American Journal of Epidemiology

Vol. 178, No. 2
DOI: 10.1093/aje/kws469
Advance Access publication:
May 31, 2013

Systematic Reviews and Meta- and Pooled Analyses

Liver Aminotransferases and Risk of Incident Type 2 Diabetes: A Systematic Review and Meta-Analysis

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Initially submitted June 11, 2012; accepted for publication November 28, 2012.

We evaluated the associations of liver aminotransferases with risk of type 2 diabetes (T2D) in general populations by conducting a systematic review and meta-analysis of published prospective studies. Studies were identified in a literature search of PubMed, EMBASE, and Web of Science from 1950 through October 2012. Of the 2,729 studies reviewed, 17 studies involving 60,359 participants and 3,890 incident T2D events were included. All of the studies assessed associations between alanine aminotransferase (ALT) level and T2D, with heterogeneous findings ($I^2 = 88\%$, 95% confidence interval (CI): 82, 92; $P < 0.001$). The pooled fully adjusted relative risk of T2D was 1.26 (95% CI: 1.14, 1.41) per 1-standard-deviation change in log baseline ALT level. This association became nonsignificant after trim-and-fill correction for publication bias. Nine studies evaluated associations between aspartate aminotransferase (AST) levels and T2D risk, with a corresponding relative risk of 1.02 (95% CI: 0.99, 1.04). The relative risk of T2D per 5-IU/L increase in ALT level was 1.16 (95% CI: 1.08, 1.25). Available data indicate moderate associations of ALT with risk of T2D events, which may be attributable to publication bias. There was no evidence for an increased risk of T2D with AST. Large prospective studies may still be needed to establish the magnitude and nature of these associations.

aminotransferases; liver enzymes; meta-analysis; risk; type 2 diabetes

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; MOOSE, Meta-analysis of Observational Studies in Epidemiology; T2D, type 2 diabetes.

The liver enzymes, namely γ-glutamyltransferase, the aminotransferases, and alkaline phosphatase, have been reported to be associated with increased risk of type 2 diabetes (T2D). Although γ-glutamyltransferase is a less specific marker of liver function, several reports have indicated that among the liver enzymes, it is the strongest risk indicator for T2D (1–6). The nature and magnitude of the associations of liver aminotransferases with T2D are less certain. The liver aminotransferases—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—are both found in the liver, in serum, and in various organ tissues. Their assays are common laboratory tests that are used to screen for liver diseases (7). ALT is found predominantly in the liver, and its serum levels become elevated whenever disease processes affect liver cells; it is considered a more specific marker for liver injury than AST (8). ALT also appears to be a better marker for accumulation of liver fat (3, 9), which is known to precede the development of T2D (10).

The aminotransferases, particularly ALT, are markers of nonalcoholic fatty liver disease, which is also known as the hepatic manifestation of the metabolic syndrome (11, 12). Nonalcoholic fatty liver disease is a syndrome characterized by hepatic steatosis with varying degrees of necroinflammation and fibrosis and which develops in the absence of substantial alcohol intake (11). It is the most common cause of elevated levels of liver enzymes (13, 14), and it is associated with insulin resistance (15). The measurement of these enzymes involves well-standardized, simple, inexpensive, and routine tests with no requirement for fasting prior to venipuncture; therefore, it is of interest to establish whether

such liver enzymes are associated with development of T2D. It has also been suggested that they could be included in diabetes risk prediction scores (16).

Several epidemiologic studies have found associations between ALT level and increased risk of future T2D events (3, 5, 6, 16, 17). In a previous review, Fraser et al. (1) attempted to quantify the association between ALT and subsequent risk of T2D by synthesizing available data from prospective studies. This review was a literature-based meta-analysis of 11 prospective population-based studies involving a total of 1,999 incident T2D events. Fraser et al. reported an 85% higher risk of incident T2D per unit increase in log ALT level in fully adjusted analyses (1). Additional prospective studies have since been published, and their results have been inconsistent. Data on the association between AST level and risk of incident T2D are limited and uncertain (3, 6, 17, 18). To our knowledge, no systematic review and meta-analysis has yet been conducted to quantify the association between AST and T2D outcomes. There is continued debate on this topic, and therefore it warrants in-depth investigation.

In this context, we carried out a systematic review and meta-analysis of prospective population-based studies to evaluate the associations between both liver aminotransferases and risk of T2D. Additionally, we also conducted dose-response meta-analyses of the associations of both liver aminotransferases with T2D risk. There is compelling evidence of a strong association between γ-glutamyltransferase and risk of incident T2D, which was confirmed in the previous meta-analysis (1); therefore, it is not addressed here. The association between alkaline phosphatase and T2D risk is also not addressed, since, to the best of our knowledge, only a few studies to date (2, 4, 19) have evaluated these associations in general populations. It is a novel research area, and more studies involving individual participant data are needed before results can be pooled.

MATERIALS AND METHODS

Data sources and search strategy

We performed a systematic review and meta-analysis of studies that evaluated the association between liver aminotransferase levels and T2D in adults, using the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (20) for the conduct of meta-analyses of observational cohort studies. We searched the PubMed, EMBASE, and Web of Science databases for prospective (cohort or "nested case-control") studies that evaluated associations between ALT and AST and the risk of T2D from January 1950 through October 2012. In our search strategy, we crossed the terms "alanine aminotransferase" and "aspartate aminotransferase" (and similar terms) with "type 2 diabetes" (and similar terms). There were no language restrictions. The reference lists of retrieved articles were manually scanned for additional studies. Studies were included if they had at least 1 year of follow-up and recruited participants from approximately general populations (i.e., did not select participants on the basis of preexisting diabetes or known liver disease). Both published and unpublished studies were eligible for inclusion.

