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Aminotransferase Levels and 20-year Risk of Metabolic Syndrome, Diabetes, and Cardiovascular Disease

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Abstract

Background & Aims—Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of obesity and metabolic syndrome. Alanine aminotransferase (ALT) levels are used to detect NAFLD and have also been associated with increased risk for metabolic syndrome, diabetes mellitus and cardiovascular disease. We studied the relationship between ALT levels and these disorders in a long-term follow-up study.

Methods—Framingham Offspring Heart Study participants (n=2812, mean age 44 years, 56% women) were followed for the development of metabolic syndrome, diabetes, cardiovascular disease, and all-cause mortality using logistic regression (metabolic syndrome, diabetes) or Cox proportional hazards models (cardiovascular disease, all-cause mortality).

Results—Among individuals at baseline, per 1 standard deviation increase in log ALT level, there were increased odds of the development of metabolic syndrome (odds ratio 1.21, $p<0.001$) and diabetes (odds ratio 1.48, $p<0.001$) over 20 years of follow-up. These findings also applied to participants with ALT levels within the normal range (metabolic syndrome odds ratio 1.17, $p=0.006$; diabetes odds ratio 1.34, $p=0.002$). There was an increased risk of cardiovascular disease in age-sex adjusted models (hazard ratio 1.23, $p<0.001$), but this was attenuated in multivariable-adjusted models (hazard ratio 1.05, $p=0.27$); no association was observed for all-cause mortality. Aspartate aminotransferase levels were found to be associated with an increased risk of only diabetes.

Conclusions—Both normal and increased levels of ALT are associated with long-term development of multiple metabolic disorders. These results indicate the potential for ALT values as biomarkers for the risk for metabolic disease.

Introduction

Nonalcoholic fatty liver disease (NAFLD) has recently been recognized as one of the leading causes of chronic liver disease and as the hepatic manifestation of obesity and the

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metabolic syndrome (MetS).^{1–4} Obesity is the primary cause of NAFLD, and it accounts for most of the abnormal aminotransferase measurements in the United States.⁵ Serum biomarkers, specifically alanine aminotransferase (ALT) levels, are sensitive in the detection of NAFLD in both obese and non-obese patients.^{6, 7}

Several cross-sectional and prospective studies have demonstrated that ALT levels are related to MetS and diabetes mellitus (DM).^{5, 8–11} Furthermore, both NAFLD and MetS are associated with an increased risk of cardiovascular disease (CVD).^{12–17} Many studies have considered 40 U/L as an upper limit of normal for both ALT and aspartate aminotransferase (AST) levels.^{18–21} These upper limits, however, might not be adequate to exclude liver disease, especially NAFLD,^{22, 23} and to predict a risk of death from liver disease.²⁴ Advanced histological damage due to NAFLD can be present even in patients with normal ALT levels.^{25, 26}

Taken together, these data suggest that ALT values, as possible manifestations of NAFLD, may be associated with increased risks for MetS, DM, and CVD. It is unclear, however, whether these increased risks occur with normal ALT values. To investigate this hypothesis, we studied the relationship between aminotransferase levels and the incidence of each of MetS, DM, CVD, and all-cause mortality in long-term follow-up of the community-based Framingham Heart Study sample. We performed these analyses in the overall sample and in those participants with normal aminotransferase values.

Methods

Study Sample

The Framingham Heart Study began in 1948;^{27, 28} in 1971, 5,124 men and women were enrolled into the Framingham Offspring Study, which included the children or spouses of the children of the original cohort. Offspring subjects underwent examinations approximately every 4 years, as previously described.²⁹ The current investigation is comprised of subjects from the Framingham Offspring Study who attended a baseline examination in 1978–1982 (second examination cycle), when aminotransferase levels were obtained. Of the 3863 participants who attended the second examination cycle, 189 were excluded because of missing AST or ALT, 27 were further excluded because of either AST or ALT levels of more than three-times normal (>120 U/L). We excluded participants with excess alcohol consumption to avoid the presence of alcoholic liver disease in our study sample: 3 were excluded because of unknown alcohol use information, 719 for excess alcohol consumption (>20 grams per day in women or >30 grams per day in men, which have been shown to cause liver damage in prior studies^{30, 31}) at baseline, and 83 were further excluded because of other evidence of alcohol abuse at any time during follow-up. An additional 30 participants were excluded because of missing covariates at baseline, resulting in a final sample size of 2812.

