Ultrasonographic Hepatic Steatosis Increases Prediction of Mortality Risk From Elevated Serum Gamma-Glutamyl Transpeptidase Levels

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The aim of the present study was to investigate the association of serum gamma-glutamyltransferase (GGT) levels with all-cause mortality and to assess the impact of ultrasonographic findings of hepatic hyperechogenicity in that association. We used data from 4,160 subjects (2,044 men and 2,116 women) recruited for the population-based Study of Health in Pomerania (SHIP) without baseline hepatitis B and C infections or liver cirrhosis. GGT was divided into age- and sex-dependent quintiles to calculate overall and sex-specific crude incidence mortality rates. Hepatic steatosis was defined by elevated GGT levels (>80%) and the presence of hyperechogenic liver ultrasound. We used multiple-adjusted Cox proportional hazards regression models, first, to assess the direct effect of GGT on all-cause mortality, second, to stratify according to the ultrasonographic finding, and third, to investigate potential mediating effects of cardiometabolic risk factors. During 29,810 person-years (7.3 years, median) of follow-up, 307 individuals (7.5%) died, resulting in a death rate of 0.86 deaths per 1000 person-years. Elevated GGT levels were associated with increased risk of mortality in men (hazard ratio [HR] 1.49; 95% confidence interval [CI], 1.08-2.05), but not in women (HR 1.30; 95% CI, 0.80-2.12). This association was even stronger in men with hepatic steatosis (HR 1.98; 95% CI, 1.21-3.27). Cause-specific mortality analysis by cardiovascular disease deaths confirmed the sex-specific association. Adjustment for cardiometabolic risk factors did not affect the estimates. *Conclusion:* In the case of increased GGT levels, liver ultrasound should be performed, not only for diagnosis, but also for further risk stratification. (HEPATOLOGY **2009;50:1403-1411.**)

Serum gamma-glutamyltransferase (GGT) has been widely used as an index of liver dysfunction and a marker of alcohol intake. But despite its clinical use, several recent epidemiological studies cast light on elevated GGT levels as an independent predictor of morbidity and mortality. Among 7,613 middle-aged British men followed for 11.5 years, elevated GGT levels were independently associated with a significant increase in all-cause and cardiovascular disease (CVD) mortality.[1] Two prospective population-based studies in Austrian adults also reported an association of elevated GGT levels with increased risk of CVD mortality.[2,3] However, pathways linking GGT to mortality risk are not fully understood and several mediating variables have been proposed. For example, GGT was found to be positively associated with metabolic syndrome,[4,5] type 2 diabetes,[6,7] hypertension,[8] and stroke.[9,10] Hepatic steatosis or fatty liver, as a common finding in the general population,[11] has also been discussed as a possible indirect mechanism to explain the GGT/mortality association.[12,13] Given the links between GGT, hypertension, carotid atherosclerosis, type 2 diabetes,[14-16] features of the metabolic syndrome,[17] and hepatic

Abbreviations: CVD, cardiovascular disease; GGT, gamma-glutamyltransferase; HBsAg, hepatitis B surface antigen; hs-CRP, high-sensitivity C-reactive protein; ICD-10, International Classification of Diseases, 10th revision SHIP, Study of Health in Pomerania; WC, waist circumference.

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steatosis,[18] we assumed that these meditational variables might, at least in part, account for the relation of GGT with mortality.

The aim of the present study was two-fold. First, to investigate the direct effects of GGT levels on all-cause mortality in a population-based sample of 4160 subjects (2044 men and 2,116 women). And second, to assess the impact of ultrasonographic finding of hepatic hyperechogenicity in that association, as well as potential mediating effects of cardiometabolic variables.

Patients and Methods

Study Population. The Study of Health in Pomerania (SHIP) is a population-based cohort study in West Pomerania, a region in northeastern Germany. Details on the SHIP study design and data have been published.[19,20] In brief, from the 212,157 inhabitants living in the area, a representative sample of 7008 subjects aged between 20 and 79 years was selected using population registries where all German inhabitants are registered. Only individuals with German citizenship and main residency in the study area were included. The net sample (without migrated or deceased persons) comprised 6,267 eligible subjects, whereof 4,310 finally participated (response proportion 68.8%). All participants gave written informed consent. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in an *a priori* approval by the local Ethics Committee of the University of Greifswald.

