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Liver enzymes and risk of cardiovascular disease in the general population: A meta-analysis of prospective cohort studies

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ABSTRACT

Background: Gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), commonly used markers of liver dysfunction, have been implicated with risk of cardiovascular disease (CVD). However, the strength and consistency of their associations in the general population have not been reliably quantified.
Methods: We synthesized available prospective epidemiological data on the associations of baseline levels of GGT, ALT, AST, and ALP with CVD [composite CVD, coronary heart disease (CHD), or stroke outcomes]. Relevant studies were identified in a literature search of MEDLINE, EMBASE, and Web of Science up to December 2013. Pooled relative risks (RRs) with 95% confidence intervals (CIs) were calculated using random effects models.
Results: Twenty-nine unique cohort studies with aggregate data on over 1.23 million participants and 20,406 cardiovascular outcomes were included. The pooled fully adjusted RRs (95% CIs) for CVD were 1.23 (1.16–1.29) and 1.08 (1.03–1.14) per 1-standard deviation change in log baseline levels of GGT and ALP levels respectively. There was no evidence of an association of ALT or AST with CVD, however, ALT was somewhat inversely associated with CHD 0.95 (0.90–1.00) and positively associated with stroke 1.01 (1.00–1.02) in stratified analysis. Tests for nonlinearity were suggestive of linear relationships of GGT and ALP levels with CVD risk.
Conclusions: Baseline levels of GGT and ALP are each positively associated with CVD risk and in a log-linear fashion. There may be variations in the associations of ALT with cause-specific cardiovascular endpoints, findings which require further investigation.

1. Introduction

Liver enzymes -gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) – are commonly used as markers of liver dysfunction. Over the past decade, these enzymes have sparked great interest as emerging markers for cardiovascular risk, but uncertainty exists because important questions pertaining to their aetiological relationships with cardiovascular disease (CVD) remain unresolved. Whereas several studies have observed associations of these markers of liver dysfunction with risk of CVD [1–5], others have shown threshold effects or even no association at all [1,6–13]. While some of these studies have reported log-linear associations, others have reported nonlinear relationships or have failed to evaluate nonlinearity, leaving great uncertainty regarding the aetiological nature of these associations. Although GGT is a less specific marker of liver dysfunction, several reports suggest that among the liver enzymes, it is the strongest risk indicator for CVD. Fraser and colleagues have previously reported positive independent associations between GGT levels and subsequent risk of CVD outcomes [coronary heart disease (CHD), stroke, and a combined outcome of CHD or stroke] by synthesizing data from available prospective studies [6]. In the same review, they also pooled the results of the only two studies that evaluated the association of ALT with incident vascular outcomes and reported no significant associations. Since this review, several large prospective studies evaluating the associations of GGT and ALT levels with risk of cardiovascular outcomes have been published and their results have been inconsistent [7,10,14,15]. Data on the association of AST and ALP levels with risk of CVD are comparatively limited and also

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inconsistent, and no reviews quantifying their aetiological associations have been performed to date.

Evaluation of all four common liver enzymes is important, because their assays are sensitive, well standardised, simple, inexpensive, do not require a fasting state prior to venepuncture, are commonly measured together, and are emerging risk markers for CVD. Furthermore, they may hold potential for CVD risk prevention, either as validated causal therapeutic targets or as markers of risk prediction. In this context, we have carried out a comprehensive systematic literature review and study-level meta-analysis of available prospective epidemiological data to quantify the aetiological associations of baseline circulating levels of GGT, ALT, AST and ALP with risk of CVD in the general population.

2. Methods

2.1. Data sources and searches

This systematic review and meta-analysis of studies was conducted using a predefined protocol and in accordance with PRISMA and MOOSE guidelines [16,17](Appendix Supplements 1,2). We searched MEDLINE, EMBASE, and Web of Science for prospective (cohort or "nested case control") population-based studies that evaluated associations of baseline circulating levels of GGT, ALT, AST, or ALP with risk of composite CVD, CHD or stroke outcomes among adults up to December 2013. The computer-based searches combined free and MeSH search terms and combination of key words related to the exposures (e.g., "gamma glutamyltransferase", "alanine aminotransferase", "aspartate aminotransferase", "alkaline phosphatase", etc) and outcomes (e.g., "cardiovascular disease", "coronary heart disease", "stroke", etc). There were no restrictions on language or the publication date. Reference lists of retrieved articles were manually scanned for all relevant additional studies and review articles. We searched and contacted several investigators for unpublished studies on the associations. We restricted the search to studies of humans. Further details on the search strategy are presented in Appendix Supplement 3.

2.2. Study selection

Observational cohort studies were included if they had at least 1 year of follow-up, assessed associations of GGT, ALT, AST, or ALP with risk of composite CVD, CHD, or stroke in adults, with samples measured at baseline, and recruited participants from approximately general populations (i.e., they did not select participants on the basis of confirmed pre-existing medical conditions such as CVD, diabetes mellitus, liver disease, or chronic kidney disease at baseline). Retrospective cohort studies were not included. For findings published only in abstract form, we contacted the investigators to determine if the results were still considered to be valid.

2.3. Data extraction, endpoints, and quality assessment

Data were abstracted, where available, on study, publication date, geographical location, population source, time of baseline survey, sample population, study design, sample source (plasma/serum), nature of sample (fresh or frozen and storage temperature), assay type and source, case definition, sample size, number of cases, number of participants, mean age, duration of follow-up, degree of adjustment for potential confounders (defined as '+' when RRs were adjusted for age and/or sex; '++' further adjustment for established risk factors such as smoking status, body mass index, blood pressure, lipids; and '+++' additional adjustment for alcohol consumption, other liver markers, or inflammatory markers) and risk estimates reported for greatest adjustment for potential confounders. Two authors (H.K. and T.A.A.) independently abstracted data and performed quality assessments. A standardized predesigned data collection form was used for data extraction. Each article was assessed using the inclusion criteria above and any disagreement regarding eligibility of an article was discussed, and agreement reached by consensus with a third reviewer (S.K.K.). In the case of multiple publications involving the same cohort, the most up-to-date study or study with the most comprehensive information was abstracted. We contacted authors of eligible studies where the published data were insufficient, to provide relevant missing information. The primary outcome of this analysis was a composite endpoint of CVD (i.e., a combined outcome of CHD, stroke, cardiovascular death, angina, heart failure, and other CVDs). If a composite endpoint of CVD was not reported or indeterminable, CHD or stroke outcomes were used as reported. Studies that reported on only heart failure as a distinct primary outcome were not included. Appendix Supplement 4 provides details of study-specific outcome definitions. Study quality was assessed based on the nine-star Newcastle–Ottawa Scale (NOS) [18] using pre-defined criteria namely: selection (population representativeness), comparability (adjustment of confounders), and ascertainment of outcome. The NOS assigns a maximum of four points for selection, two points for comparability, and three points for outcome. Nine points on the NOS reflects the highest study quality.

