Longitudinal Change in Serum Gamma-Glutamyltransferase and Cardiovascular Disease Mortality. A Prospective Population-Based Study in 76 113 Austrian Adults

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Objective—The purpose of this study was to investigate the association of longitudinal change in serum γ-glutamyltransferase (GGT) with mortality from cardiovascular disease (CVD).

Methods and Results—A population-based cohort of 76 113 Austrian men and women with 455 331 serial GGT measurements was prospectively followed-up for a median of 10.2 years after assessment of longitudinal GGT change during an average period of 6.9 years. Cox proportional hazards regression with time-varying covariates was used to evaluate GGT change as an independent predictor for CVD death. Independently of baseline GGT and other classical CVD risk factors, a pronounced increase in GGT (7-year change >9.2 U/L) was significantly associated with increased total CVD mortality in men ($P=0.005$); the adjusted hazard ratio (95% confidence interval) in comparison to stable GGT (7-year change −0.7 to 1.3 U/L) was 1.40 (1.09 to 1.81). Similarly, total CVD risk was elevated for increasing GGT in women, although effects were less pronounced and statistically significant only in subanalyses regarding coronary heart disease. Age of participants significantly modified the relation between GGT change and CVD mortality, with markedly stronger associations to be observable for younger individuals.

Conclusion—Our study is the first to demonstrate that a longitudinal increase in GGT, independently of baseline GGT and even within its normal range, significantly increases risk of fatal CVD. (***Arterioscler Thromb Vasc Biol.* 2008;28:000-000**)

Key Words: cardiovascular disease mortality ■ γ-glutamyltransferase ■ longitudinal change ■ risk factor ■ epidemiology

Gamma-glutamyltransferase (GGT), present on the external surface of most cells and in serum, is the enzyme responsible for the extracellular catabolism of glutathione (GSH), the main thiol antioxidant in mammalian cells.[1–3] Despite its well established clinical use as an indicator of hepato-biliary diseases and marker of excessive alcohol intake,[1,4,5] in recent years, several epidemiological studies have sparked further interest in elevated GGT as an independent predictor for morbidity and mortality from causes other than liver disease.[6]

Specifically, it was reported that GGT is related to incidence of and mortality from cardiovascular disease (CVD),[7–14] and correlated with most cardiovascular risk factors, including diabetes, hypertension, dyslipidemia, and the metabolic syndrome.[15–18] It has been speculated that GGT may be a sensitive and reliable marker of oxidative stress,[7] which has been shown to be a key mechanism in the pathogenesis of many metabolic disorders, CVD, and other degenerative and lifestyle-related diseases.[19–22]

Despite a growing body of epidemiological evidence on the predictive significance of baseline GGT for prevalent health conditions or mortality, few data have been published on longitudinal changes in GGT. Although GGT displays a considerable intraindividual stability and strong "tracking" pattern,[23] evidence from large-scale population studies indicates also a perceptible increase in GGT during the past few decades, with a strong secular trend.[19,24] It has been reported that an increase in GGT over time is positively correlated with an increase in body mass index in both men and women, although other CVD risk factors, including systolic blood pressure, total and HDL-cholesterol, and triglycerides were associated differently in the genders.[24] A recent study re-

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ported a three-year increase of GGT (>5 U/L), even within its normal range, to be significantly related to insulin resistance and risk of incident type 2 diabetes, independently of baseline GGT, which is itself a diabetes risk factor.[25]

In the present study, we prospectively investigated the association of longitudinal change in serum GGT during an average period of 7 years with subsequent all-cause mortality, mortality from total CVD, coronary heart disease (CHD), congestive heart failure (CHF), and stroke in a population-based cohort of 76 113 Austrian men and women. Although previous studies have investigated the association of baseline GGT with morbidity and mortality from CVD, to our knowledge, the present study constitutes the first investigation to assess the predictive value of GGT change over time for mortality from overall CVD and all major subforms.

Methods

Study Population

The Vorarlberg Health Monitoring and Promotion Program [VHM&PP][8,23,26] started in 1985 and conducted by the Agency for Social and Preventive Medicine in Vorarlberg, the westernmost province of Austria, is one of the world's largest ongoing population-based risk factor surveillance programs. All adults of the region are invited to participate by a combination of different measures including written invitations, television, radio, and newspaper reports. Active follow-up of study participants is performed through a recall-system of written biennial reinvitation letters. Sociodemographic data are recorded, and a voluntary physical examination is conducted regularly in a standardized manner by trained local physicians and internists. During the examination, a fasting blood sample is taken. Costs are covered by the participant's (compulsory) health insurance. A more detailed description of the program methodology has been reported elsewhere.[23]

Between 1985 and 2005, a total of 184 774 male and female Vorarlberg residents (aged >18 years) were enrolled in the VHM&PP. Participants had between 1 and 19 routine health examinations. The current investigation was restricted to individuals with at least 2 examinations over a 5- to 9-year time interval, during which longitudinal GGT change from the first (baseline) visit was assessed. To minimize possible effects of reverse causality attributable to preexisting disease, we further excluded participants dying within the first year after GGT change assessment. Consequently, to avoid attrition/censoring bias in our analyses, we also excluded participants with follow-up periods <1 year, not experiencing a fatal event, yielding a total of 32 365 men and 43 748 women with complete and valid data on longitudinal GGT change eligible for analyses. Baseline characteristics of the present study cohort were very similar in comparison to the total VHM&PP cohort (supplemental Table I); however, because participants suffering from early deaths were not routinely eligible for the present investigation, mortality rates were slightly lower in both men and women.

