DOI:10.1158/0008-5472.CAN-07-6686

Cancer Research

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*Cancer Res* 2008;68:3970-3977. Published online May 15, 2008.

DOI:10.1158/0008-5472.CAN-07-6686

Research Article

# Association of γ-Glutamyltransferase and Risk of Cancer Incidence in Men: A Prospective Study

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## Abstract

**Although several epidemiologic studies have shown that γ-glutamyltransferase (GGT) is independently associated with cardiovascular disease and all-cause mortality, its relationship with cancer incidence remains widely unexplored. In several experimental models, the ability of cellular GGT to modulate crucial redox-sensitive functions has been established, and it thus may play a role in tumor progression, as has been repeatedly suggested. We prospectively investigated the association between GGT and risk of overall and site-specific cancer incidence in a large population-based cohort of 79,279 healthy Austrian men with serial GGT measurements. Median follow-up was 12.5 years. Adjusted Cox proportional hazards models were calculated to evaluate GGT as an independent predictor for cancer incidence, and nonparametric regression splines were fitted to flexibly capture the dose-response relationship. Elevated GGT significantly increased overall cancer risk, showing a clear dose-response relationship (*P* for GGT log-unit increase < 0.0001; *P* for trend < 0.0001). In comparison with the reference GGT concentration (25 units/L), we found adjusted relative risks (95% confidence intervals) equalling 1.19 (1.15-1.22) for GGT concentrations of 60 units/L, 1.32 (1.28-1.36) for 100 units/L, 1.67 (1.60-1.75) for 200 units/L, and 2.30 (2.14-2.47) for 400 units/L. In cancer site-specific models, GGT was significantly associated with malignant neoplasms of digestive organs, the respiratory system/intrathoracic organs, and urinary organs (all *P* < 0.0001). Age of participants significantly modified the association of GGT and cancer risk (*P* < 0.001), revealing markedly stronger associations in participants ages ≤65 years. Our findings, for the first time, show that elevated GGT is significantly associated with increased cancer risk in men.** [Cancer Res 2008;68(10):3970–7]

## Introduction

In clinical practice, measurement of serum γ-glutamyltransferase (GGT) is a commonly used diagnostic procedure, mainly seen as an indicator for hepatobiliary disease and a biological marker of excessive alcohol intake (1–5). However, in recent years, several epidemiologic studies have sparked further interest in elevated GGT as an independent predictor for morbidity and mortality from causes other than liver disease. Particularly, it was reported that GGT is independently associated with cardiovascular disease (6–11) and most cardiovascular risk factors (12–15), and more recently, an association with chronic kidney disease was found (16). In addition, several large-scale studies indicate an independent role of GGT for premature death from all causes (9, 12, 17).

The association of GGT with cancer incidence, however, remains largely unexplored to date. Several experimental models have elucidated the ability of cellular GGT to modulate crucial redox-sensitive functions, such as antioxidant/antitoxic defenses and cellular proliferative/apoptotic balance, and its role in tumor progression, invasion, and drug resistance has been proposed (18–21). In addition, a potentially interesting interpretation subsumes GGT as a biomarker of exposure to certain cancer-causing xenobiotics, including persistent organic pollutants (22, 23).

We are aware of only two epidemiologic investigations related to the topic, both failing to detect an association between GGT and cancer mortality in middle-aged men from the general population (9, 24). In a recent retrospective, hospital-based study, however, Kazemi-Shirazi et al. (17) could show a significant association of GGT and cancer death in men and women. In the present, prospective, 19-year follow-up study, we aimed to investigate the association of GGT and cancer incidence in a large population-based cohort of 79,279 apparently healthy Austrian men across a wide age range. To our knowledge, this is the first epidemiologic investigation of the association of GGT and cancer incidence in men.

## Materials and Methods

**Study population.** The Vorarlberg Health Monitoring and Promotion Program (VHM&PP; refs. 25–27), conducted by the Agency for Social and Preventive Medicine in Vorarlberg since 1985, the westernmost province of Austria, is one of the world's largest ongoing population-based risk factor surveillance programs. All adults of the regions are invited to participate by a combination of different measures, such as 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 exam, 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 (25).

Between 1985 and 2003, 80,224 male Vorarlberg residents (ages >18 y) were enrolled in the VHM&PP, resulting in a total of 271,871 routine health

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi:10.1158/0008-5472.CAN-07-6686