2.4. Data synthesis and analysis

Analyses involved only within-study comparisons (i.e., cases and controls were only directly compared within each study) to limit potential biases. The relative risk (RR) with 95% confidence intervals (CIs) was used as the common measure of association across studies. To enable a consistent approach to the meta-analysis and enhance interpretation of the findings, reported study-specific risk estimates (per-unit change, quintiles, quartiles, thirds, or other groupings) were transformed using standard statistical methods [19,20]. As there is evidence of linear associations of some of these markers with cardiovascular risk and to ensure consistency, we pooled estimates per 1-standard deviation (SD) change in logarithmically transformed baseline levels of these enzymes. Briefly, the log risk ratio for a 1-SD change being equivalent to the log risk ratio for a comparison of extreme thirds divided by 2.18 (equivalently, as the log risk ratio for a comparison of extreme quarters divided by 2.54 or as the log risk ratio for a comparison of extreme quintiles divided by 2.80). Log risk estimates were transformed assuming a normal distribution (or that a transformation of the explanatory variable for which the risk ratio is based was normally distributed). In parallel analyses, risk estimates where appropriate, were also transformed and pooled to involve comparisons between the top third and bottom third of the baseline levels of GGT, ALT, AST and ALP. Standard errors of the log risk estimates were calculated using published confidence limits and were standardised in the same way (Appendix Supplement 5 provides details of the statistical methods used and Stata command used). Authors of studies that reported risk estimates that could not be transformed were contacted to provide standardized estimates. We calculated summary RRs by pooling study-specific estimates (Stata command –metan-) using random effects models that accounted for between-study heterogeneity. When studies published more than one estimate of the association according to subgroups (e.g., by sex), a within-study summary estimate was obtained using a fixed effect analysis. Where appropriate and possible, we estimated dose–response associations of these liver enzyme levels with risk of CVD. A 2-step generalized least-squares trend estimation (GLST) analysis (Stata command –glst-) as described by Greenland and Orsini [20,21] was used to compute study-specific slopes (linear trends) from the correlated natural logs

of the RRs across categories of exposures. Only studies that reported the number of cases, person-years of follow-up or non-cases, and the RRs with the variance estimates for at least three quantitative exposure categories were included. To examine potential nonlinear dose–response relationships between liver enzymes levels and CVD risk, we modeled levels of liver enzymes using restricted cubic splines. A *P*-value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero.

Statistical heterogeneity across studies was quantified using standard chi-square tests and the I^2 statistic [22,23]. Several study-level characteristics were pre-specified as characteristics for assessment of heterogeneity, which was conducted using stratified analyses and random effects meta-regression (Stata command –metareg-) [24]. In analysis specified post-hoc, there was further stratified analysis to examine the difference in pooled RRs by baseline average age of participants. We performed sensitivity analyses to assess the influence of each individual study by omitting one study at a time and calculating a pooled estimate for the remainder of the studies (Stata command –metaninf-). We assessed the potential for publication bias through formal tests, namely Begg's funnel plots [25] (Stata command –metafunnel-) and Egger's regression symmetry test (Stata command –metabias-) [26]. Publication bias was not evaluated for studies of any liver enzyme with less than 10 studies, as funnel plots are unlikely to be useful in such instances [27]. All analyses were conducted using Stata version 12 (Stata Corp, College Station, Texas). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Literature search

Our initial search identified 5671 potentially relevant citations. After screening of titles and abstracts, 79 articles remained for further evaluation. Following detailed assessments, 50 articles were excluded. Overall, 31 articles based on 29 unique prospective cohort studies were included in the meta-analysis (Fig. 1 and Appendix Supplement 11). In aggregate, the included studies comprised 1,230,779 non-overlapping participants and 20,406 cardiovascular events.

3.2. Study characteristics and quality

Table 1 provides details of the eligible studies that evaluated baseline circulating GGT, ALT, AST, or ALP levels with risk of cardiovascular outcomes. All included studies were prospective cohort studies carried out in United States, Europe (United Kingdom, Netherlands, Germany, Italy, and Austria), and Asia (Korea and Japan) with average baseline ages ranging from 19 to 79 years. Average duration of follow-up for cardiovascular outcomes ranged from 3 to 24 years. Cardiovascular endpoints were validated by

