Atherosclerosis xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Serum gamma-glutamyl transferase and mortality in persons undergoing coronary angiography—The Ludwigshafen Risk and Cardiovascular Health Study

Tatjana Stojakovic[a,*], Hubert Scharnagl[a], Michael Trauner[b], Burkert Pieske[c], Britta Wellnitz[d], Ursula Seelhorst[d], Dieter Schilling[e], Bernhard R. Winkelmann[f], Bernhard O. Boehm[g], Winfried März[a,h]

[a] *Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbruggerplatz 15, A-8036 Graz, Austria*
[b] *Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Austria*
[c] *Department of Cardiology, Medical University of Graz, Austria*
[d] *LURIC Study Nonprofit LLC, Freiburg, Germany*
[e] *Department of Gastroenterology and Metabolism, Diakonie Hospital, Mannheim, Germany*
[f] *Cardiology Group Frankfurt-Sachsenhausen, Frankfurt, Germany*
[g] *Division of Endocrinology and Diabetes, Department of Internal Medicine I, Ulm University, Germany*
[h] *Synlab Centre of Laboratory Diagnostics Heidelberg, Germany*

ARTICLE INFO

Article history:
Received 23 May 2009
Received in revised form 13 July 2009
Accepted 16 July 2009
Available online xxx

Keywords:
Cardiovascular disease
Gamma-glutamyl transferase
Mortality
Risk factor

ABSTRACT

Objective: Serum gamma-glutamyl transferase (GGT) seems to be a predictor for coronary artery disease (CAD). The objective of this study was to elucidate the relationship between GGT and total as well as cardiovascular mortality.
Methods: Serum levels of GGT were determined in 2556 subjects with and 699 subjects without angiographic evidence of CAD in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.
Results: Serum GGT was positively associated with male gender, alcohol consumption and markers of the metabolic syndrome (triglycerides, blood pressure, waist circumference and insulin resistance). It was positively related to aspartate aminotransferase, alanine aminotransferase, C-reactive protein, interleukin-6, and negatively related to glutathione and increased age. During a mean follow-up period of 7.75 years, 754 subjects died. Compared with subjects in the lowest quartile of GGT, the unadjusted hazard ratios (95% CI) for all-cause death were 1.2 (0.9–1.5), 1.4 (1.1–1.8) and 1.9 (1.5–2.3), respectively, in other GGT quartiles. Hazard ratios (CI) for death from cardiovascular causes were 1.4 (1.0–2.0), 1.8 (1.4–2.5) and 2.2 (1.6–2.9). After adjustment for age, gender and cardiovascular risk factors GGT remained a significant predictor for total and cardiovascular mortality. In angiographic CAD the predictive value of GGT was also significant and similar to that in the entire cohort.
Conclusion: Serum GGT is predictive of all-cause and cardiovascular mortality in individuals with CAD independently of other cardiovascular risk factors.

1. Introduction

Gamma-glutamyl transferase (GGT) is located on plasma membranes of several cells and tissues with a predominance of hepatocytes [1]. As such it is a well-established marker for hepatobiliary diseases of various etiologies [2]. GGT is directly involved in the extracellular catabolism of glutathione (GSH), the main thiol antioxidant in humans, by hydrolysis of the gamma-glutamyl bond between glutamate and cysteine [1]. The extracellular catabolism of GSH may exert prooxidant effects [3,4]. GGT is bound to LDL particles [5] and might thus be associated with the incidence of cardiovascular disease. Moreover, GGT has been found in atherosclerotic plaques [6,7].

Common causes of an elevated GGT include cholestatic liver diseases, alcohol and various drugs which induce GGT in hepatocytes [1]. In addition, serum GGT may also be a marker of non-alcoholic fatty liver disease (NAFLD) which encompasses a disease spectrum ranging from "simple" steatosis to non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular cancer (HCC) [8]. NAFLD is emerging as an independent cardiovascular risk factor [9,10] although the mechanism(s) linking fatty liver to atherosclerosis are still poorly understood. Potential mechanisms include oxidative stress and altered hepatic lipoprotein metabolism in NAFLD. Moreover, there is a strong association

* Corresponding author. Tel.: +43 316 385 7816; fax: +43 316 385 3430.
E-mail address: tatjana.stojakovic@medunigraz.at (T. Stojakovic).

0021-9150/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2009.07.026

between the severity of liver histopathology in NAFLD and carotid artery intima-media thickness and plaques as well as lower endothelial flow-mediated vasodilation (as markers of subclinical atherosclerosis), independent of obesity and other components of the metabolic syndrome [10,11]. Thus, NAFLD with elevated GGT can be regarded as the hepatic manifestation of the metabolic syndrome [12,13].