Of the 4,310 SHIP participants, 54 had an uncertain diagnosis of liver echography, 14 tested positive for hepatitis B surface antigen (HBsAg), 20 for hepatitis C virus antibody (anti-HCV), and 11 had a known history of liver cirrhosis. Furthermore, 51 participants lacked measured GGT. In all, 150 participants were excluded from further analysis. This resulted in a study population of 4,160 subjects (2,044 men and 2,116 women) who were available for the present analysis.

Measures. Baseline data included socioeconomic characteristics, behavioral risk factors, individual medical history, as well as sonographic, laboratory, and somatometric examinations. Behavioral risk factors and socioeconomic characteristics were assessed by computer-aided personal interviews and included sex, age, educational level (<10, $=10$, or >10 years at school), civil status (living alone versus married or cohabiting), and "equalized" household income (in Euros). Because income is a household-level variable, the "equivalence" criteria allow for economies of scale at the household level. We used the commonly adopted procedure of the Luxembourg Income Study to divide the household income by the square root of the number of household members, thus assuming an equivalence parameter of 0.5.[21] Subjects who participated in physical training during summer or winter for at least 1 hour a week were classified as being physically active. Alcohol consumption was evaluated as beverage-specific alcohol consumption (beer, wine, and distilled spirits) on the last weekend and last weekday preceding the examination, and the mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions.[22] Medical history included a recall of physician's diagnoses of diabetes mellitus, stroke, and myocardial infarction. Peripheral artery disease was defined according the four stages proposed by Rose et al.[23] Definition of heart failure was based on recommendations of the New York Heart Association,[24] and angina pectoris on the Rose Angina Questionnaire.[23] Comorbid health status was measured by the Functional Comorbidity Index, which is a summary measure of comorbid diseases selected and weighted according to their association with physical functioning.[25]

Sonographic examinations were performed by trained physicians who were unaware of the participant's clinical and laboratory characteristics. Liver ultrasound was performed using a 5 MHz transducer and a high-resolution instrument (Vingmed VST Gateway, Santa Clara, CA). A hyperechogenic liver ultrasound was defined as the presence of an ultrasound pattern of a bright liver, with evident density differences between hepatic and renal parenchyma.[26] In SHIP, ultrasound examinations and readings underlie strict quality standards.[27-30] For the laboratory examinations, nonfasting blood samples were drawn from the cubital vein in the supine position. The laboratory takes part quarterly in the official national German external proficiency testing programs. In addition, internal quality controls were analyzed daily. Markers of HBsAg and anti-HCV were determined by enzyme-linked immunosorbent assays (AxSym HBSAG and AxSym HCV, Abbott, Abbott Park, IL). High-sensitivity C-reactive protein (hs-CRP) was determined immunologically on a Behring Nephelometer II with commercially available reagents from Dade Behring (Eschborn, Germany). The assay was performed according to the manufacturer's recommendations with a test sensitivity of 0.2 mg/L. Serum GGT levels were measured photometrically (Hitachi 717; Roche Diagnostics, Mannheim, Germany), and categorized by sex-specific quintiles, expressed as μmol/L $\times$ s, which corresponds to (μmol/L $\times$ s) $\times$ 60 = IU/L. Assays were performed according to the manufacturers' recommendations. Hepatic steatosis was defined by elevated GGT levels ($>$80%) together with hyperechogenic liver ultrasound. Waist circumference (WC) was measured to the nearest 0.1 cm using an inelas-

tic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the subject standing comfortably with weight distributed evenly on both feet. Information on vital status was acquired at regular intervals from the time of enrollment into the study through August 31, 2007. The mean duration of follow-up was 7.2 years (25th, 6.6; 75th, 8.0). Subjects were censored at the time of death, based on death certificates requested from the local health authority of the place of death. The number of months between baseline examination and censoring was used as follow-up length. Causes of deaths were coded by a certified nosologist according to the International Classification of Diseases, 10th revision (ICD-10). Additionally, two internists (H.W. and M.D.) independently validated the underlying cause of death and performed a joint reading in cases of disagreement. A third internist (H.V.) finally decided in cases of still existing disagreement.

