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Original Contribution

Use of A Mendelian Randomization Approach to Assess the Causal Relation of γ-Glutamyltransferase with Blood Pressure and Serum Insulin Levels

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Elevated levels of γ-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 γ-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: β −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, γ-glutamyltransferase; *GGT1*, γ-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 γ-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 γ-glutamyltransferase 1 (*GGT1*) gene (±100 kilobases) and were therefore considered for the present analysis.

Baseline characteristics were compared across sex-specific GGT quartiles using Kruskal-Wallis tests for continuous 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 $(m)^2$), 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 $\times$ 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 $\times$ log GGT interaction in OLS analyses, we also present results stratified by age group. This age $\times$ 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 $\times$ 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 γ-Glutamyltransferase Level, CoLaus Study, Lausanne, Switzerland, 2003–2006

Characteristic	Sex-Specific Quartile of γ-Glutamyltransferase												*P* Value[a]
	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.	%	
γ-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[b]	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, μU/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, μg/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, μmol/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*, γ-glutamyltransferase 1; IQR, interquartile range.

[a] Kruskal-Wallis test for continuous variables and chi-squared test for categorical variables.

[b] Weight (kg)/height $(m)^2$.

Table 2. Change in Systolic and Diastolic Blood Pressures of Participants (mm Hg) According to Sex-Specific Quartile of γ-Glutamyltransferase, CoLaus Study, Lausanne, Switzerland, 2003–2006

Variable and Regression Model	Sex-Specific Quartile of γ-Glutamyltransferase							*P* for Trend[a]
	1 (*n* = 1,068)	2 (*n* = 1,077)		3 (*n* = 1,115)		4 (*n* = 1,100)		
	(β = 0)	β	*P* Value	β	*P* Value	β	*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[b]	Referent	1.5	0.02	2.6	0.0001	4.4	<0.0001	<0.0001
Multivariable-adjusted 2[c]	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[b]	Referent	1.3	0.003	1.9	<0.0001	2.6	<0.0001	<0.0001
Multivariable-adjusted 2[d]	Referent	0.9	0.03	1.0	0.02	1.2	0.01	0.08

Abbreviation: CoLaus, Cohorte Lausannoise.

[a] *P* value for linear trend across quartiles of γ-glutamyltransferase.

[b] Adjusted for age, sex, body mass index, alcohol consumption, and smoking.

[c] Adjusted for age, sex, body mass index, alcohol consumption, smoking, C-reactive protein, albumin, aspartate aminotransferase, antihypertensive treatment, lipid-lowering treatment, and total cholesterol.

[d] 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 × 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 (μU/L) According to Sex-Specific Quartile of γ-Glutamyltransferase, CoLaus Study, Lausanne, Switzerland, 2003–2006

Regression Model	Sex-Specific Quartile of γ-Glutamyltransferase							*P* for Trend[a]
	1 (*n* = 1,068)	2 (*n* = 1,077)		3 (*n* = 1,115)		4 (*n* = 1,100)		
	(β = 0)	β	*P* Value	β	*P* Value	β	*P* Value	
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[b]	Referent	0.08	0.0005	0.15	<0.0001	0.23	<0.0001	<0.0001
Multivariable-adjusted 2[c]	Referent	0.04	0.08	0.08	0.0005	0.09	0.0002	<0.0001

Abbreviation: CoLaus, Cohorte Lausannoise.

[a] *P* value for linear trend across quartiles of γ-glutamyltransferase.

[b] Adjusted for age, sex, body mass index, alcohol consumption, and smoking.

[c] 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 γ-Glutamyltransferase Level Using an Instrumental-Variable Approach, CoLaus Study, Lausanne, Switzerland, 2003–2006[a]

Variable and Regression Model	No. of Participants	Ordinary Least Squares			2-Stage Least Squares				*P* for Difference[b]
		β	95% CI	*P* Value	β	95% CI	*P* Value	*F* Value (First Stage)	
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[c]	4,359	2.52	1.73, 3.31	<0.0001	−4.82	−10.61, 0.96	0.102		0.012
Multivariable-adjusted 2[d]	4,359	1.30	0.32, 2.03	0.007	−5.68	−11.51, 0.16	0.056		0.020
Age group									
≥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
≥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.

