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Alanine aminotransferase and the 6-year risk of the metabolic syndrome in Caucasian men and women: the Hoorn Study

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Abstract

Aims To study the association between alanine aminotransferase (ALT) and the 6-year risk of the metabolic syndrome in a population-based study in Caucasian men and women.

Methods The association of ALT with the 6-year risk of the metabolic syndrome in 1097 subjects, aged 50–75 years, was assessed in the Hoorn Study with logistic regression analysis. Subjects with the metabolic syndrome at baseline, defined according to the Adult Treatment Panel III of the National Cholesterol Education Program, were excluded.

Results After 6.4 (range 4.4–8.1) years follow-up, 226 subjects (20.6%) had developed the metabolic syndrome. The odds ratio (95% confidence interval) for developing the metabolic syndrome, adjusted for age, sex, alcohol intake and follow-up duration was 2.25 (1.50–3.37) for subjects in the upper tertile compared with those in the lower tertile of ALT. This association persisted after additional adjustment for all the baseline metabolic syndrome features [1.62 (1.02–2.58)]. Among the individual components of the metabolic syndrome, ALT was significantly associated only with fasting plasma glucose at follow-up.

Conclusions These data suggest that ALT is associated with risk of the metabolic syndrome in a general population of middle-aged Caucasian men and women, further strengthening the role of ALT as an indicator for future metabolic derangement. These findings warrant further studies to elucidate the role of non-adipose tissue fat accumulation in the pathogenesis of complications related to the metabolic syndrome.

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Keywords metabolic syndrome, alanine aminotransferase, epidemiology, elderly

Abbreviations ALT, alanine aminotransferase; CI, confidence interval; CRP, C-reactive protein; DM2, Type 2 diabetes mellitus; HDL, high-density lipoprotein; OR, odds ratio

Introduction

The metabolic syndrome, characterized by abdominal obesity, hyperglycaemia, hypertension and dyslipidaemia, confers a

high risk of the development of Type 2 diabetes mellitus (DM2) [1,2] and cardiovascular disease (CVD) [3]. The prevalence of elevated liver enzymes, including alanine aminotransferase (ALT), is higher in individuals with DM2 or the metabolic syndrome [4,5]. Several cross-sectional studies have demonstrated associations of ALT with individual components of the metabolic syndrome, including obesity and dyslipidaemia [6,7]. To date, a limited number of studies have addressed the prospective

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relation of liver enzymes with the future risk of the metabolic syndrome. Nakanishi *et al.* found that, of the liver enzymes studied, ALT was associated with risk of metabolic syndrome, but this study was limited to middle-aged Japanese men and used body mass index instead of waist circumference to define the metabolic syndrome [8]. Hanley *et al.* studied the relation of four different liver enzymes (including ALT) with the development of the metabolic syndrome in a multi-ethnic cohort and demonstrated that ALT was positively associated with risk of the metabolic syndrome [9]. In a Japanese cohort of male and female health-care employees, Suzuki *et al.* studied the sequential order of elevated transaminases (ALT and aspartate aminotransferase) with features of the metabolic syndrome, and found that elevated transaminases are preceded by weight gain, while the other features are followed by elevated transaminases [10]. To date, no study has assessed the prospective relationship of ALT and the metabolic syndrome in a population-based study in Caucasian men and women.

In the present study, we addressed the prospective association of ALT with the 6-year risk of the metabolic syndrome, defined according to the Adult Treatment Panel III of the National Cholesterol Education Program, and with the individual components of the metabolic syndrome in a population-based study of glucose tolerance and related complications in Caucasian men and women aged 50–75 years at baseline.

Subjects and methods

Subjects and follow-up

The subjects were participants in the Hoorn Study, a prospective population-based cohort study of glucose metabolism and diabetes complications [11]. Briefly, in 1989, a random sample of all man and women aged 50 to 75 years was taken from the municipal registry of the town of Hoorn in the Netherlands. Of the 3552 individuals who were invited to take part in the study, 2540 agreed to participate (71%). Baseline data were collected from October 1989 to February 1992. After excluding non-Caucasians ($n = 56$), the study cohort consisted of 2484 men and women. Between January 1996 and December 1998, 2086 participants were invited to take part in a follow-up examination; the other members of the original study population were not invited for logistical reasons ($n = 140$), or because they had died ($n = 150$) or had moved out of Hoorn ($n = 108$). In total, 1513 subjects (72.5%) took part in the follow-up measurements [12]. Metabolic syndrome at baseline and at follow-up was defined according to the Adult Treatment Panel III of the National Cholesterol Education Program, i.e. three or more of the following: fasting glucose ≥ 6.1 mmol/l, high-density lipoprotein (HDL) cholesterol < 1.0 mmol/l (men) or < 1.3 mmol/l (women), triglycerides ≥ 1.7 mmol/l, waist circumference ≥ 102 cm (men) or ≥ 88 cm (women) and hypertension $\geq 130/85$ mmHg [13]. For the present study, subjects with the metabolic syndrome and/or DM2 at baseline were excluded ($n = 329$). Furthermore, subjects with missing data on alcohol intake, missing data on ALT enzyme activity, insulin, smoking status and physical activity at baseline were also excluded ($n = 22$). Finally, subjects with