Statistical Analysis. Data on quantitative characteristics are expressed as mean with standard deviation. Data on qualitative characteristics are expressed as percent values. A χ^2 test (qualitative data) or Mann-Whitney U test (quantitative data) were performed for intergroup comparisons by sex. GGT was divided by age- (in decades) and sex-specific quintiles (according cutoffs provided in Supporting Table 1) to calculate overall and sex-specific crude mortality incidence rates (per 1000 person-years). To assess the association between GGT and mortality we used Cox proportional hazards regression models. Three successive regression models were estimated accounting for different covariates: the first only included GGT quintiles as predictors, the second added age (in decades), and the third additionally adjusted for WC, alcohol consumption, physical activity, educational level, civil status, equalized income, and Functional Comorbidity Index. Next, the association of GGT with mortality was stratified by ultrasonographic finding of hepatic hyperechogenicity, assessing interaction by a product term of continuous GGT and bivariate liver ultrasound. To test for trend across quintiles of GGT, we estimated Cox models in which the categorical GGT variable appeared as a continuous predictor. Kaplan-Meier survival curves were used to illustrate the association of hepatic steatosis and mortality, with differences tested by a log-rank test. To compare the predictability of GGT with liver ultrasound we estimated area under receiver operating characteristic curves (AUCs area) from full-adjusted Cox models and tested their difference. Sensitivity analyses were performed excluding heavy drinkers (>60 g/day for men and >30 g/day for women), or nondrinkers (<10 g/day for men and women). Finally, models testing for mediation estimated the association of GGT with mortality in multivariate Cox models, controlling for the intermediate variable each in a separate model. We analyzed mediating effects of diabetes, a summative CVD score[31] comprising angina pectoris, peripheral artery disease, heart failure, stroke, myocardial infarction, and hs-CRP. We used both graphical and hypotheses testing methods for examining the proportional hazard assumption.[32] Schoenfeld's tests as well as visual inspection of smoothed estimates of the hazard ratio (HR) against time confirmed the proportional hazard assumption for all variables. HRs were calculated with a 95% confidence interval (95% CI). Two-sided probability values <0.05 were considered statistically significant. This article was written in accordance with the STROBE statement, giving guidelines for reporting of observational studies.[33] All statistical analyses were performed using Stata 9 (Stata, College Station, TX).

Results

Baseline characteristics stratified by sex are presented in Table 1. Compared to women, men were older, less often living alone, had higher income, and fewer years of schooling. Mean baseline levels of GGT and the prevalence of hyperechogenic liver ultrasound were significantly higher among men than women (Table 1). The overall prevalence of hepatic steatosis was 10%; 12.3% in men and 7.8% in women. Differences between men and women were also found in WC, alcohol consumption, hs-CRP, and prevalence of peripheral artery disease, heart failure, stroke, and myocardial infarction (Table 1).

During 29,810 person-years (7.3 years, median) of follow-up, 307 individuals (7.5%) died, resulting in a death rate of 0.86 deaths per 1000 person-years. Overall and sex-specific crude mortality incidence rates by quintiles of GGT are presented in Table 2. Overall rates tended to increase over GGT quintiles, whereas sex-specific rates showed differences only for subjects in the upper quintiles (Table 2). Crude, age-, and multiple-adjusted HRs are presented in Table 3. In men, Cox proportional hazard regression models revealed a positive association between elevated GGT levels and mortality in the crude (HR 1.52; 95% CI, 1.01-2.26) and age-adjusted model (HR 1.58; 95% CI, 1.06-2.36), but not in the multiple-adjusted model (HR 1.46; 95% CI, 0.94-2.05). We detected no evidence of trend over quintiles of GGT. Cox models with the highest GGT quintile versus all lower revealed positive associations between elevated GGT levels and mortality throughout all models (Table 3). In women, we detected no association between elevated GGT levels and mortality in any applied model, whereas a significant trend across GGT quintiles in crude and age-adjusted models was found (Table 3).

