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Liver enzymes and risk of all-cause mortality in general populations: a systematic review and meta-analysis

Setor K Kunutsor,[1,2]* Tanefa A Apekey,[2] Dorothy Seddoh[3] and John Walley[2]

[1]Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK, [2]Institute of Health Sciences, Faculty of Medicine and Health, University of Leeds, Leeds, UK and [3]Maranatha University, P.O. Box AN 10320, Accra, Ghana

*Corresponding author. Department of Public Health and Primary Care, Strangeways Research Laboratory, Worts Causeway, University of Cambridge, Cambridge CB1 8RN, UK. E-mail: skk31@cantab.net

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Background Gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), commonly used as markers of liver dysfunction, have been implicated with risk of all-cause mortality. The prospective evidence on the associations in general populations has not been reliably quantified.

Methods We conducted a systematic review and meta-analysis of published prospective cohort studies evaluating the associations of baseline levels of these enzymes with all-cause mortality in general populations. Relevant studies were identified in a literature search of MEDLINE, EMBASE and Web of Science up to March 2013. Authors of unpublished studies provided data on request.

Results Nineteen unique cohort studies with aggregate data on over 9.24 million participants and 242 953 all-cause mortality outcomes were included. In a comparison of extreme thirds of baseline GGT and ALP levels, relative risks (RRs) (95% confidence intervals) for all-cause mortality were 1.60 (1.42-1.80) and 1.38 (1.17-1.63), respectively. The corresponding RRs for ALT were 0.82 (0.78-0.86) and 1.43 (1.08-1.90) in North American and Asian populations, respectively. There was no strong evidence of an association of AST with all-cause mortality: RR 1.23 (0.80-1.88). The pooled RRs per 5 U/l increment in GGT and ALP levels were 1.07 (1.04-1.10) and 1.03 (1.01-1.06), respectively.

Conclusions Available data indicate positive independent associations of baseline levels of GGT and ALP with all-cause mortality, consistent with linear dose-response relationships. There were geographical variations in the association of ALT with all-cause mortality which require further investigation. The potential incremental prognostic values of GGT and ALP in mortality risk assessment need evaluation.

Keywords Gamma glutamyltransferase, aminotransferases, alkaline phosphatase, mortality, meta-analysis

Introduction

Assays for gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are the most common laboratory tests used for the detection of liver diseases. Circulating GGT is present on the external surfaces of most cells, particularly hepatocytes, and in serum, and is used as a biological marker of excessive alcohol intake.[1] The liver aminotransferases (ALT and AST) are found abundantly within hepatocytes and they catalyze the transfer of amino groups to generate products in gluconeogenesis and amino acid metabolism.[2, 3] Alkaline phosphatase is a hydrolase enzyme that catalyzes the hydrolysis of inorganic pyrophosphate, which is a vascular calcification inhibitor. Serum levels of ALP are commonly used in clinical practice as a marker of liver or bone disease.[4]

There have been important advances in the understanding of the physiological functions of these liver enzymes and several epidemiological associations have been reported. Several prospective epidemiological associations have been demonstrated between these markers of liver dysfunction and risk of type 2 diabetes mellitus,[5–7] cardiovascular disease (CVD)[8–11] or mortality from vascular and nonvascular causes,[4,12,13] after accounting for important risk factors. Furthermore, baseline circulating levels of GGT, ALT, AST and ALP have been reported to be associated with future risk of all-cause mortality (which is a more ultimate indicator of health than cause-specific outcomes[14]).[11,13,15–22] However, the majority of these studies were conducted in selected populations such as in the elderly, participants at high vascular risk or those with pre-existing disease.[17–20,22] Therefore the nature of the individual associations of these enzymes with risk of all-cause mortality among general populations is not very clear. A number of prospective studies have been published reporting on the associations between baseline levels of these enzymes and risk of all-cause mortality in general populations, but their results have been inconsistent.[9,11,13,15,17,20,21] Although they generally have low specificities for the liver, assays for these liver enzymes are sensitive, well standardized, simple, inexpensive and commonly used laboratory tests. These enzymes may be useful for the prediction of all-cause mortality[23] and the identification of individuals at high risk of dying from all-causes. Although, among the liver enzymes, GGT is a less specific indicator of liver dysfunction, research has largely focused on GGT and the evidence suggests that it may be a strong risk indicator for all-cause mortality. The aminotransferases and ALP (which are often measured simultaneously with GGT) have received less attention and their significance for all-cause mortality is less certain. We therefore, for the very first time, aimed to quantify precisely the nature and magnitude of the associations between baseline levels of GGT, ALT, AST and ALP with the risk of all-cause mortality in general populations using a meta-analytic approach.

Methods

Data sources and search strategy

This systematic review and meta-analysis of studies was conducted using a predefined protocol and in accordance with PRISMA and MOOSE guidelines[24,25](Supplementary Appendices 1 and 2, available as Supplementary data at *IJE* online). We searched MEDLINE, EMBASE and Web of Science for prospective (cohort or 'nested case-control') population-based studies that evaluated associations between baseline circulating levels of GGT, ALT, AST and ALP with all-cause mortality risk up to March 2013. The computer-based searches combined free and MeSH search terms and combinations of keywords related to the exposures (e.g. 'gamma glutamyltransferase', 'alanine aminotransferase', 'aspartate aminotransferase', 'alkaline phosphatase') and outcomes (e.g. 'all-cause mortality', 'death'). 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 authors for unpublished studies on the associations. We restricted the search to studies of humans. Further details on the search strategy are presented in Supplementary Appendix 3 (available as Supplementary data at *IJE* online).

Eligibility criteria

Observational cohort studies were included if they had at least 1 year of follow-up, assessed associations of GGT, ALT, AST or ALP with all-cause mortality in adults (aged over 18 years), with samples measured at baseline and reported recruitment of participants representative of or 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.

Data extraction 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, numbers of all-cause mortality outcomes, numbers of controls, mean age, duration of follow-up and degree of adjustment for potential confounders (defined as: '+' when RRs were adjusted for age and/or sex; '++' with further adjustment for established vascular risk factors such as smoking status,

body mass index, blood pressure, lipids or physical activity; and '+++' with additional adjustment for alcohol consumption, liver enzymes or inflammatory markers). The literature search, data extraction and quality assessment were conducted by two independent reviewers (T.A.A., D.S.). A standardized predesigned data collection form was used for data extraction. Each article was assessed using the inclusion criteria above; 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 both unpublished studies and those with missing information, to conduct re-analyses of data and provide the required information.[26–28] Study quality was assessed based on the nine-star Newcastle–Ottawa Scale (NOS)[29] using pre-defined criteria namely: selection (population representativeness), comparability (adjustment for confounders) and ascertainment of outcome. The NOS assigns a maximum of four points for selection, two points for comparability (two points were awarded for studies that reported estimates for the highest degree of adjustment defined above +++ and one point for ++), and three points for outcome. Nine points on the NOS reflect the highest study quality.