All participants signed informed consents to have personal data stored and processed. For this study, institutional review board approval was obtained by the Ethics Committee of the province of Vorarlberg.

Data Collection

Measurements of height, weight, systolic and diastolic blood pressure, total cholesterol, triglycerides, blood glucose, GGT, and smoking status (current, former, never) are routinely obtained for each study participant. Individuals who reported smoking of at least one cigarette per day during the year before examination were classified as current smokers. Occupational status (blue collar, white collar, or self-employed) was determined by the insurance number of participants and used as a surrogate measure of socioeconomic status. Participants who were retired at baseline were classified according to their former occupation, and housewives were classified according to their husband's job.

Laboratory Measurements

Two central laboratories undergoing regular internal and external quality procedures enzymatically determined GGT concentrations on fasting blood samples. Within 60 to 240 minutes after venous blood sample collection from a cubital vein, serum was obtained by centrifugation for 15 minutes at 4000 rotations per minute. Subsequently, GGT concentrations were measured at 37°C and were given as units per liter (U/L). To check calibration, 3 daily control samples were included. If average values of the control samples of each run were not within 3% of the true value, the run was repeated. Day-by-day variation had to be within 5%.

End Points

By the end of 2005, a total of 4551 deaths were recorded in our database of which 1955 (43.0%) were cardiovascular- or cerebrovascular-related deaths. Date and cause of death information was provided by the local health authority and was linked in the database with the use of a validated procedure. All deaths were identified from death certificates that were confirmed by authorized physicians only. In cases of unclear causes of death, autopsies were performed. For analyses, deaths from total CVD (ICD-9 401 to 443 [except 415 to 417]; ICD-10 I10 to I79 [except I26 to I28])[8,27] were grouped into the following CVD subcategories: CHD (ICD-9 410 to 414; ICD-10 I20 to I25 [except I25.5]), CHF (ICD-9 425, 428, 429.0, 429.1, 429.3; ICD-10 I25.5, I42, I43, I50, I51.5, I51.7) and stroke (ICD-9 430 to 438; ICD-10 I60 to I69).

Statistical Analyses

A 2-stage regression approach, including (1) linear regression models to calculate least-square estimates of slopes for assessing longitudinal within-subject change in serum GGT per year[28–30] and (2) extended Cox proportional hazards models with time-varying covariates[31] to calculate hazard ratios with their 95% confidence intervals for the association of GGT change with all-cause and CVD mortality, was used. Because the number of examinations and the time between examinations differed among study participants, we chose a 5- to 9-year time period (mean 6.9 years) to assess longitudinal GGT change to balance the length of the assessment period against the length of the follow-up period after GGT change assessment. Specifically, the time period for GGT change assessment ended at the study visit that occurred closest to 7 years after baseline. All measurements obtained during the period of GGT change assessment were used to construct linear regression models for each participant, using classical least-squares estimation of the slope of the trend in GGT (measured by the size of the regression coefficients) against time as an indicator of their individual trend for GGT change, with each data point weighted equally.[28–30,32] Consequently, subjects were stratified into 7 equally-sized gender-specific categories according to the distribution of slopes of GGT change with 3 categories for each, increasing and decreasing GGT (mild, moderate, pronounced) and stable GGT.[33] The assumption of linearity in longitudinal GGT change in the individual linear regression models was checked by visual inspection of residual plots in a randomly selected subsample of 10% of study participants with at least 3 GGT measurements and was found to be approximately fulfilled. Additionally, because the variability between person's slopes was considerably greater than the residual or within person's variability, the estimates of the individual slopes have been shown to be approximately the same regardless of the method of estimation used.[32,34]

Follow-up started after assessment of longitudinal GGT change and ended at incidence of cardiovascular death or at censoring. Censoring events were noncardiovascular death, loss to follow-up, and end of study. We first estimated hazard ratios with 95% confidence intervals for the association of baseline GGT with all-cause and CVD mortality by calculating Cox proportional hazards models, adjusted for baseline values of age, body mass index, smoking status, occupational status, triglycerides, total cholesterol,

Table 1. Characteristics of Study Population, VHM&PP 1985 to 2005

Characteristic	Males	Females
Eligible participants with complete and valid data for longitudinal GGT-Change assessment, n*	32 365	43 748
Routine health examinations with longitudinal GGT measurements, n	187 635	267 696
Period of longitudinal GGT-Change assessment, mean±SD (range), y	6.9±0.9 (5.0–9.0)	6.9±0.9 (5.0–9.0)
Follow-up after longitudinal GGT-Change assessment, mean±SD (median), y	9.1±3.8 (9.8)	9.7±3.7 (10.6)
Person years at risk after longitudinal GGT-Change assessment	293 259	422 830
Age, mean±SD (range), y†	42.1±13.6 (18–89)	42.0±14.4 (19–90)
Body-mass-index, mean±SD (median), kg/m^2†	25.3±3.4 (25.0)	24.1±4.3 (23.3)
Glucose, mean±SD (median), mg/dL†	86.4±22.0 (84.0)	84.3±18.4 (83.0)
Triglycerides, mean±SD (median), mg/dL†	152.4±105.4 (123.0)	111.4±64.9 (95.0)
Total Cholesterol, mean±SD (median), mg/dL†	220.9±46.8 (218.0)	216.1±46.5 (211.0)
Gamma-glutamyltransferase, mean±SD (median), U/L†	42.2±55.7 (28.6)	23.7±29.2 (17.9)
Systolic Blood Pressure, mean±SD (median), mm Hg†	131.9±18.0 (130.0)	127.6±20.8 (125.0)
Diastolic Blood Pressure, mean±SD (median), mm Hg†	81.8±10.7 (80.0)	79.3±11.0 (80.0)
Occupational status†		
Blue collar, %	31.7	36.4
White collar, %	58.3	56.5
Self-employed, %	10.0	7.1
Current or Former Cigarette Smoking, %†	31.5	23.4
Total Mortality, n (%)	2304 (7.1)	2247 (5.1)
Cardiovascular/cerebrovascular deaths, n (%)	986 (3.0)	969 (2.2)
Other death cause, n (%)	1318 (4.1)	1278 (2.9)

