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ORIGINAL ARTICLE

Gamma-glutamyl-transferase is associated with incident hip fractures in women and men ≥ 50 years: a large population-based cohort study

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Abstract

Summary The association of serum gamma-glutamyl-transferase (GGT) with hip fracture risk has not been examined in women and men ≥ 50 years. We show that elevated GGT was associated with increased hip fracture risk, particularly in men. GGT could be a candidate serum marker of long-term hip fracture risk in the elderly.

Introduction We herein examined a possible relation between serum levels of GGT and hip fracture risk in women and men aged ≥ 50 years, which has not been investigated before.

Methods In this population-based prospective cohort study, approximately 41,000 women and nearly 33,000 men ≥ 50 years participating in a medical prevention program 1985–2005 in western Austria were followed up for the occurrence of osteoporotic hip fractures during 2003–2013. ICD-10 based discharge diagnoses for hip fracture included S72.0, S72.1, and S72.2 available from all regional hospitals. GGT-related hip fracture risk was ascertained at each participant´s first and last examination during the prevention program. In a subset of 5445 participants, alcohol consumption could be included as a covariate.

Results In men, hip fracture risk rose significantly by 75% and 86% for every tenfold increase of GGT measured at the first and last examination, respectively, and in women, hip fracture risk rose by 22% from the last examination. Elevated GGT (≥ 36 U/l in women, ≥ 56 U/l in men) at the first examination was associated with increased hip fracture risk only in men (HR 1.51, 95% CI 1.25–1.82), and at the last examination in both women (HR 1.14, 95% CI 1.02–1.28) and men (HR 1.61, 95% CI 1.33–1.95). Alcohol consumption had no significant influence on GGT-mediated hip fracture risk in women and men.

Conclusions Our findings identified an association of elevated GGT and hip fracture in women and men ≥ 50 years and suggest GGT as a candidate serum marker of long-term hip fracture risk in an elderly population.

Keywords Alcohol · Gamma-glutamyl-transferase · Hip fracture · Osteoporosis · Vorarlberg Health Monitoring and Promotion Program

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Introduction

The risk of osteoporotic bone fractures rises with advancing age, as does the proportion of incident hip fractures relative to other fractures [1, 2]. Hip fracture patients face substantial disability and mortality, and considerable economic costs are incurred for the public [2, 3]. In this regard, identification of risk factors as well as predictive biomarkers contributes toward preventing hip fractures and reducing disease burden, particularly in the elderly. Only a few clinical serum markers have though been associated with and shown to predict future hip fracture risk. Whereas the prognostic values for hip fractures of the serum bone formation markers osteocalcin, P1NP, and alkaline phosphatase and of the bone resorption marker CTX are disputed [4–7], a correlation between low serum 25-(OH)-vitamin D levels and long-term hip fracture risk could be established [8]. Moreover, it has been shown that hyperglycemia, hypertriglyceridemia, and hyperuricemia are linked with increased [9–11] and that high cholesterol is associated with reduced long-term hip fracture risk [9].

GGT is a membrane enzyme that effectuates extracellular hydrolysis of the antioxidant glutathione, thereby enabling transport of its components into the cell where re-synthesis takes place [12, 13]. GGT is expressed in a number of tissues and also occurs in serum where elevated concentrations are indicative of alcohol consumption and hepatobiliary disorders reflecting destruction of liver tissue [12, 13]. Besides its established role in favoring the resupply of intracellular antioxidant glutathione, GGT has also been ascribed pro-oxidant properties, ensuing from the cleavage of extracellular glutathione and leading to the promotion of free radicals and oxidative stress [12, 14, 15]. Accordingly, elevated GGT serum levels are implicated with disorders where oxidative stress plays a crucial pathogenic role, like cardiovascular disease [15, 16] and cancer [15, 17, 18].

Epidemiological evidence links GGT also to bone. In several cross-sectional investigations, serum GGT levels were inversely associated with bone mineral density at various skeletal sites [19–22]. Furthermore, a cohort study in Korean men linked increasing serum GGT levels with the risk of osteoporotic fractures [23]. Likewise, analysis of a Swedish cohort of middle-aged women and men provided evidence that increased GGT is a risk factor for low-trauma fractures [24] and specifically also for hip fractures [24, 25]. While oxidative stress has been implicated with damage to bone resulting in increased fracture risk [26], which the pro-oxidative property of GGT could account for, it is also conceivable that, in particular, alcohol consumption, affecting bone and GGT serum levels alike represents a major risk modifying confounding influence [27].

In this large population-based cohort study, we hypothesized that serum GGT is associated with the risk of future hip fractures in postmenopausal women and men after the age of 50 years. We monitored the stability of effects along two different follow-up times and furthermore specifically addressed the role of alcohol as a possible confounding factor in a subset of our cohort.

Patients and methods

Data acquisition and study design

The Vorarlberg Health Monitoring and Promotion Program (VHM&PP) is a voluntary population-based medical prevention program in Vorarlberg, the westernmost Austrian province. Within its framework, 185,459 individuals, representing more than half of the inhabitants in the province, were recruited to 716,679 health examinations from January 1, 1985, until June 30, 2005. Upon exclusion of all examinations with implausible or missing data entries of at least one variable, of all examinations at < 50 years, and of subjects who died prior to January 1, 2003, a cohort of 73,880 individuals was arrived at who participated in 301,183 examinations (Fig. 1). For each participant, prospective follow-up started at two time points, i.e., from his/her first and last examination 1985–2005, for sustaining a hip fracture within the observation interval from January 1, 2003 to December 31, 2013. The rationale for including two baseline points of reference was to monitor the stability of possible effects along different follow-up times. If an individual participated in only one examination 1985–2005, the same baseline was used in both analyses. If a fracture occurred before the baseline examination, the patient was excluded from further analysis, giving rise to 41,025 women and 32,854 men in the final cohort of first examinations, and 40,987 women and 32,837 men in the final cohort of the last examinations (Fig. 1).