DOI:10.1158/0008-5472.CAN-07-6686



**Table 1.** Characteristics of study population

| | |
|---|---|
| All male VHM&PP participants, 1985-2003 ($n$) | 80,224 |
| Participants with complete and valid data on GGT ($n$) | 79,363 |
| Eligible participants for analyses ($n$) | 79,279* |
| Total number of visits ($n$) | 270,281 |
| No. visits, mean ± SD (range) | 3.5 ± 2.8 (1-19) |
| Age (y), mean ± SD (range) | 41.6 ± 14.6 (18-96) |
| BMI (kg/m$^2$), mean ± SD (median) | 25.3 ± 3.6 (24.9) |
| GGT (units/L), median (range) | 28.6 (2.0-798.0) |
| Current cigarette smoking (%) | 30.0 |
| Former cigarette smoking (%) | 8.5 |
| Occupational status | |
| Blue collar (%) | 37.6 |
| White collar (%) | 51.9 |
| Self-employed (%) | 10.5 |
| Follow-up (y), mean ± SD (median) | 11.3 ± 5.6 (12.5) |
| Total person-years at risk | 894,231 |
| Incident cancers, $n$ (%) | 5,305 (6.7) |
| Age at cancer diagnosis (y), mean ± SD (range) | 57.0 ± 12.2 (19-93) |

NOTE: GGT values are shown as averages of each participant during individual follow-up and before eventual cancer diagnoses. All other characteristics pertain to baseline values (i.e., measurements at first visit).

*Participants with GGT values >800 units/L or with history of cancer before enrollment were excluded.

examinations. Approximately 70% of participants had two or more health examinations. After excluding 861 participants (1.1%) with a history of malignancies before enrollment or with no GGT measurements, the current investigation was restricted to 79,363 male participants, free of cancer at baseline. To eliminate possible effects of severe preclinical disease, undiagnosed at time of enrollment, we further excluded participants with baseline GGT values >800 units/L ($n$ = 84), resulting in a total of 79,279 initially healthy men, with 270,281 longitudinal GGT measurements eligible for analyses.

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, GGT, and smoking status (current, former, or never) routinely are obtained for each VHM&PP 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.

**Cancer ascertainment.** Cancers were identified by the Vorarlberg cancer registry, which has been accepted for IARC publication since 1993 (28) and has high completeness of recording (29). Nearly all cancers (96.7%) were histologically confirmed. Cohort data were linked with the Vorarlberg Death Index to identify deaths and to calculate person-years at risk. For analyses, cancers were grouped into the following subgroups according to the International Classification of Diseases, 9th and 10th Revision (ICD-9 and ICD-10)[9]: malignant neoplasms of digestive organs (ICD-9 150-157; ICD-10 C15-C26); malignant neoplasms of respiratory system and intrathoracic organs (ICD-9 160-165; ICD-10 C30-C39); malignant neoplasms of bone, connective tissue, soft tissue, and skin (ICD-9 170-173; ICD-10 C40-C49); malignant neoplasms of male genital organs (ICD-9 185-187; ICD-10 C60-C63); malignant neoplasms of urinary organs (ICD-9 188-189; ICD-10 C64-68); malignant neoplasms of nervous system and unspecified sites (ICD-9 190-199; ICD-10 C69-C72); and malignant neoplasms of lymphoid, hematopoietic, and related tissue (ICD-9 200-208; ICD-10 C81-C96).

**Laboratory measurements.** Two central laboratories undergoing regular internal and external quality procedures enzymatically determined GGT concentrations on fasting blood samples. Within 60 to 240 min after venous blood sample collection from a cubital vein, serum was obtained by centrifugation for 15 min at 4,000 rotations per minute. Subsequently, GGT concentrations were measured at 37°C and given as units per liter (units/L). Because it has been shown that several serum analytes, including GGT, are sufficiently stable, even for 4 d in separated serum stored at +9°C (30), there was no difference in the same samples measured between 1 and 4 h. To check calibration, three 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%.

**Statistical analyses.** Follow-up started at enrollment in the cohort and ended at cancer diagnosis or at censoring. Censoring events were death, loss to follow-up, and emigration. To estimate hazard ratios with 95% confidence intervals (95% CI), we first calculated Cox proportional hazards models, adjusted for age, body mass index (BMI), smoking status, occupational status, and year of examination, which included log GGT at first examination as the exposure variable on a time scale. We used the same Cox models to estimate adjusted cumulative cancer incidence according to quintiles of the log-GGT distribution. To assess dose-response relationships of GGT and cancer incidence, trends across quintiles of logarithmically transformed GGT levels were tested using the median GGT level for each quintile as an ordinal variable in the Cox models. To test for effect modification of GGT by age, we included multiplicative interaction terms in the same models.