*Participants diagnosed with malignancies before enrolment, during assessment of longitudinal GGT-change, or within 1 year after longitudinal GGT-change assessment were excluded.

†Values referring to baseline (i.e. measurement at first visit).

systolic and diastolic blood pressure, and blood glucose. Because of skewed distributions, GGT and triglycerides were transformed using logarithm based 10. To incorporate repeated measurements and to further estimate hazard ratios for GGT as a time-varying variable, we recalculated all hazard ratios, using extended Cox proportional hazards models simultaneously adjusting for the above covariates as time-varying variables.[31] To estimate hazard ratios with 95% confidence intervals for the association of GGT change (in 7 categories) with all-cause and CVD mortality, we fitted the same extended Cox models with time-varying covariates and additional adjustment for baseline GGT (logarithmically transformed) and the period of GGT change assessment. The proportional hazards assumption was checked using Schoenfeld residuals[31] and visual inspection of the hazards plots. To assess dose-response relationships of GGT change and risk of CVD and total mortality, log-linear trends across categories of increasing and decreasing GGT, in respect to stable GGT as the reference category, were tested using the median GGT change for each category as an ordinal variable in our Cox models.

In sensitivity analyses, we evaluated whether the GGT change–CVD association was confounded by severe preexisting illness excluding the first 2 years of follow-up. To investigate possible effect modification by age, we included multiplicative interaction terms in our Cox models and used stratified analyses. We further recalculated all results, excluding (1) participants with elevated GGT at baseline (>60 U/L in men and >36 U/L in women) or participants increasing into the abnormal range of GGT. To investigate possible effects of within-subject variability in GGT change over time, we included the root mean square error, obtained from linear regression analyses, as an additional covariate in our regression runs. Two-sided probability values <0.05 were considered statistically significant. All statistical analyses were conducted using STATA 9.0 and SPSS 15.0 statistical software.

Results

Characteristics of Study Population and Categories of GGT Change

Demographic and clinical characteristics of the study population are shown in Table 1. The average period of longitudinal GGT change assessment was 6.9±0.9 years, and median follow-up after GGT change assessment corresponded to 9.8 years in men and 10.6 years in women, with a total of 716 098 person-years at risk. Most participants (96.7%) were followed-up for at least 2 years after GGT change assessment, and 72.5% had follow-up times of 7 or even more years. Mean age at study entry was 42.1 years in men and 42.0 years in women. During follow up, 1955 (2.6%) CVD deaths were recorded in our database. On average, 6 GGT measurements were obtained for each participant, with median baseline GGT concentrations of 28.6 U/L in men and 17.9 U/L in women. Stratification of subjects into 7 equally-sized gender-specific categories according to the distribution of slopes of GGT change yielded the following cut-off values: "stable" (7-year change −0.68 to 1.26 U/L in men; −0.55 to 0.79 U/L in women), "mild increase" (7-year change 1.27 to 3.76 U/L in men; 0.80 to 2.27 U/L in women), "moderate increase" (7-year change 3.77 to 9.16 U/L in men; 2.28 to 5.15 U/L in women), "pronounced increase" (7-year change >9.17 U/L in men; >5.15 U/L in women), "mild decrease" (7-year change −3.02 to −0.69 U/L in men; −1.97 to −0.56 U/L in women), "moderate

Table 2. Estimated Adjusted Hazard Ratios With 95% Confidence Intervals for the Association of Gamma-Glutamyltransferase, Modeled as Baseline and Time-Varying Covariate, With All-Cause Mortality and Mortality From Cardiovascular Disease in 76 113 Austrian Adults, VHM&PP 1985 to 2005