For a subgroup of 5445 study participants, information on self-reported alcohol consumption was available from other medical and health survey records in Vorarlberg. These include the following:

1) Three surveys of the CINDI (countrywide integrated non-communicable diseases intervention) program of the WHO for the prevention of chronic diseases. In 1986, 1991, and 1998, respectively, 2401, 2400, and 2794 subjects of all ages randomly selected from the general population of Vorarlberg were interviewed about lifestyle and nutritional habits by professional interviewers. Besides other questions, participants were asked about their weekly consumed amount of beer (glasses and bottles of 0.5 l), wine (glasses of 0.25 l), spirits

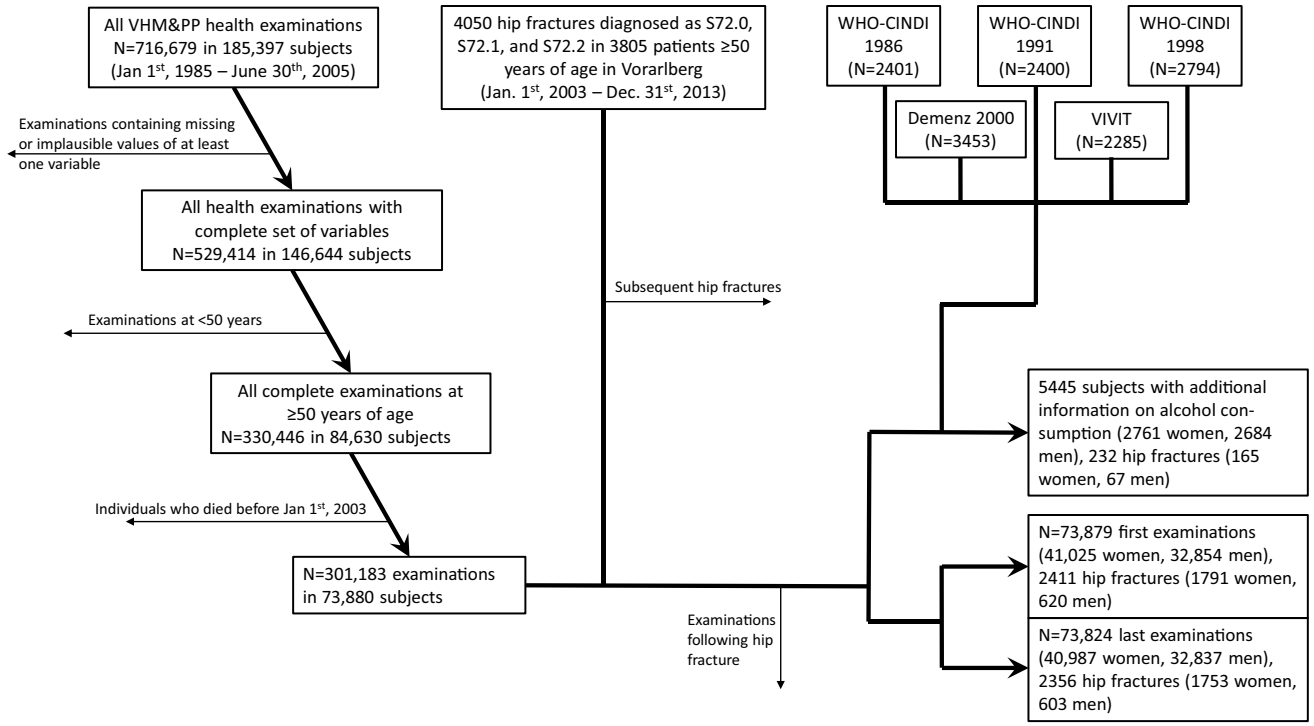

Fig. 1 Study flow chart

(glasses of 0.02 l), must (glasses of 0.5 l), and aperitifs (glasses of 0.02 l). From the three surveys, 555, 697, and 394 subjects, respectively, were identified also in the entire study population and recruited to the sub-cohort.

2) Records of a risk factor surveillance program for early diagnosis of dementia ("Demenz 2000") to which 3453 subjects ≥ 65 years were recruited from among participants of the VHM&PP health examinations in 2000 and 2001. Participants answered additional lifestyle- and health-related questions at their health examination, including whether their daily consumption exceeded one beer (0.5 l), one glass of wine (0.25 l), or one glass of spirits (0.02 l) each. Drinking habits were documented for 2938 participants who were included in the sub-cohort.
3) A surveillance program for patients with cardiovascular and peripheral artery disease conducted by the Vorarlberg Institute of Vascular Investigation and Treatment (VIVIT) between 1999 and 2012 including 2285 subjects of all ages, from which 861 individuals were recruited to the sub-dataset. Records of this program contained information on weekly alcohol intake [g].

Written consent was obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used. The Ethics Committee of Vorarlberg approved the evaluation of the data (EK-Nr. 2006–6/3), and all procedures were carried out in agreement with the Helsinki Declaration of 1975, as revised in 2013.

Covariates

At each medical examination, participants´ body height and weight were acquired according to a standard protocol, from which the BMI was calculated. Blood pressure was measured and participants answered a questionnaire on smoking habits (classification as ex-, current, and non-smoker). Finally, blood was collected either post-prandially (1985–1987) or after a minimum of eight hours of fasting (1988–2005). All blood samples were analyzed at the medical central laboratories Feldkirch and Dornbirn according to consistent protocols with regular internal and external quality controls. The blood was centrifuged for 15 min at 4000 rotations per minute. Blood chemistries were measured using the Dade Behring Dimension RXL Chemistry Analyzer or Roche Hitachi Chemistry Analyzer until 2000, and afterward using the Roche Cobas Integra Automated Biochemistry Analyzer (Roche Diagnostics, Rotkreuz, Switzerland). Serum parameters were measured at 37 °C, including gamma-glutamyl-transferase (GGT), uric acid, glucose, cholesterol, and triglycerides. Impaired fasting glucose was defined as blood glucose levels from 100 to 125 mg/dl for fasting glucose (1988–2005) and from 180 to 199 mg/dl for post-prandial glucose (1985–1987). Individuals with

fasting and post-prandial blood glucose levels ≥ 126 mg/dl and ≥ 200 mg/dl, respectively, were classified as suffering from diabetes mellitus. In the subgroup, consumption of 10 g of alcohol was defined as 1 standard drink in accordance with the WHO guidelines [28]. Thus 0.5 l of beer and must, and 0.25 l of wine corresponded each to 2 standard drinks, and 0.02 l of spirits and aperitifs to 1 and 0.5 standard drink, respectively. The variable was categorized according to (i) no reported alcohol consumption, (ii) up to 2 standard drinks a day (1–140 g /week), and (iii) more than 2 daily standard drinks (> 140 g /week), for both women and men. For each subject in the subgroup, parameters of the one health examination with the least temporal offset to the date of alcohol inquiry were chosen as baseline.