To flexibly capture the dose-response association of GGT with cancer incidence, we further modeled GGT using a b-spline expansion in binary logistic models (31). In these models, we calculated relative risks for each value $x$ with respect to a reference value $x_{\text{ref}}$ as

$$rr(x, x_{\text{ref}}) = \exp\left(\sum_{i=1}^{n} \beta_i [s_i(x) - s_i(x_{\text{ref}})]\right)$$

where $n$ is the number of degrees of freedom (df) of the spline expansion, $\beta_i$ is the coefficient of the $i^{\text{th}}$ spline basis function, and $s_i(x)$ is the value of the $i^{\text{th}}$ spline basis function at $x$. Ninety-five percent confidence intervals were computed based on asymptotic normality of the estimates. The calculations are based on an algorithm previously described in detail by Cao et al. (32) and were implemented by us in a Statistical Analysis System (SAS) macro that is available from the authors on request. Because participants underwent unequal numbers of routine health examinations and GGT is known to display a considerable long-term stability with a strong tracking pattern (tracking coefficient = 0.72; refs. 25, 33), we used the average GGT concentration (arithmetic mean) during individual follow-up in the spline analyses. For all other covariates, baseline values were used in the spline models. A GGT concentration of 25 units/L, as the midpoint of the laboratory range of normal GGT values (0-50 units/L in men), was used as reference value with a relative risk equalling 1.00. Model selection was based on Akaike information criterion (AIC; ref. 34), a penalized likelihood that takes into account the number of variables estimated in the model, compromising between a good fit and a parsimonious model. All models were adjusted for age, BMI, smoking status, occupational status, and year of examination. To account for potential effects of length of follow-up, in a sensitivity analysis, we additionally adjusted our spline models for follow-up time. Two-sided $P$ values of <0.05 were considered statistically significant. All statistical analyses were conducted using Statistical Package for the Social Sciences 15.0 and SAS 9.1 standard software.

[9] http://www.who.int/classifications/icd/en

DOI:10.1158/0008-5472.CAN-07-6686



**Table 2.** Association of GGT with overall and site-specific cancer incidence, VHM&PP, 1985-2003

| | All cancers ($n$ = 5,305) | Site-specific cancers | | |
|---|---|---|---|---|
| | | Digestive organs ($n$ = 1,242) | Respiratory system and intrathoracic organs ($n$ = 877) | Bone, connective tissue, soft tissue, and skin ($n$ = 447) |
| Hazard ratio for GGT log-unit increase* | 1.58 (1.45-1.72) | 2.00 (1.67-2.39) | 2.20 (1.80-2.69) | 1.04 (0.75-1.44) |
| $P$ for GGT log-unit increase* | <0.0001 | <0.0001 | <0.0001 | 0.84 |
| $P$ for trend across log-GGT quintiles † | <0.0001 | <0.0001 | <0.0001 | 0.65 |

NOTE: Participants with GGT values >800 units/L or with history of malignancies before enrollment were excluded. Baseline GGT values (i.e., measurements at first visit) were used in the Cox models.

*Hazard ratios and $P$ values for GGT log-unit increases were estimated from Cox proportional hazards models adjusted for age, BMI, smoking status, occupational status, and year of examination.

†$P$ values for trend across quintiles of logarithmically transformed GGT levels were tested using the median GGT level for each quintile as an ordinal variable in a Cox proportional hazards model adjusted for age, BMI, smoking status, occupational status, and year of examination.

## Results

**Characteristics of study population.** Demographic and clinical characteristics of the study population are shown in Table 1. Median follow-up time was 12.5 years, corresponding to a total of 894,231 person-years. Most participants (92.1%) were followed-up for at least 2 years after baseline GGT measurement and 61.5% had follow-up times of 10 or even more years. Mean age at study entry was 41.6 years. During follow-up, 5,305 (6.7%) incident cancers were observed. On average, 3.5 GGT measurements were obtained for each participant (range, 1-19). GGT levels ranged from 2.0 to 798.0 units/L, with a median of 28.6 units/L.

**Determination of best-fitting spline models.** The optimal number of segments was selected by putting an increasing number of knots at subsequent tertiles, quartiles, and quintiles of the GGT distribution, and the best-fitting model was defined as the one minimizing AIC. It has been shown that AIC may perform poorly for small samples or in the presence of too many variables in relation to the size of the sample (35, 36); however, none of these conditions affected our study. Fitting characteristics of different linear and quadratic spline models for the association of GGT and risk of overall and site-specific cancer incidence, according to AIC, are shown in Supplementary Table S1. We found quadratic spline models, including either three or four knots, at consecutive quartiles/quintiles of the GGT distribution to have the best model fit for the association of GGT with risk of overall cancer incidence, malignant neoplasms of digestive organs, and respiratory system/

