

University of Groningen

## Nutritional and metabolic aspects of the hepatorenal axis

Deetman, Petronella Elisabeth

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Deetman, P. E. (2015). *Nutritional and metabolic aspects of the hepatorenal axis*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

# 6

## **ALANINE AMINOTRANSFERASE AND MORTALITY IN PATIENTS WITH TYPE 2 DIABETES (ZODIAC-38)**

Petronella E. Deetman<sup>1</sup>, Alaa Alkhalaf<sup>2</sup>,  
Gijs W.D. Landman<sup>3,4</sup>, Klaas H. Groenier<sup>4,5</sup>,  
Jenny E. Kootstra-Ros<sup>6</sup>, Gerjan Navis<sup>1</sup>,  
Henk J.G. Bilo<sup>1,4</sup>, Nanne Kleefstra<sup>4,7</sup>,  
Stephan J.L Bakker<sup>1</sup>

<sup>1</sup>Department of Medicine, University of Groningen,  
University Medical Center Groningen, the Netherlands;

<sup>2</sup>Department of Gastroenterology, Isala Clinics,  
Zwolle, the Netherlands;

<sup>3</sup>Department of Medicine, Gelre Hospital, Apeldoorn,  
the Netherlands;

<sup>4</sup>Diabetes Centre, Isala Clinics, Zwolle, the Netherlands;

<sup>5</sup>Department of General Practice, University  
of Groningen, University Medical Center Groningen,  
the Netherlands;

<sup>6</sup>Department of Laboratory Medicine, University  
Medical Center Groningen, the Netherlands;

<sup>7</sup>Langerhans Medical Research Group,  
Zwolle, the Netherlands.

## ABSTRACT

**Background.** Combined data suggest a bimodal association of alanine aminotransferase (ALT) with mortality in the general population. Little is known about the association of ALT with mortality in patients with type 2 diabetes. We therefore investigated the association of ALT with all-cause, cardiovascular, and non-cardiovascular mortality in patients with type 2 diabetes.

**Methods.** A prospective study was performed in patients with type 2 diabetes, treated in primary care, participating in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study. Cox regression analyses were performed to determine associations of  $\log_2$ -transformed baseline ALT with all-cause, cardiovascular, and non-cardiovascular mortality.

**Results.** In 1,187 patients with type 2 diabetes ( $67 \pm 12$  years, 45% female), ALT levels were 11 (8–16) U/l. During median follow-up for 11.1 (6.1–14.0) years, 553 (47%) patients died, with 238 (20%) attributable to cardiovascular causes. Overall, ALT was inversely associated with all-cause mortality (Hazard Ratio [HR] 0.81; 95% Confidence Interval [CI] 0.72–0.92), independently of potential confounders. This was less attributable to cardiovascular mortality (HR 0.87; 95% CI 0.72–1.05), than to non-cardiovascular mortality (HR 0.77; 95% CI 0.65–0.90). Despite the overall inverse association of ALT with mortality, it appeared that a bimodal association with all-cause mortality was present with increasing risk for levels of ALT above normal ( $P=0.003$ ).

**Conclusions.** In patients with type 2 diabetes, low levels of ALT are associated with an increased risk of all-cause mortality, in particular non-cardiovascular mortality, compared to normal levels of ALT, while risk again starts to increase when levels are above normal.

## INTRODUCTION

The prevalence of type 2 diabetes is on the rise, with already more than 382 million patients affected worldwide (1). Type 2 diabetes and the metabolic syndrome are associated with hepatic fat accumulation (2,3). Hepatic fat accumulation, in the absence of alcohol abuse, is known as non-alcoholic fatty liver disease (NAFLD). NAFLD is a heterogeneous disease that ranges from simple steatosis to steatohepatitis. Approximately 50–75% of the patients with type 2 diabetes will develop some form of NAFLD during their life (4,5). Because NAFLD is highly prevalent among subjects with the metabolic syndrome, NAFLD is considered the hepatic component of the metabolic syndrome (2,6).

The presence of NAFLD has been associated with an increased risk of microvascular and macrovascular complications in patients with type 2 diabetes (5,7). One of those complications may be chronic kidney disease (CKD). It has been shown that the prevalence of CKD is higher among patients with NAFLD (8,9). Patients with type 2 diabetes and NAFLD had a 2-fold increased risk of mortality compared to type 2 diabetes patients without NAFLD (7). In most cases of NAFLD, the enzyme alanine aminotransferase (ALT) is elevated (10). Elevated levels of ALT are therefore regarded as a marker of NAFLD, and have been independently associated with an increased risk of coronary heart disease in population-based studies (11,12). However, there are now also indications that low ALT is a risk factor for mortality, suggesting that there could be a bimodal, U-shaped association of ALT with mortality (13–15).