The study was approved by the institutional review board of the Boston University Medical Center. All subjects provided written informed consent.

Baseline Measurements and Definitions

AST and ALT were measured on fasting morning serum samples using the Kinetic method (Beckman Liquid-Stat Reagent Kit).³² Coefficients of variation for AST and ALT, respectively, were 10.7 and 8.3%.

Covariates and Risk Factor Assessment

Details regarding risk factor measurement and laboratory analysis have been described.³³ Each examination included a CVD assessment and blood testing. Hypertension was defined as a systolic blood pressure >140 mm Hg, a diastolic blood pressure >90 mm Hg (average of 2 readings taken by the examining physician) or use of blood pressure lowering medications. Fasting lipid measures included total and HDL cholesterol and triglycerides. The presence of diabetes and glucose levels were assessed at baseline. Smoking status was defined as smoking 1 or more cigarettes/day in the year preceding the examination. Body mass index (BMI) was defined as weight (kilograms) divided by the square of height (meters). Waist circumference was measured at the level of the umbilicus at the follow-up examination only, with cut-points of 88 cm for women, and 102 cm for men. Excess alcohol use was defined as at least 20 grams per day in women or 30 grams per day in men as self-reported to a Framingham Heart Study physician. Alcohol abusers were defined based on chart review of all hospitalizations and medical encounters, and were identified via ICD-9 codes. Menopause was defined as cessation of menses ≥ 1 year.

Outcomes

Diabetes and MetS outcomes were examination-based and determined at 20-year interval follow-up, corresponding to the seventh (1998–2001) examination cycle. Incident DM was defined as participants with fasting plasma glucose levels ≥ 126 mg/dL (7.0 mmol/L) and/or receiving oral hypoglycemic or insulin treatment for diabetes among those free of diabetes at baseline. MetS was defined by the presence of at least 3 out of 5 modified ATPIII criteria based on the following components:³⁴ participants with systolic blood pressure of at least 130 mm Hg, or diastolic blood pressure of at least 85 mm Hg, or use of anti-hypertensive treatment fulfilled the blood pressure criterion; those with triglyceride levels of at least 150 mg/dL or using lipid treatment fulfilled the triglycerides criterion; for the HDL criterion, levels of <40 mg/dL (men) and <50 mg/dL (women) were used as cut-offs; the impaired fasting glucose criterion was defined as a fasting plasma glucose level of 100–125 mg/dl in the absence of diabetes treatment; patients with diabetes were excluded from the MetS analysis. Because waist circumference was not measured at the second examination cycle, we used a BMI ≥ 30 kg/m² (obese) to fulfill the waist circumference criterion in order to exclude participants with MetS at baseline; BMI has been shown to have a strong correlation with waist circumference in our study sample, with correlation coefficients of $r = 0.89$ (men) and $r = 0.86$ (women).³⁵ To diagnose MetS at follow-up, standard waist circumference criteria were utilized. For analyses involving the incidence of MetS or its components, participants had to be free of MetS, diabetes, or the component of interest at baseline to be considered in the sample. Similarly, for analyses involving diabetes, participants had to be free of diabetes at baseline to be considered in the sample.

Incident CVD events included fatal and non-fatal myocardial infarction, coronary insufficiency, congestive heart failure, and stroke; all-cause mortality included all deaths ascertained over follow-up. All events were adjudicated by a three-member physician panel based on medical record review.³³ All participants analyzed for the incidence of CVD were CVD-free at baseline.

Loss to Follow-up

For diabetes and MetS, of the 2812 participants in our sample, 2274 attended exam 7. For all-cause mortality, of the 2812 participants in our sample, 2274 attended exam 7, while 261 participants died before Exam 7 and an additional 22 participants died after exam 7, resulting in 2557 participants with known status at approximately the 20 year time point. This calculates to a rate of 91% for participants where long-term follow-up is available.

