

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart
Association®



Learn and Live SM

Plasma γ -Glutamyltransferase, Cysteinyl-Glycine, and Oxidized Low-Density Lipoprotein : A Pathway Associated With Myocardial Infarction Risk?

Dagmar Drohan, Cornelia Weikert, Jutta Dierkes, Kerstin Klipstein-Grobusch, Brian Buijsse, Matthias Möhlig, Andreas F. H. Pfeiffer, Tobias Pischon, Joachim Spranger and Heiner Boeing

Arterioscler Thromb Vasc Biol 2010, 30:2053-2058: originally published online July 29, 2010

doi: 10.1161/ATVBAHA.110.209346

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2010 American Heart Association. All rights reserved. Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://atvb.ahajournals.org/content/30/10/2053>

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at

<http://atvb.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:

journalpermissions@lww.com

Reprints: Information about reprints can be found online at

<http://www.lww.com/reprints>

Plasma γ -Glutamyltransferase, Cysteinyl-Glycine, and Oxidized Low-Density Lipoprotein

A Pathway Associated With Myocardial Infarction Risk?

Dagmar Drogan, Cornelia Weikert, Jutta Dierkes, Kerstin Klipstein-Grobusch, Brian Buijsse, Matthias Möhlig, Andreas F. H. Pfeiffer, Tobias Pischon, Joachim Spranger, Heiner Boeing

Objective—To investigate the interrelation between plasma γ -glutamyltransferase (GGT) activity, cysteinyl-glycine (Cys-Gly) (ie, a thiol originating from GGT-mediated cleavage of glutathione), and oxidized low-density lipoprotein (oxLDL) with regard to myocardial infarction (MI) risk in a prospective study.

Methods and Results—Incident cases of MI were identified among European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam participants without prior MI during 6.0 years of follow-up. Baseline levels of Cys-Gly and oxLDL and GGT activity in plasma were measured in a case-cohort study comprising 837 subjects without incident MI and 116 subjects with incident MI. The relation of GGT, Cys-Gly and oxLDL to MI risk was assessed using Cox proportional hazards regression analysis. After adjustment for established risk factors, hazard ratios associated with a 1-SD unit increase in the log-transformed biomarker were 1.63 (95% CI, 1.30 to 2.05) for GGT, 1.36 (95% CI, 1.07 to 1.72) for Cys-Gly, and 1.37 (95% CI, 1.00 to 1.86) for oxLDL. Cys-Gly and oxLDL accounted for 2.3% of the relation between GGT and MI risk.

Conclusion—The positive association between GGT activity and MI risk appears to be independent of circulating Cys-Gly and oxLDL levels. With Cys-Gly, we found a potential new predictor of MI risk whose impact needs to be further elucidated. (*Arterioscler Thromb Vasc Biol.* 2010;30:2053-2058.)

Key Words: γ -glutamyltransferase ■ cysteinyl-glycine ■ oxidized LDL ■ myocardial infarction ■ prospective study

Elevated blood γ -glutamyltransferase (GGT) activity, an indicator of hepatobiliary dysfunction and alcohol misuse, has been recognized as a predictor of cardiovascular diseases (CVDs).¹⁻⁴ GGT is present in blood and on the surface of most cell types, where it catalyzes the cleavage of extracellular glutathione to a γ -glutamyl moiety and cysteinyl-glycine (Cys-Gly). The latter triggers iron-dependent oxidation of low-density lipoprotein (LDL).⁵ In human atherosclerotic plaques, catalytically active GGT colocalizes with oxidized LDLs (oxLDLs) and CD68⁺ foam cells, suggesting that the pro-oxidative action of Cys-Gly may causally link GGT activity with CVD risk.⁶

To our knowledge, the hypothesis of the previously discussed metabolic relation has not been studied so far in observational studies. Specifically, prospective data relating Cys-Gly status to risk of myocardial infarction (MI) are lacking. Accordingly, the aims of this study were 2-fold: (1) to assess the distinct relations between plasma GGT activity, Cys-Gly, and oxLDL to risk of

MI in a prospective cohort study and (2) to investigate to what extent circulating Cys-Gly and oxLDL account for the association between GGT activity and MI risk.

Methods

Study Design and Population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study is part of the large-scale EPIC cohort and includes 10,904 male and 16,644 female participants recruited from the general population of Potsdam and surrounding areas. The preferred age range for recruitment was from 35 to 65 years. Study procedures were approved by the ethics committee of the medical association of Brandenburg, Germany; and all participants gave their written informed consent. A baseline examination was conducted from 1994 through 1998 and included blood sampling, measurements of blood pressure (BP) and anthropometric parameters, self-administered questionnaires on diet and lifestyle, and personal computer-assisted interviews.⁷

Incident MI cases were identified by death certificate or self-report on the biennial follow-up questionnaires.⁸ The diagnosis was verified

Received on: January 29, 2010; final version accepted on: July 12, 2010.

