Liver Enzymes as a Predictor for Incident Diabetes in a Japanese Population: The Hisayama Study

Yasufumi Doi, Michiaki Kubo,* Koji Yonemoto,† Toshiharu Ninomiya,† Masanori Iwase,* Yumihiro Tanizaki,† Kentaro Shikata,† Mitsuo Iida,* and Yutaka Kiyohara†*

Abstract

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Objective: We studied the relationship between liver enzymes and the development of diabetes in a general Japanese population.

Research Methods and Procedures: A total of 1804 nondiabetic subjects 40 to 79 years of age were followed-up prospectively for a mean of 9.0 years.

Results: During the follow-up, 135 subjects developed diabetes. In both sexes, the age-adjusted cumulative incidence of diabetes increased significantly with elevating quartiles of serum γ-glutamyltransferase (GGT) and alanine aminotransferase (ALT) levels. This pattern was also observed in aspartate aminotransferase (AST) quartiles for men but not for women. In multivariate analyses after adjusting for comprehensive risk factors and other liver enzymes, the risk of developing diabetes was significantly higher in the highest GGT quartile than in the lowest quartile [odds ratio (OR), 2.54; 95% confidence interval (CI), 1.03 to 6.26 for men; OR, 5.73; 95% CI, 1.62 to 20.19 for women]. Similar results were observed in ALT quartiles (OR, 2.32; 95% CI, 0.91 to 5.92 for men; OR, 4.40; 95% CI, 1.38 to 14.06 for women) but not in AST quartiles in either sex. Significant positive associations of GGT and ALT with diabetes were seen within each stratified category of risk factors, namely fasting insulin, BMI, waist-to-hip ratio, high-sensitivity C-reactive protein, and alcohol consumption. In receiver operating characteristic analyses, the areas under the receiver operating characteristic curve of GGT and ALT were significantly larger than that of AST, fasting insulin, waist-to-hip ratio, or C-reactive protein.

Discussion: Our findings suggest that serum GGT and ALT concentrations are strong predictors of diabetes in the general population, independent of known risk factors.

Key words: liver, longitudinal, C-reactive protein, diabetes, visceral obesity

Introduction

The liver, a major site of insulin clearance, plays an important role in maintaining normal glucose concentrations during fasting and postprandially (1). Recently, several cohort studies have shown that serum γ-glutamyltransferase (GGT)[1] (2–6), alanine aminotransferase (ALT) (7–9), and aspartate aminotransferase (AST) (10) levels are predictors of diabetes. In one of these reports, a study on Pima Indians (8) found that high serum ALT levels were a significant risk factor for diabetes, although no clear association between serum GGT and diabetes was seen. On the other hand, serum GGT levels, but not AST levels, have been identified as an independent predictor of incident diabetes in British men selected from lists of general practitioners (2). Moreover, the Mexico City Diabetes Study

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*Department of Medicine and Clinical Science and †Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.
Address correspondence to Yusufumi Doi, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: doi@intmed2.med.kyushu-u.ac.jp

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[1] Nonstandard abbreviations: GGT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; OR, odds ratio; CI, confidence interval; ROC, receiver operating characteristic.

found that serum AST is an independent risk factor for future diabetes in multivariable adjustment, whereas no association was observed between serum GGT or ALT and the development of diabetes (10). These reports suggest that the liver is associated with the development of diabetes; however, to the best of our knowledge, there have been no studies to date to determine which of these three enzymes is the best marker for incident diabetes. Furthermore, it also remains unknown whether liver enzyme markers are stronger predictors of future diabetes than well-known risk factors for diabetes, such as adiposity, insulin resistance, and inflammation. The purpose of this study is to examine the effects of serum liver enzymes, i.e., GGT, ALT, and AST, on the development of diabetes in a prospective study of a defined Japanese population, taking into account comprehensive risk factors, including BMI, waist-to-hip ratio, fasting insulin, and high-sensitivity C-reactive protein (HS-CRP) levels.

