <60 years old without hypertension.

## Genetic Covariation between Serum $\gamma$ -Glutamyltransferase Activity and Cardiovascular Risk Factors

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**Background:** Several studies have shown that variation in serum  $\gamma$ -glutamyltransferase (GGT) in the population is associated with risk of death or development of cardiovascular disease, type 2 diabetes, stroke, or hypertension. This association is only partly explained by associations between GGT and recognized risk factors. Our aim was to estimate the relative importance of genetic and environmental sources of variation in GGT as well as genetic and environmental sources of covariation between GGT and other liver enzymes and markers of cardiovascular risk in adult twin pairs.

**Methods:** We recruited 1134 men and 2241 women through the Australian Twin Registry. Data were collected through mailed questionnaires, telephone interviews, and by analysis of blood samples. Sources of variation in GGT, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and of covariation between GGT and cardiovascular risk factors were assessed by maximum-likelihood model-fitting.

**Results:** Serum GGT, ALT, and AST were affected by additive genetic and nonshared environmental factors, with heritabilities estimated at 0.52, 0.48, and 0.32, respectively. One-half of the genetic variance in GGT was shared with ALT, AST, or both. There were highly significant correlations between GGT and body mass

index; serum lipids, lipoproteins, glucose, and insulin; and blood pressure. These correlations were more attributable to genes that affect both GGT and known cardiovascular risk factors than to environmental factors.

**Conclusions:** Variation in serum enzymes that reflect liver function showed significant genetic effects, and there was evidence that both genetic and environmental factors that affect these enzymes can also affect cardiovascular risk.

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In addition to its diagnostic uses, serum  $\gamma$ -glutamyltransferase (GGT)<sup>5</sup> activity has substantial epidemiologic significance (1). Prospective studies have shown a significant relationship between increased GGT and subsequent mortality and morbidity (2–7) and between GGT and development of specific conditions, including myocardial infarction (4), stroke (8), non-insulin-dependent diabetes (9), and hypertension (10). Major effects of body mass index (BMI) on serum GGT have been found, and associations between GGT and multiple cardiovascular risk factors, including serum lipids, blood pressure, smoking, and impaired glucose tolerance or insulin resistance, have been reported [summarized in Ref. (1)]. The known associations with other risk factors account for some, but not all, of the predictive value of GGT, so that it must in part be considered an independent risk factor or a marker of some type of risk that has not yet been characterized.

GGT shows significant correlations within the general population with both serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Some of the epidemiologic studies that showed high GGT to be a risk

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<sup>5</sup> Nonstandard abbreviations: GGT,  $\gamma$ -glutamyltransferase; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL-C and LDL-C, HDL- and LDL-cholesterol, respectively; and apo, apolipoprotein.

factor or to be associated with risk factors also measured and evaluated AST or ALT. In a German study of construction workers (6), AST and ALT, as well as GGT, were significant predictors of disability and death. In a British study of predictors of type 2 diabetes (9), AST was significant, but less predictive than GGT. Both AST and ALT, as well as GGT, are positively associated with BMI (11–14), which may be a reflection of the increased prevalence of fatty liver in more obese individuals.

For all these reasons, the sources of variation in GGT and their overlap with sources of variation in aminotransferases as well as the sources of covariation with cardiovascular risk factors are important. In this study, we assessed the genetic and environmental factors affecting variation in serum GGT activity and the degree to which similar factors affect AST or ALT. We also examined the genetic and environmental causes of covariation between the concentrations of these enzymes in serum and multiple biochemical or physiologic cardiovascular risk factors.

## Participants and Methods

### PARTICIPANTS

The characteristics of the participants in this study were described in a previous report (15). They completed a questionnaire in 1989, a telephone interview in 1993–1994, and provided a blood sample in 1993–1996. All participants were twins, born between 1903 and 1964, but in some cases only one member of a twin pair provided blood. Zygosity was determined from responses to questions about physical similarity and the inability of others to tell them apart, supplemented by blood group information. Participants gave informed consent to the questionnaire, interview, and blood collection, and the studies were approved by the appropriate Ethics Committees.

### PROCEDURES

Blood was collected from 1134 men and 2241 women. Immediately before blood collection, participants filled in a brief questionnaire that included a table asking how many drinks containing alcohol (10 g) they had consumed on each of the preceding 7 days, divided into beer, wine, spirits, fortified wine, or "other". The numbers of drinks were summed to obtain a total for the past week. Participants also reported the time of their last meal, and the time of blood collection was noted. At the same visit, their height and weight was measured. BMI was calculated from weight and height as: weight (kg)/[height (m)]<sup>2</sup>. Systolic and diastolic blood pressures were measured, with the participants sitting, by use of an automated blood pressure recorder (Dynamap 845 Vital Signs Monitor; Critikon Inc.). The mean of two results taken at 1-min intervals was calculated. Blood pressure results were available for 1666 of the participants.

Serum was separated from the blood and stored at –70 °C until analyzed. Serum GGT, AST, ALT, glucose, urate, total cholesterol, and triglycerides were measured by Boehringer Mannheim reagents and methods on a

Hitachi 747 analyzer. Ferritin, transferrin, and iron were measured using Roche Diagnostics reagents and methods on a Hitachi 917 analyzer. HDL-cholesterol (HDL-C) was measured by precipitation of non-HDL lipoproteins with dextran/MgSO<sub>4</sub> followed by enzymatic cholesterol assay. Apolipoproteins A-I, A-II, B, and E were measured by immunonephelometry using a Behring nephelometer and Behring reagents. Plasma insulin was measured by RIA (Diagnostic Products).

### STATISTICAL METHODS

Several of the measured variables were log-transformed because their frequency distributions were skewed. All references to serum GGT, AST, ALT, triglycerides, ferritin, and insulin and to the quantity of alcohol consumed per week are to the log-transformed values unless specified otherwise. LDL-cholesterol (LDL-C) was calculated from the total cholesterol, HDL-C, and triglyceride values by the Friedewald equation if triglycerides were ≤4.5 mmol/L. If the serum triglyceride concentration was above this limit, LDL-C was treated as missing. The samples were not taken in the fasting state, but participants reported the time of their last meal, and the triglyceride, glucose, and insulin results were adjusted for the elapsed time between the last meal and blood collection.

Initial analysis of the results revealed highly significant ( $P < 0.001$ ) correlations between GGT results and numerous biochemical, physiologic, and alcohol-related characteristics. Because the participants were twins and therefore not genetically independent, the effective number of individuals for any characteristic with substantial heritability would be less than the actual number of participants, and therefore, the significance (but not the magnitude) of correlations may be overestimated. More detailed examination of the sources of variation in GGT and of the reasons for covariation with the variables that showed significant correlations in the exploratory analysis was performed using the Mx program, Ver. 1.50 (16), which is designed for analysis of twin and family data and overcomes this problem.

This analysis, like all studies based on twin pairs reared together, depends on the assumption that environments are equally similar for monozygotic and dizygotic co-twins. For biochemical and physiologic characteristics and for individuals ≥30 years of age and living independently, the equal-environments assumption is generally accepted.

After allowing for the effects of demographic variables such as age and sex, the residual correlations between co-twins, by zygosity, were estimated. The data were fitted to models of sources of variation in GGT, ALT, and AST. These models may contain additive and dominance genetic variation and shared and nonshared environmental variation; models that show a significantly worse fit with the data are rejected, and the most parsimonious model that does not yield a worse fit than the full model is chosen. For example, the model containing only addi-

tive genetic and nonshared environmental sources of variation (AE model) will be accepted if the model containing only shared environmental and nonshared environmental sources of variation (CE model) gives a significantly worse fit to the data and if addition of either shared environmental or dominance genetic sources of variation fails to produce significant improvement in the goodness of fit.

Because of the significant correlations between GGT and many other variables, the sources of covariation between them were modeled in a series of multivariate analyses. This led to estimates of the common and unique paths from genetic and environmental sources of variation for the variables included. This was an extension of the univariate model fitting, and in addition to estimating the proportions of variance attributable to genetics and environment, it provided estimates of the extent to which the genetic or environmental effects were specific to one variable (e.g., GGT) and the extent to which they affected more than one variable (e.g., GGT and ALT, or GGT and ALT and AST). Because simultaneous analysis of a large number of variables is computationally intensive, several separate analyses were conducted. The covariation between GGT, ALT, and AST was first analyzed, followed by the covariation between GGT, BMI, and the lipid and lipoprotein concentrations [triglycerides, HDL- and LDL-C, apolipoprotein (apo) A-II, B, and E]; between GGT, BMI, glucose, and insulin; between GGT, BMI, and ferritin; between GGT, BMI, and urate; and between GGT, BMI, and systolic and diastolic blood pressure.

## Results

### HERITABILITY OF SERUM GGT, ALT, AND AST

Adjustments were made for demographic and sample-related variables that affected GGT, ALT, and AST activity. Mean values were higher in men than in women, and age had significant effects in both sexes. Mean values for these enzymes and for the other variables measured are shown in Data Supplement Table DS1. (All data supplement tables are available with the online version of the article at <http://www.clinchem.org/content/vol48/issue8/>.) The adjusted within-pair correlations by zygosity for GGT, ALT, and AST are shown in Data Supplement Table DS2, with the higher correlations in monozygotic than in dizygotic pairs suggesting significant genetic effects.

The results of testing of models of sources of variation, including additive genetic effects (A), dominance genetic effects (D), common environmental effects shared by members of a twin pair regardless of zygosity (C), and nonshared environmental effects (E), are shown in Data Supplement Table DS3. The models containing only C and E were rejected because the goodness-of-fit between the model and the data was significantly worse, for each of the enzymes, than for a model containing A and E only. Models containing A, C, and E or A, D, and E as sources of variation showed no significant improvements over the

AE models, and under the ACE models, the C component was estimated as zero. Therefore, we conclude that there is no evidence that nonadditive genetic effects or shared environmental effects contribute to interindividual variation in GGT, ALT, or AST. The age- and sex-adjusted heritabilities for log-transformed enzyme activities were 0.52 for GGT, 0.48 for ALT, and 0.32 for AST.

### PHENOTYPIC CORRELATIONS BETWEEN SERUM GGT, ALT, AND AST ACTIVITIES AND OTHER CHARACTERISTICS

Highly significant correlations were found between GGT values and many other physiologic or biochemical characteristics. These included other liver enzymes (AST and ALT), alcohol intake and smoking status (but only in men), BMI and biochemical aspects of the metabolic syndrome (triglycerides; HDL- and LDL-C; apo A-II, B, and E; urate; glucose; and insulin), blood pressure (systolic and diastolic), and iron status (ferritin). Similarly, there were significant associations between ALT and AST and many of the variables that showed correlations with GGT. The phenotypic correlations, taking the individual as the unit and making no allowance for gene-sharing within twin pairs, are shown in Data Supplement Table DS4.

The correlations with cardiovascular risk factors tended to be stronger for GGT than for ALT or (even more so) AST. This trend was apparent for BMI, lipids, apolipoproteins [except apo A-I and apo(a), which showed little association with the enzyme values], glucose and insulin, urate, blood pressures, and ferritin. It therefore seems that there are two components to the associations between GGT and the other measurements: one a general hepatic effect shared with ALT and AST, and the other more specific to GGT.

It is also noteworthy that although apo A-II was significantly and positively associated with GGT, ALT, and AST in both men and women, apo A-I was not, and HDL-C tended to show negative correlations. This dissociation between the components of HDL was not seen for the lipids and apoproteins associated with LDL or VLDL.

### COMMON AND UNIQUE GENETIC AND ENVIRONMENTAL INFLUENCES ON GGT, AST, AND ALT

Because of the large number of variables associated with GGT as well as with ALT and AST, the relationships between these enzymes and the genetic or environmental causes of these relationships were explored in a series of analyses. The relationships between genetic and environmental sources of variation acting on GGT, AST, and ALT are illustrated in Fig. 1. This path diagram shows additive genetic (A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>) and nonshared environmental (E<sub>1</sub>, E<sub>2</sub>, and E<sub>3</sub>) effects on these variables. The paths between the sources of variation and the observed values are shown in Fig. 1 and consist of A<sub>1</sub> and E<sub>1</sub>, which influence all three variables; A<sub>2</sub> and E<sub>2</sub>, which affect ALT and GGT, but not AST; and A<sub>3</sub> and E<sub>3</sub>, which affect GGT only. Values next to these paths show the proportion of

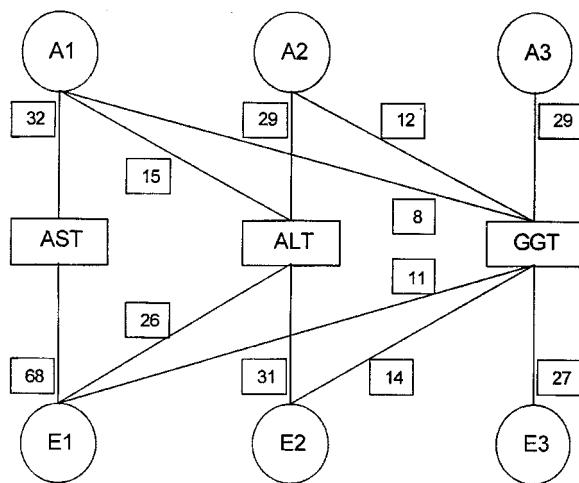


Fig. 1. Genetic and environmental sources of variation and covariation in GGT, AST, and ALT after adjustment for covariates.

The covariates are sex, age, weekly alcohol intake, BMI, weekly alcohol intake  $\times$  BMI interaction, lifetime alcohol dependence, smoking status, triglycerides, HDL, ferritin, and systolic and diastolic blood pressures. The numbers in boxes are the percentages of variance in GGT, AST, and ALT accounted for by additive genetic (A) and nonshared environmental (E) factors influencing all three enzymes (A1 and E1), ALT and GGT only (A2 and E2), and GGT only (A3 and E3). Note that there are minor differences from the estimated heritabilities in Table DS3 because of adjustment for extra variables (listed above) in this analysis.

variance: e.g., the path from A1 to ALT labeled "15" shows that 15% of the variance in ALT is attributable to genes that also affect AST. For GGT, 29% of the observed variance is attributable to genes that affect GGT but not ALT or AST, whereas 20% (12% + 8%) is attributable to genes that also affect ALT or AST or both. Twenty-seven percent of the variance in GGT is attributable to environmental effects not shared with AST and ALT, whereas 25% is attributable to environmental factors that also affect ALT and/or AST. Therefore, GGT is subject to both general "liver enzyme" factors and GGT-specific factors. It is slightly more closely related to ALT than to AST. Fig. 1 considers only the relationships between GGT and the aminotransferases; the genetic and environmental connections with cardiovascular risk factors are considered below. Loci affecting cardiovascular risk factors are expected to affect both the genetic effects unique to GGT and those shared with the other enzymes.

#### GENETIC AND ENVIRONMENTAL CORRELATIONS BETWEEN GGT AND VARIABLES RELATED TO CARDIOVASCULAR RISK

As stated above, there were multiple highly significant correlations between GGT and known or suspected cardiovascular risk factors. Of these, the strongest relationships were between GGT and variables associated with VLDL (triglycerides, apo B, and apo E), obesity, blood pressure (diastolic and systolic), and insulin resistance (insulin and glucose). There were also significant correlations with HDL-C and apo A-II (but not apo A-I), with urate, and with ferritin. The degrees to which these phenotypic correlations were attributable to genes affect-

ing multiple variables from this list or to nongenetic (environmental) factors with similar multifaceted effects can be seen from the genetic and environmental correlations with GGT, which are shown in Table 1.

Because the phenotypic correlations were the outcome of both genetic and environmental effects, the correlations in Table 1 are broadly similar to those in Table DS4. There was a tendency for the genetic correlations to be greater than the environmental ones, in part because environmental factors included measurement errors and short-term biological variation and these would not usually be correlated across variables. The genetic correlations with GGT were notably stronger than the environmental correlations for triglycerides, apo B and E, and BMI, and to a lesser extent for blood pressure and HDL-C.

Many of the cardiovascular risk factors that showed phenotypic, genetic, and environmental correlations with GGT were also correlated with each other. The strongest GGT correlation was with triglyceride concentration. This led us to consider whether there might be a single underlying cardiovascular factor associated with GGT, largely based on triglycerides and VLDL. This was examined by ordering the multivariate analysis so that genetic factors affecting both triglycerides and GGT were estimated and that, for other lipid or lipoprotein variables, only the components not affecting triglycerides and GGT would appear. The outcome is shown in Table 2. Although each of the variables listed had reasonably strong genetic correlations with GGT (Table 1), the genes affecting both triglycerides and GGT (including genes that also

**Table 1. Genetic and environmental correlations between logGGT and other variables.**

Variable	Genetic correlation	Environmental correlation
ALT (log)	0.35	0.34
AST (log)	0.42	0.49
BMI	0.34	0.23
Triglycerides (log)	0.45	0.29
apo B	0.37	0.17
apo AI	-0.04	-0.03
apo AI	0.22	0.19
apo E	0.35	0.19
LDL-C	0.25	0.16
HDL-C	-0.25	-0.15
apo(a) (log)	-0.08	0.00
Glucose <sup>a</sup>	0.20	0.11
Insulin (log) <sup>a</sup>	0.25	0.22
Uric acid	0.22	0.28
Diastolic BP <sup>b</sup>	0.27	0.16
Systolic BP	0.25	0.15
Iron	0.05	-0.12
Transferrin	0.10	0.07
Ferritin (log)	0.24	0.24

<sup>a</sup> Glucose and insulin results have been adjusted for time since last meal, collection-to-processing time, and storage time.

<sup>b</sup> BP, blood pressure.