Table 1. Baseline Characteristics by Sex

Characteristic	Men (N = 2044)	Women (N = 2116)	*P* Value*
Age, years	50.56 (16.6)	48.74 (16.2)	<0.001
Civil status, living alone	19.7	29.3	<0.001
Equalized household income, Euros	1003.6 (495.7)	931.8 (456.9)	<0.001
Educational level, years of schooling			
<10 years	42.2	37.2	
=10 years	41.7	48.2	
>10 years	16.1	14.6	<0.001
Serum gamma-glutamyl transpeptidase, μmol/L s	0.80 (1.69)	0.37 (0.51)	<0.001
Hyperechogenic ultrasound pattern	38.1	22.2	<0.001
Hepatic steatosis	12.3	7.8	<0.001
Waist circumference, cm	95.6 (11.7)	83.2 (13.1)	<0.001
Alcohol consumption, g/day	19.6 (23.0)	5.3 (9.0)	<0.001
High-sensitivity C-reactive protein, mg/L	2.79 (6.31)	2.99 (4.43)	<0.001
Physical activity	41.4	43.4	0.184
Functional Comorbidity Index	1.2 (1.3)	1.3 (1.4)	0.668
Diabetes mellitus	8.8	7.3	0.070
Angina	3.8	4.6	0.165
Peripheral artery disease	1.0	1.8	0.016
Heart failure	10.3	13.7	<0.001
Stroke	3.1	1.4	<0.001
Myocardial infarction	5.5	1.4	<0.001

Data are percentages or means (standard deviation).
Functional Comorbidity Index is a summary measure of comorbid diseases selected and weighted according to their association with physical functioning.
*P values were calculated with χ^2 test for categorical and Mann-Whitney U test for continuous variables.

We further ran Cox models for the association of GGT and mortality, stratified by ultrasonographic finding of hepatic hyperechogenicity (Table 4). The results indicate that elevated GGT was strongly associated with an increased risk of mortality in men with a hyperechogenic liver ultrasound (HR 3.01; 95% CI, 1.28-3.64). Furthermore, among men with a hyperechogenic liver, P for trend statistics revealed a significant trend for increased mortality risk across GGT quintiles. Additionally, Fig. 1 shows that subjects with hepatic steatosis had significantly shorter survival times compared to subjects without hepatic steatosis (log-rank test, $P < 0.001$). Hyperechogenic liver alone was not a predictor for mortality risk (age-adjusted model: men: HR, 0.92; 95% CI, 0.70; 1.21; women: HR, 1.22; 95% CI, 0.80; 1.84). Full adjusted models confirmed the missing association (men: HR, 0.77; 95% CI, 0.57; 1.04; women: HR, 0.98; 95% CI, 0.63; 1.53). Analyzing predictability, we found that GGT has the best predictive ability in terms of mortality risk with AUCs area of 0.774, compared to 0.609 for liver ultrasound. Test of differences in AUCs area revealed a P-value of <0.001. We further investigated the mediating effect of diabetes, CVD, and hs-CRP in the association of GGT with all-cause mortality (Table 5).

Table 2. Overall and Sex-Specific Crude Incidence Rates of All-Cause Mortality by Quintiles of Gamma-Glutamyl Transpeptidase (GGT)

	GGT Levels in Quintiles (μmol/L s)				
	1. Quintile	2. Quintile	3. Quintile	4. Quintile	5. Quintile
Men and women					
N	907	837	802	806	808
Person-years	6483	6064	5749	5801	5712
Number of deaths (crude incidence rate per 1000 person-years)	58 (8.9)	59 (9.7)	51 (8.9)	59 (10.2)	80 (14.0)
Men					
N	427	413	401	405	398
Person-years	3007	2948	2813	2880	2775
Number of deaths	41 (12.4)	43 (14.6)	39 (13.9)	35 (12.2)	57 (20.5)
Women					
N	480	424	401	401	410
Person-years	3476	3116	2936	2921	2938
Number of deaths	17 (4.9)	16 (5.1)	12 (4.1)	24 (8.2)	23 (7.8)

Table 3. Association of Gamma-Glutamyl Transpeptidase (GGT) in Quintiles with All-Cause Mortality Stratified by Sex