A limited number of previous studies revealed that serum GGT levels predict cardiac mortality or non-fatal myocardial infarction, in particular in individuals with past coronary events [14–16]. Recent prospective studies pointed out that high GGT is positively associated with increased mortality or incidence of cardiovascular disease [17–23]. In this study we examined the relationship between GGT levels and mortality in subjects undergoing coronary angiography.

2. Methods

2.1. Study design and participants

We studied participants of the Ludwigshafen Risk and Cardiovascular Health (LURIC) [24] study. LURIC is an ongoing prospective study investigating risk factors for coronary artery disease (CAD). A coronary angiography was performed in all patients who had to be in a stable clinical condition (except for acute coronary syndromes) without major concomitant non-cardiovascular diseases. The study was approved by the ethics committee at the "Landesärztekammer Rheinland-Pfalz" and informed written consent was obtained from all participants.

CAD was assessed by angiography using the maximum luminal narrowing estimated by visual analysis. Clinically relevant CAD was defined as the occurrence of at least one stenosis 20% or greater in at least one of fifteen coronary segments. Individuals with stenoses less than 20% were considered as not having CAD. We alternatively used another definition of CAD, namely stenoses of $\geq$50% and stratified the study participants according the number of vessels (1, 2 or 3) with stenosis $\geq$50%.

Diabetes mellitus was diagnosed if plasma glucose was greater than 1.25 g/L in the fasting state or greater than 2.00 g/L two hours after the oral glucose load, respectively, or if individuals were treated with oral anti-diabetics or insulin. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mm Hg or if the current medication included antihypertensive drugs. The metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment Panel III [25]. Alcohol consumption was defined based on self-reported average daily/weekly intake.

Measurements of GGT, aspartate aminotransferase (AST), ALT, lipoproteins, C-reactive protein (CRP) and creatinine were complete in 3255 out of 3316 individuals with coronary angiograms. Among these, 2556 persons had angiographic CAD, 1525 were in a clinically stable condition while 1031 presented within seven days after onset of symptoms of either unstable angina, non-ST-elevation myocardial infarction (NSTEMI; troponin T >0.1 μg/L) or ST-elevation myocardial infarction (STEMI; troponin T >0.1 μg/L).

Information on vital status was obtained from local registries. Of the 3255 persons studied, 754 (23.2%) died during a median time of follow-up of 7.75 years. Death certificates were missing for 15 decedents (2%) who were included in the total mortality analysis but excluded from the cardiovascular mortality analysis. Cardiovascular death included sudden death, fatal myocardial infarction, death due to congestive heart failure, death immediately following intervention to treat CAD, fatal stroke and other causes of death due to CAD.

2.2. Laboratory analysis

GGT, AST, ALT and glucose were measured using enzymatic reagents (Roche Diagnostics, Mannheim, Germany) [24]. Transferrin was determined by immunoturbidimetry and ferritin by an electrochemoluminescence enzyme immunoassay (Roche Diagnostics, Mannheim, Germany). Glutathione (GSH) was measured by HPLC (Chromsystem Instruments & Chemicals GmbH, Martinsried, Germany). Lipoproteins were separated using a combined ultracentrifugation–precipitation method [26,27]. "Sensitive" CRP was measured by immunonephelometry (N Latex CRP mono, Dade Behring, Marburg, Germany) and interleukin-6 (IL-6) was determined using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, United States of America). IR was calculated using the homeostasis model assessment (HOMA) as (fasting insulin [μU/mL] × fasting glucose [mg/dL])/405. Glomerular filtration rate (GFR) was calculated as GFR (mL/min/1.73 m^2) = 186 × creatinine$^{-1.154}$ × age$^{-0.203}$ and GFR (mL/min/1.73 m^2) = 142 × creatinine$^{-1.154}$ × age$^{-0.203}$ in males and females, respectively.

2.3. Statistical analysis

GGT, triglycerides, glutathione, CRP and ferritin were transformed logarithmically before being used in parametric statistical procedures. We established quartiles of continuous variables according to the values in subjects without angiographic CAD. Clinical and biochemical characteristics of all study participants are presented as percentages for categorical variables and mean ± SD or median and 25th through 75th percentile for continuous variables. Associations of categorical and continuous variables were analysed by logistic regression and univariate analysis of variance (ANOVA), respectively, with covariables as indicated (Table 1). We studied the effects of age, gender, CAD and cardiovascular risk factors on GGT using ANOVA models in which we included those factors not under examination as covariables (Table 2). Cox proportional hazards models were used to examine the relationship of GGT with total and cardiovascular mortality. Multivariable adjustment was carried out for age, gender, CAD and cardiovascular risk factors (Tables 3 and 4). A value of $P < 0.05$ was considered statistically significant. The SPSS 15.0 statistical package (SPSS Inc, Chicago, USA) was used for all analyses.