**Table 2. Genetic and environmental paths from lipids to GGT.<sup>a</sup>**

	Triglycerides	apo B	BMI	apo E	LDL-C	HDL-C	apo A-II	Unique
A	9.6	1.7	1.7	0.9	0.2	0.3	0.9	36.6
E	3.9	0.2	0.9	0.2	0.5	0.1	1.7	40.8

<sup>a</sup> Each column shows the percentage of variance in GGT that can be accounted for by the additive genetic (A) and environmental (E) effects that affect the variable (and also those in columns to the right). The last column shows the contributions of genetic and environmental variance unique to GGT after covariance with lipids is taken into account. The genetic effects sum to 52% and the environmental effects to 48%, consistent with the univariate estimate of GGT heritability.

affect apo B, BMI, and so forth) accounted for 9.6% of the variance in GGT, the genes affecting both apolipoprotein B and GGT (including genes that also affect BMI, apo E, and so forth, but not triglycerides) accounted for 1.7% of the variance in GGT, and the other variables listed had only minor effects on GGT except through pathways shared with triglycerides. Genetic effects on GGT that were not shared by any of the other variables listed accounted for 36.6% of the GGT variance. Similarly, the nonunique environmental effects on GGT mainly affected triglycerides, and paths between other variables and GGT that excluded effects on triglycerides were negligible.

### Discussion

There are only limited previous data on the heritability of serum GGT and the aminotransferases. In our previous work (17), all participants were between 18 and 35 years of age with a mean age of 23 years. AST and ALT were affected by genetic and nonshared environmental factors, some of which affected both enzymes, whereas GGT was affected by both shared and nonshared environmental factors but not genetic ones. A recent study on Danish twins (18) reported data from individuals 73–102 years of age and found a mixture of genetic and nonshared environmental influences on GGT and ALT; AST was not measured. Our current results are consistent with the latter report and confirm the presence of genetic effects on these serum enzymes in adults after the age of 30.

This result implies that either the release of enzymes from liver cells or the rate of clearance of the enzymes from the circulation is subject to genetic effects. Because a considerable proportion of the genetic effect is shared by the three enzymes, which probably have different rates of clearance, we favor the former explanation. The genetic component unique to GGT may reflect variation in GGT activity at the hepatocyte surface exposed to the circulation.

However, the major topics of interest are not so much the variations in enzyme activities in the serum as the variations in the underlying physiologic or pathophysiological processes and the ways in which these processes interact with cardiovascular risk. All three enzymes show associations with cardiovascular risk factors, although these associations are strongest for GGT and only GGT has a convincing body of evidence connecting it with

outcomes in prospective studies (2–10). The pattern of relationships among the three liver enzymes suggests that GGT in part reflects the same processes as ALT or AST and in part is independently determined.

Although all three enzymes are associated with cardiovascular risk factors, the role of GGT in replenishing intracellular glutathione, and possibly in controlling apoptosis and proliferation in atherosomatous plaques (19, 20), may give it added significance. It is clear that increased GGT is associated with an increased probability of death from cardiovascular causes, development of type 2 diabetes, and development of hypertension and stroke (4, 8–10). Most probably, it is associated with fatty liver, insulin resistance, and oxidative stress (21, 22). Because it is possible that GGT plays a role in the proliferation of atherosomatous plaques, some of the circulating GGT may come from such plaques. Thus, there are two possible (but not necessarily exclusive) explanations for the association between serum GGT and cardiovascular risk: either GGT comes in part from the atherosomatous plaques, which will be more common and extensive in patients with adverse cardiovascular risk profiles, or GGT is associated with the risk factors even before the plaques are fully developed. Given the age range and essentially healthy status of our participants, the latter seems more likely.

It was notable that the associations between serum GGT and variables related to cardiovascular risk, reported by others and confirmed by us, had a strong genetic component and were less influenced by environmental variation (see Tables 1 and 2). It seems likely that a genetic predisposition to abdominal obesity and insulin resistance is associated with fatty liver, lipid abnormalities (particularly increased VLDL), and increased liver enzymes. The genetic component in the underlying factor of abdominal obesity or metabolic syndrome will lead to genetic correlations between its multiple consequences. Similar reasoning in relation to blood pressure (also associated with insulin resistance) could explain the genetic correlations between GGT and systolic and diastolic blood pressures.

Two other analytes are worthy of comment because of their correlations with both the liver enzymes and the cardiovascular risk factors. Urate was significantly correlated (all  $P < 0.01$ ; data not shown) with GGT, ALT, and AST as well as with BMI, blood pressures, triglycerides, apo B, and apo E in both women and men. Ferritin was significantly correlated with the three enzymes and with BMI, triglycerides, and apo A-II in both sexes and additionally with blood pressures, LDL-C, apo B, and apo E in the women. The associations of serum urate with liver enzymes and with cardiovascular risk are not unexpected (21, 23, 24), although they are unexplained. The association between ferritin and liver enzymes may reflect adverse effects of iron on the liver or the release of ferritin from hepatocytes in situations that also cause release of enzymes, but the additional association with cardiovascular risk factors suggests that either iron overload in-

creases the probability of liver dysfunction, insulin resistance, and lipid changes or vice versa. The relationships between iron overload and components of the insulin resistance syndrome have been investigated and discussed recently (25–27), but which is cause and which is effect is still uncertain.

In conclusion, clarification of the genetic associations between liver function (as indicated by the three enzymes studied) and the combined cardiovascular risk factors requires incorporation of a large number of variables and leads to a computationally intensive analysis. These relationships, which raise such issues as the effect of liver function in the broad sense on cardiovascular risk, will be the subject of further work.

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## **$\gamma$ -Glutamyltransferase, but not markers of hepatic fibrosis, is associated with cardiovascular disease in older people with type 2 diabetes mellitus: the Edinburgh Type 2 Diabetes Study**

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### **Abstract**

**Aims/hypothesis** We examined the association of prevalent and incident cardiovascular disease (CVD) with chronic liver disease in a cohort of community-based people with type 2 diabetes, in order to clarify the relationship between these two important conditions.

**Methods** 1,066 participants with type 2 diabetes aged 60–75 years underwent assessment of a range of liver injury markers (non-specific injury, steatosis, steatohepatitis, fibrosis, portal hypertension). Individuals were followed up for incident cardiovascular events.

**Results** At baseline there were 370/1,033 patients with prevalent CVD, including 317/1,033 with coronary artery disease

(CAD). After a mean follow-up of 4.4 years there were 44/663 incident CVD events, including 27/663 CAD events. There were 30/82 CVD-related deaths. Risk of dying from or developing CVD was no higher in participants with steatosis than in those without (HR 0.90; 95% CI 0.40, 2.00;  $p>0.05$ ). The only notable relationship was with  $\gamma$ -glutamyltransferase (GGT) (incident CVD: adjusted HR for doubling GGT 1.24 [95% CI 0.97, 1.59]  $p=0.086$ ; incident CAD: adjusted HR 1.33 [95% CI 1.00, 1.78]  $p=0.053$ ), suggesting that in our study population, chronic liver disease may have little effect on the development of, or mortality from, CVD.

**Conclusions/interpretation** An independent association between GGT and CVD warrants further exploration as a potentially useful addition to current cardiovascular risk prediction models in diabetes. However, overall findings failed to suggest that there is a clinical or pathophysiological association between chronic liver disease and CVD in elderly people with type 2 diabetes.

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**Keywords** Cardiovascular diseases · Community-based · Epidemiology · Fatty liver ·  $\gamma$ -Glutamyltransferase · Type 2 diabetes mellitus

### **Abbreviations**

ALT	Alanine aminotransferase
APRI	Aspartate to platelet ratio index
AST	Aspartate aminotransferase
CAD	Coronary artery disease
CK18	Cytokeratin-18
CVD	Cardiovascular disease
dBp	Diastolic blood pressure
eGFR	Estimated glomerular filtration rate

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ELF	Enhanced Liver Fibrosis panel
ET2DS	Edinburgh Type 2 Diabetes Study
FIB4	Fibrosis-4 score
GGT	$\gamma$ -Glutamyltransferase
HA	Hyaluronic acid
MI	Myocardial infarction
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NFS	NAFLD fibrosis score
OPCS	Office for Population Censuses and Surveys
P3NP	Aminoterminal peptide of procollagen III
sBP	Systolic blood pressure
TIA	Transient ischaemic attack
TIMP-1	Tissue inhibitor of metalloproteinases-1

## Introduction

Reports of higher cardiovascular mortality rates in people from the general population with non-alcoholic fatty liver disease (NAFLD) [1, 2] raise the possibility that there may be a pathophysiological relationship between NAFLD and the development of cardiovascular disease (CVD). In people with type 2 diabetes, such a relationship could help to explain the higher prevalences of both conditions. However, the association between CVD and NAFLD has not been well researched in diabetic populations, such that the true relationship between these two important conditions remains uncertain. Epidemiological knowledge of the relationship between NAFLD and CVD in diabetes is particularly limited: current studies are restricted to ultrasound scan-detected NAFLD and the secondary care end of the diabetes spectrum [3, 4]. We therefore aimed to determine the association of CVD with a range of biomarkers of chronic liver injury in a large cohort representative of the full spectrum of elderly people with type 2 diabetes.

Biologically, an association between NAFLD and CVD is plausible. Many of the pathogenic factors proposed for NAFLD and atherosclerosis are shared (e.g. insulin resistance, dyslipidaemia, systemic inflammation) and are closely linked to type 2 diabetes. The concept of the liver–vessel axis hypothesis [5] could also explain the biological mechanisms linking the liver directly to the accelerated atherosclerosis proposed in NAFLD. There is evidence indicating that a consequence of advanced NAFLD (non-alcoholic steatohepatitis [NASH]) includes enhanced atherosclerosis via further insulin resistance leading to atherogenic hyperlipidaemia (low HDL-cholesterol, high triacylglycerol and high LDL-cholesterol levels) and systemic inflammation through pro-inflammatory and pro-atherogenic factors (IL-6, TNF- $\alpha$ , nuclear factor kappa-light-chain-enhancer of activated B cells [6, 7]).

One of the challenges in exploring the association between CVD and NAFLD or chronic liver disease in general in

human epidemiological studies is the lack of validated methods to diagnose the various stages of chronic liver disease using non-invasive tests which can be ethically applied to large groups of people who are mostly asymptomatic in terms of liver disease. Attempts to categorise people as ‘diseased’ or ‘not diseased’ based on findings of such non-invasive tests in an epidemiological setting are likely to lead to considerable bias. Therefore, we chose to explore the direct association of a wide range of different liver injury biomarkers with CVD rather than attempt to categorise chronic liver disease based on what would be arbitrary cut-points. We examined the association of prevalent and incident CVD with an array of biomarkers, including those measuring non-specific liver injury (plasma liver enzymes), steatosis (ultrasound), steatohepatitis (cytokeratin-18 [CK18] [8]), surrogate of advanced portal hypertension (platelet count), and liver fibrosis (aspartate to platelet ratio index [APRI] [9], aspartate aminotransferase [AST] to alanine aminotransferase [ALT] ratio, fibrosis-4 score [FIB4] [10], enhanced liver fibrosis panel [ELF] [11] and NAFLD fibrosis score [NFS] [12]).

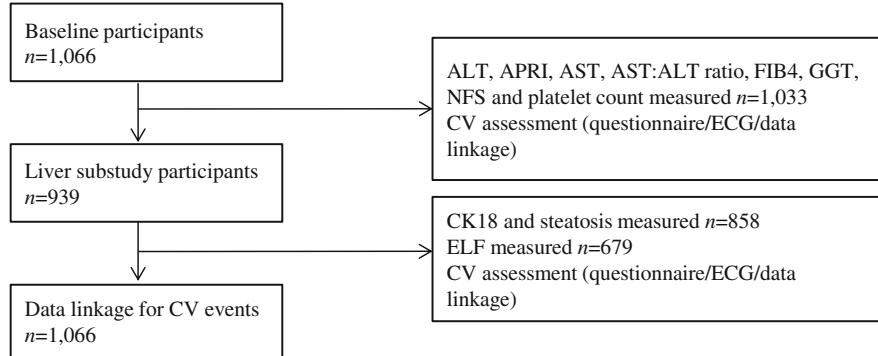
## Methods

### The Edinburgh type 2 Diabetes study

Full methods of the Edinburgh Type 2 Diabetes Study (ET2DS) have been published elsewhere [13]. Patients with type 2 diabetes aged 60–75 years at baseline were selected at random from the Lothian Diabetes Register, a comprehensive register of patients with diabetes living in Lothian, Scotland, UK. Baseline attendees ( $n=1,066$ ) have previously been shown to be representative of all those randomly selected to participate ( $n=5,454$ ), and therefore representative of the target population of older people with type 2 diabetes living in the general population [14]. The liver assessment clinic was attended by 939 participants at year 1 (Fig. 1).

### Clinical examination

Research clinics were held at the Wellcome Trust Clinical Research Facility, Western General Hospital, Edinburgh, UK, at baseline, year 1 and at follow-up and have been described previously [13, 15]. Briefly, attendees underwent fasting venous blood sampling for measurement of plasma liver enzymes (including ALT, AST and  $\gamma$ -glutamyltransferase [GGT]) and platelets; height and weight recording; blood pressure measurement; and a self-administered questionnaire including standard questions on current medications (including diabetes treatment, defined as diet-controlled, oral antihyperglycaemic agent only or insulin±oral antihyperglycaemic agent), alcohol consumption, smoking (categorised as ever or never), history of liver disease and CVD, as well as the Edinburgh Claudication and

**Fig. 1** Participant flowchart

WHO chest pain questionnaires. A 12-lead ECG was also recorded, using recognised standard operating procedures and a MAC 1200 resting ECG analysis system (GE Medical Systems, Milwaukee, Wisconsin, USA), and coded using The Minnesota Code manual [16]. Imaging included abdominal ultrasound scan. Average alcohol intake per week over the previous year and a history of alcohol excess were determined by questionnaire using questions adapted from the Alcohol Use Disorders Identification Test Consumption screening tool. Alcohol excess was defined as >14 units/week in women and >21 units/week in men [17] or self-reported history of an alcohol problem.

NAFLD was defined as the presence of hepatic steatosis on ultrasound scan, without alcohol excess or use of hepatotoxic medication, and a negative liver screen [18].

Alcohol excess was as defined above. Hepatotoxic medication use was defined as the use of non-topical glucocorticoids (isoniazid, methotrexate, amiodarone or tamoxifen) for >2 weeks within the 6 months prior to ultrasound scan. A positive liver screening included any of positive autoantibodies (any of anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody), ferritin >2,247 pmol/l,  $\alpha$ -fetoprotein >6  $\mu$ g/l, or positive hepatitis B or C serology. Clinically significant positive immunology titres were defined as anti-smooth muscle antibody titre >1:160 or anti-mitochondrial antibody titre >1:40 [19].

### Biomarkers of chronic liver injury

Biomarkers of liver injury were categorised and defined as: non-specific liver injury (liver enzyme levels: AST, ALT, GGT), steatosis (ultrasound scan), steatohepatitis (CK18), liver fibrosis (APRI, AST:ALT ratio, ELF, FIB4 and NFS) and advanced portal hypertension (platelet count).

Plasma liver enzymes, APRI, AST:ALT ratio, FIB4, NFS and platelet count were measured at baseline. CK18 and ELF were measured at year 1. All patients underwent a liver ultrasound scan at the 1 year visit. Sonographic grading of hepatic steatosis was performed using standard criteria, as described

previously, following validation against proton magnetic resonance spectroscopy [20].

ALT, AST and GGT were analysed using a Vitros Fusion chemistry system (Ortho Clinical Diagnostics, High Wycombe, UK) at the Western General Hospital, Edinburgh, UK. APRI [9], FIB4 [10] and NFS [12] were calculated as in the original publications. AST:ALT ratio was calculated as AST (U/l)/ALT (U/l). CK18 and ELF tests were undertaken on serum samples taken at the time of the liver ultrasound scan and subsequently stored at -80°C. CK18 was measured using the M30-Apoptosense ELISA (Peviva, Stockholm, Sweden) at the Biomedical Research Unit laboratory, University of Nottingham, UK. ELF scores were derived from the serum hyaluronic acid (HA), aminoterminal peptide of procollagen III (P3NP) and tissue inhibitor of metalloproteinases-1 (TIMP-1) equation as in the original publication [11] and measured using the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, New York, NY, USA) at the iQur laboratory, London, UK.

Given that biomarkers of fibrosis (e.g. ELF) could potentially be influenced by the presence of arthropathies [21] and renal disease, the presence of joint diseases (osteoarthritis, rheumatoid arthritis and others) was actively sought through self-administered questionnaire. Estimated glomerular filtration rate (eGFR) was measured at the time of clinic attendance and analysed using a Vitros Fusion chemistry system (Ortho Clinical Diagnostics) at the Western General Hospital, Edinburgh, UK.

### Identifying CVD

Information on cardiovascular events at baseline and at follow-up clinics was collected from multiple sources including patient- and/or general practitioner-completed questionnaires, 12-lead ECG, and linkage to hospital discharge and death certification data. Data linkage was undertaken, via the National Health Service National Services Scotland, to Scottish Morbidity Record (SMR01) general and acute inpatient discharge records using ICD-10 ([www.who.int/classifications/icd/en/](http://www.who.int/classifications/icd/en/)) (and related ICD-9 [[www.icd9data.com](http://www.icd9data.com)]).

[com/2007/Volume1](http://com/2007/Volume1)) codes and to Office for Population Censuses and Surveys (OPCS) version 4 codes for cardiovascular interventions. A fatal or non-fatal cardiovascular event was recorded if predetermined criteria based on the multiple data sources were met.

**Myocardial infarction** (1) ICD-10 code for myocardial infarction (MI) on discharge/death record, plus either self-report of a doctor diagnosis of MI, positive WHO chest pain questionnaire for MI, report of MI on general practitioner questionnaire or new ECG codes for MI; or (2) clinical criteria for MI met following scrutiny of clinical notes.