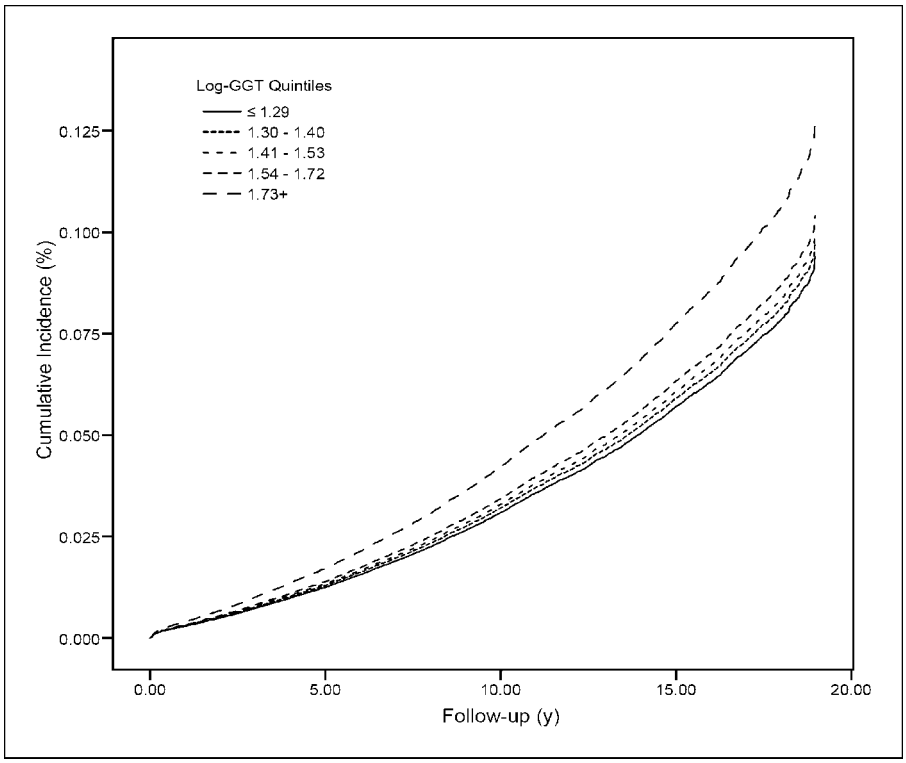


**Figure 1.** Adjusted cumulative cancer incidence according to quintiles of log GGT at first examination among 79,279 male Austrian adults (mean age, 41.6 y) in the VHM&PP estimated at the average values of covariates. Survival curves were calculated from Cox proportional hazards models adjusted for age, BMI, smoking status, occupational status, and year of examination.

DOI:10.1158/0008-5472.CAN-07-6686



**Table 2.** Association of GGT with overall and site-specific cancer incidence, VHM&PP, 1985-2003 (Cont'd)

| Site-specific cancers | | | |
|---|---|---|---|
| Genital organs ($n$ = 1,858) | Urinary organs ($n$ = 446) | Nervous system and unspecified sites ($n$ = 115) | Lymphoid, hematopoietic, and related tissue ($n$ = 320) |
| 1.11 (0.95-1.30) | 1.60 (1.19-2.17) | 1.10 (0.58-2.11) | 1.04 (0.70-1.53) |
| 0.20 | 0.002 | 0.77 | 0.85 |
| 0.21 | <0.0001 | 0.87 | 0.81 |

intrathoracic organs. Linear models, with knot numbers ranging from two to four, were found to minimize AIC values for all other site-specific cancers.

**Association of GGT with overall cancer incidence and site-specific cancers.** The association of GGT with overall and site-specific cancer incidence is shown in Table 2. An adjusted Cox regression, modeling the association of GGT and risk of overall cancer incidence, using log-transformed GGT concentrations at first examination, yielded a hazard ratio of 1.58 (95% CI, 1.45-1.72; $P < 0.0001$) per GGT log-unit increase. Adjusted cumulative cancer incidence according to quintiles of the log-GGT distribution is shown in Fig. 1. The highest log-GGT quintile was significantly associated with increased overall cancer incidence, showing a clear dose-response relationship ($P$ for trend < 0.0001). In cancer site-specific Cox models, GGT was an independent predictor for malignant neoplasms of digestive organs, the respiratory system/intrathoracic organs, and urinary organs ($P$ for GGT log-unit increase, all < 0.005; $P$ for trend across log-GGT quintiles, all < 0.0001; Table 2).

Relative risk estimates for the association of GGT with overall and site-specific cancer incidence from best-fitting spline models are shown in Table 3 and Fig. 2. In the best-fitting model, adjusted for age, BMI, smoking status, occupational status, and year of examination, even moderately elevated GGT (60 units/L) was significantly associated with increased risk of overall cancer incidence with a relative risk (95% CI) of 1.19 (1.15-1.22) in comparison with the reference GGT concentration of 25 units/L. This risk substantially increased to 1.32 (1.28-1.36) for a GGT concentration equalling 100 units/L, 1.67 (1.60-1.75) for 200 units/L, 2.30 (2.14-2.47) for 400 units/L, and 2.59 (2.25-2.97) for 600 units/L. After additionally adjusting for follow-up time in our models, all reported relative risks even slightly increased (data not shown). Very low GGT concentrations were significantly associated with moderately decreased risk of overall cancer incidence; for a GGT value of 10 units/L, we observed a relative risk equalling 0.83 (0.76-0.92), in comparison with the reference concentration of 25 units/L (Table 3; Fig. 2).