missing data to define the metabolic syndrome at follow-up ($n = 63$) and subjects with ALT levels > 2.5 times the upper level of the reference value ($n = 2$) were also excluded, the latter to reduce confounding as a result of subjects with ALT elevation caused by viral or toxic agents. Thus, this analysis includes 1097 subjects. The Ethical Review Committee of VU University Medical Centre approved the Hoorn Study and written informed consent was obtained from all participants.

Methods

Measurements

Fasting blood samples were collected after an overnight fast from 20.00 h the evening before. Serum ALT enzyme activity was measured according to the 1985 method of the International Federation of Clinical Chemistry [14]. Glucose was measured in plasma with the glucose dehydrogenase method (Merck, Darmstadt, Germany) at the baseline measurement and with the hexokinase method (Boehringer, Mannheim, Germany) at the follow-up measurement. Immuno-specific insulin was measured in serum with a double-antibody RIA (antibody SP21; Linc Research, St Louis, MO, USA). Triglycerides, total and HDL cholesterol were determined in serum by enzymatic techniques (Boehringer, Mannheim, Germany). Systolic and diastolic blood pressure was measured twice on the right arm with a random-zero sphygmomanometer (Hawksley-Gelman, Lancing, UK) and the mean of both measurements used for the analyses. Information on alcohol intake was assessed by a validated semiquantitative food frequency questionnaire [15]. Subjects were asked if they drink alcohol and, if so, how many glasses they consume in a week. This information was converted to alcohol intake in g/day with the computerized version of the Dutch Food Composition Table. Physical activity was assessed as described previously [11]. Weight and height were measured in subjects wearing light clothes only, and the body mass index was calculated as weight divided by height squared (kg/m^2). Waist and hip circumference were measured according to a standardized method [16]. Smoking status was assessed by questionnaire.

Statistical analysis

Data are presented as mean (SD) or as median (interquartile range) for variables with a skewed distribution according to tertiles of ALT. For the prospective data, logistic regression analyses were performed to calculate the odds ratios (ORs) and the 95% confidence intervals (CIs) for the metabolic syndrome. The upper tertile of ALT was compared with the lower tertiles of ALT. The first model was adjusted for age, sex, alcohol intake and follow-up duration, because follow-up differed between subjects. In the final model, additional adjustments were made for the individual components of the metabolic syndrome criteria entered as continuous variables. To assess the prospective relation of ALT with the individual components of the metabolic syndrome, we used multivariate linear regression models. In these models, the individual metabolic syndrome component at follow-up was entered as the outcome variable and ALT as the determinant. These models were adjusted for age, sex and follow-up duration and subsequently adjusted for the metabolic syndrome component at baseline. These associations were expressed as

Table 1 Baseline characteristics of the participants ($n = 1097$) stratified by ALT tertiles

Variables	Alanine aminotransferase activity (U/l)			P_{trend}^*
	Tertile 1	Tertile 2	Tertile 3	
n	329	418	350	
ALT (U/l)	10.4 ± 2.1	15.6 ± 1.5	27.3 ± 10.1	
Age (years)	60.9 ± 6.9	60.4 ± 6.8	58.6 ± 6.3	<0.001
Male (%)	27.1	41.9	57.4	<0.001
Body mass index (kg/m^2)	25.2 ± 2.9	25.5 ± 2.7	26.6 ± 3.0	<0.001
Waist (cm)	84.5 ± 9.5	87.4 ± 8.7	91.0 ± 8.8	<0.001
Fasting glucose (mmol/l)	5.2 ± 0.5	5.3 ± 0.5	5.4 ± 0.5	<0.001
Plasma lipids (mmol/l)				
Total cholesterol	6.5 ± 1.1	6.5 ± 1.0	6.6 ± 1.1	0.016
HDL cholesterol	1.42 ± 0.35	1.41 ± 0.36	1.38 ± 0.39	0.020
Triglycerides	1.2 (0.9–1.5)	1.2 (0.9–1.5)	1.3 (1.0–1.6)	0.054
Systolic BP (mmHg)	127 ± 18	130 ± 17	131 ± 18	<0.001
Diastolic BP (mmHg)	78 ± 10	79 ± 9	82 ± 9	<0.001

ALT, alanine aminotransferase; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data are means $\pm$ SD or median (interquartile range) for variables with a skewed distribution.
*Tested for trend with regression analyses adjusted for age and sex.

standardized betas with the 95% CI. A standardized beta of 0.5 means that, if the independent variable increases by 1 SD, the dependent variable increases by 0.5 SD. Variables with a skewed distribution were entered in the models after logarithmic transformation. The statistical analyses were performed with SPSS for Windows version 11.0.5 (SPSS Inc., Chicago, IL, USA). A two-sided P -value < 0.05 was considered to indicate statistical significance. Effect modification by age and sex were tested by entering interaction terms (age times ALT tertile or sex times ALT tertile) into the models. We choose a two-sided P -value < 0.1 to indicate effect modification.

