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Higher Concentrations of Alanine Aminotransferase within the Reference Interval Predict Nonalcoholic Fatty Liver Disease

Yoosoo Chang,[1] Seungho Ryu,[2*] Eunju Sung,[3] and Yumi Jang[4]

Background: **In nonalcoholic fatty liver disease (NAFLD), increased alanine aminotransferase (ALT) concentrations are considered to be a consequence of hepatocyte damage. We performed a prospective study to examine the association between ALT within its reference interval and risk for subsequent development of NAFLD.**

Methods: **The study cohort comprised 5237 healthy men without diagnosed NAFLD and without increases of either ALT (≥35 U/L) or γ-glutamyltransferase (GGT; ≥40 U/L) above the reference intervals. We assessed alcohol intake via self-reporting (questionnaire) and performed biochemical tests for liver and metabolic function and abdominal ultrasonography. We used the Cox proportional hazards model to calculate the adjusted hazard ratios (aHRs) in the model for NAFLD.**

Results: **During 13 276.6 person-years of follow-up over a 4-year period, 984 new incident cases of NAFLD developed. We adjusted for age, weight change, body mass index, glucose, blood pressure, triglycerides, HDL cholesterol, smoking, alcohol consumption, regular exercise, homeostasis model assessment of insulin resistance, C-reactive protein, and incident diabetes. Compared with an ALT concentration of <16 U/L, aHR values (95% confidence intervals) for ALT concentrations were 1.53 (1.18–1.98), 1.66 (1.29–2.13), 1.62 (1.26–2.08), and 2.21 (1.73–2.81) for ALT concentrations of 16–18, 19–21, 22–25, and 26–34 U/L, respectively. This relationship remained significant even among normal-weight participants who were still within the reference interval of ALT and GGT at all follow-up examinations.**

Conclusions: **In apparently healthy, nondiabetic Korean men, increased ALT concentration, even within the reference interval, was an independent predictor of incident NAFLD.**

Hepatic steatosis unrelated to excessive alcohol consumption is termed nonalcoholic fatty liver disease (NAFLD).[5] NAFLD encompasses the entire spectrum of liver conditions, ranging from simple steatosis through steatohepatitis to advanced fibrosis and cirrhosis *(1)*. Concurrent with the worldwide epidemic of obesity, NAFLD is considered to be the most common cause of unexplained abnormal results of liver function tests *(2)*. Although hepatic steatosis was long believed to be a benign disease, NAFLD has recently gained much interest *(3)*. Percentage hepatic fat has been reported to be a feature highly associated with insulin resistance *(4, 5)*. Furthermore, several studies have suggested an association between NAFLD and features of the metabolic syndrome, including dyslipidemia and obesity, thereby stressing the association with insulin resistance as an important feature of NAFLD *(6, 7)*. Currently, NAFLD is recognized as a pathogenic factor of insulin resistance and type 2 diabetes *(3)*.

Several prospective epidemiological studies have demonstrated that increased concentrations of hepatic enzyme in serum, even within the reference interval, may be related to increased risk of type 2 diabetes and the metabolic syndrome, as well as death *(8–10)*. Among the hepatic enzymes aspartate aminotransferase (AST), ala-

[1] Health Screening Center, [2] Department of Occupational Medicine, and [3] Department of Family Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea.

[4] Department of Radiology, Asan Medical Center, University of Ulsan College of Medicine, Ulsan, Korea.

* Address correspondence to this author at: Kangbuk Samsung Hospital, 108 Pyung dong, Jongro-Gu, Seoul, Korea 110-746. Fax 82-2-2001-2626; e-mail sh703.yoo@samsung.com.