Statistical analysis

The relative risks (RRs) with 95% confidence intervals (CIs) were used as the common measure of association across studies. Reported study-specific RRs were converted to a consistent comparison using standard statistical methods.[30,31] Briefly, risk estimates for all the studies included in the analyses, where appropriate, were transformed to involve comparisons between the top third and bottom third of the study population's baseline distribution of GGT, ALT, AST or ALP levels. Log risk estimates were transformed assuming a normal distribution (or assuming that a transformation of the explanatory variable for which the risk ratio is based was normally distributed), with the comparison between top and bottom thirds being equivalent to 2.18 times the log risk ratio for a 1 standard deviation increase (or equivalently, as 2.18/2.54 times the log risk ratio for a comparison of extreme quarters and as 2.18/2.80 times the log risk ratio for a comparison of extreme quintiles). Standard errors of the log risk estimates were calculated using published confidence limits and were standardized in the same way. 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. Hazard ratios, relative risks and odd ratios were assumed to approximate the same measure of relative risk. Summary RRs were pooled using a random-effects model to minimize the effect of between-study heterogeneity.[32]

For the dose-response meta-analyses of the associations of liver enzyme levels with risk of all-cause mortality, we used generalized least-squares trend estimation (GLST) analysis as described by Greenland and Orsini[31,33] 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. Potential nonlinear dose-response relationships were examined by modelling levels of liver enzymes using restricted cubic splines.

Statistical heterogeneity across studies was quantified using Cochran χ^2 and the I^2 statistics, with I^2 >50% considered to be important.[34,35] Study-level characteristics including geographical location, sex differences, average duration of follow-up, number of cases, sample source and state, degree of adjustment, and study quality were pre-specified as characteristics for assessment of heterogeneity, which was conducted using stratified analysis and random-effects meta-regression.[36] In analysis specified *post hoc*, there was further stratified analysis to examine the difference in pooled RRs by baseline average age of participants. Sensitivity analyses were performed to assess the influence of each individual study by omitting one study at a time and calculating pooled estimates for the remainder of the studies. We assessed the potential for small study effects such as publication bias through formal tests, namely Begg's funnel plots[37] and Egger's regression symmetry test.[38] We also calculated absolute risk differences based on available summary estimates and incidence rates from the general US and European populations using the formula: risk difference = background incidence rate X (RR-1).[39] All analyses were conducted using Stata version 12 (Stata Corp, College Station, TX).

Results

Study identification and selection

Our initial search identified 3286 potentially relevant citations. After screening of titles and abstracts, 55 articles remained for further evaluation. Following detailed assessments, 36 articles were excluded of which 6 involved study populations with pre-existing disease.[18,19,22,40–42] Overall, 19 articles based on 19 unique prospective cohort studies were included in the meta-analysis (Figure 1). One study combined results of three independent cohorts.[43] In aggregate, the included studies comprised 9 238 201 non-overlapping participants and 242 953 all-cause mortality outcomes. A single study which provided results of unpublished data contributed over 90% of data (comprising 8 922 358 participants and 220 216 events) to the present review.[28]

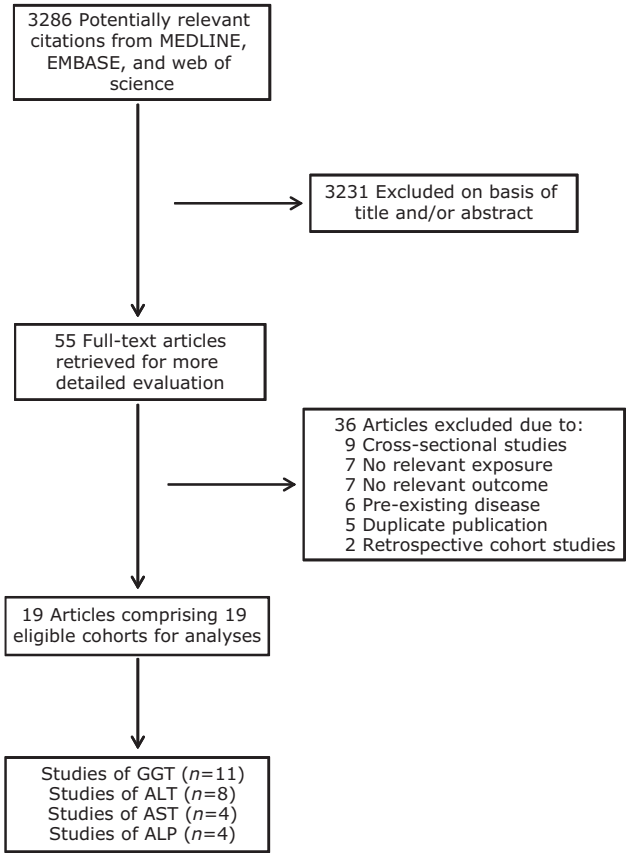

Figure 1 Selection of studies included in the meta-analysis. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase

Study characteristics and study quality

Table 1 provides details of the eligible studies that evaluated baseline circulating GGT, ALT, AST and ALP levels with risk of all-cause mortality outcomes[4,9,11,13,15,16,20,21,26,27,43–50] and their quality assessment scores. All included studies were prospective cohort studies carried out in USA, Europe (UK, The Netherlands, Germany, Italy and Austria), and Asia (Korea and Japan) with an average baseline age of 51 years. Duration of follow-up for all-cause mortality outcomes ranged from 5 to 20 years, with a median follow-up of 10 years. The majority of studies achieved the highest score for quality. Table 2 provides assay characteristics of measured levels of liver enzymes from studies contributing to the analysis. All studies evaluated associations in approximately general populations.