Statistical Analysis

The primary outcomes of interest were incident MetS and its components, DM, CVD, and all-cause mortality. AST and ALT were natural log-transformed to normalize their skewed distribution. All analyses were performed at 20 years of follow-up. As an additional analysis, AST and ALT were limited to the normal range (up to 40 U/L for each).³⁶ Covariates were outcome-specific and included:

1. *MetS*: age, sex, smoking, menopausal status, alcohol use (g/day).
2. *Diabetes*: age, sex, smoking, menopausal status, alcohol use (g/day), BMI.
3. *Cardiovascular disease*: age, sex, systolic blood pressure, hypertension treatment, smoking, BMI, diabetes, total/HDL cholesterol, lipid treatment, alcohol use (g/day).
4. *All-cause mortality*: age, sex, systolic blood pressure, hypertension treatment, smoking, BMI, diabetes, total/HDL cholesterol, lipid treatment, alcohol use (g/day), prevalent CVD.

For examination-based outcomes, we used multivariable logistic regression to model the change in odds of the event per 1 sex-specific standard deviation (SD) increase in log-AST or log-ALT; this was calculated by multiplying the regression coefficient by the SD ($OR = e^{\text{coefficient} \times SD}$). For CVD and all-cause mortality, we used multivariable Cox proportional hazards analysis to model the change in hazard of the event per 1 sex-specific SD increase in log AST or log ALT.

Secondary analyses included additional adjustment for interim weight change, calculated as the difference between baseline weight and weight at follow-up, for MetS and diabetes; adjustment for baseline fasting plasma glucose for diabetes; and removal of the exclusion of alcohol abusers and self-reported excess alcohol drinkers for incident CVD and all-cause mortality. We also examined the risk of developing MetS and its components at 8-years of follow-up. Lastly, we used the generalized estimating equations to account for sibling correlations.

SAS version 8.0 was used to perform all computations;³⁷ a two-tailed p-value < 0.05 was considered significant.

Results

Baseline Characteristics

The baseline characteristics of the study participants are summarized in Table 1. The mean age of the participants at baseline was 44 years, and 56% were women. The baseline prevalence of obesity was 14.2%, of MetS 20.1%, and of CVD 4.0%. The age-sex adjusted correlation between log ALT and log AST was 0.54 (p-value < 0.001).

Incident Metabolic Syndrome and its Components

Among individuals free of MetS and diabetes at baseline, 621 developed MetS at 20 years of follow-up (Table 2). The results for individuals in the overall sample are shown in Table 3. AST was not significantly associated with incident MetS or its components, with the exception of the development of low HDL levels (OR 1.23, 95% CI 1.06–1.43, p=0.006). ALT, however, was highly correlated with incident MetS: for each 1 SD (0.55) increase in log-ALT (mean 3.05), the overall odds ratio of developing MetS was 1.21 (95% CI 1.09–1.34, p<0.001); results did not change appreciably when models were additionally adjusted for interim weight change.

When the analysis was restricted to those individuals with normal aminotransferases (Table 3), higher ALT remained associated with the development of MetS, even after adjustment for interim weight change (mean log-ALT 2.94, SD 0.47). The results for the individual components of MetS are shown in Table 3. Results were similar when adjusted only for age and sex (Online Supplemental Table 1).

In order to model shorter-term risks associated with higher levels of ALT, we examined the 8-year risk of developing MetS and its components (Online Supplemental Table 2). Overall, a 30% increased risk of developing MetS was observed, as well as increases in the risk of developing obesity, elevated triglycerides, elevated blood pressure, and impaired fasting glucose.

Incident Diabetes

Among individuals free of diabetes at baseline, 208 developed the condition at 20 years of follow-up. In the overall sample (Table 4), the OR for every 1 SD (0.48) change in log-AST (mean 2.89) was 1.33 (95% CI 1.16–1.52, $p<0.001$). These statistically significant relations remained significant when further adjusted for baseline blood glucose levels or interim weight change. Higher ALT was more significantly associated with an increased risk of DM (OR 1.48, 95% CI 1.30–1.69, $p<0.001$). These associations remained statistically significant when further adjusted for baseline blood glucose levels or interim weight change.