Exposure

GGT values were either ^{10}log transformed because of their distinct right-skewed distribution and treated as a continuous variable, or defined as a discrete variable upon the categorization of untransformed values. Discrete categorization was gender specific and according to quartiles as well as to classification as elevated vs. normal values. Quartile cut-off values at each participant´s first health examination were 14.3, 19.7, and 30.4 U/l for women and 23.3, 32.2, and 51.9 U/l for men, and at last examination, 16.1, 22.0, 32.2 U/l for women and 23.0, 32.2, 50.1 U/l for men. Owing to the exploratory scope of our study, reference quartiles were defined as the ones with the lowest outcome risk, i.e., the 2nd quartile for the first and the 3rd quartile for the last health examination. Categorization as per quartiles was omitted in the sub-cohort, including alcohol consumption as a covariate because of its reduced sample size. GGT levels were considered elevated at ≥ 36 U/l and ≥ 56 U/l in women and men, respectively [29], and highly elevated in women at ≥ 56 U/l.

Outcome

Study end point was discharge diagnosis of hip fracture in one of the hospitals in Vorarlberg (Landeskrankenhaus Bregenz, Landeskrankenhaus Bludenz, Krankenhaus Dornbirn, Landeskrankenhaus Feldkirch, Landeskrankenhaus Hohenems, and Sanatorium Schruns) between January 1, 2003 and December 31, 2013. Hip fracture diagnosis was defined according to the ICD-10 code of S72, including subcodes S72.0, fracture of femoral head and neck, S72.1, pertrochanteric fracture, and S72.2, sub-trochanteric fracture [30]. Only patients´ first hip fractures in the study period were accounted for, and data were censored by date of death or last day of the study period (December 31, 2013), whichever came first.

Statistical analysis

Cox proportional hazards regression analysis was applied to obtain hazard ratios (HRs) with 95% confidence intervals (CIs) for hip fracture incidence in models adjusted for age only and fully adjusted for all available covariates in the full cohort, and additionally for alcohol consumption in the subgroup analysis. Triglycerides and GGT values displayed a right-skewed distribution and were thus ^{10}log transformed for analyses as continuous variables. HRs for cholesterol, systolic, and diastolic blood pressure are each based on increments of 10 units owing to the wide distribution range of data. In the subgroup analysis, alcohol consumption was coded as discrete variable. Baseline parameters under normal and elevated GGT levels were compared using the chi-square test for discrete variables, whereas, for comparison of continuous variables, Student's *t*-test was applied in case of normal distribution, the Mann–Whitney *U* test otherwise. Shapiro–Wilk and Kolmogorov–Smirnov tests served as checks for normality of distributions. HRs were considered statistically significant at the 95% confidence level. All analyses were conducted using MedCalc, version 19.7.2 (MedCalc Software Ltd., Ostend, Belgium) and IBM SPSS Statistics, version 25 (IBM Corp., Armonk, NY).

Results

Table 1 shows the baseline parameters at first examination in 41,025 women and 32,854 men, and Table 2 those at last examination in 40,987 women and 32,837 men. Median follow-up time was longer starting from the first health examination (18.7 and 16.9 years in women and men, respectively) than from the last one (10.0 and 9.7 years in women and men, respectively), and consistently, women and men were on average younger at their first (57.1 ± 7.5 years and 56.4 ± 6.6 years, respectively) than at their last examination (64.2 ± 9.4 years and 62.8 ± 8.8 years, respectively). Following the first examination, 1791 women sustained a hip fracture at a mean age of 81.3 ± 8.3 years and 620 men at a mean age of 77.6 ± 9.2 years, and from the last examination, 1753 women at 81.5 ± 8.2 years and 603 men at 77.9 ± 9.2 years.

In the entire study population, women´s risk of future hip fracture was increased with rising and at elevated GGT levels at the first health examination, but statistical significance was not reached in the fully adjusted regression models (Fig. 2; Supplementary Table 1). In contrast, a rise of GGT concentrations by a factor of ten measured at the last examination in the program was associated with a statistically significant increase in hip fracture risk by 22% in the fully adjusted regression model (HR 1.22, 95% CI 1.02–1.46). Also, hip fracture risk was significantly increased at GGT levels of the highest (4th) quartile vs. the

Table 1 Female participants´ baseline characteristics at their first and last health examination in the study, according to normal and elevated levels of GGT. Baseline parameters were compared between categories of normal and elevated GGT levels using appropriate statistical methods (cf. statistical analysis in Patients and methods)