3. Results

3.1. Study participants

Patients with angiographic CAD were significantly older than control subjects (Table 1). Diabetes mellitus, hypertension, current or past smoking, peripheral vascular and cerebrovascular disease were more prevalent in CAD patients. In contrast, the proportion of patients consuming potentially hepatotoxic amounts of alcohol was significantly less frequent in the CAD patient group. More than half of the CAD patients had a history of myocardial infarction. CAD patients had higher waist circumference, systolic blood pressure, fasting glucose, insulin resistance, triglycerides and ferritin but lower high-density lipoprotein cholesterol (HDL-C) and glutathione. Low-density lipoprotein cholesterol (LDL-C) was lower in CAD patients than in controls even after adjustment for lipid-lowering medication. CAD patients had higher levels of CRP and IL-6 which may be due to the presence of individuals presenting with acute coronary syndromes or the greater burden of metabolic risk factors in this patient group. Diastolic blood pressure, AST, ALT, transferrin and glomerular filtration rate did not differ significantly between both groups.

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Table 1
Clinical and biochemical characteristics of study participants at baseline.

	No CAD ($n=699$)	CAD ($n=2556$)	P[a]
Age, years	58 ± 12	64 ± 10	<0.001
Male sex	52	75	<0.001[b]
Waist circumference, cm	97 ± 12	100 ± 12	0.031
Diabetes mellitus	18	36	<0.001
Insulin resistance by HOMA	2.9 ± 3.3	3.9 ± 4.7	<0.001
Alcohol consumption[f]	24	21	0.001
Systemic hypertension	63	75	0.002
Angiographic CAD			
Stenosis 20–49%	–	13	–
1 vessel ≥50% stenosis	–	24	–
2 vessels ≥50% stenosis	–	24	–
3 vessels ≥50% stenosis	–	39	–
Smoking			
Never	52	32	
Past	30	48	
Current	18	20	<0.001
Previous myocardial infarction	–	53	–
Peripheral vascular disease	2	12	<0.001
Cerebrovascular disease	5	9	0.026
Systolic blood pressure, mm Hg	136 ± 22	143 ± 24	0.008[c]
Diastolic blood pressure, mm Hg	80 ± 11	81 ± 11	0.441[c]
Fasting blood glucose, g/L	1.05 ± 0.28	1.16 ± 0.37	<0.001
LDL cholesterol, g/L	1.20 ± 0.31	1.16 ± 0.35	0.002[d]
HDL cholesterol, g/L	0.43 ± 0.12	0.38 ± 0.10	<0.001[d]
Triglycerides, g/L[f]	1.33 (0.97–1.94)	1.50 (1.13–2.02)	<0.001[d,e]
Aspartate aminotransferase, U/L	12 ± 9	12 ± 7	0.295
Alanine aminotransferase, U/L	17 ± 16	17 ± 20	0.634
Glutathione, μmol/L[g]	4.3 (3.4–5.4)	4.0 (3.0–5.1)	0.026[e]
C-reactive protein, mg/L[g]	2.2 (1.0–5.9)	3.8 (1.5–9.2)	<0.001[d,e]
Interleukin-6, pg/mL[g]	2.4 (1.4–4.4)	3.4 (2.0–6.7)	<0.001[d,e]
Ferritin, μg/L[g]	148 (87–268)	159 (91–275)	0.030[e]
Transferrin, g/L	2.55 ± 0.41	2.51 ± 0.40	0.700
Glomerular filtration rate, mL/min	83 ± 18	81 ± 19	0.866

Values are mean ± SD and %, respectively.
[a] ANOVA or logistic regression, respectively, adjusted for age and gender.
[b] Logistic regression, adjusted for age only.
[c] Adjusted for use of beta blockers, ACE inhibitors, AT1 receptor antagonists, calcium channel blockers, diuretics and lipid-lowering drugs.
[d] Adjusted for use of lipid-lowering drugs.
[e] ANOVA of logarithmically transformed values.
[f] Thresholds of 20 and 30 g alcohol per day apply to females and males, respectively.
[g] Median and 25th through 75th percentile.