Gamma glutamyltransferase and all-cause mortality

The combined RR (95% CI) for all-cause mortality outcomes in a comparison of individuals in the top thirds with those in the bottom thirds of baseline GGT level for the 11 studies in fully adjusted analysis was 1.60 (1.42–1.80) with substantial heterogeneity between studies (I^2 >70%) (Figure 2). Exclusion of any single study at a time from the meta-analysis had minimal effect on the pooled RRs, which ranged from 1.53 (1.36–1.73) to 1.65 (1.46–1.87). The combined RR excluding the single largest study was 1.60 (1.37–1.86), very similar to the main finding. Little of the heterogeneity in the contributing studies was explained by differences in several study level characteristics other than study size (P for meta-regression = 0.05; Figure 3). A stronger association was observed in studies with ⩾500 outcomes: 1.73 (1.49–2.00) compared with smaller studies: 1.31 (1.15–1.50). Between-study heterogeneity was substantial in several study characteristic categories. There was less heterogeneity in studies conducted in North American populations, studies of small size and studies of the highest quality. In further exploration of heterogeneity, this was substantially reduced when analysis was restricted to studies of the highest quality. The strong positive association was consistently observed across several subgroups. The Egger test for publication bias was not significant (P = 0.52), consistent with observed funnel plot symmetry (Supplementary Figure 1, available as Supplementary data at *IJE* online).

Aminotransferases and all-cause mortality

The pooled RR (95% CI) for all-cause mortality in a comparison of extreme thirds of baseline level of ALT was 1.03 (0.76–1.41), with substantial heterogeneity (I^2 > 70%) across the eight studies (Figure 2). Analysis examining the influence of a single study on the combined RR showed similar results. The combined RR on exclusion of the single largest study, which could have potentially influenced our findings, was 1.07 (0.82–1.40). The inconsistency across the studies that evaluated ALT was partially explained by geographical location (P for meta-regression = 0.0002; Supplementary Figure 2, available as Supplementary data at *IJE* online). The pooled RRs for studies conducted in North America, Europe and Asia were 0.82 (0.78–0.86), 0.95 (0.84–1.07) and 1.43 (1.08–1.90), respectively. Similarly as for studies of GGT, heterogeneity was also less among studies conducted in Europe and North America, smaller studies and studies of the highest quality. In further exploration of heterogeneity, this was substantially reduced when we restricted the analysis to studies of the highest quality. Among the four remaining studies, the pooled RR also did not show evidence of an association of ALT with all-cause mortality, at 0.94 (0.82-1.07).

In pooled results of the only four studies of AST, there was no strong evidence of an association with all-cause mortality outcomes [RR 1.23 (0.80–1.88)] (Figure 2). The limited number of studies for AST precluded us from investigating the potential sources

Table 1 Characteristics of prospective studies evaluating associations between liver enzymes and all-cause mortality, 1995–2013

Lead author, publication date, reference no.	Study	Location	Year of baseline survey	Baseline age range (yrs)	% male	Follow up years	Total participants	No. of cases	Covariates adjusted for	Study quality
Gamma glutamyltransferase										
Wannamethee, 1995[46]	BRHS	UK	1978–80	40–59	100	11.5	5309	462	Age, social class, smoking, PA, HTN treatment, use of other medications, DM, BMI, TC, HDL-C, blood glucose, heart rate, FEV_1	7
Calori, 2011[26]	Cremona	Italy	1990–91	>40	44	15	2074	495	Age, sex, alcohol use, fibrinogen, DM, AST, ALT, LDL-C, TC, HDL-C, SBP	9
Fulks, 2008[28]	CRL	USA	1991–2007	⩾18	59.7	9.6	8 922 358	220 216	Age, fructosamine, TC/HDL-C, ALT, AST, ALP	6
Lee, 2007[44]	FHS	USA	1978–82	44[a]	48	19	3451	362	Age, sex, BMI, DM, SBP, TC/HDL-C ratio, smoking, alcohol use, CRP	9
Kengne, 2012[43]	HSE/SHeS	UK	1994/95/98	53.6[a]	45	12.7	17 269	2859	Age, sex, DM, SBP, HTN, Hx of CVD, PA, smoking, alcohol use, TC, lipid medication	9
Ruhl, 200[45]	NHANES III	USA	1988–94	-	-	12	14 950	2189	Age, sex, race-ethnicity, BMI, WHR, glucose, TC, HDL-C, SBP, DBP, smoking, alcohol use, caffeine, PA, CRP, transferrin saturation, education	9
Loomba, 2012[49]	RBS	USA	1984–87	70[a]	46	13.7	2364	1329	Age, sex, alcohol use, BMI, TC, HDL-C, TG, smoking, SBP, DM, IL-6, CRP	9
Koehler, 2011[50]	Rotterdam	Netherlands	1990–93	⩾55	-	18	3867	1825	Age, sex, alcohol use, BMI, HTN, DM, TC, smoking, educational level	9
Haring, 2009[16]	SHIP	Germany	1997–2001	20–79	49	7.3	4160	307	Age, WC, alcohol use, PA, educational level, civil status, equalized income, FCI	9
Strasak, 2008[48]	VHM&PP	Austria	1985–2005	42[a]	42.5	10.2	76 113	4551	Age, BMI, smoking, occupational status, TG, TC, SBP, DBP, blood glucose	8
Breitling, 2011[15]	WCB	Germany	1986–92	25–64	100	20	19 090	2170	Age, nationality, occupation, DM, IHD, HT, BMI, smoking, elevated blood glucose, TG, TC, alcohol	7
Subtotal							9 071 005	236 765		
Alanine aminotransferase										
Fulks, 2008[28]	CRL	USA	1991–2007	⩾18	59.7	9.6	8 911 194	219 869	Age, fructosamine, TC/HDL-C, GGT, AST, ALP	6

(continued)