	All-Cause Mortality	Total Mortality From Cardiovascular Disease	Mortality From Coronary Heart Disease	Mortality From Congestive Heart Failure	Stroke Mortality
Males (n=32 365)					
Fatal Events, n (%)	2304 (7.1)	986 (3.0)	541(1.7)	91 (0.3)	228 (0.7)
HR (95% CI, *P* value) per baseline GGT log-unit increase*	2.18 (1.89–2.53, *P*<0.0001)	1.87 (1.48–2.36, *P*<0.0001)	1.80 (1.32–2.46, *P*<0.0001)	3.04 (1.45–6.23, *P*=0.003)	2.25 (1.40–3.61, *P*=0.001)
HR (95% CI, *P* value) per GGT log-unit increase with GGT as time-varying covariate†	2.53 (2.22–2.89, *P*<0.0001)	2.13 (1.73–2.62, *P*<0.0001)	2.22 (1.68–2.93, *P*<0.0001)	1.74 (0.89–3.41, *P*=0.11)	2.29 (1.51–3.48, *P*<0.0001)
Females (n=43 748)					
Fatal Events, n (%)	2247 (5.1)	969 (2.2)	442 (1.0)	97 (0.2)	266 (0.6)
HR (95% CI, *P* value) per baseline GGT log-unit increase*	1.53 (1.31–1.80, *P*<0.0001)	1.21 (0.94–1.56, *P*=0.13)	1.40 (0.97–2.02, *P*=0.07)	0.89 (0.38–2.07, *P*=0.79)	1.29 (0.80–2.08, *P*=0.30)
HR (95% CI, *P* value) per GGT log-unit increase with GGT as time-varying covariate†	1.92 (1.65–2.24, *P*<0.0001)	1.72 (1.35–2.18, *P*<0.0001)	1.45 (1.01–2.09, *P*=0.046)	3.18 (1.62–6.22, *P*=0.001)	2.12 (1.37–3.28, *P*=0.001)

*Estimated from Cox proportional hazards models adjusted for baseline values of age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose. GGT and triglycerides were log-transformed.

†Estimated from extended Cox proportional hazards models adjusted for age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose as time-varying covariates. GGT and triglycerides were log-transformed.

decrease" (7-year change −7.98 to −3.03 U/L in men; −4.49 to −1.98 U/L in women) and "pronounced decrease" (7-year change <−7.99) U/L in men; <−4.50) U/L in women).

Association of Baseline and Time-Varying GGT With All-Cause and CVD Mortality

The association of GGT, modeled as baseline and time-varying covariate, with all-cause mortality, total CVD mortality, and mortality from CHD, CHF, and stroke is shown in Table 2. In Cox proportional hazards models adjusted for baseline values of age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose, baseline GGT was independently associated with all-cause mortality in both men and women; the adjusted hazard ratios (95% CI) per baseline GGT log-unit increase were 2.18 (1.89 to 2.53) and 1.53 (1.31 to 1.80), respectively. In men, but not in women, baseline GGT was further significantly associated with total CVD mortality, mortality from CHD, CHF and stroke (all $P<0.01$, Table 2).

When GGT was modeled as time-varying covariate, with additional adjustment for all above confounding factors as time-varying variables, associations with all-cause mortality markedly increased in both men and women. Similarly, time-varying GGT became an independent predictor for total CVD mortality, mortality from CHD, CHF, and stroke also in women (all $P<0.05$), whereas the association of GGT with mortality from CHF turned out to be nonsignificant in men ($P=0.11$, Table 2).

Association of Longitudinal GGT Change With All-Cause and CVD Mortality

The association of longitudinal GGT change with all-cause and CVD mortality in men and women is shown in Tables 3 and 4, respectively. In extended Cox proportional hazards models, adjusted for baseline log-GGT, period of GGT change assessment and age, body mass index, smoking status, occupational status, log-triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose as time-varying covariates, a pronounced 7-year increase in GGT was independently related to increased risk of all-cause mortality in both sexes (*P* for trend both <0.01); the hazard ratios (95% CI) in comparison to stable GGT were 1.36 (1.16 to 1.61) and 1.26 (1.06 to 1.49), respectively.

In men, a pronounced increase in GGT was further independently associated with increased risk of total CVD mortality; the hazard ratio (95% CI) in comparison to stable GGT was 1.40 (1.09 to 1.81, *P* for trend 0.005). Although total CVD risk similarly was elevated for all categories of increasing GGT also in women, effects were less pronounced in comparison to men and not statistically significant. For a pronounced decrease in GGT concentrations over time, we observed a marked increase in CVD death for both genders; however, after full adjustment for confounding factors as time-varying covariates in our multivariate regression runs, these associations proved to be nonsignificant in both men and women (Tables 3 and 4). In CVD subcategories, a pronounced increase in GGT was significantly associated with stroke mortality in men (hazard ratio in comparison to stable GGT 1.89 (1.11 to 3.22), *P* for trend 0.002, Table 3),

Table 3. All-Cause Mortality and Mortality From Cardiovascular Disease According to Categories of Longitudinal GGT-Change in 32 365 Men, VHM&PP 1985 to 2005