3.2. Association of GGT with cardiovascular risk factors and CAD status

We examined the relationship of GGT to established cardiovascular risk factors using factors not under consideration as covariables (Table 2). GGT was significantly higher in men than in women and decreased with age. GGT was positively associated with diabetes mellitus, insulin resistance, waist circumference, alcohol consumption and hypertension. GGT was increased in the highest quartile of LDL-C, and also in increasing quartiles of triglycerides. However, the differences in GGT levels between the groups with higher and lower cardiovascular risk factors were modest. AST, ALT, CRP, IL-6 and ferritin were positively and glutathione was inversely related to GGT ($P<0.001$). Angiographic CAD, intake of lipid lowering drugs, smoking, transferrin saturation and glomerular filtration rate were not significantly associated with GGT. In addition, GGT was also not related to other markers of inflammation such as fibrinogen and serum amyloid A (data not shown).

Multiple linear regression using forward selection of independent variables revealed the most important predictors of GGT as following: AST > glutathione > gender > CRP > triglycerides > alcohol consumption > ferritin > diabetes mellitus.

3.3. GGT and mortality from all causes

Compared with subjects in the lowest quartile of GGT, the unadjusted hazard ratios (HR) for death at GGT concentrations in the second, third and fourth quartile were 1.19 (95% CI, 0.92–1.54; $P=0.194$), 1.42 (95% CI, 1.13–1.77; $P=0.002$) and 1.86 (95% CI, 1.50–2.32; $P<0.001$), respectively (model 1, Table 3). Inclusion of age and gender as covariables did not influence these estimates (model 2, Table 3). Although HRs slightly decreased, GGT remained predictive after adjustment for cardiovascular risk factors (model 3, Table 3). An increase of 1 SD in GGT was associated with unadjusted and incrementally adjusted HRs of death from any cause by 1.23 (95% CI, 1.15–1.32), 1.27 (95% CI, 1.19–1.36) and 1.17 (95% CI, 1.08–1.26), respectively (Table 3). Among the 2556 subjects with angiographic CAD, 667 (26.1%) died during follow-up. In this subgroup, HRs for death were similar to those in the entire cohort (Table 3). Among the subjects with angiographic CAD, 1525 patients had stable CAD and 1031 patients presented with unstable CAD (unstable angina, NSTEMI or STEMI). In these subgroups of patients, 427 (28.0%) and 240 deaths (23.3%), respectively, occurred. In particular in unstable CAD subjects, we found consistent and robust associations of GGT with mortality from all causes (Table 3). Only 87 deaths (12.4%) occurred among the 699 subjects with coro-

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Table 2
Association of GGT with cardiovascular risk factors and coronary artery disease.

	GGT, U/L[a]	Difference, %[b]	P[c]
Gender			
Men	21 (20–21)		
Women	15 (14–15)	−28.6	<0.001
Age, years			
<60	19 (18–20)		
60–70	19 (18–20)	0.0	0.714
>70	17 (17–18)	−10.5	0.012
Coronary artery disease			
None	18 (17–20)		
Stable CAD	19 (19–20)	+5.6	0.303
Unstable CAD (Troponin T−)	18 (17–19)	0.0	0.301
NSTEMI or STEMI (Troponin T+)	17 (16–19)	−5.6	0.220
Waist circumference, cm			
≤88 or 102[d]	18 (17–19)		
>88 or 102[d]	19 (18–20)	+5.6	0.039
Diabetes mellitus			
No	17 (17–18)		
Yes	21 (20–22)	+19.0	<0.001
Insulin resistance by HOMA			
≤2.5	18 (17–18)		
>2.5	19 (19–20)	+5.6	0.001
Alcohol consumption, g/d			
≤20 or 30[e]	18 (17–18)		
>20 or 30[e]	22 (20–23)	+22.2	<0.001
Hypertension			
No	18 (17–18)		
Yes	19 (18–19)	+5.6	0.021
Lipid lowering drugs			
No	19 (18–19)		
Yes	18 (18–19)	−5.3	0.618
Smoking			
Never	18 (18–19)		
Former	18 (17–19)	0.0	0.472
Current	20 (18–21)	+11.1	0.122
LDL cholesterol, g/L			
1st quartile (<1.00)	18 (17–19)		
2nd quartile (1.00–1.19)	18 (17–19)	0.0	0.849
3rd quartile (1.20–1.40)	18 (17–19)	0.0	0.883
4th quartile (≥1.41)	20 (19–21)	+11.1	0.031
HDL cholesterol, g/L			
1st quartile (<0.34)	18 (17–19)		
2nd quartile (0.34–0.41)	19 (18–20)	+5.6	0.111
3rd quartile (0.42–0.49)	19 (18–20)	+5.6	0.042
4th quartile (≥0.50)	19 (18–21)	+5.6	0.058
Triglycerides, g/L			
1st quartile (<0.97)	16 (15–17)		
2nd quartile (0.97–1.32)	18 (17–19)	+12.5	0.001
3rd quartile (1.33–1.94)	18 (17–19)	+12.5	<0.001
4th quartile (≥1.95)	21 (20–22)	+31.3	<0.001
Aspartate aminotransferase, U/L			
1st quartile (<7)	14 (13–14)		
2nd quartile (7–9)	16 (15–17)	+14.3	<0.001
3rd quartile (10–12)	19 (18–20)	+35.7	<0.001
4th quartile (≥13)	26 (25–28)	+85.7	<0.001
Alanine aminotransferase, U/L			
1st quartile (<8)	13 (12–14)		
2nd quartile (8–12)	16 (16–17)	+23.1	<0.001
3rd quartile (13–18)	20 (19–21)	+53.8	<0.001
4th quartile (≥19)	26 (24–27)	+100.0	<0.001
Glutathione, μmol/L			
1st quartile (<3.4)	25 (24–26)		
2nd quartile (3.4–4.3)	19 (18–20)	−24.0	<0.001
3rd quartile (4.4–5.4)	15 (14–16)	−40.0	<0.001
4th quartile (≥5.5)	13 (12–14)	−48.0	<0.001