**Angina** (1) ICD-10 code for angina as primary diagnosis on discharge record; or (2) at least two of (a) self-report of a doctor diagnosis of angina or of starting angina medication, (b) ECG codes for ischaemia, and (c) positive WHO chest pain questionnaire; or (3) clinical diagnosis of angina on scrutiny of hospital notes.

**Stroke** (1) ICD-10 code for stroke as discharge/death record; or (2) clinical criteria for stroke met on scrutiny of clinical notes in individuals with either self-report of stroke or with non-primary ICD-10 hospital discharge/death code for stroke.

**Transient ischaemic attack** (1) ICD-10 code for transient ischaemic attack (TIA) on discharge record; or (2) clinical criteria for TIA met on scrutiny of clinical notes in individuals with either self-report of stroke or with non-primary ICD-10 hospital discharge code for stroke or TIA.

**Coronary intervention** OPCS-4 code for coronary intervention on discharge record.

**Intermittent claudication** (1) ICD-10 code for intermittent claudication on discharge record; or (2) clinical criteria for intermittent claudication met on scrutiny of clinical notes in individuals with either self-report of intermittent claudication or positive Edinburgh Claudication Questionnaire.

**Peripheral vascular intervention** OPCS-4 code for peripheral vascular intervention on discharge record.

**Carotid endarterectomy** OPCS-4 code for carotid endarterectomy on discharge record.

Prevalent CVD at baseline (for ALT, AST, GGT, AST:ALT ratio, APRI, FIB4, NFS and platelets) or year 1 (for steatosis, CK18, ELF) was defined as any of MI, angina, coronary intervention, intermittent claudication, peripheral vascular intervention, stroke, TIA or carotid endarterectomy at any time prior to this point. Prevalent coronary artery disease (CAD) at baseline/year 1 was defined as any of MI, angina or coronary intervention at any time.

Incident CVD was defined as any of MI, angina, coronary intervention, intermittent claudication, peripheral vascular intervention, stroke, TIA or carotid endarterectomy occurring between baseline/year 1 and end of August 2011, for both non-fatal and fatal events, in those patients without prevalent CVD at baseline. Incident CAD was defined as any of MI, angina or coronary intervention occurring between baseline/year 1 and end of August 2011, for non-fatal and fatal events, in those patients without prevalent CAD at baseline.

## Data analysis

The primary outcome measures were prevalent cardiovascular events and incident cardiovascular events. The secondary outcome measures were prevalent and incident CAD events. Fatal and non-fatal events were combined for analysis.

Data were assessed for normality and where necessary non-normal variables (APRI, CK18 and GGT) were transformed on the log<sub>2</sub> scale.

The follow-up time for each individual for incident disease was from the date of the baseline/liver substudy research clinic attendance until the first of: cardiovascular event, death or end of August 2011.

Analysis was undertaken using a listwise approach for three scenarios—measurements taken at baseline (ALT, APRI, AST, AST:ALT ratio, FIB4, GGT, NFS and platelets), measurements taken at the initial liver substudy clinic (CK18 and steatosis on ultrasound scan) and ELF.

Univariate analysis with normal continuous variables was carried out using Student's *t* test (ALT, AST, AST:ALT ratio, ELF FIB4, NFS and platelets), non-normal continuous variables (APRI, CK18 and GGT) using the Mann–Whitney *U* test, and categorical variables (steatosis) using the  $\chi^2$  test, examining for both the presence of prevalent and incident CVD and CAD.

Logistic regression for the association with prevalent CVD and CAD, and Cox proportional hazards regression for the association with incident CVD and CAD, were undertaken for all markers of liver injury. Both were performed unadjusted, adjusted for age and sex, and additionally adjusted for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation (Scottish Index of Multiple Deprivation quintile), smoking status, excess alcohol consumption, BMI, systolic blood pressure (sBP), diastolic blood pressure (dBp), HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR. Analysis of prevalent disease was undertaken for all participants; analysis of incident disease was undertaken for participants free of CVD at baseline.

Sensitivity analyses of the incident cardiovascular events were undertaken: (1) for participants with NAFLD (defined as the presence of hepatic steatosis on ultrasound scan without alcohol excess or use of hepatotoxic medication and a

negative liver screen); and (2) following inclusion of all participants and adjusted for prevalent CVD at baseline.

Data were analysed using SPSS version 19.0 (SPSS, Chicago, IL, USA).

Ethics approval was obtained from the Lothian Research Ethics Committee and all participants gave written informed consent.

## Results

### Patient characteristics

The baseline research clinic was attended by 1,066 patients, 939 (88%) of whom returned for the liver assessment at 1 year. Figure 1 shows the participant flow. There were no significant differences between attenders at baseline and attenders at the liver assessment (reported previously [22]); participant characteristics are described in Table 1.

Full data from baseline were available for 1,033 participants. From the 1 year liver assessment, steatosis and CK18 data were available for 858 participants. ELF data were available on a random subgroup of 679 participants; there were no significant differences between participants with and without available ELF scores (Table 1).

### Prevalent CVD

At baseline there were 370/1,033 (35.8%) patients with prevalent CVD and 317/1,033 (30.7%) with prevalent CAD. A significantly higher proportion of those with CVD and CAD were male (both 61.8%,  $p<0.001$ ) compared with those free of disease. Those with CVD and CAD were older (mean 68.4 vs 67.6 years,  $p=0.004$ , and 68.6 vs 67.6 years,  $p<0.001$ , respectively) than those without. Results were similar for the 1 year assessment: at baseline there were 303/858 (35.3%) patients with prevalent CVD and 260/858 (30.3%) with prevalent CAD. Again, those with CVD and CAD were significantly more likely to be male and to be older than those without.

There were no significant differences in the distribution of joint disease potentially influencing fibrosis biomarkers between those with and those without CVD (osteoarthritis 22.3% vs 23.8%,  $p=0.785$ ; rheumatoid arthritis 5.3% vs 3.2%,  $p=0.173$ ; other joint disease 15.6% vs 12.5%,  $p=0.440$ , respectively). Mean eGFR was lower in those with prevalent CVD than in those without (62.1 vs 65.7  $\text{mL}^{-1} \text{min}^{-1} 1.73 \text{ m}^{-2}$ ,  $p<0.001$ ).

Participants with prevalent CVD had marginally lower ALT (mean 41.9 vs 43.7 U/l,  $p=0.048$ ) and higher GGT measures (median 20.0 vs 17.0 U/l,  $p<0.001$ ) compared with

**Table 1** Characteristics of all ET2DS participants, those undergoing CK18 and steatosis assessment and subgroups with ELF measurements

Characteristic	All participants (n=1,033)	CK18 and steatosis participants (n=858)	ELF participants (n=679)
Age, years	67.9 (4.2)	67.9 (4.2)	67.8 (4.2)
Sex, % male	51.2 (530)	53.8 (462)	52.6 (357)
Duration of diabetes, years	6.0 (3.0–11.0)	6.0 (3.0–11.0)	6.0 (3.0–10.0)
HbA <sub>1c</sub> , %	7.39 (1.1)	7.38 (1.1)	7.36 (1.1)
HbA <sub>1c</sub> , mmol/mol	57.0 (12.1)	57.2 (12.3)	57.0 (11.9)
Fasting glucose, mmol/l	7.54 (2.1)	7.48 (2.0)	7.49 (2.0)
Diet-controlled, % yes	19.8 (197)	19.3 (161)	19.2 (127)
Oral antihyperglycaemic agent use, % yes	63.0 (628)	64.8 (541)	65.4 (432)
Insulin therapy, % yes	17.3 (172)	15.9 (133)	15.4 (102)
BMI, kg/m <sup>2</sup>	31.3 (5.6)	31.2 (5.7)	31.2 (5.7)
Waist circumference, cm	106.7 (12.7)	106.6 (12.8)	106.5 (12.7)
Serum cholesterol, mmol/l	4.30 (0.9)	4.31 (0.9)	4.33 (0.9)
sBP, mmHg	133.2 (16.4)	133.3 (16.1)	133.5 (16.3)
dBP, mmHg	69.1 (9.0)	69.3 (8.9)	69.4 (8.9)
Alcohol excess <sup>a</sup> , % yes	8.1 (84)	7.6 (65)	8.4 (57)
Ever smoked, % yes	60.7 (527)	59.6 (455)	60.0 (366)

Values are mean (SD), median (interquartile range) or proportion ( $n$ )

All variables were measured concurrently at year 1 examination of the ET2DS, except for BMI and waist circumference, which were measured at baseline

<sup>a</sup> Defined as women >14 units/week, men >21 units/week or patient disclosed history of a current or prior alcohol problem

those without. Patients with prevalent CAD also had significantly higher GGT values than those without (median 20.0 vs 17.0 U/l,  $p<0.001$ ), although all median levels were within the normal range. The proportion of participants with steatosis was lower in those with CVD than in those without (CVD 54.1% vs 57.5%,  $p=0.350$ ; CAD 51.2% vs 58.5%,  $p=0.051$ ). Full data are given in Table 1 of the electronic supplementary material (ESM).

Multivariable analysis of the relationship between liver markers and prevalent cardiovascular events, adjusting for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation, smoking status, excess alcohol consumption, BMI, systolic blood pressure, HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR, is shown in Table 2. GGT was the only liver marker independently associated with prevalent CVD (OR for a doubling of GGT 1.18; 95% CI 1.03, 1.36;  $p=0.021$ ) or CAD (OR 1.21; 95% CI 1.05, 1.40;  $p=0.008$ ).

## Incident CVD

There were 663 participants without CVD. After a mean follow-up of 4.4 years from baseline attendance there were 44/663 (6.6%) patients with incident CVD and 27/663 (4.1%) with incident CAD events. A significantly higher proportion of those with incident CVD were male (59.1% vs 44.3%,  $p=0.061$ ) and they were significantly older (68.9 vs 67.5 years,  $p=0.024$ ), with no differences in those with incident CAD compared with those without incident CAD. Similar results were obtained for those patients followed up from the 1 year assessment (mean follow-up 3.5 years), with 35/561 (6.2%) incident CVD and 19/561 (3.4%) incident CAD events and with a similar age/sex distribution.

There were 82/1,033 (7.9%) deaths in the follow-up period from baseline, with 30/82 (36.6%) attributable to CVD, of which 20 were attributable to CAD.

**Table 2** Multivariable association between liver markers and prevalent cardiovascular events

Liver marker	Model 1	<i>p</i> value	Model 2	<i>p</i> value	Model 3	<i>p</i> value
<b>All CVD</b>						
ALT, U/l	0.99 (0.98, 1.00)	0.079	0.99 (0.98, 1.00)	0.028	0.99 (0.98, 1.00)	0.088
AST, U/l	0.99 (0.98, 1.01)	0.341	0.99 (0.98, 1.01)	0.174	0.99 (0.98, 1.01)	0.385
GGT, log <sub>2</sub> <sup>a</sup>	1.21 (1.07, 1.37)	0.002	1.20 (1.06, 1.35)	0.005	1.18 (1.03, 1.36)	0.021
Steatosis, % yes	0.91 (0.68, 1.22)	0.518	0.96 (0.71, 1.30)	0.774	0.84 (0.60, 1.17)	0.296
CK18, log <sub>2</sub> <sup>a</sup>	1.08 (0.90, 1.30)	0.421	1.09 (0.90, 1.31)	0.405	0.99 (0.81, 1.22)	0.926
APRI, log <sub>2</sub> <sup>a</sup>	0.98 (0.78, 1.23)	0.833	0.85 (0.67, 1.08)	0.189	0.90 (0.70, 1.67)	0.439
AST:ALT ratio	1.34 (0.56, 3.21)	0.509	1.39 (0.56, 3.44)	0.473	1.51 (0.56, 4.07)	0.419
ELF score	1.00 (0.83, 1.21)	0.984	1.01 (0.82, 1.23)	0.964	0.94 (0.74, 1.19)	0.604
FIB4	1.13 (0.90, 1.41)	0.289	1.01 (0.80, 1.28)	0.921	1.03 (0.80, 1.33)	0.801
NFS	1.09 (0.96, 1.24)	0.174	1.07 (0.93, 1.22)	0.350	1.01 (0.85, 1.19)	0.915
Platelets, $\times 10^9/l$	1.00 (1.00, 1.00)	0.569	1.00 (1.00, 1.00)	0.499	1.00 (1.00, 1.00)	0.734
<b>CAD</b>						
ALT, U/l	0.99 (0.98, 1.00)	0.200	0.99 (0.98, 1.00)	0.124	0.99 (0.98, 1.01)	0.390
AST, U/l	0.99 (0.98, 1.01)	0.383	0.99 (0.98, 1.01)	0.230	1.00 (0.98, 1.01)	0.588
GGT, log <sub>2</sub> <sup>a</sup>	1.22 (1.08, 1.39)	0.002	1.22 (1.07, 1.38)	0.002	1.21 (1.05, 1.40)	0.008
Steatosis, % yes	0.75 (0.55, 1.02)	0.064	0.79 (0.58, 1.08)	0.140	0.66 (0.46, 0.94)	0.019
CK18, log <sub>2</sub> <sup>a</sup>	1.05 (0.86, 1.28)	0.650	1.05 (0.86, 1.28)	0.610	0.96 (0.78, 1.18)	0.707
APRI, log <sub>2</sub> <sup>a</sup>	1.00 (0.79, 1.27)	0.987	0.88 (0.67, 1.13)	0.320	0.95 (0.73, 1.24)	0.720
AST:ALT ratio	1.01 (0.40, 2.53)	0.981	0.93 (0.36, 2.42)	0.887	0.94 (0.33, 2.66)	0.912
ELF score	0.98 (0.80, 1.20)	0.848	0.96 (0.77, 1.19)	0.726	0.88 (0.69, 1.13)	0.324
FIB4	1.20 (0.95, 1.50)	0.123	1.07 (0.84, 1.36)	0.599	1.11 (0.85, 1.43)	0.441
NFS	1.11 (0.97, 1.27)	0.138	1.07 (0.93, 1.23)	0.328	1.03 (0.86, 1.22)	0.765
Platelets, $\times 10^9/l$	1.00 (1.00, 1.00)	0.386	1.00 (1.00, 1.00)	0.788	1.00 (1.00, 1.00)	0.967

Values are ORs (95% CI)

<sup>a</sup>APRI, CK18 and GGT analysed on the log<sub>2</sub> scale for linearisation; therefore, ORs relate to a doubling of the marker

Model 1, unadjusted; model 2, adjusted for age and sex; model 3, adjusted for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation (Scottish Index of Multiple Deprivation quintile), smoking status, excess alcohol consumption, BMI, sBP, dBp, HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR at baseline

Mean (or median) liver injury marker levels were largely similar between participants with and without incident CVD (ESM Table 2) and after multivariable adjustment (Table 3). Only GGT appeared to have some independent association with either incident CVD (HR for a doubling of GGT 1.24; 95% CI 0.97, 1.59;  $p=0.086$ ) or incident CAD (HR 1.33; 95% CI 1.00, 1.78;  $p=0.053$ ). None of the individual covariates added to the multivariable model had a major attenuating effect on the HR estimating the GGT–outcome association (ESM Table 3). In further analyses performed on all participants with either a first or subsequent cardiovascular event occurring after baseline (i.e. including those with prevalent CVD at baseline, but with adjustment for prevalent cases), an association between GGT and events was confirmed (ESM Tables 4 and 5). HRs with similar magnitudes were observed with increased statistical significance ( $p<0.05$ ), likely due to the increase in sample size.

When restricted to patients with NAFLD ( $n=319$ ) there were 38 incident cardiovascular events, with 23 attributable

to CAD. Of all the liver injury markers investigated, GGT alone showed an independent association with incident CVD in this subgroup (fully adjusted HR for a doubling of GGT 1.56; 95% CI 1.08, 2.28;  $p=0.019$ ) (ESM Tables 6 and 7).