	Longitudinal GGT Change								
	Decrease				Increase			*P* for Trend GGT Decrease*	*P* for Trend GGT Increase*
	Pronounced n=4625	Moderate n=4625	Mild n=4625	Stable n=4625	Mild n=4625	Moderate n=4625	Pronounced n=4615		
Longitudinal 7-year GGT-change (U/L) - range	<(−7.99)	−7.98–(−3.03)	−3.02–(−0.69)	−0.68–1.26	1.27–3.76	3.77–9.16	>9.17		
Longitudinal 7-year GGT-change - mean±SD (median) (U/L)	−30.1±52.7 (−15.8)	−5.0±1.4 (−4.8)	−1.8±0.7 (−1.7)	0.3±0.6 (0.2)	2.5±0.7 (2.4)	6.0±1.5 (5.8)	31.8±52.9 (17.9)		
Baseline GGT - mean±SD (median) (U/L)	100.2±115.8 (69.0)	37.8±20.2 (34.0)	28.9±16.6 (25.1)	25.6±15.7 (21.5)	25.1±16.2 (21.5)	29.5±20.3 (25.1)	48.3±49.0 (34.0)		
All-cause mortality									
Fatal events, n (%)	490 (10.6)	385 (8.3)	283 (6.1)	266 (5.8)	243 (5.3)	242 (5.2)	395 (8.6)		
HR (95% CI)†	0.93 (0.78–1.11)	1.01 (0.86–1.18)	0.89 (0.75–1.06)	1.00 (Ref)	1.05 (0.88–1.25)	1.07 (0.90–1.28)	1.36 (1.16–1.61)	0.54	<0.0001
Total CVD mortality									
Fatal events, n (%)	221 (4.8)	159 (3.4)	139 (3.0)	117 (2.5)	102 (2.2)	113 (2.4)	135 (2.9)		
HR (95% CI)†	1.01 (0.78–1.32)	0.97 (0.76–1.25)	0.92 (0.71–1.18)	1.00 (Ref)	1.04 (0.79–1.37)	1.16 (0.88–1.51)	1.40 (1.09–1.81)	0.85	0.005
Coronary heart disease									
Fatal events, n (%)	125 (2.7)	87 (1.9)	80 (1.7)	60 (1.3)	58 (1.3)	65 (1.4)	66 (1.4)		
HR (95% CI)†	0.94 (0.66–1.34)	0.92 (0.66–1.29)	0.92 (0.65–1.30)	1.00 (Ref)	1.09 (0.76–1.56)	1.23 (0.87–1.76)	1.13 (0.79–1.61)	0.70	0.36
Congestive heart failure									
Fatal events, n (%)	21 (0.5)	13 (0.3)	13 (0.3)	11 (0.2)	7 (0.2)	10 (0.2)	16 (0.3)		
HR (95% CI)†	0.97 (0.42–2.25)	0.77 (0.34–1.76)	1.07 (0.48–2.36)	1.00 (Ref)	0.62 (0.23–1.68)	0.81 (0.31–2.11)	1.57 (0.70–3.50)	0.69	0.35
Stroke									
Fatal events, n (%)	54 (1.2)	38 (0.8)	29 (0.6)	26 (0.6)	22 (0.5)	24 (0.5)	35 (0.8)		
HR (95% CI)†	1.29 (0.74–2.26)	1.19 (0.70–2.00)	0.86 (0.48–1.52)	1.00 (Ref)	0.98 (0.53–1.80)	1.32 (0.74–2.34)	1.89 (1.11–3.22)	0.67	0.002

*Log-linear trends across categories of increasing or decreasing GGT, in respect to stable GGT, were tested using the median GGT change for each category as an ordinal variable in an extended Cox proportional hazards model adjusted for age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose as time-varying covariates. All models are additionally adjusted for baseline GGT and the period of GGT-change assessment. GGT and triglycerides were log-transformed.

†Estimated from extended Cox proportional hazards models adjusted for age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose as time-varying covariates. All models are additionally adjusted for baseline GGT and period of GGT change assessment. GGT and triglycerides were log-transformed.

whereas we found no such association in women. In women, however, pronounced increasing GGT slightly increased risk for fatal CHD (hazard ratio in comparison to stable GGT 1.42[0.96 to 2.10], *P* for trend 0.06), while this association was not observable for men.

Exclusion of the first 2 years of follow-up in all above analyses did not change our findings. Including the root mean square error of the slope of GGT change for participants with at least 3 GGT measurements as an additional covariate in our regression models did not indicate an independent effect of variability of GGT on risk of total or CVD mortality in neither men nor women. When recalculating all results, excluding participants with elevated GGT at baseline (>60 U/L in men and >36 U/L in women) or participants increasing into the abnormal range of GGT, the above reported associations of increasing GGT with CVD mortality remained stable in magnitude of effect in both men and women (Figure 1A and 1B; supplemental Tables II and III). Additionally, for the association of GGT change with CHD mortality in women, the hazard ratio for a pronounced GGT increase in comparison to stable GGT markedly increased to 1.80 (1.15 to 2.81), with a test for trend across categories of increasing GGT becoming highly significant (*P*=0.008, supplemental Table III).

Age of participants significantly modified the association between GGT change and CVD mortality for both men and women (*P* for multiplicative interaction age*GGT change, both <0.001). In stratified analyses, the association of GGT change with total CVD mortality markedly increased in male participants aged ≤60 years at baseline (hazard ratio for pronounced increasing GGT versus stable GGT 1.72 (1.10 to 2.69, *P* for trend 0.009; Figure 2A) and was attenuated to a nonsignificant level in men aged >60 years at baseline (hazard ratio for pronounced increasing GGT versus stable

Table 4. All-Cause Mortality and Mortality From Cardiovascular Disease According to Categories of Longitudinal GGT Change in 43 748 Women, VHM&PP 1985 to 2005