Among individuals with aminotransferases within the normal range (Table 4), there was a significant association of higher AST levels with a greater risk of incident DM (mean log-AST 2.82, SD 0.44). The OR of 1.24 per SD increment (95% CI 1.04–1.48, $p=0.02$) remained statistically significant after further adjustment for interim weight change. ALT levels in this group were significantly associated with incident DM (OR 1.34 per SD increment, 95% CI 1.11–1.61, $p=0.002$), remaining statistically significant after further adjustment for baseline blood glucose levels and interim weight change. Age-sex adjusted results are included in Tables 3 and 4 and presented in detail in Supplemental Table 1.

Incident Cardiovascular Disease and All-cause Mortality

Among individuals free of CVD at baseline, 365 developed the condition at 20 years of follow-up. There were 283 deaths at 20 years of follow-up. In individuals in the overall sample (Table 5), higher ALT levels were significantly associated with incident CVD in age-sex adjusted analyses; these findings were attenuated upon multivariable adjustment. Similar results were observed when analyses were limited to AST and ALT levels within the normal range (Table 5). When exclusions were relaxed to allow inclusion of excess alcohol drinks and alcohol abusers, results were unchanged (data not shown). No significant association was observed between AST and ALT levels and all-cause mortality.

Secondary Analyses

When the generalized estimating equations were used to account for familial correlations, results were not materially different (data not shown).

Combined Effects of Body Weight and ALT Levels

In age-sex adjusted models, there was a significant interaction between the BMI category and ALT levels for the development of DM (Figure 1a; p -value for interaction=0.01), but not for the development of MetS (Figure 1b; p -value for interaction=0.47). Individuals with normal weight and ALT levels in the highest tertile had 5.9-fold increased odds of developing DM after 20 years compared to those normal weight individuals in the lowest third of ALT levels. After 20 years of follow-up, overweight and obese individuals within the lowest tertile of ALT levels had 6.8 and 14.4-fold increased odds for DM, respectively,

compared to normal weight individuals in the lowest third of ALT levels (referent). For participants with highest ALT levels, this risk was increased 20 and 30-fold for overweight and obese participants, respectively.

Discussion

The most important finding in our study is the association between ALT levels and the development of incident MetS and DM over 20 years of follow-up. These results were observed when analyzed in the overall cohort, including individuals with elevated ALT levels to 3 times the upper limit of normal. The results remained significant, however, for individuals with ALT values within the normal range. A significant interaction was observed between obesity status, ALT levels, and the development of diabetes, suggesting that with respect to the development of diabetes, the combination of both conditions is more adverse than having either one alone.

These findings suggest that ALT levels within the “normal” range are associated with adverse metabolic outcomes. As individuals with normal ALT values can exhibit histological changes of NAFLD,²⁵ these biomarkers may represent a sensitive indicator for NAFLD.

Metabolic Syndrome

Our study reveals a significant association between circulating ALT levels and incident MetS. The association between NAFLD, ALT levels, and MetS has been studied previously. A recent cross-sectional study by Jeong et al. found correlations between ALT levels and the prevalence of MetS.³⁸ Among 9771 adults in the Korean National Health and Nutrition Examination Surveys, an association was observed between increasing aminotransferase levels, including values in the normal range, and the presence of MetS.³⁹ In a recent study of 5586 adolescents, there was a correlation between ALT levels and both waist circumference and circulating insulin levels.⁴⁰ Our findings demonstrate an association between ALT levels and the long-term development of MetS among individuals free of the condition at baseline. This is of particular importance because cross-sectional studies cannot address the question of whether abnormalities in ALT levels precede the development of MetS, or whether the MetS components themselves can lead to the increase in ALT levels. The findings in the present study suggest that elevations in ALT levels precede the clinical development of MetS.