Where available, data were abstracted on the name of the study, publication date, geographical location, population source, time of the baseline survey, sample population, study design, sample source (plasma/serum), nature of the sample (fresh or frozen and storage temperature), assay type and source, case definition, sample size, numbers of T2D events, numbers of controls, mean age at baseline, sex, mean duration of follow-up, degree of adjustment for potential confounders, and summary statistics, using a standardized abstraction form. Reported risk estimates from fully adjusted (age; sex; classical diabetes risk factors, including alcohol consumption; and/or levels of liver enzymes) analyses were extracted. The literature search and data extraction were conducted by 2 authors. Each article was assessed using the inclusion criteria above; any disagreement regarding eligibility of an article was discussed, and agreement was reached by consensus with a third author. Study authors were contacted for additional data when required.

Statistical analysis

The relative risk (and its 95% confidence interval) was used as the common measure of association across studies. Reported study-specific relative risks were converted to a consistent comparison using a standard statistical method (21). Because there was evidence for a linear association of the liver aminotransferases with T2D events (6, 22), risk estimates for each prospective study were calculated per 1-standard-deviation change in the log of the baseline distribution of aminotransferase values. The standard deviations for baseline log of aminotransferase values were equivalent to approximately 2-fold higher circulating aminotransferase levels. Log risk estimates were transformed assuming a normal distribution, with the log risk ratio for a 1-standard-deviation 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). Because most of the studies presented relative risks for quantiles of ALT or AST compared with the lowest quantile, we also calculated the corresponding pooled estimates involving comparisons between the top third and bottom third of the population's baseline distribution of aminotransferase values. Hazard ratios, relative risks, and odds ratios were assumed to approximate the same measure of relative risk. When risk estimates were reported separately for men and women in a study, the overall estimate for the study was pooled using fixed-effects analysis. Summary relative risks were pooled using a random-effects model to minimize the effects of heterogeneity (23).

For the dose-response meta-analyses of the associations of ALT and AST with T2D risk, we used the method described by Greenland and Longnecker (24) and Orsini et al. (25) to compute study-specific slopes from the correlated natural logs of the relative risks across categories of aminotransferase levels. Only studies that reported the number of cases and person-years or noncases and the relative risk with its variance estimate for at least 3 quantitative exposure categories were included. The median or mean level of ALT and AST for each category was assigned to

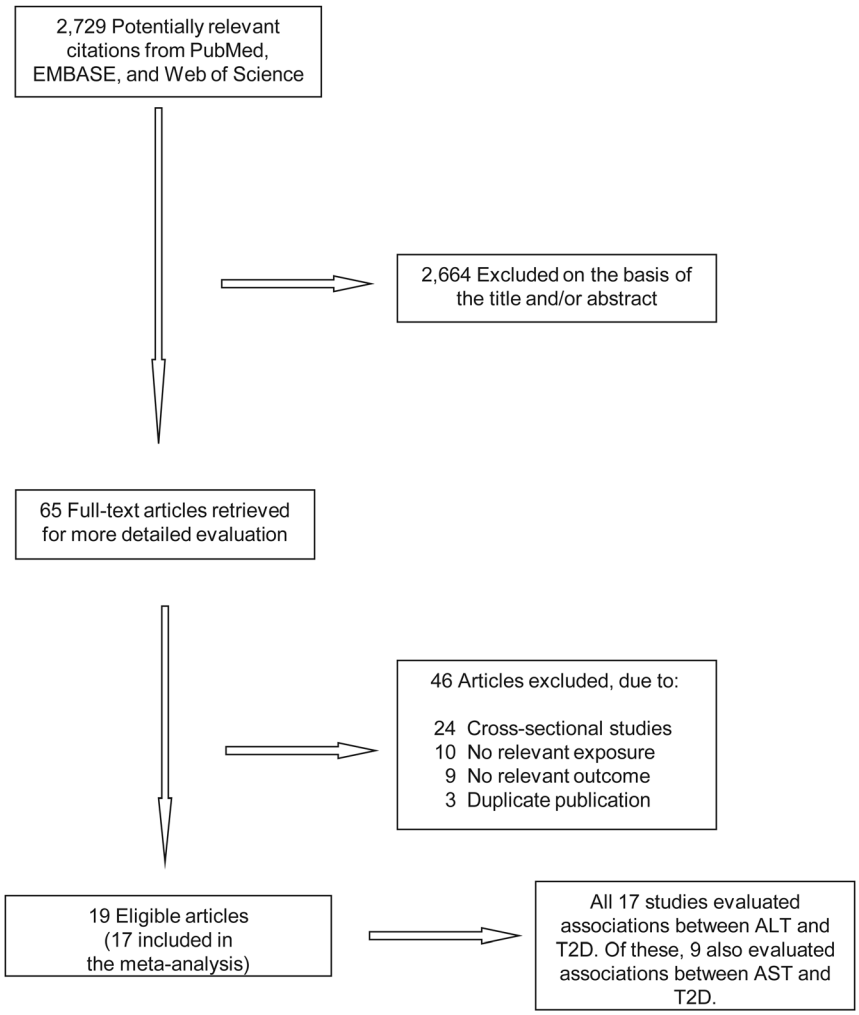

Figure 1. Search strategy used to select studies on the association between liver aminotransferase levels and type 2 diabetes (T2D) for the current review, 2003–2009. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

each corresponding relative risk. If data were not available, we estimated the median value using the midpoint of each category. When the highest or lowest category was open, we assumed it to have the same amplitude as the adjacent category. Dose-response slopes for an increment of 5 IU/L were estimated for both liver aminotransferases.