	First examination in the study				Last examination in the study			
	All	Normal GGT (< 36 U/l)	Elevated GGT (≥ 36 U/l)	*p*	All	Normal GGT (< 36 U/l)	Elevated GGT (≥ 36 U/l)	*p*
n	41,025	33,733	7292		40,987	32,734	8253	
GGT (U/l), median (IQR)	19.7 (14.3–30.4)	17.9 (14.3–23.2)	53.7 (43.0–78.8)		22.0 (16.1–32.2)	19.7 (15.0–25.0)	53.0 (42.0–77.0)	
Follow-up (years), median (IQR)	18.7 (13.3–23.9)	18.6 (13.4–23.8)	18.9 (13.2–24.1)	0.75	10.0 (9.0–13.1)	10.0 (9.0–13.1)	9.9 (8.9–12.8)	< 0.0001
Age at health examination (years), mean ± SD	57.1 ± 7.5	56.9 ± 7.5	57.8 ± 7.4	< 0.0001	64.2 ± 9.4	64.2 ± 9.5	64.2 ± 9.0	0.06
BMI (kg/m^2), mean ± SD	26.3 ± 4.7	26.0 ± 4.6	27.7 ± 5.1	< 0.0001	26.8 ± 4.9	26.5 ± 4.8	27.9 ± 5.2	< 0.0001
Systolic blood pressure (mm Hg), mean ± SD	139.1 ± 21.7	138.1 ± 21.6	143.7 ± 21.8	< 0.0001	141.6 ± 21.7	140.9 ± 21.6	144.3 ± 21.6	< 0.0001
Diastolic blood pressure (mm Hg), mean ± SD	84.0 ± 11.0	83.5 ± 10.8	86.0 ± 11.2	< 0.0001	83.6 ± 10.7	83.3 ± 10.6	85.0 ± 10.9	< 0.0001
Triglycerides (mg/dl), median (IQR)	108 (79–152)	104 (77–145)	130 (92–188)	< 0.0001	111 (81–154)	107 (79–147)	129 (92.5–183)	< 0.0001
Total cholesterol (mg/dl), mean ± SD	238.7 ± 45.0	236.8 ± 43.8	247.4 ± 49.4	< 0.0001	233.3 ± 43.0	232.3 ± 41.9	237.3 ± 47.0	< 0.0001
Serum uric acid (mg/dl), mean ± SD	4.5 ± 1.2	4.4 ± 1.2	4.9 ± 1.4	< 0.0001	4.8 ± 1.3	4.7 ± 1.2	5.1 ± 1.4	< 0.0001
Diabetes status, *n* (%)				< 0.0001				< 0.0001
Blood glucose normal	31,982 (77.96%)	27,022 (80.11%)	4960 (68.02%)		28,244 (68.91%)	23,496 (71.78%)	4748 (57.53%)	
Impaired fasting glucose	6679 (16.28%)	5178 (15.35%)	1501 (20.58%)		9653 (23.55%)	7296 (22.29%)	2357 (28.56%)	
Diabetes	2364 (5.76%)	1533 (4.54%)	831 (11.40%)		3090 (7.54%)	1942 (5.93%)	1148 (13.91%)	
Smoking status, *n* (%)				0.22				< 0.0001
Non-smoker	32,621 (79.51%)	26,854 (79.61%)	5767 (79.09%)		32,588 (79.51%)	26,195 (80.02%)	6393 (77.46%)	
Ex-smoker	2858 (6.97%)	2363 (7.00%)	495 (6.79%)		3527 (8.60%)	2754 (8.41%)	773 (9.37%)	
Current smoker	5546 (13.52%)	4516 (13.39%)	1030 (14.12%)		4872 (11,89%)	3785 (11.56%)	1087 (13.17%)	
Hip fractures, *n* (%)	1791 (4.37%)	1422 (4.22%)	369 (5.06%)	< 0.01	1753 (4.28%)	1375 (4.20%)	378 (4.58%)	0.13

Abbreviations: *IQR*, interquartile range; *SD*, standard deviation; *IFG*, impaired fasting glucose

3rd quartile (HR 1.17, 95% CI 1.03–1.34), at elevated vs. normal GGT concentrations (HR 1.14, 95% CI 1.02–1.28), as well as at highly elevated (≥ 56 U/l) vs. normal + moderately elevated (< 56 U/l) GGT levels (HR 1.29, 95% CI 1.11–1.50) in the fully adjusted models (Fig. 2; Supplementary Table 1).

In men, a significant increment in hip fracture risk by 75% (HR 1.75, 95%-CI 1.33–2.32) and 86% (HR 1.86, 95% CI

Table 2 Male participants´ baseline characteristics at their first and last health examination in the study, according to normal and elevated levels of GGT. Baseline parameters were compared between categories of normal and elevated GGT levels using appropriate statistical methods (*cf.* statistical analysis in Patients and methods)

	First examination in the study				Last examination in the study			
	All	Normal GGT (< 56 U/l)	Elevated GGT (≥ 56 U/l)	*p*	All	Normal GGT (< 56 U/l)	Elevated GGT (≥ 56 U/l)	*p*
n	32,854	25,843	7011		32,837	25,849	6988	
GGT (U/l), median (IQR)	32.2 (23.3–52.0)	28.6 (21.5–37.6)	82.3 (66.2–121.7)		32.2 (23.0–50.1)	28.0 (21.0–37.6)	83.0 (66.2–125.0)	
Follow-up (years), median (IQR)	16.9 (12.3–22.4)	17.1 (12.4–22.6)	16.5 (11.8–21.9)	< 0.0001	9.7 (8.9–12.3)	9.7 (8.9–12.2)	9.7 (8.8–12.4)	0.09
Age at health examination (years), mean ± SD	56.4 ± 6.6	56.5 ± 6.8	55.9 ± 6.0	< 0.01	62.8 ± 8.8	63.1 ± 8.9	61.7 ± 8.2	< 0.0001
BMI (kg/m^2), mean ± SD	26.6 ± 3.6	26.3 ± 3.5	27.6 ± 3.7	< 0.0001	26.9 ± 3.8	26.6 ± 3.7	27.9 ± 4.0	< 0.0001
Systolic blood pressure (mm Hg), mean ± SD	138.6 ± 20.1	137.3 ± 19.7	143.5 ± 20.7	< 0.0001	140.5 ± 20.1	139.6 ± 20.0	143.9 ± 20.2	< 0.0001
Diastolic blood pressure (mm Hg), mean ± SD	84.8 ± 11.0	84.1 ± 10.8	87.2 ± 11.6	< 0.0001	84.1 ± 10.7	83.6 ± 10.5	86.0 ± 11.0	< 0.0001
Triglycerides (mg/dl), median (IQR)	130 (91–193)	122 (87–178)	169 (114–259)	< 0.0001	123 (86–181)	116 (83–168)	152 (105–233)	< 0.0001
Total cholesterol (mg/dl), mean ± SD	231.1 ± 44.4	228.0 ± 42.4	242.7 ± 49.5	< 0.0001	219.3 ± 43.2	217.0 ± 41.6	227.6 ± 47.9	< 0.0001
Serum uric acid (mg/dl), mean ± SD	5.8 ± 1.3	5.6 ± 1.3	6.3 ± 1.5	< 0.0001	5.9 ± 1.4	5.8 ± 1.3	6.3 ± 1.5	< 0.0001
Diabetes status, *n* (%)				< 0.0001				< 0.0001
Blood glucose normal	23,182 (70.56%)	18,916 (73.20%)	4266 (60.85%)		20,392 (62.10%)	16,792 (64.96%)	3600 (51.52%)	
Impaired fasting glucose	7089 (21.58%)	5300 (20.51%)	1789 (25.52%)		9219 (28.08%)	6980 (27.00%)	2239 (32.04%)	
Diabetes	2583 (7.86%)	1627 (6.29%)	956 (13.63%)		3226 (9.82%)	2077 (8.04%)	1149 (16.44%)	
Smoking status, *n* (%)				< 0.0001				< 0.0001
Non-smoker	18,209 (55.42%)	14,800 (57.27%)	3409 (48.62%)		18,203 (55.43%)	14,799 (57.25%)	3404 (48.71%)	
Ex-smoker	7277 (22.15%)	5555 (21.50%)	1722 (24.56%)		8382 (25.53%)	6382 (24.69%)	2000 (28.62%)	
Current smoker	7368 (22.43%)	5488 (21.23%)	1880 (26.82%)		6252 (19.04%)	4668 (18.06%)	1584 (22.67%)	
Hip fractures, *n* (%)	620 (1.89%)	458 (1.77%)	162 (2.31%)	< 0.01	603 (1.84%)	450 (1.74%)	153 (2.19%)	< 0.05

