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Gamma-Glutamyltransferase Is Associated With Incident Vascular Events Independently of Alcohol Intake
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Objective—To investigate the association of γ-glutamyltransferase (GGT) with incident CHD and stroke. GGT is a marker of alcohol intake but may also reflect oxidative stress and nonalcoholic fatty liver disease. Alanine aminotransferase (ALT) is the enzyme most closely associated with liver fat content.

Methods and Results—Associations of GGT and ALT with incident CHD, stroke, and a combined outcome of CHD or stroke were examined in the British Women's Heart and Health study (n=2961), and a meta-analysis of population based studies examining these associations was performed. In pooled analyses of fully adjusted results of 10 prospective studies, a change of 1 U/L of GGT was associated with a HR=1.20 (95% CI: 1.02, 1.40) for CHD; a HR=1.54 (95% CI: 1.20, 2.00) for stroke; and HR=1.34 (95% CI: 1.22, 1.48) for CHD or stroke. Heterogeneity was substantially decreased when 2 studies in Asian populations were excluded. In a subgroup of nondrinkers results were similar to the main analysis. Meta analyses of the only 2 studies that examined the association of ALT with incident cardiovascular events found a HR=1.18 (95% CI: 0.99, 1.41) for CHD and a HR=1.10 (95% CI: 0.89, 1.36) for CHD or stroke (combined).

Conclusion—GGT is associated with incident vascular events independently of alcohol intake. The mechanisms underlying this association remain unclear and require future study. (*Arterioscler Thromb Vasc Biol*. 2007;27:2729-2735.)

Key Words: nonalcoholic fatty liver disease ■ alanine-aminotransferase ■ γ-glutamyltransferase ■ cardiocascular diseases ■ meta-analysis

S everal population-based studies have found positive associations of GGT with incident cardiovascular events.[1,2] Yet the magnitude of associations differs between studies, and one study, which examined associations stratifying by age, found no strong evidence for an association of GGT with a combined outcome of nonfatal MI and fatal CHD in older men and women (≥60 years old).[3]

γ-glutamyltransferase (GGT) has long been used as a marker of excessive alcohol intake.[4] However additional, not necessarily mutually exclusive mechanisms have been proposed as explanations of observed associations between GGT and cardiovascular and diabetes risk: oxidative stress[5,6] and nonalcoholic fatty liver disease (NAFLD).[4,7]

We studied the associations of GGT with fatal and nonfatal coronary heart disease (CHD), stroke, and a combined outcome of CHD or stroke in a random sample of older British women (60 to 79 years old at baseline). We then performed a systematic review and meta-analysis of prospective population-based studies of GGT and incident CHD and stroke. Including both GGT and self-reported alcohol consumption in the same multivariable model may result in biased estimates of the association of GGT with outcomes attributable to measurement error in alcohol consumption levels by self-report,[8] as heavy drinkers may under-report their consumption.[9] Therefore, to determine whether GGT is related to cardiovascular events independently of alcohol intake we examined the associations of GGT with incident CHD and stroke in a subgroup of nondrinkers. We also compared results of the meta-analyses of GGT and incident cardiovascular events to pooled results of studies examining the association of alanine-aminotransferase (ALT) with incident CHD and stroke. ALT is the most commonly used marker of NAFLD and appears to be the liver enzyme most closely correlated to liver fat content.[10]

Materials and Methods

British Women's Heart and Health Study
Full details of the selection of participants and measurements have been previously reported.[11–13] Women aged 60 to 79 years were randomly selected from general practitioner lists in 23 British towns.

AA total of 4286 women participated and baseline data (self-completed questionnaire, research nurse interview, physical examination and medical record review) were collected between April 1999 and March 2001.

These women have been followed-up over a median of 4.6 years, to December 2004, by a detailed review of their medical records, conducted every 2 years, to establish nonfatal cardiovascular disease events and by flagging with the NHS central register (NHSCR) for mortality data. Informed consent was obtained from the women to examine their medical records and link them to the NHSCR, and both local and multicenter ethics committees' approvals were obtained for the study.

Levels of GGT and ALT in serum were determined using an automated analyzer (Technicon Sequential Multiple Analyzer; Technicon Instruments Corporation). Incident cases of CHD (in those without baseline CHD) were defined as any of CHD death (ICD10 codes I20-I25, I51.6) or a nonfatal myocardial infarction, diagnosis of angina, or coronary artery by-pass or angioplasty identified in the follow-up medical record reviews. Incident cases of stroke (in those without baseline stroke) were defined as either stroke death (ICD10 codes I60-I69, G45) or occurrence of a nonfatal stroke identified in the follow-up medical record reviews. Baseline CHD and stroke (for exclusion of these cases) were defined as having either a self-report of a doctor diagnosis of myocardial infarction or angina (CHD) or stroke, or having evidence of these in the baseline medical record review.