Table 1 Continued

Lead author, publication date, reference no.	Study	Location	Year of baseline survey	Baseline age range (yrs)	% male	Follow up years	Total participants	No. of cases	Covariates adjusted for	Study quality
Goessling, 200[21]	FHS Offspring	USA	1978–82	44[a]	44	20	2812	283	Age, sex, SBP, HTN, smoking, BMI, DM, lipid txt, alcohol use	9
Schindhelm, 2007[9]	Hoorn	Netherlands	1989–92	50–75	60.9	10	1439	174	Age, sex, alcohol use, smoking, PA, waist, TGs, SBP, FPG, HDL-C	9
Ruhl, 2009[45]	NHANES III	US	1988–94	-	-	12	14 950	2189	Age, sex, race-ethnicity, BMI, WHR, glucose, TC, HDL-C, SBP, DBP, smoking, alcohol use, caffeine, PA, CRP, transferring saturation, education	9
Nakamura, 2006[47]	NHI	Japan	1989–91	40–69	-	10	4524	214	Age, sex, BMI, smoking, alcohol use, SBP, medication for HTN, TC, DM	8
Kim, 2004[13]	KMIC	Korea	1990/1992	35–59	66.5	8	142 055	3786	Age, BMI, smoking, alcohol use, plasma glucose, TC, BP, FHx liver disease	8
Koehler, 2011[50]	Rotterdam	Netherlands	1990–93	⩾55	-	18	3867	1825	Age, sex, alcohol use, BMI, HTN, DM, TC, smoking, educational level	9
Ford, 2011[20]	WOSCOPS	Scotland	-	45–64	100	15	6595	1293	Country, treatment allocation, age, sex, smoking, DM, HTN, BMI, SBP, DBP, HDL-C, LDL-C, TGs, glucose, alcohol	7
Subtotal							9 087 436	229 633		
Aspartate aminotransferase										
Fulks, 2008[28]	CRL	USA	1991–2007	⩾18	59.7	9.6	8 897 875	219 669	Age, fructosamine, TC/HDL-C, ALT, GGT, ALP	6
Goessling, 2008[21]	FHS Offspring	USA	1978–82	44[a]	44	20	2812	283	Age, sex, SBP, HTN, smoking, BMI, DM, lipid txt, alcohol use	9
Kim, 2004[13]	KMIC	Korea	1990/1992	35–59	66.5	8	142 055	3786	Age, BMI, smoking, alcohol use, plasma glucose, TC, BP, FHx liver disease	8
Koehler, 2011[50]	Rotterdam	Netherlands	1990–93	⩾55	-	18	3867	1825	Age, sex, alcohol use, BMI, HTN, DM, TC, smoking, educational level	9
Subtotal							9 046 609	225 563		

(continued)

Table 1 Continued

Lead author, publication date, reference no.	Study	Location	Year of baseline survey	Baseline age range (yrs)	% male	Follow up years	Total participants	No. of cases	Covariates adjusted for	Study quality
Alkaline phosphatase										
Wannamethee, 2013[11]	BRHS	UK	1998–2000	60–79	100	11	3381	984	Age, smoking, alcohol use, PA, social class, BMI, antihypertensive medication, DM, lung function, SBP, estimated GFR, CRP, vWF	7
Fulks, 2008[28]	CRL	USA	1991–2007	⩾18	59.7	9.6	8 897 875	219 669	Age, fructosamine, TC/HDL-C, ALT, AST, GGT	6
Filipowicz, 2013[27]	NHANES 1999–2004	USA	1999–2004	⩾20	48.9	4.6	9771	438	Age, sex, race, SBP, DBP, WC, estimated GFR, liver disease, Ca, Ph, AST, ALT, GGT, bilirubin, albumin, Hb	9
Tonelli, 2009[4]	NHANES III	USA	1988–94	⩾20	49	12	14 716	2064	Age, sex, race, smoking, SBP, antihypertensive medication, GFR $< 60\,mL\,min^{-1}\ 1.73\,m^{-2}$, albuminuria, Hb, RBC distribution width, serum albumin, HDL-C, Ca, Ph, 25-hydroxyvitamin D, DM, bilirubin, CRP ⩾ 3 mg/L, alcohol use, ALT, AST	9
Subtotal							8 848 155	221 127		
Total[b]							9 238 201	242 953		

BRHS, British Regional Heart Study; CRL, Clinical Reference Laboratory; FHS, Framingham Heart Study; HSE, Health Survey of England; KMIC, Korea Medical Insurance Corporation; NHANES, National Health and Nutrition Examination Survey; NHI, National Health Insurance; RBS, Rancho Bernardo Study; SHeS, Scottish Health Survey; SHIP, Study of Health in Pomerania; VHM&PP, Vorarlberg Health Monitoring and Promotion Program; WCB, Workmen's Compensation Board; WOSCOPS, West of Scotland Coronary Prevention Study.

ALT, aspartate aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Ca, calcium; CRP, C-reactive protein; DBP, diastolic blood pressure; DM, diabetes mellitus; FCI, Functional Comorbidity Index; FEV_1, forced expiratory volume in 1 s; FHx, family history; FPG, fasting plasma glucose; GFR, glomerular filtration rate; GGT, gamma glutamyltransferase; Hb, haemoglobin; HDL-C, high-density lipoprotein cholesterol; HTN, hypertension; IHD, ischaemic heart disease; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; PA, physical activity; Ph, phosphorus; RBC, red blood cell; SBP, systolic blood pressure; TC, total cholesterol; ; TG, triglycerides; vWF, von Willebrand factor; WC, waist circumference; WHR, waist-hip-ratio.

[a]Mean ages at baseline.

[b]Total numbers for the unique studies.

Table 2 Study and assay characteristics of studies contributing data to current analysis, 1995–2013

Lead author, year, reference no.	Study	Sampling method	Year of sample collection	Sample source	Fasting samples	Sample state before analysis, storage, temperature(C) if frozen	Assay method	Assay source (manufacturer)
Wannamethee, 1995[46]	BRHS	Random	1978–80	Serum	No	Fresh	NS	Technicon SMA 12/60 Autoanalyzer (Technicon Instruments Corp., Tarrytown, NY)
Wannamethee, 2013[11]	BRHS	Complete	1998–2000	Serum	Yes	Frozen, −70	Colorimetric	Hitachi autoanalyzer (Roche Diagnostics, Indianapolis, IN)
Calori, 2011[26]	Cremona	Random	1990–91	Serum	Yes	Fresh	NS	Hitachi 705 autoanalyser (Hitachi, Tokyo, Japan)
Fulks, 2008[28]	CRL	Complete	1991–2007	Serum	No	Fresh	NS	Hitachi autoanalyzer (Roche Diagnostics, Indianapolis, IN)
Lee, 2007[44]	FHS	Complete	1978–82	Serum	Yes	Frozen, −20	Spectrophotometry	Quest Diagnostics (MedpaTH, SC)
Kengne, 2012[43]	HSE/SHeS	Random	1994/95/98	Serum	No	Frozen, −70	DAX nitroanilide	NS
Ruhl, 2009[45]	NHANES III	Random	1988–94	Serum	Yes	Frozen, −20	NS	Hitachi 737 analyzer (Boehringer-Mannheim Diagnostics, Indianapolis,IN)
Tonelli, 2009[4]	NHANES III	Random	1988-–94	Serum	Yes	Frozen, −20	NS	Hitachi 737 analyzer (Roche Diagnostics, Indianapolis, IN)
Filipowicz, 2013[26]	NHANES	Random	1999–2004	Serum	Yes	Frozen, −20	Beckman Access Ostase	(Beckman Coulter Inc, Carlsbad, CA)
Loomba, 2012[49]	RBS	Complete	1984–87	Serum	Yes	Fresh	Colorimetric	In-house
Strasak, 2008[48]	VHM&PP	Complete	1985–2005	Serum	Yes	Fresh	NS	NS
Breitling, 2011[15]	WCB	Complete	1986–92	Serum	NS	Fresh	NS	Hitachi 705/717 instrument
Goessling, 2008[21]	FHS Offspring	Complete	1978–82	Serum	Yes	Fresh	Kinetic method	Beckman Liquid-Stat Reagent Kit
Schindhelm, 2007[9]	Hoorn	Random	1989–92	Serum	Yes	Fresh	Enzymatic Photometry	NS
Nakamura, 2006[47]	NHI	Complete	1989–91	Serum	NS	NS	Ultraviolet method	NS
Kim, 2004[13]	KMIC	Random	1990–92	Serum	Yes	Fresh	NS	NS
Koehler, 2011[50]	Rotterdam		1990–93	Serum	No	Fresh	-	-
Haring, 2009[16]	SHIP	Random	1997–2001	Serum	No	Fresh	Spectrophotometry	Hitachi 717 (Roche Diagnostics, Mannheim, Germany)
Ford, 2011[20]	WOSCOPS	Random	NS	Serum	NS	Fresh	Reaction rate	Hitachi 737 automated analyser