It is unknown whether ALT is associated with long-term outcome in patients with type 2 diabetes. Therefore, the aim of the present study was to investigate the association of ALT with progression of renal function, all-cause, cardiovascular, and non-cardiovascular mortality in a large prospective cohort of patients with type 2 diabetes, and to investigate whether these associations are U-shaped rather than linear.

## PATIENTS AND METHODS

### STUDY DESIGN AND POPULATION

The Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study was initiated in 1998 in the Zwolle region of the Netherlands. The design and details of this study have been published elsewhere (16,17). In brief, this is a prospective study in primary care patients with type 2 diabetes. In 1998, 1,143 patients with type 2 diabetes were included and in 2001, an additional 546 patients with type 2 diabetes were enrolled, resulting in a combined cohort of 1,689 patients (18). ALT was measured in 1,187 patients (70%). The ZODIAC study was approved by the local medical ethics committee, and all patients provided informed consent.

## DATA COLLECTION AND MEASUREMENTS

Baseline data, collected in 1998 and 2001, consisted of a full medical history, including a history of cardiovascular disease (CVD), use of medication, and tobacco consumption. Subjects were considered to have a positive history of CVD if they had a history of angina pectoris, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke, or transient ischemic attack. The RAND-36 questionnaire was taken. According to the Fried frailty criteria (19), physical weakness is a component of the frailty phenotype. The physical functioning subscale of the RAND-36 questionnaire can serve as the physical weakness component of the Fried frailty criteria (20). Baseline laboratory and physical assessment data were collected and included gamma-glutamyltransferase (GGT), nonfasting lipid profile, HbA1c, serum creatinine, urinary albumin, and blood pressure. Every year, data on serum creatinine and urine albumin concentration were collected.

Samples were stored at  $-80^{\circ}\text{C}$  until analyzed for ALT. In a previous study it was shown that the level of ALT was stable after three months of storage at  $-20^{\circ}\text{C}$  (21). The stability of ALT was confirmed by data from the manufacturer of the assay, which indicate that ALT levels are stable when stored at  $-70^{\circ}\text{C}$  (unpublished data). ALT was measured with an UV test with pyridoxal phosphate activation according to IFCC recommendations (Roche Modular, Roche, Mannheim, Germany) (22). In our laboratory, the coefficients of variation (CVs) were determined for ALT levels of 51 U/L and 164 U/L. At these specific levels, the inter-assay CVs for the entire measurement period are 1.9% and 1.4%, respectively. The intra-assay CVs are 2.1% and 1.2%, respectively. In our laboratory, the upper limit of normal of ALT is  $<45$  U/L for men and  $<34$  U/L for women (22). Blood pressure was measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 min of rest. The eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation (23).

## CLINICAL END-POINTS

In this study, associations were examined of baseline ALT with three clinical end points: progression of renal function, all-cause, cardiovascular, and non-cardiovascular mortality. Progression of renal function was defined as a confirmed doubling of serum creatinine or incident micro- or macroalbuminuria. Microalbuminuria was defined as an albumin to creatinine ratio (ACR) of  $>2.5$  mg/mmol for men and  $>3.5$  mg/mmol for women. In 2012, vital status and cause of death were retrieved from medical records. Cause of death was coded according to the ICD-9. Cardiovascular death was defined as any death in which the principal cause of death was cardiovascular in nature (ICD-9 codes 390–459) (16,17).

## STATISTICAL ANALYSIS

Baseline characteristics of the study population were calculated according to sex-stratified tertiles of ALT. Results are expressed as mean  $\pm$  SD for normally distributed data and median (interquartile range [IQR]) for skewed data. Categorical distributed data are presented as the total number of subjects with percentage (n [%]). *P*-values for a linear trend across sex-stratified tertiles of ALT were determined with linear regression analyses. Residuals were checked for normality and variables were log-transformed when appropriate.

Univariable survival analyses were performed using log-rank tests. Adjustment for potential confounders was performed in multivariable Cox regression analyses. Cox regression analyses were performed with ALT as a continuous variable. ALT was  $\log_2$ -transformed. Therefore, HRs and 95% CI are shown per each doubling of ALT. We also tested for potential interactions of ALT with age and sex for associations with mortality by means of product-terms of the respective variables. The final model included age, sex, BMI, smoking, systolic blood pressure, cholesterol/HDL ratio, diabetes duration, serum creatinine, HbA1c, cardiovascular history, use of ACE inhibitors, and ACR. The proportionality of hazards was tested by checking Schoenfeld's residuals. Finally, we tested for potential non-linearity of the prospective associations of ALT with mortality (24). For the analyses in which we investigated the association of ALT with incident micro or macroalbuminuria, patients with albuminuria at baseline were excluded.