Statistical Analysis

Cox proportional hazard models were used to examine associations of exposures with incident CHD, stroke (fatal and nonfatal) and CHD, or stroke among those with no evidence of these outcomes at baseline. In the Cox proportional hazards models, the participant's age was the time axis and risk was assessed from the date of baseline examination for each woman. Contributions to risk were censored at the date of first outcome event of interest, death from any other cause or the end of the follow-up period (December 31, 2004) for those who remained alive and free of CHD or stroke.

Systematic Review and Meta-Analysis

We systematically searched Medline and EMBASE (March 2007) for prospective population-based studies evaluating the association between ALT, GGT, and CHD or stroke events. Studies conducted in populations restricted to patients with diagnosed CHD or previous stroke were excluded. We crossed the terms "alanine-aminotransferase" or "γ-glutamyltransferase" (and similar) with cardiovascular diseases, coronary disease, myocardial infarction, stroke (and similar terms). No language restrictions were applied. One reviewer scanned abstracts, obtained relevant full text publication, and applied inclusion criteria. Two independent reviewers extracted data into a standardized spreadsheet.

Because different studies presented results on different scales, eg, risk ratios for quantiles of GGT compared with the lowest quantile or per category, we used a standard statistical method to estimate the log hazard ratio (HR) per log unit increase in GGT, together with its standard error, for each outcome (incident CHD, stroke, and a combined end point).[14,15] Results are presented as the HR per 1 U/L change in GGT on a log scale. In other words, the reported HRs are per GGT×2.718 (the base of the natural logarithm).

When data were presented according to quantiles or categories of GGT, we used the median or mean in each group, when they were reported. When these were not reported, we estimated the mean in each group based on the distribution of subjects across groups, as outlined by Chêne and Thompson.[14] This method deals with the problem of unbounded upper or lower categories by assuming a normal distribution of the exposure in the population. All exposure (GGT, ALT) levels were converted to the lognormal scale if not already presented as such. We then estimated the log HR per 1 U/L increase in GGT using the method of Greenland and Longnecker.[15] This allows for the correlations between HRs that are related to the same reference group.

Table 1. Hazard Ratios (95% CI) of Incident CHD, Stroke, and Combined CHD or Stroke per 1 U/L Change in Naturally Logged ALT and GGT

	Incident CHD (n=151 Cases)	Incident Stroke (n=40 Cases)	Incident CHD or Stroke (n=186 Cases*)
GGT			
Model 1	1.28 (1.01, 1.62)	1.56 (1.02, 2.39)	1.31 (1.06, 1.62)
Model 2	1.22 (0.96, 1.55)	1.45 (0.93, 2.25)	1.24 (1.00, 1.54)
Model 3	1.15 (0.88, 1.48)	1.45 (0.90, 2.34)	1.17 (0.93, 1.48)
ALT			
Model 1	1.01 (0.68, 1.50)	0.88 (0.40, 1.97)	0.97 (0.67, 1.39)
Model 2	1.06 (0.72, 1.57)	0.92 (0.42, 2.02)	1.03 (0.72, 1.47)
Model 3	0.95 (0.63, 1.43)	0.88 (0.38, 2.02)	0.94 (0.65, 1.37)

*5 women had both an incident CHD and incident stroke event.

Model 1: Adjusted for age.

Model 2: Model 1 plus potential confounder (childhood and adult social class, physical activity, smoking, and alcohol consumption).

Model 3: Model 2 plus diabetes/insulin resistance, and other components of the metabolic syndrome: BMI, triglycerides, HDL-c, systolic blood pressure.

When possible, data were extracted separately for men and women. Studies were pooled using a random effect model and heterogeneity was assessed using the I^2 measure with a value of 50% used to denote moderate levels of heterogeneity.[16] We also conducted a subgroup analysis of the association between GGT and outcomes among nondrinkers. In addition, the effect of sex, duration of follow-up, the proportion of fatal events on study results were assessed by meta-regression. Publication bias was formally tested for using the both the Begg and Mazumdar, and Egger tests. All analyses were conducted in Stata version 9 (Stata Corporation, 2005).