Statistical heterogeneity across studies was quantified using the χ^2 and I^2 statistics (26, 27). The I^2 statistic, which provides a measure of the percentage of overall variation attributable to between-study heterogeneity, was reported as a measure of consistency across the studies. Study-level characteristics, including geographical location, population source, sex differences, sample source, duration of follow-up, number of incident T2D events, and degree of adjustment, were prespecified as characteristics for assessment of heterogeneity, which was conducted using random-effects meta-regression (28). We also performed a sensitivity analysis by repeating the analysis after excluding the studies that had the largest effect on the overall result. We conducted a cumulative meta-analysis of the studies ordered chronologically to assess the sequential contributions of studies published over time (29). Furthermore, we assessed the potential for publication bias through formal tests, namely Begg's funnel plots (30) and Egger's regression symmetry test (31). Finally, we adjusted for the effect of publication bias by means of Duval and Tweedie's nonparametric trim-and-fill method (32), which imputes data for hypothetical small missing null or negative studies. All analyses were conducted using Stata, version 12 (StataCorp LP, College Station, Texas).

RESULTS

Characteristics of studies

Our initial search identified 2,729 potentially relevant citations. After screening of titles and abstracts and more

Table 1. Characteristics of Published Prospective Studies Evaluating the Association Between Liver Aminotransferase Levels and Incident Type 2 Diabetes, 2003–2009

First Author, Year (Reference No.)	Name of Study or Source of Participants	Location of Study	Year(s) of Baseline Survey	Baseline Age Range, years	% Male	Duration of Follow-up, years	Total No. of Participants	No. of Cases	Covariates
Fraser, 2009 (1)	British Women's Heart Health Study	United Kingdom	1999–2001	60–79	0	7	3,041	112	Age, alcohol, childhood and adult social class, physical activity, smoking, HOMA-IR, waist:hip ratio, triglycerides, HDL cholesterol, SBP
Wannamethee, 2005 (5)	British Regional Heart Study	United Kingdom	1998–2000	60–79	100	5	3,500	100	Age, social class, physical activity, smoking, alcohol, preexisting coronary heart disease/ stroke, use of statins, BMI, HOMA-IR
Cho, 2007 (33)	Korean Health and Genome Study	South Korea	2001–2002	40–69	47	2	8,750	542	Age, BMI, SBP, FHxD, smoking, alcohol, exercise status, FPG, triglycerides, HDL cholesterol, HOMA-IR, high-sensitivity CRP
Andre, 2005 (3)[a]	DESIR Study	France	1994–1996	30–65	49	3	4,201	89	Age and GGT
Ford, 2008 (22)	EPIC-Potsdam Study	Germany	1994–1998	35–65	43	7	3,011	787	Age, sex, educational status, smoking, alcohol, occupational and sports activity, WC, BMI, SBP, total cholesterol, HDL cholesterol, CRP, glucose
Goessling, 2008 (34)[a]	FHS Offspring Study	United States	1978–1982	44[b]	44	20	2,138	208	Age, sex, smoking, menopause, alcohol, BMI, glucose, interim weight change
Monami, 2008 (35)[a]	FIBAR Study	Italy	2001–2003	40–75	43	3	2,662	36	Age, sex, alcohol, smoking, FPG
Doi, 2007 (36)[a]	Hisayama Study	Japan	1988	40–79	40	9	1,804	135	Age, FHxD, fasting insulin, BMI, waist:hip ratio, total cholesterol, HDL cholesterol, triglycerides, CRP, hypertension, alcohol, smoking, physical activity, other liver enzymes
Schindhelm, 2005 (37)	Hoorn Study	Netherlands	1989–1992	50–74	NA	6	1,289	123	Age, sex, follow-up duration, WC, BMI, alcohol, fasting plasma insulin, 2-hour postload glucose

Table continues

Table 1. Continued

First Author, Year (Reference No.)	Name of Study or Source of Participants	Location of Study	Year(s) of Baseline Survey	Baseline Age Range, years	% Male	Duration of Follow-up, years	Total No. of Participants	No. of Cases	Covariates
Kim, 2009 (38)	Health promotion center	South Korea	2000	20–79	62	5	3,556	92	Age, sex, FHxD, BMI, SBP, alcohol, smoking, exercise, GGT
Hanley, 2004 (17)[a]	Insulin Resistance Atherosclerosis Study	United States	1992–1994	40–69	74	5	906	148	Age, sex, ethnicity, clinical center, alcohol, smoking, WC, triglycerides, HDL cholesterol, impaired glucose tolerance, insulin sensitivity index, acute insulin response
Sato, 2008 (18)[a]	Kansai Healthcare Study	Japan	2000–2001	40–55	100	4	8,576	878	Age, BMI, FPG, smoking, parental history of diabetes, walking to work, regular leisure-time physical activity
Nannipieri, 2005 (4)[a]	Mexico City Diabetes Study	Mexico	1987–1992	35–64	40	7	1,233	94	Age, BMI, WC, fasting insulin, alcohol
Nakanishi, 2004 (6)[a]	Office workers	Japan	1994	35–59	100	7	3,260	276	Age, FHxD, BMI, alcohol, smoking, physical activity, FPG, white blood cell count, other liver enzymes
Lee, 2003 (39)	Steelworkers	South Korea	1994; 1998	25–55	100	4	4,088	83	Age, BMI, smoking, physical activity, FHxD, fasting serum glucose, alcohol
Jiamjarasrangsi, 2008 (19)[a]	University hospital	Thailand	2001–2005	35–60	19	3	2,370	48	Age, sex, education, alcohol, smoking, FHxD, BMI, SBP, diastolic blood pressure, cholesterol, triglycerides, FPG, blood urea nitrogen, uric acid, follow-up time, AST (or ALT)
Sattar, 2004 (16)	WOSCOPS	United Kingdom	NA	45–64	100	5	5,974	139	Age, BMI, smoking, SBP, total cholesterol:HDL cholesterol ratio, pravastatin treatment, triglycerides, alcohol, FPG
Total							60,359	3,890	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DESIR, Data from Epidemiological Study on the Insulin Resistance Syndrome; EPIC, European Prospective Investigation into Cancer and Nutrition; FHS, Framingham Heart Study; FHxD, family history of diabetes; FIBAR, Firenze Bagno a Ripoli; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; NA, not available; SBP, systolic blood pressure; WC, waist circumference; WOSCOPS, West of Scotland Coronary Prevention Study.