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Table 2 (*Continued*)

	GGT, U/L[a]	Difference, %[b]	*P*[c]
C-reactive protein, mg/L			
<3	16 (16–17)		
3–10	19 (18–20)	+18.8	<0.001
>10	24 (22–25)	+50.0	<0.001
IL-6, pg/mL			
1st quartile (<1.33)	16 (15–17)		
2nd quartile (1.33–2.27)	18 (17–19)	+12.5	0.019
3rd quartile (2.28–4.05)	18 (17–19)	+12.5	0.008
4th quartile (≥4.06)	20 (19–21)	+25.0	<0.001
Ferritin, μg/L			
≤300	18 (17–18)		
>300	21 (20–22)	+16.7	<0.001
Transferrin saturation, %			
≤45	18 (18–19)		
>45	19 (18–21)	+5.6	0.385
Glomerular filtration rate, mL/min			
≥90	18 (17–19)		
60–89	19 (18–19)	+5.6	0.513
<59	19 (17–20)	+5.6	0.557

[a] Estimated marginal means and 95% confidence intervals obtained in a general linear model (ANOVA), adjusted for sex, age, coronary artery disease, waist circumference, diabetes mellitus, alcohol consumption, hypertension, lipid lowering drugs, smoking, LDL cholesterol, HDL cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, C-reactive protein, glomerular filtration rate.
[b] Compared to the first category of each variable.
[c] Post hoc pairwise comparisons with the first category of each variable.
[d] Thresholds of 102 and 88 cm apply to males and females, respectively.
[e] Thresholds of 20 and 30 g alcohol per day apply to females and males, respectively.

nary stenoses less than 20%. An increment of 1 SD in GGT revealed an unadjusted HR of death of 1.49 (95% CI, 1.22–1.82; $P<0.001$), and this association was robust against adjustment for age and gender (HR 1.47, 95% CI, 1.20–1.80; $P<0.001$), and full adjustment (HR 1.26, 95% CI, 1.01–1.57; $P=0.040$), respectively. Finally, HRs in all models were materially unchanged using the definition of CAD with ≥50% instead of ≥20% stenosis (Supplemental Tables 5 and 6).

Table 3
Hazard ratios (HR) for death from all causes according to GGT. The study population consisted of controls (stenosis <20%) and individuals with angiographic CAD (stenosis ≥20%).