Study acronyms are provided in Table 1; NS, not stated.

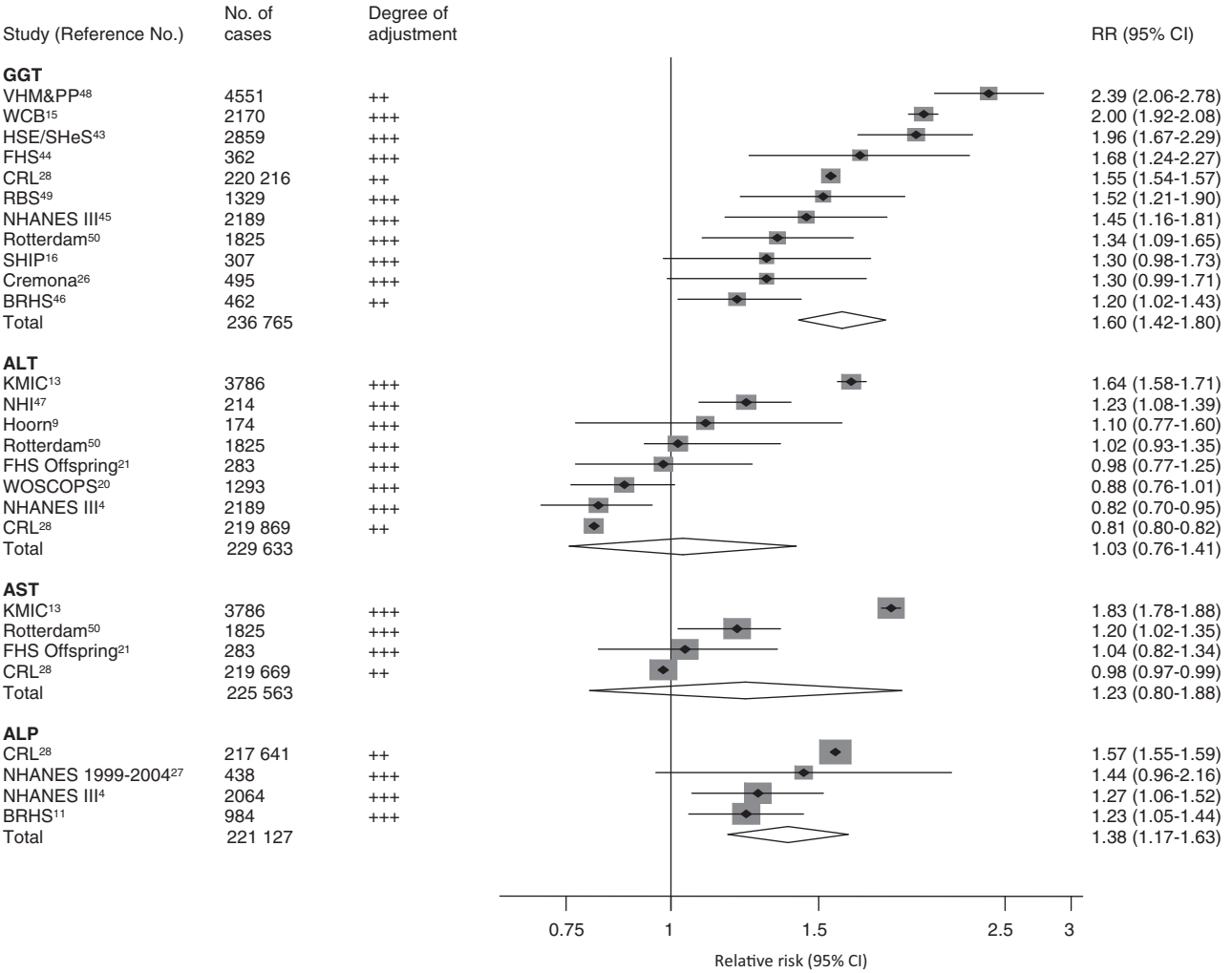

Figure 2 Relative risk for all-cause mortality in individuals in the top compared with the bottom third of levels of liver enzymes in eligible studies, 1995–2013. Study acronyms are provided in Table 1. The summary estimate presented was calculated using a random-effects model. Degree of adjustment: +, adjusted for age and/or sex; ++, further adjustment for established vascular risk factors; +++, additional adjustment for alcohol consumption, other liver markers or inflammatory markers; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval (bars); GGT, gamma glutamyltransferase; RR, relative risk

of heterogeneity. As funnel plots are unlikely to be useful in meta-analysis containing fewer than 10 studies,[51] publication bias was not evaluated for studies of the aminotransferases.