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[5] Nonstandard abbreviations: NAFLD, nonalcoholic fatty liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; HOMA-IR, homeostasis model assessment of insulin resistance; CRP, C-reactive protein; BMI, body mass index; CI, confidence interval; aHR, adjusted hazard ratio.

nine aminotransferase (ALT), and γ-glutamyltransferase (GGT), ALT is most closely related to liver fat accumulation *(11)*. In cross-sectional studies *(12)*, participants with NAFLD often have increased circulating concentrations of ALT. Paradoxically, the complete spectrum of NAFLD was reported in patients with normal ALT activity, even after the cutoff value was decreased to ≤19 U/L *(13)*. Moreover, increased ALT not associated with fatty liver was observed frequently in obese participants *(14)*. ALT actually is a glucogenic enzyme, and increased ALT has been demonstrated to be an indicator of impaired insulin signaling, which might not necessarily be associated with liver injury due to hepatic steatosis *(3, 15)*. To date, although an increased ALT concentration is considered a consequence of hepatocyte damage in NAFLD *(3)*, it is unclear what underlies the relationship between ALT and NAFLD. Of more recent interest, an inverse correlation between ALT concentrations and adiponectin concentrations has been demonstrated *(16–18)*. As these previous observational findings are consistent with a biological link between ALT and development of NAFLD, we performed a prospective study to test whether higher concentrations of ALT within its reference interval predict future NAFLD in apparently healthy men in Korea.

Materials and Methods

STUDY POPULATION

We conducted a prospective cohort study of nondiabetic Korean men at a large semiconductor manufacturing company and its 13 affiliates. All employees participate in either annual or biennial health examinations, as required by Korea's Industrial Safety and Health Law. The study population included men ≥40 years old who underwent an annual comprehensive health examination and men 30 to 39 years old who underwent a biennial comprehensive health examination. In 2002, 15 347 workers, ages 30 to 59 years, participated in the comprehensive health examinations at a university hospital in Seoul, Korea. We excluded 9462 men based on the following exclusion criteria that might influence insulin resistance or ultrasonography findings of the liver as a result of other liver disease (because some individuals met more than 1 exclusion criterion, the following data total more than 9462): (*a*) 27 had a history of a malignancy; (*b*) 16 had a history of cardiovascular disease; (*c*) 125 reported current use of antihyperlipidemics; (*d*) 279 had fasting glucose concentrations ≥7.0 mmol/L or current use of blood glucose–lowering agents; (*e*) 2498 reported an alcohol intake ≥20 g/day; (*f*) 5053 had fatty liver based on ultrasonography; (*g*) 6928 had (*i*) a positive serologic finding for hepatitis B or C virus, (*ii*) chronic liver disease or liver cirrhosis based on ultrasonography, (*iii*) increased ALT (≥35 U/L) or GGT (≥40 U/L), or (*iv*) a reported history of known liver disease, including viral, genetic, autoimmune, and drug-induced liver disease; and (*h*) 337 had missing data in medical histories or serum aminotransferase concentrations.

The NAFLD-free cohort thus comprised 5888 men. They were reexamined at the same hospital annually or biennially until July 2006. After excluding 651 men who did not complete their follow-up examinations, 1222 participants with annual health examinations (mean follow-up 3.02 years, SD 1.14 years) and 4015 with biennial health examinations (mean follow-up 2.39 years, SD 1.01 years) were successfully followed and observed for the development of NAFLD. Altogether, these 5237 men were included in the final analysis, and their mean (SD) follow-up period was 2.54 (1.08) years. This study was approved by the Institutional Review Board at Kangbuk Samsung Hospital and was in accordance with the principles of the Helsinki II Declaration.

MEASUREMENTS

Initial health examinations performed in 2002 included a medical history, physical examination, questionnaire about health-related behavior, anthropometric measurements, and biochemical measurements. Medical history and history of prescription drug use were assessed by the examining physicians. All participants were asked to respond to a questionnaire on health-related behavior. Questions about alcohol intake included the frequency of alcohol consumption on a weekly basis and the usual amount consumed on a daily basis. We considered persons reporting that they smoked to be current smokers. In addition, participants were asked about their weekly frequency of physical activity such as jogging, bicycling, and swimming that lasted long enough to produce perspiration.