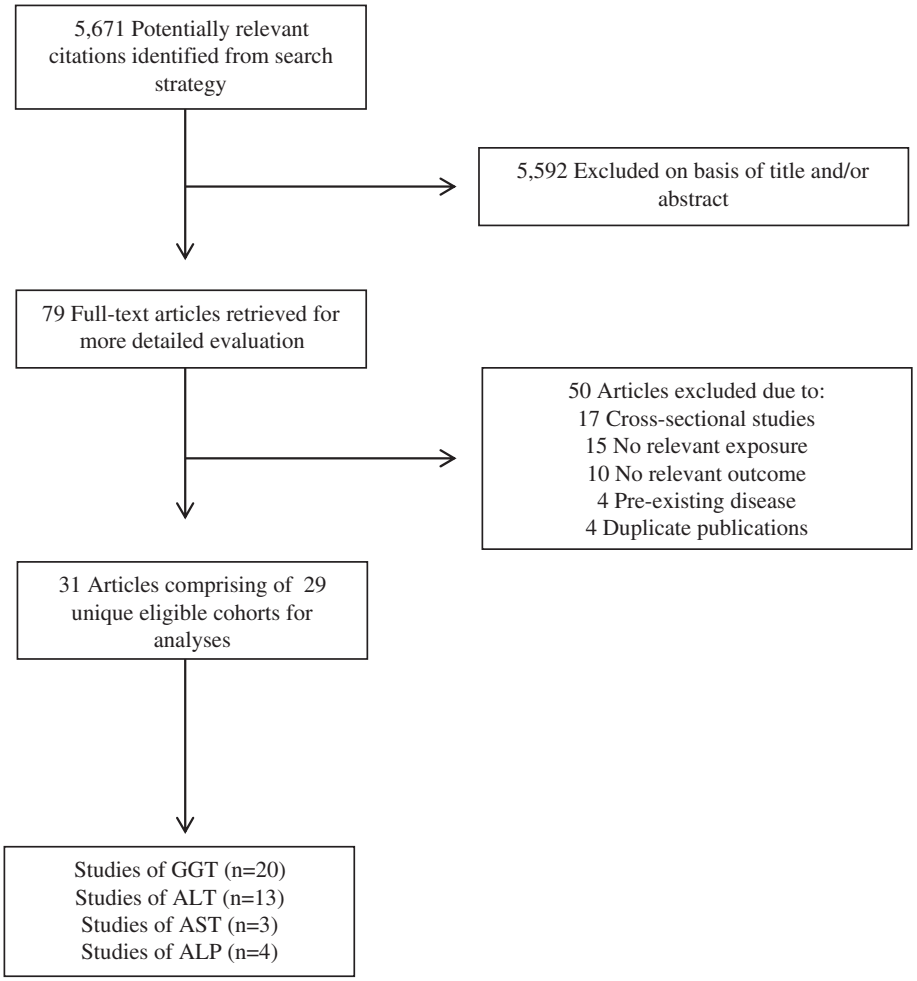


Fig. 1. Selection of studies included in the meta-analysis. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase.

Table 1
Prospective studies based in general populations of liver enzymes and cardiovascular disease risk contributing to pooled analyses.

Lead Author, year	Study	Location	Year of baseline survey	Age range at baseline (years)	% Male	Mean follow-up (years)	Outcome	No. of cases	No. of participants	Covariates adjusted for	Study quality
Gamma glutamyltransferase											
Bots, 2002	Caerphilly	UK	1979–1983	45–59	100	≤12[a]	Stroke	57	181	Age and sex	6
Bots, 2002	KIHD	Finland	1984–1989	42–60	100	3–9[a]	Stroke	66	200	Age and sex	7
Bots, 2002	Rotterdam	The Netherlands	1990–1993	≥55	36	1–4[a]	Stroke	108	429	Age and sex	8
Ruttmann, 2005	VHM&PP	Austria	1985–2001	≥19	46	12	CVD death	3026	163,944	Age, BMI, systolic blood pressure, cholesterol, triglycerides, glucose, smoking, work status and year of examination	8
Ebrahim, 2006	KMIC	Korea	1986–1990	30–64	84	10	MI plus stroke	4563	787,442	Age, sex, BMI, height, glucose, hypertension, ethanol consumption, smoking, physical activity, monthly pay and area of residence	8
Meisinger, 2006	MONICA-KORA	Germany	1984–1985	25–64	100	16	CHD	150	1878	Age, education, history of diabetes, hypertension, total cholesterol/HDL ratio, regular smoking, physical activity, alcohol intake and BMI	9
Lee, 2006	North Karelia/ Kuopio	Finland	1982/1987/1992/ 1997	25–64	48	12	CHD	1467	28,838	Age, study year, study area, BMI, smoking, alcohol consumption, physical activity, SBP, total cholesterol, HDL-cholesterol and diabetes	9
Fraser, 2007	BWHHS	UK	1999–2001	60–79	0	5	CHD or Stroke	186	2961	Age, social class, physical activity, smoking, alcohol consumption, diabetes/insulin resistance, BMI, triglycerides, HDL-cholesterol and SBP	8
Lee, 2007	FHS Offspring	USA	1978–1982	44[b]	48	19	CVD	535	3451	Age, sex, diabetes, SBP, total cholesterol, HDL-cholesterol, current smoking, alcohol consumption	9
Hozawa, 2007	NIPPON DATA90	Japan	1990	≥30	40	9.6	CVD death	165	6846	Age, alcohol consumption, smoking, HDL-cholesterol, total cholesterol, triglyceride, ALT, AST, BMI, habitual exercise, SBP, use of antihypertensives and diabetes	9
Wannamethee, 2008	BRHS	UK	1978–1980	40–59	100	24	CHD plus stroke	1803	6997	Age, social class, smoking, alcohol intake, physical activity, pre-existing evidence of undiagnosed CHD, BMI, SBP, cholesterol, blood glucose and HDL-cholesterol	8
Monami, 2008	FIBAR	Italy	2001–2003	40–75	42	3	CVD	20	2617	Age, sex, alcohol, smoking status and fasting glucose	9
Lee, 2009	MHS	USA	1990–1992 & 1995 –1997	28–65	56	5–12[a]	CVD death	137	386	Age, sex, smoking, alcohol use, physical activity, total cholesterol, HDL-cholesterol, SBP and nonfasting glucose	9
Ruhl, 2009	NHANES III	USA	1988–1994	≥20	47	9	CVD death	479	10,514	Age, sex, race-ethnicity, BMI, WHR, glucose status, total cholesterol, HDL cholesterol, SBP, DBP, smoking, alcohol, caffeine, physical activity, C-RP, transferrin saturation, education and time of blood draw	9
Haring, 2009	SHIP	Germany	1997–2001	20–79	49	7	CVD death	153[c]	4160	Age, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalized income and Functional Comorbidity Index	9
Shimizu, 2010	CIRCS	Japan	1986–1993	40–69	36	18	Stroke	432	9752	Age, community, BMI, smoking, alcohol intake, total cholesterol, triglycerides, albumin, AST, ALT, SBP, antihypertensive medication use, DM	9
Onat, 2011	TARFS	Turkey	2003–2004	33–84	49	4	CHD	118	1533	Age, sex, BMI and menopause	8
Kengne, 2012	HSE/SHeS	UK	1994/1995/1998	53.6[b]	45	12.7	CVD death	719	17,269	Age, sex, diabetes status, SBP, hypertension, history of CVD, physical activity, smoking status, alcohol consumption, total	9