Results

British Women's Heart and Health Study

Of 3511 women who did not have CHD or stroke at baseline, complete data on exposures (GGT and ALT), outcomes (CHD and stroke), and potential confounders (childhood and adult social class, physical activity, smoking, alcohol consumption, diabetes, fasting insulin, BMI, triglycerides, HDL-c, and systolic blood pressure) were available for 2961 women (84%). Of these women 151 experienced an incident case of CHD over 12 985 woman years of follow-up giving a rate of 11.6 per 1000 woman years (95% CI: 9.9, 13.6 per 1000 woman years) and 40 women had an incident case of stroke over 13 283 woman years of follow-up giving a rate of 3.0 per 1000 woman years (95% CI: 2.2, 4.1 per 1000 woman years).

Table 1 shows the age-adjusted and multivariable associations of (natural logged) ALT and GGT with CHD, stroke and a combined outcome of CHD, or stroke. The positive age-adjusted associations of GGT and outcomes (model 1) were attenuated when adjusting for childhood and adult social class social class, physical activity, smoking, and alcohol consumption (model 2). Associations were further attenuated when adjusting for diabetes, insulin resistance in nondiabetic women, BMI, triglycerides, HDL-c, and systolic blood pressure. No strong evidence of associations of ALT with CHD, stroke or a combined outcome was found.

We also examined the association of GGT (categorized into fourths of the distribution) with outcomes. Relative to the
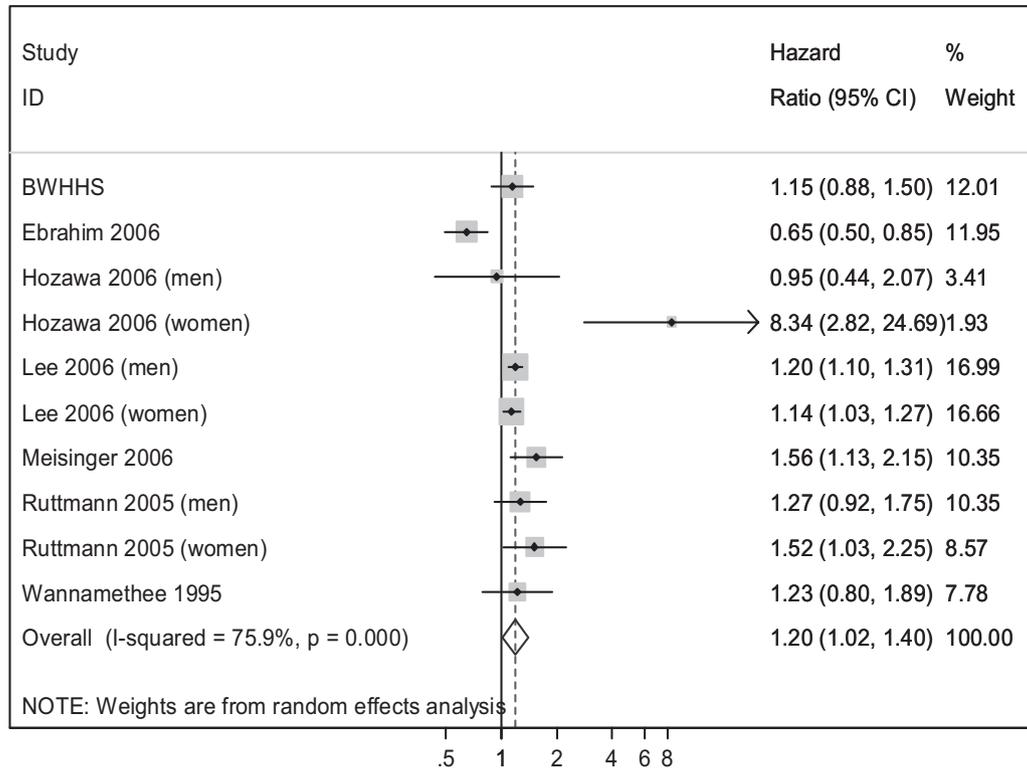
Figure 1. Forest plot of meta-analysis of fully adjusted study results of the association of GGT with incident CHD.

bottom fourth, the top fourth of the GGT distribution was associated with an age-adjusted increased risk of CHD (HR=1.47, 95% CI: 0.97, 2.34), stroke (HR=2.03, 95% CI: 0.80, 5.17) and with a combined end point of CHD or stroke (HR=1.49, 95% CI: 1.01, 2.19). However, these associations all attenuated to the null with control for established cardiovascular risk factors commonly used for risk stratifications in clinical settings: age, total cholesterol: HDL-c ratio, systolic blood pressure, smoking, and diabetes. Age-adjusted associations were also attenuated to the null when adjusting for covariables included in the main analysis (equivalent to model 3, Table 1).