From the Department of Epidemiology (D.D., C.W., K.K.-G., B.B., T.P., and H.B.), German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; the Department of Clinical Chemistry and Pathobiochemistry (J.D.), Otto-von-Guericke University, Magdeburg, Germany; the Institute of Medicine (J.D.), Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway; the Faculty of Health Sciences (K.K.-G.), School of Public Health, University of the Witwatersrand, Johannesburg, South Africa; the Department of Clinical Nutrition (M.M., A.F.H.P., and J.S.), German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; and the Department of Endocrinology, Diabetes and Nutrition (M.M., A.F.H.P., and J.S.), Charité-Universitätsmedizin Berlin, Berlin, Germany.

Correspondence to Dagmar Drogan DrPH, Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany. E-mail drogan@dife.de

© 2010 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.110.209346

by review of medical records from the hospital, by contacting the patients' attending physicians, or by review of death certificates according to World Health Organization criteria.⁹ After excluding subjects with a history of MI, we identified 159 cases of incident MI (*International Statistical Classification of Diseases, 10th Revision [ICD-10]* code I21) between the baseline examination and April 30, 2004 (mean±SD follow-up, 6.0±1.5 years). By using a case-cohort design, a subcohort (n=851) was randomly drawn among those participants of the EPIC-Potsdam cohort who were free of MI at baseline and who had provided baseline plasma samples. Consistent with the case-cohort design, the subcohort included 5 subjects who sustained an incident MI during the study period. We excluded 5 noncases with missing follow-up information, 1 noncase and 19 cases with incomplete biomarker data, and 3 cases with missing information on relevant covariates. Because diabetes mellitus may be both a consequence and a cause of high GGT levels, we excluded 8 noncases and 21 cases with prevalent diabetes at baseline. Thus, the final data set comprised 953 individuals, with 842 belonging to the subcohort (5 internal cases and 837 noncases) and 111 external cases.

Assessment of Covariates

The baseline measurement of anthropometric parameters was obtained by trained personnel with the participants dressed in light clothes and without shoes.¹⁰ The body mass index of participants was calculated as weight in kilograms divided by height in meters squared. Smoking habits, physical activity, educational attainment, and medical history were assessed during a standardized interview. Dietary habits, including alcohol consumption during the previous year, were assessed at baseline by using a validated self-administered food frequency questionnaire.¹¹ Measurements of systolic and diastolic BP were obtained after a resting period of 15 to 30 minutes.¹⁰

Biomarker Assessment

During the baseline examination, peripheral venous citrate blood samples were taken and subsequently centrifuged at 1000g for 10 minutes at 4°C. Plasma was fractionated and stored in liquid nitrogen at -196°C. Total cholesterol (TC, ABX Diagnostics Cholesterol), high-density lipoprotein cholesterol (HDL-C, ABX Diagnostics HDL Cholesterol Direct), GGT (ABX Diagnostics GGT CP) activity, and C-reactive protein (CRP, ABX Diagnostics CRP CP) levels were determined on an ABX Pentra 400 using standard reagents from Horiba ABX, Montpellier, France. The Cys-Gly level was determined by high-performance liquid chromatography with fluorescence detection, as described for homocysteine.¹² The concentration of oxLDL was measured with a competitive ELISA (Mercodia AB, Uppsala, Sweden). The intra-assay and interassay coefficients of variation were 0.9% and 4.7% for TC, 1.2% and 5.2% for HDL-C, 0.8% and 5.7% for GGT, 7.3% and 9.1% for Cys-Gly, 9.0% and 17.0% for oxLDL, and 0.9% and 4.7% for CRP, respectively.

Statistical Analysis

Statistical analysis was performed using the SAS software package, release 9.1 (SAS Institute Inc, Cary, NC). All tests performed were 2-sided, with $P < 0.05$ considered statistically significant.

The major baseline characteristics of cases and subjects of the subcohort are presented as mean±SD or frequency. GGT, Cys-Gly, and oxLDL were log transformed and are displayed as geometric means with corresponding 95% CIs. Within subjects of the subcohort, we calculated geometric mean values and 95% CIs of GGT, Cys-Gly, and oxLDL across baseline characteristics using the general linear model. To examine the correlation among GGT, Cys-Gly, and oxLDL, we calculated Spearman correlation coefficients (r_s) within subcohort members.

To investigate the association of GGT, Cys-Gly, and oxLDL with risk of MI, we conducted Cox proportional hazards regression analysis adapted for the case-cohort design using the weighting method described by Prentice.¹³ We categorized the biomarkers into sex-specific quartiles based on their distribution within the subcohort and calculated hazard ratios (HRs) using participants within the lowest category as the reference group. Because of the dependence

among observations introduced by the case-cohort design, we used robust SEs obtained from the robust sandwich covariance estimates for calculating 95% CIs. For the counting process style of input, the subjects' age at recruitment was used as the entry time and age at MI diagnosis or censoring as the exit time. All models were stratified according to age at recruitment to reduce sensitivity against violations of the proportional hazards assumption.