Research Methods and Procedures

Study Population and Follow-up Survey

A population-based prospective study of cardiovascular disease has been underway since 1961 in the town of Hisayama, a suburb in the Fukuoka metropolitan area on Kyushu Island in Japan. The age and occupational distributions of the town population were almost identical to those of Japan as a whole from 1961 to the present based on data from the national census. A screening survey for this study was performed in 1988. A detailed description of this survey has been published previously (11,12). Briefly, of all 3227 residents 40 to 79 years of age listed in the town registry, 2587 (80.2%) consented to take part in a comprehensive assessment, including an interview covering medical history (including diabetes, hypertension, and other chronic diseases) and current medical treatment with insulin and oral anti-diabetic agents. The baseline classification of subjects as either having or not having diabetes was based on the fasting criteria of the American Diabetes Association (13): subjects with a fasting plasma glucose level of $\geq$7.0 mM or those who were taking anti-diabetic medications were defined as having diabetes. A total of 2274 subjects (963 men and 1311 women) were enrolled in the baseline examination after the exclusion of 1 subject for whom no blood sample was obtained, 75 subjects who had already taken breakfast before the examination, 233 subjects with diabetes, and 4 subjects who had died before starting our follow-up.

After the initial screening in 1988, fasting glucose levels were again measured between 1993 and 1998. Of the 2274 subjects, 1804 (719 men and 1085 women) underwent a follow-up examination (follow-up rate, 79.3%). We considered a subject to have developed diabetes when his/her fasting glucose level met the above-mentioned American Diabetes Association criteria or if the subject started taking anti-diabetic medication during the follow-up period. During this period, 135 subjects (71 men and 64 women) developed diabetes.

Clinical Evaluation and Laboratory Measurements

Blood samples were collected after at least 12 hours of fasting for the determination of serum liver enzymes, plasma glucose, and other parameters. Serum GGT concentrations were measured using a modified version of the method of Orlowski and Meister (14). Both serum ALT and AST concentrations were determined by a kinetic ultraviolet ray method based on the rate of reduced nicotinamide adenine dinucleotide oxidation. Plasma glucose levels were determined by a glucose-oxidase method, and serum insulin levels were measured by double-antibody, solid-phase radioimmunoassay. Hemoglobin A_{1c} levels were measured by high-pressure liquid chromatography. Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were determined enzymatically. HS-CRP concentrations were analyzed using a modified latex-enhanced HS-CRP assay (Behring Diagnostics, Westwood, MA). Serum hepatitis B surface antigen was detected by an immunoprecipitation method (Shino-test, Tokyo, Japan), and presence of hepatitis C virus antibody was assessed by both particle agglutination assay (Serodia-HCV; Fujirebio, Tokyo, Japan) and recombinant immunoblot assay (RIBA 2.0; Ortho Diagnostic Systems, Raritan, NJ).

Blood pressure was obtained three times using a mercury sphygmomanometer with the subject in a sitting position; the averages of the three values were used in this analysis. Hypertension was defined as a systolic blood pressure of $\geq$140 mm Hg and/or a diastolic blood pressure of $\geq$90 mm Hg and/or current treatment with anti-hypertensive agents. The height and weight of each subject were recorded with the subject wearing light clothes but no shoes, and BMI (kg/m^2) was calculated. Abdominal girth at the umbilical level and hip circumference at 5 cm below the spina iliaca anterior superior were measured and used to calculate the waist-to-hip ratio.

On baseline examination, each participant completed a self-administered questionnaire covering medical history, anti-hypertensive treatment, alcohol intake, and smoking habits, and the questionnaire was checked by trained interviewers at the screening. Diabetes in first- or second-degree relatives was taken to indicate a family history of diabetes. Subjects engaging in sports at least three times per week during their leisure time were defined as the regular exercise group. Alcohol intake and smoking habits were used to classify subjects as having current habits or not.

Statistical Analysis

Because the distributions of GGT, ALT, AST, fasting insulin, HS-CRP, and triglycerides were skewed, these variables were natural log-transformed for statistical analyses.