To investigate the discriminative and predictive capabilities of ALT, we determined the Harrell's C, the Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI). The Harrell's C is a measure of how well a model distinguishes subjects who will get the outcome from subjects who will not get the outcome. The Harrell's C was calculated for crude and multivariable-adjusted Cox regression models and for a Cox regression model without ALT. To estimate to which extent ALT improves risk classification, the NRI and IDI were calculated. With the NRI procedure, subjects are classified into low, intermediate and high risk categories. Cut off points for risk classification, as applied for NRI were 15, 30 and 40 for all-cause mortality and 10, 20, and 30 for non-cardiovascular mortality. IDI is a continuous version of the NRI (25). These analyses were done for all-cause and non-cardiovascular mortality only, because ALT was significantly associated with these outcomes. Finally, a goodness-of-fit test, as proposed by Grønnesby and Borgan, was performed for Cox regression analyses.

As sensitivity analyses, Cox regression analyses were performed after excluding subjects using statins, and after excluding subjects with ALT levels above the upper limit of normal. Data on the physical functioning subscale of the RAND-36 questionnaire were available in approximately two-thirds of patients (n = 735, 62%). As additional sensitivity analyses, we repeated the Cox regression analyses with additional adjustment for the physical functioning subscale of the RAND-36 questionnaire. We performed these analyses only in patients with complete data on this measure.

Statistical analyses were performed with SPSS version 20.0 for Windows (IBM Corp., Armonk, NY), and STATA version 11 (StataCorp LP, College Station, TX, USA). A  $P < 0.05$  was considered to indicate statistical significance.

## RESULTS

### PATIENTS CHARACTERISTICS

A total of 1,187 patients with type 2 diabetes (mean age  $67 \pm 12$  years, 54% female) were included. At baseline, the median (IQR) duration of diabetes was 4 (2–9) years. The median ALT level was 11 (8–16) U/l. A total of 23 patients had an ALT level above the upper limit of normal. Baseline characteristics are shown according to sex-stratified tertiles of ALT (Table 1). ALT was positively associated with GGT, HbA1c, BMI, physical functioning, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, lipid lowering drugs, eGFR, and albuminuria. There were inverse relationships of ALT with age, duration of diabetes, HDL, and serum creatinine. ALT was not associated with smoking status, use of ACE inhibition, and ACR.

### ALT AND OUTCOMES

During a median follow-up of 11.1 (6.1–14.0) years, 553 (47%) patients died. Of those subjects, 238 (43%) subjects died of CVD. All-cause mortality rates were 221 (62%), 211 (46%), and 121 (32%) in the respective tertiles of ALT ( $P < 0.001$ , Figure 1). A total of 127 patients (16%) developed a 50% increase in serum creatinine. Of the patients with normoalbuminuria at baseline, 126 (32%) of the patients developed albuminuria.

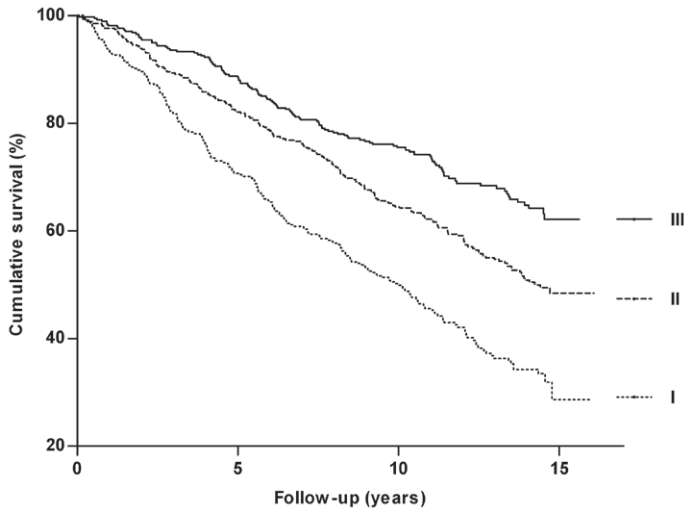
In a univariable Cox regression analysis, ALT was not associated with 50% increase in serum creatinine (Hazard Ratio [HR], 1.24, 95% CI 0.88–1.73;  $P = 0.22$ ) or incident albuminuria (HR, 1.03, 95% CI 0.79–1.34;  $P = 0.81$ ). These associations remained nonsignificant after adjustment for potential confounders. ALT was inversely associated with all-cause mortality (Table 2), independently of potential confounders. No interactions were found of ALT with age and sex. ALT was not associated with cardiovascular mortality, but ALT was associated with non-cardiovascular mortality. In addition, despite the overall inverse association of low to normal levels of ALT with mortality, there was a U-shaped association of ALT and all-cause mortality with again increasing risk for levels of ALT above normal ( $P = 0.003$ , Figure 2).

**Table 1.** Baseline characteristics of the study population presented for the whole study population, and for sex stratified tertiles of alanine aminotransferase (ALT).