For each biomarker, we present 3 regression models. The first model includes age and sex. In addition to the variables in the first model, the second model also includes the following variables: body mass index, smoking status (never smokers, former smokers >5 years, former smokers ≤5 years, smokers <20 U/d, and smokers ≥20 U/d), educational attainment (vocational school or less, technical school, or university), physical activity (mean duration of leisure time physical activities during the summer and winter seasons [in hours per day]), and sex-specific categories of alcohol consumption (men: <2, 2–14.99, or ≥15 g/d; and women: 1, 1–7.49, or ≥7.5 g/d). In addition to the variables in the first 2 models, the third model also includes the TC to HDL-C ratio (sex-specific quartiles) and history of hypertension. The latter was defined as a systolic BP of 140 mm Hg or higher, a diastolic BP of 90 mm Hg or higher, a self-reported hypertension diagnosis, or use of antihypertensive medication. We calculated the probability value for linear trend across quartiles by using the participants' assigned quartile as a continuous variable in the respective Cox model.

We also analyzed MI risk per 1-SD increase in log-transformed GGT, Cys-Gly, and oxLDL. Effect modification by sex was tested by including cross-product terms of the respective log-transformed predictor variables with sex in the third regression model. In additional analyses, we controlled for log-transformed Cys-Gly and/or oxLDL levels to study the extent by which adjustment for these potential mediators attenuated the association between GGT (log transformed) and risk of MI. The contribution of each of the 2 biomarkers to this risk relation was quantified using effect decomposition.¹⁴ The corresponding 95% CI was calculated based on the Fieller theorem.¹⁵

Results

Subjects with incident MI had higher plasma levels of GGT, Cys-Gly, and oxLDL than individuals of the subcohort (Table 1). In addition, MI cases tended to be older and were more likely to be male, to be current smokers, and to have hypertension at baseline.

In participants of the subcohort, plasma GGT activity was significantly, although modestly, correlated with circulating Cys-Gly ($r_s=0.20$, $P < 0.001$) and oxLDL ($r_s=0.24$, $P < 0.001$). A similar correlation was observed between oxLDL and Cys-Gly ($r_s=0.24$, $P < 0.001$). There was a strong correlation between oxLDL levels and TC/HDL-C ratio ($r_s=0.59$, $P < 0.001$). Levels of GGT, Cys-Gly, and oxLDL were higher in men compared with women and in participants with prevalent hypertension (Table 2). Mean levels of all 3 biomarkers were highest among subjects who smoke ≥20 U/d.

In Cox models stratified by age and adjusted for sex, body mass index, smoking status, educational attainment, physical activity, alcohol consumption, prevalent hypertension, and TC/HDL-C ratio (model 3), GGT activity in plasma was positively associated with risk of MI (Table 3). Relative to the lowest quartile, subjects within the top quartile of GGT had a 3.71-fold increased risk of future MI (95% CI, 1.68 to 8.23; $P=0.002$ for trend). The relative risk for MI for the comparison of extreme Cys-Gly quartiles was 1.80 (95% CI, 1.00 to 3.23; $P=0.03$ for trend). Regarding oxLDL, we found a positive association to MI risk in models adjusted for age, sex, and several lifestyle factors (model 2). However, the HRs were

Table 1. Baseline Characteristics of the Study Population*

Characteristics	Cases (n=116)†	Subcohort (n=842)
Male sex	75.9	39.8
Age, y‡	56.2±6.5	49.8±8.5
Educational attainment		
Vocational school or less	37.9	36.1
Technical school	25.0	25.2
University	37.1	38.7
Smoking status		
Never smokers	22.4	46.4
Past smokers >5 y	19.8	25.4
Past smokers ≤5 y	6.9	7.7
Current smokers <20 U/d	25.0	15.0
Current smokers ≥20 U/d	25.9	5.5
BMI‡	27.4±3.7	25.7±3.6
Physical activity, h/d‡	1.5±1.1	1.5±1.0
Alcohol consumption		
<2.00 g/d (males) and <1.00 g/d (females)	19.0	9.6
2.00–14.99 g/d (males) and 1.00–7.49 g/d (females)	40.5	45.4
≥15.00 g/d (males) and ≥7.50 g/d (females)	40.5	45.0
Hypertension	69.8	44.8
TC/HDL-C ratio‡	5.0±1.2	3.9±1.0
GGT, U/L§	26.1 (23.2–29.4)	16.5 (15.8–17.2)
Cys-Gly, μmol/L§	28.1 (27.1–29.1)	25.2 (24.8–25.6)
oxLDL, U/L§	50.6 (47.0–54.5)	37.4 (36.4–38.5)

BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared).

*Data are given as percentages unless otherwise indicated.

†Cases are subjects with incident MI; 5 of them are subcohort members.

‡Data are given as mean±SD.