## Discussion

In this large-scale epidemiological study, we have shown that raised GGT is independently associated with an increase in both prevalent and incident cardiovascular events in older people with type 2 diabetes. Previous studies, predominantly in younger samples of the general population, have found similar results for this plasma liver enzyme; we have now shown that findings are consistent in a high-risk (diabetic) and older subgroup of the population. Despite the availability of a wide range of other liver injury markers, we found no evidence that markers of hepatic steatosis, steatohepatitis, portal hypertension or fibrosis were associated with higher levels

**Table 3** Multivariable association between liver markers and any incident CVD events

Liver marker	Model 1	<i>p</i> value	Model 2	<i>p</i> value	Model 3	<i>p</i> value
<b>All CVD</b>						
ALT, U/l	1.00 (0.97, 1.02)	0.754	1.00 (0.97, 1.02)	0.836	0.99 (0.97, 1.02)	0.669
AST, U/l	1.01 (0.98, 1.04)	0.526	1.01 (0.98, 1.04)	0.544	1.01 (0.97, 1.04)	0.700
GGT, log <sub>2</sub> <sup>a</sup>	1.25 (0.99, 1.59)	0.062	1.26 (0.99, 1.60)	0.059	1.24 (0.97, 1.59)	0.086
Steatosis, % yes	0.78 (0.36, 1.67)	0.525	0.84 (0.39, 1.80)	0.654	0.90 (0.40, 2.00)	0.787
CK18, log <sub>2</sub> <sup>a</sup>	1.05 (0.64, 1.70)	0.857	1.13 (0.68, 1.85)	0.643	1.02 (0.60, 1.75)	0.931
APRI, log <sub>2</sub> <sup>a</sup>	0.88 (0.505, 1.525)	0.644	0.79 (0.43, 1.46)	0.448	0.76 (0.40, 1.45)	0.408
AST:ALT ratio	3.63 (0.61, 21.61)	0.156	2.85 (0.475, 17.06)	0.252	3.58 (0.53, 28.12)	0.183
ELF score	1.220 (0.91, 1.64)	0.185	1.19 (0.85, 1.66)	0.312	1.15 (0.81, 1.64)	0.443
FIB4	1.01 (0.54, 1.91)	0.966	0.82 (0.40, 1.68)	0.586	0.83 (0.39, 1.76)	0.625
NFS	0.81 (0.58, 1.14)	0.226	0.76 (0.54, 1.06)	0.109	0.78 (0.57, 1.09)	0.143
Platelets, $\times 10^9/l$	1.00 (1.00, 1.01)	0.162	1.01 (1.00, 1.01)	0.061	1.00 (1.00, 1.01)	0.110
<b>CAD</b>						
ALT, U/l	1.00 (0.98, 1.03)	0.771	1.01 (0.98, 1.04)	0.497	1.01 (0.98, 1.04)	0.611
AST, U/l	1.02 (0.99, 1.05)	0.213	1.03 (0.99, 1.06)	0.135	1.02 (0.99, 1.06)	0.220
GGT, log <sub>2</sub> <sup>a</sup>	1.27 (0.95, 1.69)	0.103	1.31 (0.88, 1.75)	0.060	1.33 (1.00, 1.78)	0.053
Steatosis, % yes	0.82 (0.32, 2.14)	0.688	0.87 (0.33, 2.27)	0.774	0.91 (0.33, 2.53)	0.858
CK18, log <sub>2</sub> <sup>a</sup>	1.07 (0.58, 1.99)	0.822	1.10 (0.60, 2.01)	0.748	0.96 (0.49, 1.90)	0.908
APRI, log <sub>2</sub> <sup>a</sup>	1.07 (0.56, 2.06)	0.839	1.15 (0.56, 2.34)	0.709	1.10 (0.52, 2.32)	0.804
AST:ALT ratio	4.36 (0.51, 37.18)	0.178	3.40 (0.37, 31.13)	0.278	4.25 (0.39, 46.73)	0.237
ELF score	1.24 (0.85, 1.80)	0.269	1.15 (0.76, 1.74)	0.508	1.12 (0.69, 1.82)	0.642
FIB4	1.28 (0.64, 2.60)	0.486	1.22 (0.57, 2.64)	0.611	1.25 (0.56, 2.79)	0.583
NFS	0.84 (0.55, 1.28)	0.416	0.81 (0.53, 1.23)	0.323	0.76 (0.51, 1.17)	0.225
Platelets, $\times 10^9/l$	1.00 (1.00, 1.01)	0.301	1.00 (1.00, 1.01)	0.286	1.00 (1.00, 1.01)	0.297

Values are HRs (95% CI)

<sup>a</sup>APRI, CK18 and GGT analysed on the log<sub>2</sub> scale for linearisation; therefore, ORs relate to a doubling of the marker

Model 1, unadjusted; model 2, adjusted for age and sex; model 3, adjusted for age, sex, duration of diabetes, treatment of diabetes, lipid-lowering drugs, blood pressure-lowering drugs, deprivation (Scottish Index of Multiple Deprivation quintile), smoking status, excess alcohol consumption, BMI, sBP, dBp, HbA<sub>1c</sub>, HDL-cholesterol, total cholesterol and eGFR at baseline

of prevalent or incident CVD, suggesting that liver disease may have little effect on the development of vascular complications in our study population.

A major strength of this study is its representation of the full spectrum of people with type 2 diabetes, not just those attending secondary care or receiving advanced treatment modalities. This population is of particular interest as it may show an accelerated progression of liver disease due to the combined effects of age and metabolic risk factors. Community-based populations of people with type 2 diabetes represent the vast majority of all people with type 2 diabetes and, as such, require special attention given the impact of their longer term care on health service provision.

Our findings are consistent with previous findings of a significant association between GGT and both prevalent and incident CVD in the general population [3, 23–30], contributing to the paucity of literature in diabetic populations. In addition, contrary to previous findings, we found that this association persists into older age [26], independently of a wide range of cardiovascular risk factors. There is a biological plausibility for this relationship: GGT degrades glutathione to glutamate, which via cysteinylglycine is involved in iron reduction, allowing lipoprotein oxidation within atherosomatous plaques [31]. What is unclear is whether GGT is a pathogenic factor in atherogenesis or simply a surrogate biomarker of the microinflammatory, plaque-associated inflammatory response. Given that no liver injury markers other than GGT were independently associated with CVD, this strengthens the argument for the GGT association being driven by systemic inflammation as opposed to a direct consequence of chronic liver disease. Whatever the underlying mechanism, our findings indicate that further investigation is warranted into whether or not GGT could add predictive ability to existing vascular risk prediction models in type 2 diabetes [32].

In terms of the association between CVD and other liver injury markers, previous studies are limited and inconclusive. Significant associations between transaminases and both increased and decreased CVD in the general population have been reported [33, 34]. Investigations into the relationship between NAFLD (defined as the presence of hepatic steatosis on ultrasound scan) and cardiovascular events [35, 36], in populations comprised exclusively of patients with type 2 diabetes [3, 4, 37, 38], have reported significant associations between NAFLD and incident CVD (OR 1.53 [3], HR 1.96 [38], after controlling for cardiovascular risk factors), but no association with liver enzymes (including GGT). Although the present study failed to find a similar relationship between sonographic hepatic steatosis and CVD, our cohort differs from diabetic cohorts studied previously, mainly in its broad spectrum of patients with type 2 diabetes. Targher et al used a study population derived exclusively from secondary care diabetes settings (therein limiting generalisability), where the influence of hepatic steatosis may be stronger in the context

of more severe diabetes, consistent with other studies looking at more general populations and cardiovascular mortality [37]. Whilst our findings may also be affected by specific cohort effects, the size and follow-up time are comparable to those of several other similar studies [3, 26].

Our finding of a lower prevalence of CVD in people with steatosis could be explained, at least in part, by regression of hepatic steatosis with advancing liver disease [39]; or it may reflect survival bias, in that those with the most severe NAFLD had already died prior to participation in the ET2DS.

In patients with NAFLD, relative concentrations of serum CK18 can discriminate between steatosis and NASH [8]. However, there are no previous studies examining the relationship between CK18 levels and cardiovascular events in either general or diabetic populations. Several previous studies diagnosing NASH using different methods (such as biopsy or elevated ALT levels) showed mixed results for the association with cardiovascular risk (e.g. risk scores, lipid levels). Both Soderberg et al [40] and Ekstedt et al [2] found associations of all-cause and cardiovascular mortality with the presence of biopsy-proven NASH, but no association with steatosis. Conversely, Lazo et al [41] found no association between NASH and cardiovascular mortality in patients diagnosed by ultrasound scan and elevated hepatic enzymes, suggesting that the criteria for NAFLD and NASH classification may have a significant impact on findings.

Data on the relationship between hepatic fibrosis and CVD are also limited. Kim et al found significant associations between the NFS, APRI and FIB4 with cardiovascular mortality in a general population [42]. Our study used all these, as well as the ELF score, an extracellular matrix-related multi-component panel (HA, P3NP and TIMP-1), validated for use in patients with NAFLD [20], and found no relationship.

It should be noted that the utility of different liver injury biomarkers may be determined by the context in which they are used. For example, there is a body of evidence validating non-invasive liver biomarkers for the cross-sectional stratification of liver disease in secondary care and predicting future liver-related clinical outcomes [43, 44]. Results from this study do not suggest that most of the markers investigated would add prognostic value to existing risk scores used to predict cardiovascular endpoints in diabetes [32]. The exception to this is GGT, which is generally not considered useful for stratifying active liver disease, but which may prove beneficial in predicting CVD. Given the results presented here, further investigation into this question in diabetes is warranted.

The strengths and limitations of this study should be acknowledged. The large size, population-based approach, prospective design with intensive investigation for incident cardiovascular events, and wide range of liver biomarkers investigated are key strengths of the current study. The modest follow-up duration is partially offset by the large sample size, resulting in a significant number of person-years at risk, and

by the high-risk population under study, which resulted in a high number of incident events. Without a liver biopsy it is not currently possible to accurately identify NAFLD. However, we believe that our comprehensive approach of using ultrasound scan, assessment of alcohol consumption and hepatotoxic medication use, and liver screen will identify the vast majority of patients with NAFLD, potentially missing only those with minimal hepatic steatosis due to regression of steatosis in the advanced stages of the disease process.

In conclusion, our study provides evidence that GGT may independently associate with CVD and that its potential prognostic value for CVD in people with type 2 diabetes would be usefully investigated. However, lack of association between CVD and other markers of liver injury (non-specific injury, steatosis, steatohepatitis, significant portal hypertension, fibrosis) suggests that chronic liver disease per se may not have a major influence on the development of CVD, at least in older diabetic populations.

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**Access to research materials** Applications to access the underlying research materials will be considered via the ET2DS standard data sharing procedures. Please contact the corresponding author for details.

**Duality of interest** MWJS has received fees for speaking from Novo Nordisk, Eli Lilly and Pfizer. JRM, JAF, RMW, CMR, SG, ING and JFP report no disclosures.

**Contribution statement** JRM, JAF, RMW, SG, ING, MWJS and JFP are responsible for the conception and design of the study. The data were acquired by JRM, RMW, CMR and SG, analysed by JRM, and interpreted by JRM, JAF, ING, MWJS and JFP. The article was drafted by JRM, JAF, ING and JFP, and critically revised by all the authors. All authors approved the final version. JRM is the guarantor of this work.

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# Relationship Between Serum Gamma-Glutamyltransferase Levels and Prehypertension in Chinese Adults: The Cardiometabolic Risk in Chinese Study

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The authors aimed to investigate the relationship between serum gamma-glutamyltransferase (GGT) and prehypertension, as well as the modification of other metabolic risk factors in a large cohort of Chinese individuals. The data were collected via a community-based health examination survey in central China. Blood pressure, body mass index (BMI), and levels of GGT, fasting blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lipid indicators were measured. In total, data from 18,302 patients with available biomarkers were included in the present study. Elevated blood pressure was associated with increased GGT concentration ( $P<.001$ ). After adjusting for age, sex, BMI, fasting blood glucose, lipid indicators, AST, and family history of hypertension, the association between GGT levels and prehypertension remained significant ( $P=.021$ ). The adjusted

odds ratios (95% confidence interval) for prehypertension across quintiles of GGT level were 1.00, 1.057 (1.012–1.334), 1.068 (0.916–1.254), 1.024 (0.851–1.368), and 1.272 (1.027–1.593), respectively. In stratified analyses, the association between GGT levels and prehypertension was significant in women but was not significant in men. Moreover, additive effect of BMI and age on the effect of GGT levels on prehypertension (both  $P$  for interaction  $<.001$ ) was observed. In summary, GGT levels were positively associated with prehypertension in women, independent of other metabolic factors. Furthermore, BMI and age may amplify the effects of GGT levels on prehypertension. These findings suggest that monitoring the levels of GGT could help in the diagnosis and monitoring of prehypertension. *J Clin Hypertens (Greenwich)*. 2014;16:760–765. © 2014 Wiley Periodicals, Inc.

Several observational studies have reported on the association of elevated gamma-glutamyltransferase (GGT) levels with diabetes,<sup>1</sup> metabolic syndrome,<sup>2</sup> and cardiovascular disease.<sup>3</sup> Similarly, several cross-sectional and longitudinal studies have also noted a relatively independent association between elevated serum GGT levels and hypertension.<sup>4–6</sup> However, data on the association between GGT levels and prehypertension are limited, particularly in Chinese patients. In the present study, we aimed to examine the association between GGT levels and prehypertension in a large cohort of Chinese individuals with a normal range of blood pressure (BP) and to assess the interactions between GGT levels and other cardiovascular metabolic risk factors.

## METHODS

### Study Population

In 2012–2013, we conducted a community-based health examination survey for individuals who were randomly

selected from 22,726 residents living in the urban area of central China. Written informed consent was obtained from all participants. The study was reviewed and approved by the ethics committee of the Central Hospital of Xuzhou, Jiangsu, China. Patients with viral hepatitis, autoimmune liver disease, liver cirrhosis, malignant tumors of the liver, biliary tract disease, and history of heavy drinking (alcohol intake: men,  $>20$  g/d; women,  $>10$  g/d) were excluded. In addition, patients with systemic disease leading to fatty liver, diabetes, fasting glucose level  $\geq 7.0$  mmol/L, 2-hour oral glucose tolerance test value  $\geq 11.1$  mmol/L, those who could potentially develop hypertension (systolic BP [SBP]  $\geq 140$  mm Hg and/or diastolic BP [DBP]  $\geq 90$  mm Hg), and those who were taking or had recently taken medication that would result in elevating serum alanine transaminase (ALT) and GGT levels were excluded. Thus, in total, 18,302 patients with appropriate and sufficient data were finally enrolled in this study.

### Assessment of BP and Prehypertension

Using a standard mercury sphygmomanometer, physicians recorded BP values 3 times consecutively on the right arm, which was placed at the level of the heart, with the patient sitting still for more than 5 minutes. The 3 measurements were recorded at 60-second intervals. The average of the 3 values of SBP and DBP was used in our analyses. Prehypertension was defined

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as SBP between 120 mm Hg and 139 mm Hg or DBP between 80 mm Hg and 89 mm Hg according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).<sup>7</sup>

### Assessment of Biomarkers and Covariates

Height and body weight were measured with the participants in a standing position without shoes or heavy outer garments. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Biomarkers were measured in all participants. A venous blood sample was drawn from all patients after an overnight fast (at least 10 hours). After blood was drawn, samples were transferred into glass tubes and allowed to clot at room temperature for 1 to 3 hours. Immediately after clotting, serum was separated by centrifugation for 15 minutes at 3000 rpm. Fasting blood samples were collected for measuring the levels of GGT, fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and aspartate aminotransferase (AST), and ALT. All biochemical assays were performed enzymatically on an autoanalyzer (Type 7600, Hitachi Ltd, Tokyo, Japan).

### Statistical Analyses

In all analyses, parameters with non-normal distributions were used after log-transformation. The measured data are expressed as mean±standard deviation. The relationship between GGT levels and metabolic markers was examined using one-way analysis of variance. We used unconditional logistic regression for estimating the odds ratios (ORs) for prehypertension risk, after adjusting for covariates including age, sex, BMI, and biomarkers. The interactions between GGT levels

and other factors were also assessed using logistic regression. The joint effects between GGT levels and BMI on the risk of prehypertension were examined using the linear regression models. All reported *P* values were two-tailed. A *P* value of <.05 was considered statistically significant. Data management and statistical analysis were conducted using SAS statistical software (version 9.1.3; SAS Institute, Inc., Cary, North Carolina).

## RESULTS

### Correlation Between GGT Concentration and the Clinical Characteristics of the Study Population

The study participants had an average age of 41.7±2.24 (range, 18–91) years and a mean BMI of 23.7 kg/m<sup>2</sup>. The mean SBP and DBP values were 118 mm Hg (range, 80–139 mm Hg) and 75.9 mm Hg (range, 49–89 mm Hg), respectively. A total of 12,040 (65.8%) individuals had prehypertension. Table I presents the baseline characteristics of the study participants according to quintiles of the GGT levels. Weight, height, BMI, and levels of fasting blood glucose, log triglyceride, total cholesterol, LDL-C, log AST, and log ALT showed statistically significant differences between the GGT quintile groups. As the GGT level increased, an increasing trend in all these values was noted (*P*<.001), except for HDL-C, which showed a decreasing trend (*P*<.001).

### Correlations Between GGT Concentration and BP

Table II shows that the risk of prehypertension increased along with the elevated levels of GGT, which remained significant even in a multivariate-adjusted model. In an age-adjusted and sex-adjusted model, the ORs (95% confidence interval [CI]) of prehypertension across increasing quintiles of GGT were 1.00, 1.338

**TABLE I.** Baseline Characteristics of Participants According to Quintile Groups of Serum GGT Level (N=18,302)

Variables	GGT Level (Quintiles), U/L					<i>F</i> Value	<i>P</i> Value for Trend
	Q1 (GGT≤13.0)	Q2 (13.0<GGT≤17.0)	Q3 (17.0<GGT≤23.0)	Q4 (23.0<GGT≤36.0)	Q5 (GGT>36.0)		
No.	5162	3712	3610	3106	2712		
Age, y	40.49±10.59	41.74±12.15	42.22±12.48	41.25±12.59	40.04±12.05	1.36	.064
Weight, kg	58.46±8.32	63.78±9.85	68.67±10.56	72.56±10.78	75.68±10.91	10.25	<.001
BMI, kg/m <sup>2</sup>	22.18±2.67	22.90±2.95	23.73±3.06	24.53±3.04	25.54±3.04	8.67	<.001
Height, cm	161.83±6.25	163.68±7.27	167.48±8.00	170.47±7.37	172.33±6.39	12.65	<.001
Fasting glucose, mmol/L	5.16±1.11	5.24±1.15	5.31±1.16	5.32±1.02	5.29±0.80	9.65	<.001
HDL-C, mmol/L	1.47±0.32	1.42±0.31	1.31±0.31	1.24±0.30	1.18±0.28	14.52	<.001
LDL-C, mmol/L	2.71±0.74	2.91±0.80	3.01±0.80	3.10±0.80	3.18±0.82	25.65	<.001
Log triglyceride	-0.08±0.21	-0.01±0.24	0.07±0.25	0.13±0.26	0.23±0.26	7.64	<.001
Total cholesterol, mmol/L	4.45±0.83	4.60±0.88	4.67±0.92	4.71±0.89	4.84±0.93	15.25	<.001
Log AST	1.23±0.12	1.26±0.13	1.28±0.14	1.30±0.13	1.33±0.14	16.42	<.001
Log ALT	1.17±0.21	1.23±0.22	1.29±0.23	1.35±0.22	1.43±0.24	9.87	<.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

**TABLE II.** Association Between Serum GGT Levels and the Risk of Prehypertension by Logistic Regression Analysis

	Normotensive Controls, No. (%)	Prehypertension Cases, No. (%)	Model 1		Model 2		Model 3	
			OR	95% CI	OR	95% CI	OR	95% CI
Q1	2725 (52.8)	2437 (47.2)	1	1	1	1	1	1
Q2	1401 (37.7)	2311 (62.3)	1.338	1.221–1.467	1.183	1.075–1.301	1.057	1.012–1.334
Q3	1027 (28.4)	2583 (71.6)	1.594	1.438–1.766	1.223	1.099–1.361	1.068	0.916–1.254
Q4	685 (22.1)	2421 (77.9)	2.025	1.805–2.272	1.362	1.206–1.538	1.024	0.851–1.368
Q5	424 (15.6)	2288 (84.4)	2.974	2.607–3.393	1.736	1.510–1.996	1.272	1.027–1.593
P for trend			<.001		<.001		.021	

Abbreviations: CI, confidence interval; GGT, gamma-glutamyltransferase; OR, odds ratio. Model 1: Adjusted for age and sex. Model 2: Adjusted for sex, age, fasting glucose levels, and body mass index. Model 3: Adjusted for age, sex, body mass index, and levels of fasting glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, aspartate aminotransferase, and family history of hypertension.