GGT	HR (95% CI) for All-Cause Mortality					
	Men			Women		
(Ref.: 1.Quintile)	Crude	Age-Adjusted	Multiple-Adjusted†	Crude	Age-Adjusted	Multiple-Adjusted†
2. Quintile	1.06 (0.69; 1.63)	1.06 (0.69; 1.62)	1.06 (0.68; 1.64)	1.04 (0.53; 2.06)	0.92 (0.47; 1.83)	0.97 (0.48; 1.95)
3. Quintile	1.02 (0.66; 1.58)	1.00 (0.65; 1.55)	1.00 (0.64; 1.57)	0.83 (0.40; 1.74)	0.82 (0.39; 1.71)	0.68 (0.32; 1.47)
4. Quintile	0.89 (0.57; 1.40)	0.91 (0.58; 1.42)	0.87 (0.54; 1.41)	1.67 (0.90; 3.10)	1.45 (0.78; 2.70)	1.22 (0.63; 2.36)
5. Quintile	1.52 (1.01; 2.26)*	1.58 (1.06; 2.36)*	1.46 (0.94; 2.24)	1.60 (0.86; 3.00)	1.62 (0.87; 3.04)	1.27 (0.65; 2.46)
5. vs. 1.-4. Quintile	1.52 (1.13; 2.06)*	1.59 (1.18; 2.16)*	1.49 (1.08; 2.05)*	1.42 (0.89; 2.28)	1.54 (0.96; 2.47)	1.30 (0.80; 2.12)
P for Trend	0.115	0.072	0.200	0.045	0.044	0.296

†Adjusted for age in decades, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalized income, and Functional Comorbidity Index.

HR, hazard ratio; 95% CI, 95% confidence interval.

*$P < 0.05$.

Comparing models with and without the intermediate variables revealed that the addition of diabetes, CVD, or hs-CRP to the models predicting all-cause mortality by elevated GGT levels affected the GGT estimates only slightly.

Cause-specific mortality analysis revealed a more than 2-fold increased risk of CVD mortality from elevated GGT levels for both sex in crude and age-adjusted models, but only for men in the multiple-adjusted model (Table 6). Among these, we also detected a positive trend across quintiles of GGT, whereas Cox models with the highest GGT quintile versus all lower confirmed the aforementioned results only for men (Table 6). Stratified by ultrasonographic finding, elevated GGT was strongly associated with an increased risk of CVD mortality in men with hepatic steatosis (HR 6.22; 95% CI, 1.22-31.62), with a significant trend of increased mortality risk across GGT quintiles ($P = 0.022$). In women, further stratification by liver ultrasound did not improve risk prediction considerably (Table 7).

Sensitivity analysis with study population stratified by sex and riskful alcohol consumption (<10/>10 g/day in women and <20/>20 g/day in men), revealed no subgroup differences concerning risk of mortality from elevated GGT among both men (riskful alcohol consumption, HR, 1.62, 95% CI, 0.97-2.33; no riskful alcohol consumption, HR, 1.83, 95% CI, 1.06-3.19) and women (riskful alcohol consumption, HR, 1.46, 95% CI, 0.83-2.56; no riskful alcohol consumption, HR, 1.68, 95% CI, 0.97-3.12). Additional sensitivity analyses excluded subjects due to their alcohol consumption habits. Excluding heavy drinkers did not change the estimates at all. Conversely, excluding nondrinkers increased the risk of mortality from hepatic steatosis, but only for males (data not shown).

Table 4. Association of Gamma-Glutamyl Transpeptidase (GGT) in Quintiles with All-Cause Mortality Stratified by Sex and Ultrasound Pattern

	HR (95%-CI) for All-Cause Mortality†							
	Men				Women			
GGT in Quintiles	N	Normal Ultrasound Pattern (N = 1,265)	N	Hyperechogenic Ultrasound Pattern (N = 779)	N	Normal Ultrasound Pattern (N = 1,646)	N	Hyperechogenic Ultrasound Pattern (N = 470)
1. Quintile	373	1.00 (ref.)	67	1.00 (ref.)	447	1.00 (ref.)	34	1.00 (ref.)
2. Quintile	304	0.82 (0.48; 1.40)	108	2.17 (0.89; 5.30)	364	1.14 (0.47; 2.76)	39	0.70 (0.22; 2.26)
3. Quintile	234	0.94 (0.55; 1.62)	141	1.60 (0.64; 3.97)	314	1.08 (0.42; 2.80)	103	0.34 (0.10; 1.26)
4. Quintile	205	0.96 (0.53; 1.74)	211	1.44 (0.57; 3.64)	289	1.71 (0.71; 4.09)	130	0.76 (0.27; 2.15)
5. Quintile	149	1.10 (0.63; 1.93)	252	3.10 (1.28; 7.50)*	232	1.52 (0.61; 3.78)	164	0.82 (0.27; 2.46)
5. vs. 1.-4. Quintile		1.18 (0.74; 1.91)		1.98 (1.21; 3.27)*		1.27 (0.61; 2.65)		1.25 (0.59; 2.67)
P for trend		0.658		0.046		0.224		0.973

†Adjusted for age in decades, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalized income, Functional Comorbidity Index, and multiplicative interaction term of continuous GGT with liver ultrasound.