[a] Also evaluated AST levels and type 2 diabetes.

[b] Mean age at baseline.

detailed evaluation of full texts, we identified 19 studies as potentially relevant for our analyses, but only the results of 17 studies were meta-analyzed due to missing information and failure to obtain additional data from authors in 2 studies (Figure 1). All the studies meta-analyzed in the previous review (1) were included in our review, with the addition of other published studies.

Table 1 provides details on all of the reviewed studies (1, 3–6, 16–19, 22, 33–39), which comprised a total of 60,359 participants and 3,890 incident T2D outcomes. All 17 studies were prospective cohort studies published in 2003–2009 and carried out in Europe, Asia, North America, or South America. Study participants' ages ranged from 25 years to 79 years. Duration of follow-up for T2D endpoints ranged from 3 years to 20 years, with a median duration of follow-up of 5 years. All studies reported on ALT levels and incident T2D. Eleven of the studies also reported on AST levels and T2D risk, but the results of 9 studies (3, 4, 6, 17–19, 34–36) were meta-analyzed because of failure to obtain missing information for 2 studies. All studies but one adjusted for age and classical diabetes risk factors, with 4 additionally adjusting for levels of other liver enzymes. Appendix Table 1 details the assay characteristics of measured liver aminotransferase levels from studies contributing to the analysis.

ALT and risk of incident T2D

The combined relative risk of T2D per 1-standard-deviation change in log baseline ALT levels for the 17 studies in fully adjusted analysis was 1.26 (95% confidence interval (CI): 1.14, 1.41) (Figure 2). Alternatively expressed, the relative risk in a comparison between extreme thirds of baseline ALT values was 1.66 (95% CI: 1.31, 2.09). There was evidence of substantial heterogeneity among the findings of the contributing studies ($I^2 = 88\%$, 95% CI: 82, 92; $P < 0.001$), with little of it being explained by geographical location, population source, sex differences, average duration of follow-up, number of T2D cases, sample source, or level of adjustment (Figure 3). Exclusion of any single study from the meta-analysis had little effect on the results. In cumulative meta-analysis, there was little change in the relative risks associated with ALT on T2D endpoints with the addition of new studies after 1988, the year the first prospective study to evaluate the ALT-T2D association was published (40). The funnel plot revealed evidence of small-study effects which was further confirmed by Egger's regression symmetry test ($P < 0.001$). The trim-and-fill technique, which was used to adjust for publication bias, imputed data for 9 missing studies, which produced a symmetrical funnel plot. The pooled analysis incorporating the hypothetical studies showed a nonsignificant association between ALT and T2D events (relative risk = 1.03, 95% CI: 0.94, 1.13).

AST and risk of incident T2D

The combined relative risk of T2D per 1-standard-deviation change in log baseline AST values for the 9 studies in fully adjusted analysis was 1.02 (95% CI: 0.99, 1.04) (Figure 4). The corresponding relative risk in a comparison of extreme thirds of baseline AST values was 1.03 (95% CI: 0.98, 1.09). There was also evidence of substantial heterogeneity among the findings of the contributing studies ($I^2 = 65\%$, 95% CI: 30, 83; $P = 0.003$) and small-study effects (Egger's test: $P = 0.042$).

Dose-response meta-analyses

Eleven studies reporting relative risks for ALT exposure on at least 3 levels were included in the dose-response analysis. With a total of 3,169 T2D cases among 49,090 participants, the pooled relative risk of T2D per 5-IU/L increment in ALT level was 1.16 (95% CI: 1.08, 1.25), with evidence of substantial heterogeneity ($I^2 = 88\%$, 95% CI: 80, 93; $P < 0.001$) (Figure 5). Five studies (1,426 T2D cases among 20,211 participants) were included in our analysis of dose-response between AST and T2D risk. An increment of 5 IU/L in AST levels was not significantly associated with increased T2D risk (relative risk = 1.00, 95% CI: 0.99, 1.01), and there was no evidence of heterogeneity ($P = 0.55$).

DISCUSSION

In our updated meta-analysis of published prospective studies assessing the associations between ALT levels and subsequent risk of T2D in general populations, approximately 2-fold higher circulating baseline ALT levels were associated with an approximately 30% higher risk of T2D events in fully adjusted results. In a dose-response analysis of 11 studies, there was a 16% increased risk of T2D per 5-IU/L increase in ALT levels. Our results showed evidence of small-study effects and substantial heterogeneity among contributing studies. However, the association became nonsignificant after correction for publication bias using trim-and-fill techniques. Our pooled estimate indicated a modest association of ALT level with T2D risk as compared with the pooled estimate from the previous review (1). When we corrected for publication bias, the effect became statistically nonsignificant, and the effect size was substantially reduced. The trim-and-fill method makes numerous assumptions (41), and therefore it requires careful interpretation, especially given the fact that there was considerable heterogeneity between the contributing studies. In light of this and the strong evidence of publication bias, our meta-analysis should be interpreted as indicating no compelling association between ALT levels and risk of incident T2D events.