[a] Systolic blood pressure was the dependent variable, and γ-glutamyltransferase was the independent variable of interest. Data on γ-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 γ-glutamyltransferase was <0.0001 for ordinary least squares and 0.055 for 2-stage least squares.

[b] *P* value for the difference between ordinary least squares and 2-stage least squares estimates (Durbin-Hausman test).

[c] Adjusted for age, sex, body mass index, smoking, and alcohol consumption.

[d] 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 (μU/L) According to γ-Glutamyltransferase Level Using an Instrumental-Variable Approach, Lausanne, Switzerland, 2003–2006[a]

Variable and Regression Model	No. of Participants	Ordinary Least Squares			2-Stage Least Squares				*P* for Difference[b]
		β	95% CI	*P* Value	β	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[c]	4,359	0.14	0.12, 0.17	<0.0001	0.18	−0.01, 0.36	0.055		0.713
Multivariable-adjusted 2[d]	4,307	0.07	0.04, 0.09	<0.0001	0.19	0.01, 0.37	0.042		0.177
Body mass index[e]									
<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
≥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
≥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.

[a] Insulin was the dependent variable, and γ-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 γ-glutamyltransferase was <0.0001 for ordinary least squares and 0.814 for 2-stage least squares.

[b] *P* value for the difference between ordinary least squares and 2-stage least squares estimates (Durbin-Hausman test).

[c] Adjusted for age, sex, body mass index, smoking, and alcohol consumption.

[d] 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.

[e] Weight (kg)/height $(m)^2$.

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 pathophysiologic mechanisms.

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(Appendix tables follow)

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.	%	Median (IQR)	No.	%	
γ-Glutamyltransferase, U/L	20 (14–32)			23 (15–36)			25 (18–42)			<0.0001
Age, years	53 (45–62)			54 (45–63)			53 (44–62)			0.29
Sex		798	46.1		991	48.8		301	50.4	0.11
Body mass index[a]	25.6 (22.9–28.5)			25.7 (23.2–28.6)			25.7 (23.1–28.6)			0.49
% body fat	29 (23–37)			29 (23–36)			28 (23–36)			0.42
Diabetes mellitus		127	7.3		125	6.2		47	7.9	0.20
Antidiabetic treatment		70	4.0		67	3.3		30	5.0	0.13
Systolic blood pressure, mm Hg	127 (117–140)			128 (117–140)			126 (117–138)			0.71
Diastolic blood pressure, mm Hg	79 (73–87)			80 (73–87)			79 (73–86)			0.89
Hypertension		659	38.1		783	38.5		222	37.2	0.83
Antihypertensive treatment		321	18.5		406	20.0		119	19.9	0.51
History of lipid-lowering treatment		209	12.1		253	12.5		76	12.7	0.90
Triglycerides, mmol/L	1.2 (0.8–1.7)			1.1 (0.8–1.7)			1.2 (0.9–1.6)			0.41
Total cholesterol, mmol/L	5.6 (4.9–6.3)			5.6 (4.9–6.2)			5.5 (4.9–6.3)			0.69
High density lipoprotein cholesterol, mmol/L	1.6 (1.3–1.9)			1.6 (1.3–1.9)			1.5 (1.3–1.9)			0.26
Current smoking		477	27.6		507	25.0		147	24.6	0.14
Alcohol consumption, drinks/day	0.6 (0.0–1.4)			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)			0.8 (0.6–1.0)			0.66
Aspartate aminotransferase, U/L	27 (23–33)			27 (23–34)			27 (23–33)			0.23
Alanine aminotransferase, U/L	23 (17–32)			23 (18–33)			23 (18–34)			0.32
Insulin, μU/L	7.0 (4.9–10.0)			7.0 (5.0–11.0)			7.0 (4.8–11.3)			0.16
Glucose, mmol/L	5.4 (5.0–5.9)			5.4 (5.1–5.8)			5.4 (5.1–5.8)			0.91
Adiponectin, μg/mL	8.30 (5.05–12.54)			7.93 (5.04–12.43)			8.24 (5.09–12.62)			0.62
Uric acid, μmol/L	308 (253–368)			313 (259–373)			308 (261–369)			0.14
C-reactive protein, mg/L	1.4 (0.6–2.9)			1.3 (0.7–2.9)			1.4 (0.7–2.9)			0.80
Albumin, g/L	44 (43–46)			44 (43–46)			44 (43–46)			0.29
Protein, g/L	75 (72–77)			75 (72–78)			75 (72–77)			0.70