Abbreviations: *IQR*, interquartile range; *SD*, standard deviation; *IFG*, impaired fasting glucose

1.42–2.45) was observed for every tenfold increase in GGT concentrations recorded at the first and last examination, respectively, with adjustment for all covariates (Fig. 3; Supplementary Table 2). Moreover, adjusted for all covariates, risk rose by 42% in the highest (4th) vs. the 2nd quartile of GGT concentrations at the first examination (HR 1.42, 95% CI 1.13–1.78), and by 70% in the highest versus the 3rd quartile of measurements at the last examination (HR 1.70,

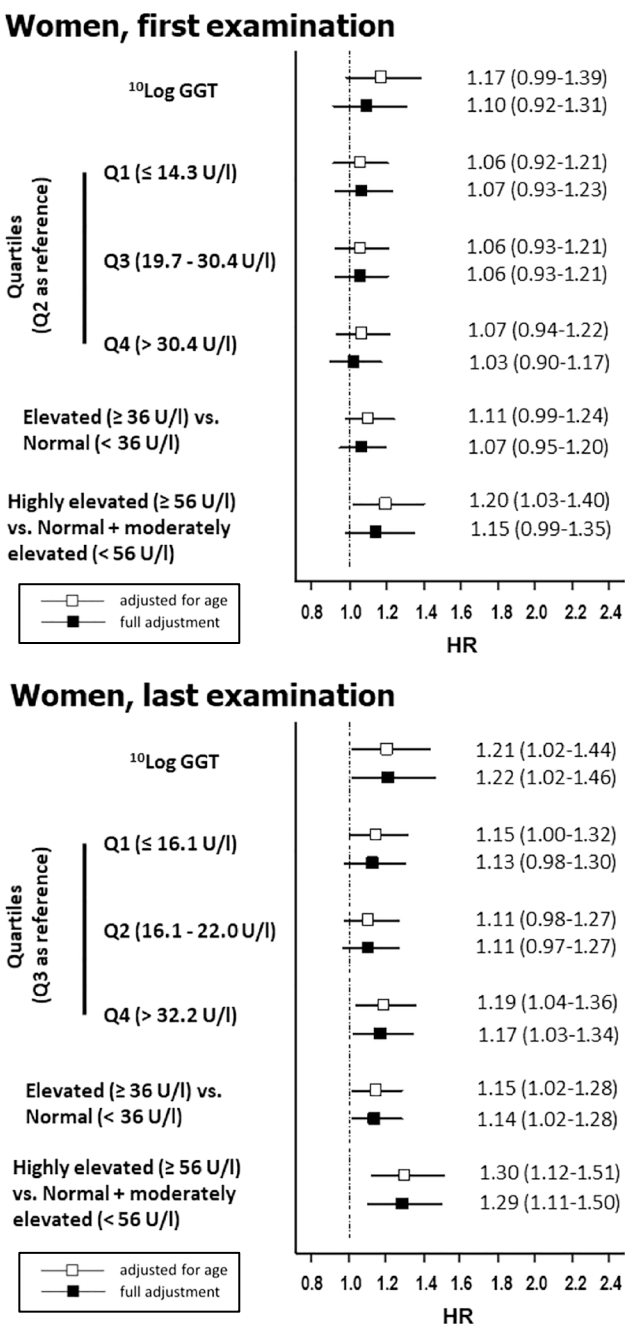

Fig. 2 Forest plots showing age-adjusted (open squares) and fully adjusted (filled squares) hazard ratios with 95% confidence intervals of future hip fracture risk in women for increasing levels of GGT as continuous ^{10}Log transformed variable as well as in quartiles (Q1–Q4), and according to both female and male cut-off values of normal versus elevated GGT levels (36 U/l and 56 U/l, respectively), measured at each participant´s first (top) and last (bottom) health examination 1985–2005. Hazard ratios are also provided numerically, including 95% confidence intervals in parentheses. The reference category for the evaluation according to quartiles was Q2 at the first and Q3 at the last examination. The full adjustment was for age at baseline, BMI, systolic blood pressure, diastolic blood pressure, triglycerides, cholesterol, serum uric acid, diabetes, and smoking status

95% CI 1.35–2.16). Consistently, elevated GGT levels at both the first and last examination were also associated with significantly increased risk of a future hip fracture in fully adjusted regression models (HR 1.51, 95% CI 1.25–1.82, and HR 1.61, 95% CI 1.33–1.95, respectively) (Fig. 3; Supplementary Table 2). Results of the Cox regression models containing all covariates in women and men from either their first or last health examination are provided in Supplementary Tables 3, 4, 5 and 6.