(1.221–1.467), 1.594 (1.438–1.766), 2.025 (1.805–2.272), and 2.974 (2.607–3.393) (*P* for trend <.001). In model 3, after further adjusting for BMI and the levels of fasting glucose, total cholesterol, triglycerides, HDL-C, LDL-C, AST, and family history of hypertension, the ORs (95% CI) were 1.00, 1.057 (1.012–1.334), 1.068 (0.916–1.254), 1.024 (0.851–1.368), and 1.272 (1.027–1.593) (*P* for trend=.021).

### Interactions Between GGT Level and Prehypertension According to Age and Sex

After stratified analysis, the associations between GGT level and prehypertension remained significant in women, but were not significant in men. Furthermore, we examined the associations between GGT level and prehypertension in different age groups: younger than 35 years, 35 to 46 years, and older than 46 years (Table III). We noted that the associations were significant in individuals older than 46 years (*P*=.019), but not in those younger than 35 years and those between 35 and 46 years. The interaction between GGT levels and age in relation to prehypertension risk was significant (*P* for interaction <.001).

### BMI Modifies the Associations Between GGT Concentration and Prehypertension

We assessed the interactions between GGT levels and other metabolic factors (BMI and levels of fasting blood glucose, LDL-C, and total cholesterol) and performed stratified analyses (Table III). While considering the power of the present study for the stratified analyses, we grouped the strata factors, fasting blood glucose levels, and lipid indicator values into 3 categories (tertiles): low, median, and high levels. We found significant interactions between GGT levels and BMI in relation to prehypertension risk (*P* for interaction <.001). Moreover, the association between GGT levels and prehypertension was significant in the groups with high (*P*<.001) and median BMI levels (*P*=.016); however, this association was not statistically significant in those with low BMI levels (*P*=.290). The tests for the interaction of fasting blood glucose, LDL-C, and total cholesterol

levels did not yield significant results (*P* for interaction=.202, .501, and .895, respectively).

### Joint Effects of GGT Levels and BMI on Prehypertension

We also examined the joint effects of GGT levels and BMI on prehypertension (Figure). These 2 markers showed an additive pattern in relation to the risk of prehypertension. Compared with patients in the group with the lowest levels for both markers, individuals in the group with highest levels of the 2 markers had a 2.29-fold higher prevalence risk ratio of prehypertension.

## DISCUSSION

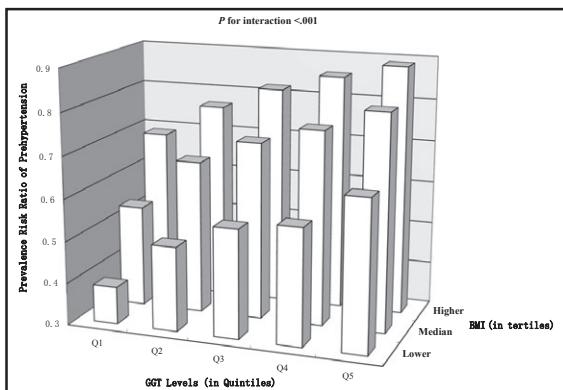
In clinical and epidemiological studies, GGT has been found to be positively associated with metabolic syndrome.<sup>8,9</sup> Shankar and colleagues<sup>10</sup> reported a clear positive association between GGT levels and prehypertension among adult men and women in the United States. Previous studies indicated this association in Korean and Japanese men but not in women.<sup>11,12</sup> However, no large-scale studies have assessed whether GGT is independently associated with prehypertension in the Chinese population. Our data showed a significant association between GGT level and the risk of prehypertension in women in a large Chinese population. In the stratified analyses, there were significant interactions between GGT level and risk of prehypertension according to age and BMI.

Previous studies have indicated that elevated serum GGT levels are associated with an increased risk of metabolic syndrome and type 2 diabetes.<sup>13–16</sup> Insulin resistance may play an important role in the pathophysiological mechanism of these findings. Compensatory hyperinsulinemia can activate the mitogen-activated protein kinase pathway, resulting in enhancement of vasoconstriction, proinflammation, increased sodium and water retention, and the elevation of BP.<sup>16</sup> In addition, insulin increases sodium reabsorption in the kidney and promotes sympathetic nerve activity, which can cause hypertension.<sup>17</sup> Moreover, the evidence of a

**TABLE III.** Adjusted ORs of Prehypertension According to GGT Level Quintiles by Stratification for Sex, Age, BMI, and Levels of Fasting Glucose and Lipids

Variables	GGT Level (Quintiles), U/L					<i>P</i> Value for Interaction	<i>R</i> <sup>2</sup>
	Q1 (GGT≤13.0)	Q2 (13.0<GGT≤17.0)	Q3 (17.0<GGT≤23.0)	Q4 (23.0<GGT≤36.0)	Q5 (GGT>36.0)		
Sex							
Women; n=8234	1	1.078 (0.927-1.25)	1.120 (0.913-1.37)	1.254 (0.950-1.65)	1.663 (1.130-2.44)	.01	0.418
Men; n=10,068	1	1.316 (1.018-1.70)	1.128 (0.880-1.44)	1.125 (0.863-1.46)	1.303 (0.961-1.76)	.508	0.096
Age, y							
Low (<35); n=6555	1	1.171 (0.935-1.46)	1.067 (0.812-1.40)	1.056 (0.758-1.47)	1.360 (0.882-2.09)	.379	<0.001
Median (35-46); n=6054	1	1.070 (0.872-1.31)	1.097 (0.862-1.39)	1.088 (0.826-1.43)	1.304 (0.934-1.82)	.198	0.157
High (>46); n=5690	1	1.224 (0.973-1.54)	1.165 (0.910-1.49)	1.321 (0.991-1.76)	1.553 (1.096-2.20)	.019	
BMI, kg/m <sup>2</sup>							
Low (<22.2); n=6112	1	1.094 (0.909-1.31)	0.929 (0.734-1.17)	0.796 (0.594-1.06)	0.988 (0.655-1.49)	.29	<0.001
Median (22.2-24.9); n=6102	1	1.227 (0.995-1.51)	1.244 (0.983-1.57)	1.300 (0.982-1.72)	1.550 (1.104-2.17)	.016	
High (>24.9); n=6088	1	1.410 (1.040-1.91)	1.532 (1.119-2.09)	1.837 (1.307-2.58)	2.258 (1.522-3.35)	<.001	
Fasting glucose, mmol/L							
Low (<4.88); n=6176	1	1.119 (0.915-1.36)	1.264 (0.990-1.61)	1.322 (0.987-1.77)	1.616 (1.107-2.36)	.008	0.202
Median (4.88-5.29); n=5943	1	1.187 (0.961-1.46)	1.097 (0.861-1.39)	0.961 (0.723-1.27)	1.181 (0.825-1.69)	.757	0.134
High (>5.29); n=5951	1	1.142 (0.883-1.47)	0.960 (0.727-1.26)	1.146 (0.836-1.57)	1.314 (0.911-1.89)	.234	
Total cholesterol, mmol/L							
Low (<4.21); n=5852	1	1.128 (0.919-1.38)	1.093 (0.856-1.39)	0.959 (0.703-1.30)	1.409 (0.911-2.17)	.434	0.895
Median (4.21-4.94); n=5783	1	1.337 (1.081-1.65)	1.292 (1.010-1.65)	1.617 (1.214-2.15)	1.764 (1.235-2.52)	.001	
High (>4.94); n=5777	1	0.985 (0.770-1.26)	0.948 (0.723-1.24)	0.931 (0.691-1.25)	1.092 (0.772-1.54)	.834	
LDL-C, mmol/L							
Low (<2.59); n=4086	1	1.195 (0.987-1.44)	1.150 (0.913-1.44)	1.101 (0.815-1.48)	1.223 (0.825-1.81)	.272	0.501
Median (2.59-3.25); n=4032	1	1.094 (0.880-1.35)	1.129 (0.879-1.45)	1.342 (1.009-1.78)	1.800 (1.257-2.57)	.002	0.152
High (>3.25); n=4054	1	1.197 (0.917-1.56)	1.053 (0.789-1.40)	1.000 (0.730-1.37)	1.116 (0.772-1.61)	.957	

Abbreviations: GGT, gamma-glutamyltransferase; LDL-C, low-density lipoprotein cholesterol. Analyses were adjusted for the following covariates: age, body mass index (BMI), sex, and biomarkers when they were not the strata variables.



**FIGURE.** The association between gamma-glutamyltransferase (GGT) level (in quintiles), body mass index (BMI) (lower, median, and higher levels), and the risk of prehypertension. The multivariate-adjusted prevalence risk ratios are presented.

link between insulin resistance and an inappropriately overactive renin-angiotensin system has been implicated in the pathogenesis of hypertension.<sup>18</sup> Recent studies have shown that insulin increases the expression of arterial angiotensinogen and angiotensin type 1 receptor in cultured vascular smooth muscle cells,<sup>18</sup> which may represent another mechanism through which hyperinsulinemia promotes the development of hypertension.

In stratified analyses, our data showed that the association between GGT and prehypertension was significant only in women. There were substantial sex-based differences in fat distribution,<sup>19,20</sup> and the differences in free fatty acid (FFA) metabolism between men and women<sup>21</sup> may explain the sex differences observed. GGT levels are a potentially important indicator of abdominal fat distribution.<sup>22</sup> Women have a greater amount of adipose tissue in the total abdominal and abdominal subcutaneous regions,<sup>19</sup> and have greater rates of nonoxidative FFA disposal<sup>21</sup> compared with men. In addition, female sex was reported to be positively associated with mean neutrophil counts.<sup>23</sup> In the present study, we noted that women with increased adiposity had higher circulating neutrophil counts; neutrophils are a major component of the inflammatory process contributing to endothelial dysfunction activated by cytokines.<sup>24</sup> Furthermore, an elevated GGT level could reflect subclinical inflammation,<sup>25</sup> which could also represent the underlying mechanism. In addition, we observed that the association between GGT level and prehypertension was not significant in individuals younger than 46 years and in those with low BMI levels. GGT levels and BMI showed an additive pattern in their effect on elevated BP. In particular, the age-dependent increases in visceral adipose tissue may be a speculative reason for the age disparity. Increasing age is associated with a marked number of changes in body composition and a progressive increase in the amount of body fat mass.<sup>26</sup> The

age-related shifts in body composition with an increase in body fat mass, particularly the accumulation of more internalized fat deposits, was associated with an increased risk of developing chronic disorders, including insulin resistance, and cardiovascular disease.<sup>26,27</sup> A sex difference in the association between GGT levels and prehypertension was found in the present study. We found that the association between GGT levels and prehypertension was significant only in women. However, a few studies have shown disparate outcomes in non-Chinese adults.<sup>11–13</sup> The ethnic and regional differences in the population studies may be speculative reasons for the difference between the studies.

Our results suggest that monitoring the levels of GGT could help in the diagnosis and monitoring of prehypertension. Through these measures, such individuals may adopt an intervention at an early stage, such as effective lifestyle modifications or use of medication to decrease GGT levels and the risk of prehypertension.

### Study Limitations and Strengths

The large sample size was a major strength of the present study and ensured sufficient power for the investigation of complex interactions between serum GGT levels and other metabolic factors. However, our study has the following limitations. First, the cross-sectional nature of our study does not allow us to infer about the causality of the effects. To confirm that the associations between GGT levels and BP are independent of other metabolic risk factors, a prospective study is needed. Second, the individuals were restricted to those who were administered a community-based health examination survey; hence, the results of this study may not be applicable to the general population in China. Third, although we carefully adjusted for the potential confounding variables in the analyses, we did not collect information on dietary intake and lifestyle habits. Therefore, the residual confounding of these unmeasured variables may influence the associations.

### CONCLUSIONS

In our study, GGT levels were positively associated with increased risk of prehypertension in Chinese women, independent of other metabolic risk factors. BMI and age showed a significant interaction with GGT levels in relation to prehypertension risk.

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## REVIEW PAPER

# Gamma-Glutamyl Transferase: A Novel Cardiovascular Risk BioMarker

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*Gamma-glutamyl transferase (GGT) is a second-generation enzymatic liver function test available for several decades, initially used as a sensitive indicator of alcohol ingestion, hepatic inflammation, fatty liver disease, and hepatitis. Longitudinal and cross-sectional investigational studies since 1990 have associated GGT with an increase in all-cause mortality, as well as chronic heart disease events such as congestive heart failure and components of the metabolic syndrome (abnormal body mass index and levels of high-density lipoprotein cholesterol, glucose, triglycerides, and systolic and diastolic blood pressure). In the upper reference range, GGT was found to be an independent biomarker of the metabolic syndrome, with a 20% per GGT quartile trend rise. Additionally, GGT was positively correlated with an 18% per quartile risk of cardiovascular events and a 26% per quartile increased risk of all-cause mortality. Furthermore, it may be considered a biomarker for "oxidative stress" associated with glutathione metabolism and possibly a "proatherogenic" marker because of its indirect relationship in the biochemical steps to low-density lipoprotein cholesterol oxidation. GGT is becoming an important addition to the multimarker approach to cardiovascular risk evaluation. It should be considered a valuable adjunct in stratifying patient risk and in assessing the aggressiveness of appropriate treatment, with hopes of preventing unnecessary cardiac events and deaths in future years.* Prev Cardiol. 2010;13:36–41. ©2009 Wiley Periodicals, Inc.

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**G**amma-glutamyl transferase (GGT) is a second-generation enzymatic liver function test (LFT) that became commercially available about 30 years ago and was used initially as a sensitive marker of hepatic inflammation. Originally called GGTP (gamma-glutamyl transpeptidase), it was felt to be the most sensitive LFT indicator of alcohol toxicity. It is in the class of hepatic enzymes similar to aspartic aminotransferase (AST), previously called serum glutamic oxaloacetic transaminase; alanine aminotransferase (ALT), previously called serum glutamic pyruvic transaminase; and alkaline phosphatase (ALP).

GGT is a glycoprotein with a molecular weight of 68,000 daltons, consisting of 2 proteins, the larger chain with a molecular weight of 46,000 daltons and the smaller one with a molecular weight of 22,000 daltons.

At higher than normal serum levels, liver function tests measure the severity of hepatic inflammation, cellular injury, or obstruction. ALT is found mainly in the liver and is elevated with hepatic inflammation or injury. AST is located in both hepatocytes and muscle cells and is elevated in the serum with hepatic cell involvement, skeletal muscle fiber inflammation, and myocardial cell injury. ALP is found in the hepatobiliary track and is markedly elevated with biliary track obstruction.

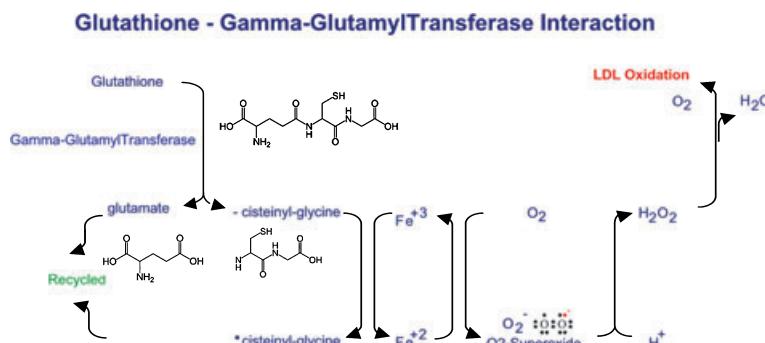
GGT is found not only in the liver but in other organ tissues, including the kidney,<sup>1</sup> lung, pancreas, and vascular endothelium, as well as in the extracellular fluid attached to  $\alpha$  and  $\beta$  lipoproteins<sup>2</sup> and albumin carrier molecules.<sup>3</sup> It is a sensitive indicator of hepatic cell inflammation and hepatic intracellular triglyceride accumulation as seen in obesity,<sup>4</sup> nonalcoholic fatty liver disease (NAFLD),<sup>5</sup> non-insulin dependent diabetes mellitus (NIDDM),<sup>6</sup> and insulin resistance.<sup>7</sup>

## BACKGROUND

The GGT enzymatic test has been available for several decades and was initially viewed as a sensitive indicator of ethanol ingestion. However, in 1990, the Tromso study<sup>8</sup> from Scandinavia described basic distribution and population patterns of GGT. In 1993, Conigrave,<sup>9</sup> working on alcohol-related



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*Figure 1. Steps in the gamma-glutamyl transferase enzyme reaction and its relationship to the oxidation of low-density lipoprotein (LDL) cholesterol.*

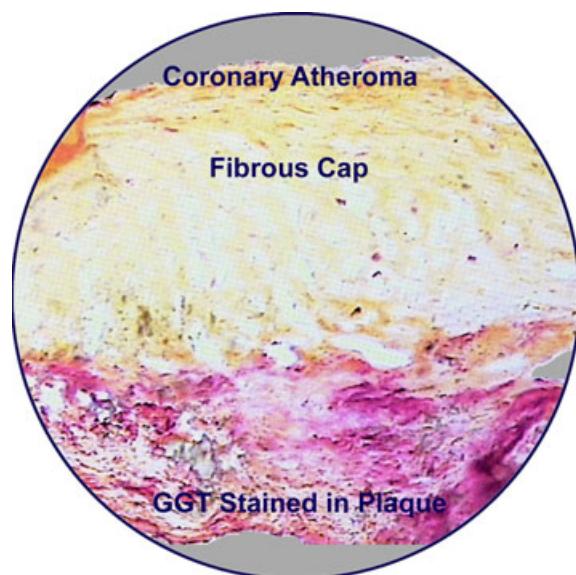
research and associated testing, described an unexpected increase in GGT levels associated with cardiac mortality. Recent research described below has focused increasing attention on the usefulness of GGT as a predictor of cardiovascular disease.