Fasting blood samples were drawn from an antecubital vein after participants had fasted for ≥12 h. We measured fasting serum glucose, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, GGT, ALT, AST, and alkaline phosphatase (ALP) concentrations enzymatically with an automatic analyzer (Advia 1650, Bayer Diagnostics). We measured fasting serum glucose with the hexokinase method; total cholesterol and serum triglycerides with enzymatic colorimetric tests; LDL cholesterol with the homogeneous enzymatic colorimetric test; HDL cholesterol with the selective inhibition method (Bayer Diagnostics); and insulin concentrations with immunoradiometric assays (Biosource), with intra- and interassay CVs of 4.7% to 12.2%. We estimated insulin resistance by use of the homeostasis model assessment (HOMA-IR), as described by Matthews et al. *(19)*. We analyzed high-sensitivity C-reactive protein (CRP) by use of particle-enhanced immunonephelometry with the BN System (Dade Behring). The minimum detectable CRP concentration was 0.175 mg/L after 1:20 sample dilution. The Korean Association of Quality Assurance for Clinical Laboratories assessed the quality control of the laboratory, both internally and externally, on a regular basis.

Because the reference interval and cutoff value of ALT are controversial *(20)*, increased serum ALT and increased serum GGT were defined as values in their

respective highest quartiles of our study population (≥35 U/L for serum ALT and ≥40 IU/L for serum GGT) *(21)*. The reference interval used at the Kangbuk Samsung Hospital for serum concentrations of ALT in men was 0 to 40 U/L.

Trained nurses obtained sitting blood pressure readings with a standard mercury sphygmomanometer. The 1st and 5th Korotkoff sounds were used to estimate systolic and diastolic blood pressure. Height and weight were measured with the participants wearing a lightweight hospital gown and no shoes. Body mass index (BMI) was calculated as the patient's weight (in kilograms) divided by the square of the patient's height (in meters). The rate of weight change was calculated as follows: weight change = (last weight − baseline weight)/follow-up period (years).

The diagnosis of fatty liver was based on the results of abdominal ultrasonography with a 3.5-MHz transducer (Logic Q700 MR, GE). Ultrasound studies were carried out by 3 experienced radiologists who were unaware of the aims of the study and blinded to laboratory values. Images were captured in a standard fashion with the patient in the supine position, right arm raised above the head. All ultrasonographic images were stored in the image server and also taken with instant film for later inspection by the radiologists and physicians. Of 4 known criteria [hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring *(22)*], a diagnosis of fatty liver required the participant to have hepatorenal contrast and liver brightness. Based on computer-generated random samples among the stored images, there was excellent agreement on fatty liver diagnosis between the 3 radiologists (agreement 99%, κ 0.98).

The ATP III proposed the following 5 abnormalities to define the metabolic syndrome *(23)*: (*a*) abdominal obesity; (*b*) high fasting glucose: ≥6.1 mmol/L; (*c*) hypertriglyceridemia: triglycerides ≥1.69 mmol/L; (*d*) low HDL cholesterol: <1.04 mmol/L; and (*e*) high blood pressure: ≥130/85 mmHg. Because waist measurements were not available for the entire study sample, we substituted BMI ≥25 kg/m^2 for all participants as an index of obesity, because this cutoff has been proposed for the diagnosis of obesity in Asian people *(24)*. Individuals with at least 3 of the 5 abnormalities were considered to have the metabolic syndrome.

statistical analysis

The χ^2-test and 1-way ANOVA were used to analyze statistical differences among the characteristics of the study participants at the time of enrollment in relation to serum ALT concentrations within the reference interval. Serum ALT was categorized into the following quintiles: <16, 16–18, 19–21, 22–25, and 26–34 U/L. Incidence density was expressed as the number of cases divided by the person-years from baseline until development of NAFLD, by assuming a date of diagnosis in the middle of the follow-up period or until the final physical examination. Incidence densities were compared by calculating hazard ratios with the 95% confidence interval (CI). We used the Cox proportional hazards model to calculate each adjusted hazard ratio (aHR) in the model for NAFLD. The data were first adjusted for age alone and then for the multiple covariates. In the multivariate models, we included the variables (age, BMI, weight change, fasting serum glucose, systolic blood pressure, triglycerides, HDL cholesterol, HOMA-IR, CRP, smoking, alcohol consumption, and regular exercise) that might confound the relationship between the serum ALT and NAFLD. For linear trends of risk, the number of quintiles was used as a continuous variable and tested on each model. Analyses were done with SPSS version 13.0 (SPSS Inc.). All the reported *P* values were 2-tailed, and those <0.05 were considered to be statistically significant.