Systematic Review and Meta-Analysis

Study characteristics of the 11 prospective studies[1–3,17–23] (including the present analysis of the British Women's Heart and Health Study) included in the systematic review are summarized in supplemental Table I (available online at http://atvb.ahajournals.org). Ten studies reported results for GGT and 2 for ALT. Results for stroke events were available from 6 studies, and for CHD from 7 studies. Of the 7 studies that reported on CHD, 1 study provided data separately for (1) acute and subacute forms of CHD, (2) chronic forms of congestive heart failure (CHD), and (3) other cardiovascular diseases.[2] The results for acute and subacute forms of CHD were included in the meta-analysis. Of the 6 studies in which stroke data were available, ischemic and hemorrhagic were analyzed separately in 4 studies.

CHD

Six publications (8 comparisons) provided age or age and sex (2 studies) adjusted HRs of the association between GGT and CHD. Pooled analysis of these studies resulted in a HR=1.53 (95% CI: 1.34, 1.76, I²=72%) per unit increase in (natural logged) GGT. Pooling fully adjusted results (see supplemental Table I for confounders included in the final model in each study) resulted in a HR=1.20 (95% CI: 1.02, 1.40, I²=76%, Figure 1).

Meta-analysis of the 2 studies that assessed the association of ALT with CHD resulted in a pooled age adjusted HR=1.25 (95% CI: 1.06, 1.48, I²=0%) and fully adjusted HR=1.18 (95% CI: 0.99, 1.41, I²=0%).
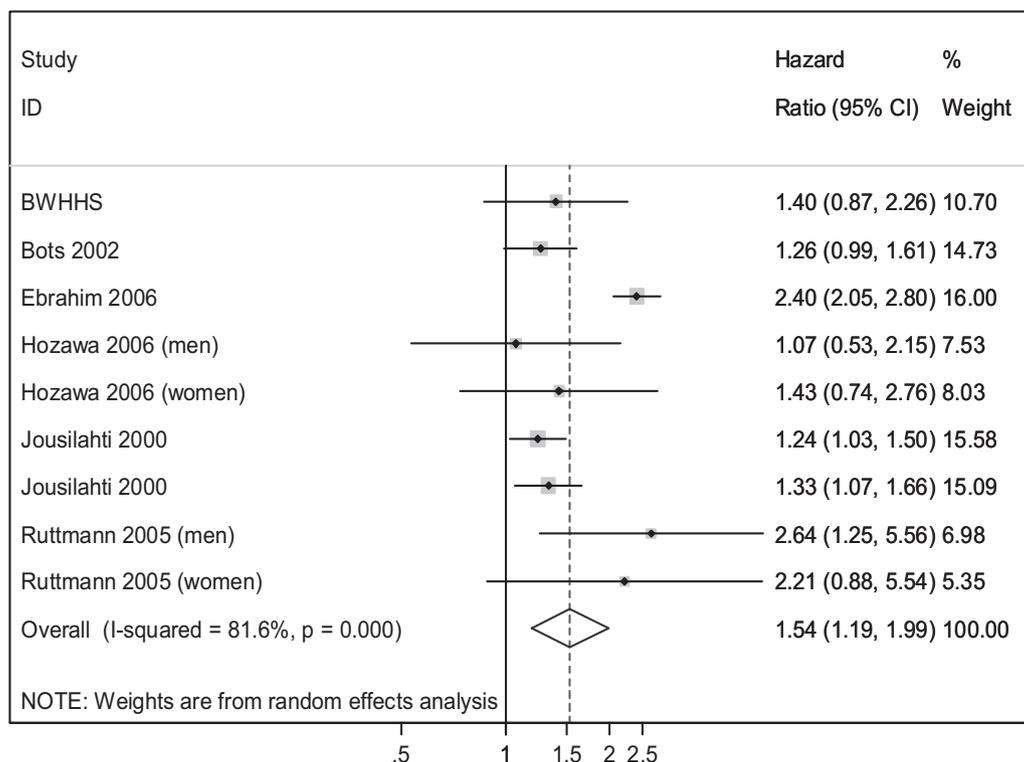
Stroke

Age adjusted risk estimates for GGT were available from 5 publications (7 comparisons). Pooling data from these studies resulted in a HR=1.62 (95% CI: 0.91, 2.87, I²=97%). The pooled analysis of fully adjusted estimates (HR=1.54, 95% CI: 1.20, 2.00; I²=82%, 6 publications, 9 comparisons) is presented in Figure 2. When fully adjusted results for hemorrhagic stroke and ischemic stroke were pooled separately, the HRs associated with a 1 U/L change in (natural logged) GGT were 2.11 (95% CI: 1.30, 3.43, I²=79%, 4 studies) and 1.45 (1.24, 1.69, I²=44%, 4 studies), respectively.

ALT as a predictor of stroke was assessed only in the BWHHS and no strong evidence of an association was found (see Table 1).