GGT (U/L)	Deaths *n*, %	Model 1 HR (95% CI)[a]	*P*	Model 2 HR (95% CI)[b]	*P*	Model 3 HR (95% CI)[c]	*P*
All individuals ($n=3255$)							
1st quartile (<9)	112 (17)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	114 (20)	1.19 (0.92–1.54)	0.194	1.15 (0.89–1.50)	0.287	1.09 (0.84–1.42)	0.532
3rd quartile (14–25)	237 (23)	1.42 (1.13–1.77)	0.002	1.42 (1.13–1.79)	0.003	1.24 (0.98–1.57)	0.074
4th quartile (≥26)	291 (29)	1.86 (1.50–2.32)	<0.001	1.99 (1.59–2.49)	<0.001	1.57 (1.23–2.00)	<0.001
1 SD		1.23 (1.15–1.32)	<0.001	1.27 (1.19–1.36)	<0.001	1.17 (1.08–1.26)	<0.001
Angiographic CAD ($n=2556$)							
1st quartile (<9)	98 (20)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	103 (24)	1.20 (0.91–1.59)	0.189	1.18 (0.89–1.56)	0.245	1.10 (0.83–1.46)	0.496
3rd quartile (14–25)	215 (25)	1.31 (1.03–1.66)	0.029	1.39 (1.09–1.77)	0.008	1.25 (0.97–1.61)	0.082
4th quartile (≥26)	251 (31)	1.66 (1.31–2.09)	<0.001	1.90 (1.50–2.41)	<0.001	1.59 (1.23–2.07)	<0.001
1 SD		1.18 (1.10–1.27)	<0.001	1.25 (1.16–1.34)	<0.001	1.17 (1.08–1.28)	<0.001
Stable CAD ($n=1525$)							
1st quartile (<9)	68 (23)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	66 (24)	1.10 (0.79–1.55)	0.567	1.02 (0.73–1.44)	0.890	1.02 (0.72–1.43)[e]	0.934
3rd quartile (14–25)	135 (27)	1.23 (0.92–1.64)	0.168	1.19 (0.89–1.60)	0.248	1.08 (0.80–1.48)[e]	0.608
4th quartile (≥26)	158 (35)	1.74 (1.31–2.31)	<0.001	1.86 (1.39–2.49)	<0.001	1.40 (1.01–1.93)[e]	0.042
1 SD		1.21 (1.10–1.33)	<0.001	1.25 (1.14–1.38)	<0.001	1.12 (1.01–1.25)[e]	0.029
Unstable CAD ($n=1031$)							
1st quartile (<9)	30 (17)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	37 (24)	1.44 (0.89–2.32)	0.142	1.56 (0.96–2.53)	0.071	1.40 (0.86–2.29)[e]	0.180
3rd quartile (14–25)	80 (24)	1.50 (0.99–2.28)	0.059	1.85 (1.21–2.82)	0.005	1.58 (1.01–2.46)[e]	0.045
4th quartile (≥26)	93 (26)	1.64 (1.09–2.47)	0.019	2.12 (1.40–3.22)	<0.001	1.86 (1.19–2.90)[e]	0.007
1 SD		1.15 (1.02–1.30)	0.025	1.25 (1.11–1.42)	<0.001	1.21 (1.06–1.38)[e]	0.006

[a] Model 1: unadjusted.
[b] Model 2: adjusted for age and gender.
[c] Model 3: additionally adjusted for coronary artery disease (none, stable CAD, unstable CAD, NSTEMI, STEMI), waist circumference, diabetes mellitus, alcohol consumption, hypertension, smoking status, aspartate aminotransferase, alanine aminotransferase, LDL cholesterol, HDL cholesterol, triglycerides, C-reactive protein, glomerular filtration rate.
[d] Reference.
[e] Coronary artery disease (none, stable CAD, unstable CAD, NSTEMI, STEMI) not used as a covariable.

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Table 4
Hazard ratios (HR) for death from cardiovascular causes according to GGT. The study population consisted of controls (stenosis <20%) and individuals with angiographic CAD (stenosis ≥20%).