To eliminate possible effects of severe preclinical disease, likely to confound our results, in a first subgroup analysis, we excluded all participants, diagnosed with malignancies within the first 2 years after enrollment. With this reanalysis, however, relative risks and statistical significance for the association of GGT and

**Table 3.** Relative risk estimates with 95% CIs for the association of GGT with overall and site-specific cancer incidence from best-fitting regression spline models, VHM&PP, 1985-2003

| | All cancers ($n$ = 5,305) | Site-specific cancers | | |
|---|---|---|---|---|
| | | Digestive organs ($n$ = 1,242) | Respiratory system and intrathoracic organs ($n$ = 877) | Bone, connective tissue, soft tissue, and skin ($n$ = 447) |
| Spline model | df = 2, knots = 4 | df = 2, knots = 2 | df = 2, knots = 2 | df = 1, knots = 2 |
| GGT (units/L) | | | | |
| 10 | 0.83 (0.76-0.92) | 0.72 (0.59-0.88) | 0.81 (0.65-1.01) | 1.20 (0.97-1.48) |
| 15 | 0.96 (0.91-1.00) | 0.87 (0.81-0.93) | 0.85 (0.79-0.92) | 1.11 (0.98-1.26) |
| 25 | 1.00 (Reference) | 1.00 (Reference) | 1.00 (Reference) | 1.00 (Reference) |
| 40 | 1.07 (1.04-1.10) | 1.07 (1.03-1.12) | 1.20 (1.14-1.26) | 1.23 (1.11-1.35) |
| 60 | 1.19 (1.15-1.22) | 1.21 (1.16-1.27) | 1.37 (1.31-1.44) | 1.29 (1.17-1.42) |
| 100 | 1.32 (1.28-1.36) | 1.53 (1.46-1.61) | 1.75 (1.66-1.86) | 1.43 (1.30-1.57) |
| 200 | 1.67 (1.60-1.75) | 2.51 (2.32-2.72) | 2.84 (2.59-3.11) | 1.85 (1.66-2.06) |
| 400 | 2.30 (2.14-2.47) | 4.74 (4.23-5.31) | 4.13 (3.59-4.74) | 3.10 (2.58-3.72) |
| 600 | 2.59 (2.25-2.97) | 5.57 (4.44-6.99) | 2.78 (1.95-3.95) | 5.16 (3.92-6.80) |

NOTE: Participants with GGT values >800 units/L or with history of malignancies before enrollment were excluded. Mean GGT values of each participant during individual follow-up and before eventual cancer diagnoses were used in the spline models. All models are adjusted for age, BMI, smoking status, occupational status, and year of examination.

DOI:10.1158/0008-5472.CAN-07-6686



**Table 3.** Relative risk estimates with 95% CIs for the association of GGT with overall and site-specific cancer incidence from best-fitting regression spline models, VHM&PP, 1985-2003 (Cont'd)

| Site-specific cancers | | | |
|---|---|---|---|
| Genital organs ($n$ = 1,858) | Urinary organs ($n$ = 446) | Nervous system and unspecified sites ($n$ = 115) | Lymphoid, hematopoietic, and related tissue ($n$ = 320) |
| df = 1, knots = 3 | df = 1, knots = 3 | df = 1, knots = 4 | df = 1, knots = 2 |
| 0.78 (0.69-0.89) | 0.95 (0.75-1.20) | 0.88 (0.66-1.17) | 1.29 (1.03-1.63) |
| 0.91 (0.85-0.97) | 0.92 (0.82-1.04) | 0.87 (0.72-1.03) | 1.16 (1.01-1.34) |
| 1.00 (Reference) | 1.00 (Reference) | 1.00 (Reference) | 1.00 (Reference) |
| 1.05 (1.01-1.08) | 1.41 (1.32-1.49) | 0.98 (0.83-1.16) | 1.27 (1.14-1.42) |
| 1.14 (1.08-1.20) | 1.65 (1.51-1.79) | 1.63 (1.42-1.88) | 1.35 (1.21-1.50) |
| 1.19 (1.13-1.25) | 1.78 (1.64-1.92) | 1.83 (1.59-2.10) | 1.52 (1.37-1.69) |
| 1.32 (1.24-1.41) | 2.15 (1.96-2.36) | 2.42 (2.10-2.80) | 2.06 (1.83-2.31) |
| 1.64 (1.44-1.86) | 3.17 (2.67-3.75) | 4.25 (3.48-5.18) | 3.76 (3.12-4.52) |
| 2.02 (1.65-2.48) | 4.63 (3.57-6.02) | 7.40 (5.60-9.77) | 6.81 (5.18-8.96) |

overall cancer incidence virtually remained unchanged. When considering interaction terms in our regression models, age of participants proved to be a significant effect modifier for the association of GGT and risk of overall cancer incidence ($P$ for multiplicative interaction = 0.001) and malignant neoplasms of digestive organs ($P$ = 0.005). In stratified analyses, the initial observed significant association of GGT with risk of overall cancer incidence fairly diminished in participants ages >65 years, leveling off at a relative risk of $\sim$1.15, also for highly elevated GGT (Fig. 3). Likewise, we found a markedly stronger association of GGT with malignant neoplasms of digestive organs in younger individuals (≤65 years), in comparison with older participants (data not shown).