Combined Outcome: CHD or Stroke

The pooled overall age-adjusted HR=1.53 (95% CI: 1.34, 1.76, I²=72%, 9 publications, 12 comparisons) and fully adjusted HR=1.34 (1.22, 1.48, I²=73%, 10 publications, 14 comparisons, Figure 3).

Figure 2. Forest plot of meta-analysis of fully adjusted study results of the association of GGT with incident stroke. Results for all stroke types (combined) were unavailable for Ruttman 2005. Therefore, results for hemorrhagic stroke are included and presented; however, results were virtually unaltered when data on ischemic stroke were included.

Meta-analysis of the 2 studies that assessed the association of ALT with CHD or stroke resulted in a pooled age-adjusted HR=1.21 (95% CI: 0.90, 1.62, I^2=45%) and fully adjusted HR=1.10 (95% CI: 0.89, 1.36, I^2=0%).

Subgroup Analyses and Heterogeneity

There was marked heterogeneity in all of the main meta-analyses of GGT presented above (I^2>70%), except for the association with ischemic stroke (I^2=44%). We examined the association between GGT and outcomes, restricting the analyses to nondrinkers. Results for this subgroup were available from 5 studies. For the British Women's Heart and Health study we restricted the analysis to life-long abstainers. In other studies[3,18,19,22] it was only possible to restrict the analyses to current nondrinkers. As can be seen from results (Table 2), the degree of heterogeneity among studies that examined GGT remained high and the point estimates for nondrinkers were higher than those obtained in the main analyses, although confidence intervals were wider. We also repeated the main analysis excluding the 2 studies (3 comparisons) conducted in Asian (rather than European) populations.[18,19] The degree of heterogeneity was reduced in all outcomes and in most analyses heterogeneity was nearly eliminated (Table 2). Among European populations there were positive associations of GGT with all outcomes.

We found no strong evidence of associations between the following study characteristics and study results: percent of fatal events, percent of women, and duration of follow-up by metaregression in studies assessing GGT. Furthermore, no evidence of an association of study outcome with study

power was found according to both the Egger test (P=0.41) and the Begg and Mazumdar test (P=0.70).

Discussion

An increase of 1 U/L of natural logged GGT was associated with a 20% increase in the risk of CHD, a 54% increase in the risk of stroke, and a 34% increase in the risk of CHD or stroke (combined), when pooling fully adjusted results (albeit variably adjusted) of 10 prospective studies. Increasing levels of GGT within the normal range were consistently associated with an increasing risk of both incident CHD and stroke in a majority of published studies. In one study,[18] increasing GGT levels were associated with an increase in the risk of MI in the age-adjusted model, but inversely associated with MI in the fully-adjusted model which controlled for a number of cardiovascular risk factors including alcohol consumption. In this study cholesterol levels and hypertension accounted for the greatest reductions in the hazard of MI associated with increasing GGT levels, with alcohol consumption accounting for relatively little of the attenuation. In another study no evidence was found for an association of increasing GGT levels with mortality from CHD and stroke in men, although a strong association was found among women.[19] These were the only studies (of 10 prospective studies) conducted in Asian populations. Excluding these studies from the meta-analysis of fully adjusted results did not substantially change the overall estimate but did substantially reduce the degree of heterogeneity between study results.