GGT (U/L)	Deaths *n* (%)	Model 1 HR (95% CI)[a]	*P*	Model 2 HR (95% CI)[b]	*P*	Model 3 HR (95% CI)[c]	*P*
All individuals (*n* = 3240)							
1st quartile (<9)	59 (9)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	73 (13)	1.44 (1.03–2.04)	0.036	1.40 (0.99–1.98)	0.055	1.37 (0.97–1.94)	0.078
3rd quartile (14–25)	161 (16)	1.82 (1.35–2.45)	<0.001	1.82 (1.35–2.47)	<0.001	1.63 (1.19–2.23)	0.002
4th quartile (≥26)	180 (18)	2.18 (1.62–2.92)	<0.001	2.31 (1.71–3.11)	<0.001	1.88 (1.36–2.59)	<0.001
1 SD		1.28 (1.17–1.39)	<0.001	1.31 (1.20–1.43)	<0.001	1.22 (1.10–1.34)	<0.001
Angiographic CAD (*n* = 2541)							
1st quartile (<9)	55 (12)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	65 (15)	1.35 (0.94–1.94)	0.100	1.33 (0.93–1.91)	0.123	1.29 (0.89–1.86)	0.174
3rd quartile (14–25)	148 (18)	1.60 (1.17–2.18)	0.003	1.70 (1.24–2.32)	0.001	1.55 (1.12–2.15)	0.009
4th quartile (≥26)	157 (20)	1.84 (1.35–2.50)	<0.001	2.09 (1.53–2.86)	<0.001	1.77 (1.26–2.48)	0.001
1 SD		1.21 (1.10–1.33)	<0.001	1.27 (1.16–1.40)	<0.001	1.20 (1.08–1.33)	0.001
Stable CAD (*n* = 1517)							
1st quartile (<9)	42 (14)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	45 (17)	1.21 (0.80–1.85)	0.359	1.13 (0.74–1.72)	0.573	1.18 (0.77–1.82)[e]	0.446
3rd quartile (14–25)	96 (19)	1.41 (0.98–2.03)	0.063	1.36 (0.94–1.96)	0.105	1.29 (0.88–1.90)[e]	0.196
4th quartile (≥26)	99 (22)	1.76 (1.22–2.52)	0.002	1.84 (1.27–2.66)	0.001	1.46 (0.96–2.20)[e]	0.074
1 SD		1.20 (1.08–1.35)	0.001	1.24 (1.10–1.39)	<0.001	1.13 (0.99–1.28)[e]	0.070
Unstable CAD (*n* = 1024)							
1st quartile (<9)	13 (7)	1.0[d]		1.0[d]		1.0[d]	
2nd quartile (9–13)	20 (13)	1.79 (0.89–3.60)	0.103	1.98 (0.98–3.99)	0.056	1.75 (0.86–3.58)[e]	0.123
3rd quartile (14–25)	52 (15)	2.24 (1.22–4.11)	0.009	2.80 (1.52–5.17)	0.001	2.32 (1.22–4.40)[e]	0.010
4th quartile (≥26)	58 (16)	2.35 (1.29–4.29)	0.005	3.10 (1.68–5.69)	<0.001	2.72 (1.43–5.16)[e]	0.002
1 SD		1.26 (1.07–1.49)	0.005	1.38 (1.17–1.63)	<0.001	1.34 (1.12–1.60)[e]	0.001

[a] Model 1: unadjusted.
[b] Model 2: adjusted for age and gender.
[c] Model 3: additionally adjusted for coronary artery disease (none, stable CAD, unstable CAD, NSTEMI, STEMI), waist circumference, diabetes mellitus, alcohol consumption, hypertension, smoking status, aspartate aminotransferase, alanine aminotransferase, LDL cholesterol, HDL cholesterol, triglycerides, C-reactive protein, glomerular filtration rate.
[d] Reference.
[e] Coronary artery disease (none, stable CAD, unstable CAD, NSTEMI, STEMI) not used as a covariable.

3.4. GGT and mortality from cardiovascular causes

During the follow-up 754 subjects deceased (missing death certificates in 15 persons). Among these, 473 (14.6% of the study population) died from cardiovascular causes. HRs for death from cardiovascular causes according to GGT were higher compared to those obtained for mortality from all causes in all models and subgroups (Table 4). An increase of 1 SD in GGT resulted in unadjusted and fully adjusted HRs for death from cardiovascular causes of 1.28 (95% CI, 1.17–1.39) and 1.22 (95% CI, 1.10–1.34), respectively. The most striking HRs were found in the subgroup of unstable CAD patients (Table 4). However, this was the smallest subgroup (*n* = 1024) and thus, these results should be interpreted with caution. Similar HRs were obtained in patients with CAD diagnosed at 1 stenosis ≥50% or the number of vessels with stenoses ≥50% as covariables (Supplemental Tables 5 and 6).

4. Discussion

This study demonstrates that high GGT levels predict all-cause and cardiovascular mortality in patients scheduled for coronary angiography, independent of established and emerging risk factors. Consistently, there was a tendency towards an increased risk of death at high GGT concentrations in subjects without CAD but this association disappeared after adjustment for confounding variables.

GGT is associated with several traditional cardiovascular risk factors and components of the metabolic syndrome. High GGT has been positively related to insulin resistance, diabetes mellitus, metabolic syndrome and hypertension [12,18,28–31]. Consistently, in the current study we observed elevated GGT in patients with diabetes mellitus, insulin resistance, hypertension and waist circumference. As shown previously, men had significantly higher concentrations of GGT than women after controlling for other confounding factors [18,32]. In our study, however, GGT was negatively associated with age which was not observed in other studies [17–19]. This finding might be due to the higher mean age in our cohort. Individuals consuming alcohol had increased GGT levels and there was a positive correlation with LDL-C and triglycerides [18,21]. In addition, smoking was not independently associated with GGT levels and this finding contrasts other reports [17–19,21]. Furthermore, elevated levels of GGT seem to be a marker of systemic inflammation and oxidative stress [33,34] and may even predict the development of chronic kidney disease [35].