	Study population	ALT <sup>a</sup>			P-value <sup>b</sup>
		I	II	III	
<b>Liver Function</b>					
ALT (U/L)	11 (8–16)	7 (6–8)	11 (10–12)	19 (16–25)	
GGT (U/L)	30 (21–47)	23 (16–34)	28 (21–41)	45 (30–75)	<0.001
<b>Demographics</b>					
Age (years)	67 ± 12	71 ± 11	67 ± 11	63 ± 11	<0.001
Male sex, n(%)	540 (46)	162 (46)	205 (45)	173 (46)	
Current smoker, n(%)	225 (19)	72 (20)	89 (20)	64 (17)	0.73
Duration of diabetes (yr)	4 (2–9)	5 (2–10)	4 (2–9)	3 (2–8)	0.002
HbA1c, (%)	7.0 (6.2–8.0)	6.7 (6.1–7.7)	6.9 (6.2–7.9)	7.4 (6.5–8.5)	<0.001
HbA1c, (mmol/mol)	53 (44–64)	50 (43–61)	52 (44–63)	57 (48–69)	<0.001
History of CVD, n(%)	342 (29)	113 (32)	135 (30)	94 (25)	0.014
<b>Body Composition</b>					
BMI (kg/m <sup>2</sup> )	29 ± 5	28 ± 5	29 ± 4	30 ± 5	<0.001
Physical functioning <sup>c</sup>	60 (30–85)	55 (25–80)	60 (35–85)	70 (35–90)	0.012
<b>Blood pressure</b>					
Systolic BP (mmHg)	152 ± 24	150 ± 24	152 ± 23	154 ± 24	0.034
Diastolic BP (mmHg)	83 ± 11	80 ± 11	84 ± 10	86 ± 11	<0.001
Use of ACE inhibition, n(%)	320 (27)	97 (27)	125 (27)	98 (26)	0.73
<b>Lipids</b>					
Total cholesterol (mmol/L)	5.51 ± 1.12	5.4 ± 1.1	5.5 ± 1.1	5.7 ± 1.1	0.002
HDL (mmol/L)	1.1 (1.0–1.4)	1.2 (1.0–1.5)	1.2 (1.0–1.4)	1.1 (0.9–1.3)	<0.001
Triglycerides (mmol/L)	2.1 (1.5–3.0)	1.6 (1.1–2.2)	2.0 (1.4–2.7)	2.8 (1.9–4.1)	<0.001
Lipid lowering drugs, n(%)	191 (16)	36 (10)	79 (17)	76 (20)	0.007
<b>Renal function</b>					
Serum creatinine (µmol/L)	92 (82–103)	94 (84–107)	91 (81–103)	90 (81–100)	<0.001
eGFR (ml/min/1.73m <sup>2</sup> )	69 ± 17	64 ± 16	69 ± 16	72 ± 17	<0.001
ACR (mg/mmol)	1.9 (0.9–6.5)	2.1 (1.0–6.8)	1.6 (0.8–6.2)	2.0 (0.9–6.7)	0.98
Albuminuria, n(%)	452 (38)	137 (39)	159 (35)	156 (42)	0.048

<sup>a</sup>Range of ALT (U/L) in men: I ≤9; II 10–14; III ≥ 15. Range of ALT (U/L) in women: I ≤8; II 9–13; III ≥ 14. <sup>b</sup>P for linear trend is shown. <sup>c</sup>Physical functioning was estimated using the physical functioning subscale of the RAND-36 questionnaire. ALT, GGT, duration of diabetes, HDL, triglycerides, serum creatinine, and ACR were log-transformed. ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; CVD, cardiovascular disease; BP, blood pressure; ACR, albumin to creatinine ratio.





**Figure 1.** Kaplan Meier curve for sex-stratified tertiles of alanine aminotransferase (ALT) and cumulative survival (%). Range of ALT (U/L) in men: I  $\leq 9$ ; II 10–14; III  $\geq 15$ . Range of ALT (U/L) in women: I  $\leq 8$ ; II 9–13; III  $\geq 14$ . Log-rank test  $P < 0.001$ .

**Table 2.** Cox regression analyses of  $\log_2$ -transformed alanine aminotransferase (ALT) with all-cause, cardiovascular and non-cardiovascular mortality.