HR, hazard ratio; 95% CI, 95% confidence interval.

*$P < 0.05$.

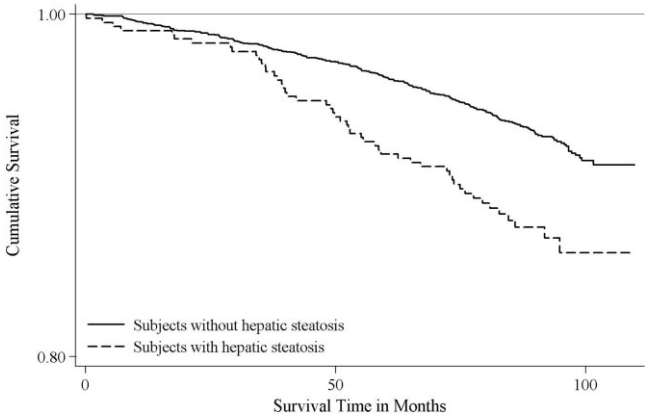

Fig. 1. Unadjusted Kaplan-Meier survival curves for subjects with and without hepatic steatosis. Subjects with hepatic steatosis, defined by GGT levels in the highest quintile and presence of a hyperechogenetic liver ultrasound pattern, had a significantly shorter survival than subjects without hepatic steatosis (log-rank test, $P \leq 0.001$).

Discussion

The present study revealed a direct association of elevated GGT levels with all-cause and CVD mortality in men. Furthermore, this association was even stronger among those with a hyperechogenic liver ultrasound. To our knowledge this is the first population-based cohort study to further stratify the association between elevated GGT levels and mortality according to the ultrasonographic finding of hepatic hyperechogenicity. Our finding of a sex-specific pattern is in agreement with previous prospective studies reporting that women had lower overall GGT levels,[34] and showed weaker associations of elevated GGT levels with mortality than men.[3] Possible explanations account for the positive correlation of longitudinal GGT change with an increase in CVD risk factors like body mass index, total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and number of cigarettes per day in men.[35] This explanation is supported by our finding of a sex-specific positive association of GGT with CVD mortality. Although cause-specific estimates for risk of mortality from CVD showed much wider confidence intervals compared to all cause analysis, our findings suggest sex-specific interactions among CVD risk factors, GGT, and mortality. However, due to the small number of CVD deaths in our sample, analyses should be replicated in larger samples with more events.

Men with high GGT levels have higher mortality, partly because of the association between GGT and other risk factors, and partly because GGT itself might act as an independent predictor of mortality. The latter is supported by studies linking elevated GGT levels directly to increased risk of CVD and all-cause mortality.[1-3,36,37] Parallel evidence for indirect effects emerges from studies

Table 5. Mediation Analyses for the Association of Gamma-Glutamyl Transpeptidase (GGT) Levels with All-Cause Mortality Stratified by Sex

GGT	HR (95% CI) for All-Cause Mortality									
	Men					Women				
(Ref.: 1.Quintile)	Multiple-Adjusted†	+ Diabetes	+ CVD Score	+ hs-CRP	Multiple-Adjusted†	+ Diabetes	+ CVD Score	+ hs-CRP
2. Quintile	1.06 (0.68; 1.64)	1.06 (0.68; 1.64)	0.99 (0.63; 1.55)	1.05 (0.68; 1.64)	0.97 (0.48; 1.95)	0.97 (0.48; 1.95)	0.93 (0.46; 1.91)	0.98 (0.47; 2.04)
3. Quintile	1.00 (0.64; 1.57)	1.00 (0.64; 1.57)	0.99 (0.63; 1.54)	0.96 (0.60; 1.52)	0.68 (0.32; 1.47)	0.65 (0.30; 1.42)	0.70 (0.32; 1.50)	0.70 (0.47; 1.57)
4. Quintile	0.87 (0.54; 1.41)	0.87 (0.54; 1.41)	0.85 (0.52; 1.37)	0.85 (0.52; 1.39)	1.22 (0.63; 2.36)	1.14 (0.58; 2.24)	1.24 (0.64; 2.40)	1.28 (0.64; 2.55)
5. Quintile	1.46 (0.94; 2.24)	1.46 (0.94; 2.24)	1.39 (0.90; 2.15)	1.37 (0.87; 2.14)	1.27 (0.65; 2.46)	1.20 (0.61; 2.36)	1.27 (0.65; 2.49)	1.26 (0.62; 2.56)
5. vs. 1.-4. Quintile	1.49 (1.08; 2.05) *	1.49 (1.08; 2.05) *	1.46 (1.06; 2.02) *	1.43 (1.02; 2.00) *	1.30 (0.80; 2.12)	1.27 (0.78; 2.08)	1.30 (0.79; 2.15)	1.27 (0.76; 2.11)
P for Trend	0.200	0.201	0.243	0.341	0.296	0.415	0.277	0.302