In addition to longitudinal data, our study further provides evidence for an association between ALT levels in the “normal range” and the development of MetS. Among 633 participants in the Insulin Resistance Atherosclerosis Study, a significant correlation between ALT levels and MetS was observed after 5 years of follow-up; the range of ALT values was not included.¹⁰ Schindhelm et al. recently reported a progressive risk between increasing ALT levels up to 2.5 times the upper limit of normal, and the development of MetS after 6.4 years of follow-up in the Hoorn study.¹¹ The results of our study do not only support the concept of NAFLD as the hepatic manifestation of MetS, but point to the importance of the subtle hepatic damage underlying even mild ALT elevations as a sensitive and early indicator for the risk of developing MetS.

Diabetes

Our data demonstrate that both AST and ALT elevations to up to 3 times the upper normal limit are associated with an increased risk for developing DM. When restricted to normal values, only ALT was associated with incident DM. Interestingly, our findings persisted with adjustment for interim weight change and baseline glucose values, strong predictors for

the development of diabetes. Our findings extend the results of others by demonstrating associations with aminotransferase concentrations within the normal range and incident DM. Several studies, including the British Regional Heart Study (3500 older men followed for 20 years),⁴¹ the Pima Indians (451 individuals followed for 7 years),⁴² and others⁴³ have revealed an increased risk for DM related to higher baseline levels of ALT. We now show that ALT levels even within the normal range are predictive for the development of DM, above and beyond baseline body weight, weight change, or glucose levels. Taken together, these findings suggest that the pathophysiologic mechanism between aminotransferases and incident DM may occur above and beyond traditional diabetes risk factors. Elevated ALT levels may reflect the potential existence of a unique biologic mechanism leading to DM that cannot be explained by traditional measures of obesity alone.

Incident Cardiovascular Disease

Although we found associations between ALT and CVD in age-sex-adjusted analyses, our study did not demonstrate an association of AST or ALT levels with CVD risk or with all-cause mortality in multivariable analyses. This suggests that shared risk factors between elevated ALT levels and CVD may account for our observed findings. The findings in the present study are in contrast to a recent report from the Hoorn Study, which demonstrated an association between higher ALT levels and CVD events after 10 years of follow-up.⁴⁴ The different results may be due to the inclusion of the full range of ALT values in the Hoorn Study, rather than a limitation of ALT values to 3 times the normal range, and inclusion of long-term alcohol abusers. In a recent study, Lee et al. report results from the Framingham Heart Study linking GGT levels to CVD.⁴⁵ This may be explained by the reflection of GGT as a marker of oxidative stress,⁴⁶ rather than as a specific marker of NAFLD.

Biological importance of AST and ALT levels and Potential Mechanisms Linking NAFLD to Cardiometabolic Risk

Hepatic insulin resistance may be the underlying pathogenic factor linking obesity to NAFLD.⁴⁷ A “two-hit” hypothesis has been described resulting in NAFLD, a combination of insulin resistance and fatty acid accumulation in the liver. In addition to insulin resistance, there are many additional mechanisms that may mediate the risk between NAFLD and cardiometabolic disease. Adipokines, such as adiponectin, leptin, and resistin, are secreted from adipose tissue. Lower levels of adiponectin are associated with NAFLD,⁴⁸ and are also associated with the development of DM, MetS, and CVD.⁴⁹ Additional mechanisms linking NAFLD to cardiometabolic risk factors and vascular disease may include body fat distribution,⁵⁰ endothelial dysfunction,⁵¹ endocannabinoids,⁵² and inflammation.⁵³ Further research is necessary to uncover the specific mechanisms linking obesity, NAFLD, and CVD risk.

Strong associations between ALT values and MetS and DM are consistent with the fact that ALT is a highly specific liver enzyme, restricted to the cytosolic component of the hepatocytes.⁵⁴ AST is less liver-specific, as it is released by damage to the liver, but also to the heart, skeletal muscle, kidney, brain, pancreas, and erythrocytes.