	All-cause mortality		Cardiovascular mortality		Non-cardiovascular mortality	
	HR (95%CI)	<i>P</i> -value	HR (95%CI)	<i>P</i> -value	HR (95%CI)	<i>P</i> -value
Number of events	515		219		296	
Model 1	0.61 (0.54–0.68)	<0.001	0.63 (0.53–0.75)	<0.001	0.59 (0.51–0.68)	<0.001
Model 2	0.81 (0.72–0.91)	<0.001	0.84 (0.70–1.01)	0.062	0.78 (0.67–0.92)	0.002
Model 3	0.79 (0.70–0.88)	<0.001	0.82 (0.69–0.98)	0.032	0.76 (0.65–0.89)	0.001
Model 4	0.81 (0.72–0.92)	0.001	0.87 (0.72–1.05)	0.15	0.77 (0.65–0.90)	0.001

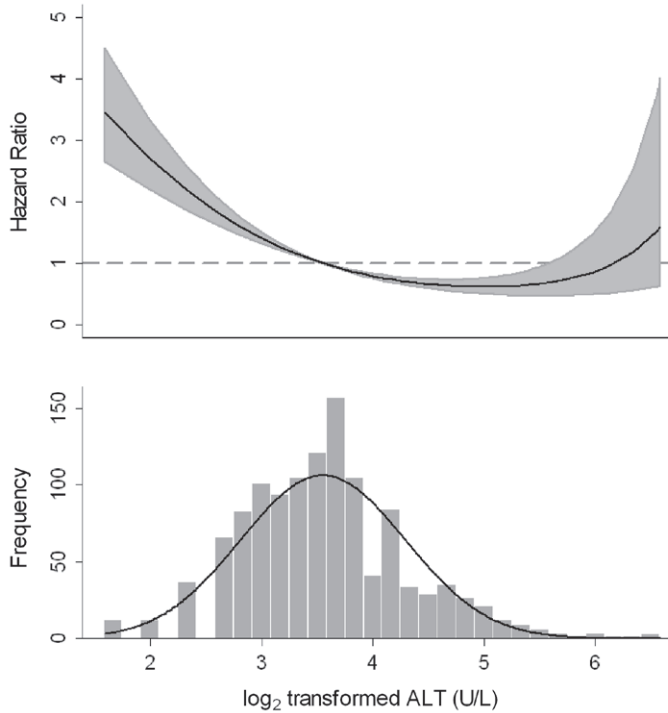
HRs per each doubling in ALT.

Model 1; crude model

Model 2; model 1 + age, sex

Model 3; model 2 + BMI, cholesterol/HDL ratio

Model 4; model 3 + smoking, systolic blood pressure, diabetes duration, serum creatinine, glycated hemoglobin, cardiovascular history, ACE inhibition, albumin to creatinine ratio



**Figure 2.** Fractional polynomial of the hazard ratio for all-cause mortality, relative to the distribution of  $\log_2$ -transformed alanine aminotransferase (ALT). In this figure,  $\log_2$ -transformed values of ALT are shown. Data of four patients with extreme values of  $\log_2$ -transformed ALT ( $\leq 1$  and  $> 7$ ) is not shown

### PREDICTIVE VALUE OF ALT AND GOODNESS OF FIT

The discriminative capabilities of ALT for all-cause and non-cardiovascular mortality, as determined by the Harrell's C, are shown in Table 3. As expected, when more variables were added to the model in addition to ALT, the discriminative ability increased. Discriminative abilities were similar in final multivariable-adjusted models with and without ALT. This indicates that the addition of ALT to Cox regression models does not materially improve the discriminative ability of saturated survival models. In addition, both the NRI and IDI were relatively low Table 3 indicating small additional predictive value of ALT on top of established predictors in Cox regression models. Furthermore, the goodness-of-fit tests for cardiovascular and non-cardiovascular mortality were not statistically significant.

**Table 3.** Additional predictive value of  $\log_2$ -transformed alanine aminotransferase (ALT) on all-cause and non-cardiovascular mortality.

	Harrell's C (95%CI)	IDI (95%CI)	NRI (95%CI)	Gronnesby & Borgan test
<b>All-cause mortality</b>				
Model 1	0.61 (0.58–0.63)	NA	NA	0.21
Model 2	0.77 (0.75–0.78)	0.51 (0.06–0.95)	4.28 (1.28–7.28)	0.22
Model 3	0.77 (0.75–0.79)	0.77 (0.24–1.29)	2.00 (-1.51–5.52)	0.10
Model 4	0.79 (0.77–0.81)	0.52 (0.07–0.97)	3.03 (-0.17–6.22)	0.63
Confounders only	0.79 (0.77–0.81)	NA	NA	0.34
<b>Non-cardiovascular mortality</b>				
Model 1	0.61 (0.58–0.64)	NA	NA	0.70
Model 2	0.77 (0.75–0.80)	0.30 (-0.08–0.68)	0.73 (-3.14–4.61)	0.46
Model 3	0.77 (0.75–0.80)	0.35 (-0.06–0.76)	0.60 (-3.45–4.66)	0.14
Model 4	0.79 (0.76–0.81)	0.41 (-0.06–0.88)	2.72 (-0.90–6.34)	0.73
Confounders only	0.78 (0.76–0.81)	NA	NA	0.48

*Model 1: crude model*

*Model 2: model 1 + age, sex*

*Model 3: model 2 + BMI, cholesterol/HDL ratio*

*Model 4: model 3 + smoking, systolic blood pressure, diabetes duration, serum creatinine, glycated hemoglobin, cardiovascular history, ACE inhibition, albumin to creatinine ratio*