Several related reasons might explain the decrease in heterogeneity when excluding the 2 studies conducted in
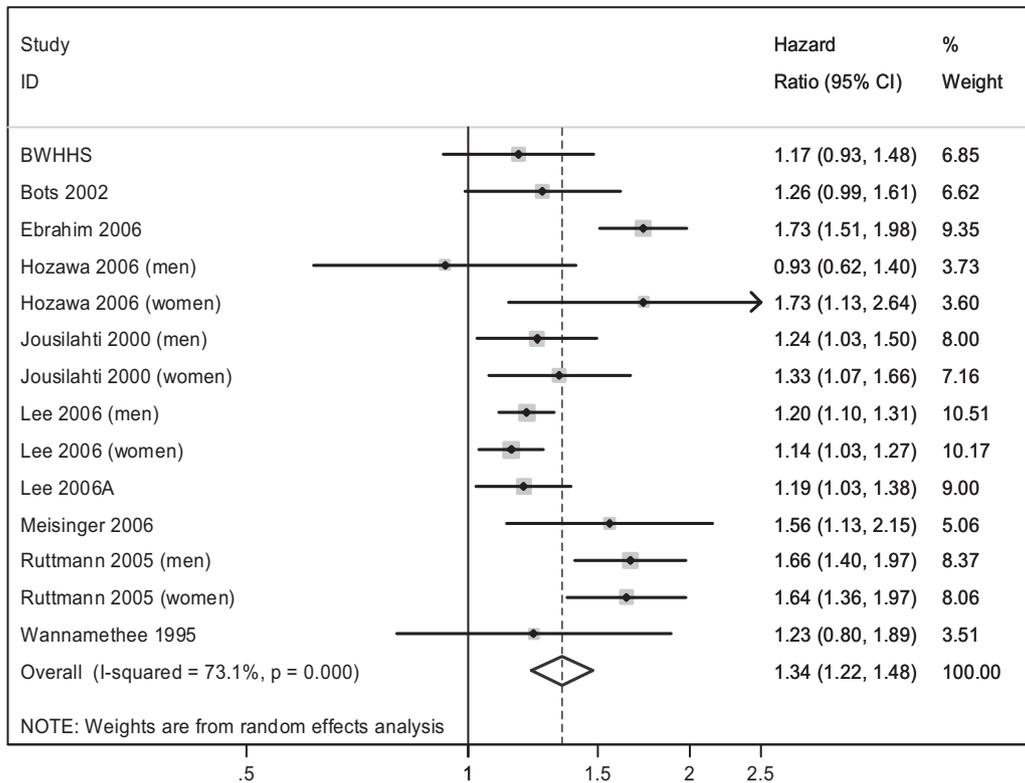
Study ID		Hazard Ratio (95% CI)	% Weight
BWHHS		1.17 (0.93, 1.48)	6.85
Bots 2002		1.26 (0.99, 1.61)	6.62
Ebrahim 2006		1.73 (1.51, 1.98)	9.35
Hozawa 2006 (men)		0.93 (0.62, 1.40)	3.73
Hozawa 2006 (women)		1.73 (1.13, 2.64)	3.60
Jousilahti 2000 (men)		1.24 (1.03, 1.50)	8.00
Jousilahti 2000 (women)		1.33 (1.07, 1.66)	7.16
Lee 2006 (men)		1.20 (1.10, 1.31)	10.51
Lee 2006 (women)		1.14 (1.03, 1.27)	10.17
Lee 2006A		1.19 (1.03, 1.38)	9.00
Meisinger 2006		1.56 (1.13, 2.15)	5.06
Ruttmann 2005 (men)		1.66 (1.40, 1.97)	8.37
Ruttmann 2005 (women)		1.64 (1.36, 1.97)	8.06
Wannamethee 1995		1.23 (0.80, 1.89)	3.51
Overall (I-squared = 73.1%, p = 0.000)		1.34 (1.22, 1.48)	100.00
NOTE: Weights are from random effects analysis			

Figure 3. Forest plot of meta-analysis of fully adjusted study results of the association of GGT with incident CHD or stroke.

Asian populations from the analyses. Chronic hepatitis is more prevalent in Asian populations compared with European population. However, Hozawa et al excluded participants with possible liver dysfunction defined as aspartate-aminotransferase (AST) or ALT levels ≥50 U/L, and Ebrahim et al excluded participants based on Hepatitis B virus antigen seropositivity.[18,19] Another possible explanation is that alcohol consumption may be lower in these populations because of a greater prevalence of ALDH2 genetic variants that are known to decrease alcohol consumption.[24,25] However, in the study by Hozawa et al,[19] an association between GGT and incident cardiovascular events was found in women of whom 93% were lifelong abstainers from alcohol. In contrast, no evidence of an association between GGT and cardiovascular disease was found in men in whom the prevalence of drinking was high. In light of these somewhat conflicting results, further studies are required to determine the magnitude and even direction of

Table 2.   Pooled HR (95% CI) per 1 U/L Change in Naturally Logged GGT From Subgroup Analyses of Studies Restricted to (1) Nondrinkers and (2) Restricted to European Population

	Incident CHD	Incident Stroke	Incident CHD or Stroke
Nondrinkers			
Age-adjusted	1.61 (1.36, 1.91)	1.75 (0.53, 5.82)	1.60 (0.62, 4.08)
	$I^2=40\%$	$I^2=93\%$	$I^2=96\%$
	6 publications[a], 8 comparisons	3 publications[b], 4 comparisons	3 publications[b], 4 comparisons
Fully adjusted	1.33 (1.00, 1.77)	1.76 (0.98, 3.16)	1.45 (0.97, 2.17)
	$I^2=72\%$	$I^2=58\%$	$I^2=67\%$
	6 publications[a], 8 comparisons	3 publications[b], 4 comparisons	3 publications[b], 4 comparisons
European population			
Age-adjusted	1.48 (1.31, 1.67)	1.44 (1.29, 1.61)	1.45 (1.36, 1.56)
	$I^2=66\%$	$I^2=0\%$	$I^2=38\%$
	4 publications[c], 5 comparisons	3 publications[e], 4 comparisons	7 publications[g], 9 comparisons
Fully adjusted	1.20 (1.13, 1.27)	1.32 (1.17, 1.49)	1.30 (1.20, 1.42)
	$I^2=0\%$	$I^2=3\%$	$I^2=60\%$
	5 publications[d], 7 comparisons	4 publications[f], 6 comparisons	8 publications[h], 11 comparisons