Results

At baseline, the mean (SD, range) age of the 5237 participants was 36.6 years (4.8, 30–59); the BMI was 22.7 kg/m^2 (2.4, 15.6–33.3). Of the 5888 eligible participants at baseline, 651 who did not have a follow-up examination by the end of July 2006 were more likely to be current smokers than the remaining participants; all other variables were not different between the participants lost to follow-up and those with successful follow-up.

During 13 276.6 person-years of follow-up, 984 new incident cases of NAFLD developed. In contrast to participants without incident NAFLD, those with incident NAFLD were slightly older and more likely to have the metabolic syndrome and incident diabetes. As expected, BMI, glucose, blood pressure, lipid profiles, and hepatic enzymes, except ALP, were significantly different between the 2 groups. In addition, HOMA-IR and CRP were higher for those with incident NAFLD (data not shown).

The baseline characteristics of the study sample according to the quintiles of serum ALT concentrations are shown in Table 1. Tests for differences of variables across the quintiles of serum ALT found that BMI, glucose, blood pressure, lipid profiles, CRP, and HOMA-IR showed a linear trend in relation to serum ALT concentration, even within its reference interval, whereas age, current smoking, alcohol consumption, regular exercise, and incident diabetes did not.

An increase across the serum ALT quintiles predicted the incidence of NAFLD in a graded and dose-responsive manner (Table 2). After adjustment for age, weight change, BMI, glucose, systolic blood pressure, triglycerides, HDL, smoking, alcohol consumption, regular exercise, HOMA-IR, CRP, and incident diabetes, and in comparison with concentrations <16 U/L, aHR values (95% CI) for ALT concentrations of 16–18, 19–21, 22–25, and 26–34 U/L were 1.53 (1.18–1.98), 1.66 (1.29–2.13), 1.62 (1.26–2.08), and 2.21 (1.73–2.81), respectively (*P* for trend <0.001), in overall participants. The relationship of ALT with incident NAFLD remained significant even after further adjustment for serum GGT or AST. Similar asso-

Table 1. Baseline characteristics of the study participants by concentration of ALT within the reference interval.[a]