Abbreviations: CoLaus, Cohorte Lausannoise; *GGT1*, γ-glutamyltransferase 1; IQR, interquartile range.
[a] Weight (kg)/height $(m)^2$.

Appendix Table 2. Change in Blood Pressure (mm Hg) or Insulin Level (μU/L) According to Sex-Specific Quartile of γ-Glutamyltransferase in Subgroup Analyses, CoLaus Study, Lausanne, Switzerland, 2003–2006

Characteristic	Sex-Specific Quartile of γ-Glutamyltransferase							*P* for Interaction[a]
	1	2		3		4		
	(β = 0)	β	*P* Value	β	*P* Value	β	*P* Value	
Age group								
≥55 years	*n* = 325	*n* = 449		*n* = 600		*n* = 635		
SBP[b]	Referent	1.0	0.41	−0.2	0.89	0.9	0.47	0.001
DBP[b]	Referent	0.2	0.76	0.2	0.76	−0.2	0.80	<0.0001
Insulin level[c]	Referent	0.02	0.54	0.07	0.04	0.07	0.07	0.16
<55 years	*n* = 743	*n* = 628		*n* = 515		*n* = 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	
Sex								
Male	*n* = 481	*n* = 541		*n* = 529		*n* = 539		
SBP	Referent	0.9	0.34	1.3	0.17	2.8	0.008	0.62
DBP	Referent	0.8	0.20	1.2	0.08	1.5	0.04	0.79
Insulin level	Referent	0.03	0.31	0.04	0.30	0.09	0.02	0.37
Female	*n* = 587	*n* = 536		*n* = 586		*n* = 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	
Body mass index[d]								
≥25	*n* = 400	*n* = 559		*n* = 712		*n* = 771		
SBP	Referent	0.4	0.67	0.6	0.57	1.3	0.22	0.25
DBP	Referent	0.5	0.42	0.6	0.38	0.9	0.18	0.32
Insulin level	Referent	0.08	0.01	0.13	<0.0001	0.16	<0.0001	<0.0001
<25	*n* = 668	*n* = 517		*n* = 404		*n* = 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	
Alcohol consumption								
≥1 drink/day	*n* = 721	*n* = 779		*n* = 831		*n* = 842		
SBP	Referent	0.5	0.56	1.6	0.04	2.7	0.002	0.77
DBP	Referent	1.0	0.04	1.5	0.004	1.7	0.002	0.70
Insulin level	Referent	0.06	0.01	0.06	0.03	0.08	0.005	0.40
<1 drink/day	*n* = 347	*n* = 298		*n* = 284		*n* = 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.

[a] For every individual trait, multiplicative interaction tests were conducted using nonstratified multivariable regression models.

[b] Adjusted for age, sex, body mass index, alcohol consumption, smoking, C-reactive protein, albumin, aspartate aminotransferase, antihypertensive treatment, lipid-lowering treatment, and total cholesterol.

[c] 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.

[d] Weight (kg)/height $(m)^2$.