Implications for further research and clinical impact

ALT is a strong predictor for the development of MetS and diabetes. This holds true for moderately elevated ALT levels, but also those within the normal range. Prior studies have called the current upper normal limits for ALT into question,^{22, 23} and have demonstrated that high normal ALT levels confer an increased mortality from liver disease.²⁴ These studies, however, have not led to a change in the recommendations for the evaluation of abnormal liver tests and the upper limit of normal, considered to be two standard deviations above the mean.⁵⁵

The present study goes beyond liver disease-specific outcomes and identifies ALT as a biomarker for the development of MetS and DM. ALT levels, therefore, appear to reflect a continuum of risk rather than a definable healthy state, similar to serum cholesterol, HDL, or LDL levels. These findings also raise the important question of whether the upper normal limit for ALT needs to be reassessed. This had been proposed by Prati et al.,²³ although this assertion has been questioned.⁵⁶ Ioannou et al. found the prevalence of aminotransferase elevation to be 9.8%, based on NHANES 1999–2002 data.⁵⁷ Applying the lower cut-point as suggested by Prati et al. would increase the prevalence substantially. Further research is warranted to determine whether a lower threshold for aminotransferase levels and increased awareness of hepatic steatosis as a potential underlying cause would optimize the detection of patients at risk for metabolic disorders.

Strengths and Limitations

The strengths of our study are the large sample size and the follow-up interval of 20 years. A three-physician panel adjudicates CVD events, allowing an excellent assessment of endpoints. Cardiac risk factors have been carefully measured over time. In addition, alcohol consumption is well documented, allowing the exclusion of heavy drinkers to focus on NAFLD. Furthermore, our study was able to assess the relations of aminotransferases to outcomes both in a sample of moderately elevated and of normal enzyme levels.

Possible limitations are that the Framingham Heart Study comprises a homogenous, all white sample, which is not truly population-based, raising the question whether our findings would be applicable to other ethnic groups. Waist circumference was not measured at the baseline examination, and only BMI was used to exclude MetS at this examination. While a recent study in the Framingham Heart Study demonstrated a strong correlation between BMI and waist circumference,³⁵ we may have potentially misclassified individuals with respect to MetS diagnosis and thereby underestimated the magnitude of the association between ALT and MetS. Insulin levels were also not measured at the baseline examination. As insulin levels are thought to be a key etiologic factor linking obesity to hepatic steatosis⁴⁷ as well as the development of diabetes, we can therefore not evaluate its potential mediating role. Aminotransferase levels were only assessed once, leading to possible misclassification. Aminotransferase levels were performed at a single reference laboratory shortly after the blood draw. Potential misclassification would bias results towards the null, and is unlikely to account for our significant findings. Serologic data to exclude participants with viral hepatitis were not available; however, given the low seroprevalence of HCV in the general population (2%), especially among those with normal aminotransferase levels (<1%)⁵⁷, the possibility of confounding our results by HCV infection is minimal. Histological data from liver biopsies or imaging data assessing the degree of hepatic steatosis were not available. Participants with NAFLD, therefore, were not specifically identified in this study. While this only allows us to speculate about the underlying liver pathology in the individuals with elevated liver enzymes, it was not the focus of the current work. Finally, while our data point to a biological mechanism connecting elevated aminotransferase levels and metabolic complications, the data are not designed to predict risk for individual patients.

Conclusions

Normal and increased levels of ALT are associated with long-term development of multiple metabolic disorders. These results indicate the potential for ALT values as biomarkers for the risk for metabolic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

WG and CSF designed and conducted the study, analyzed and interpreted the data and prepared the manuscript. They had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JMM performed all the statistical analyses. All authors reviewed and approved the manuscript. The authors report no conflicts of interest. This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (N01-HC-25195). WG is supported by K08-DK071940 (NIDDK/NIH). RSV is supported in part by 2K24HL04334 (NHLBI/NIH).

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Figure 1.

1) Figure 1a.— Age-sex adjusted odds ratios of diabetes by sex-specific tertiles of ALT (tertile 1, light grey bars; tertile 2, medium grey bars, tertile 3, dark grey bars) and by clinical BMI categories (normal weight [BMI <25], overweight [BMI 25–29.9], obese [BMI 30+]) over 20-years of follow-up. Ranges for ALT tertiles were as follows (U/L): men: 1–21; 22–32; 33–104; women: 2–15; 16–22; 23–118.