	Longitudinal GGT Change								
	Decrease				Increase			*P* for Trend GGT Decrease*	*P* for Trend GGT Increase*
	Pronounced n=6251	Moderate n=6253	Mild n=6250	Stable n=6252	Mild n=6251	Moderate n=6253	Pronounced n=6238		
Longitudinal 7-year GGT-change (U/L) - range	<(−4.50)	−4.49–(−1.98)	−1.97–(−0.56)	−0.55–0.79	0.80–2.27	2.28–5.15	>5.15		
Longitudinal 7-year GGT-change - mean±SD (median) (U/L)	−15.4±27.0 (−8.2)	−3.1±0.7 (−3.0)	−1.2±0.4 (−1.2)	0.1±0.3 (0.0)	1.5±0.4 (1.4)	3.5±0.9 (3.4)	19.3±32.1 (9.9)		
Baseline GGT - mean±SD (median) (U/L)	49.4±55.9 (34.0)	21.4±10.7 (19.7)	18.1±8.6 (16.1)	16.6±7.4 (14.3)	16.1±8.3 (14.3)	16.9±10.7 (14.3)	26.6±26.6 (19.7)		
All-cause mortality									
Fatal events, n (%)	512 (8.2)	331 (5.3)	246 (3.9)	240 (3.8)	239 (3.8)	258 (4.1)	421 (6.7)		
HR (95% CI)†	0.99 (0.83–1.18)	1.03 (0.87–1.22)	0.96 (0.80–1.15)	1.00 (Ref)	1.03 (0.86–1.24)	1.14 (0.95–1.37)	1.26 (1.06–1.49)	0.72	0.003
Total CVD mortality									
Fatal events, n (%)	214 (3.4)	153 (2.4)	113 (1.8)	117 (1.9)	114 (1.8)	106 (1.7)	152 (2.4)		
HR (95% CI)†	0.91 (0.70–1.19)	0.97 (0.75–1.26)	0.85 (0.64–1.13)	1.00 (Ref)	1.02 (0.78–1.34)	1.13 (0.86–1.48)	1.18 (0.91–1.51)	0.91	0.23
Coronary heart disease									
Fatal events, n (%)	103 (1.6)	74 (1.2)	44 (0.7)	50 (0.8)	53 (0.8)	53 (0.8)	65 (1.0)		
HR (95% CI)†	1.15 (0.77–1.73)	1.24 (0.84–1.84)	0.81 (0.51–1.27)	1.00 (Ref)	1.24 (0.81–1.88)	1.32 (0.87–2.00)	1.42 (0.96–2.10)	0.33	0.06
Congestive heart failure									
Fatal events, n (%)	19 (0.3)	15 (0.2)	10 (0.2)	15 (0.2)	10 (0.2)	11 (0.2)	17 (0.3)		
HR (95% CI)†	0.77 (0.34–1.74)	0.62 (0.27–1.45)	1.01 (0.46–2.21)	1.00 (Ref)	0.78 (0.33–1.85)	1.12 (0.50–2.49)	1.06 (0.46–2.21)	0.87	0.99
Stroke									
Fatal events, n (%)	59 (0.9)	36 (0.6)	40 (0.6)	30 (0.5)	33 (0.5)	26 (0.4)	42 (0.7)		
HR (95% CI)†	0.69 (0.41–1.11)	0.71 (0.43–1.16)	0.92 (0.57–1.49)	1.00 (Ref)	0.79 (0.47–1.31)	0.92 (0.56–1.51)	0.91 (0.57–1.46)	0.14	0.70

*Log-linear trends across categories of increasing or decreasing GGT, in respect to stable GGT, were tested using the median GGT-change for each category as an ordinal variable in an extended Cox proportional hazards model adjusted for age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose as time-varying covariates. All models are additionally adjusted for baseline GGT and the period of GGT change assessment. GGT and triglycerides were log-transformed.

†Estimated from extended Cox proportional hazards models adjusted for age, body mass index, smoking status, occupational status, triglycerides, total cholesterol, systolic and diastolic blood pressure, and blood glucose as time-varying covariates. All models are additionally adjusted for baseline GGT and period of GGT change assessment. GGT and triglycerides were log-transformed.

GGT 1.29 (0.93 to 1.79, *P* for trend 0.065; supplemental Table IV). A similar age-interaction was observable for women, although effects were still less pronounced and did not reach statistical significance (Figure 2B, supplemental Table V). Likewise, also the predictive value of GGT, modeled as baseline variable only or as time-varying covariate, was attenuated in participants aged >60 years at enrolment (supplemental Tables IV and V) and entirely disappeared when using a cut-off value of 75 years (data not shown).

Discussion

The present study, involving more than 76 000 apparently healthy Austrian men and women across a wide age range, constitutes the first epidemiological investigation of the association of GGT change over time with risk of subsequent CVD death. As suggested by previous investigations,[7–14] our data confirm an independent effect of baseline GGT on CVD, especially in younger men. However, after adjustment for baseline GGT and other classical CVD risk factors as time-varying covariates, we still found male individuals, even with baseline GGT concentrations within the normal range (<60 U/L), exhibiting a longitudinal 7-year increase in GGT >9.17 U/L, to have a 36% greater risk of fatal cardiovascular events, in comparison to male individuals with stable GGT concentrations. This risk substantially increased to 72% for men ≤60 years at baseline, strongly suggesting that not only GGT per se, but also a longitudinal increase in GGT, even within its normal range, may be related to adverse cardiovascular/cerebrovascular outcome in men. Although similar associations of longitudinally increasing GGT with CVD mortality were observable in women, these effects were less pronounced in comparison to men. In line with this, sex-specific differences in trends of CVD have previously been well established, although the precise underlying mechanisms remain unclear.[35,36] Mortality rates from heart disease differ