In a subgroup with information on alcohol consumption, 2761 women who were followed up for a median of 12.9 years were on average 66.7 ± 9.3 years old at baseline, and 2684 men followed up for a median of 12.9 years had a mean age at baseline of 64.9 ± 9.2 years (Supplementary Table 7). The mean age at hip fracture was 81.5 ± 6.6 years in women and 79.0 ± 8.2 years in men.

In this subset, alcohol consumption did not significantly alter GGT-related hip fracture risk (Table 3). In men, hip fracture risk was increased with rising and at elevated GGT levels (HR 2.60, 95% CI 1.50–4.53 with full adjustment except for alcohol consumption), and additional adjustment for alcohol intake even enhanced this effect (HR 2.74, 95% CI 1.57–4.78) (Table 3). Results of the Cox regression models containing all covariates in women and men are provided in Supplementary Tables 8 and 9, respectively.

Discussion

In this large, population-based cohort study, we demonstrate that rising and elevated serum GGT levels after the age of 50 years are associated with increased future hip fracture risk, in particular in men and that this effect is still observed after correction of data for alcohol intake. These findings portend an impact of GGT on hip bone independent of possible alcohol-related effects on bone and/or fall risk.

Epidemiologic evidence confirms the association between high serum GGT concentrations and unfavorable bone-related outcomes. A large Korean study in men ≥ 50 years reported a significant rise in the risk of future osteoporotic fractures with increasing GGT baseline levels upon adjustment for a number of covariates including alcohol drinking habits [23]. Moreover, prospective study results of a prevention program from Sweden showing greater hip fracture risk in men than in women with increasing GGT baseline concentrations [24, 25] are in line with our finding of a more pronounced effect in men. However, information on alcohol consumption was lacking, and participants were fairly young as reflected by their mean age at end of follow-up of 65 and 63.5 years in women and men, respectively [25]. Several cross-sectional studies also revealed an inverse correlation of GGT with bone mineral density (BMD) at various skeletal sites. In one of these,

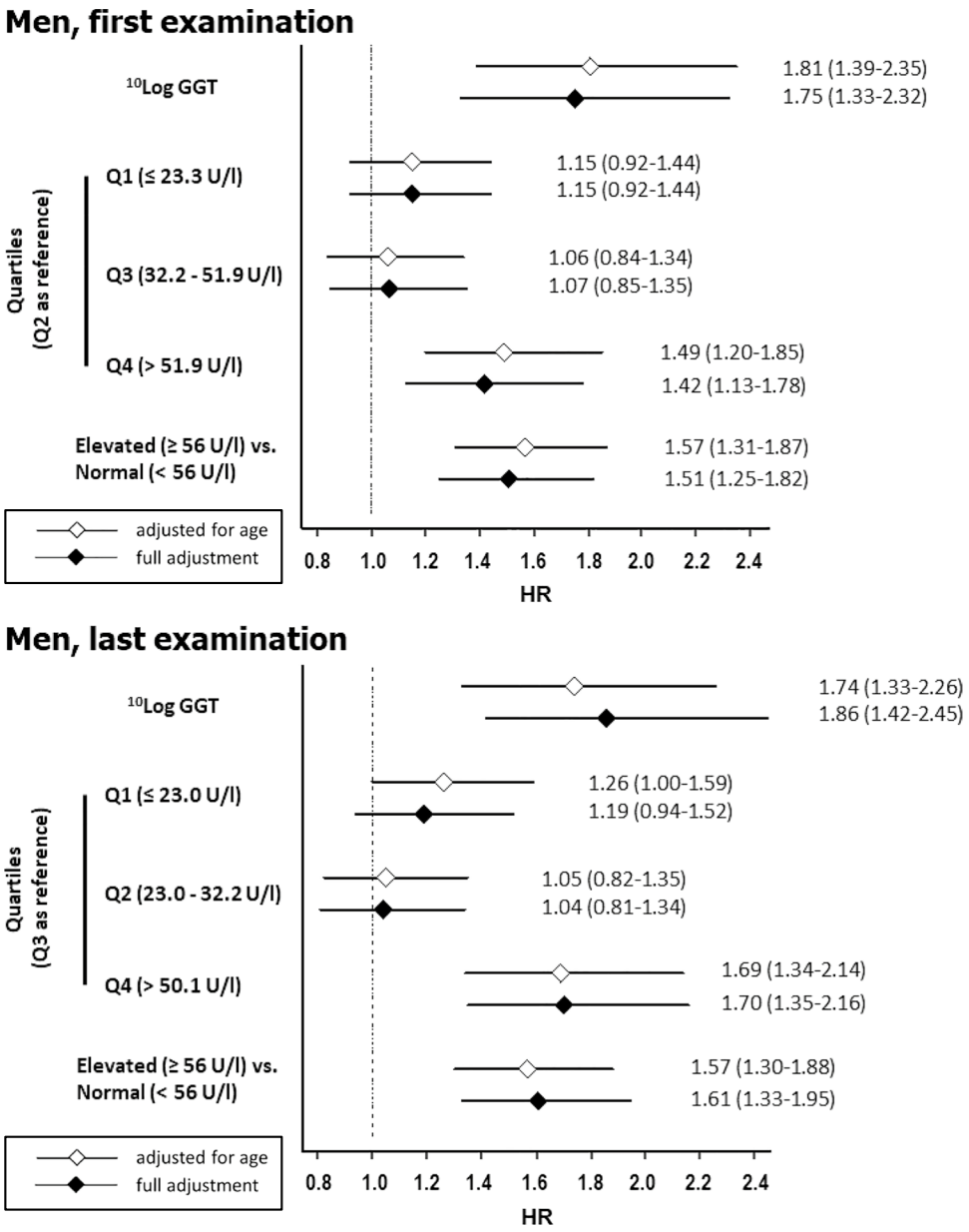

Fig. 3 Forest plots showing age-adjusted (open rhombuses) and fully adjusted (filled rhombuses) hazard ratios with 95% confidence intervals of future hip fracture risk in men for increasing levels of GGT as continuous ^{10}Log transformed variable as well as in quartiles (Q1–Q4), and according to the cut-off value of normal vs. elevated GGT levels for men (56 U/l), measured at each participant´s first (top) and last (bottom) health examination 1985–2005. Hazard ratios are also provided numerically including 95% confidence intervals in parentheses. The reference category for the evaluation according to quartiles was Q2 at the first and Q3 at the last examination. The full adjustment was for age at baseline, BMI, systolic blood pressure, diastolic blood pressure, triglycerides, cholesterol, serum uric acid, diabetes, and smoking status