									cholesterol and lipid lowering medication	
Loomba, 2012	RBS	USA	1984–1987	≥30	46	13.7	CVD death	657	2364	Age, sex, alcohol intake, BMI, total cholesterol, HDL-cholesterol, triglycerides, smoking, SBP, DM, IL-6, and CRP	9
Weikert, 2013	EPIC-Potsdam	Germany	1994–1998	35–65	38.9	8.2	Stroke	353	2066	Age, sex, BMI, waist circumference, education, sports activity, smoking, alcohol intake, prevalent hypertension, prevalent diabetes, total cholesterol, HDL-cholesterol, hsCRP	9
Sub-total								**15,194**	**1,054,181**		
Alanine aminotransferase											
Kim, 2004	KMIC	Korea	1990 & 1992	35–59	100	8	CVD death	676	142,055	Age, BMI, smoking status, alcohol consumption, plasma glucose, total cholesterol, blood pressure and family history of liver disease	8
Fraser, 2007	BWHHS	UK	1999–2001	60–79	0	4.6	CHD or Stroke	186	2961	Age, social class, physical activity, smoking, alcohol consumption, diabetes/insulin resistance, BMI, triglycerides, HDL-cholesterol and SBP	8
Schindhelm, 2007	Hoorn	Netherlands	1989–1992	50–75	45.2	10	CVD	355	1439	Age, sex, alcohol intake, smoking, physical activity, waist, triglycerides, SBP, fasting glucose, HDL-cholesterol	9
Goessling, 2008	FHS Offspring	USA	1978–1982	44[b]	44	20	CVD	365	2699	Age, sex, SBP, hypertension treatment, smoking, BMI, DM, total cholesterol/HDL, lipid treatment and alcohol use	9
Monami, 2008	FIBAR	Italy	2001–2003	40–75	41.6	3	CVD	20	2617	Age, sex, alcohol, smoking status and fasting glucose	9
Olynyk, 2009	BHS	Australia	1980–1994	25–84	41.5	10	CVD	428	3719	Age, smoking, alcohol use, SBP, fasting glucose, cholesterol, HDL-cholesterol, triglycerides and waist circumferencel	9
Yun, 2009	HPC	Korea	2000–2001	≥40	56.7	5	CVD death	79	37,085	Age, sex, past medical history, smoking, alcohol consumption, exercise, education, income, BMI, SBP, DBP, fasting glucose, total cholesterol, triglycerides, HDL-cholesterol and calculated GFR	8
Ford, 2011	WOSCOPS	UK	1989–1991	45–64	100	15	CVD	2459	6595	Treatment allocation, age, history of angina, history of diabetes, history of hypertension, smoking status, BMI, SBP, DBP, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, nitrate use, socio-economic deprivation and alcohol	7
Schooling, 2012	NHANES III	USA	1988–1994	≥20	47.3	13.2	CVD death	1657	16,854	Age, gender, race/ethnicity, education, smoking status, alcohol use, BMI, WHR, HDL-cholesterol, cholesterol, SBP, DBP and HbA1c	9
Kim, 2013	Beijing Cohort	China	1996	35–59	46.2	10	ICH	44	5614	Age, blood pressure, diabetes, cholesterol, smoking and alcohol intake	8
Weikert, 2013	EPIC-Potsdam	Germany	1994–1998	35–65	38.9	8.2	Stroke	353	2419	Age, sex, BMI, waist circumference, education, sports activity, smoking, alcohol intake, prevalent hypertension, prevalent diabetes, total cholesterol, HDL-cholesterol, hsCRP	9
Kim, 2013	MJ	Taiwan	1994–1996	≥20	48.2	12	ICH	579	107,379	Age, blood pressure, diabetes, cholesterol, smoking and alcohol intake	8
Kim, 2013	NIPPON-DATA90	Japan	1990	≥30	41.4	15	ICH	27	7578	Age, blood pressure, diabetes, cholesterol, smoking and alcohol intake	9
Sub-total								**7228**	**339,014**		
Aspartate aminotransferase											
Kim, 2005	KMIC	Korea	1990 & 1992	35–59	100	10	Stroke	2779	108,464	Age, BMI, blood pressure, fasting glucose, total cholesterol, smoking and alcohol consumption	8
Goessling, 2008	FHS Offspring	USA	1978–1982	44[b]	44	20	CVD	365	2699	Age, sex, SBP, hypertension treatment, smoking, BMI, DM, total cholesterol/HDL, lipid treatment and alcohol use	9
Monami, 2008	FIBAR	Italy	2001–2003	40–75	41.6	3	CVD	20	2617	Age, sex, alcohol, smoking status and fasting glucose	9
Sub-total								**3164**	**113,780**		

(*continued on next page*)

Table 1 (*continued*)

Lead Author, year	Study	Location	Year of baseline survey	Age range at baseline (years)	% Male	Mean follow-up (years)	Outcome	No. of cases	No. of participants	Covariates adjusted for	Study quality
Alkaline phosphatase											
Tonelli, 2009	NHANES III	USA	1988–1994	35–65	49	8.2	CVD death	939	14,716	Age, sex, race, smoking status, SBP, antihypertensive medication, GFR <60 Ml min^{-1}.1.73 m^{-2}, albuminuria, haemoglobin, red blood cell distribution width, albumin, HDL-cholesterol, serum calcium, serum phosphorus, serum 25-hydroxyvitamin D, DM, serum bilirubin, C-RP $\geq$ 3 mg/L, alcohol use, ALT and AST	9
Wieberdink, 2011	Rotterdam	Netherland	1990–1993	≥55	–	13.9	Stroke	64	4876	Age, sex and potential confounders	8
Shimizu, 2012	CIRCS	Japan	1985–1990	40–69	38.1	16	Stroke	489	10,754	Age, sex, community, BMI, smoking, alcohol intake, total cholesterol, triglycerides, albumin, AST, ALT, SBP, antihypertensive medication use, estimated GFR, DM and menopausal status	9
Wannamethee, 2013	BRHS	UK	1998–2000	60–79	100	11	CVD	605	3381	Age, smoking, alcohol intake, physical activity, social class, BMI, use of antihypertensive drugs, DM, lung function, SBP, estimated GFR, C-RP and von Willebrand factor	8
Sub-total								**2097**	**33,727**		
Total[c]								**20,406**	**1,230,779**		

Reference list of included studies in Appendix Supplement 11.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHS, Busselton Health Survey; BWHHS, British Womens Heart Health Study; BMI, body mass index; BRHS, British Regional Health Study; CHD, coronary heart disease; CIRCS, Circulatory Risk in Communities Study; CVD, cardiovascular diseases; DBP, diastolic blood pressure; DM, diabetes mellitus; EPIC, European Prospective Investigation of Cancer; FHS, Framingham Heart Study; FIBAR, Firenze Bagno a Ripoli; GFR, glomerular filtration rate; GP, general practitioner; HDL, high-density lipoprotein; HPC, Health Promotion Center; hsCRP, high sensitivity C-reactive protein; ICH, intracerebral haemorrhage; IL-6, interleukin-6; KMIC, Korea Medical Insurance Corporation; LDL, low-density lipoprotein; MHS, Minnesota Heart Survey; MI, Myocardial Infarction; MJ, MJ Health Screening Centre; NHANES III, Third National Health and Nutrition Examination Survey; RBS, Rancho Bernado Study; SHIP, Study of Health in Pomerania; SHeS, Scottish Health Surveys; SBP, systolic blood pressure; TARFS, Turkish Adult Risk Factor Study; UK, United Kingdom; USA, United States of America; VHM&PP, Voralberg Health Monitoring and Promotion Program; WHR, waist-to-hip ratio; WOSCOPS, West of Scotland Coronary Prevention Study.