Alkaline phosphatase and all-cause mortality

Circulating ALP level was associated with a 38% higher risk of all-cause mortality outcomes (pooled RR: 1.38, 1.17–1.63) in a comparison of extreme thirds of baseline level of ALP (Figure 2). Substantial heterogeneity ($I^2 > 70\%$) was observed across the four studies. Exclusion of any single study at a time (including the largest study) from the meta-analysis did not change the direction of the association, yielding pooled RRs which ranged from 1.26 (1.13–1.41) to 1.44 (1.23–1.70). Meta-regression, stratified analyses and evaluation of publication bias were not conducted because of the limited number of studies.

Dose-response analyses

Figure 4 shows the dose-response relationships of levels of liver enzymes with risk of all-cause mortality outcomes for pooled results of studies providing relevant data. For the dose-response relation between baseline GGT level and all-cause mortality risk, examination of the figure did not suggest substantial departure from linearity though the test for a nonlinear relation was marginally significant (P for nonlinearity = 0.06; Figure 4A). The pooled RR per 5 U/l increment in GGT levels was 1.07 (1.04–1.10). In dose-response analysis stratified by geographical

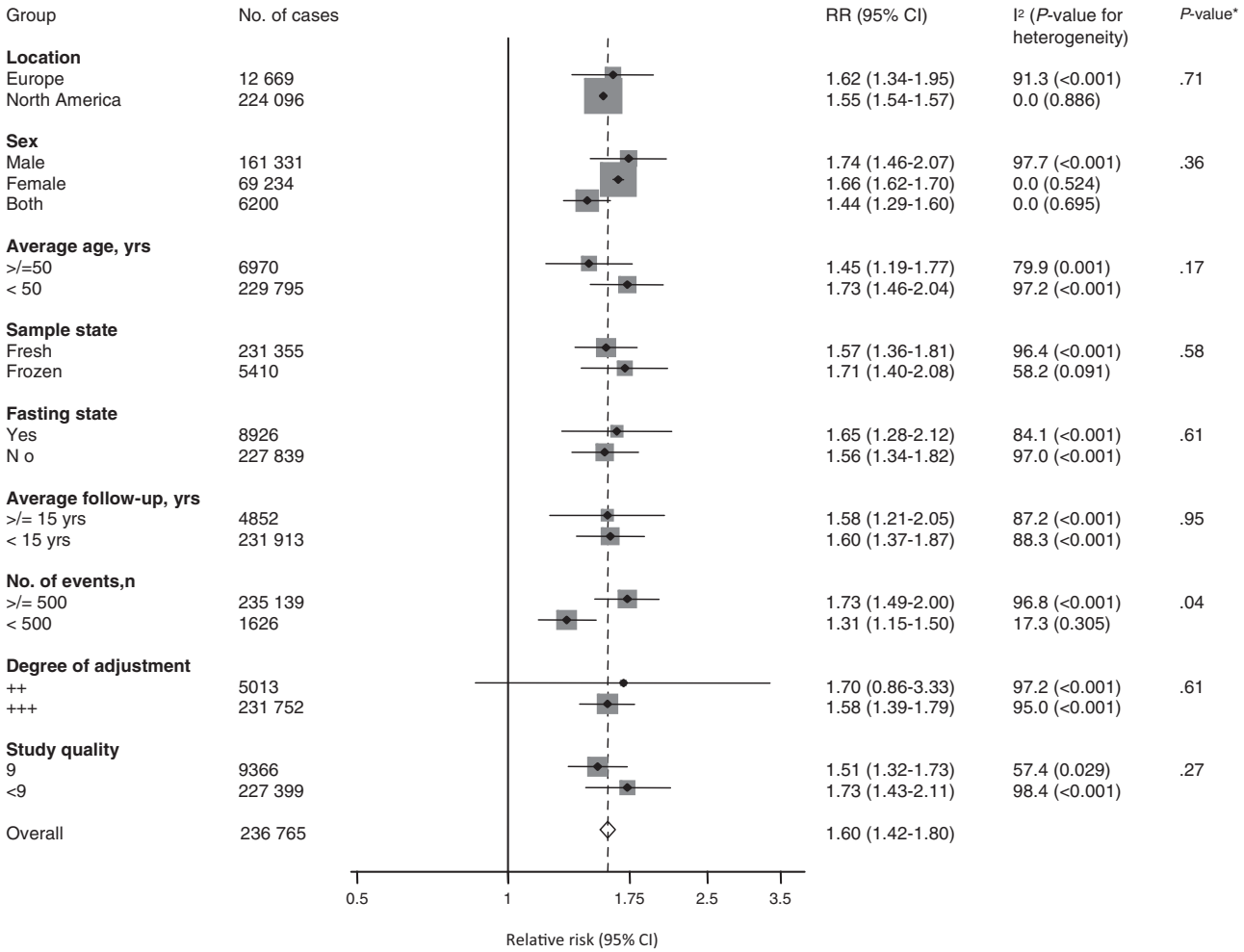

Figure 3 Prospective studies of GGT levels with all-cause mortality risk, grouped according to several study characteristics, 1995–2012. The summary estimate presented was calculated using a random-effects model; Sizes of data markers are proportional to the inverse of the variance of the relative ratio; CI, confidence interval (bars); GGT, gamma glutamyl-transferase; RR, relative risk; *, *P*-value for meta-regression

location, monotonous risk increases for all-cause mortality across increasing GGT levels were apparent in both North American and European populations (Figure 4B and C).

There was evidence for a linear relation between ALP levels and all-cause mortality risk (*P* for nonlinearity = 0.61; Figure 4D). A 5-U/l increment in ALP levels conferred a RR of 1.03 (1.01–1.06). These findings suggest that the pooling of dose-response estimates using GLST analysis for GGT and ALP with all-cause mortality outcomes was appropriate. In contrast, significant nonlinearity (*P* for nonlinearity < 0.0001; Figure 4E) was detected for ALT levels and all-cause mortality, with no effect of levels of ALT up to about 17 U/l, followed by an increase in risk beyond these levels. In analysis restricted to Asian populations (two studies), the pooled RR for all-cause mortality per 5-U/l increase in ALT level was 1.11 (1.09–1.12) and examination of the dose-response figure did not suggest substantial departure from linearity though the test for nonlinearity was marginally significant (*P* for nonlinearity = 0.05; Figure 4F). The few data points precluded us from assessing the dose-response associations in European and North American populations. The cubic spline model that included two studies on AST levels also indicated a nonlinear relation between all-cause mortality risk and AST levels (*P* for nonlinearity < 0.0001; Figure 4G).