In the study of Lee et al. [18], 3451 participants of the Framingham Heart Study were followed for more than 20 years which was a period of time about threefold of our study. Of the entire cohort 535 participants developed cardiovascular disease and 362 died. The proportional increments of GGT concentrations were similar to our four groups. The risk of death increased across GGT quartiles accounting for conventional risk factors and CRP (HR for 1 SD in GGT 1.23) which was very close to our HR of death of 1.18 per increase of 1 SD in GGT in the fully adjusted model for HR (Table 3). In our subgroup of patients with proven angiographic CAD nearly identical values of HRs for all-cause mortality were observed. Meisinger et al. [19] examined the associations of quartiles of GGT with incident coronary events in 1878 men who were free of coronary heart disease at baseline. A total of 150 acute coronary events occurred, comparing the highest versus the lowest quartile of GGT the HR was then 2.34. Finally, compared with the results of Ruttmann et al. [21] who analyzed cardiovascular mortality in a cohort of 163,944 Austrian adults, we found slightly lower HR for cardiovascular death (1 SD in GGT 1.23, Table 4).

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The mechanisms linking GGT to increased risk for cardiovascular death have not been elucidated so far. Higher serum GGT has been associated with an increased incidence of the metabolic syndrome [18]. A recent study reported that elevation of liver enzymes and hepatic insulin resistance as reflected by fasting insulin is independent of diabetes status and occurs in the early stages of insulin resistance, highlighting the central role of the liver in insulin resistance [12]. Furthermore, Lim et al. [36] revealed an interaction between GGT and body mass index which was not associated with the risk of prevalent diabetes indicating that obesity itself may not be a sufficient risk factor for type 2 diabetes. GGT is a marker for NAFLD and was proposed as an indicator of insulin resistance [8,10]. Our findings were in agreement with this hypothesis. We found a strong association with markers of the metabolic syndrome (triglycerides, blood pressure, abdominal obesity and insulin resistance by HOMA). Elevated GGT levels most likely reflect associated NAFLD in our patients. Hemochromatosis could be ruled out as there was no association with an increase of the transferrin saturation. The presence of other liver diseases leading to elevated concentrations of GGT could not be excluded, but is unlikely to be a major contributor in this patient population.

There is strong evidence that inflammation plays a substantial role in the development of atherosclerosis and CAD [37,38]. Lee and Jacobs reported an association between serum levels of GGT and CRP, a marker of low-grade systemic inflammation [39]. This is in agreement with our results that GGT was strongly associated with the inflammatory markers CRP and IL-6. Moreover, GGT may be involved in the oxidation process of lipoprotein particles [4]. Cell membrane associated GGT hydrolyzes extracellular GSH and the resulting thiol can initiate the oxidation of LDL suggesting that GGT may promote atherogenesis. Whether GGT in serum may be an indicator of increased activity of the membrane bound enzyme is unclear. In the present study the concentrations of GSH, the substrate of GGT, was inversely associated with GGT. However, we found no association with other markers of the oxidative stress like MDA or oxLDL (data not shown).

As a matter of fact, it is one of the limitations of the manuscript that the observational character of our study does not allow final conclusions as to a potential causal role of GGT in the development of atherosclerosis, although several pathophysiological considerations would suggest such a role. This of course applies to the relationship between GGT and subclinical inflammation.

The strengths of this study are its prospective design, the standardized protocol and the amount of obtained variables. The study population consisted of middle-aged to elderly Caucasians referred to cardiac catheterization. Thus, generalizability to younger individuals and other ethnicities is limited. However, this referral bias could also be regarded as strength of this investigation. The prevalence of clinically asymptomatic coronary atherosclerosis has been reported to be high in subjects 50 years old or older [40]. Therefore, angiography-based recruitment avoids inadvertent allocation to the control-group of individuals with remarkable, clinically inapparent CAD. Moreover, GGT was measured only once at baseline. Hence, intra-individual variability over time cannot be excluded.

In summary, the present study shows that high GGT levels independently predict all-cause and cardiovascular mortality in subjects with CAD. This finding may have major implications for use and interpretation of serum GGT level in daily clinical practice. Further studies are needed to address the causality of our findings. In the meantime, elevated GGT levels should prompt physicians and patients to rigorously pursue a healthy life-style.

Grant/funding support

None.

Financial disclosures

None.

Acknowledgments

The authors extend appreciation to the participants of the Ludwigshafen Risk and Cardiovascular Health Study without their collaboration this article would not have been written. We thank the LURIC study team either temporarily or permanently involved in patient recruitment, sample and data handling and the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg and Ulm, Germany and the German registration offices and local public health departments for their assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2009.07.026.

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