Several factors may be responsible for the difference between our results and those from the previous meta-analysis (1). There were 2 larger earlier studies (33, 37) that were not included in the previous meta-analysis. In addition, prior to publication of the previous meta-analysis or since its publication, 4 comparatively larger prospective cohort studies evaluating the associations of ALT level with T2D risk have been published (18, 19, 34, 38). Altogether, these studies cumulatively doubled the number of T2D cases in our review in comparison with the previous meta-analysis. The publication of these large-scale prospective studies may be responsible for our pooled results.

Not all studies identified in the systematic review could be included in the meta-analysis as a result of missing information and failure to obtain additional data from the authors involved.

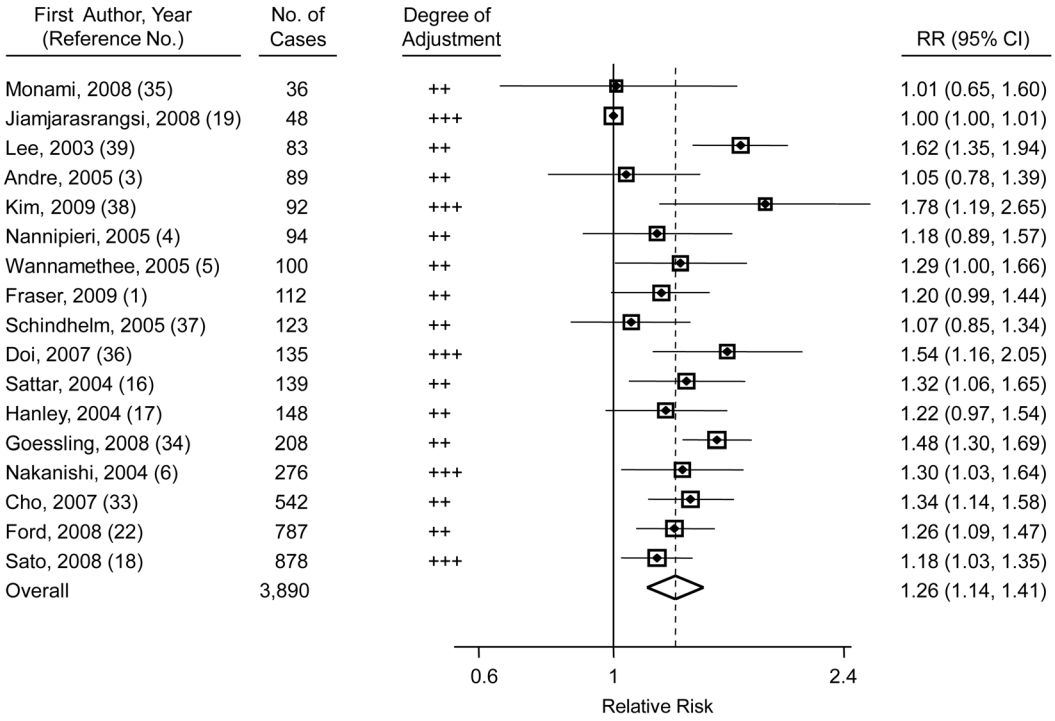

Figure 2. Results from prospective studies of alanine aminotransferase level and type 2 diabetes risk, 2003–2009. The summary estimate presented (diamond) was calculated using a random-effects model. Assessment of heterogeneity: $\chi^2_{16} = 128.39$, $P < 0.001$, $I^2 = 88\%$ (95% confidence interval (CI): 82, 92). Degree of adjustment: ++, adjusted for age, sex, diabetes risk factors, and alcohol consumption; +++, additionally adjusted for other liver enzymes. RR, relative risk. Bars, 95% CI.

No evidence of a significant association was found for AST level and T2D risk in the pooled analysis of 9 studies. There was also substantial heterogeneity among contributing studies and evidence of small-study effects. These findings and the diverging results from several prospective population-based studies cast doubts about the true nature of the association between serum aminotransferase levels and risk of T2D. The associations appear to have been overestimated in previous studies. Therefore, additional studies may be required to investigate further and clarify these associations. In evaluating the association between ALT level and T2D, little of the heterogeneity among the contributing studies was explained by any of the study characteristics, including duration of follow-up, as demonstrated by the previous review. There are speculative suggestions that duration of follow-up may play a role in the association between liver aminotransferases and T2D risk. This probably reflects the duration of elevated liver enzyme levels and the long-term effect of these enzymes on the dysglycemic process. Analysis of primary data may be required to confirm this.

Several mechanisms have been implicated as underlying the association between serum liver enzymes and T2D. An elevated ALT level, even within the normal range, correlates strongly with accumulation of liver fat (3, 9) and is considered a marker of nonalcoholic fatty liver disease (15), a disorder thought to be part of the pathogenic mechanism for diabetes development (16). Elevated liver fat is linked to hepatic insulin resistance (42, 43), which is known to cause an increase in hepatic glucose output (15). These pathophysiological changes antedate the development of T2D. The mechanism underlying the association of AST with T2D risk has been difficult to identify, though it has been reported that AST is also associated with liver fat accumulation, albeit to a lesser extent than ALT. Kim et al. (38) demonstrated significant associations between serum levels of liver enzymes (ALT and γ-glutamyltransferase) and T2D risk after excluding participants with ultrasonographically diagnosed fatty liver. This suggests that fatty liver may be an independent risk factor for T2D development and that other mechanisms could be underlying the associations between liver enzymes and T2D risk. It has also been suggested that elevated liver enzyme levels reflect underlying chronic inflammation, which may impair insulin signaling in the liver and other organs (10, 44). The mechanisms of liver enzymes, especially aminotransferase levels, in relation to T2D development need to be reviewed, and this is a topic for continued investigation.