a, BWHHS[1,3,18,19,22]; b, BWHHS[18,19]; c, BWHHS[1,3,22]; d, BWHHS[1–3,22]; e, BWHHS[17,20]; f, BWHHS[2,17,20]; g, BWHHS[1,3,17,20–22]; h, BWHHS.[1–3,17,20–22]

the association of GGT with incident CHD and stroke in Asian populations.

In a subgroup analysis of the associations of GGT and cardiovascular outcomes in nondrinkers, HRs were similar to those obtained in the main analysis. Thus alcohol is unlikely to fully explain the association of GGT with cardiovascular outcomes. Indeed, GGT varies considerably even among people reporting little and no alcohol consumption.[26,27] Furthermore, the documented inverse association between moderate alcohol consumption and CHD risk also contrasts with the positive association of GGT with incident CHD and stroke events.[28]

Several mechanisms have been implicated as underlying the association of GGT and cardiovascular disease. Both GGT and ALT are considered markers of NAFLD,[29] with ALT being more closely related to liver fat content,[10] in a limited number of studies thus far conducted on this topic. Indeed, GGT is potentially more strongly associated with incident diabetes than ALT,[30] so further studies are needed to compare the 2 parameters in their association with liver fat. We found only 1 published study of the association of ALT and incident cardiovascular events.[23] Pooled analysis of the results of this study and results from the British Women's Heart and Health Study suggest that ALT may also be associated with cardiovascular risk. Therefore it is possible that the association between GGT and outcomes reflects the common association of both ALT and GGT with NAFLD. Of note, in the British Women's Heart and Health Study there was stronger evidence of an association of GGT with outcomes than of ALT with outcomes. Additional studies with measures of both ALT and GGT, and ultrasound diagnosed NAFLD would provide more definite evidence as to whether the GGT association with cardiovascular outcomes reflects the association of NAFLD with cardiovascular risk. Mendelian randomization studies, combining genetic and prospective datasets,[31,32] could help unpick causality of relationships between liver enzymes and vascular events.

Oxidative stress, both localized within plaques and systemic, may also link GGT with cardiovascular disease. GGT is present in atherosclerotic plaques and may catalyze oxidation of LDL lipoproteins and thereby contribute to plaque evolution and rupture.[5] GGT is also present on the surface of most cell types and is the enzyme responsible for the extracellular catabolism of antioxidant glutathione.[33] Ectoplasmic GGT has been implicated in the generation of reactive oxygen species, and consistent evidence supports its role as a marker of systemic oxidative stress, as recently reviewed.[6]

In light of this evidence it has been suggested that GGT may be useful for risk stratification (ie, as a predictor of those at high risk of future cardiovascular disease).[5] However, given the arguably modest magnitude of associations (eg, a 34% increase in the risk of CHD or stroke per more than doubling of GGT), its value in cardiovascular disease risk stratification remains debatable and will require further studies and formal prediction analyses.

Several important caveats deserve attention. In the British Women's Heart and Health Study outcomes were determined after a relatively short follow-up period (median 4.6 years) compared with other studies included in the meta-analysis

and participants were older than participants in other studies. These differences may explain the null results found in the British Women's Heart and Health Study, compared with the overall associations found in the meta-analyses. In the meta-analyses we combined results from models that adjusted for different sets of covariables. This in itself may explain some of the heterogeneity between studies. With regard to the subgroup of nondrinkers, it should be noted that in 4 of the 6 studies included in this analysis, nondrinkers were not necessary lifelong abstainers and therefore reverse causality cannot be ruled out. Finally, it is not clear why results of the 2 studies conducted in Asian populations account for much of the heterogeneity observed between studies or why their results are inconsistent with each other.

In conclusion, GGT is positively associated with incident CHD and stroke in both women and men in European populations and among self-reported nondrinkers. Therefore, GGT levels probably also reflect other biological processes or indeed lifestyle or dietary behaviors that are linked to cardiovascular disease. Future studies, including more in Asians populations, will help provide insight into the nature of these processes.