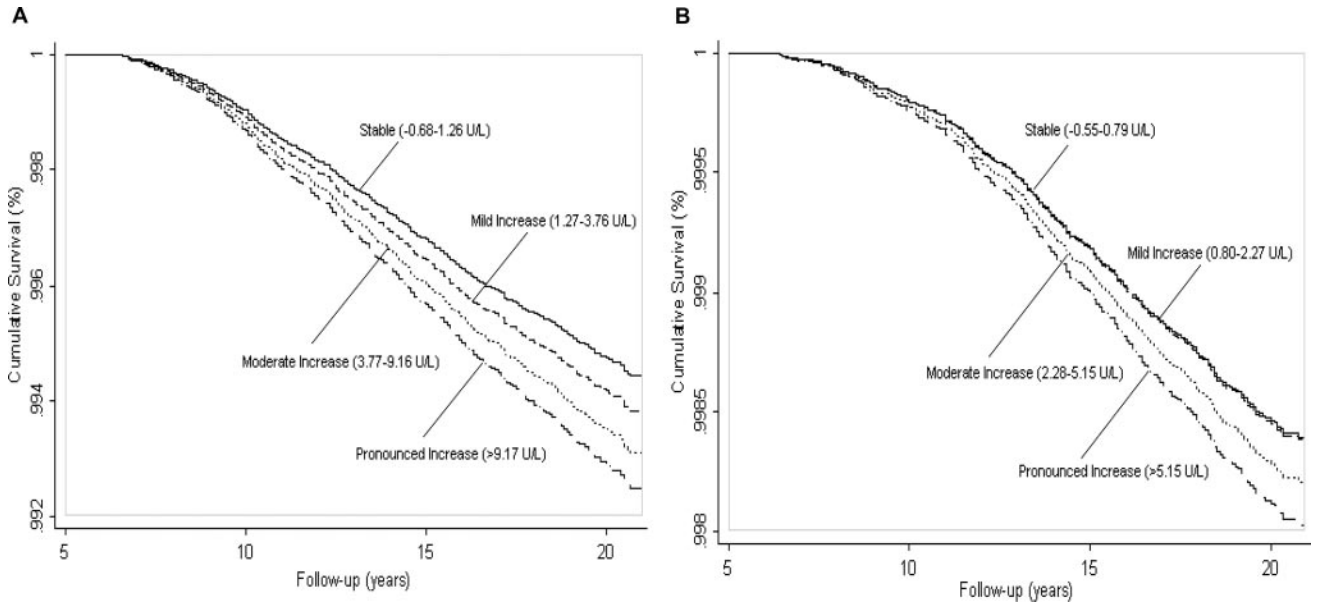

Figure 1. Adjusted cumulative survival from all cardiovascular events according to categories of 7-year increase of serum γ-glutamyltransferase (GGT) among 26 105 male (A) and 36 822 female (B) Austrian adults. Participants with baseline GGT >60 U/L in men and >36 U/L in women or participants increasing into the abnormal range of GGT were excluded.

across the life course of men and women, both in timing of incidence and in clinical presentation, attributed by some investigators to alterations in lipoprotein and hemostatic systems occurring in the postmenopausal period in women.[37] Additionally, women in our cohort had substantially lower absolute levels of GGT, resulting in lower differences among categories of GGT change and experienced fatal CVD events lagged by almost a decade after men, with 10-year follow-up analyses possibly not capturing this fully.

In the only epidemiological investigation related to the topic, André and colleagues[25] recently demonstrated that a 3-year increase in GGT (>5 U/L), in comparison to decreasing GGT during the same time period, was associated with a significant increase of risk for incident type 2 diabetes in both sexes, after adjusting for age and baseline GGT. After further adjustment for several confounding factors, including body mass index, smoking habits, and fasting insulin, this association was attenuated to borderline significance in women, while even more pronounced in men.

It has been shown that a change in GGT over time is correlated with a longitudinal change of other CVD risk factors. However, these associations proved to exhibit a strong sex-specific pattern.[24] In males, a 7-year increase in GGT was reported to positively correlate with an increase in