*IDI, integrated discrimination improvement; NRI, net reclassification improvement; BMI, body mass index; HDL, high density lipoprotein; ACE, angiotensin converting enzyme.*

## SENSITIVITY ANALYSES

In a sensitivity analysis, we investigated whether ALT was associated with all-cause and non-cardiovascular mortality, irrespective of statin treatment. After the exclusion of subjects using statins ( $n = 191$ ), results remained essentially similar. Furthermore, after the exclusion of subjects with ALT levels above the ULN ( $\geq 45$  U/l for men and  $\geq 34$  U/l for women,  $n = 23$ ), we found similar results for the association with all-cause and non-cardiovascular mortality. The U-shape of the association of ALT with all-cause mortality disappeared after exclusion of these subjects ( $P$  for non-linearity = 0.32). Finally, results remained materially unchanged after additional adjustment for an indicator of physical functioning.

## DISCUSSION

In this prospective cohort of patients with type 2 diabetes, we found an inverse association of serum levels of ALT with all-cause mortality. Furthermore, ALT was not associated with cardiovascular mortality, but particularly with non-cardiovascular mortality. These associations were independent of potential confounders. ALT was not associated with progression of renal function. Furthermore, there was a bimodal, U-shaped, association of ALT with all-cause mortality.

To our knowledge, the association of ALT with progression of renal function has not been investigated to date. In previous studies it has been shown that the prevalence of CKD is significantly higher among patients with both diabetes and NAFLD compared to those without NAFLD (8,9). In those studies, the majority of patients had ALT levels within the normal range (8,9). Thus, ALT may be an imperfect marker for NAFLD. This may explain why we did not find an association of ALT with progression of renal function.

It is established that elevated levels of ALT are associated with NAFLD. Besides other components of the metabolic syndrome, NAFLD is associated with increased cardiovascular mortality (26,27). In contrast with our findings of an inverse association of ALT with mortality, three population-based cohort studies have reported positive associations of ALT with mortality among subjects with levels of ALT above the ULN (12,28,29). Like mentioned, our study differs from these studies in that we investigated the association of ALT with mortality comparing subjects within the normal range. Several population-based studies that mainly compared subjects within the normal range of ALT also found an inverse association of ALT with mortality (13–15,30–32).

Apparently, studies that focused on high versus normal ALT found positive associations of ALT with mortality, whereas studies that focused on ALT within the normal range found inverse associations of ALT with mortality. It is tempting to speculate that both high and low levels of ALT are associated with mortality. Such a trend would be reflected in a U-shaped relationship or bimodal association. Indeed, other studies found bimodal associations of ALT with all-cause mortality (13,28). In line, the results of the present study also showed a significant bimodal association of ALT with all-cause mortality. It might also be speculated that ALT above the normal range heralds NAFLD (10) and might therefore be a risk factor of cardiovascular mortality, whereas low ALT increases the risk of non-cardiovascular mortality. Because few subjects in our study had ALT levels above the normal range, this might explain why we did not find an association of ALT with cardiovascular mortality whereas previous studies that focused on elevated levels of ALT indeed found a positive association of ALT with cardiovascular mortality (29).

There are several potential explanations for the inverse association of ALT with all-cause mortality in the present study. Firstly, ALT levels are associated with age (33–

35). Elinav et al. reported the highest ALT levels at 50 years, with decreasing ALT levels with advancing age (35). In the present study, ALT and age were also correlated. Interestingly, in studies that are in disagreement with our findings, mean age was approximately 13 years lower compared to our study population (12,36). However, we did not find evidence for an interaction of ALT with age and adjustment for age did not alter the association of ALT with mortality. It has been suggested that ALT levels decrease with advancing age because of hepatic aging (14). During aging, hepatic blood flow decreases, which subsequently reduces the number of functional hepatocytes (14). Because hepatocytes are the main site for production of ALT, a decrease in functional hepatocytes is reflected by a decrease in ALT (37,38). A decrease in functional hepatocytes has detrimental effects, since hepatocytes are important mediators in detoxification, lipid metabolism, and glucose regulation (39,40).

Frailty may be another potential explanation. In our analyses, we used the physical functioning subscale of the RAND-36 questionnaire, which has been used to serve as the physical weakness component of the Fried frailty criteria (19,20), rather than the complete set of Fried frailty criteria. This means that, although we found the association of ALT with mortality to be independent of physical functioning, we cannot exclude the possibility that frailty actually mediates the association of ALT with mortality, as has been found in a previous study on this subject (15). Furthermore, although ALT is generally thought of in relation to the liver, ALT is also found in other tissues including skeletal muscle, albeit in smaller quantities (41). It may be possible that increased mortality found with low ALT could result from smaller muscle mass. Because we do not have data available about muscle mass we, unfortunately, could not take this possibility into consideration.