†Adjusted for age in decades, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalized income, and Functional Comorbidity Index. CVD score and summative score are based on the diagnosis of angina pectoris, peripheral artery disease, heart failure, stroke, and myocardial infarction.
HR, hazard ratio; 95% CI, 95% confidence interval. *$P < 0.05$.

Table 6. Association of GGT in Quintiles with CVD Mortality Stratified by Sex

GGT	HR (95% CI) for CVD Mortality					
	Men			Women		
(Ref.: 1. Quintile)	Crude	Age-Adjusted	Multiple-Adjusted†	Crude	Age-Adjusted	Multiple-Adjusted†
2. Quintile	1.52 (0.68; 3.38)	1.47 (0.66; 3.28)	1.67 (0.72; 3.88)	1.83 (0.44; 7.65)	1.54 (0.37; 6.46)	1.52 (0.36; 6.43)
3. Quintile	1.62 (0.73; 3.61)	1.59 (0.71; 3.54)	1.86 (0.81; 4.30)	1.94 (0.46; 8.11)	1.84 (0.44; 7.71)	1.22 (0.28; 5.33)
4. Quintile	1.46 (0.65; 3.29)	1.42 (0.63; 3.19)	1.48 (0.61; 3.60)	3.52 (0.95; 12.99)	2.92 (0.79; 10.79)	1.85 (0.47; 7.21)
5. Quintile	2.44 (1.15; 5.15)*	2.58 (1.22; 5.45)*	2.80 (1.24; 6.31)*	3.90 (1.08; 14.19)*	3.78 (1.04; 13.73)*	2.34 (0.61; 8.96)
5. vs. 1.-4. Quintile	1.75 (1.06; 2.87)*	1.89 (1.14; 3.10)*	1.86 (1.10; 3.14)*	1.93 (0.91; 4.08)	2.07 (0.98; 4.36)	1.64 (0.74; 3.66)
P for Trend	0.032	0.022	0.027	0.013	0.014	0.166

†Adjusted for age in decades, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalized income, and Functional Comorbidity Index.

95% CI, 95% confidence interval; CVD, cardiovascular disease; GGT, gamma-glutamyl transpeptidase; HR, hazard ratio.

*$P < 0.05$.

suggesting that higher GGT levels are associated with CVD risk factors, including metabolic syndrome, diabetes, hypertension, and dyslipidemia.[4-10] GGT levels also correlate positively with novel cardiovascular risk factors, such as CRP,[38] fibrinogen, F2-isoprostanes,[39] and inversely with antioxidant levels.[40] Thus, high GGT levels are suggested as an early marker of oxidative stress and inflammation, explaining various associations in the pathway of GGT-disease-mortality. However, considering GGT as a multifunctional protein,[37] mechanisms that explain the contribution and underlying factors of GGT to CVD and mortality have not been fully elucidated.