an inverse association of serum GGT was found with a BMD T score in the osteoporotic range derived from the lumbar spine, femoral neck, and total femur in subjects 72 years of age on average [19]. Another investigation comprising individuals 55 years on average described an association of the highest tertile of GGT values in the study with low bone mass, encompassing both osteopenic and osteoporotic T scores from the lumbar spine, femoral neck, and total hip BMD, and an inverse correlation between GGT and BMD was demonstrated at all three skeletal sites [20]. In a further study including subjects at a mean age of approximately 48 years, femoral neck, lumbar spine, total femur, and whole-body BMD were each significantly lower at GGT levels > 32 U/l vs. ≤ 13 U/l [21]. A significant inverse correlation between serum GGT levels and BMD measured at the femoral neck was also reported from participants of the NHANES III study [22]. Our results are thus in line with other notions of a relation between high serum GGT and lower bone mass as well as risk for fractures. Specifically, our findings extend this relation to hip fracture risk as the exclusive endpoint in women and men ≥ 50 years, the target population at highest risk for fragility fractures. Also, separate analyses according to time of baseline indicated an increased effect size for each patient´s last baseline examination in the study, especially in women. Since follow-up was markedly shorter than from the first examination, we assume dilution of the effect with advancing time from baseline. In

Table 3 GGT-related risk of future hip fracture (hfx) in participants with information on alcohol intake

			Adjusted for age at baseline	Adjusted for age at baseline + alcohol intake	Fully adjusted [a]	Fully adjusted [a] + alcohol intake
Exposure variable	*n* (all subjects)	*n* (subjects with hfx)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Women						
^{10}Log GGT	2761	165	0.93 (0.49–1.77)	0.93 (0.49–1.77)	0.91 (0.47–1.77)	0.92 (0.47–1.79)
Normal vs. elevated GGT						
Normal (< 36 U/l)	2274	137	Reference	Reference	Reference	Reference
Elevated (≥ 36 U/l)	487	28	1.08 (0.72–1.62)	1.08 (0.72–1.62)	1.05 (0.70–1.60)	1.06 (0.70–1.60)
Normal + moderately elevated (< 56 U/l)	2535	150	Reference	Reference	Reference	Reference
Highly elevated (≥ 56 U/l)	226	15	1.39 (0.81–2.36)	1.39 (0.82–2.37)	1.38 (0.80–2.36)	1.38 (0.81–2.37)
Men						
^{10}Log GGT	2684	67	2.33 (1.04–5.25)	2.61 (1.16–5.87)	2.76 (1.18–6.45)	3.02 (1.29–7.06)
Normal vs. elevated GGT						
Normal (< 56 U/l)	2179	46	Reference	Reference	Reference	Reference
Elevated (≥ 56 U/l)	505	21	2.39 (1.41–4.03)	2.55 (1.50–4.33)	2.60 (1.50–4.53)	2.74 (1.57–4.78)

[a] Adjusted for age at baseline, BMI, systolic blood pressure, diastolic blood pressure, triglycerides, cholesterol, serum uric acid, diabetes, and smoking status

general, however, effects proved to be stable over different times of follow-up, which demonstrates the utility of GGT as a potential marker of long-term hip fracture risk.

Several modes of action have been suggested how GGT might be implicated with negative outcomes on bone. First, increased GGT serum levels are known to mirror oxidative stress triggered e.g., by alcohol or inflammation [14]. Alternatively, aside from providing the cell with glutathione as an intracellular defense against oxidative stress [31], GGT itself could assume a pro-oxidant role in the extracellular milieu due to the cleavage of extracellular glutathione, giving rise to redox cascades that result in the production of radicals and H_2O_2 [15]. Oxidative stress has been shown to impinge upon bone remodeling by promoting pre-osteoclastic differentiation to osteoclasts on the one hand, and by stimulating apoptosis of osteoblasts and osteocytes and reducing osteoblast activity and differentiation on the other [32]. Indeed, hip fracture risk in postmenopausal women correlated with biomarkers of oxidative stress in blood plasma [26]. In addition, serum levels of homocysteine could be correlated with those of GGT [33, 34], and elevated homocysteine has been associated with adverse effects on bone, including osteoporotic BMD [19] and hip fractures [35], mediated by oxidative stress, but also by inhibition of collagen cross-link formation [36]. Next, there is evidence for a direct stimulatory effect of GGT on osteoclasts. Purified GGT protein was able to induce osteoclast formation in murine bone marrow cells and increased expression of the RANK (receptor activator of nuclear factor-κB) ligand, a pivotal factor for osteoclastogenesis, in murine bone marrow stromal cells, notably also when the enzymatic activity of GGT was blocked, which demonstrates that GGT acts as a cytokine [37]. This osteoclastogenic action was shown to involve binding of extracellular GGT to transmembrane TLR (Toll-like receptor) 4 and further MyD88 dependent cellular downstream signaling [38]. In human stenotic aortic valve tissue, Cappelli et al. observed expression of GGT on osteoclast-like cells as well as a negative association between the enzymatic activity of GGT extracted from the tissue and the extent of valve calcifications [39]. Furthermore, the severity of valve stenosis was negatively correlated with GGT concentrations in both valve tissue and patients´ sera in this study [39]. GGT could thus act as a resorptive regulator of tissue calcification in an autocrine/paracrine manner in aortic valve tissue and analogously also in bone.