## Discussion

The present, prospective, 19-year follow-up study is the first epidemiologic investigation to explore the association of GGT with risk of subsequent cancer incidence in men. After adjustment for several confounding factors, we found elevated GGT to be significantly associated with increased risk of overall cancer incidence and several site-specific cancers. Our estimates proved to be stable under several modeling strategies, using different analytic approaches, and after exclusion of participants diagnosed with malignancies within the first 2 years after enrollment, strongly indicating an independent role of GGT on carcinogenesis, particularly in men ages <65 years.

Our results are in line with recent findings from Kazemi-Shirazi et al. (17), reporting a 2.3-fold risk increase for cancer death in the highest GGT quintile, in comparison with normal low GGT in >120,000 Austrian men. Anyway, in that investigation, data were obtained retrospectively from previous hospital attendants, requesting laboratory analyses of GGT for any reason. Therefore, their study population is in no way comparable with the present cohort, rather consisting of apparently healthy men, undergoing GGT measurement for routine health examination. In the only other previous epidemiologic studies related to the topic, Wannamethee et al. (9) and Petersson et al. (24) failed to detect an association between GGT and risk of cancer mortality in middle-aged men from general populations of the United Kingdom and Sweden. Notably, in both investigations, sample sizes of the study cohorts were <10% of the present investigation and insufficient events in both studies may have resulted in inadequate power.

Given the epidemiologic nature of our observations, the underlying biological mechanisms causing elevated GGT to be significantly associated with increased cancer risk, particularly in men ages <65 years, cannot be certainly answered in the present investigation. However, in respect to our finding of a more prominent association in younger individuals, in comparison with their older counterparts, from a statistical point of view, one might argue that with increasing age more individuals are at risk for the development of cancer in general, possibly attenuating the excess effect of elevated GGT.

Experimental evidence has elucidated the ability of cellular GGT to modulate crucial redox-sensitive functions, such as antioxidant/antitoxic defenses and cellular proliferative/apoptotic balance, and its role in tumor progression, invasion, and drug resistance has repeatedly been suggested (18–21). GGT is constitutively expressed in several organs and is often significantly increased in malignant or premalignant lesions, where it is considered a factor conferring growth and survival advantages for the rapidly dividing neoplastic cells (37).

In our analyses, GGT was consistently associated with malignancies of digestive organs, the respiratory system, and urinary organs, whereas we found no significant dose-response association of GGT with other site-specific cancers. It is well known that the first three entities of cancers are predominantly influenced by lifestyle-related factors (including smoking, dietary habits, and alcohol consumption) and environmental hazards, whereas other cancer entities, not significantly associated with GGT in our study, are only influenced by endogenous effects and non–lifestyle factors such as age, genetics, or immunologic variables (38). In line with our finding of a significant dose-response association of GGT with several lifestyle-triggered malignancies, it was shown that GGT is greatly influenced by dietary factors (39, 40). GGT was reported to decrease with higher consumption of fruit or plant foods, whereas GGT increased with higher consumption of meat. When nutrients in plant foods and meat were examined, dietary constituents

DOI:10.1158/0008-5472.CAN-07-6686



related to oxidative stress were associated with GGT. Whereas GGT was inversely correlated with several dietary antioxidants, including vitamin C and β-carotene, dietary heme iron was positively associated with GGT.

Lee et al. (22) and Lee and Jacobs (23) recently showed that several environmental pollutants, such as lead, cadmium, dioxins, or organochlorine pesticides, are positively and monotonically associated with serum GGT levels in the general U.S. population. Because cellular GGT is indispensable for metabolism of extracellular reduced glutathione (GSH), higher serum GGT plausibly reflects increased cellular GGT activity to metabolize extracellular GSH conjugates. GSH has an important function in conjugating xenobiotics such as lead, cadmium, dioxins, or organochlorine pesticides to facilitate their excretion in the urine or bile, by rendering them more water soluble. Thus, serum GGT increases with increasing exposure to xenobiotics with need to be conjugated to GSH (22, 23). In an experiment with carcinogen-treated rats, Stark et al. (41) found that metabolism of GSH by GGT in preneoplastic