2) Figure 1b. - Age-sex adjusted odds ratio of MetS by sex-specific tertiles of ALT and by clinical BMI categories (normal weight [BMI <25], overweight [BMI 25–29.9], obese [BMI 30+]) over 20-years of follow-up. Ranges for ALT tertiles were as follows (U/L): men: 1–21; 22–32; 33–104; women: 2–15; 16–22; 23–118.

Table 1

Baseline Study Characteristics stratified by sex-specific quartiles of ALT*

	Mean (SD) n=2812	Q1	Q2	Q3	Q4
Age (years)	44 (10)	43 (11)	44 (10)	44 (10)	45 (10)
%Women	55.6	56.8	55.8	53.7	56.1
Systolic Blood Pressure (mm Hg)	121 (16)	118 (15)	119 (15)	121 (16)	125 (16)
AST (U/L)	20 (9)	15 (7)	18 (6)	20 (7)	27 (11)
ALT (U/L)	24 (14)	12 (4)	19 (4)	26 (5)	41 (15)
AST≤40 U/L (%)	96.9	99.6	99.6	98.4	90.2
ALT≤40 U/L (%)	89.5	100.0	100.0	100.0	58.4
Hypertension treatment (%)	8.9	4.6	6.8	8.5	15.9
Current Smoking (%)	32.0	34.2	32.7	30.1	31.0
Body Mass Index (kg/m ²)	25.6 (4.4)	24.4 (3.7)	24.8 (3.6)	25.7 (4.3)	27.4 (5.2)
Diabetes (%)	2.5	2.1	1.4	1.5	5.0
Total/HDL Cholesterol	4.6	4.3 (1.5)	4.5 (1.7)	4.6 (1.7)	5.0 (1.9)
Current alcohol use (%)	5.5	5.4	5.1	6.1	5.4
Lipid Treatment (%)	0.7	0.4	1.1	0.1	1.0
Obesity (BMI ≥30kg/m ²) (%)	14.2	6.9	7.6	14.9	27.3
Metabolic Syndrome (%)	20.1	11.9	14.4	18.2	35.7
Prevalent Cardiovascular Disease (%)	4.0	3.3	4.3	4.1	4.4

* sex-specific cut-points for ALT per quartile are 1–19 [men]; 2–13 [women] for Q1; 20–26[men]; 14–18 [women] for Q2; 27–35 [men]; 19–24 [women] for Q3; 36–104 [men]; 25–118 [women] for Q4.

Table 2

Incident Case Counts over 20 years of Follow-up

	Overall Sample		AST and ALT in the Normal Range	
	Case count/persons at risk	Follow- up time (person- years)	Case count/persons at risk	Follow- up time (person- years)
Metabolic Syndrome	621/1692	-	569/1579	-
Diabetes	208/2138	-	154/1918	-
CVD	365/2699	43916	299/2390	39210
All-cause mortality	283/2812	48023	238/2488	42658

Table 3

Baseline ALT and AST and the Odds Ratio of Developing Incident Metabolic Syndrome (and its components) per 1 sex-specific SD Increase in log-transformed aminotransferase levels over 20 years of follow-up

	Overall Sample		AST or ALT in the Normal Range	
	OR (95% CI)	p-value	OR (95% CI)	p-value
AST				
MetS – age, sex adjusted	1.04 (0.94–1.15)	0.45	0.99 (0.89–1.10)	0.86
MetS – MV adjusted*	1.05 (0.95–1.16)	0.38	1.02 (0.92–1.14)	0.68
+interim weight change	1.06 (0.95–1.18)	0.33	1.05 (0.93–1.18)	0.42
MetS Component				
Elevated WC*	0.99 (0.90–1.10)	0.92	0.95 (0.86–1.05)	0.31
HDL*	1.23 (1.06–1.43)	0.006	1.14 (0.96–1.35)	0.13
TG*	0.97 (0.88–1.08)	0.62	0.96 (0.86–1.07)	0.43
BP*	1.03 (0.93–1.15)	0.55	1.01 (0.90–1.13)	0.92
IFG*	1.03 (0.92–1.16)	0.59	1.07 (0.94–1.21)	0.30
ALT				
MetS – age, sex adjusted	1.22 (1.10–1.35)	0.0002	1.15 (1.03–1.28)	0.01
MetS – MV adjusted*	1.21 (1.09–1.34)	<0.001	1.17 (1.05–1.31)	0.006
+interim weight change	1.22 (1.09–1.38)	<0.001	1.21 (1.07–1.37)	0.002
MetS Component				
Elevated WC*	1.10 (0.99–1.22)	0.07	1.03 (0.93–1.15)	0.53
HDL*	1.13 (0.97–1.31)	0.11	1.06 (0.89–1.27)	0.50
TG*	1.03 (0.93–1.15)	0.56	0.98 (0.88–1.10)	0.77
BP*	1.08 (0.96–1.21)	0.18	1.15 (1.02–1.29)	0.02
IFG*	1.15 (1.02–1.29)	0.02	1.22 (1.07–1.38)	0.003