The strengths and potential limitations of this review and meta-analyses merit careful consideration. Previous studies have evaluated the associations between serum liver aminotransferase levels and risk of T2D, but to our knowledge our meta-analysis was the first attempt to pull together the results of several different studies that evaluated the associations between both liver aminotransferases (ALT and

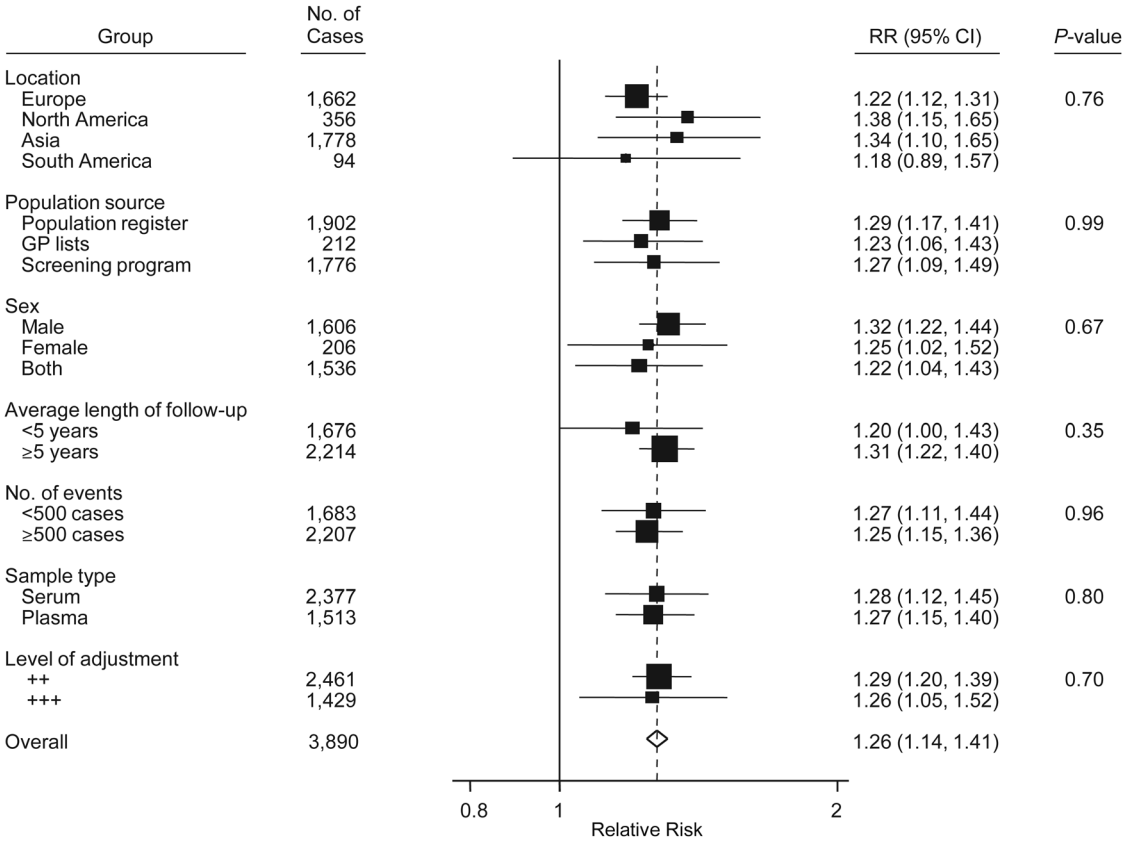

Figure 3. Results from prospective studies of alanine aminotransferase level and type 2 diabetes risk, grouped according to several study characteristics, 2003–2009. The summary estimate presented (diamond) was calculated using a random-effects model. The sizes of the data markers are proportional to the inverse of the variance of the relative risk. Numbers for sex do not add up to the total, since 1 study did not provide numbers for males and females separately. Degree of adjustment: ++, adjusted for age, sex, diabetes risk factors, and alcohol consumption; +++, additionally adjusted for other liver enzymes. GP, general practitioner; RR, relative risk. Bars, 95% confidence interval.

AST) and T2D risk. This was the first meta-analysis to evaluate the association between serum AST levels and risk of T2D, possibly because of the limited number of studies available and the novel nature of the topic. Our updated meta-analysis of the association between ALT and T2D was larger than the previous one, with the addition of several important new studies, and it clarified the conflicting results among previous studies. The contributing studies used standardized assay methods for the measurement of liver aminotransferase levels (Appendix Table 1); therefore, biases due to the use of different laboratory methods were avoided.

We were unable to fully examine the impact of adjustment for all known and potential T2D risk factors and also combine models in studies that adjusted for the same set of confounders, because we relied on published data and studies varied in their degree of confounder adjustment. However, we combined results from fully adjusted models in our meta-analyses, and grouping studies by degree of reported adjustment did not indicate major differences. The included studies were carried out in several different geographical locations in Europe, Asia, North America, and South America, which increases the likelihood that these results can be generalized to white and mixed populations. However, further studies are still needed in other ethnic populations (such as cohorts from black populations). Our meta-analyses included studies that had recruited participants from approximately general populations (i.e., persons with a history of T2D at baseline were excluded), thereby reducing any effects of clinically evident preexisting disease on aminotransferase levels. It was not possible to correct the estimates for within-individual variation in liver aminotransferase levels over time, because data involving repeat measurements were not reported in the contributing studies. Given the substantial heterogeneity among the available prospective studies, it was arguable whether we should present a summary estimate rather than report estimates for relevant subgroups, since pooling of relative risk data is controversial in the presence of heterogeneity. In addition to the summary estimate, we have also reported risk estimates for the various subgroups assessed, though little of the heterogeneity was explained by any of the study characteristics.