At physiologic serum levels, GGT acts as a protein catalyst in the degradation of glutathione, the major thiol antioxidant in the body (Figure 1). Glutathione is a molecule consisting of glutamic acid, cysteine, and glycine, synthesized within the cell, and may be present both in the reduced state and in the oxidized dimer form by thiol bonding.

As an antioxidant, single glutathione molecules are formed and are metabolically inactive and require degradation. The oxidized form of glutathione is reduced by the action of glutathione reductase in preparation for recycling. GGT hydrolyzes this glutathione into glutamate and a cisteinyl-glycine dipeptide, and inside the cell, the amino acids are subsequently reused, producing additional reduced glutathione.

However, on the cellular membrane and in the extracellular space, the cisteinyl-glycine moiety can act as a strong reducing agent of iron, with the stepwise development of the super-oxide ion and hydrogen peroxide. Unintended oxidation of low-density lipoprotein cholesterol particles may occur, which is felt to participate in the formation of inflammatory atheroma within the vascular endothelial wall.<sup>10</sup>

An eloquent study by Drs. Paolicchi and Emdin<sup>11</sup> at the University of Pisa in 2004 specifically identified GGT in coronary atheroma removed at the time of surgical atherectomy (Figure 2). The enzymatically active GGT identification in the plaque was done by an azo-coupling reaction using gamma-glutamyl-4-methoxy-2-naphthylamide as a substrate for GGT activity, stained with fast garnet GBC as the chromogen. The images of GGT stained in an atheromatous plaque with a fibrous cap were felt significant in the direct participation of GGT and low-density lipoprotein cholesterol oxidation within that plaque. They felt the "pathogenic mechanism proposed for the role of GGT



*Figure 2. Photomicrograph of a coronary atheroma (20× magnification) identifying active enzyme within the plaque by an enzyme histochemical reaction to human gamma-glutamyl transferase (GGT), stained red with the diazonium salt fast garnet GBC, as the chromogen. Used with permission from Lippincott, Williams & Wilkins.*

should be considered independent, complementary, and synergistic to conventional determinates."<sup>11</sup>

## Biomarker for Cardiovascular Risk

To be a unique biomarker for cardiac and metabolic risk evaluation, GGT must meet certain stringent characteristics.<sup>12</sup> It must measure a single specific entity, either physiologic or pathologic, and offer additional information over presently used determinants. It must also add to the clinical assessment of a specific problem and correlate with known cardiovascular disease risk factors.<sup>13</sup> Demographically, it must be applicable to both men and women of differing ages and varying ethnicities. It must be easily standardized, with both a high sensitivity and

specificity, and have automated testing readily available in most regions. GGT enzyme analysis has been available for many years, meets all of these strict measures, and thus would appear to pass accepted criteria as defined by Vasan,<sup>12</sup> as a biomarker for increased cardiovascular risk.

## RECENT CLINICAL STUDIES

GGT levels were first associated with cardiovascular disease and all-cause mortality in a British Regional Heart Study by Wannamethee<sup>14</sup> reported in October of 1995. This study evaluated 7613 British men over 11.5 years in England, Wales, and Scotland. The study plan included personal history questionnaires, history and physical exams, and laboratory screening for GGT, total cholesterol, high-density lipoprotein cholesterol, and non-fasting glucose. Increasing GGT levels were strongly associated with all-cause mortality, particularly in patients with ischemic heart disease, and a strong positive correlation was also noted with body mass index, total cholesterol, and diabetes mellitus<sup>15,16</sup> (some of the present criteria for the metabolic syndrome).<sup>17</sup> A lesser correlation was seen in relation to blood pressure, heart rate, and cigarette smoking. There was no correlation with acute cardiac events, cancer, or other causes of noncardiac deaths including alcohol ingestion.

A second important cross-sectional and longitudinal study reported by Ruttmann<sup>18</sup> in 2005 involved the Vorarberg Health Monitoring and Promotion Program in western Austria, with the participation of 163,944 adults and GGT evaluated as a risk factor for cardiovascular mortality. GGT was positively associated with significant risk factors for cardiovascular disease including body mass index,<sup>19</sup> serum triglycerides, total cholesterol, systolic and diastolic blood pressure, and glucose (most of those criteria are now part of the metabolic syndrome). Other less positive correlates included ethanol ingestion and smoking, whereas physical activity, education and high-density lipoprotein cholesterol were negatively correlated with GGT. Using the Cox hazard ratio analysis and GGT values partitioned in quintiles, cardiovascular survival rates decreased significantly with each increasing group. Within the highest quintile, there was a 64% increase in all vascular-related deaths in men and 51% in women. A 162% increase in congestive heart failure was also identified in men within the highest grouping. This study concluded that GGT is an independent biomarker for cardiovascular mortality correlating with deaths from chronic heart disease (ischemic heart disease and congestive heart failure) but not acute cardiac events. Patients with higher GGT values had a more than 1.5-fold risk increase of total mortality from cardiovascular disease, and adults younger than 60 had an additional 2- to 2.6-fold increase in risk. Positive correlations in this group included body mass index, total cholesterol, blood

pressure, low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol, triglycerides, glucose, ethanol ingestion, and cigarette smoking.

A cross-sectional paper published by Onat and colleagues<sup>20</sup> in October 2006 from the Turkish Adult Risk Factor Study showed that waist circumference is the major determinant in GGT activity and that a doubling of GGT increases the odds ratio of metabolic syndrome developing by 74% and of coronary heart disease developing by 45%.

A third large strictly longitudinal study reported by Kazemi<sup>21</sup> in 2007 involved 283,438 patients over a 12-year period. This study evaluated both inpatients and outpatients seen at a general hospital who had GGT tests ordered at initial visit and were followed over time to relate GGT levels with cause of death. Using the Cox hazard ratio, an analysis of GGT for all-cause mortality, cancer-related deaths, and non-cancer-related deaths, including subsets of vascular, cerebrovascular, and ischemic heart disease, was performed. Significant relationships developed even in patients with relatively normal GGT values. In the highest quintile for all-cause mortality, a 100% risk increase was observed. These findings provided a strong predictive value of long-term survival even with GGT values within the considered reference range. GGT values were significantly associated with all-cause mortality, cancer-related deaths, non-cancer-related deaths, and vascular mortality. Younger patients evaluated in decreasing decade intervals had increasing mortality risk, with up to 3.3 times the risk if younger than 30. GGT levels found significantly above the normal cutoff were a gross predictor of hepatobiliary disease and death.

The most recent complete study, reported in January 2007, evaluated 3451 patients in the Framingham Offspring Study<sup>22</sup> looking at cross-sectional correlation of GGT with multiple variables and longitudinal GGT correlation with the metabolic syndrome, coronary heart disease risk factors, onset of congestive heart failure, peripheral vascular disease, cardiovascular disease, or death. Participants were evaluated every 4 years over a 20-year span between 1971 and 1991. Body mass index  $\geq 30 \text{ kg/m}^2$  was used as a proxy for abdominal circumference in the National Cholesterol Education Program Adult Treatment Panel III criteria for metabolic syndrome.

Cross-sectional analysis disclosed significant positive GGT correlation, in decreasing order of significance, to triglycerides, male sex, and alcohol consumption (accounting for the most variation). Additional covariates included diastolic blood pressure, body mass index, age, smoking, low-density lipoprotein cholesterol, and fasting glucose. Although previously reported by other authors,<sup>23,24</sup> including a report using the National Health and Nutrition Examination Survey III population,<sup>25</sup> no significant statistical interaction was seen between

**Table I.** Framingham Offspring Study: Longitudinal Study Results Summary

GAMMA-GLUTAMYL TRANSFERASE (GGT) VALUES					
GGT values (U/L), men	1–11	12–16	17–24	25–99	Normal, ≤50 U/L
GGT values (U/L), women	1–6	7–9	10–13	14–88	Normal, ≤40 U/L
QUARTILES	1ST	2ND	3RD	4TH	QUARTILE TREND P VALUE
METABOLIC SYNDROME (MET S)					
AT 20 YEARS (968 PATIENTS)					
Met-S developed, %	n/a	n/a	n/a	n/a	28.0
HR adjusted for age and sex	Ref	1.21	1.49	1.85	1.23
HR adjusted for age, sex, and CRP	Ref	1.23	1.36	1.76	1.20
CARDIOVASCULAR DISEASE (CVD)					
AT 20 YEARS (535 PATIENTS)					
CVD developed, %	10.5	12.1	16.7	23.8	15.5
HR adjusted for age, sex, and CRP	Ref	1.28	1.53	1.88	1.23
HR adjusted for all covariables <sup>a</sup>	Ref	1.26	1.40	1.67	1.18
ALL-CAUSE MORTALITY (DEATH)					
AT 19.1 YEARS (362 PATIENTS)					
Mortality, %	6.3	7.4	12.2	16.6	10.5
HR adjusted for age, sex, and CRP	Ref	1.20	1.65	1.94	1.26
HR adjusted for all covariables <sup>a</sup>	Ref	1.21	1.67	1.95	1.26

Abbreviations: CRP, C-reactive protein; HR, hazard ratio; n/a, not available; Ref, reference quartile. <sup>a</sup>Adjustment for all covariables included age, sex, CRP, body mass index, diabetes mellitus, systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, smoking, and alcohol consumption.

GGT and C-reactive protein. This study did not report the percentage incidence of metabolic syndrome per GGT quartile, but it did permit calculation of the percentage of participants in whom the syndrome developed over the 2-decade study period.

As seen in Table I, longitudinal 20-year study results disclosed 28% of participants in whom the metabolic syndrome developed. After adjustment for age and sex, a 23% per GGT quartile increase was found, and after an additional adjustment for C-reactive protein, a 20% per GGT quartile trend was recorded regarding the metabolic syndrome. With still further adjustment for all additional identified covariables, including age, sex, C-reactive protein, body mass index, fasting glucose, triglycerides, systolic and diastolic blood pressure, alcohol consumption, and smoking status, a 9% per GGT quartile trend still remained (data not shown) over the 20-year study. The stepwise GGT quartile increase, adjusted for all covariables, unfortunately, was not reported. This was the first reputable study to demonstrate the separate correlation of GGT with the metabolic syndrome after all confounding variables were taken into account.

Over the same period, cardiovascular disease developed in 15.5% of participants, with an 18% per GGT quartile increase, and in relation to all-cause mortality, 10.5% of patients died during the study, with a 26% per quartile trend. This study reported GGT in the 4th quartile to be an independent predictor of development of the metabolic

syndrome, with a 1.76-fold risk increase in its incidence over 20 years.<sup>22,26</sup>

These and other more recent articles regarding GGT published by experts in the field point to GGT as an important biomarker for prognostic cardiovascular risk evaluation. In 2007, Grundy,<sup>26</sup> in his editorial regarding the Framingham Offspring Heart Study, discussed GGT as another good biomarker for metabolic syndrome and cardiovascular risk. Even more recently, Emdin and colleagues<sup>27</sup> from the University of Pisa reported in their article regarding the additive prognostic value, along with C-reactive protein and fasting glucose, of GGT in coronary artery disease.

## CLINICAL UTILIZATION

In a landmark consensus paper in 2003, Nagahashi, Libby,<sup>28,29</sup> and others presented a 2-part risk assessment compendium entitled “From Vulnerable Plaque to Vulnerable Patient.” Risk detection was subdivided into tests and biomarkers regarding “the vulnerable plaque, the vulnerable blood, and the vulnerable myocardium.” The “vulnerable vessel” is included here to help better identify early occult vascular changes.

Evaluation of the “vessel at risk” would include clinical inflammatory markers such as high-sensitivity C-reactive protein, lipoprotein-associated phospholipase A<sub>2</sub>, and fibrinogen, while homocysteine could be used as a proxy for endothelial function.<sup>13</sup> The GGT biomarker would fall under a new classification of “oxidative stress,” in view of its role in the

degradation of the antioxidant glutathione. It could likewise be considered a proinflammatory marker<sup>30</sup> in view of its generation of cysteinyl-glycine, which has an indirect effect causing low-density lipoprotein cholesterol oxidation in the presence of iron. Its independent correlation with the metabolic syndrome makes the biomarker valuable, along with blood glucose, insulin levels, and hemoglobin A<sub>1c</sub> in evaluating patients at risk for diabetes or insulin resistance or who have diabetes or insulin resistance.

Measurements of the "plaque at risk" would include lipid profile evaluation, with low-density lipoprotein cholesterol concentration; low-density lipoprotein particle size measurement; small, dense low-density lipoprotein; and apolipoprotein B. The high-density lipoprotein (HDL) cholesterol measurement and high-density lipoprotein particle size determinations, especially HDL 2b, are considered part of plaque risk determination. Apolipoprotein A1 can also be grouped as part of this component.

Laboratory testing to evaluate the "blood clotting mechanism at risk" or the "hypercoagulable state" would include fibrinogen; lipoprotein(a), in view of its structural similarity to plasminogen; and possibly homocysteine.

Evaluation of the "myocardium at risk" includes creatine phosphokinase and, more importantly, the biomarker N-terminal prohormone brain natriuretic peptide to identify both ventricular and atrial myocardial cell stress.

## CONCLUSIONS

The GGT enzymatic assay at abnormally high serum levels is an established liver function test for alcoholic toxicity, inflammation, fatty liver disease (hepatitis), and hepatitis.

However, in the upper reference range, GGT has recently been found to be a strong independent biomarker for the metabolic syndrome (insulin resistance syndrome). A 20% per GGT quartile trend was seen in relation to the development of the metabolic syndrome.

GGT is also an independent risk marker for the development of cardiovascular disease, with an 18% per quartile increase in risk, and shows independent correlation for all-cause mortality, with a 26% per quartile increase over baseline values. It was positively correlated with age, sex, triglycerides, blood pressure, body mass index, low-density lipoprotein cholesterol, fasting glucose, ethanol ingestion, and smoking, as expected for increasing cardiovascular risk. Negative correlation included physical activity, education, and high-density lipoprotein cholesterol.

In addition, GGT can be considered a biomarker for oxidative stress associated with glutathione regulation and degradation and possibly a proatherogenic marker because of its indirect relationship in the biochemical steps leading to low-density lipoprotein oxidation.

Further academic studies will need to be done to evaluate GGT's overall importance, independent from high-sensitivity C-reactive protein and other presently accepted biomarkers and relative to the traditional risk factors used for predicting the development of the metabolic syndrome and cardiovascular disease. However, because of its wide availability and inexpensive cost for screening, identifying higher than expected GGT levels in otherwise healthy individuals should alert the physician to study those patients in more detail, with the hopeful outcome of preventing unnecessary cardiac-related events and deaths in future years.

GGT is a unique biomarker in the continuum of cardiovascular disease risk. GGT is thus a potentially valuable addition to the growing list of clinically available tests useful in initially stratifying patient risk associated with well-known cardiovascular conditions and should be considered in assessing the appropriate aggressiveness of treatment.

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## $\gamma$ -Glutamyltransferase Is a Predictor of Incident Diabetes and Hypertension: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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**Background:**  $\gamma$ -Glutamyltransferase (GGT), which maintains cellular concentrations of glutathione, may be a marker of oxidative stress, and GGT itself may produce oxidative stress. We performed a prospective study to examine whether serum GGT predicts diabetes and hypertension.

**Methods:** Study participants were 4844 black and white men and women 18–30 years of age in 1985–1986; they were reexamined 2, 5, 7, 10, and 15 years later. Year 0 GGT cutpoints were 12, 17, 25, and 36 U/L (overall 25th, 50th, 75th, and 90th percentiles; the laboratory cutpoints for abnormal are 40 U/L in women and 50 U/L in men). We deleted 32 participants with prevalent diabetes and 140 participants with prevalent hypertension from the respective incidence analyses.

**Results:** After adjustment for study center, race, sex, and age in proportional hazards regression, the hazard ratios across year 0 GGT categories were 1.0, 1.6, 1.7, 4.0 (95% confidence interval, 2.0–8.1), and 5.5 (2.7–11.1) for 15-year incident diabetes and 1.0, 1.2, 1.7 (1.2–2.2), 2.3 (1.7–3.2), and 2.3 (1.7–3.2) for hypertension. Additional adjustment for year 0 alcohol consumption, body mass

index, cigarette smoking, and physical activity attenuated this relationship, but GGT remained a significant predictor.