	ALT, U/L					
	<16	16–18	19–21	22–25	26–34	*P* for trend
n	1189	1014	1018	978	1038	
Age, years	36.2 (4.7)	36.8 (4.9)	36.6 (4.8)	36.9 (5.0)	36.5 (4.8)	0.111
BMI, kg/m^2	21.7 (2.2)	22.4 (2.2)	22.7 (2.3)	23.3 (2.2)	23.7 (2.3)	<0.001
Fasting serum glucose, mmol/L	4.91 (0.47)	4.94 (0.46)	4.95 (0.48)	5.02 (0.49)	5.00 (0.48)	<0.001
Systolic blood pressure, mmHg	111.8 (11.4)	113.4 (12.2)	113.7 (11.8)	114.9 (12.1)	114.7 (11.9)	<0.001
Diastolic blood pressure, mmHg	72.3 (9.4)	73.1 (9.3)	73.4 (9.5)	74.3 (9.6)	74.3 (9.4)	<0.001
Total cholesterol, mmol/L	4.81 (0.76)	4.91 (0.83)	5.04 (0.81)	5.10 (0.81)	5.18 (0.84)	<0.001
HDL cholesterol, mmol/L	1.45 (0.31)	1.39 (0.29)	1.38 (0.28)	1.36 (0.30)	1.33 (0.28)	<0.001
LDL cholesterol, mmol/L	2.85 (0.68)	2.93 (0.73)	3.01 (0.70)	3.06 (0.68)	3.12 (0.72)	<0.001
Triglyceride, mmol/L	1.04 (0.81–1.38)	1.13 (0.87–1.54)	1.23 (0.94–1.65)	1.28 (0.99–1.72)	1.36 (1.01–1.85)	<0.001
CRP, mg/L	0.30 (0.20–0.70)	0.40 (0.20–0.70)	0.30 (0.20–0.70)	0.40 (0.20–0.80)	0.45 (0.30–0.90)	<0.001
Insulin, pmol/L	39.4 (32.5–49.2)	42.2 (34.0–53.1)	43.5 (34.9–55.3)	46.2 (37.1–60.0)	47.6 (37.3–62.7)	<0.001
HOMA-IR	1.24 (1.02–1.59)	1.32 (1.04–1.73)	1.36 (1.09–1.77)	1.50 (1.16–1.93)	1.52 (1.17–2.01)	<0.001
Current smoker, %	44.7	41.3	41.6	39.7	44.6	0.658
Light drinker, %[b]	24.1	25.0	26.5	25.4	24.6	0.696
Regular exerciser, %[c]	49.4	50.7	51.2	54.0	50.3	0.298
Metabolic syndrome, %	1.3	2.9	3.4	4.6	7.2	<0.001
Incident diabetes, %	0.8	0.5	1.4	0.5	0.5	0.890

[a] Data are mean (SD) or median (interquartile range) unless otherwise noted.
[b] Ethanol, 10–20 g per day.
[c] One time or more per week.

ciations were also observed in the stratified subgroup analyses according to drinking habit (alcohol drinking of <10 g per day or nondrinker), nonobese (BMI <25 kg/m^2), and even normal weight (BMI <23 kg/m^2). Moreover, even in participants without any features of the metabolic syndrome, any increase of serum ALT, despite being within the reference interval, continued to predict the incidence of NAFLD.

To explore whether the risk for NAFLD in relation to serum ALT within its reference interval was mediated by the subsequent increase of serum ALT and serum GGT, we fit an additional model, excluding participants who showed an increase of serum ALT ≥35 U/L and serum GGT ≥40 U/L at follow-up. Even after these exclusions, the linear association between NAFLD and the serum ALT quintiles within the reference interval remained statistically significant (*P* for trend <0.001). In addition, even after exclusion of participants who reported ethanol intake of ≥20 g/day only at follow-up, the relationship of ALT on incident NAFLD remained statistically significant (*P* for trend <0.001). During follow-up, 4 new incident cases of hepatitis B virus (serologically positive) were found, and no cases of hepatitis C virus were found. Furthermore, these 4 new incident cases of hepatitis B virus did not have NAFLD at follow-up.

Baseline GGT also predicted the incidence of NAFLD, but this association was weaker than the gradient for ALT, and the associations across GGT quintiles seemed to be nonlinear (models 4 and 5 in Table 3). Furthermore, serum GGT was not significantly related to the incidence of NAFLD in nondrinkers and normal-weight participants (BMI <23 kg/m^2). The ratio of baseline ALT to AST also predicted the incidence of NAFLD, although again this association was weaker than the gradient for ALT. Neither AST nor ALP was significantly related to the incidence of NAFLD.

Discussion

In this prospective study of apparently healthy, nondiabetic Korean men, increasing ALT concentration, even within its reference interval, was an independent predictor of incident NAFLD, irrespective of various potential confounders, including BMI, alcohol consumption, CRP, HOMA-IR, and the metabolic syndrome components of fasting glucose, lipids, and blood pressure. Moreover, this relationship of ALT on incident NAFLD remained even after adjustment for incident diabetes and weight gain. The strength of this study was the large sample size, which allowed us to identify the effect among stratified subgroup analyses. Even in normal-weight participants, modestly increased ALT continued to predict incident NAFLD, as was apparent among our participants whose ALT remained within the reference interval at follow-up.

To our knowledge, this is the first study to demonstrate a relationship between ALT and incident NAFLD based on ultrasonography, although several cross-sectional studies have already demonstrated that higher ALT, even within the currently accepted normal reference interval, is associated with NAFLD *(25)*. A previous study also showed that ALT appears to have associations with both hepatic insulin resistance and later decline in hepatic insulin sensitivity *(26)*. Moreover, a recent study has

Table 2. Adjusted hazard ratios of incidence of nonalcoholic fatty liver disease in relation to ALT concentrations within the reference interval

	ALT, U/L					
	<16	16–18	19–21	2–25	26–34	*P* for trend
All participants (n = 5237)						
Cases	102	156	188	237	301	
Person-years	3226.9	2639.1	2605.7	2400.3	2404.6	
ID (per 1000 person-years)[a]	31.6	59.1	72.2	98.7	125.2	
aHR, 95% CI						
Model 1[b]	1.00	1.82 (1.42–2.33)	2.23 (1.76–2.84)	3.11 (2.39–3.80)	3.89 (3.11–4.87)	<0.001
Model 2[c]	1.00	1.56 (1.21–2.01)	1.70 (1.33–2.18)	1.70 (1.33–2.18)	2.29 (1.81–2.89)	<0.001
Model 3[d]	1.00	1.56 (1.21–2.00)	1.68 (1.32–2.15)	1.68 (1.32–2.15)	2.26 (1.79–2.86)	<0.001
Model 4[e]	1.00	1.53 (1.18–1.98)	1.67 (1.30–2.15)	1.62 (1.26–2.08)	2.20 (1.73–2.80)	<0.001
Model 5[f]	1.00	1.53 (1.18–1.98)	1.66 (1.29–2.13)	1.62 (1.26–2.08)	2.21 (1.73–2.81)	<0.001
Ethanol <10 g/day (n = 3925)						
aHR (95% CI), Model 5	1.00	1.60 (1.18–2.17)	1.74 (1.30–2.33)	1.96 (1.47–2.60)	2.36 (1.79–3.11)	<0.001
Nondrinkers (n = 1119)						
aHR (95% CI), Model 5	1.00	1.60 (0.92–2.81)	1.54 (0.88–2.67)	2.70 (1.59–4.60)	2.53 (1.51–4.22)	<0.001
BMI <25 kg/m^2 (n = 4333)						
aHR (95% CI), Model 5	1.00	1.51 (1.12–2.04)	1.60 (1.20–2.13)	1.89 (1.42–2.52)	2.13 (1.61–2.80)	<0.001
BMI <23 kg/m^2 (n = 3226)						
aHR (95% CI), Model 5	1.00	1.53 (1.05–2.24)	1.61 (1.12–2.29)	1.64 (1.13–2.39)	1.92 (1.34–2.76)	0.001
Without metabolic syndrome (n = 5037)						
aHR (95% CI), Model 5	1.00	1.47 (1.13–1.92)	1.63 (1.26–2.10)	1.54 (1.19–2.00)	2.29 (1.79–2.92)	<0.001
No metabolic syndrome traits (n = 2795)						
aHR (95% CI), Model 5	1.00	2.05 (1.37–3.06)	1.80 (1.21–2.69)	2.32 (1.54–3.48)	2.07 (1.39–3.07)	0.001
ALT <35 U/L and GGT <40 U/L during the study period (n = 2959)						
aHR (95% CI), Model 5	1.00	1.69 (1.10–2.59)	2.27 (1.50–3.45)	2.55 (1.68–3.87)	3.07 (2.04–4.63)	<0.001
Ethanol <20 g /day during the study period (n = 4535)						
aHR (95% CI), Model 5	1.00	1.67 (1.25–2.22)	1.79 (1.35–2.36)	1.85 (1.40–2.45)	2.42 (1.85–3.16)	<0.001

[a] ID, incidence density.
[b] Model 1: adjustment for age.
[c] Model 2: model 1 plus adjustment for weight change, fasting serum glucose, $\log_e$ triglyceride, HDL cholesterol, BMI, systolic blood pressure, smoking, exercise, and alcohol intake.
[d] Model 3: model 2 plus adjustment for HOMA-IR.
[e] Model 4: model 3 plus adjustment for CRP.
[f] Model 5: model 4 plus adjustment for incident diabetes.

demonstrated that subtle alterations in glucose tolerance and lipid metabolism exist in those patients with higher ALT, and that this is not necessarily accompanied by hepatic steatosis *(27)*. In our prospective study, even ALT concentrations in the upper portion of the reference interval predicted NAFLD, and they did so in a dose-dependent manner. Therefore, although the mechanism through which serum ALT is related to the risk for NAFLD remains to be elucidated, ALT might be not only an indicator of liver injury due to hepatic steatosis but also an early indicator of impaired insulin signaling.

Among the hepatic enzymes in the present study, serum ALT concentration was more closely associated with the development of NAFLD than either AST or GGT concentrations. This finding could be explained by the higher specificity of ALT for liver injury *(11)*, as well as by the contribution of ALT as a glucogenic enzyme. We have shown that the predictive effect of ALT on incident NAFLD was independent of the degree of adiposity and that ALT, but not GGT, predicted the development of NAFLD, even in normal-weight participants (BMI <23 kg/m^2). Our results appear to agree with the findings of several previous studies that ALT is more closely associated than either AST or GGT with both hepatic insulin resistance and later decline in hepatic insulin sensitivity *(26)*. GGT might also be involved in the pathogenesis of NAFLD through other mechanisms, such as oxidative stress *(28)*.

With respect to other risk factors, our findings support an association between weight gain and NAFLD *(6)*. In addition, the link between ALT and other confounders, such as triglycerides, CRP, and HOMA-IR could be related to incident NAFLD. Even after the potential confounders in the present study were adjusted for, the predictive ability of ALT persisted across every ALT category compared with the referent group of the lowest

Table 3. Adjusted hazard ratios of incidence of nonalcoholic fatty liver disease in relation to GGT concentrations within the reference interval.

	GGT, U/L					
	<15	**15–17**	**18–21**	**22–26**	**27–39**	***P* for trend**
Total participants (n = 5237)						
Cases / person-years	144 / 3,333.0	134 / 2,432.7	229 / 2,888.0	229 / 2,320.1	248 / 2,302.8	
ID (per 1000 person-years)[a]	43.2	55.1	79.3	98.7	107.7	
aHR, 95% CI						
Model 1[b]	1.00	1.26 (0.99–1.59)	1.81 (1.47–2.22)	2.22 (1.80–2.74)	2.38 (1.93–2.92)	<0.001
Model 2[c]	1.00	1.12 (0.88–1.42)	1.40 (1.13–1.74)	1.46 (1.18–1.82)	1.23 (0.98–1.54)	0.019
Model 3[d]	1.00	1.12 (0.89–1.42)	1.38 (1.11–1.70)	1.44 (1.16–1.80)	1.21 (0.96–1.52)	0.030
Model 4[e]	1.00	1.16 (0.91–1.48)	1.37 (1.10–1.71)	1.47 (1.17–1.84)	1.18 (0.93–1.50)	0.064
Model 5[f]	1.00	1.16 (0.91–1.48)	1.37 (1.10–1.72)	1.47 (1.18–1.85)	1.18 (0.93–1.50)	0.058
Ethanol <10 g /day (n = 3925)						
aHR (95% CI), Model 5	1.00	1.17 (0.89–1.54)	1.53 (1.19–1.95)	1.60 (1.24–2.06)	1.29 (0.99–1.68)	0.010
Non-drinkers (n = 1119)						
aHR (95% CI), Model 5	1.00	1.24 (0.79–1.93)	1.25 (0.82–1.91)	1.52 (0.94–2.45)	0.96 (0.58–1.60)	0.662
BMI <25 kg/m^2 (n = 4333)						
aHR (95% CI), Model 5	1.00	1.10 (0.83–1.46)	1.48 (1.14–1.92)	1.57 (1.21–2.05)	1.37 (1.05–1.79)	0.003
BMI <23 kg/m^2 (n = 3226)						
aHR (95% CI), Model 5	1.00	1.05 (0.72–1.53)	1.38 (0.97–1.95)	1.44 (1.00–2.07)	1.38 (0.95–1.98)	0.026
Without metabolic syndrome (n = 5037)						
aHR (95% CI), Model 5	1.00	1.15 (0.90–1.48)	1.34 (1.06–1.69)	1.46 (1.16–1.84)	1.20 (0.93–1.53)	0.044
No metabolic syndrome traits (n = 2795)						
aHR (95% CI), Model 5	1.00	1.21 (0.83–1.76)	1.54 (1.09–2.19)	1.68 (1.16–2.43)	1.13 (0.76–1.66)	0.157
ALT <35 U/L and GGT <40 U/L during the study period (n = 2959)						
aHR (95% CI), Model 5	1.00	1.25 (0.87–1.78)	1.38 (0.97–1.95)	1.51 (1.06–2.16)	1.41 (0.91–2.20)	0.032
Ethanol <20 g /day during the study period (n = 4535)						
aHR (95% CI), Model 5	1.00	1.08 (0.83–1.41)	1.36 (1.07–1.73)	1.485 (1.16–1.89)	1.19 (0.92–1.53)	0.045

[a] ID, incidence density.
[b] Model 1: adjustment for age.
[c] Model 2: model 1 plus adjustment for weight change, fasting serum glucose, $\log_e$ triglyceride, HDL cholesterol, BMI, systolic blood pressure, smoking, exercise, and alcohol intake.
[d] Model 3: model 2 plus adjustment for HOMA-IR.
[e] Model 4: model 3 plus adjustment for CRP.
[f] Model 5: model 4 plus adjustment for incident diabetes.

quintile. Therefore, increased ALT, despite remaining within the reference interval, might be an important preclinical marker of NAFLD, possibly as a component of the early phase of NAFLD.

Our study had several limitations. First, NAFLD was not assessed by biopsy. Although in the diagnosis of liver steatosis ultrasonography is a useful method with a reasonable sensitivity and specificity *(29, 30)*, it may underestimate the actual rate of NAFLD, because ultrasonography changes do appear at a hepatocyte fat content of ≥15% to 30% *(29)*. Another possible explanation could be that a slightly increased serum ALT concentration might reflect subclinical (or ultrasonography-undetectable) early fatty changes in the liver (hepatic steatosis), which predate the overtly detectable NAFLD, i.e., ALT might be a preclinical marker of NAFLD. In addition, because ultrasonography cannot accurately differentiate steatosis from fibrosis *(29)*, in the present study incident NAFLD on ultrasonography might have represented the simple hepatic steatosis as well as another NAFLD spectrum condition, such as nonalcoholic steatohepatitis, defined as steatosis plus any stage of fibrosis or steatosis plus inflammation *(31)*. A second possible limitation is that serum ALT during follow-up was not included in the analysis. However, an association between ALT and incident NAFLD remained even after exclusion of participants who showed an increase of serum ALT ≥35 U/L and serum GGT ≥40 U/L at follow-up. Finally, the information on alcohol drinking was self-reported and thus likely to have been underreported. Nevertheless, the association of ALT with incident NAFLD in our study remained

even after exclusion of participants with ethanol intake of >20 g/day first reported at follow-up.

In conclusion, in apparently healthy, nondiabetic Korean men, higher ALT concentrations within the reference interval were an independent predictor of incident NAFLD. This relation remained significant even among normal weight participants still within the reference interval of ALT and GGT at all follow-up examinations, irrespective of potential confounders. Further studies on the relation between ALT within its reference interval and NAFLD might help to elucidate the underlying mechanism of the relationship between ALT and increased NAFLD.

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