§Data are given as geometric mean (95% CI).

substantially attenuated and no longer significant after adjustment for hypertension and TC/HDL-C ratio, with subjects in the top compared with the bottom quartile exhibiting an HR of 1.31 (95% CI, 0.56 to 3.05; $P=0.31$ for trend across quartiles).

To test the hypothesis that Cys-Gly and oxLDL explain a significant proportion of the association between GGT activity and MI risk, we first analyzed the 3 biomarkers as continuous predictor variables for the end point. Based on model 3, HRs corresponding to a 1-SD increase in the log-transformed biomarker levels were 1.63 (95% CI, 1.30 to 2.05) for GGT, 1.36 (95% CI, 1.07 to 1.72) for Cys-Gly, and 1.37 (95% CI, 1.00 to 1.86) for oxLDL (Figure). The strengths of these associations were not significantly different between men and women (P values for interaction between biomarker levels and sex: GGT, $P=0.54$; Cys-Gly, $P=0.68$; and oxLDL, $P=0.36$). Further control for Cys-Gly and oxLDL had a minor influence on the positive relation between GGT and MI risk (HR, 1.60; 95% CI, 1.27 to 2.02) (Figure). Overall, both biomarkers explained 2.3% of the association between GGT and MI risk, with Cys-Gly accounting for 1.8% (95% CI, -0.3% to 4.8%) and oxLDL for an additional 0.5% (95% CI, -0.2% to 1.8%). In comparison, Cys-Gly levels were significantly associated with MI risk after adjustment for GGT activity but not after adjustment for oxLDL. In contrast, there was no statistically significant association between oxLDL

and MI risk after control for GGT and/or Cys-Gly. Additional adjustment for CRP levels did not notably alter any of the associations described (data not shown).

Discussion

In this case-cohort study nested within the EPIC-Potsdam cohort, plasma GGT activity and circulating Cys-Gly were positively associated with MI risk. Although the formation of Cys-Gly is believed to link GGT activity with LDL oxidation in atherosclerotic plaques, plasma levels of Cys-Gly and oxLDL statistically accounted for only 2.3% of the relation between plasma GGT activity and MI risk.

In line with our findings, there is growing epidemiological evidence of a positive association of GGT with coronary heart disease (CHD)^{16,17} and CVD mortality.^{3,4,18–20} A meta-analysis¹ of prospective studies suggested that an elevation of GGT by 1 U/L was associated with an HR of 1.20 (95% CI, 1.02 to 1.40) for CHD. Low-grade inflammation has been proposed as 1 mechanism by which GGT activity may contribute to atherosclerosis and CVD risk.²¹ However, in participants of the Framingham Study, serum GGT was positively associated with incident CVD even after accounting for CRP.² Consistently, adjustment for CRP had a minor impact on MI risk associated with GGT activity in our study (data not shown). Alternatively, GGT-mediated oxidative stress has been hypothesized as a pathway linking GGT activity with CVD risk.⁶ Specifically, the action of GGT on glutathione leads to an increase in Cys-Gly, which can trigger LDL oxidation in the presence of transition metals.⁵ Because there is evidence for such a pathway in human atherosclerotic plaques,²² we aimed to study the interrelation between circulating Cys-Gly and oxLDL and GGT activity with regard to MI risk in an observational study.

To our knowledge, we present the first prospective data on the association between circulating Cys-Gly and MI risk. Conflicting results were reported for the comparison of Cys-Gly levels between patients with CHD and healthy controls.^{23,24} However, these studies were cross-sectional and did not adjust for potentially confounding factors that may have biased the observed associations. In our data, Cys-Gly was positively associated with MI risk after controlling for a variety of risk factors (Figure). Adjustment for plasma GGT activity had a minor influence on this risk relation, suggesting that, despite its metabolic link, circulating Cys-Gly is not merely a reflection of GGT activity. By including oxLDL as a covariate, we also attempted to eliminate all pathways by which circulating oxLDL may mediate the relation between Cys-Gly and MI risk. Although the impact of oxLDL was not strong, the resulting risk estimate lost statistical significance, suggesting a weak interrelation between levels of Cys-Gly and LDL oxidation in plasma (Figure). This interrelation is presumably stronger in the pro-oxidative environment of the subendothelial space, and recent data support the hypothesis that circulating Cys-Gly reflects or even determines its content in the atherosclerotic plaque.²⁵ In addition, Cys-Gly is a component of plasma oxidation-reduction thiol status²⁶; thus, oxidation-reduction and disulphide exchange reactions with other thiols may further contribute to the positive relation between Cys-Gly and MI risk beyond GGT activity. If other

Table 2. Plasma GGT Activity and Levels of Cys-Gly and oxLDL Across Baseline Study Characteristics in 842 Subjects of the Subcohort