**Conclusions:** Serum GGT within a range regarded as physiologically normal is associated with incident diabetes and hypertension. Considering known functionality of GGT, these associations are consistent with a role for oxidative stress in risk for diabetes and hypertension.

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Serum  $\gamma$ -glutamyltransferase (GGT),<sup>8</sup> even within reference intervals, is associated with several cardiovascular disease risk factors and components of the insulin resistance syndrome (1–3). In addition, in several prospective studies (4–10), the baseline serum GGT concentration has been an independent risk factor for the development of cardiovascular or cerebrovascular diseases.

In our previous prospective studies in healthy Korean men (10), serum GGT concentrations within the reference interval showed a strong dose-response relationship with incident diabetes. This strong relationship was observed even in nondrinkers and individuals without increased concentrations of any other liver enzymes. Therefore, although GGT has been widely used as a marker of alcohol consumption or liver disease (11), neither alcohol nor hepatic dysfunction explained the observed relationships between GGT and diabetes. GGT is also a modest risk factor for hypertension (9). Therefore, the mechanism underlying these observations is not fully understood. An

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<sup>8</sup> Nonstandard abbreviations: GGT,  $\gamma$ -glutamyltransferase; CARDIA, Coronary Artery Risk Development in Young Adults; AST, aspartate aminotransferase; CRP, C-reactive protein; BMI, body mass index; RR, relative risk; and 95% CI, 95% confidence interval.

interesting ancillary observation is that the well-known strong association of either obesity or age with diabetes was observed only among individuals with high-normal GGT at baseline (10).

At present, experimental and epidemiologic studies of GGT are in an early stage; therefore, confirmation of recent findings in other population-based cohort studies is important. We performed a prospective study to examine whether GGT is a predictor of incident diabetes and hypertension among young adult black and white men and women, and to analyze whether the relationships of diabetes and hypertension with obesity or age were modified by baseline GGT concentration.

## Materials and Methods

### STUDY POPULATION

Coronary Artery Risk Development in Young Adults (CARDIA) is a longitudinal, multicenter epidemiologic study of the impact of lifestyle and other factors on the evolution of coronary heart disease risk factors during young adulthood. The study design, recruitment of participants, and methods have been described elsewhere (12). In 1985–1986, 5115 black and white men and women 18–30 years of age were recruited and examined at four clinical sites in the US: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Participants were reexamined at 2, 5, 7, 10, and 15 years post-baseline, with reexamination rates among surviving cohort members of 90%, 86%, 81%, 79%, and 74%, respectively.

For this study, we excluded 65 study participants in whom GGT was not measured at year 0 and 212 who never returned for a follow-up examination, leaving 4844 participants for analysis. Participants who never returned had higher baseline GGT and were more likely to be black than those who returned. In addition, for analyses of diabetes, 32 participants who had type 1 or type 2 diabetes (defined below) at year 0 were excluded. For analyses of hypertension, 140 participants with hypertension (defined below) at year 0 were excluded. The final sample sizes entering proportional hazards life table regression analyses with the outcome of incident diabetes or incident hypertension were 4812 and 4704, respectively.

### QUESTIONNAIRES

Standard questionnaires were used to maintain consistency in the assessment of demographic and behavioral information across CARDIA examination visits. Sex, race, date of birth, weekly alcohol consumption, and cigarette smoking were determined by structured interview or by self-administered questionnaire. A physical activity score was derived from the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire (13). Alcohol intake (mL/day) was computed from the self-reported frequency of beer, wine, and liquor consumed per week.

### CLINICAL MEASUREMENTS

All participants were asked to fast at least 12 h and to avoid smoking and heavy physical activity at least 2 h before the examination. After a 5-min rest, blood pressure was measured on the right arm in the sitting position. First and fifth-phase Korotkoff sounds were recorded three times at 1-min intervals, using a random zero sphygmomanometer (WA Baum Company). The mean of the second and third measurements was used in the analyses. Blood was then collected with minimal stasis for GGT, glucose, insulin, lipids, blood cell counts, uric acid, fibrinogen, C-reactive protein (CRP), and F2-isoprostanes. In this analysis, we used year 0 and year 10 GGT, year 0 glucose, year 0 insulin, year 0 lipids, year 0 blood cell counts, year 5 fibrinogen, year 15 uric acid, year 15 CRP, and year 15 F2-isoprostanes. After plasma or serum separation, aliquots were stored at  $-70^{\circ}\text{C}$  until shipped on dry ice to a central laboratory.

Serum GGT was measured at year 0 and year 10. At year 0, liver-related enzymes, including GGT and aspartate aminotransferase (AST) were measured with a SMA-CII continuous-flow analyzer (Technicon Instruments Corp.) at American Bio-science Laboratories (now Smith-Kline Beecham). At year 10, GGT was measured colorimetrically by the nitroanilide method on a Roche Cobas Mira Plus chemistry instrument at Linco Research Inc. (St. Louis, MO). The methodology for measuring GGT was not comparable between year 0 and year 10. To identify an appropriate recalibration formula, GGT was remeasured at Linco Research with the year 10 methodology in 103 baseline samples with original GGT values of 3–228 U/L that had been stored at  $-70^{\circ}\text{C}$  for 17 years (since 1985–1986). The correlation between measurements made in year 0 and those measured with the year 10 methodology was 0.995; accordingly, the year 0 values reported here are  $2.7618 + 1.9004$  times the original year 0 values.

Year 0 glucose was measured by the hexokinase-ultraviolet method. Year 0 fasting insulin was measured by RIA on sera frozen for 8 years from year 0. Year 0 lipids were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Total triglycerides and total HDL-cholesterol were measured by enzymatic procedures. HDL-cholesterol was measured after dextran sulfate-magnesium precipitation. LDL-cholesterol was calculated with use of the Friedewald equation. Year 0 complete blood cell count was performed at each local clinical center with a Coulter counter. Year 5 fibrinogen was measured by the Clauss method at Medlantic Research Foundation (Washington, DC). Year 15 uric acid was measured at Linco Research by the uricase method. CRP was measured at the Pathology Laboratory at the University of Vermont with high-sensitivity ELISAs. Year 15 F2-isoprostanes, a free-radical-dependent oxidative damage product of arachidonic acid metabolism, was measured at the Molecular Epidemiology and Biomarker Research Laboratory in the University of Minnesota by a gas chromatography-mass spectrometry-based method

(14). Body weight with light clothing was measured to the nearest 0.2 pounds, and body height without shoes was measured to the nearest 0.5 cm. Body mass index (BMI) was computed as weight divided by height squared ( $\text{kg}/\text{m}^2$ ).

#### STATISTICAL ANALYSIS

We first examined the distribution and change in GGT, using the natural logarithmic transformation to account for skewness. Year 0 serum GGT concentrations were classified into five groups with use of cutpoints of 12, 17, 25, and 36 U/L (the 25th, 50th, 75th, and 90th percentiles computed over the entire sample) for study of GGT in relation to baseline and follow-up correlates, as well as GGT as a predictor of incidence of diabetes or hypertension during 15 years. The definition of diabetes incidence was serum fasting glucose  $\geq 1260 \text{ mg/L}$  or taking diabetes medication. Of 109 participants who ever reported taking antidiabetic medication during the study, insulin was the only drug in only 29 cases. Because individuals with type 1 diabetes always require insulin medication exclusively, most diabetic participants had type 2 diabetes. The definition of hypertension was systolic blood pressure  $\geq 140 \text{ mmHg}$ , diastolic blood pressure  $\geq 90 \text{ mmHg}$ , or the use of antihypertensive medication.

For calculation of incidence density, length of follow-up was calculated as time from the baseline exam to the exam at which disease outcome first occurred or the time of the last follow-up exam. Diabetes or hypertension was assumed to be present starting at the first examination at which it was diagnosed. Thus, "events" could occur after exactly 2, 5, 7, 10, or 15 years of follow-up. Before this diagnosis, the outcome was assumed to be absent, even if the earlier examination was missed. Participants were censored after their last examination. Cox proportional hazard models were used to calculate multivariate-adjusted hazard ratios in separate models for diabetes or hypertension. Covariates were the baseline values of study center, sex, race, age, BMI, alcohol consumption, cigarette smoking, and physical exercise. In some models, baseline values of systolic blood pressure, fasting plasma glucose, or insulin were included as additional covariates. In addition, we examined associations after stratification by race-sex or alcohol consumption.

We next looked at the short-term risk of GGT measured at year 10 when participants were 28–40 years of age. Year 10 serum GGT cutpoints of 12, 18, 29, and 50 U/L (the 25th, 50th, 75th, and 90th percentiles computed over the entire sample) were used for study of association of year 10 GGT with year 15 uric acid, CRP, and F2-isoprostanes and for prediction of incident diabetes and hypertension, the latter using proportional hazards regression. Among 3950 individuals who attended a year 10 examination, we excluded 80 study participants in whom GGT was not measured at year 10 and 540 who did not return for a year 15 follow-up examination, leaving 3352

participants for analysis. We excluded 98 and 388 participants with any diagnosis of diabetes or hypertension, respectively, at or before year 10, the baseline for incident analysis. This analysis provided both a shorter term follow-up and a baseline for GGT at a mean age of 35 years.

Finally, we assessed whether the associations between age, BMI, and disease outcomes were modified by baseline serum GGT concentration. We evaluated both long-term risk during 15 years (baseline to year 15) and short-term risk during 5 years (year 10 to year 15). The median serum GGT value at either year 0 or year 10 was used as the cutoff point in these stratified analyses.

## Results

### BASELINE CHARACTERISTICS

Year 0 serum GGT was strongly associated with year 10 GGT ( $r = 0.67$ ). GGT increased during 10 years [mean (SD) change, 1.1 (1.7) U/L]. At baseline, most cardiovascular risk factors showed clear positive or negative relationships with serum GGT concentration (Table 1). In addition to alcohol consumption, black race, male gender, older age, lower educational attainment, cigarette smoking, and higher BMI were positively associated with baseline serum GGT concentration. Among clinical variables, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, insulin, triglycerides, LDL-cholesterol, red blood cell count, hematocrit, hemoglobin, and white blood cell count showed a positive association with baseline GGT level, whereas HDL-cholesterol showed a negative association.

Year 0 and year 10 GGT also showed positive associations with known markers of oxidative stress or inflammation, including year 5 fibrinogen, year 15 uric acid, year 15 CRP, and year 15 F2-isoprostanes (Table 2). These associations were shown among nondrinkers, drinkers, and individuals with AST concentrations within the reference interval ( $< 30 \text{ U/L}$ ; data not shown).

### INCIDENCE OF DIABETES AND HYPERTENSION BY BASELINE GGT CONCENTRATION

Incidence densities of diabetes and hypertension were 2.4 (157 cases) and 11.6 (708 cases) per 1000 person-years, respectively. There were positive associations between baseline GGT and both incident diabetes and hypertension, but the association with diabetes was the stronger of the two (Table 3). After minimal adjustment for study center, race, sex, and age, the relative risks (RRs) of incident diabetes were 1.0, 1.6, 1.7, 4.0, and 5.5, respectively. Additional adjustment for alcohol consumption did not change the relationship. Further adjustment for known risk factors for diabetes attenuated this relationship, but GGT remained a significant risk factor in the model that included BMI, smoking, and physical activity. Additional adjustment for baseline fasting serum glucose or baseline fasting insulin concentration did not materi-

**Table 1.** Adjusted mean<sup>a</sup> values for demographic, health behavior, and clinical characteristics by serum GGT at baseline in the CARDIA Study, 1985–1986.

	GGT at 0 year					<i>P</i> <sup>b</sup>
	<25% (n = 847)	25% to <50% (n = 1465)	50% to <75% (n = 1503)	75% to <90% (n = 557)	≥90% (n = 472)	
Demographic variables						
Black, %	28.3	39.7	56.8	72.9	75.4	<0.001
Male, %	21.7	37.4	52.8	63.5	68.1	<0.001
Age, years	24.3	24.4	25.0	25.5	26.4	<0.001
Education, years	14.0	13.9	13.9	13.7	13.3	<0.001
Health behavior variables (year 0)						
Alcohol, g/day	7.6	9.5	12.6	15.1	22.5	<0.001
Smoker, %	22.4	26.4	30.9	32.8	47.5	<0.001
Physical activity, exercise units	414.9	421.4	422.8	423.9	401.5	0.643
BMI, kg/m <sup>2</sup>	23.3	23.9	24.6	26.0	26.2	<0.001
Clinical variables (year 0)						
SBP, <sup>c</sup> mmHg	108.9	109.1	110.7	112.4	113.8	<0.001
DBP, mmHg	67.3	67.9	68.7	69.9	71.0	<0.001
Fasting plasma glucose, mg/L	824	819	822	843	835	0.019
Insulin, μIU/L	10.1	10.8	11.6	13.6	13.9	<0.001
Triglycerides, <sup>d</sup> mg/L	568	589	644	738	828	<0.001
LDL-C, mg/L	1034	1069	1099	1158	1152	<0.001
HDL-C, mg/L	538	532	530	513	542	0.002
RBC, × 10 <sup>10</sup> /L	477	478	480	485	485	<0.001
Hematocrit, %	41.7	42.0	42.3	42.6	42.8	<0.001
Hemoglobin, g/L	141	142	143	143	144	<0.001
WBC, × 10 <sup>9</sup> /L	5.7	6.0	6.2	6.4	6.5	<0.001
Platelets, × 10 <sup>9</sup> /L	265	271	274	280	280	<0.001

<sup>a</sup> Adjusted for study center, race, sex, and age.<sup>b</sup> *P* values are based on *F*-test for any difference among GGT categories.<sup>c</sup> SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; RBC, red blood cells; WBC, white blood cells.<sup>d</sup> Geometric mean.

ally alter the association with GGT; because it is possible that increased GGT was in the causal pathway for hyperinsulinemia at baseline, this model may be an overadjustment. Incident hypertension was also associated with baseline GGT, with RRs of 1.0, 1.2, 1.5, 2.0, and 1.9 after adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, respectively (Table 3). The positive association between baseline GGT and incident diabetes and/or hypertension was observed in both nondrinkers and drinkers (data not shown).

After adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, RRs for AST with cutpoints of 18, 23, 28, and 37 U/L (the 25th, 50th, 75th, and 90th percentiles computed over the entire sample) were 1.0, 1.0 [95% confidence interval (95% CI), 0.6–1.6], 1.2 (95% CI, 0.8–2.0), 1.4 (95% CI, 0.8–2.4), and 2.0 (95% CI, 1.1–3.5) for prediction of diabetes and 1.0, 1.2 (95% CI, 0.9–1.5), 1.0 (95% CI, 0.8–1.3), 1.2 (95% CI, 0.9–1.5), and 1.4 (95% CI, 1.1–1.8) for prediction of hypertension. The dose-response relationship between GGT concentration and incidence of diabetes and/or hyperten-

sion was observed among individuals with AST concentrations within the reference interval (data not shown).

The positive association between baseline GGT and incident diabetes and/or hypertension was observed among all race and sex subgroups, although power was reduced in the subgroup analyses and the subgroup relationship did not always reach statistical significance. For example, compared with individuals with GGT below the median in each race-sex group, after adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, RRs for diabetes among individuals with year 0 GGT at or above the 75th percentile were 2.5 (95% CI, 1.1–5.7) in black men, 1.8 (95% CI, 1.0–3.3) in black women, 1.6 (95% CI, 0.6–3.7) in white men, and 8.8 (95% CI, 1.1–72.7) in white women. Similarly, RRs for hypertension among individuals with GGT at or above the 75th percentile were 1.6 (95% CI, 1.1–2.2) in black men, 1.5 (95% CI, 1.1–2.1) in black women, 1.6 (95% CI, 1.0–2.5) in white men, and 1.4 (95% CI, 0.7–2.5) in white women.

The RRs for year 10 serum GGT with incident diabetes or hypertension at year 15 (Table 4) were higher than the RRs for the 15 year risk that started with year 0 GGT as

**Table 2. Adjusted mean<sup>a</sup> values for inflammatory and/or oxidative stress markers by year 0 and/or year 10 GGT in the CARDIA Study, 1985–2001.**

	GGT at year 0					Difference, <sup>b</sup> %	P for trend
	<25%	25% to <50%	50% to <75%	75% to <90%	≥90%		
Fibrinogen (year 5), mg/L	2607 (n = 758)	2607 (n = 1268)	2634 (n = 1297)	2674 (n = 471)	2728 (n = 414)	5	<0.001
Uric acid (year 15), mg/L	46 (n = 639)	48 (n = 1099)	50 (n = 1083)	52 (n = 411)	52 (n = 339)	13	<0.001
CRP (year 15), <sup>c</sup> mg/L	1.3 (n = 642)	1.4 (n = 1101)	1.4 (n = 1085)	1.6 (n = 411)	1.7 (n = 340)	31	<0.001
F2-Iso prostanes (year 15), ng/L	56.0 (n = 535)	59.1 (n = 921)	59.3 (n = 909)	60.9 (n = 332)	63.3 (n = 282)	13	0.003
GGT at year 10							
	<25%	25% to <50%	50% to <75%	75% to <90%	≥90%	Difference, <sup>b</sup> %	P for trend
Uric acid (year 15), mg/L	45 (n = 677)	47 (n = 901)	50 (n = 868)	52 (n = 511)	54 (n = 311)	20	<0.001
CRP (year 15), <sup>c</sup> mg/L	1.2 (n = 676)	1.4 (n = 905)	1.5 (n = 869)	1.7 (n = 512)	1.6 (n = 311)	33	<0.001
F2-Iso prostanes (year 15), ng/L	54.7 (n = 572)	57.6 (n = 757)	59.3 (n = 738)	62.4 (n = 427)	68.2 (n = 253)	25	<0.001

<sup>a</sup> Adjusted for study center, race, sex, and age.<sup>b</sup> Lowest vs highest.<sup>c</sup> Geometric mean.

baseline (Table 3). Because year 10 serum GGT was increased, the minimally adjusted RRs for diabetes were 1.0, 6.0, 10.4, 24.4, and 28.3, and those for hypertension were 1.0, 2.5, 2.5, 3.8, and 3.4 (Table 4). After adjusting for

study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, the RRs were 1.0, 4.5, 6.3, 11.0, and 15.5 for diabetes and 1.0, 2.4, 2.2, 3.1, and 2.8 for hypertension.