Absolute risk differences associated with increasing levels of liver enzymes

Using the most recent mortality statistics for the USA and Europe,[52,53] the estimated absolute risk differences for all-cause mortality were 41.4 and 37.7 cases per 100 000 individuals per year for USA and Europe, respectively, for every 5-U/l increment in GGT levels. The corresponding estimates for every

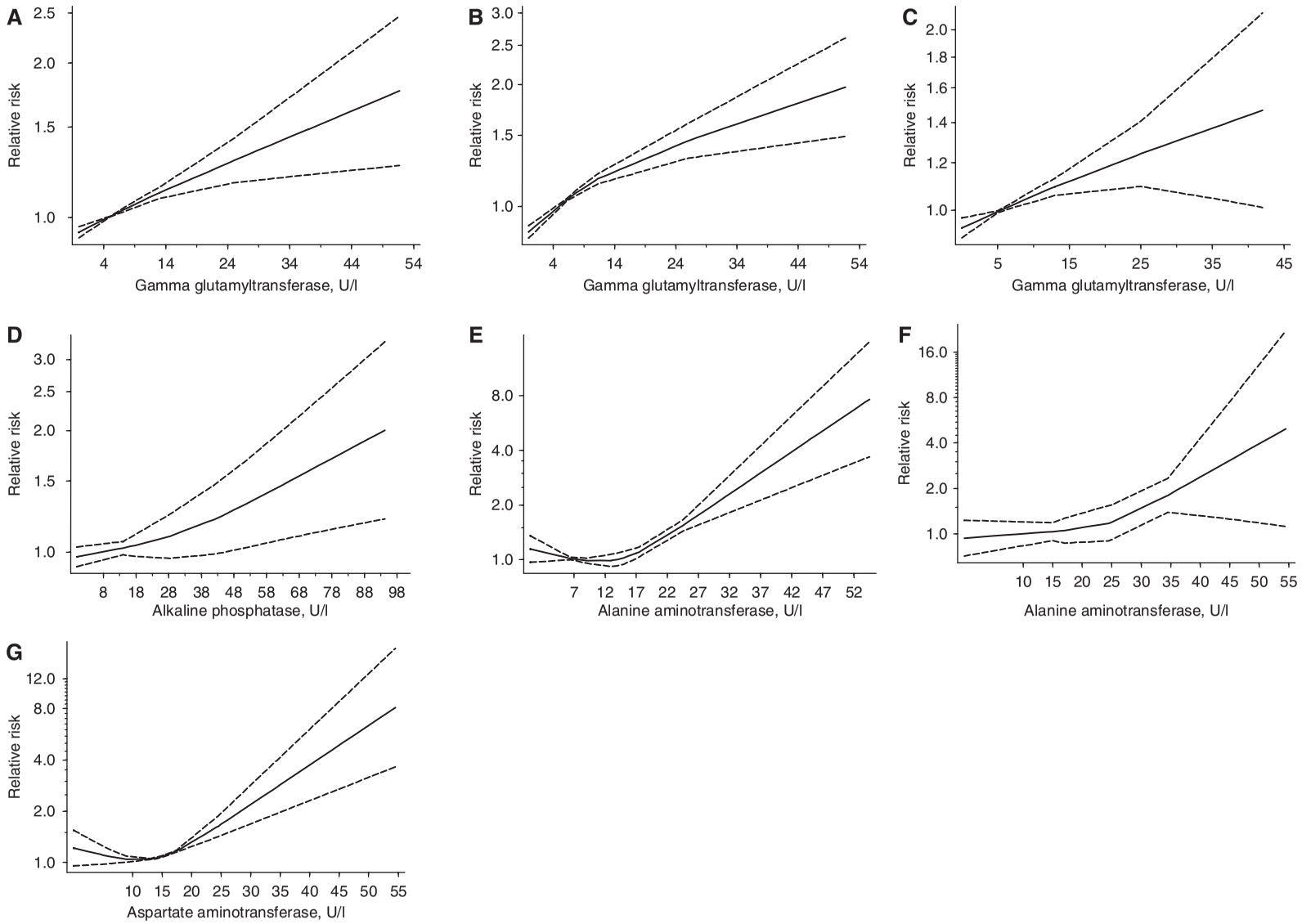

Figure 4 Dose-response relations between levels of liver enzymes and relative risks of all-cause mortality. (A) Gamma glutamyltransferase; (B) gamma glutamyltransferase in North American populations; (C) gamma glutamyltransferase in European populations; (D) alkaline phosphatase; (E) alanine aminotransferase; (F) alanine aminotransferase in Asian populations; (G) aspartate aminotransferase. Adjusted relative risks and 95% confidence intervals (CIs; dashed lines) are reported. Data were modelled with restricted cubic splines with 3 knots in random-effects dose-response models. The median values of the lowest reference ranges were used to estimate all relative risks. The vertical axes are on log scales

5-U/l increment in ALP levels were 27.6 and 6.3 cases per 100 000 individuals per year, respectively.

Discussion

We have conducted the first ever review and meta-analysis in an attempt to reliably quantify the prospective associations between baseline levels of GGT, ALT, AST and ALP with risk of all-cause mortality outcomes in general populations. This review, which involves a total of 19 unique prospective cohorts, provides the most comprehensive assessment and robust evidence to date of the associations between four commonly measured liver enzymes and the risk of future all-cause mortality outcomes. Our results show that elevated baseline levels of GGT and ALP are associated with increased risk of all-cause mortality. There was no strong evidence of any associations of the aminotransferases with all-cause mortality outcomes in pooled analyses of all the eligible studies. However, stratified analysis by geographical location showed a strong positive association of ALT for Asian populations, and the inverse for North American populations. All associations observed were largely within normal ranges of these enzymes, as the majority of the studies included in the review involved participants with normal reference levels of these enzymes measured at baseline. Risk of all-cause mortality increased monotonically with increasing levels of GGT in pooled analyses of all studies contributing relevant data and in analysis stratified by geographical location. The associations of the aminotransferases with all-cause mortality were shown to be nonlinear, with risk of mortality starting to increase at levels above 17 U/l. Pooled dose-response analysis of studies restricted to Asian populations however showed evidence of a linear association between ALT levels and all-cause mortality, with risk starting to increase significantly at ALT levels of 30 U/L and beyond.