Characteristics	GGT Data		Cys-Gly Data		oxLDL Data	
	Geometric Mean (95% CI), U/L	P Value	Geometric Mean (95% CI), μ mol/L	P Value	Geometric Mean (95% CI), U/L	P Value
Sex		<0.001		<0.001		<0.001
Male	24.7 (23.3–26.1)		27.1 (26.5–27.8)		43.3 (41.6–45.0)	
Female	13.2 (12.5–13.9)		24.3 (23.9–24.8)		35.6 (34.3–36.8)	
Educational attainment		0.07		0.07		0.02
Vocational school or less	17.4 (16.2–18.7)		25.8 (25.2–26.5)		40.7 (38.9–42.5)	
Technical school	16.1 (14.8–17.5)		24.7 (24.0–25.5)		37.3 (35.4–39.3)	
University	18.3 (17.1–19.6)		25.8 (25.1–26.4)		38.0 (36.4–39.7)	
Smoking status		<0.001		0.07		<0.001
Never smokers	14.8 (13.9–15.8)		25.0 (24.4–25.6)		36.6 (35.2–38.1)	
Past smokers >5 y	20.3 (18.7–22.1)		26.1 (25.3–26.9)		39.8 (37.7–42.0)	
Past smokers \leq 5 y	17.6 (15.1–20.5)		26.0 (24.6–27.5)		40.9 (37.2–45.0)	
Current smokers <20 U/d	18.5 (16.7–20.5)		25.2 (24.3–26.2)		38.8 (36.3–41.4)	
Current smokers \geq 20 U/d	22.6 (19.5–26.2)		26.8 (25.4–28.3)		46.3 (42.2–50.9)	
Alcohol consumption		<0.001		0.45		0.09
<2.00 g/d (males) and <1.00 g/d (females)	15.9 (14.0–18.1)		25.2 (24.0–26.4)		40.9 (37.7–44.3)	
2.00–14.99 g/d (males) and 1.00–7.49 g/d (females)	15.4 (14.5–16.4)		25.3 (24.8–25.9)		37.6 (36.1–39.1)	
\geq 15.00 g/d (males) and \geq 7.50 g/d (females)	20.1 (18.8–21.4)		25.8 (25.2–26.4)		39.5 (38.0–41.1)	
Hypertension		<0.001		<0.001		<0.001
No	15.4 (14.6–16.4)		24.5 (24.0–25.1)		35.7 (34.3–37.0)	
Yes	19.9 (18.7–21.1)		26.7 (26.1–27.3)		42.5 (40.9–44.1)	

prospective studies confirm Cys-Gly as a predictor of MI, its role as either a marker or a causal risk factor needs to be elucidated.

Because high concentrations of oxLDL in plasma and plaque are correlated with the vulnerability of atherosclerotic lesions,²⁷ we also analyzed the impact of circulating oxLDL on MI risk. Each 1-SD increase in log-transformed oxLDL was associated with a 37% increased MI risk after adjustment for several cardiovascular risk factors (Figure). This estimate is comparable to Cys-Gly, although the CIs were wider. Subsequently, adjustment for GGT and/or Cys-Gly attenuated the association between oxLDL and MI risk to statistical nonsignificance, although the shift in the risk estimate was not strong. In view of the strong correlation between oxLDL and TC/HDL-C ratio, it appeared that this lipid ratio, rather than GGT or Cys-Gly, determines the impact of circulating oxLDL on MI risk. Comparable to our own study, at least 3 prospective studies^{28–30} have used a monoclonal antibody 4E6-based competitive ELISA for analyzing oxLDL levels in relation to coronary events. However, only 2 of them have included standard lipids as potential confounders in their regression models. In data from the Health Professionals Follow-up Study and the Nurses' Health Study, the positive relation between oxLDL and CHD risk was strongly attenuated on adjustment for the TC/HDL-C ratio.³⁰ In contrast, oxLDL predicted CHD risk in male participants of the Multinational MONItoring of trends and determinants in CARDiovascular disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) Augsburg studies, independent of other lipids, including TC/HDL-C ratio.²⁹ These contradictory data suggest that the degree of correlation between oxLDL and standard lipids hampers the possibility to disentangle their independent contributions to the end point.

In line with the modest correlation among the biomarkers, our study did not confirm that much of the association between plasma GGT activity and the end point was accounted for by circulating Cys-Gly and oxLDL. Unexpectedly, the 2 biomarkers explained only a small fraction of the increased MI risk associated with higher GGT activity. Considering the antioxidant properties of plasma, this finding suggests that the hypothesized pathway has minor relevance in plasma, whereas GGT-mediated cleavage of glutathione may still promote LDL oxidation in the intima. Alternatively, GGT has been hypothesized to predict CVD as a marker of oxidative stress³¹ or exposure to xenobiotics that need to be conjugated to glutathione.³² In both cases, the increased demand for glutathione would result in a compensatory increase in GGT, thereby contributing to excretion of conjugates and supply with precursors for intracellular glutathione synthesis. In plasma, glutathione and GGT are negatively correlated,³³ reflecting either an overconsumption of glutathione because of GGT-induced oxidative stress or an increase in GGT activity in response to higher glutathione turnover. GGT and glutathione are also involved in the formation of Cys-leukotrienes that may contribute to atherosclerosis and ischemia.³⁴ In addition, GGT may reflect fat accumulation and insulin resistance in liver tissue and is, thus, considered as a biomarker for the presence of metabolic syndrome.³⁵