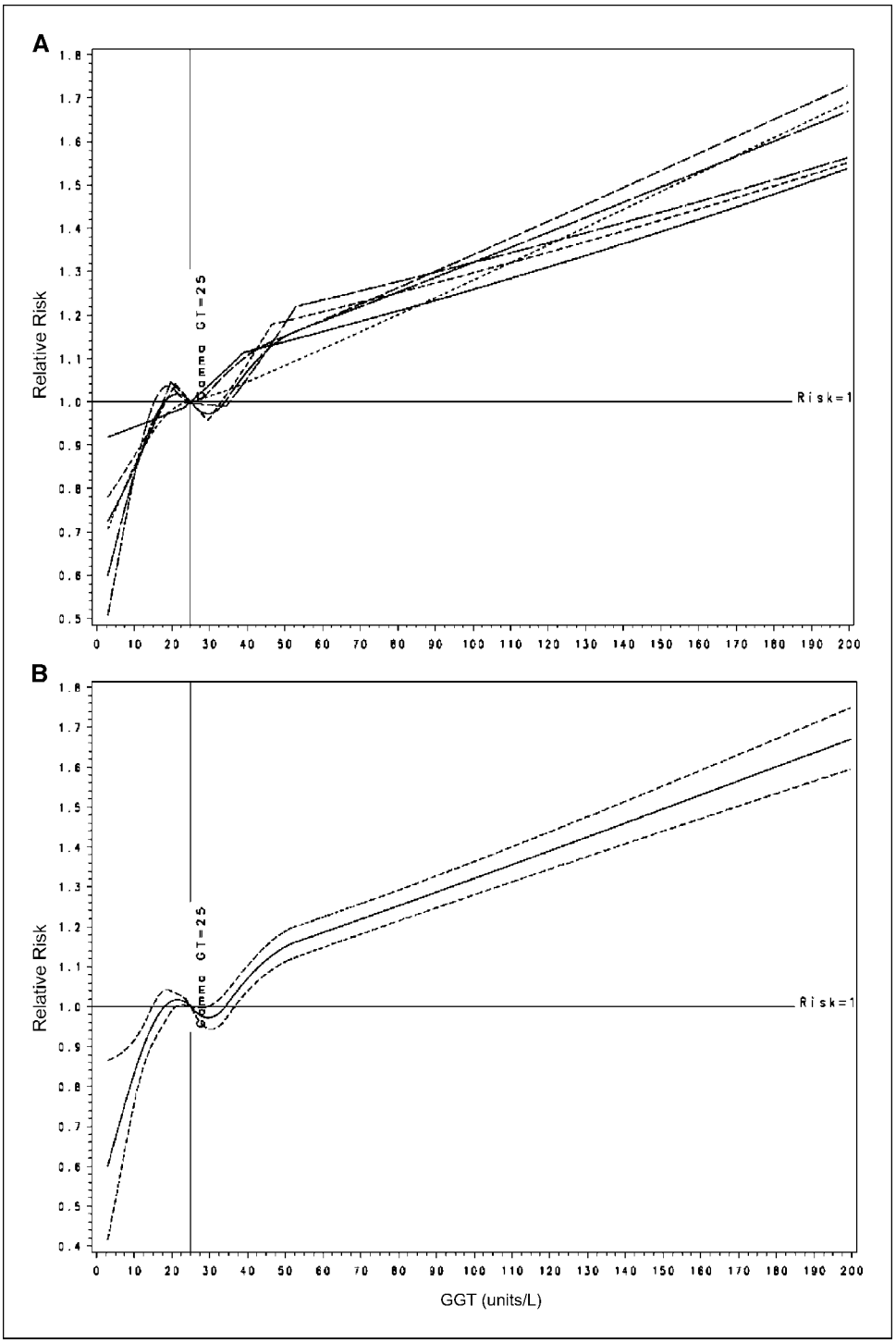


**Figure 2.** Relative risk estimates for the association of GGT (range, 0-200 units/L) and overall cancer incidence in 79,279 male Austrian adults from different linear and quadratic regression spline models for two to four knots (*A*) and AIC-minimizing, best-fitting spline model with df = 2, knots = 4, and 95% CI (*dashed lines*; *B*). All models are adjusted for age, BMI, smoking status, occupational status, and year of examination. A GGT concentration of 25 units/L was used as reference value with a relative risk of 1.00.

DOI:10.1158/0008-5472.CAN-07-6686



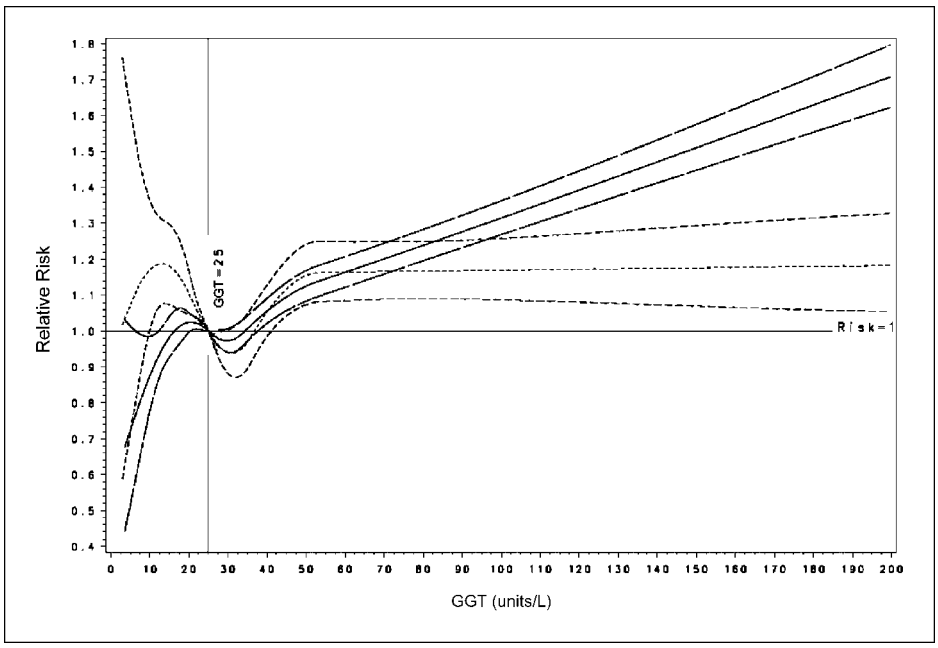


**Figure 3.** Relative risk estimates with 95% CIs for the association of GGT (range, 0-200 units/L) and overall cancer incidence in participants ages ≤65 y ($n$ = 73,278; 3,979 incident cancers; *solid lines*) and participants ages >65 y ($n$ = 6,001; 1,326 incident cancers; *dashed lines*). Relative risks were estimated from individual, best-fitting regression spline models. Models are adjusted for age, BMI, smoking status, occupational status, and year of examination. A GGT concentration of 25 units/L was used as reference value with a relative risk of 1.00.

liver foci can initiate an oxidative process leading to a radical-rich environment and to oxidative damage. Such damage may contribute to the processes by which cells within such foci progress to malignancy.

Our study had several strengths and potential limitations that should be considered. Major strengths were the prospective design, large sample size, length of follow-up, and standardized protocol performed by experienced physicians. Beyond analyzing the effect of GGT on risk of cancer incidence, using baseline GGT concentrations in our Cox models, we further considered the average of all GGT measurements, longitudinally obtained from each participant in our spline models. This approach should help to further minimize possible influence of short-term fluctuation in GGT, thereby reducing potential bias in our risk estimates. A further analytic strength of our study was retaining GGT as a continuous variable and applying nonparametric spline regression to flexibly capture its dose-response association with cancer incidence. The problems of categorizing continuous exposure variables in epidemiologic studies have been widely discussed and it has been shown that these models often provide a poor approximation of the true relationship by not using the full range of exposure data available to estimate associations (42, 43).

Potential limitations of our study include the inability, although information on major risk factors was collected, to account for additional factors that further might have residually confounded the relationship between GGT and cancer incidence, including physical activity, diet, genetic and psychosocial variables, and, most notably, alcohol consumption. Chronic and excessive alcohol consumption was reported to increase the risk for cancer of the organs and tissues of the respiratory tract and the upper digestive tract, liver, colon, rectum, and breast (44). Because alcohol intake was only measured in a randomly selected subsample of 731 VHM&PP participants, we were unable to directly examine for confounding or effect modification by alcohol use. However, in this subsample, providing self-reported alcohol data, only a weak although statistically significant age-adjusted correlation of $r$ = 0.15 of GGT with the average number of alcoholic units per week was observed. This correlation was considerable weaker than the association of GGT with BMI, which we adjusted for in all analyses. Anyway, the extent of alcohol-induced elevation of cancer risk, coreflected by elevated GGT as a marker for excessive alcohol consumption, remains to be quantified in further investigations.

In summary, the present study investigated the association of GGT and cancer incidence in a population-based cohort of 79,279 apparently healthy men across a wide age range. Our results, for the first time, show that elevated GGT is significantly related to increased risk of overall cancer incidence and several site-specific cancers, particularly in men ages <65 years. Although our findings need to be confirmed in other populations, they emphatically suggest the clinical importance of monitoring and intervention based on the presence of elevated GGT. Because GGT is greatly influenced by dietary factors and can be decreased with higher consumption of fruit and various plant foods, its normalization could be one goal of future dietary recommendations for primary cancer prevention.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

Received 12/16/2007; revised 2/19/2008; accepted 3/18/2008.

**Grant support:** Austrian National Bank Grant OENB-12737 (H. Ulmer); National Institute on Aging intramural program (L.J. Brant); and Government of the State of Vorarlberg, Austria.

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We thank all the participants and physicians of the VHM&PP and Elmar Bechter, M.D., and Hans-Peter Bischof, M.D., at the Health Department of the Vorarlberg State Government.

Members of the VHM&PP study group are Guntram Hinteregger, M.D., Karin Parschalk, M.D., Wolfgang Metzler, M.D., Elmar Stimpfl (Agency for Preventive and Social Medicine, Bregenz, Austria), Jochen Klenk, M.Sc., and Stephan K. Weiland, M.D., M.Sc. (Department of Epidemiology, University of Ulm, Ulm, Germany).

DOI:10.1158/0008-5472.CAN-07-6686



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