Original Article

# 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 fluorooxalated 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:

$$\mathrm{FLI} = \frac{e^{L}}{(1+e^{L})} \times 100$$

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 standardized 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 ($6$–$6^2H_2$)

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 ± 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 (*rSp*) 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 ± 9 | 48 ± 9 | <0.0001 |
| Men (%) | 38% | 54% | 0.0001 |
| BMI (kg/m$^2$) | 23.2 ± 3.0 | 24.8 ± 3.2 | <0.0001 |
| Waist circumference (cm) | 78 ± 9 | 84 ± 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 ± 0.5 | 5.4 ± 0.8 | <0.0001 |
| Fasting insulin (pmol/l)[a] | 39.9 (20.8) | 45.3 (23.3) | 0.0001 |
| HOMA-IR[a] | 1.11 (0.7) | 1.35 (0.9) | <0.0001 |
| FLI | 16.1 ± 18.2 | 29.4 ± 24.2 | <0.0001 |
| GGT (IU/l)[a] | 18 (13) | 23 (21) | <0.0001 |
| ALT (IU/l)[a] | 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.
[a]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

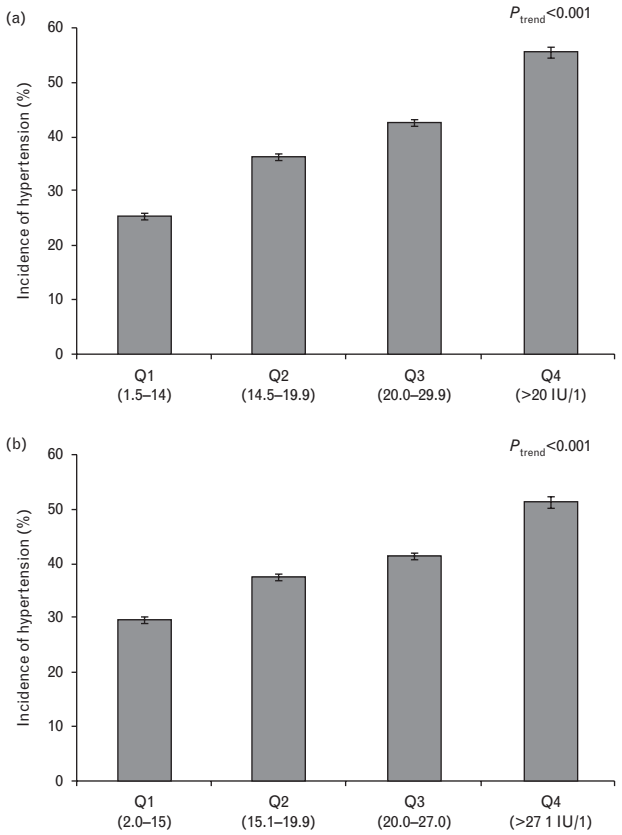


**FIGURE 1** Crude 9-year incidence of hypertension (standard error) in the data from an epidemiological study on the insulin resistance cohort, according to quartile groups of (a) gamma-glutamyltransferase and (b) alanine aminotransferase.

**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.

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 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 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 $\left(r_{S_p} = 0.36, P < 0.0001\right)$ and DBP $\left(r_{S_p} = 0.28, P < 0.0001\right)$ 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 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 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 ≥ 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).

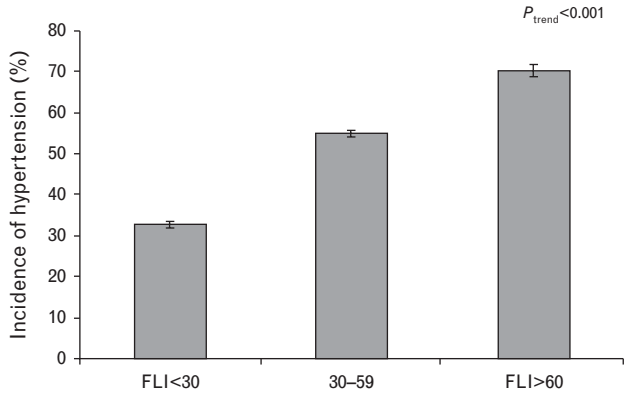


**FIGURE 2** Crude 9-year incidence of hypertension (standard error) in the data from an epidemiological study on the insulin resistance cohort, according to categories of the fatty liver index.