It should also be noted that the level of ALT was lower than expected. Because ALT is stable when stored at  $-20^{\circ}\text{C}$  (21), and because in the present study samples were stored at  $-80^{\circ}\text{C}$ , we believe that it is unlikely that frozen storage has affected the results concerning the measurement of ALT. In addition, pyridoxal phosphate (vitamin B6) serves as a co-enzyme for the activation of ALT. Therefore, a deficiency of vitamin B6 may result in lower activity of circulating ALT *in vivo* (14,42). Because we used an assay with activation by pyridoxal phosphate, (vitamin B6), and because pyridoxal phosphate activation causes activation of all available ALT, it is unlikely that vitamin B6 deficiency is involved in the associations of ALT with outcomes that we observed. It may be hypothesized that the relatively low level of ALT that we observed is a consequence of healthy survivor bias.

This study has some limitations. First, no data of alcohol use were available. ALT levels can be elevated in the presence of alcoholic liver disease, which might have caused unmeasured confounding. However, if associations of ALT and mortality are as we report in patients with type 2 diabetes, this would have caused underestimation rather than overestimation of our results. Second, no ultrasonography or biopsies were

performed to determine whether subjects had NAFLD. Thirdly, since ALT was only measured at baseline, variation in ALT over time cannot be taken into account. The strengths of this study are the prospective design, the large number of events and the length of follow-up period.

In conclusion, our results show an inverse association of ALT with all-cause mortality and particularly with non-cardiovascular mortality in patients with type 2 diabetes. Furthermore, there appears to be a U-shaped, bimodal relationship of ALT with all-cause mortality. The association of ALT and mortality seems different when ALT is mainly studied within the normal range rather than with inclusion of a large number of subjects that have levels of ALT above the normal range. ALT was not associated with progression of renal function in this population of patients with type 2 diabetes.

## ACKNOWLEDGEMENTS

This research was supported by GlaxoSmithKline, the Netherlands. The funding source had no further involvement in study design, data collection, or interpretation of the data. No other potential conflicts of interest were reported.

## REFERENCES

1. International Diabetes Federation. Available at: <http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures>. Accessed April, 2013.
2. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* 2005 Nov 15;143(10):722–728.
3. Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, et al. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab* 1999 May;84(5):1513–1517.
4. Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. *Diabetes Care* 2007 Aug;30(8):2119–2121.
5. Targher G, Byrne CD. Clinical Review: Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* 2013 Feb;98(2):483–495.
6. Kotronen A, Yki-Jarvinen H. Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2008 Jan;28(1):27–38.
7. Adams LA, Harmsen S, St Sauver JL, Charatcharoenwitthaya P, Enders FB, Therneau T, et al. Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. *Am J Gastroenterol* 2010 Jul;105(7):1567–1573.

8. Targher G, Pichiri I, Zoppini G, Trombetta M, Bonora E. Increased prevalence of chronic kidney disease in patients with Type 1 diabetes and non-alcoholic fatty liver. *Diabet Med* 2012 Feb;29(2):220–226.
9. Targher G, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C, et al. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. *Diabetologia* 2008 Mar;51(3):444–450.
10. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000 Apr 27;342(17):1266–1271.
11. Ioannou GN, Weiss NS, Boyko EJ, Mozaffarian D, Lee SP. Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. *Hepatology* 2006 May;43(5):1145–1151.
12. Lee TH, Kim WR, Benson JT, Therneau TM, Melton LJ, 3rd. Serum aminotransferase activity and mortality risk in a United States community. *Hepatology* 2008 Mar;47(3):880–887.
13. Ruhl CE, Everhart JE. The Association of Low Serum Alanine Aminotransferase Activity With Mortality in the US Population. *Am J Epidemiol* 2013 Sep 26;178(12):1702–11.
14. Elinav E, Ackerman Z, Maaravi Y, Ben-Dov IZ, Ein-Mor E, Stessman J. Low alanine aminotransferase activity in older people is associated with greater long-term mortality. *J Am Geriatr Soc* 2006 Nov;54(11):1719–1724.
15. Le Couteur DG, Blyth FM, Creasey HM, Handelsman DJ, Naganathan V, Sambrook PN, et al. The association of alanine transaminase with aging, frailty, and mortality. *J Gerontol A Biol Sci Med Sci* 2010 Jul;65(7):712–717.
16. Drion I, Kleefstra N, Landman GW, Alkhalaf A, Struck J, Groenier KH, et al. Plasma COOH-terminal proendothelin-1: a marker of fatal cardiovascular events, all-cause mortality, and new-onset albuminuria in type 2 diabetes? (ZODIAC-29). *Diabetes Care* 2012 Nov;35(11):2354–2358.
17. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med* 2005 Mar;63(3):103–110.
18. Lutgers HL, Gerrits EG, Graaff R, Links TP, Sluiter WJ, Gans RO, et al. Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus. *Diabetologia* 2009 May;52(5):789–797.
19. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001 Mar;56(3):M146–56.
20. Sirola J, Pitkala KH, Tilvis RS, Miettinen TA, Strandberg TE. Definition of frailty in older men according to questionnaire data (RAND-36/SF-36): The Helsinki Businessmen Study. *J Nutr Health Aging* 2011 Nov;15(9):783–787.
21. Cuhadar S, Koseoglu M, Atay A, Dirican A. The effect of storage time and freeze-thaw cycles on the stability of serum samples. *Biochem Med (Zagreb)* 2013;23(1):70–77.