Results

Baseline characteristics

The mean age of the 1097 participants (465 men and 632 women) was 60.0 years (SD 6.7). Table 1 shows the baseline characteristics of the participants stratified by ALT divided into tertiles. Subjects in the upper tertile were younger, more likely to be male, had higher fasting glucose and cholesterol levels, lower HDL cholesterol levels and a higher blood pressure, compared with those in the lower tertile. The difference in fasting triglyceride levels was not statistically significant.

Risk for development of the metabolic syndrome

Table 2 shows the OR of development of the metabolic syndrome in 1097 men and women who were free of the metabolic syndrome at baseline after a mean of 6.4 (range 4.4–8.1) years of follow-up with ALT divided into tertiles. Of these, 226 (20.6%) subjects developed metabolic syndrome at follow-up. In the model adjusted for age, sex and follow-up duration and alcohol intake, subjects in the upper tertile had an OR of

2.25 (95% CI 1.50–3.37) compared with those in the lower tertile. The subsequent models in which components of the metabolic syndrome were entered as continuous variables showed that this relation was most strongly attenuated by waist, followed by glucose and to a lesser extent by blood pressure, but the associations remained statistically significant [OR 1.62 (1.02–2.58)]. Lifestyle factors, including physical activity and smoking, and fasting insulin did not affect the relationship to a significant extent. No effect modification for sex or age was observed (both $P > 0.1$). To further address the effect of alcohol intake as a major potential confounder, we excluded subjects ($n = 152$) with an alcohol intake of more than 20 g per day. However, this only led to a small attenuation of model 1 [2.10 (1.36–2.54)].

The models in which the components of the metabolic syndrome were entered as dichotomised variables (according to the Adult Treatment Panel III of the National Cholesterol Education Program) did not materially change the associations presented in Model 1 (data not shown); the OR of Model 2 was slightly higher [OR 2.02 (1.31–3.12)].

The analyses assessing the relation of ALT with the individual components of the metabolic syndrome showed that ALT was associated with all the components at follow-up except for HDL cholesterol. After adjustment for the baseline values of the individual components, ALT was significantly associated with glucose only (Table 3).

Discussion

The main finding of this study is that, in an elderly population of non-diabetic Caucasian men and women free of the metabolic syndrome at baseline, higher ALT was associated with an increased risk of conversion to the metabolic syndrome. This

Table 2 The associations of ALT with development of the metabolic syndrome ($n = 1097$)

Models* <i>n</i>	ALT enzyme activity		
	Tertile 1 329	Tertile 2 418	Tertile 3 350
Model 1	1	1.43 (0.96–2.13)	2.25 (1.50–3.37)
Model 1 + waist	1	1.30 (0.89–1.95)	1.70 (1.11–2.58)
Model 1 + glucose	1	1.34 (0.89–2.01)	2.01 (1.33–3.03)
Model 1 + HDL cholesterol	1	1.55 (1.03–2.32)	2.60 (1.72–3.95)
Model 1 + triglycerides	1	1.54 (1.03–2.35)	2.18 (1.43–3.33)
Model 1 + systolic BP	1	1.39 (0.93–2.08)	2.08 (1.38–3.14)
Model 1 + diastolic BP	1	1.41 (0.95–2.10)	2.11 (1.40–3.18)
Model 1 + lifestyle factors†	1	1.45 (0.97–2.18)	2.29 (1.51–3.48)
Model 1 + insulin	1	1.46 (0.97–2.20)	2.19 (1.44–3.35)
Model 2	1	1.40 (0.90–2.18)	1.62 (1.02–2.58)

*Model 1 adjusted for age, sex, alcohol intake and follow-up duration; Model 2, Model 1 + adjusted for waist, glucose, high-density lipoprotein (HDL) cholesterol, triglycerides and systolic and diastolic blood pressure (BP).
†Lifestyle factors: smoking and physical activity.