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Table I: Characteristics of prospective population based studies of ALT, GGT and incident CHD and stroke

Study ID, design	Study design	Country	Ages	Total N	% women (N)	Length of follow up (years)	Relevant outcomes	Covariables in fully-adjusted model
GGT Bots 2002 [17]	Case control	The Netherlands and United Kingdom	42 and above	231 cases, 570 controls	34 (276)	Not applicable	• Fatal & non-fatal stroke (all) • Fatal & non-fatal hemorrhagic stroke • Fatal & non-fatal ischemic stroke	Age, sex, any medication, MI, TC, hypertension
BWHHS	Cohort	UK	60-79	2,961	100 (2,961)	Median: 4.6	• Fatal & non-fatal CHD or stroke • Fatal & non-fatal CHD • Fatal & non-fatal stroke	Age, childhood and adult social class, physical activity, smoking diabetes/insulin resistance, BMI, triglycerides, HDL-C, SBP, alcohol consumption
Ebrahim 2006 [18]	Cohort	Korea	30-64	787,442	16 (125,742)	11	• Fatal and non-fatal stroke or MI • Fatal & non-fatal ischemic stroke • Fatal & non-fatal hemorragic stroke • Fatal & non-fatal MI	Age, sex, BMI, height, fasting glucose, hypertension, smoking physical activity, monthly pay, area of residence, alcohol consumption
Hozawa	Cohort	Japan	>=30	6,846	60 (4,122)	9.6	• Fatal CHD or stroke	Age, alcohol

2006[19]							• Fatal CHD • Fatal stroke	consumption, smoking, HDL-c, total cholesterol, triglycerides, GOT, GPT, BMI, physical activity, SBP, antihypertensive medication, diabetes
Jousilahti 2000 [20]	Cohort	Finland	25-64	14,874	52 (7,698)	Mean: 10.4	• Fatal & non-fatal stroke (all) • Fatal & non-fatal ischemic stroke • Fatal and non-fatal intracerebral hemorrhage • Fatal and non-fatal subarachnoid hemorrhage	Age study year, smoking, cholesterol, SBP, DBP, BMI
Lee 2006 [3]	Cohort	Finland	25-74	28,838	52 (15,027)	Median: 11.9	• Fatal CHD or non-fatal MI • Fatal CHD • Non-fatal MI	Age, study year, study area, BMI, smoking, physical activity, SBP, TC, HDL-C, diabetes, alcohol consumption
Lee 2006 A[21]	Cohort	USA	Mean: 44 (SD=10)	3,451	52 (1790)	Mean: 19.1	Fatal and non fatal cardiovascular disease (CHD, peripheral vascular disease, cerebrovascular disease , heart failure)	Age, sex, BMI, disbetes, SBP, antihypertensive treatment, total/HDL-c ratio, current smoking, alcohol consumption
Meisinger	Cohort	Germany	25-64	1,878	0	Mean:	Fatal & non-fatal MI or sudden cardiac	Age, education,

Study	Design	Country	Age	N	Cases	Follow-up	Outcomes	Adjustments
2006[22]						15.7	death	diabetes, hypertension, TC/HDL-C ratio, regular smoking, physical activity, BMI, alcohol consumption
Ruttmann 2005 [2]	Cohort	Austria	19-95	163,944	54 (89,114)	Median: men 11.1 Women 12	• Fatal cardiovascular or cerebrovascular events • Fatal acute and subacute forms of CHD • Fatal ischemic stroke • Fatal hemorrhagic stroke	Age, BMI, SBP, cholesterol, triglycerides, glucose, smoking, work status, year of examination
Wannamethee 1995 [1]	Cohort	UK	40-59	7,080	0	Median: 11.5	Fatal CHD	Age, social class, physical activity, BMI, diabetes, any medication, antihypertensive treatment, SBP, TC, HDL-c, glucose, FEV1, heart rate, alcohol consumption
ALT BWHHS	Cohort	UK	60-79	2,944	100 (2,944)	Median: 4.6	• Fatal & non-fatal CHD or stroke • Fatal & non-fatal CHD • Fatal & non-fatal stroke	Age, childhood and adult social class, physical activity, smoking diabetes/insulin resistance, BMI, triglycerides, HDL-c, SBP, alcohol consumption

Schindhelm 2006 [23]	Cohort	The Netherlands	50-75	1439	55 (788)	10	• Fatal & non-fatal CHD • Fatal and non fatal cardiovascular disease (CHD, congestive heart failure, stroke, transient ischemic attack, peripheral disease, sudden death of unknown cause)	Age, sex, smoking, physical activity, waist, triglycerides, SBP, fasting glucose, HDL-c, alcohol consumption

TC – total cholesterol; HDL-c – high density lipoprotein cholesterol; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure