Low alanine aminotransferase and higher cardiovascular events in type 2 diabetes: analysis of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study

Running title: Low alanine aminotransferase and cardiovascular events in diabetes

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Abstract

Aims

Non-alcoholic fatty liver disease (NAFLD) is common in type 2 diabetes and associated with higher risk of cardiovascular disease. This study aimed to determine whether alanine aminotransferase (ALT) or gamma-glutamyltransferase (GGT), as markers of liver health and NAFLD, might predict cardiovascular events in this population.

Methods

Data from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study were analysed to examine the relationship between liver enzymes and incident cardiovascular events (nonfatal myocardial infarction, stroke, coronary and other cardiovascular death, coronary or carotid revascularization) over 5 years.

Results

ALT had a linear inverse relationship with the first cardiovascular event on study. After adjustment, for every standard deviation higher baseline ALT (13.2U/L), the risk of an event was 7%(95%CI 4–13, *P*=0.02) lower. Participants with ALT below and above the reference range 8–41 U/L for women and 9–59 U/L for men, had a hazard ratio of an event of 1.86(95%CI, 1.12–3.09) and 0.65(95%CI, 0.49–0.87), respectively (*P*=0.001). No relationship was found for GGT.

Conclusions

The data may indicate that in type 2 diabetes — associated with higher ALT due to prevalent NAFLD — lower ALT is a marker of hepatic or systemic frailty rather than health.

Introduction

Non-alcoholic fatty liver disease (NAFLD), ranging from simple steatosis to cirrhosis, is strongly associated with diabetes and the metabolic syndrome [1]. While not universally accepted, the potential systemic implications of NAFLD are increasingly being recognized [1]. This would include the clinically important, independent association between NAFLD and cardiovascular disease that has been asserted by many, although not all, groups [1-3]. A logical step to explore this association, has been to examine liver enzymes as predictors of cardiovascular events [4], and their use as part of risk-algorithms for cardiovascular disease has been proposed [5]. Alanine aminotransferase (ALT), has a good correlation with liver fat, as assessed by magnetic resonance spectroscopy, and is a viewed as a valid marker of necroinflammation in NAFLD [4, 6]. Both ALT and gamma glutamyl-transferase (GGT) are associated with the metabolic syndrome, insulin resistance, and diabetes and its development, but their relationship with cardiovascular outcomes is more complex [1, 2, 4, 5, 7-14].

This study sought to examine the association of baseline ALT and GGT with time to total cardiovascular events in participants of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, designed to assess the effect of fenofibrate on cardiovascular disease events in people with diabetes [15].

Patients and Methods

The study was a subsidiary analysis of the FIELD study—a double-blind, placebo-controlled trial done in 63 centres in Australia, New Zealand, and Finland [15]. In brief, 9795 participants aged 50–75 years with type 2 diabetes according to WHO criteria [16] were randomly allocated between 1998 and 2000 to once-daily micronized fenofibrate or placebo. Participants had an initial total-cholesterol concentration 3.0–6.5 mmol/L, plus

either total-cholesterol/HDL-cholesterol ratio ≥4.0 or plasma triglyceride concentration 1.0–5.0 mmol/L, and not on lipid-modifying therapy at study entry. Exclusion criteria included: blood creatinine >130 μmol/L, chronic liver disease, a cardiovascular event within 3 months of recruitment, ALT >2 times the upper limit of normal and a recent history of alcohol abuse. Total cardiovascular events was a pre-specified secondary endpoint and included nonfatal myocardial infarction, total stroke, cardiovascular death, coronary and carotid revascularization [15].

Baseline characteristics

A full clinical assessment was performed at baseline. A history of macrovascular disease was defined as any self-reported history of myocardial infarction, angina, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke, claudication, peripheral vascular disease, or peripheral revascularization. Nephropathy was defined as the presence of albuminuria [15]. Alcohol consumption pattern was classified as none, infrequent (special occasion to once/week) or regular (≥2 times/week), as data were lacking for grams taken per week.

Laboratory parameters

All samples were analysed at one of two laboratories: SA Pathology, Adelaide, Australia or the laboratory of the National Public Health Institute, Helsinki, Finland. Both laboratories participated in national quality assurance schemes. ALT and GGT were measured 16 weeks and ALT again 6 weeks before randomization. The average of the two ALT measurements was used for analysis. All specimens were stored immediately at -20°C and shipped expediently (within 7 days) for processing. ALT and GGT assays used standard colorimetric techniques consistent with the guidelines of the International Foundation of Clinical Chemistry (IFCC) [17].

Statistical analysis

Analyses were performed on an intention-to-treat basis using SPSS v21, and confirmed on SAS v9.3. Due to the non-normal distribution of both ALT and GGT, their relationship with baseline characteristics was determined using Spearman's correlation for continuous variables and the Wilcoxon rank–sum or Kruskal–Wallis test for categorical variables. To assess the relationship between baseline ALT and GGT and time to first cardiovascular event, Cox proportional-hazards regression was used to compute hazard ratios (HRs) and 95% confidence intervals (CIs). Examined by quintiles and deciles, the data were consistent with a linear relationship between ALT and cardiovascular events *(Fig. 1)*, and so continuous ALT (per one SD) was used in predictive models. With similar techniques, GGT had a nonlinear relationship to the time to first cardiovascular event, and so the primary analysis was by quintiles (*Fig. 1)*. All models were adjusted for assignment to fenofibrate and the following pre-specified baseline variables: age, gender, diabetes duration, hypertension, nephropathy, macrovascular disease, current-smoker status, waist-hip-ratio, $HbA1_c$, triglyceride levels, HDL-cholesterol (HDL-c) and LDL-cholesterol (LDL-c).

For ALT, in *post hoc* analysis, the multivariable model was further individually adjusted for laboratory used, alcohol consumption pattern or C-peptide, fasting glucose, and diabetes treatment category (diet, oral antidiabetic therapy or insulin±oral therapy). Penalized Cox modelling with an estimate of treatment effect was then used to adjust for statin therapy use on study [18]. To explore whether ALT might be acting as a marker of systemic frailty, the model was also adjusted for reported activity (very light, light, moderate, heavy or very heavy) [19]. Interactions among all predefined baseline variables, in addition to laboratory used, alcohol consumption pattern, C-peptide, fasting glucose, and diabetes treatment

category, were tested to identify any differential effect of ALT between various categories, with $P<0.05$ considered significant. If positive interactions were found, appropriate sub-analysis was then performed.

The multivariable GGT quintile model was further adjusted individually for alcohol consumption pattern and baseline ALT. Given no clear relationship between GGT and time to first cardiovascular event after adjustment, interaction testing for GGT was limited to specific variables: assignment to fenofibrate use, age, sex, history of macrovascular disease, alcohol consumption pattern, laboratory used and ALT.

To further explore the relationships seen in primary models, the population was divided into normal, low, and high ALT and GGT by established international reference ranges, published by *Ceriotti et al* on behalf of the IFCC [17]. Kaplan–Meier graphs were produced for time to first total cardiovascular event for both ALT and GGT by category. To explore differences among these groups in baseline characteristics, including presence of the metabolic syndrome (ATP III criteria [20]) and in measures of homeostasis model assessment of insulin resistance (HOMA-IR), highly-sensitive C-reactive protein (hsCRP) and platelets as markers of insulin resistance, inflammation, and hypersplenism, respectively, the Wilcoxon rank–sum or the Kruskal–Wallis test was used for continuous data and the chi-squared test for categorical variables. HRs for the first cardiovascular event on study using these categories were calculated using Cox regression. Models were univariate only, given the limited number of events in the low ALT and GGT categories.

Ethics

All participants provided written informed consent. The study protocol was approved by local and national ethics committees and was undertaken in accordance with the

Declaration of Helsinki and Good Clinical Practice Guidelines. The original trial was registered with the International Standard Randomised Controlled Trial Number (ISRCTN) 64783481.

Results

Baseline associations with ALT and GGT

The average age of the cohort was 62±7 years. Median (interquartile range, (IQR)) diabetes duration, $HbA1_c$ and BMI were 5(2–10) years, 52(43-62) mmol/mol (6.9(6.1–7.8) %), and 29.8(26.8–33.5) kg/m^2, respectively. In regard to medication, 2608(27%) were on diet, 5841(59%) were on oral antidiabetic therapy only, and 1346(14%) were on insulin ± oral therapy.

The median baseline ALT was 24(IQR 18–33) U/L and the median baseline GGT was 29(IQR 21–44) U/L. Lower ALT, but not GGT, was associated with a prior history of macrovascular disease and current smoking. GGT had a positive correlation with hsCRP (*Table 1 and eTable 1*).

With normal ALT defined as 8–41 U/L for women and 9–59 U/L for men [17], 1%(*n*=64) had low ALT and 6%(*n*=550) had high ALT. Of participants with low, normal, and high ALT, 15/64(23%), 1230/9181(13%) and 50/550(9%) had cardiovascular events on study, respectively (*Table 2, eTable 1*). Of those with low ALT, 40% of events were death due to cardiovascular disease, compared to 17% in those with normal ALT, and 8% in those with high ALT (*Table 2)*. With normal GGT defined as 6–40 U/L for women and 12–68 U/L for men [17], 1%(*n*=76) of the cohort had low GGT and 16%(*n*=1566) had high GGT. Of participants with low, normal, and high GGT, 6/70(8%), 1100/8153(13%), and 189/1566(12%) had cardiovascular events, respectively. Data for basic demographics and

metabolic syndrome presence, HOMA-IR, hsCRP, platelet count, and cardiovascular deaths as proportions of first cardiovascular events on study for ALT and GGT categories are shown in *eTable 1*.

Baseline ALT and first cardiovascular event

In univariate analysis, the risk of a cardiovascular event was 8(3-13)% lower for each SD (13.2 U/L) higher ALT at baseline (*P*=0.004). This relationship remained significant after adjustment (*Table 3*). The unadjusted HRs for cardiovascular events on study in those above and below the reference ranges for ALT when compared to those with normal ALT were 0.65(95% CI, 0.49–0.87) and 1.86(95% CI, 1.12–3.09), respectively (*P*=0.001) (*Fig. 2, eTable 1*).

The primary model was adjusted for other possible contributing factors, which did not affect the relationship between ALT and cardiovascular events (*Table 3)*. A positive interaction was found for HDL-c and ALT for the relationship between ALT and cardiovascular events (*P*=0.03 for interaction). To further explore this, sub-analysis was performed with the Cohort divided into HDL-c quintiles (*Table 4*).

Baseline GGT and first cardiovascular event

GGT quintiles were significantly related to time to first cardiovascular event in univariate analysis (*P*=0.001). This relationship was attenuated after adjustment, although, qualitatively, the lowest GGT quintile appeared to have a lower risk of events than the remainder of the cohort (*Fig. 1*). Adjustment for alcohol consumption pattern had no effect on the model. Interestingly, statistical associations of GGT quintiles with cardiovascular events were significant after adjustment for ALT (*P*=0.04, *Table 5*). No interaction was found for assignment to fenofibrate, age, sex, alcohol consumption pattern, laboratory used,

history of macrovascular disease or ALT. The unadjusted HRs for the first cardiovascular event on study for those with low or high GGT when compared to those with normal GGT were 0.56(0.25–1.25) and 0.90(0.7–1.05), respectively (*Fig. 2*, *eTable 1)*.

Discussion

ALT had an inverse relationship with the first cardiovascular event on study in people with type 2 diabetes, without known chronic liver disease, and with ALT not greater than twice the upper limit of normal. No clear association between GGT and the time to first cardiovascular event was found.

Given that NAFLD is considered a potential risk factor for cardiovascular disease, our finding might seem counterintuitive; however, similar results have been reported in regard to both mortality and cardiovascular events in several other studies using cohorts with different characteristics and not defined by diabetes [21] [22] [9], [23] [24]. Our results are, however, contrary to the findings of other studies that have found either a positive or no association between ALT and cardiovascular disease events and mortality [2, 7, 10]. The reasons for these differences could include the population selected for study, in particular their dissimilar overall cardiovascular risk and range of ALT levels, and the duration of follow-up and the methods of analysis, including whether linear or threshold analysis was used for ALT.

ALT is commonly higher in type 2 diabetes and often presumed to represent NAFLD, which has a prevalence of 70% in diabetes overall and can be significantly higher when obesity is also present [1, 4]. ALT may also be elevated due to other factors [1, 4]. For example, higher ALT in diabetes may also be the result of upregulated gluconeogenesis through hepatic insulin resistance, in addition to the production of ALT within adipose

tissue when insulin resistance and obesity coexist (as demonstrated in mice) [4]. Perhaps similar to the increased risk for mortality that has been demonstrated in those with type 2 diabetes with low or normal body weight when compared to those who are in the overweight category [25, 26], a low ALT in our cohort may not be associated with health but may rather exist as a marker of a more severe phenotype, without significant insulin resistance or steatosis, consisting of reduced β-cell function, an at-risk genetic profile and/or overall biological frailty and co-morbidity [19, 26].

Low ALT has been linked to frailty in several studies, although our limited analysis to explore the role of ALT as a potential marker of frailty was not useful. For example, *Ruhl et al* showed that adjusted appendicular lean mass, potentially associated with sarcopenia, was lower among the lowest ALT deciles in 15 028 subjects whose body composition was measured by dual-energy X-ray absorptiometry [9]. *Couteur et al,* found that lower ALT was associated with older age and lower survival over 4.9 years of follow-up in 1673 community-dwelling men aged >70 years [19]. This finding was attenuated by adjustment for frailty, with ALT independently associated with frailty as defined by a formal frailty index.

Low ALT may also be a marker of reduced liver reserve, perhaps enhancing an environment of increased oxidative stress, as a result of biological aging and/or increased liver fibrosis due to non-alcoholic steatohepatitis [8, 27]. Indeed, while results are mixed, the severity of liver disease may increase cardiovascular risk in NAFLD [1, 28], with the presence of simple steatosis alone having no adverse implications [29]. Moreover, ALT levels are thought to be 22–64% heritable, with ALT loci near genes involved in glucose and lipid metabolism, inflammation and immunity, and the biogenesis of mitochondria

[30], as a potential link between ALT and clinically important genes that may affect cardiovascular risk [30]. The potential for greater risk reduction by statin therapy in those with a higher ALT is also possible [22],[12], perhaps through a greater reduction in small LDL particles or other adverse lipid measures [11, 12], although adjustment for statin therapy did not significantly change our results. Lower ALT has also been associated with several markers of systemic inflammation in one study [11] and with higher NT proBNP in another [31].

Clinical and biochemical associations with increasing ALT in our analysis were similar to those previously described [1, 8, 11], and included positive associations with measures of adiposity, the metabolic syndrome, insulin resistance, and poorer glycaemic control. Those with a known history of macrovascular disease and those who were current smokers or users of insulin, all factors that may potentially increase future cardiovascular event risk, had lower ALT than their respective reference group. ALT also had an inverse association with age. Despite these associations, which may link low ALT to cardiovascular events, no interaction was found for these factors, and appropriate adjustments were included in multivariable models. A positive interaction was found for HDL-c, with subsequent quintile analysis suggesting that the relationship was most pronounced in those with HDL-c ≤ 0.88 mmol/L, consistent with more significant dyslipidaemia. No significant interaction was found for fenofibrate therapy, excluding a differential treatment effect.

Surprisingly, we did not find a relationship between GGT and on-study cardiovascular events after adjustment. As GGT is presumed to be a less specific marker of liver fat and NAFLD per se, and rather may reflect the metabolic syndrome [4, 5, 13], this may be explained by the effects of adjustment for variables that would also reflect an adverse

metabolic profile. Interestingly, further adjustment for alcohol consumption pattern did not significantly affect the model. On the other hand, adjustment for ALT did strengthen the relationship between GGT and cardiovascular events, perhaps by selecting for elevated GGT due to liver fibrosis (by adjusting for steatosis), or by selecting for GGT as a marker of oxidative stress ± atherosclerotic burden, rather than liver pathology [5, 8, 14, 30]. Despite the statistically negative finding, the positive direction of the association between GGT and cardiovascular events is consistent with current literature. GGT has been positively associated with incident vascular events and mortality in several studies but not all [5, 8, 9, 13, 19].

Strengths of our analysis include the use of prospective data from a large, well-defined cohort, with pre-specified outcomes. In addition, intra-individual variation in ALT was accommodated by using an average of two independent samples. Weaknesses of our findings relate to the problems of secondary analysis. High ALT and known chronic liver disease were exclusion criteria and so the effects of comorbidity due to liver disease are likely to be attenuated in this data set. Also, follow-up was a median of 5 years, which is perhaps insufficient time to see the effects of an adverse metabolic phenotype associated with higher baseline ALT. Participants selected for study for a clinical trial may have been healthier than a community dwelling population with diabetes. ALT and GGT were measured at two laboratories, and differences in analytical technique and geographic variation may not have been fully adjusted for [17]. Alcohol consumption by grams per week, steatosis presence, or NAFLD severity could not be corrected for, nor could assessment by a formal frailty index. Reference ranges used meant that groups above and below normal had small numbers. Normal values for liver enzymes have not been

established. These reference ranges were chosen as they were developed from an international cohort, with strict attention to population selected and analytical technique [17]. The analysis using these reference ranges was illustrative only, to complement primary models.

While NAFLD has been linked to cardiovascular disease, in populations with type 2 diabetes, a lower ALT may predict cardiovascular events. Study of the mechanisms behind lower ALT being associated with cardiovascular outcomes in various at-risk groups is needed, with a particular emphasis on formal assessment of biological frailty and co-morbidity, NAFLD severity, and lipid parameters in cohorts under study. Use of ALT or GGT in predictive algorithms for cardiovascular disease is premature.

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Tables

Table 1: Clinical and biochemical associations with baseline alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) levels

	ALT		GGT	
Baseline characteristic (number, % of cohort)	**Median and IQR (U/L) or correlation coefficient**	***P***	**Median and IQR (U/L) or correlation coefficient**	***P***
Age (years)	–0.21	<0.001	–0.13	<0.001
Sex		<0.001		<0.001
Female (*n*=3657, 37%)	21 (16–29)		26 (19–40)	
Male	25 (19–35)		31 (22–46)	
Country of recruitment		<0.001		<0.001
Australia or New Zealand (*n*=8402, 86%)	23 (18–32)		29 (21–43)	
Finland	26 (19–37)		32 (22–48)	
Waist-hip ratio	0.26	<0.001	0.25	<0.001
Body mass index (kg/m^2)	0.19	<0.001	0.21	<0.001
Diabetes duration (years)	–0.04	<0.001	–0.05	<0.001
Smoking status		<0.001		<0.001
Non-smoker (*n*=3929, 40%)	23 (17–32)		27 (19–40)	
Ex-smoker (*n*=4944, 51%)	25 (19–34)		30 (22–46)	
Current smoker (*n*=922, 9%)	23 (17–31)		31 (22–47)	
Alcohol intake		<0.001		<0.001
None (*n*=2691, 28%)	23 (17–31)		27 (20–40)	
Infrequent (*n*=4604, 47%)	24 (18–32)		28 (20–42)	
Regular (*n*=2494, 25%)	25 (19–35)		34 (24–52)	
Metabolic syndrome (*n*=8101, 83%)	24 (18–34)	<0.001	30 (22–46)	<0.001
No metabolic syndrome	21 (16–28)		25 (17–36)	
Comorbidity				
Hypertension (*n*=5546, 57%)	24 (18–33)	0.34	30 (22–46)	<0.001
No hypertension	24 (18–32)		28 (20–41)	
Known macrovascular disease (*n*=2131, 22%)	23 (18–31)	<0.001	29 (21–45)	0.08
No known macrovascular disease	24 (18–33)		29 (21–44)	
Nephropathy (*n*=2508, 26%)	24 (18–34)	0.007	32 (23–48)	<0.001
No nephropathy	24 (18–32)		28 (20–42)	