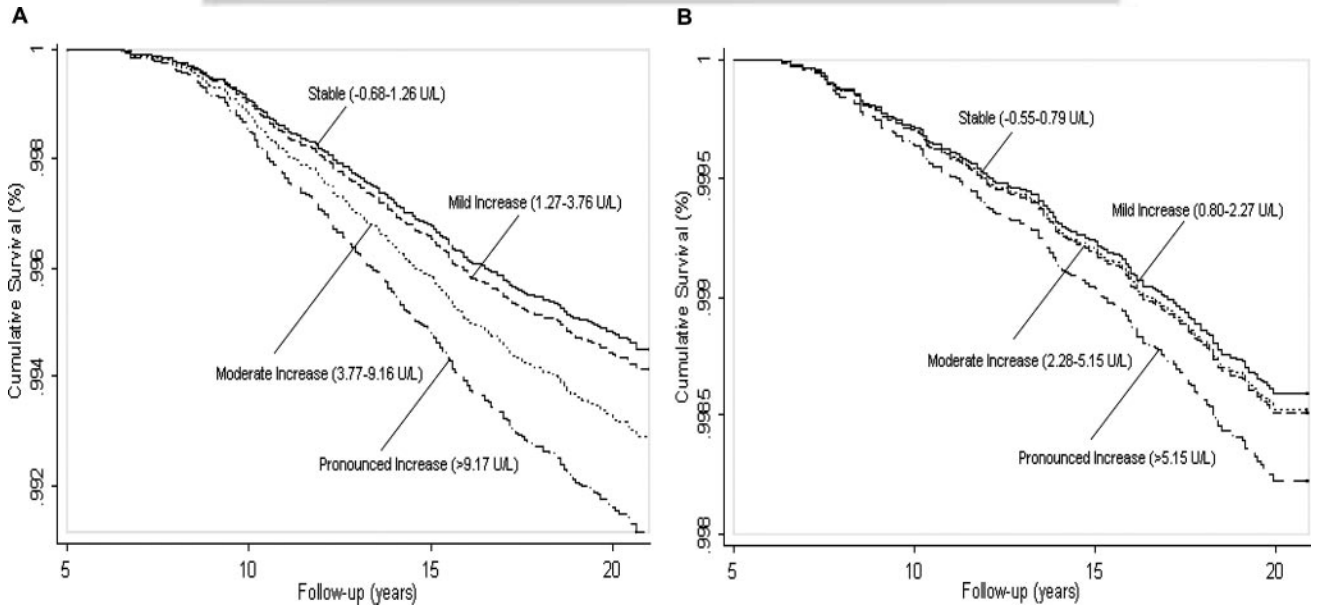

Figure 2. Adjusted cumulative survival from all cardiovascular events according to categories of 7-year increase of serum γ-glutamyltransferase (GGT) among 28 681 male (A) and 37 987 female (B) Austrian adults aged <60 years at enrolment.

body mass index, total serum cholesterol, HDL cholesterol, and number of cigarettes per day. Conversely, in women, longitudinal change of all the above reported CVD risk factors, with the exception of body mass index, showed negative correlations with increasing GGT. Increase in frequency of inebriation resulted in an increase in GGT only in males. In women, increasing GGT was positively associated with use of oral contraceptives and occurrence of menopause.[24]

Given the epidemiological nature of our observations, the mechanisms causing a longitudinal change in GGT over the 7-year assessment period cannot be directly addressed in the present study. Despite evidence for a sensible secular trend in GGT during the past few decades,[19,24,38] several cross-sectional studies have reported a positive association between age and serum GGT. However, this age-effect was estimated to be of approximately 3 U/L during a 7-year time period[19] and thus cannot sufficiently explain a pronounced variation in the extreme categories of GGT change in our cohort. Considering mild to moderate variation in GGT during the 7-year assessment period, it cannot be ruled out, however, that intraindividual variability or methodological artifacts, including measurement error, year-to-year laboratory variation, or "regression toward the mean" may have resulted in minor classification bias.

Our study had several strengths and potential limitations that should be considered. Major strengths are the prospective design, the large sample size, length of follow-up, and the standardized protocol performed by experienced physicians. Even though information on all major CVD risk factors was collected, our study was unable to account for additional factors that might have residually confounded the relationship between GGT change and CVD death, including lipid subfractions or apolipoproteins, C-reactive protein, homocysteine, alcohol consumption, physical activity, diet, and genetic and psychosocial factors. A further limitation of this study is the inability to examine the effect of medication use (eg, statins, antihypertensive drugs) on the relationship of GGT change with CVD. In regard to statins, however, there is little if any effect, as 75% of the study participants were examined before the implementation of statin therapy in Austria in 1995.

Alcohol consumption has not been documented routinely in the VHM&PP, and we therefore were unable to examine for confounding or effect modification by alcohol use. Numerous epidemiological studies have investigated the relationship of alcohol consumption to heart disease and stroke, and there is evidence supporting a moderate beneficial effect of alcohol consumption on CVD risk.[39] However, because of its multiple pathways of both positive and negative influences, there is still no conclusive evidence about its biological mechanism. It has been shown that alcohol affects lipid metabolism, as well as hemostatic and oxidative factors.[40,41] Mainly because of caloric intake, alcohol consumption has been shown to be associated with obesity and elevated blood pressure.[42] Although our findings of longitudinally increasing GGT showing more prominent effects in males suggests a role of alcohol, GGT was recently reported to increase risk for CVD in never-drinkers as well, as shown in a study of nondrinking Japanese women.[10] Moreover, the observed monotonic increasing trend of all-cause and CVD mortality with increasing GGT among both men and women is unlikely to simply reflect the effects of alcohol consumption because previous studies have rather shown U- or J-shaped relationships of alcohol drinking to cardiovascular and all-cause mortality.[39,43,44] Additionally, in a randomly selected subsample of 731 participants from the VHM&PP that provided self-reported alcohol data, only a weak though statistically significant age- and sex-adjusted correlation of GGT with the average number of alcoholic units per week was observed ($r=0.12$).[45]

In summary, the present study prospectively investigated the association of longitudinal change in serum GGT with risk of subsequent all-cause and CVD mortality in more than 76 000 Austrian men and women. Our findings for the first time demonstrate that a longitudinal increase in GGT, independently of baseline GGT and even within its normal range, significantly increases risk of fatal CVD and all-cause mortality, particularly in individuals at younger ages. Although our findings need to be confirmed in other populations, longitudinal monitoring of GGT change may be beneficial in primary prevention of CVD, especially because GGT is a low cost and widely used laboratory measurement.

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Disclosures

None.

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