[a] Range of follow-up.

[b] Mean age at baseline.

[c] Represents unique numbers of participants and cases.

medical records, death certificates and/or endpoint committees using the International Classification of Diseases diagnosis coding (Appendix Supplement 4). The degree of covariate adjustment varied, but majority of studies adjusted for established vascular risk factors such as age, sex, smoking status, body mass index, blood pressure, lipids and physical activity plus additional adjustment for alcohol consumption, liver enzymes, or inflammatory markers. Overall, majority of the studies were judged to be of high quality (quality score: 8–9).

3.3. Gamma glutamyltransferase and cardiovascular risk

The pooled RR (95% CI) per 1-SD change in baseline loge GGT levels was 1.23 (1.16–1.29) for CVD, using a random effects model (Figs. 2,3). In a comparison of individuals in the top thirds with those in the bottom thirds of baseline GGT levels, the corresponding estimate was 1.49 (1.30–1.71) (Appendix Supplements 6,7). Exclusion of any single study at a time from the meta-analysis had minimal effect on the pooled RR. There was substantial heterogeneity among studies (I^2 = 73%, 95% CI, 57–82; P = 0.001), which was not explained by any of the study-level characteristics assessed (Fig. 4). Between-study heterogeneity was substantial in several study characteristic categories. There was less heterogeneity in studies conducted in North American populations, studies that did not use fasting samples for assays and studies of the highest quality. The positive association was consistently observed across several subgroups including geographical location, sex, age, and cause-specific cardiovascular endpoints. Heterogeneity was no longer statistically significant when analysis was restricted to studies of the highest quality (I^2 = 31%, 95% CI, 0–65; P = 0.15). Among the remaining 12 studies, the pooled RR (95% CI) was 1.17 (1.12–1.23), which was comparable to the overall combined RR. The funnel plot did not suggest evidence of publication bias which was confirmed by Egger's test (P = 0.06) (Appendix Supplement 8).

3.4. Aminotransferases and cardiovascular risk

The pooled RR (95% CI) per 1-SD change in baseline loge ALT levels was 1.00 (0.99–1.02) for CVD (Figs. 2,3). The corresponding estimate in extreme thirds was 1.00 (0.97–1.03). (Appendix Supplements 6,7). There was substantial heterogeneity between studies (I^2 = 79%, 95% CI, 65–88; P = 0.001), which was to a large part explained by several study-level characteristics such as average follow-up duration (P = 0.01) and cause-specific cardiovascular outcome (P = 0.03), with geographical location approaching marginal significance (P = 0.06) (Fig. 4). The pooled estimates for CHD and stroke endpoints were 0.95 (0.90–1.00) and 1.01 (1.00–1.02) respectively. Funnel plot suggested no evidence of publication bias which was confirmed by Egger's test (P = 0.90) (Appendix Supplement 8).

Circulating AST level was not significantly associated with an increased risk of CVD in pooled analysis of the three available studies 1.03 (0.96–1.11), with substantial heterogeneity (I^2 > 70%) between the studies (Figs. 2,3). The limited number of studies precluded us from investigating the potential sources of heterogeneity.

3.5. Alkaline phosphatase and cardiovascular risk

In pooled analysis of four studies, circulating ALP was associated with an 8% (95% CI: 3–14%) higher risk of CVD per 1-SD deviation increase in baseline ALP levels. There was no evidence of significant heterogeneity between the contributing studies (I^2 = 31%, 95% CI, 0–75; P = 0.23) (Figs. 2,3).

3.6. Dose-response analyses

Nine studies (14 data points because results for males and females were reported separately for some of the studies) reported RRs for categories of GGT levels and were eligible for GLST dose–response analysis. Fig. 5 shows the dose–response relation between baseline GGT levels and CVD risk. Examination of the figure did not suggest substantial departure from linearity, though the test for a nonlinear relation was marginally significant (P-value for nonlinearity = 0.05). The pooled RR per 5 U/L increment in GGT levels was 1.05 (1.03–1.06). The few data points precluded us from assessing the dose–response shapes for the other liver enzymes, though the test for nonlinearity was suggestive of a linear relation

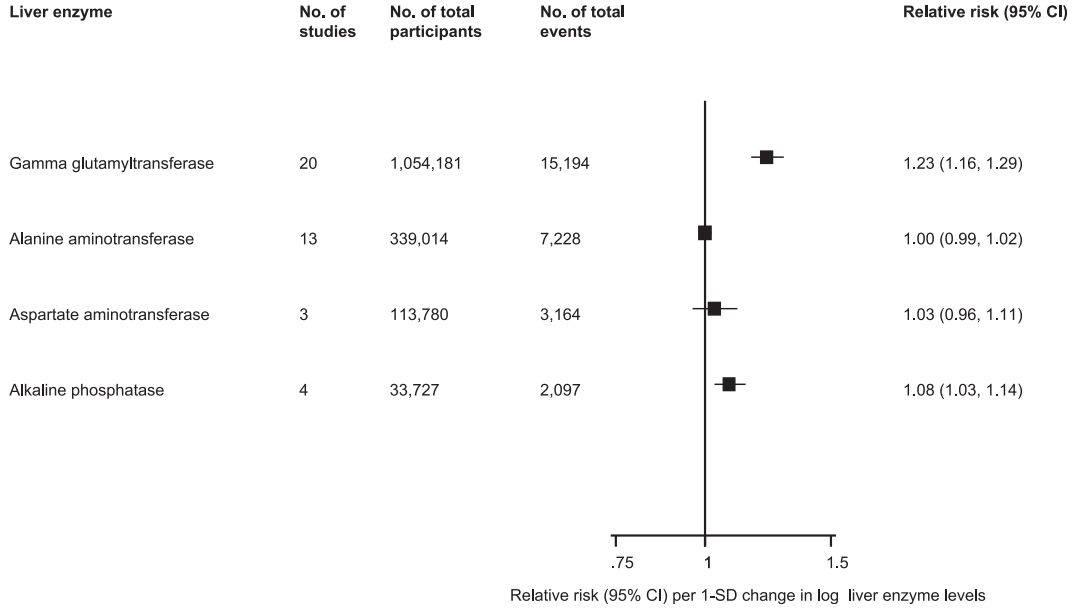


Fig. 2. Summary of pooled relative risks for cardiovascular disease per 1-standard deviation change in baseline levels of liver enzymes in eligible studies. CI, confidence interval (bars); SD, standard deviation.