When we investigated the potential role of hyperechogenic liver ultrasound in the pathway from GGT to mortality, we found that men with hepatic steatosis exposed significantly shorter survival times compared to subjects without hepatic steatosis. Although increased liver enzymes are insensitive at detecting hepatic steatosis,[41] the sensitivity of ultrasound also varies between 49% and 94%.[42] In population-based cohorts, ultrasonographic evidence of hepatic steatosis is found in almost 20% of normal weight persons and 80% of overweight persons.[43] Thus, we determined hepatic steatosis by using both laboratory markers as well as ultrasound. Doing so, risk of mortality from hepatic steatosis was higher compared to assessment of elevated GGT levels only, suggesting that in case of increased GGT levels, liver ultrasound should be performed—not only for diagnosis, but also for further risk specification. Furthermore, we compared the predictability of GGT and liver ultrasound and found that GGT is the better predictor for mortality risk compared to liver ultrasound. This finding supports our conclusion that in the need for risk assessment, GGT has the best predictive ability.

Finally, we analyzed cardiometabolic variables as mediators in the pathway of GGT-mortality. As development of diabetes is predicted by high GGT levels,[7,34] even if in its reference range, diagnosis of diabetes may mediate the revealed association between GGT and mortality. But inclusion of diabetes in a model that predicted all-cause mortality by elevated GGT levels did not affect the esti-

Table 7. Association of GGT in Quintiles with CVD Mortality Stratified by Sex and Ultrasound Pattern

	HR (95%-CI) for All-Cause Mortality†							
	Men				Women			
GGT in Quintiles	N	Normal Ultrasound Pattern (N = 1,265)	N	Hyperechogenic Ultrasound Pattern (N = 779)	N	Normal Ultrasound Pattern (N = 1,646)	N	Hyperechogenic Ultrasound Pattern (N = 470)
1. Quintile	373	1.00 (ref.)	67	1.00 (ref.)	447	1.00 (ref.)	34	1.00 (ref.)
2. Quintile	304	1.57 (0.59; 4.22)	108	2.14 (0.39; 11.81)	364	2.02 (0.18; 22.74)	39	0.93 (0.14; 6.60)
3. Quintile	234	1.65 (0.59; 4.65)	141	3.45 (0.68; 17.43)	314	2.88 (0.28; 29.76)	103	0.42 (0.05; 3.59)
4. Quintile	205	1.37 (0.43; 4.39)	211	2.92 (0.57; 15.03)	289	3.94 (0.40; 38.67)	130	0.81 (0.13; 5.03)
5. Quintile	149	2.25 (0.80; 6.31)	252	6.22 (1.22; 31.62)*	232	7.31 (0.84; 63.22)	164	0.98 (0.11; 8.84)
5. vs. 1.-4. Quintile		1.63 (0.75; 3.53)		2.41 (1.05; 5.55)*		3.13 (1.02; 9.67)*		1.41 (0.32; 6.22)
P for trend		0.193		0.022		0.030		0.991

†Adjusted for age in decades, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalized income, Functional Comorbidity Index, and multiplicative interaction term of continuous GGT with liver ultrasound.

95% CI, 95% confidence interval; CVD, cardiovascular disease; GGT, gamma-glutamyl transpeptidase; HR, hazard ratio.

*$P < 0.05$.

mates, as well as inclusion of a summative CVD score. Despite previous studies suggesting a direct association of serum GGT with CRP,[38] and growing evidence that hs-CRP acts as an independent mortality risk factor,[44,45] our mediation analyses with the inclusion of hs-CRP only modestly changed the estimates. Sensitivity analysis considering alcohol consumption also lacked an impact on the pathway from GGT to mortality, suggesting that elevated GGT levels acts as an independent risk factor for mortality.

Our study has several strengths and potential limitations that should be considered. Major strengths are the population-based design, ultrasound examinations performed by standardized protocol and trained medical staff, and the routinely documented alcohol consumption. Despite the fact that liver biopsy is the best diagnostic tool for confirming diagnosis of hepatic steatosis, the inability to perform this in a large-scale population-based study like SHIP arises from logistic concerns.

In summary, the present study reports a direct association of elevated GGT levels and ultrasonographic evidence of hepatic steatosis with all-cause mortality in men. When we assessed the potential role of hyperechogenic liver ultrasound in the pathway from GGT to mortality, we found increased risk of mortality in men with hepatic steatosis. Our findings strongly suggest that in case of increased GGT levels, liver ultrasound should be performed, not only for diagnosis, but also for further risk stratification. Although our findings need to be confirmed in other populations, longitudinal monitoring of GGT changes may be beneficial, because many subjects will experience death before they are able to develop liver disease, and especially because GGT is a low-cost and widely used laboratory measurement.

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