## 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 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

**TABLE 3. Associations between categories of fatty liver index at baseline and the risk of incident hypertension in the data from an epidemiological study on the insulin resistance cohort**

| FLI | *n* | OR (95% CI) | *P* |
|---|---|---|---|
| $FLI_{year\ 0} < 30$ | 1893 | 1 (reference) | |
| $FLI_{year\ 0}$ 30–60 | 452 | 1.73 (1.36–2.21) | 0.0001 |
| $FLI_{year\ 0} \geq 60$ | 220 | 3.02 (2.15–4.26) | <0.0001 |

Logistic regression analysis with adjustment for age, sex, smoking, fasting glucose and alcohol intake. CI, confidence interval; FLI, fatty liver index; OR; odds ratio.

**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 ± 7.9 | 47.6 ± 7.7 | <0.0001 |
| Men (%) | 49% | 62% | 0.07 |
| BMI (kg/m$^2$) | 25.5 ± 3.8 | 27.4 ± 3.7 | 0.0005 |
| Waist circumference (cm) | 87 ± 13 | 92 ± 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 ± 0.5 | 5.3 ± 0.7 | <0.0001 |
| Fasting insulin (pmol/l)[a] | 32.0 (20.0) | 39.0 (28.7) | <0.0001 |
| Hepatic insulin resistance index[a] | 0.38 (0.28) | 0.48 (0.50) | 0.003 |
| FLI | 31.3 ± 26.9 | 30.9 ± 25.2 | 0.85 |
| GGT (IU/l)[a] | 20 (12) | 25 (18) | <0.0001 |
| ALT (IU/l)[a] | 18 (11) | 21 (11) | 0.002 |

Data shown are as mean ± SD, median (interquartile range) or %. ALT, alanine aminotransferase; FLI, fatty liver index; GGT, gamma-glutamyltransferase.
[a]Log-transformed for analysis.

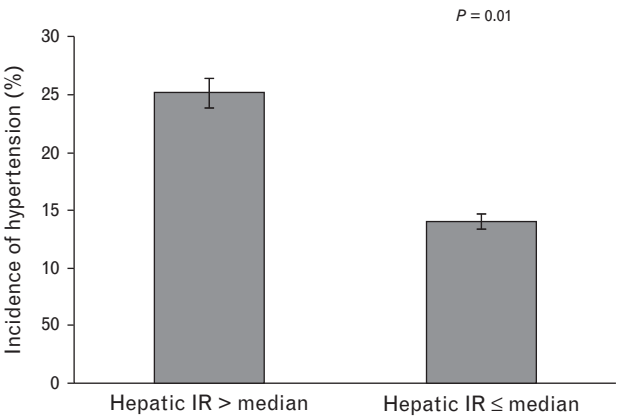


**FIGURE 3** Crude 9-year incidence of hypertension (standard error) in the RISC cohort, according to the hepatic insulin resistance index above or below the median value.

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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## Conflicts of interest

There are no conflicts of interest. All authors read and approved the final manuscript.

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## Reviewer's Summary Evaluations

### Reviewer 2

Studied was the association between liver markers, hepatic insulin resistance and incident hypertension in two longitudinal studies. This study is very clinically relevant and timely, as reports are surfacing that fatty liver disease increases the risk for cardiovascular disease. The authors used indirect measures of liver injury (circulating liver enzyme levels), fatty liver index (calculated using body mass index, waist circumference, triglycerides and liver enzyme levels), and the hepatic insulin resistance index in their correlative studies. Therefore, the direct integrative mechanisms and global physiological impact of fatty liver disease on promoting hypertension and cardiovascular disease remain unknown.