Alcohol consumption affects not only serum GGT levels but also bone both directly and via fall risk [26], and differential effects of the consumed amount of alcohol on bone have been observed. Whereas heavy drinking has consistently been associated with bone loss as well as increased risk of falls and accordingly greater hip fracture risk, low to moderate alcohol intake is generally linked with higher

BMD and lower hip fracture risk [27, 40, 41]. Notably, also sex- and age-related differences have recently been examined by Søgaard et al. [42], who found no association of alcohol consumption with hip fracture risk in men ≥ 60 years but increased risk in males < 60 years drinking frequently vs. moderately. Among women, the risk was not elevated in frequent as compared with moderate drinkers but was higher in those who never or rarely consumed alcohol, albeit less distinctly so in subjects ≥ 60 years [42]. Results of our sub-analysis confirm a subordinate role of alcohol consumption for hip fracture risk in elderly post-menopausal women and men ≥ 50 years. In men, fracture risk associated with increasing and elevated GGT concentrations even rose upon adjustment for alcohol consumption. Moreover, the hazard for hip fracture was nonsignificantly lower in men consuming > 140 g alcohol /week vs. lower amounts (Supplementary Table 9). This slight change in risk can tentatively be explained by restricted or abandoned alcohol drinking due to health reasons amongst subjects with high morbidity, which might become apparent particularly in men whose alcohol intake is generally higher compared with women.

Our results show that the GGT-related rise in hip fracture risk was more pronounced in men than in women or even absent in the latter. When the male cut-off for elevated GGT (i.e., 56 U/l) was used also for women to explore the possibility that GGT exerts bone-related effects in a concentration-dependent manner irrespective of sex, hazards were indeed increased in comparison with the female cut-off (i.e., 36 U/l) but still not as much as in men. In a comparatively young study population, Holmberg et al. also observed a higher GGT-related hip fracture risk in men than in women [24, 25]. Given that dwindling estrogen levels after menopause increase the baseline risk of hip fractures, and given that roughly 2.5 times more Austrian women than men ≥ 50 years are affected by a hip fracture [43, 44], it is conceivable that the addition of another risk factor such as elevated GGT entails a smaller relative risk effect in women than in men, not precluding, of course, a possible strong effect in absolute terms. Decreasing estrogen after menopause could also explain why fracture risk was less stable over time in women than in men. As its relative importance increases with longer follow-up (i.e., last vs. first examination), GGT could concomitantly become less predictive for hip fracture risk.

There are several limitations but also strengths of the present study to be addressed. First, not all relevant covariates with potential influence on hip fracture risk were known, including participants´ physical activity, osteoporosis, vitamin D, and mental health status, as well as data on medication history, such as anti-osteoporotic drugs, cortisone, and estrogen replacement. It should be stressed, however, that many of these variables do not affect GGT, which are thus not to be regarded as confounders. For example, supplementation-induced rise in vitamin D serum levels was not accompanied by altered GGT concentrations in a randomized controlled trial [45], and in a large cross-sectional investigation in more than 24,000 adults, GGT and vitamin D serum levels were not correlated [46]. A potential source of confounding that we could not directly adjust for is chronic liver disease that is a cause of elevated GGT levels and has been associated with osteoporosis [47]. Alcohol abuse as one major risk factor of chronic liver disease was, however, considered in our sub-analysis, and non-alcoholic fatty liver disease as another important cause was indirectly accounted for by adjustment for components of the metabolic syndrome, i.e., diabetes status, triglycerides, total cholesterol (as a surrogate for HDL), BMI (as an approximation to central obesity), and blood pressure. A further potential limitation relates to a possible effect of death competing with the outcome event (i.e., hip fracture) during the observation period. GGT is a well-known marker of all-cause mortality [48], and in our study, relative to participants with normal GGT levels at baseline, elevated GGT was associated with approximately 35% and 25% more deaths during the observation period from first and last examination, respectively (data not shown). Hence a bias could have been introduced via different lengths of the observation period, leading to overestimation of GGT-related fracture risk. Also, the number of observed fractures would be expected to be higher along with longer survival, resulting, by contrast, in underestimation of GGT-related risk. However, given the clearly etiological rather than decision-oriented alignment of our research question, the application of Cox proportional hazards regression with censoring of death competing with the outcome event, as conducted herein, has been recommended as the appropriate approach [49]. Next, the sub-cohort including participants with records on alcohol consumption could have been subject to sampling bias because of its limited size relative to the entire study population, as well as selection bias because of the inclusion criteria of the additional surveillance programs. Indeed, subjects were older at baseline than at the first examination in the entire study population but similar in age to the full cohort at the last examination. Since also other baseline features are very similar (Tables 1 and 2; Supplementary Table 7) and covariates in the sub-cohort showed the same trends for association with hip fracture risk as in the entire study population, e.g., cholesterol, diabetes, and smoking (Supplementary Tables 8 and 9), we deem the sub-cohort representative of the whole study population. Also, the time point of acquisition of drinking habits usually did not match that of the baseline VHM&PP health examination. Since most subjects took part in more than one VHM&PP health examination, we chose the one with minimal offset to the acquisition of drinking habits to mitigate this

potential limitation. Finally, whereas we are unable to rule out that individuals were lost to follow-up because of relocation, complete documentation of hip fracture cases on an individual level can be assumed because residents of Vorarlberg sustaining a hip fracture are highly unlikely to be admitted to hospitals outside the province because of political, health insurance coverage, and geographic factors [44]. Further strengths of the present population-based study encompass the extensive follow-up time and a large number of participants, as well as the possibility to analyze the effects of alcohol intake. Also, this is the first investigation to examine the association of GGT and hip fracture as a study endpoint in a population of elderly women and men ≥ 50 years.

Collectively, this large, population-based cohort study among postmenopausal women and men ≥ 50 years demonstrates a positive association of increasing and elevated serum GGT concentrations with the risk of future hip fracture, which is particularly significant in men. In a subpopulation analysis accounting for alcohol consumption as a covariate, no influence of alcohol intake on GGT-related hip fracture risk was apparent in women, and in men, alcohol even nonsignificantly covered up the association with GGT. Our findings thus support the notion of GGT affecting bone and fracture risk independently of possible alcohol-related effects in an elderly population. These results underpin the possible utility of GGT as a serum marker of future risk of hip fractures.

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Availability of data and material The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability N/A.

Declarations

Ethics approval The Ethics Committee of Vorarlberg approved the evaluation of the data (EK-Nr. 2006–6/3), and all procedures were carried out in agreement with the Helsinki Declaration of 1975, as revised in 2013.

Consent to participate Written consent was obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Consent for publication N/A

Conflict of interest The authors declare no competing interests.

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