Table 3 Multivariate linear regression analysis of ALT with the individual metabolic syndrome components at follow-up*

Metabolic syndrome component at follow-up	Standardized betas (95% CI)	
	Model 1	Model 2
Fasting glucose	0.19 (0.13; 0.25)	0.13 (0.07; 0.18)
Waist circumference	0.17 (0.11; 0.22)	0.004 (−0.03; 0.04)
Triglycerides	0.10 (0.03; 0.16)	0.05 (−0.001; 0.10)
Diastolic blood pressure	0.10 (0.04; 0.17)	0.03 (−0.02; 0.09)
Systolic blood pressure	0.09 (0.03; 0.15)	0.03 (−0.02; 0.08)
HDL cholesterol	0.03 (−0.03; 0.09)	−0.02 (−0.05; 0.02)

Models 1 and 2 adjusted for age, sex, alcohol intake and follow-up duration.
Model 2 additionally adjusted for the baseline value of the individual component.
*Standardized betas indicate SD difference per 1 SD ALT at baseline.

association was independent of age, sex, alcohol intake, follow-up duration and the baseline metabolic syndrome components. In the prospective analyses of the individual components of the metabolic syndrome, ALT was significantly associated with fasting glucose levels and to a lesser extent with HDL cholesterol, triglyceride levels and elevated blood pressure and waist circumference.

The findings of the present study are in accordance with the two previous studies, predominantly performed in Asian [8] and African-American and Hispanic [9] populations, that addressed the relation of liver enzymes including ALT with future risk of the metabolic syndrome. Correction of all the baseline metabolic syndrome criteria may result in over-correction, implying that the models in the present study may underestimate the true associations. Other risk factors for the development of the metabolic syndrome have been studied. These factors included C-reactive protein (CRP) [17], physical inactivity [18], pro-insulin [19] and the individual components themselves [19]. In the present study, physical activity did not affect the associations. Unfortunately, pro-insulin and CRP

levels were not available at baseline in the Hoorn study cohort and consequently the effect of these mediating or confounding variables could not be studied.

The chronological ordering of elevated transaminases (ALT and aspartate aminotransferase) in relation to components of the metabolic syndrome was studied by Suzuki *et al.*; they reported that weight gain, low HDL cholesterol and hypertriglyceridaemia preceded transaminase elevation, and that hypertension and glucose intolerance then followed [10]. Our study was not designed to study the chronological ordering of ALT with the individual components of the metabolic syndrome; however, our results do support some of the findings by Suzuki *et al.*—that elevated ALT precedes components of the metabolic syndrome. Indeed, glucose at follow-up, adjusted for baseline glucose values, was the strongest component associated with elevated ALT.

The mechanisms underlying the observed association between ALT (and other liver enzymes) and the metabolic syndrome are not yet fully understood. ALT is most strongly associated with liver fat accumulation measured by proton magnetic

resonance spectroscopy [20], reflecting fat deposition in the liver, which is regarded as a feature of the metabolic syndrome [21,22]. This hepatic fat deposition, as reflected by slightly elevated ALT levels, may lead to early hepatic and systemic insulin resistance [23,24]. Indeed, an earlier study demonstrated that hepatic fat content measured by proton spectroscopy is associated with insulin resistance, independent of obesity [25]. In addition, hepatic fat content was related to decreased suppression of insulin-mediated hepatic glucose output, which is in line with the observation of the present study that ALT is positively associated with fasting glucose levels in the prospective analysis. High ALT levels might also reflect hepatic and systemic inflammation, and the interplay of visceral adipose tissue, stimulating hepatic CRP production which may contribute to further metabolic derangement. The results of the present study show that subjects with slightly elevated ALT have a higher risk of developing the metabolic syndrome.

A number of potential limitations of this study should be considered. The population consisted of Caucasian individuals aged 50–75 years, and caution should be exercised in generalizing our findings to other populations. Although we controlled for alcohol as a confounder in our study, we could not account for other factors that might have caused a rise in ALT, such as viral hepatitis infections or hepatotoxic drugs. To overcome this limitation to some extent, subjects with an ALT level of 2.5 times the upper level of the reference value were excluded. In approximately one-third of hepatitis C infections, a condition associated with an increased risk for development of diabetes, subjects may have normal ALT levels [26,27]. However, the effect of hepatitis C on our results may be very limited, as the prevalence of hepatitis C infection in the Dutch general population is very low [28]. In addition, the association of other liver enzymes such as aspartate aminotransferase and γ -glutamyltransferase with risk of developing the metabolic syndrome was not assessed. A previous study, however, found that ALT was the enzyme most strongly correlated to hepatic fat accumulation [20]. In the Hoorn study, no investigations were performed to assess the cause of elevated ALT. However, all relevant baseline data was sent to the individual's general practitioner, with advice to perform further tests according to prevailing guidelines.

Despite these limitations, the findings obtained in a population-based study of elderly Caucasian men and women support the conclusion that ALT is positively associated with the metabolic syndrome and further studies are needed to elucidate and understand the pathophysiological mechanisms.

Competing interests

None declared.

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