Potential mechanisms for increased mortality in people with elevated levels of GGT have been postulated. Much of the mortality is said to be mediated by increased cardiovascular risk. Although, at normal levels, GGT counteracts oxidative stress by making cysteine available for regeneration of intracellular glutathione, recent evidence also suggests that these levels are associated with promotion of atherosclerosis through pro-oxidant and pro-inflammatory activities.[54,55] GGT enzyme activity has also been demonstrated to be directly involved in atheromatous plaque formation.[55]

Mechanisms postulated for the increased risk of all-cause mortality with elevated levels of the aminotransferases include the presence of unrecognized liver diseases which increase the risk of mortality, as liver diseases are generally asymptomatic until there are complications of advanced disease. The strong positive association of ALT with all-cause mortality demonstrated for Asians may be attributed to undiagnosed prevalent chronic liver diseases, which are comparatively more common, are leading causes of death among these populations and may have been missed during baseline recruitment. Recent reports from studies conducted in both Asian and European populations have suggested that the current reference ranges of ALT (a more sensitive indicator of liver injury) levels do underestimate the frequency of chronic liver disease, and have made recommendations for the revision of the upper limit of normal to be lowered.[56–58] As there are indications that race has no influence on ALT levels,[56,58] it is likely that these recommendations may help improve the identification of individuals with subclinical liver disease at a global level. Elevated aminotransferases may also increase the risk of mortality by increasing the risk for cardiovascular disease (CVD) and they may also reflect other serious co-morbid conditions which increase the risk of mortality.[59] Ford and colleagues[20] propose several reasons for the inverse association between ALT and mortality, including: (i) a limitation of observational studies (i.e. residual confounding); (ii) a liver-associated mechanism where low ALT levels reflect reduced functionality of the liver, which itself is associated with increased mortality; or (iii) sarcopenia, which is associated with low ALT levels and higher mortality.[60] The several mechanisms postulated remain hypothetical and may be more complex than generally appreciated, as the evidence suggests. Studies are therefore warranted to dissect the mechanistic pathways underlying these paradoxical associations. The excess mortality associated with ALP may be via promotion of vascular calcification, pro-inflammatory activities or subclinical liver dysfunction.[4]

Our findings are highly relevant and may have several implications. They underscore a potentially deleterious role of increasing levels of liver enzymes (particularly GGT and ALP) within normal ranges, on future risk of all-cause mortality outcomes in general populations. We also found the absolute risk differences for death associated with every 5-U/l increment in GGT level to be approximately 41 and 38 cases per 100 000 individuals per year for the USA and Europe, respectively, with lower corresponding risk differences for ALP. Altogether, these findings should renew epidemiological interest in the potential prognostic value of liver enzymes in risk assessment, as there has not been sufficient evidence to generate recommendations for clinical practice.

The strengths and potential limitations of this review and meta-analyses deserve mention. We employed stringent inclusion criteria and only included studies that reported recruitment of participants from approximately general populations, therefore minimizing any effects of clinically evident pre-existing disease on levels of liver enzymes. However, we cannot completely rule out the existence of

undiagnosed prevalent diseases in the study populations at baseline. We employed standardized risk estimates from all contributing studies to allow a consistent combination of estimates across studies. The very large number of total events provided high statistical power to quantitatively assess the associations between the liver enzymes and all-cause mortality risk. In meta-analysis of published studies, publication bias is of concern as small studies with null results often tend not to be published. To minimize bias due to unpublished results, we contacted several authors who provided results of their unpublished data. Visual inspection of plots and formal tests demonstrated no statistical evidence of publication bias or small study effects.

One of the main limitations of this review was the inability 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 across individual studies. However, all but three studies (including the single largest study) reported estimates for the highest degree of adjustment defined in our study (+++) and we combined fully adjusted models in our meta-analyses (Figure 2). There was a potential that the single study contributing over 90% of data to the present review may have unduly influenced our findings. However, sensitivity testing excluding this large study in all analyses yielded comparable results, indicating the robustness of our findings. 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. There are data to suggest that the levels of these enzymes in individuals can fluctuate considerably over time[61]; hence, the associations demonstrated may be even stronger. Studies are therefore needed with serial measurements of these liver enzymes to be able to adjust for regression dilution bias.

There was substantial heterogeneity among the available prospective studies. Given this, it was debatable whether pooled estimates should be presented rather than reporting estimates in relevant subgroups, as the presence of heterogeneity makes pooling of risk estimates data somewhat controversial. We however systematically explored and identified the possible sources of heterogeneity using stratified analyses, meta-regression and sensitivity analyses. The heterogeneity among the available prospective studies appeared to have been contributed by geographical location, study size and study quality. We presented pooled RRs for the relevant subgroups and the results showed that the strong positive association demonstrated for GGT and all-cause mortality risk was consistent in various subgroups and in sensitivity analyses. The limited number of studies for ALT precluded us from investigating the potential sources of heterogeneity in greater detail, but the available data showed that this inconsistency was partly due to geographical variations in the association. Additionally, limiting the analyses to only studies of the highest quality did not substantially change the overall estimate but did substantially reduce the degree of heterogeneity between study findings. Though the meta-analysis was very comprehensive, it was based on data from published reports, preventing the undertaking of more in-depth analyses. The results should therefore be interpreted in context of the limitations available. The limitations notwithstanding, this is the first prospective evaluation of the associations of liver enzymes with risk of all-cause mortality outcomes in approximately general populations, using a meta-analytic approach. More detailed analyses in a broader range of circumstances and exploration of potential sources of heterogeneity require collaborative pooling of individual participant data from prospective studies.[62]

In conclusion, available evidence suggests positive independent associations of baseline circulating levels of GGT and ALP with all-cause mortality risk, consistent with linear dose-response relationships. Any association of AST with all-cause mortality risk is comparatively moderate and nonlinear, and requires further investigation. There are geographical variations in the associations of ALT with all-cause mortality risk, which require further data for confirmation. The overall findings may have substantial implications for overall survival in populations. Measurement of these liver enzymes, particularly assays for GGT and ALP, may serve as prognostic tools for the long-term prediction of mortality in clinical practice. They may also serve as screening tools to identify individuals at high risk of dying from all-causes. Further work is required to establish these roles. In the absence of such data, however, slightly elevated levels of these enzymes even within normal ranges in individuals should be an alert for further clinical evaluation.

Supplementary Data

Supplementary data are available at *IJE* online.

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Conflict of interest: None declared.

KEY MESSAGES

- Available epidemiological data indicate positive, continuous and independent associations of baseline circulating levels of GGT and ALP with risk of future all-cause mortality.
- There are geographical variations in the association of ALT level with all-cause mortality. Further data are needed to elucidate this association.
- The current epidemiological data do not support an association between AST level and all-cause mortality outcomes.

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