There was also evidence of publication bias in our results, which may have inflated our estimates. We adjusted for publication bias using the trim-and-fill approach, but because

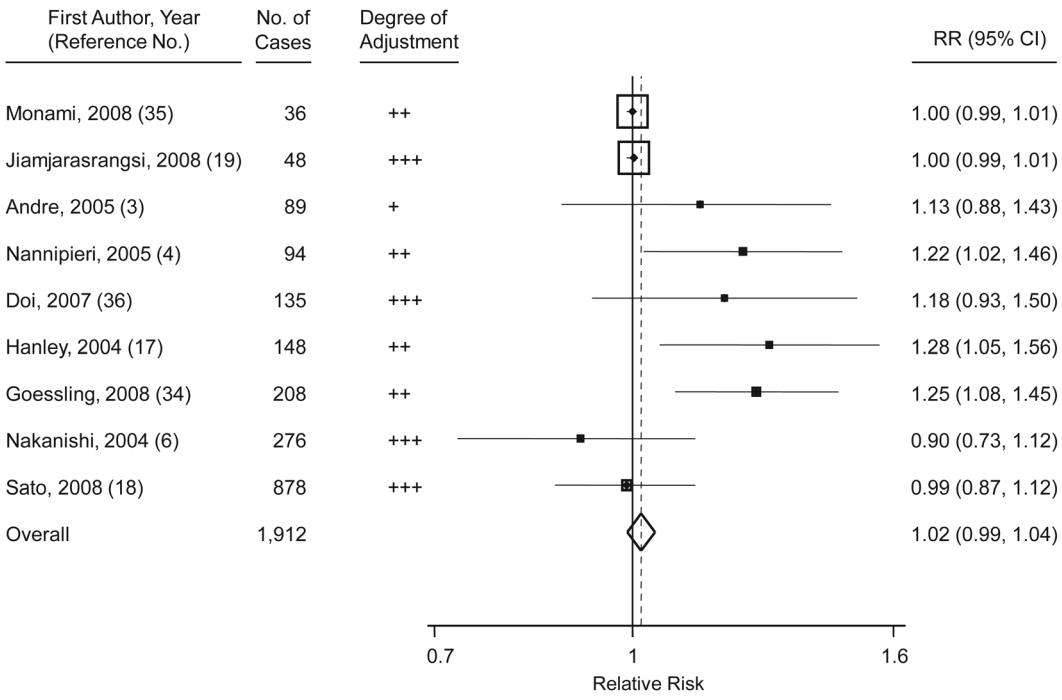

Figure 4. Results from prospective studies of aspartate aminotransferase level and type 2 diabetes risk, 2003–2009. The summary estimate presented (diamond) was calculated using a random-effects model. Assessment of heterogeneity: $\chi^2_8 = 23.12$, $P = 0.003$, $I^2 = 65\%$ (95% confidence interval (CI): 30, 83). Degree of adjustment: +, adjusted for age and sex; ++, adjusted for age, sex, diabetes risk factors, and alcohol consumption; +++, additionally adjusted for other liver enzymes. RR, relative risk. Bars, 95% CI.

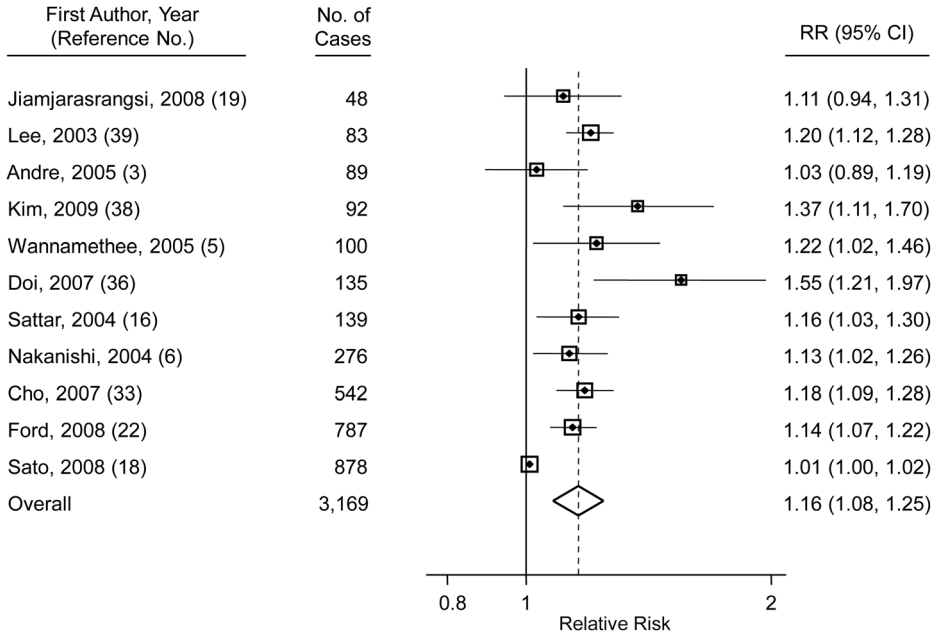

Figure 5. Relative risk (RR) of type 2 diabetes per 5-IU/L increase in alanine aminotransferase level in studies with relevant data, 2003–2009. Assessment of heterogeneity: $\chi^2_{10} = 82.37$, $P < 0.001$, $I^2 = 88\%$ (95% confidence interval (CI): 80, 93). Bars, 95% CI.

this method is known to perform poorly in the presence of substantial between-study heterogeneity (45), we have presented pooled results calculated before and after adjustment for publication bias. Although the meta-analysis was very comprehensive, it was based on data from published reports, preventing the undertaking of more in-depth analyses. In addition, residual confounding and bias cannot be addressed through pooling. Therefore, the results should be interpreted in the context of the various limitations. Collaborative pooling of individual participant data from prospective studies is needed to conduct more detailed analyses under a broader range of circumstances (46, 47).