**Table 3. Incidence density and adjusted RR for incident diabetes and hypertension from year 0 to year 15 by serum GGT at baseline in the CARDIA Study, 1985–2001.**

Outcome	GGT at year 0					P for trend
	<25%	25% to <50%	50% to <75%	75% to <90%	≥90%	
Incident cases of diabetes <sup>a</sup>						
Cases/person-years	11/11 552	32/19 729	37/19 879	35/7262	42/6127	
Incidence density, per 1000 person-years	1.0	1.6	1.9	4.8	6.9	
Adjusted RR						
Model 1 <sup>b</sup>	1.0	1.6 (0.8–3.3)	1.7 (0.9–3.4)	4.0 (2.0–8.1)	5.5 (2.7–11.1)	<0.01
Model 2 <sup>c</sup>	1.0	1.7 (0.8–3.3)	1.8 (0.9–3.6)	4.2 (2.1–8.5)	6.1 (3.0–12.3)	<0.01
Model 3 <sup>d</sup>	1.0	1.5 (0.8–3.0)	1.4 (0.7–2.7)	2.6 (1.3–5.3)	3.3 (1.6–6.8)	<0.01
Model 4 <sup>e</sup>	1.0	1.5 (0.7–3.4)	1.5 (0.7–3.4)	2.6 (1.2–6.0)	2.9 (1.2–6.9)	0.002
Incident cases of hypertension <sup>f</sup>						
Cases/person-years	65/11 271	150/19 043	240/18 762	133/6394	120/5310	
Incidence density, per 1000 person-years	5.8	7.9	12.8	20.8	22.6	
Adjusted RR						
Model 1 <sup>b</sup>	1.0	1.2 (0.9–1.6)	1.7 (1.2–2.2)	2.3 (1.7–3.2)	2.3 (1.7–3.2)	<0.01
Model 2 <sup>c</sup>	1.0	1.2 (0.9–1.6)	1.6 (1.2–2.2)	2.3 (1.7–3.2)	2.3 (1.7–3.2)	<0.01
Model 3 <sup>d</sup>	1.0	1.2 (0.9–1.6)	1.5 (1.1–2.0)	2.0 (1.5–2.7)	1.9 (1.4–2.7)	<0.01
Model 4 <sup>e</sup>	1.0	1.3 (0.9–1.8)	1.5 (1.1–2.1)	1.9 (1.4–2.8)	1.5 (1.0–2.2)	0.003

<sup>a</sup> Fasting plasma glucose ≥1260 mg/L or medication from year 0 to year 15.<sup>b</sup> Model 1: minimal adjustment for study center, race, sex, and age.<sup>c</sup> Model 2: model 1 plus adjustment for alcohol consumption.<sup>d</sup> Model 3: model 2 plus adjustment for BMI, cigarette smoking, and physical activity.<sup>e</sup> Model 4: model 3 plus adjustment for fasting serum glucose and insulin for diabetes and systolic blood pressure and insulin for hypertension.<sup>f</sup> Blood pressure ≥140/90 mmHg or medication from year 0 to year 15.

**Table 4. Incidence density and adjusted RR for incident diabetes and hypertension from year 10 to year 15 by serum GGT at year 10 in the CARDIA Study, 1995–2001.**

Outcome	GGT at year 10					P for trend
	<25%	25% to <50%	50% to <75%	75% to <90%	≥90%	
Incident cases of diabetes						
Cases/person-years	1/3789	8/5034	15/4739	19/2697	14/1610	
Incidence density, per 1000 person-years	0.3	1.6	3.2	7.0	8.7	
Adjusted RR						
Model 1 <sup>a</sup>	1.0	6.0 (0.8–48.5)	10.4 (1.4–80.4)	24.4 (3.2–187.2)	28.3 (3.6–223.4)	<0.001
Model 2 <sup>b</sup>	1.0	6.0 (0.7–48.5)	10.7 (1.4–82.0)	26.1 (3.4–199.3)	34.3 (4.3–270.02)	<0.001
Model 3 <sup>c</sup>	1.0	4.5 (0.6–36.2)	6.3 (0.8–48.8)	11.0 (1.4–85.4)	15.5 (1.9–122.9)	<0.001
Model 4 <sup>d</sup>	1.0	3.5 (0.4–28.9)	5.1 (0.7–39.6)	7.0 (0.9–55.4)	8.7 (1.1–71.3)	0.010
Incident cases of hypertension						
Cases/person-years	21/3675	72/4672	77/4202	68/2348	38/1268	
Incidence density, per 1000 person-years	5.7	15.4	18.3	29.0	30.0	
Adjusted RR						
Model 1 <sup>a</sup>	1.0	2.5 (1.5–4.1)	2.5 (1.5–4.0)	3.8 (2.3–6.4)	3.4 (1.9–6.0)	<0.001
Model 2 <sup>b</sup>	1.0	2.5 (1.5–4.1)	2.5 (1.5–4.0)	3.9 (2.3–6.4)	3.5 (2.0–6.1)	<0.001
Model 3 <sup>c</sup>	1.0	2.4 (1.5–4.0)	2.2 (1.3–3.6)	3.1 (1.8–5.3)	2.8 (1.6–5.1)	<0.001
Model 4 <sup>d</sup>	1.0	2.3 (1.4–3.9)	2.0 (1.2–3.3)	2.8 (1.6–4.8)	2.1 (1.1–3.8)	0.034

<sup>a</sup> Model 1: minimal adjustment for study center, race, sex, and age.<sup>b</sup> Model 2: model 1 plus adjustment for alcohol consumption.<sup>c</sup> Model 3: model 2 plus adjustment for BMI, cigarette smoking, and physical activity.<sup>d</sup> Model 4: model 3 plus adjustment for fasting serum glucose and insulin for diabetes and systolic blood pressure and insulin for hypertension.

#### INTERACTION BETWEEN GGT AND AGE OR BMI ON INCIDENCE OF DIABETES AND HYPERTENSION

When a 15-year risk of developing diabetes was examined, starting at a mean age of 25 years, there were no interactions between BMI and GGT on the development of diabetes and hypertension. However, when a 5-year risk was examined, starting with year 10 as the baseline

when the mean age was 35 years, the association of year 10 BMI with incident diabetes and/or hypertension was different depending on year 10 GGT (Figs. 1 and 2). Compared with individuals with year 10 GGT below the median, year 10 BMI among individuals with year 10 GGT above the median was more strongly associated with incident diabetes and/or hypertension than for those

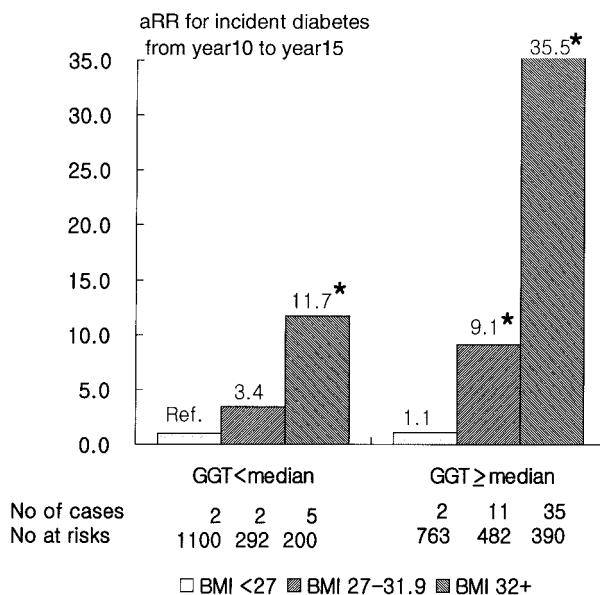


Fig. 1. Adjusted RR for incident diabetes by BMI at year 10 and serum GGT at year 10 in the CARDIA Study.

Adjusted for study center, race, sex, age, alcohol consumption, BMI, cigarette smoking, and physical activity. \*, 95% CI for the adjusted RR does not include 1.

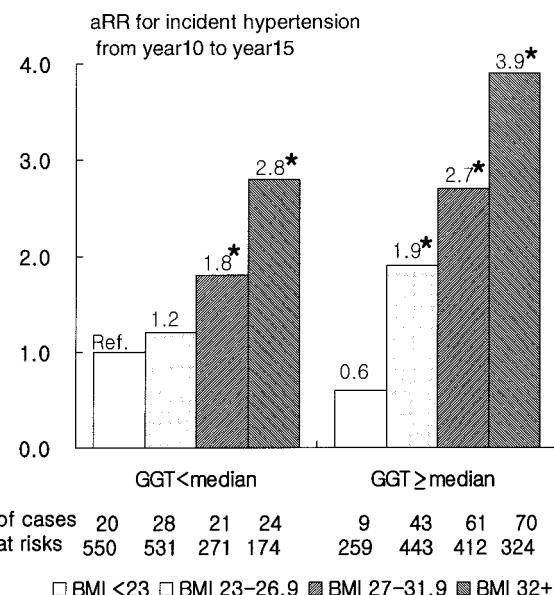


Fig. 2. Adjusted RR for incident hypertension by BMI at year 10 and serum GGT at year 10 in the CARDIA Study.

Adjusted for study center, race, sex, age, alcohol consumption, BMI, cigarette smoking, and physical activity. \*, 95% CI for the adjusted RR does not include 1.

with GGT below the median. No such interaction was seen in the 5-year risk (incident diabetes or hypertension at year 2 or 5), using year 0 GGT as baseline (data not shown). Although the stronger gradient of risk starting at age 35 than starting at age 25 (comparing Tables 3 and 4) is suggestive of an interaction between age and GGT and gradients of risk associated with age for both diabetes and hypertension were slightly higher among individuals with high-normal GGT, we observed no clear interaction of age and GGT on the development of diabetes or hypertension (data not shown).

### Discussion

In this prospective study of young black and white men and women, serum GGT concentrations measured at ages 18–30 years and mostly within the reference interval predicted the development of diabetes and/or hypertension during 15 years of follow-up in a dose-response relationship. These associations were not confounded by other lifestyle factors examined and did not differ materially by race or sex. Moreover, the coefficient for serum GGT and the 5-year risk of diabetes or hypertension, starting at a mean age of 35 years, was higher than that for long-term risk, starting at age 25 years. Our data are in agreement with results of previous prospective studies (6, 7, 9, 10), which showed that baseline serum GGT was an independent risk factor for the development of type 2 diabetes and hypertension.

Increased GGT is conventionally interpreted as a marker of alcohol abuse and/or liver damage (11). However, neither of these interpretations explains the association of GGT within its reference interval with incident diabetes and/or hypertension. In this study, the associations of GGT with incident diabetes and hypertension were independent of alcohol intake and were present in nondrinkers. Recently, fatty liver with a broad spectrum of pathologic conditions has also been linked to insulin resistance syndrome and/or type 2 diabetes (15, 16). GGT might therefore be interpreted as a marker for hepatic steatosis and hepatic insulin resistance in the pathogenesis of type 2 diabetes. However, in our participants, the dose-response relationship between GGT and incidence of diabetes and/or hypertension was observed among individuals with AST concentrations within the reference interval (<30 U/L); AST usually increases in cases of hepatic steatosis. In addition, the associations between AST and disease outcomes were weaker than those of GGT and were restricted to the highest AST concentrations; note that the highest category of AST is above the reference interval used by laboratories. Thus, neither alcohol consumption nor liver damage appear to explain the association of GGT with diabetes and/or hypertension.

Emerging evidence has shown that serum GGT might be an important enzyme in the pathogenesis of cardiovascular diseases. Consistent with such a role, population-based studies (1–3) have found a strong association

between serum GGT concentrations and many cardiovascular disease risk factors. Even in this young adult population, after adjusting for alcohol consumption, serum GGT concentration was associated with many cardiovascular disease risk factors, including black race, male gender, older age, cigarette smoking, BMI, higher blood pressure, higher fasting blood sugar, higher fasting blood triglycerides, higher blood LDL-cholesterol, and lower blood HDL-cholesterol. In addition, other variables, such as white blood cell count, red blood cell count, hematocrit, and hemoglobin, were positively associated with GGT.

At present, studies on GGT are at an early stage. Although the mechanism underlying the above associations remains largely unknown, some possible mechanisms exist. Previous experimental studies (17–19) have reported that GGT plays an important role in antioxidant systems with the primary function of maintaining intracellular concentrations of glutathione, a critical antioxidant defense for the cell. Increases in GGT activity can be a response to oxidative stress, marking increased transport of glutathione into cells. In addition, GGT is leaked into the serum, possibly as a result of normal cell turnover and cellular stresses. Thus, increased serum GGT may identify those individuals with low but persistent increases in oxidative and other cellular stresses. On the other hand, recent experimental studies (20–23) indicated that under physiologic conditions, GGT is involved directly in the generation of reactive oxygen species in the presence of iron or other transition metals. GGT might alternatively be a specific marker of oxidative stress, e.g., as a result of iron overload, because several experimental and epidemiologic studies have suggested a close relationship between iron overload and cellular or serum GGT activity (24, 25). Interestingly, a recent review (26) pointed out important influences of iron metabolism in type 2 diabetes. Diabetes and hypertension are interrelated diseases that strongly predispose affected individuals to atherosclerotic cardiovascular disease. Regardless of the type of diabetes, hypertension is two to three times more common among diabetic individuals than in nondiabetic individuals (27). Furthermore, both diabetes and hypertension are among the numerous pathologic conditions that are associated with increased vascular production of reactive oxygen species (28–30). Vascular oxidant stress, particularly interactions between NO and oxygen-derived radicals, represents a common pathologic mechanism in many risk factors for atherosclerosis.

In this study, serum GGT concentrations measured at ages 18–30 years predicted 15-year incidence of diabetes and/or hypertension and the future concentrations of oxidative stress and inflammation markers such as fibrinogen, uric acid, CRP, and F2-isoprostanes, which were measured at various times during the 15 years of follow-up. These observations suggest that GGT might be an early marker of oxidative or other cellular stress and that it is possibly directly related to the pathogenesis of

diabetes and hypertension, perhaps as an oxidative stressor itself.

Another interesting finding of this study was an interaction between BMI and GGT, both measured at a mean age of 35 years, in the prediction of 5-year incident diabetes. This analysis was motivated by our previous finding in Korean men, in whom there were interactions between age and/or BMI and GGT in the development of diabetes during 4 years (10). In partial agreement with our previous findings, we observed that BMI was a stronger risk factor for incident diabetes and/or hypertension among individuals with GGT concentrations greater than the median. No such interaction was observed between year 0 GGT and year 0 BMI, measured at a mean age of 25 years, and 15-year risk. A possible interpretation of this interaction is that obese individuals with high-normal GGT have already suffered subclinical pathologic changes as a result of obesity, whereas obese individuals with low-normal GGT are at an earlier stage of pathogenesis. According to this interpretation, the serum GGT concentration might be an intervening factor in the association between obesity and diabetes. However, relatively high GGT was not predictive of either incident diabetes or and/or hypertension among the leaner participants. Therefore, some other condition may be necessary for pathogenesis, in addition to relatively high GGT alone; among the possible conditions is high body iron (20–23). For example, GGT may play an antioxidant or a prooxidant role, depending on the presence of iron (20–23). In this regard, it is interesting that population studies have reported a positive association between BMI and/or age and serum ferritin, a marker of body iron storage (31, 32). In our previous study of Korean men (10), age was a strong risk factor of diabetes only among individuals with high-normal GGT; CARDIA data, however, failed to show an interaction of GGT and diabetes or hypertension with age. This divergence of findings may arise in part because of the difference in age distribution between the two cohorts. In our previous study, the interaction with age was largely restricted to participants who were 45 years of age or older. The oldest CARDIA participant was 45 years of age at year 15 of follow-up. Furthermore, age did play a role in the interaction of GGT with BMI and incident diabetes: the interaction was found only for GGT and BMI measured at a mean age of 35 years.

Although this report shows that GGT provides significant prognostic value above and beyond that provided by traditional risk factors, GGT alone has inadequate sensitivity to be used as a screening tool in clinical management. Furthermore, although the exact shape of the relationship between GGT and either diabetes or increased blood pressure is not known, the evidence presented is consistent with an increase in risk that is gradual as GGT increases. At present, it is uncertain whether serum GGT has a role in the causal pathway of diabetes or hypertension; GGT may be only a marker of risk. However, the information presented is consistent

with such a role and enhances the importance of further study of GGT, either as a marker of oxidative or other stress, such as iron overload, or as an etiologic agent in itself.

Single measurements of fasting blood sugar or blood pressure for diagnosis of diabetes or hypertension in our study is a limitation typical of epidemiologic studies. Although diagnosis based on a single measurement is not acceptable clinically in individual patients because of random fluctuations, it is generally accepted as a diabetes criterion in epidemiologic studies. Random error attributable to a single determination usually leads to an attenuated estimate of the strength of association.

In conclusion, this study suggests that serum GGT is a strong predictor of diabetes and hypertension. Neither alcohol consumption nor liver damage likely explains this association. We speculate that it might be involved in the pathogenesis of diabetes and hypertension through a mechanism related to oxidative stress. In addition, the well-known associations of BMI with diabetes and hypertension may be modified by serum GGT concentrations.

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