Baseline characteristic (number, % of cohort)	ALT		GGT	
	Median and IQR (U/L) or correlation coefficient	*P*	Median and IQR (U/L) or correlation coefficient	*P*
Medication				
Metformin (*n*=4794, 49%)	25 (18–35)	<0.001	30 (22–46)	<0.001
No metformin	23 (18–31)		28 (20–42)	
Sulfonylurea (*n*=4433, 45%)	25 (19–34)	<0.001	30 (22–47)	<0.001
No sulfonylurea	23 (18–31)		28 (20–42)	
Insulin (*n*=1346, 14%)	23 (17–30)	0.003	28 (19–43)	<0.001
No insulin	24 (18–33)		29 (21–44)	
Diabetes treatment overall		<0.001		<0.001
Diet only	22.5 (17.5–30.5)		28 (20–40)	
Oral hypoglycaemic	24.5 (18.0–34.0)		30 (22–46)	
Insulin ± oral hypoglycaemic	22.5 (17.0–30.0)		28 (19–43)	
Biochemistry				
HbA_{1c} (% or mmol/mol)	0.12	<0.001	0.12	<0.001
Fasting glucose (mmol/L)	0.16	<0.001	0.14	<0.001
C-peptide (nmol/L)	0.29	<0.001	0.30	<0.001
HDL-C (mmol/L)	–0.15	<0.001	–0.09	<0.001
LDL-C (mmol/L)	–0.09	<0.001	–0.09	<0.001
Triglyceride (mmol/L)	0.15	<0.001	0.21	<0.001
Apolipoprotein A1 (g/L)	–0.10	<0.001	–0.01	0.27
Apolipoprotein B (g/L)	0.03	0.001	0.07	<0.001
Lipoprotein a (g/L)	–0.10	<0.001	–0.10	<0.001
Highly-sensitive C-reactive protein (mg/L)	–0.01	0.49	0.18	<0.001
Uric acid (µmol/L)	0.11	<0.001	0.14	<0.001
HOMA insulin resistance	0.32	<0.001	0.33	<0.001
Platelets ($x10^9$/L)	–0.14	<0.001	–0.09	<0.001
ALT (U/L)	–		0.51	<0.001

Table 2: Type of first cardiovascular event on study by alanine aminotransferase (ALT) category as determined by reference ranges set by Ceriotti et al [17].

Type of cardiovascular event	ALT below reference range	ALT within reference range*	ALT above reference range
Death due to coronary heart disease	5 (33%)	154 (13%)	3 (6%)
Death due to other cardiovascular disease	1 (7%)	49 (4%)	1 (2%)
Coronary artery bypass grafting	4 (27%)	224 (18%)	6 (12%)
Percutaneous transluminal coronary angioplasty	1 (7%)	190 (15%)	14 (28%)
Nonfatal coronary infarction	3 (20%)	317 (26%)	15 (30%)
Nonfatal stroke	1 (7%)	257 (21%)	8 (16%)
Carotid revascularization	0	39 (3%)	3 (6%)
Total number of events/total in group	15/64	1230/9181	50/550

* Using these reference ranges, normal ALT, 8–41 U/L for women and 9–59 U/L for men.

Data are presented as n (%) within each group.

Table 3: Relationship between baseline alanine aminotransferase (ALT) and first cardiovascular event

Model	Hazard ratio[a]	95% confidence interval	*P*
Unadjusted (*n*=9795, 1295 events)	0.92	0.87–0.97	0.004
Adjusted (*n*=9765, 1291 events)[b]	0.92	0.87–0.98	0.01
Adjusted plus central laboratory (*n*=9765, 1291 events)	0.92	0.87–0.98	0.01
Adjusted plus alcohol consumption pattern (*n*=9759, 1288 events)	0.92	0.87–0.98	0.01
Adjusted plus C-peptide, fasting glucose (to replace $HbA1_c$) and diabetes treatment (*n*=9658, 1281 events)	0.91	0.85–0.97	0.003
Adjusted plus statin therapy (penalized Cox model) (*n*=9765, 1291 events)	0.92	0.87 – 0.98	0.01
Adjusted for reported level of activity (*n*=9648, 1273 events)	0.92	0.86 - 0.98	0.01

[a] Per 1 standard deviation higher baseline ALT.