* age, sex, smoking, menopause, alcohol use (g per day)

MetS=metabolic syndrome; MV=multivariable; WC=waist circumference; HDL=high density lipoprotein cholesterol (HDL<40mg/dl in men or <50 in women); TG=triglycerides (150 mg/dl or lipid treatment);BP=blood pressure criteria (SBP 130mm Hg or DBP 85 or hypertension treatment); IFG=impaired fasting glucose (FPG 100-125mg/dl)

Table 4

Baseline ALT and AST and the Odds Ratio of Developing Incident DM per 1 SD sex-specific Increase in log-transformed Aminotransferase Levels over 20 years of follow-up

	Overall Sample		AST or ALT in the Normal Range	
	OR (95% CI)	p-value	OR (95% CI)	p-value
AST				
age, sex adjusted	1.41 (1.25–1.60)	<0.0001	1.32 (1.12–1.55)	0.001
MV adjusted *	1.33 (1.16–1.52)	<0.001	1.24 (1.04–1.48)	0.02
+adj glucose	1.25 (1.08–1.45)	0.002	1.15 (0.96–1.39)	0.13
+interim weight change	1.33 (1.17–1.53)	<0.001	1.24 (1.04–1.48)	0.02
ALT				
age, sex adjusted	1.72 (1.51–1.94)	<0.0001	1.62 (1.36–1.94)	0.01
MV adjusted *	1.48 (1.30–1.69)	<0.001	1.34 (1.11–1.61)	0.002
+ glucose	1.42 (1.23–1.63)	<0.001	1.28 (1.05–1.55)	0.01
+interim weight change	1.48 (1.30–1.69)	0.01	1.34 (1.11–1.61)	0.002

* age, sex, smoking, menopause, alcohol use (g per day), BMI

Table 5

Baseline ALT and AST and the Odds Ratio of CVD and All-Cause Mortality per 1 sex-specific SD Increase in log-transformed Aminotransferase Levels over 20 years of follow-up

	Overall Sample		AST or ALT in the Normal Range	
	OR (95% CI)	p-value	OR (95% CI)	p-value
AST				
CVD-Age-sex adjusted	1.06 (0.97–1.17)	0.21	1.03 (0.92–1.16)	0.61
CVD-MV-adjusted*	0.97 (0.88–1.07)	0.53	1.02 (0.91–1.15)	0.70
All-Cause Mortality-age-sex	1.06 (0.95–1.19)	0.27	1.04 (0.92–1.19)	0.53
All-Cause Mortality-MV-adjusted**	1.02 (0.91–1.14)	0.76	1.06 (0.93–1.21)	0.41
ALT				
CVD-Age-sex adjusted	1.23 (1.12–1.34)	<0.001	1.15 (1.02–1.30)	0.02
CVD-MV-adjusted*	1.05 (0.96–1.16)	0.27	1.11 (0.98–1.25)	0.10
All-Cause Mortality-age-sex	1.08 (0.96–1.20)	0.19	1.00 (0.88–1.14)	0.97
All-Cause Mortality-MV adjusted**	0.99 (0.88–1.10)	0.82	0.98 (0.86–1.12)	0.77

* adjusted for age, sex, SBP, HTN Rx, smoking, BMI, DM, Total/HDL, Lipid Rx, alcohol use (g per day)

** additionally adjusted for prevalent CVD