22. Schumann G, Bonora R, Ceriotti F, Ferard G, Ferrero CA, Franck PF, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. *Clin Chem Lab Med* 2002 Jul;40(7):718–724.
23. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009 May 5;150(9):604–612.
24. Hosmer DW, Lemeshow S, May S. *Applied Survival Analysis: Regression Modeling of Time-to-Event Data*, Chapter 5. 2nd ed.: John Wiley & Sons; 2008.
25. Pencina MJ, D'Agostino RB S, D'Agostino RB, Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 2008 Jan 30;27(2):157–72; discussion 207–12.
26. Calori G, Lattuada G, Ragona F, Garancini MP, Crosignani P, Villa M, et al. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology* 2011 Jul;54(1):145–152.
27. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006 Oct;44(4):865–873.
28. Koehler EM, Sanna D, Hansen BE, van Rooij FJ, Heeringa J, Hofman A, et al. Serum liver enzymes are associated with all-cause mortality in an elderly population. *Liver Int* 2014 Feb;34(2):296–304.
29. Yun KE, Shin CY, Yoon YS, Park HS. Elevated alanine aminotransferase levels predict mortality from cardiovascular disease and diabetes in Koreans. *Atherosclerosis* 2009 Aug;205(2):533–537.
30. Schooling CM, Kelvin EA, Jones HE. Alanine transaminase has opposite associations with death from diabetes and ischemic heart disease in NHANES III. *Ann Epidemiol* 2012 Nov;22(11):789–798.
31. Hovinen SM, Pitkala KH, Tilvis RS, Strandberg TE. Alanine aminotransferase activity and mortality in older people. *J Am Geriatr Soc* 2010 Jul;58(7):1399–1401.
32. Ford I, Mooijaart SP, Lloyd S, Murray HM, Westendorp RG, de Craen AJ, et al. The inverse relationship between alanine aminotransferase in the normal range and adverse cardiovascular and non-cardiovascular outcomes. *Int J Epidemiol* 2011 Dec;40(6):1530–1538.
33. Dong MH, Bettencourt R, Brenner DA, Barrett-Connor E, Loomba R. Serum levels of alanine aminotransferase decrease with age in longitudinal analysis. *Clin Gastroenterol Hepatol* 2012 Mar;10(3):285–90.e1.
34. Dong MH, Bettencourt R, Barrett-Connor E, Loomba R. Alanine aminotransferase decreases with age: the Rancho Bernardo Study. *PLoS One* 2010 Dec 8;5(12):e14254.
35. Elinav E, Ben-Dov IZ, Ackerman E, Kiderman A, Glikberg F, Shapira Y, et al. Correlation between serum alanine aminotransferase activity and age: an inverted U curve pattern. *Am J Gastroenterol* 2005 Oct;100(10):2201–2204.



36. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. *Gastroenterology* 2009 Feb;136(2):477–85. e11.
37. Ruhl CE, Everhart JE. Upper limits of normal for alanine aminotransferase activity in the United States population. *Hepatology* 2012 Feb;55(2):447–454.
38. Matloff DS, Selinger MJ, Kaplan MM. Hepatic transaminase activity in alcoholic liver disease. *Gastroenterology* 1980 Jun;78(6):1389–1392.
39. Martin R, Fitzl G, Mozet C, Martin H, Welt K, Wieland E. Effect of age and hypoxia/reoxygenation on mRNA expression of antioxidative enzymes in rat liver and kidneys. *Exp Gerontol* 2002 Dec;37(12):1481–1487.
40. Castro Mdel R, Suarez E, Kraiselburd E, Isidro A, Paz J, Ferder L, et al. Aging increases mitochondrial DNA damage and oxidative stress in liver of rhesus monkeys. *Exp Gerontol* 2012 Jan;47(1):29–37.
41. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC, Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* 2008 Apr;47(4):1363–1370.
42. Ono K, Ono T, Matsumata T. The pathogenesis of decreased aspartate aminotransferase and alanine aminotransferase activity in the plasma of hemodialysis patients: the role of vitamin B6 deficiency. *Clin Nephrol* 1995 Jun;43(6):405–408.