In conclusion, the associations of liver aminotransferase levels and T2D risk appear to have been overestimated in previous studies. Available data indicate that there are moderate associations of ALT level with T2D risk, which may be due to publication bias. Our meta-analysis also suggests that ALT levels confer a dose-response effect on T2D risk. The risk of developing T2D increases by approximately 20% for every 5-IU/L increase in ALT levels. There was no evidence for an increased risk of T2D with AST level. Further studies in large general populations with long-term follow-up and repeat measurements of relevant exposures may still be warranted to establish the magnitude and direction of these associations.

ACKNOWLEDGMENTS

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We thank the following authors for providing additional data upon request: Dr. Soo Lim, Division of Endocrinology and Metabolism, Seoul National University College of Medicine (Seoul, South Korea); Dr. Wiroj Jiamjarasrangsi, Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University (Bangkok, Thailand); and Dr. Kyoko Kogawa Sato, Department of Preventive Medicine and Environmental Health, Osaka City University Graduate School of Medicine (Osaka, Japan).

Conflict of interest: none declared.

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(Appendix follows)

Appendix Table 1. Characteristics of Studies Included in the Current Analysis of the Association Between Liver Aminotransferase Levels and Type 2 Diabetes, 2003–2009

First Author, Year (Reference No.)	Name of Study or Source of Participants	Location of Study	Sampling Method	Year(s) of Sample Collection	Sample Source	Fasting Samples	Sample State Before Analysis, Storage, and Temperature (°C) if Frozen	Assay Method Used	Source (Manufacturer) of Assay
Fraser, 2009 (1)	British Women's Heart Health Study	United Kingdom	Random	1999–2001	Serum	Yes	Fresh	NS	Technicon Sequential Multiple Analyzer (Technicon Instruments Corporation, Tarrytown, New York)
Wannamethee, 2005 (5)	British Regional Heart Study	United Kingdom	Complete	1998–2000	Serum	Yes	NS	NS	Roche Hitachi 747 autoanalyzer (Roche Diagnostics, Basel, Switzerland)
Cho, 2007 (33)	Korean Health and Genome Study	South Korea	Random	2001–2002	Plasma	Yes	NS	NS	NS
Andre, 2005 (3)	DESIR Study	France	Complete	1994–1996	Serum	Yes	NS	NS	Technicon DAX 24 (Bayer Diagnostics, Puteaux, France)
Ford, 2008 (22)	EPIC-Potsdam Study	Germany	Random	1994–1998	Plasma	NS	NS	NS	ADVIA 1650 Chemistry System (Siemens Medical Solutions, Erlangen, Germany)
Goessling, 2008 (34)	FHS Offspring Study	United States	Complete	1978–1982	Serum	Yes	Fresh	Kinetic ultraviolet ray	Liquid-Stat Reagent Kit (Beckman Coulter Inc., Carlsbad, California)
Monami, 2008 (35)	FIBAR Study	Italy	Complete	2001–2003	Plasma	Yes	Fresh	Enzymatic photometry	Aeroset (Abbott Laboratories, Milan, Italy)
Doi, 2007 (36)	Hisayama Study	Japan	Complete	1988	Serum	Yes	Fresh	Kinetic ultraviolet ray	NS
Schindhelm, 2005 (37)	Hoorn Study	Netherlands	Random	1989–1992	Serum	Yes	Fresh	Enzymatic photometry	NS

Table continues

Appendix Table 1. Continued

First Author, Year (Reference No.)	Name of Study or Source of Participants	Location of Study	Sampling Method	Year(s) of Sample Collection	Sample Source	Fasting Samples	Sample State Before Analysis, Storage, and Temperature (°C) if Frozen	Assay Method Used	Source (Manufacturer) of Assay
Kim, 2009 (38)	Health promotion center	South Korea	Complete	2000	Serum	Yes	Fresh	Enzymatic photometry	Toshiba autoanalyzer (Toshiba Corporation, Tokyo, Japan)
Hanley, 2004 (17)	Insulin Resistance Atherosclerosis Study	United States	Complete	1992–1994	Plasma	Yes	NS	Enzymatic photometry	Paramax PLA instrument (Baxter Diagnostics, Chicago, Illinois)
Sato, 2008 (18)	Kansai Healthcare Study	Japan	Complete	2000–2001	Serum	Yes	NS	Enzymatic photometry	NS
Nannipieri, 2005 (4)	Mexico City Diabetes Study	Mexico	Complete	1987–1992	Serum	Yes	Fresh	Enzymatic photometry	Syncron CX 4 (Beckman Instruments, Fullerton, California)
Nakanishi, 2004 (6)	Office workers	Japan	Complete	1994	Serum	NS	Fresh	Enzymatic photometry	Olympus AU-5000 (International Reagents, Kobe, Japan)
Lee, 2003 (39)	Steelworkers	South Korea	Complete	1994; 1998	Serum	Yes	Fresh	NS	Hitachi 7170 autoanalyzer (Hitachi Ltd., Tokyo, Japan)
Jiamjarasrangsi, 2008 (19)	University hospital	Thailand	Complete	2001–2005	Serum	Yes	Fresh	Enzymatic photometry	NS
Sattar, 2004 (16)	WOSCOPS	United Kingdom	Complete	NA	Serum	NS	Fresh	Enzymatic photometry	NS

Abbreviations: DESIR, Data from Epidemiological Study on the Insulin Resistance Syndrome; EPIC, European Prospective Investigation into Cancer and Nutrition; FHS, Framingham Heart Study; FIBAR, Firenze Bagno a Ripoli; NA, not available; NS, not supplied; WOSCOPS, West of Scotland Coronary Prevention Study.