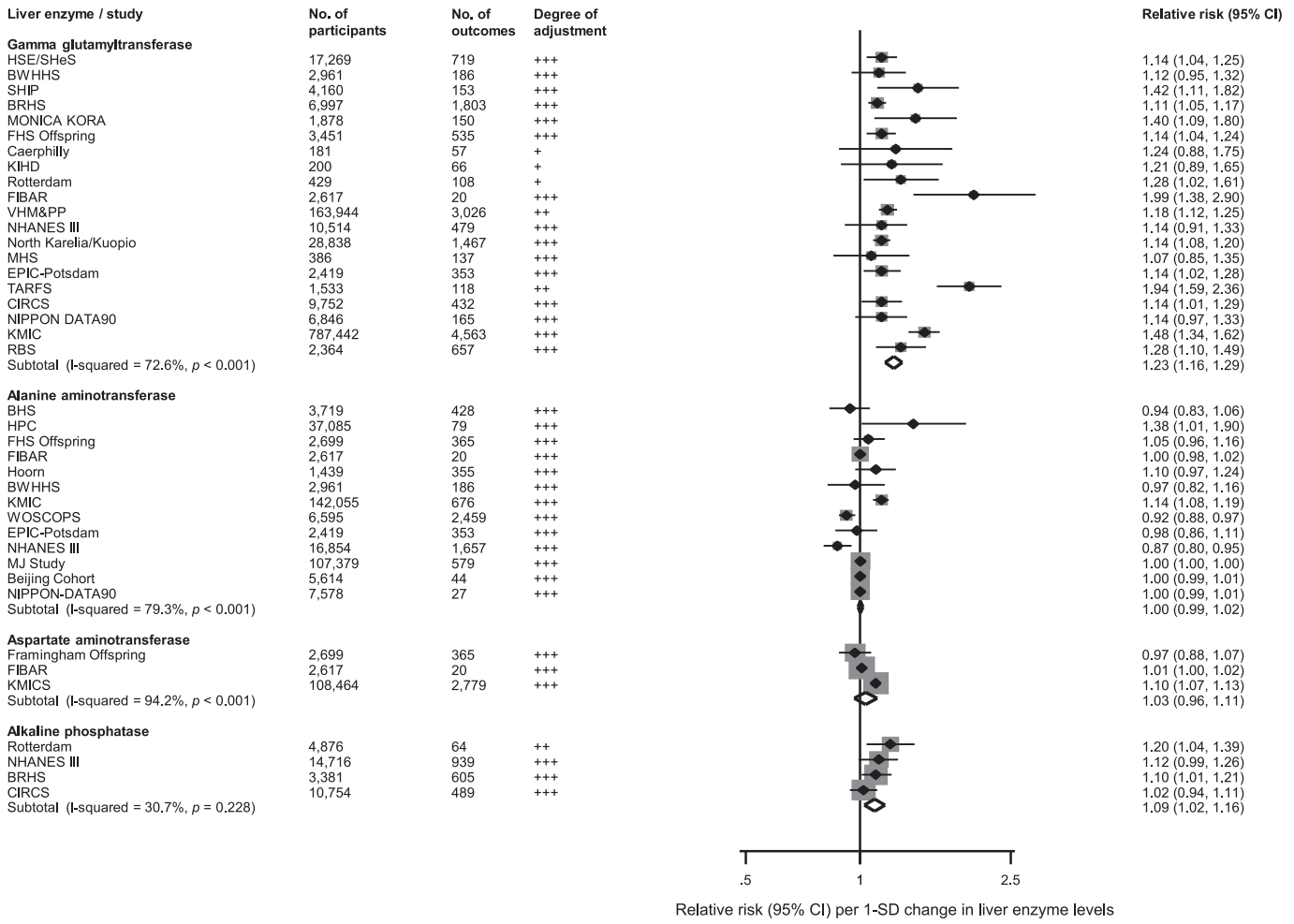


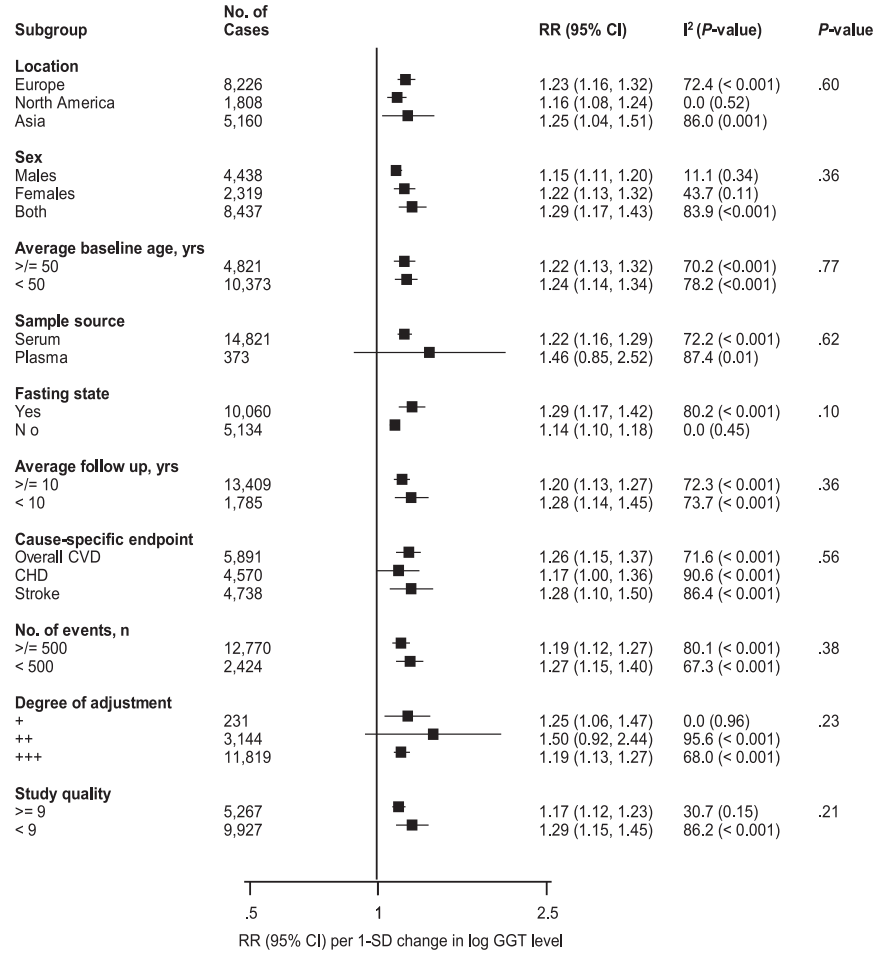
Fig. 3. Individual relative risks for cardiovascular disease per 1-standard deviation change in baseline levels of liver enzymes in eligible studies. Study acronyms are provided in Table 1. The summary estimates for studies of GGT, ALT and AST were calculated using random effects models; †, Degree of adjustment: +, adjusted for age and/or sex; ++, further adjustment for cardiovascular risk factors; +++, further adjustment for alcohol consumption, other liver markers, or inflammatory markers; CI, confidence interval (bars); RR, relative risk; SD, standard deviation.

between ALP levels and CVD risk based on only three data points (P for nonlinearity = 0.11).

4. Discussion

In this comprehensive review and meta-analysis of common markers of liver dysfunction in relation to CVD, we have shown that GGT and ALP levels are each positively and independently associated with cardiovascular risk. There was no strong evidence for any associations of the aminotransferases with CVD. However, stratified analysis by cause-specific cardiovascular endpoints showed that ALT was somewhat inversely associated with CHD and positively associated with stroke. Subgroup findings were also suggestive of a positive association of ALT with CVD in Asian populations, and possible inverse associations in North American and European populations. There was a monotonous risk increase for CVD across increasing levels of GGT, and an indication of a linear association of ALP with cardiovascular risk. This investigation which included a total of about 1.23 million participants in whom approximately 20,000 unique cardiovascular events were documented overall, provides robust evidence on the prospective associations between circulating levels of liver enzymes and CVD risk.

Our findings of a graded positive increase in cardiovascular risk with increasing levels of GGT and consistency of the association across diverse populations and cause-specific cardiovascular endpoints, extend the previous results by Fraser and colleagues [6], and is supported by epidemiological and experimental data on the independent role of GGT in the pathogenesis of CVD [28]. Potential mechanisms for increased cardiovascular risk associated with elevated levels of GGT have been postulated and these include promotion of the atherosclerotic process through its pro-oxidant and pro-inflammatory activities [29] and direct involvement in atheromatous plaque formation [30]. Establishing causality of the association requires robust evidence from randomized controlled trials (RCTs). Several pharmacological agents or interventions are available that modify levels of GGT, however, such available interventions (e.g. insulin sensitizers and antioxidants) also influence levels of other liver enzymes and lipid factors [31] (Appendix Supplements 9,10). In the absence of clinical trials however, Mendelian randomisation (MR) studies of genetic variants specifically related to GGT levels may provide another route to assess causality [32]. Gamma glutamyltransferase level is under strong genetic regulation, with heritability estimates reported to range between 50 and 77% [33,34]. There is evidence to suggest that the *GGT1* locus, which is the main protein-coding gene for GGT, may be specific for GGT levels [35,36] and therefore variants within this locus might be valid instrumental variables for MR studies. Conen and colleagues [37] using a MR approach recently found evidence



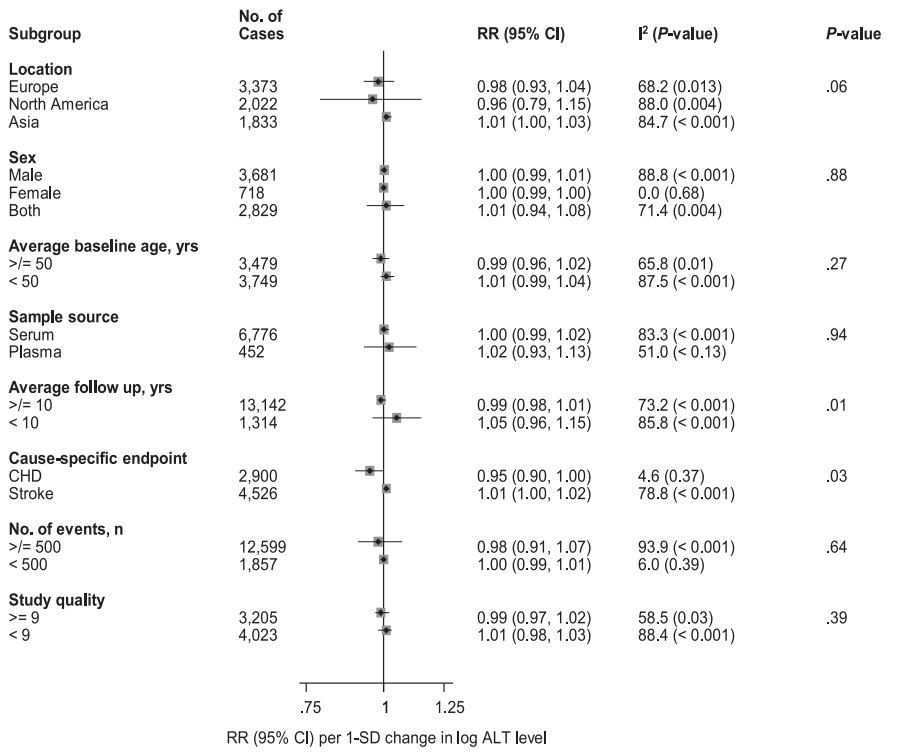


Fig. 4. Prospective Studies of GGT and ALT Levels with cardiovascular disease, grouped according to several study-level characteristics. The summary estimates presented were calculated using random effects models; ALT, alanine aminotransferase; CI, confidence interval (bars); GGT, gamma glutamyltransferase; RR, relative risk; SD, standard deviation; *, *P*-value for meta-regression.

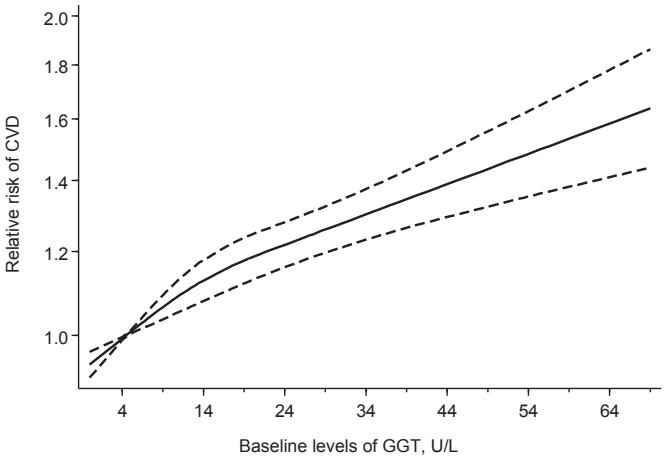


Fig. 5. Dose-response relation between GGT levels and relative risks of cardiovascular disease for pooled results of nine studies providing relevant data. GGT, gamma glutamyltransferase; adjusted relative risks and 95% confidence intervals (CIs; dashed lines) are reported. Data were modeled with restricted cubic splines with 3 knots in a random effects dose–response model. The median value (4.5 U/L) of the lowest reference range was used to estimate all relative risks. The vertical axis is on a log scale.

for a causal effect of GGT on fasting insulin, using the rs2017869 variant of the *GGT1* gene as an instrumental variable.

Whereas the numbers of studies were limited for ALP levels, our findings suggest that circulating ALP levels may be associated with the development of CVD. The limited data points precluded us from examining the actual shape of the association, but we found no evidence of a nonlinear association. We have also recently demonstrated a linear dose–response relationship between ALP levels and all-cause mortality in pooled analysis of prospective studies [38]. Findings from another recent study, however, demonstrated a U-shaped relationship between ALP level and risk of stroke [13]. Additional observational evidence is needed to better assess the shape of the association. The excess cardiovascular risk associated with ALP has been suggested to be via mechanisms related to vascular calcification through increased bone metabolism, impaired vascular homoeostasis, pro-inflammatory activities, or subclinical liver dysfunction [12,13]. Though our pooled analyses did not demonstrate statistically significant associations of ALT or AST levels with overall CVD risk, the results do suggest that ALT may be inversely associated with CHD and positively associated with stroke. This observation might be due to different effects of ALT levels on vascular risk factors (since vascular outcomes may somewhat have diverse aetiologies [39]) or to limited statistical power to detect cause-specific cardiovascular endpoints. The evidence on the association of ALT with cardiovascular risk has mostly been inconsistent. A U-shaped association of ALT with vascular risk has been reported [40], and there is emerging evidence that circulating ALT levels may be inversely related to CVD outcomes [10,41]. Elevated levels of ALT have also been implicated with an increased risk of CVD, via underlying non-alcoholic fatty liver disease (which is closely related with three important pathogenic risk factors for diabetes and CVD: i) ectopic fat deposition; ii) insulin resistance and iii) adverse profiles in adipokine levels [42]), endothelial dysfunction and vessel wall damage [43], inflammation [44], oxidative stress [44] and impaired haemostasis [45]. The mechanisms for the inverse associations are not clear, but reduced functionality of the liver in the presence of low ALT levels has been postulated or it could be that ALT may simply be a marker of an underlying aetiology [10,41]. Biases due to selection and residual confounding have also been implicated [10,41]. The current findings also suggest that there might be geographical variations in the association of ALT with CVD – positive associations in Asians and inverse associations in both North American and European populations. Similar findings have been observed in our recent review of ALT and all-cause mortality outcomes [38]. The positive associations in Asians might be attributed to the presence of unrecognised liver diseases which are more prevalent in these populations. Further evidence is needed to elucidate these paradoxical associations.

4.1. Strengths and limitations

The strengths and potential limitations of this review and meta-analyses deserve mention. Our study provides quantitative pooled estimates and dose–response relationships of the associations of baseline circulating levels of four most commonly measured liver enzymes with risk of CVD for the first time. The meta-analyses included studies that had recruited participants from approximately healthy populations, therefore reducing any effects of clinically evident pre-existing disease on levels of liver enzymes. Only prospective cohort studies with at least a year follow-up duration were eligible, limiting the possibility of selection or recall bias. We employed standardized risk estimates from all contributing studies to allow a consistent combination of estimates across studies. Selective reporting of studies was not a concern in our analyses, as our comprehensive search and contact with investigators made it unlikely that any published report was missed and visual inspection of plots and formal tests demonstrated no statistical evidence of publication bias. Dose-response relationships were evaluated using GLST analyses, however, we were unable to assess these for all exposures because of the limited data points. We were unable to fully examine the impact of adjustment for all known and potential risk factors and also combine models in studies that adjusted for the same set of confounders, because of the varying degree of confounder adjustment in individual studies. Despite using a composite endpoint of CVD to maximise comparability, we were unable to achieve this across all studies, as some studies reported cause-specific cardiovascular endpoints such as CHD, stroke, or CVD death. Given that some of these endpoints (such as stroke and CHD) have different aetiologies [39], this may have underestimated or overestimated some of the results. However, we conducted stratified analyses of cause-specific cardiovascular endpoints where possible. In addition, several of these cause-specific endpoints share many common aetiologies with the composite CVD endpoint and represented only a few of reported outcomes. It was not possible to correct the estimates for within-individual variation in levels of the liver enzymes over time which may have underestimated the associations, because data involving repeat measurements were not reported by all the contributing studies. Hence, the associations demonstrated may be even stronger. We observed significant heterogeneity among the studies than would be expected as a result of chance, which is not surprising given the substantial differences in study populations and methods. We however systematically explored and identified possible sources of heterogeneity using stratified analyses, meta-regression and sensitivity analyses. The results should therefore be interpreted in context of the limitations available. More detailed analyses under a broader range of circumstances, such as shapes of the dose–response relationships, subgroup analyses and exploration of potential sources of heterogeneity, will require collaborative pooling of individual participant data from prospective studies.

5. Conclusions

Baseline levels of GGT and ALP are each positively and independently associated with CVD risk and in a log-linear manner.

There may be variations in the associations of ALT with cause-specific cardiovascular endpoints, findings which require further investigation.

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Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2014.06.006.

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