Our study benefits from a well-characterized study population embedded into the EPIC-Potsdam cohort. Data were collected prospectively, thereby eliminating the potential for recall bias and reducing the possibility that biomarker levels change as a result of the outcome. Furthermore, follow-up proportions exceeded 90%⁸; and all self-reports on incident MI were verified through medical records, treating physicians, or death certificates. Finally, we were able to adjust for

Table 3. Data for Myocardial Infarction Across Quartiles of Plasma GGT Activity, Cys-Gly, and oxLDL

Characteristics	Quartile*				P Value for Trend
	1	2	3	4	
GGT data					
Median for males/females, U/L	12.7/7.3	18.7/11.0	27.5/14.7	47.9/23.3	NA
No. of cases, males/females	12/2	24/4	19/9	33/13	NA
Model 1†	1.00	2.09 (1.02–4.27)	2.25 (1.13–4.51)	3.97 (2.01–7.85)	<0.001
Model 2†	1.00	2.49 (1.13–5.45)	2.26 (1.01–5.06)	4.13 (1.90–8.97)	<0.001
Model 3†	1.00	2.37 (1.05–5.34)	2.07 (0.92–4.70)	3.71 (1.68–8.23)	0.002
Cys-Gly data					
Median for males/females, $\mu\text{mol/L}$	20.3/18.0	25.0/22.2	28.5/26.5	35.2/32.2	NA
No. of cases, males/females	17/1	18/7	21/10	32/10	NA
Model 1†	1.00	1.25 (0.67–2.35)	1.53 (0.82–2.85)	1.94 (1.07–3.51)	0.02
Model 2†	1.00	0.96 (0.48–1.91)	1.16 (0.59–2.25)	1.93 (1.08–3.46)	0.02
Model 3†	1.00	0.88 (0.44–1.77)	1.14 (0.60–2.19)	1.80 (1.00–3.23)	0.03
oxLDL					
Median for males/females, U/L	24.6/21.2	37.1/30.4	47.9/39.3	63.9/56.2	NA
No. of cases, males/females	11/3	8/6	28/8	41/11	NA
Model 1†	1.00	0.97 (0.44–2.17)	2.30 (1.14–4.61)	3.18 (1.58–6.39)	<0.001
Model 2†	1.00	0.85 (0.36–1.99)	1.75 (0.83–3.66)	2.08 (0.96–4.49)	0.01
Model 3†	1.00	0.78 (0.33–1.84)	1.30 (0.58–2.89)	1.31 (0.56–3.05)	0.31

NA indicates not applicable.

*Quartiles are sex specific and based on the distribution of the respective plasma biomarker level within the subcohort.

†Data are given as HR (95% CI), derived from 9 Cox proportional hazards regression models with age as the underlying time variable. Model 1 is stratified by age and adjusted for sex; model 2, stratified by age and adjusted for sex, body mass index, smoking status, educational attainment, physical activity, and alcohol consumption; and model 3, adjusted for the variables in model 2 and also for hypertension and TC/HDL-C ratio.

important risk factors that may have confounded the association between GGT, Cys-Gly, and oxLDL regarding MI risk. Because we used a case-cohort design, our findings are expected to be generalizable to the source population without the need to assess biomarker levels in the entire cohort¹³; the external generalizability may be limited to populations with similar characteristics. Compared with a recently described case-cohort study nested within EPIC-Potsdam,³⁶ the presented analyses are based on a study population with a shorter follow-up but with available data on the 3 biomarkers (GGT, Cys-Gly, and oxLDL). Although the relatively few MI cases

affect the statistical power to detect weak associations, established risk factors (eg, smoking or hypertension) remained predictive for MI in our data. In addition, a positive association between GGT and CVD has been confirmed by several prospective studies, suggesting that the conclusions drawn are valid, although the number of cases is relatively low. Because the observed associations between the biomarkers and MI risk were not significantly different between men and women ($P>0.05$), we did not stratify for sex in any of the analyses. However, we relied on a single baseline blood sample from each participant. Thus, our findings do not reflect within-subject variations in biomarker levels over time, and random measurement errors may have attenuated the true relation between the biomarkers and the end point. Given the epidemiological nature of our observations, our study can neither prove nor disprove a causal link between GGT, Cys-Gly, oxLDL, and MI risk. Rather, we have shown that our data are not consistent with a hypothetical model to explain the association between plasma GGT activity and incidence of MI.