[b] The adjusted model includes: assignment to treatment with fenofibrate and baseline: age, sex, diabetes duration, known macrovascular disease, hypertension, nephropathy, current smoker status, waist-hip-ratio, $HbA1_c$, triglyceride level, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol.

Table 4. Relationship between baseline alanine aminotransferase (ALT) and cardiovascular (CVD) events by High-density lipoprotein cholesterol (HDL-c) quintiles using Cox Proportional Hazards Regression analysis

ALT per SD (13.2 U/L)		
Variable (CVD events/total number of individuals in analysis)	**HR (95% CI)**	**p value**
HDL-c ≤ 0.88mmol/L (364/1968)	0.86 (0.77-0.97)	0.01
HDL-c > 0.88 mmol/L and ≤ 1.01 mmol/L (298/2037)	0.88 (0.78-1.00)	0.05
HDL-c > 1.01 mmol/L and ≤ 1.12 mmol/L (234/1901)	0.91 (0.79-1.04)	0.17
HDL-c > 1.12 mmol/L and ≤ 1.28 mmol/L (214/1916)	1.07 (0.94-1.22)	0.31
HDL-c > 1.28 mmol/L (181/1943)	0.99 (0.84-1.16)	0.88
P value for trend in effect of ALT by HDL-c quintiles = 0.022		

[a] model adjusted for: assignment to treatment with fenofibrate and baseline age, sex, diabetes duration, known macrovascular disease, hypertension, nephropathy, current smoker, waist-hip-ratio, $HbA1_c$, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. HR, hazard ratio; CI, confidence interval; SD, standard deviation

Table 5: Relationship between baseline alanine aminotransferase (ALT) as a linear variable and gamma glutamyltransferase (GGT) in quintiles and time to first cardiovascular event

			95.0% CI for HR	
	P	HR	Lower	Upper
ALT as linear variable in adjusted model*				
ALT per SD (per 13.2 U/L)	0.01	0.92	0.87	0.98
GGT quintiles in adjusted model				
GGT ≤19 U/L	0.10			
GGT >19 and ≤26 U/L		1.13	0.95	1.35
GGT >26 and ≤33 U/L		1.28	1.07	1.54
GGT >33 and ≤49 U/L		1.10	0.91	1.32
GGT >49 U/L		1.10	0.91	1.33
ALT (linear) and GGT (quintiles) in adjusted model and corrected for each other				
ALT per SD (per 13.2 U/L)	0.003	0.90	0.84	0.97
GGT ≤19 U/L	0.04			
GGT >19 and ≤26 U/L		1.16	0.97	1.38
GGT >26 and ≤33 U/L		1.34	1.11	1.61
GGT >33 and ≤49 U/L		1.18	0.98	1.43
GGT >49 U/L		1.24	1.01	1.52

* With adjustment for: assignment to treatment with fenofibrate and baseline age, sex, diabetes duration, known macrovascular disease, hypertension, nephropathy, current smoker, waist-hip-ratio, $HbA1_c$, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. HR, hazard ratio; CI, confidence interval; SD, standard deviation

Figure Legends

Figure 1

Adjusted beta coefficients obtained by Cox regression analysis for A. alanine aminotransferase (ALT), and B. gamma-glutamyltransferase (GGT) by quintiles (using the median of each quintile on the x axis) with line of best fit illustrated on the ALT model. Models were adjusted for the pre-specified variables—assignment to treatment with fenofibrate and baseline characteristics: age, sex, diabetes duration, known macrovascular disease, hypertension, nephropathy, current smoker status, waist-hip ratio, $HbA1_c$, triglyceride level, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. The 95%, 2-sided, confidence intervals were created using Plummer's method of constructing floating confidence intervals. Numbers refer to quintiles 1 to 5. HR, hazard ratio; U/L, units per litre.

Figure 2

Kaplan-Meier plots for time to first total cardiovascular event on study by A. alanine aminotransferase (ALT), and B. gamma-glutamyltransferase (GGT) category. Normal ALT, 8–41 U/L for women and 9–59 U/L for men. Normal GGT, 6–40 U/L for women and 12–68 U/L for men. Log-rank *P*=0.001 for difference across categories for ALT and *P*=0.16 for difference across categories for GGT. HR, hazard ratio; RR, reference range.