In summary, we demonstrated that GGT activity in plasma is positively associated with incident MI. With Cys-Gly, we found a potential new predictor of MI risk whose impact needs to be further elucidated. However, contrary to our hypothesis, plasma levels of Cys-Gly and oxLDL appear to have a negligible influence on the relationship between GGT and the end point.

Acknowledgments

We thank Mercodia for providing the oxLDL kits free of charge, Wolfgang Fleischhauer, physician, for case ascertainment, Ellen Kohlsdorf, MS, and Wolfgang Bernigau, MS, for data management,

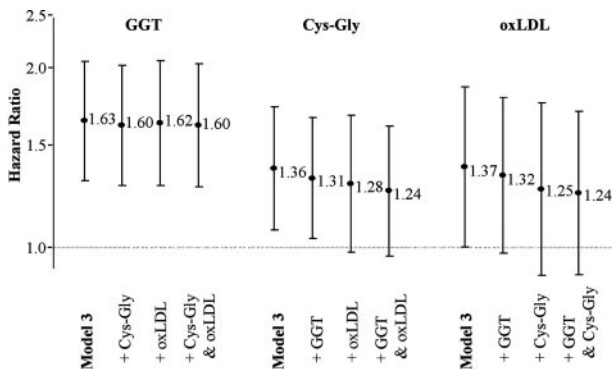


Figure. The HRs (95% CIs) of myocardial infarction per 1-SD increase in log-transformed plasma GGT activity, Cys-Gly, and oxLDL, as derived from Cox proportional hazards regression analysis, with age as the underlying time variable. Model 3 is stratified by age at recruitment and adjusted for sex, body mass index, smoking status, educational attainment, physical activity, alcohol consumption, hypertension, and TC/HDL-C ratio.

the technical assistants for performing the laboratory measurements, and all EPIC-Potsdam participants for their invaluable contributions.

Sources of Funding

The recruitment phase of the EPIC-Potsdam Study was supported by the Federal Ministry of Science of Germany (01 EA 9401) and the European Union (SOC 95201408 05F02). The follow-up was supported by German Cancer Aid (70-2488-Ha I) and the European Community (SOC 98200769 05F02). The present study was supported by the Federal Ministry of Education and Research (0312750B), clinical research groups of the German Research Foundation (KFO192/1 and 218/1), a graduate school of the German Research Foundation (GK1208) (Drs Möhlig, Pfeiffer, and Spranger), the German Research Federal Ministry (Drs Pfeiffer and Spranger), and a Heisenberg-Professorship (SP716/1-1) (Dr Spranger).

Disclosures

None.

References

- Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: analysis of the British Women's Heart and Health Study and Meta-Analysis. *Arterioscler Thromb Vasc Biol*. 2007;27:2729–2735.
- Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, Wang TJ, Benjamin EJ, D'Agostino RB, Vasan RS. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol*. 2007;27:127–133.
- Lee DH, Buijse B, Steffen L, Holtzman J, Luepker R, Jacobs DR Jr. Association between serum gamma-glutamyltransferase and cardiovascular mortality varies by age: the Minnesota Heart Survey. *Eur J Cardiovasc Prev Rehabil*. 2009;16:16–20.
- Strasak AM, Kelleher CC, Klenk J, Brant LJ, Ruttman E, Rapp K, Concin H, Diem G, Pfeiffer KP, Ulmer H. Longitudinal change in serum gamma-glutamyltransferase and cardiovascular disease mortality: a prospective population-based study in 76,113 Austrian adults. *Arterioscler Thromb Vasc Biol*. 2008;28:1857–1865.
- Paolicchi A, Minotti G, Tonarelli P, Tongiani R, De Cesare D, Mezzetti A, Dominici S, Comperti M, Pompella A. Gamma-glutamyl transpeptidase-dependent iron reduction and LDL oxidation: a potential mechanism in atherosclerosis. *J Invest Med*. 1999;47:151–160.
- Emdin M, Pompella A, Paolicchi A. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. *Circulation*. 2005;112:2078–2080.
- Boeing H, Korfmann A, Bergmann MM. Recruitment procedures of EPIC-Germany: European Investigation into Cancer and Nutrition. *Ann Nutr Metab*. 1999;43:205–215.
- Bergmann MM, Bussas U, Boeing H. Follow-up procedures in EPIC-Germany: data quality aspects: European Prospective Investigation into Cancer and Nutrition. *Ann Nutr Metab*. 1999;43:225–234.
- WHO MONICA Project. MONICA manual, part IV: event registration. Section 1: coronary event registration data component. 1999. Available at <http://www.ktl.fi/publications/monica/manual/part4/iv-2.htm>. Accessed December 8, 2009.
- Kroke A, Bergmann MM, Lotze G, Jeckel A, Klipstein-Grobusch K, Boeing H. Measures of quality control in the German component of the EPIC study: European Prospective Investigation into Cancer and Nutrition. *Ann Nutr Metab*. 1999;43:216–224.
- Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the German part of the EPIC project: European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol*. 1997;26:S59–S70.
- Dierkes J, Jeckel A, Ambrosch A, Westphal S, Luley C, Boeing H. Factors explaining the difference of total homocysteine between men and women in the European Investigation into Cancer and Nutrition Potsdam study. *Metabolism*. 2001;50:640–645.
- Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *BioMetrika*. 1986;73:1–11.
- Chen C, Wang H, Snapinn SM. Proportion of treatment effect (PTE) explained by a surrogate marker. *Stat Med*. 2003;22:3449–3459.
- Fieller EC. The biological standardization of insulin. *J R Stat Soc*. 1940;7(suppl):1–15.
- Lee DH, Silventoinen K, Hu G, Jacobs DR Jr, Jousilahti P, Sundvall J, Tuomilehto J. Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women. *Eur Heart J*. 2006;27:2170–2176.
- Meisinger C, Doring A, Schneider A, Lowel H. Serum gamma-glutamyltransferase is a predictor of incident coronary events in apparently healthy men from the general population. *Atherosclerosis*. 2006;189:297–302.
- Hozawa A, Okamura T, Kadowaki T, Murakami Y, Nakamura K, Hayakawa T, Kita Y, Nakamura Y, Okayama A, Ueshima H. Gamma-glutamyltransferase predicts cardiovascular death among Japanese women. *Atherosclerosis*. 2007;194:498–504.
- Ruttman E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation*. 2005;112:2130–2137.
- Wannamethee G, Ebrahim S, Shaper AG. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all causes. *Am J Epidemiol*. 1995;142:699–708.
- Lee DH, Jacobs DR Jr. Association between serum gamma-glutamyltransferase and C-reactive protein. *Atherosclerosis*. 2005;178:327–330.
- Franzini M, Corti A, Martinelli B, Del Corso A, Emdin M, Parenti GF, Glauber M, Pompella A, Paolicchi A. Gamma-glutamyltransferase activity in human atherosclerotic plaques: biochemical similarities with the circulating enzyme. *Atherosclerosis*. 2009;202:119–127.
- Mendis S, Athauda SB, Takashi K. Association between hyperhomocysteinemia and ischemic heart disease in Sri Lankans. *Int J Cardiol*. 1997;62:221–225.
- Ozkan Y, Ozkan E, Simsek B. Plasma total homocysteine and cysteine levels as cardiovascular risk factors in coronary heart disease. *Int J Cardiol*. 2002;82:269–277.
- Zinella A, Lepedda A Jr, Sotgia S, Zinella E, Scanu B, Turrini F, Spirito R, Deiana L, Formato M, Carru C. Evaluation of low molecular mass thiols content in carotid atherosclerotic plaques. *Clin Biochem*. 2009;42:796–801.
- Ueland PM, Mansoor MA, Guttormsen AB, Muller F, Aukrust P, Refsum H, Svardal AM. Reduced, oxidized and protein-bound forms of homocysteine and other aminothiols in plasma comprise the redox thiol status: a possible element of the extracellular antioxidant defense system. *J Nutr*. 1996;126:1281S–1284S.
- Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K, Nagahiro S. Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler Thromb Vasc Biol*. 2002;22:1649–1654.
- Holvoet P, Kritchevsky SB, Tracy RP, Mertens A, Rubin SM, Butler J, Goodpaster B, Harris TB. The metabolic syndrome, circulating oxidized LDL, and risk of myocardial infarction in well-functioning elderly people in the health, aging, and body composition cohort. *Diabetes*. 2004;53:1068–1073.
- Meisinger C, Baumert J, Khuseynova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation*. 2005;112:651–657.
- Wu T, Willett WC, Rifai N, Shai I, Manson JE, Rimm EB. Is plasma oxidized low-density lipoprotein, measured with the widely used antibody 4E6, an independent predictor of coronary heart disease among US men and women? *J Am Coll Cardiol*. 2006;48:973–979.
- Lee DH, Blomhoff R, Jacobs DR Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radic Res*. 2004;38:535–539.
- Lee DH, Jacobs DR Jr. Is serum gamma-glutamyltransferase a marker of exposure to various environmental pollutants? *Free Radic Res*. 2009;43:533–537.
- Sedda V, De Chiara B, Parolini M, Caruso R, Campolo J, Cighetti G, De Maria R, Sachero A, Donato L, Parodi O. Plasma glutathione levels are independently associated with gamma-glutamyltransferase activity in subjects with cardiovascular risk factors. *Free Radic Res*. 2008;42:135–141.
- Back M. Leukotriene signaling in atherosclerosis and ischemia. *Cardiovasc Drugs Ther*. 2009;23:41–48.
- Grundy SM. Gamma-glutamyl transferase: another biomarker for metabolic syndrome and cardiovascular risk. *Arterioscler Thromb Vasc Biol*. 2007;27:4–7.
- Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost HG, Haring HU, Boeing H, Fritsche A. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. *Circulation*. 2008;118:2555–2562.