Table 1. Characteristics of subjects by sex

	Men (n = 719)	Women (n = 1085)
Age (yrs)	58 ± 10	58 ± 10
GGT (units/L)	22 (11 to 95)	13 (8 to 35)
ALT (units/L)	14 (7 to 38)	11 (6 to 24)
AST (units/L)	22 (14 to 45)	19 (12 to 33)
Fasting plasma glucose (mM)	5.6 ± 0.5	5.5 ± 0.5
Hemoglobin A_{1c} (%)	5.5 ± 0.5	5.4 ± 0.5
Family history of diabetes (%)	9.2	7.2
Fasting insulin (pM)	30.0 (18.0 to 72.0)	36.0 (18.0 to 72.0)
BMI (kg/m^2)	22.9 ± 2.9	23.0 ± 3.1
Waist-to-hip ratio	0.92 ± 0.05	0.91 ± 0.07
Total cholesterol (mM)	5.07 ± 1.03	5.54 ± 1.04
HDL-cholesterol (mM)	1.25 ± 0.30	1.34 ± 0.29
Triglycerides (mM)	1.24 (0.57 to 3.49)	1.02 (0.49 to 2.32)
HS-CRP (mg/L)	0.49 (0.07 to 7.14)	0.36 (0.06 to 3.22)
Systolic blood pressure (mm Hg)	131 ± 17	130 ± 20
Diastolic blood pressure (mm Hg)	82 ± 11	76 ± 11
Hypertension (%)	42.8	33.2
Current drinking (%)	60.8	8.6
Current smoking (%)	47.3	5.5
Regular exercise (%)	15.9	4.9

HDL, high-density lipoprotein; HS-CRP, high-sensitivity C-reactive protein; GGT, γ-glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Variables of GGT, AST, ALT, fasting insulin, triglycerides, and CRP are median values (95% confidence intervals). All other values are given as mean ± standard deviation or as a percentage.

To analyze liver enzyme levels as categorical variables, these levels were divided into four groups on the basis of quartiles by sex: GGT, men, 6 to 16, 17 to 22, 23 to 37, and 38 to 529 U/L; GGT, women, 6 to 10, 11 to 13, 14 to 17, and 18 to 261 U/L; ALT, men, 5 to 10, 11 to 13, 14 to 18, and 19 to 354 U/L; ALT, women, 5 to 8, 9 to 11, 12 to 14, and 15 to 153 U/L; AST, men, 8 to 17, 18 to 21, 22 to 27, and 28 to 424 U/L; AST, women, 7 to 16, 17 to 18, 19 to 22, and 23 to 273 U/L. The age-adjusted cumulative incidences of diabetes were calculated by the direct method using all subjects, and the results were compared by the Mantel-Haenszel χ^2 test using 10-year age-groupings. Age- and multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression analysis. The sensitivity of cut-off points was defined as their ability to correctly identify individuals who later developed diabetes, and their specificity was defined as their ability to correctly identify individuals who did not develop diabetes. To compare the prognostic abilities of risk factors including liver enzymes and to detect the presence or absence of future diabetes across a range of the values for each risk factor, we plotted receiver operating characteristic (ROC) curves and compared the areas under them (15,16). The diagnostic properties of specific cut-off levels of each risk factor were defined by maximizing the sensitivity and specificity to identify future diabetes. A value of $p < 0.05$ was considered statistically significant in all analyses.

This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, Kyushu University, and written informed consent was obtained from all participants.

Results

The clinical characteristics of all subjects by sex are shown in Table 1. The mean age was 58 years for both sexes. The mean values of GGT, ALT, AST, fasting plasma glucose, hemoglobin A_{1c}, waist-to-hip ratio, triglycerides, HS-CRP, systolic and diastolic blood pressures, frequency of hypertension, alcohol intake, smoking habits, and regular exercise were higher in men than in women, whereas women had higher concentrations of fasting insulin, total cholesterol, and HDL-C. The frequency of family history of diabetes and mean BMI levels did not differ between the sexes.

The age-adjusted cumulative incidence of diabetes was 9.6% for men and 5.9% for women, giving a statistically significant difference ($p = 0.002$). Figure 1 shows the age-adjusted cumulative incidence of diabetes according to quartiles of each liver enzyme level by sex. The cumulative incidence in the third and forth GGT quartiles was significantly higher compared with that of the first quartile in both sexes. A similar tendency was observed for ALT quartiles: there were significant differences between the first and fourth quartiles in both sexes. This pattern was also found in AST quartiles for men but not for women.

The age-adjusted OR for the development of diabetes increased significantly with elevating quartiles of each liver enzyme concentrations in both sexes (Table 2, Model 1). In the multivariate analyses after adjustment for age, family history of diabetes, fasting insulin, BMI, waist-to-hip ratio, total cholesterol, HDL-C, triglycerides, HS-CRP, hypertension, current drinking, current smoking, and physical activity, the ORs of future diabetes increased significantly with elevating quartiles of serum GGT and ALT (Model 2). These trends were also observed in AST quartiles for men but not for women. As shown in Model 3 of Table 2, after additional adjustment for the other liver enzymes, these relationships remained substantially unchanged in both GGT and ALT quartiles but not in AST in either sex.

To examine the influence of insulin resistance-related factors, inflammation and alcohol intake on the development of diabetes, we estimated the age- and sex-adjusted ORs and 95% CIs of diabetes by increments of 1 log in each liver enzyme in men and women together in accordance with other risk factor levels (Table 3). Analyses were performed dividing the subjects into three groups according to tertiles of fasting insulin, BMI, waist-to-hip ratio, and HS-CRP or to alcohol intake levels (0, 1 to 30, and $\geq$30 g/d). Significant positive associations of GGT and ALT with diabetes were observed in all stratified categories of each risk factor; however, we found no significant associations between AST and diabetes in the third tertile of BMI, in the third tertile of waist-to-hip ratio, or in the second level of alcohol intake.

To compare the ability of each risk factor to predict future diabetes over a mean of 9 years of follow-up, we plotted ROC curves and calculated optimal cut-off points, sensitivities, specificities, and the area under the ROC curves (Table 4). Both maximum sensitivity and specificity exceeded 60% only for GGT and ALT, and the areas under the ROC curve of GGT and ALT were significantly larger than that of AST, fasting insulin, waist-to-hip ratio, or HS-CRP and were slightly but not significantly larger than that of BMI. The difference in the area under the ROC curve between GGT and ALT was not significant.

Viral hepatitis infection can increase liver enzyme levels without liver fat accumulation. Thus, hepatitis B and C virus markers were examined in 1583 of the 1804 subjects in 1998. We found 13 viral hepatitis subjects (3 subjects with hepatitis B virus and 10 with C virus; 10.7%) in 122 subjects of the group developing diabetes and 104 viral hepatitis subjects (25 subjects with hepatitis B virus and 79 with C virus; 7.1%) in 1461 subjects of the group that did not develop diabetes: the difference was not significant ($\chi^2 = 2.1$; $p = 0.15$).

Discussion

We have shown, in a prospective study of a general Japanese population, that elevated levels of GGT and ALT, but not AST, are independent predictors of diabetes for both sexes after adjustment for age, family history of diabetes, fasting insulin, BMI, waist-to-hip ratio, total cholesterol, HDL-C, triglycerides, HS-CRP, hypertension, current drinking, current smoking, physical activity, and the other liver enzymes. In our stratified analyses, associations of both GGT and ALT with the development of diabetes were observed in all layers of other risk factors, such as fasting insulin, BMI, waist-to-hip ratio, HS-CRP, and alcohol intake. ROC analyses showed that the predictive power of GGT and ALT was similar to that of BMI but stronger than that of AST, fasting insulin, waist-to-hip ratio, and HS-CRP. To the best of our knowledge, this study is the first report to indicate that liver enzymes are independent risk factors for developing diabetes in a general Japanese population in either sex, taking into account comprehensive risk factors for diabetes. Several prospective studies have found that high levels of hepatic enzymes, including GGT (2–6), ALT (7–9), and AST levels (10), are associated with later development of diabetes. These findings, together with these results, strongly suggest that the liver plays an important role in the development of diabetes in relatively lean Asian populations who may have smaller fat content in the liver, as it does in Western populations.

A recent study using a fatless mouse model has shown that ectopic fat accumulation in the liver is associated with severe insulin resistance (17). In normal weight and moderately overweight subjects, directly determined liver fat content has also been shown to correlate with several features of insulin resistance, independent of BMI and intra-abdominal or overall obesity (18). These findings indicate that hepatic fat accumulation is a critical manifestation of insulin resistance. However, direct measurement of liver fat requires ultrasound, computed tomography, or proton spectroscopy, and such techniques are unlikely to be recommended in routine clinical practice. Some circulating variables, including serum ALT, GGT, and AST, provide insight into the extent of liver fat accumulation. Among these, ALT is found primarily in the liver, whereas AST and GGT are also found in other tissues and are, therefore, less specific markers of liver function. Therefore, ALT is the most specific marker of liver pathology and seems to be the best marker for liver fat accumulation: serum ALT is cor-

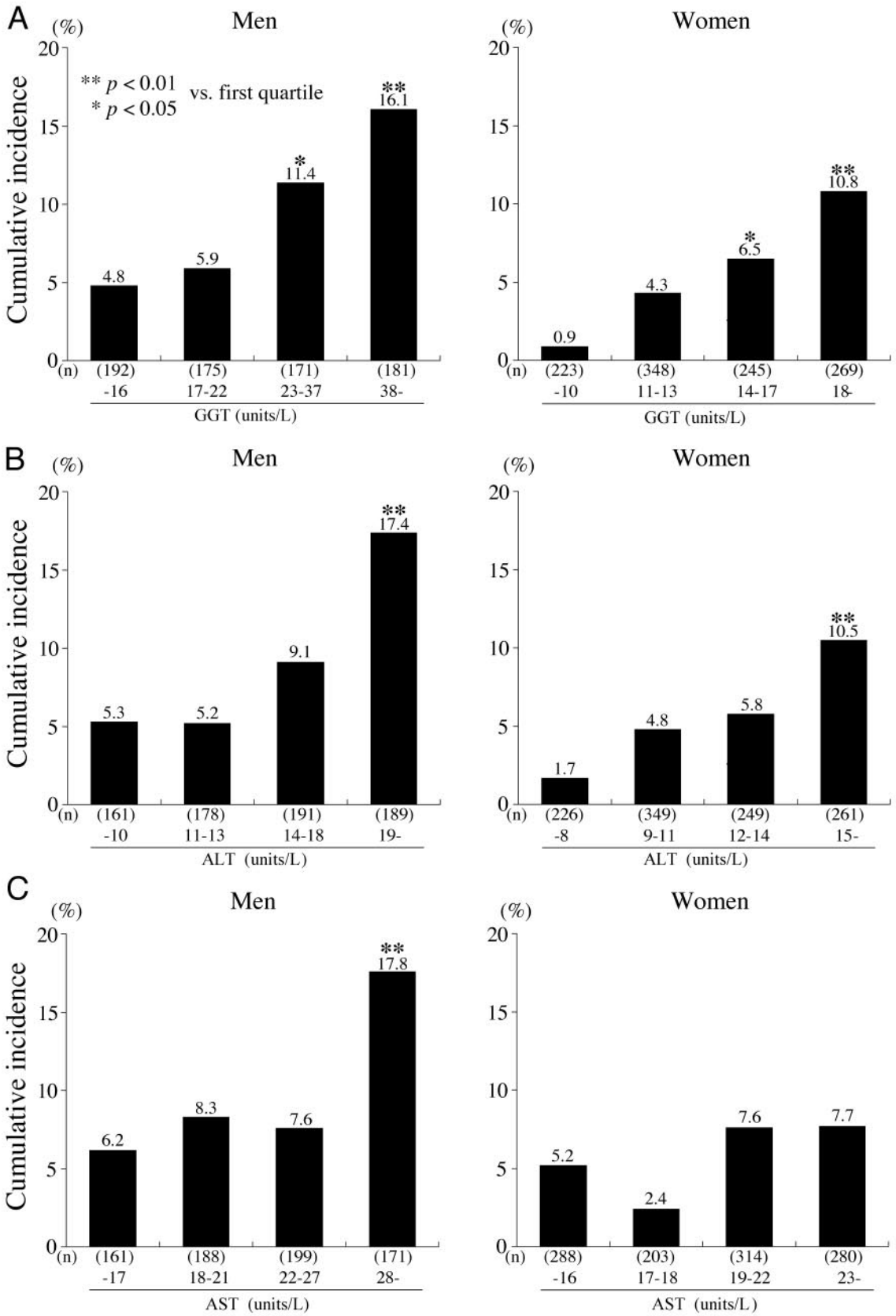

Figure 1: The age-adjusted cumulative incidences of diabetes according to quartiles of serum liver enzymes. (A) GGT, γ-glutamyltransferase; (B) ALT, alanine aminotransferase; (C) AST, aspartate aminotransferase.

Table 2. Age- and multivariate-adjusted ORs and 95% CIs for the development of diabetes according to quartiles of each liver enzyme by sex during mean 9 years of follow-up

	Range	Population at risk (*n*)	Number of events (*n*)	Model 1: OR (95% CI)	*p* for trend	Model 2: OR (95% CI)	*p* for trend	Model 3: OR (95% CI)	*p* for trend
GGT (U/L)									
Men	0 to 16	192	10	1 (referent)		1 (referent)		1 (referent)	
	17 to 22	175	10	1.10 (0.45 to 2.71)		0.85 (0.32 to 2.28)		0.85 (0.31 to 2.27)	
	23 to 37	171	20	2.39 (1.09 to 5.28)		2.02 (0.84 to 4.88)		1.99 (0.82 to 4.80)	
	38 to	181	31	3.71 (1.75 to 7.87)	0.0001	2.71 (1.13 to 6.52)	0.0040	2.54 (1.03 to 6.26)	0.0088
Women	0 to 10	223	3	1 (referent)		1 (referent)		1 (referent)	
	11 to 13	348	15	3.29 (0.94 to 11.48)		2.65 (0.74 to 9.47)		2.64 (0.74 to 9.42)	
	14 to 17	245	16	5.10 (1.46 to 17.74)		3.72 (1.04 to 13.29)		3.66 (1.02 to 13.09)	
	18 to	269	30	8.98 (2.70 to 29.87)	0.0001	5.80 (1.67 to 20.12)	0.0011	5.73 (1.62 to 20.19)	0.0017
ALT (U/L)									
Men	0 to 10	161	9	1 (referent)		1 (referent)		1 (referent)	
	11 to 13	178	9	0.90 (0.35 to 2.33)		0.71 (0.25 to 1.98)		0.68 (0.24 to 1.92)	
	14 to 18	191	17	1.65 (0.71 to 3.83)		1.31 (0.52 to 3.32)		1.18 (0.46 to 3.03)	
	19 to	189	36	3.98 (1.83 to 8.64)	0.0001	2.85 (1.17 to 6.92)	0.0017	2.32 (0.91 to 5.92)	0.016
Women	0 to 8	226	5	1 (referent)		1 (referent)		1 (referent)	
	9 to 11	349	17	2.18 (0.79 to 6.00)		2.28 (0.74 to 7.02)		2.26 (0.73 to 6.99)	
	12 to 14	249	14	2.60 (0.92 to 7.34)		2.83 (0.90 to 8.92)		2.86 (0.90 to 9.07)	
	15 to	261	28	5.15 (1.95 to 13.59)	0.0001	4.53 (1.50 to 13.64)	0.0027	4.40 (1.38 to 14.06)	0.0077
AST (U/L)									
Men	0 to 17	161	10	1 (referent)		1 (referent)		1 (referent)	
	18 to 21	188	16	1.43 (0.63 to 3.24)		0.96 (0.40 to 2.31)		0.91 (0.38 to 2.19)	
	22 to 27	199	15	1.26 (0.55 to 2.89)		0.88 (0.37 to 2.10)		0.81 (0.33 to 1.95)	
	28 to	171	30	3.27 (1.54 to 6.94)	0.0016	2.30 (1.01 to 5.21)	0.030	1.87 (0.77 to 4.53)	0.17
Women	0 to 16	288	12	1 (referent)		1 (referent)		1 (referent)	
	17 to 18	203	5	0.56 (0.19 to 1.62)		0.40 (0.12 to 1.29)		0.40 (0.12 to 1.28)	
	19 to 22	314	24	1.79 (0.86 to 3.73)		1.69 (0.80 to 3.58)		1.70 (0.79 to 3.62)	
	23 to	280	23	1.91 (0.91 to 4.04)	0.019	1.49 (0.68 to 3.24)	0.073	1.26 (0.55 to 2.92)	0.17

OR, odds ratio; CI, confidence interval; GGT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Model 1: adjustment was made for age.

Model 2: adjustment was made for age, family history of diabetes, fasting insulin, BMI, waist-to-hip ratio, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, high-sensitivity C-reactive protein, hypertension, current drinking, current smoking, and physical activity.

Model 3: adjustment was made for the variables used in Model 2 and for the other liver enzymes

Table 3. Age- and sex-adjusted ORs and 95% CIs for the occurrence of diabetes by increments of 1 log in each liver enzyme according to risk factor levels during mean of 9 years of follow-up

	Range	Population at risk (*n*)	Number of events (*n*)	Age- and sex-adjusted [OR (95% CI) for GGT]	*p*	Age- and sex-adjusted [OR (95% CI) for ALT]	*p*	Age- and sex-adjusted [OR (95% CI) for AST]	*p*
Fasting insulin (pM)	0 to 24.0	605	32	2.93 (1.87 to 4.59)	0.0001	2.78 (1.45 to 5.35)	0.0022	2.26 (1.08 to 4.74)	0.031
	24.1 to 36.0	547	36	2.21 (1.38 to 3.52)	0.0009	2.53 (1.39 to 4.63)	0.0025	2.50 (1.26 to 4.96)	0.0087
	36.1 to	651	67	1.86 (1.22 to 2.82)	0.0039	2.07 (1.31 to 3.26)	0.0018	1.93 (1.09 to 3.44)	0.025
BMI (kg/m^2)	0 to 21.5	601	29	2.91 (1.82 to 4.64)	0.0001	2.87 (1.50 to 5.48)	0.0014	3.32 (1.57 to 7.04)	0.0017
	21.6 to 24.2	602	36	2.09 (1.30 to 3.38)	0.0025	3.43 (1.81 to 6.49)	0.0002	2.54 (1.26 to 5.13)	0.0090
	24.3 to	601	70	1.99 (1.32 to 3.00)	0.0010	1.71 (1.10 to 2.65)	0.016	1.72 (0.97 to 3.03)	0.063
Waist-to-hip ratio	0 to 0.88	586	24	3.71 (2.10 to 6.54)	0.0001	2.19 (1.15 to 4.17)	0.017	2.38 (1.09 to 5.22)	0.030
	0.89 to 0.94	583	59	2.28 (1.55 to 3.35)	0.0001	3.19 (1.86 to 5.49)	0.0001	2.83 (1.53 to 5.23)	0.0009
	0.95 to	590	50	1.78 (1.15 to 2.75)	0.01	2.24 (1.37 to 3.67)	0.0014	1.73 (0.91 to 3.30)	0.093
HS-CRP (mg/L)	0 to 0.25	586	21	2.12 (1.21 to 3.73)	0.009	2.49 (1.31 to 4.74)	0.0056	2.68 (1.29 to 5.54)	0.0081
	0.26 to 0.64	587	46	2.62 (1.60 to 4.27)	0.0001	2.78 (1.50 to 5.15)	0.0011	2.45 (1.11 to 5.40)	0.026
	0.65 to	586	64	2.09 (1.45 to 3.02)	0.0001	2.51 (1.59 to 3.96)	0.0001	2.35 (1.32 to 4.17)	0.0038
Alcohol intake (g/day)	0	1,274	79	2.81 (1.90 to 4.15)	0.0001	2.56 (1.73 to 3.79)	0.0001	2.19 (1.29 to 3.72)	0.0036
	1 to 29	289	24	2.86 (1.50 to 5.44)	0.001	3.04 (1.45 to 6.35)	0.0032	2.20 (0.91 to 5.31)	0.079
	30 to	241	32	1.69 (1.08 to 2.64)	0.02	2.49 (1.24 to 5.00)	0.011	2.31 (1.12 to 4.74)	0.023

OR, odds ratio; CI, confidence interval; GGT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS-CRP, high-sensitivity C-reactive protein.

Table 4. Optimal cut-off points of risk factors defined by maximizing sensitivity and specificity to predict future diabetes and their ROC curve areas

	GGT (units/L)	ALT (units/L)	AST (units/L)	Fasting insulin (pM)	BMI (kg/m^2)	Waist-to-hip ratio	HS-CRP (mg/L)
Cut-off point	18	13	19	30.0	24.1	0.91	0.44
Sensitivity (%)	63.3	63.4	48.6	50.7	66.2	47.8	54.8
Specificity (%)	63.7	65.9	71.1	65.2	56.3	69.9	67.9
ROC curve area (%)	67.9	67.4	62.3*†	61.1*†	64.6	60.0*†	61.6*†
(95% CI)	(63.1 to 72.3)	(62.4 to 72.1)	(57.0 to 67.4)	(56.0 to 65.9)	(59.5 to 69.6)	(55.4 to 64.5)	(56.9 to 66.2)

ROC, receiver operating characteristic; GGT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS-CRP, high-sensitivity C-reactive protein; CI, confidence interval.
* $p < 0.05$ vs. GGT.
† $p < 0.05$ vs. ALT.

related with liver fat measured by proton spectroscopy and, after weight loss, the change in serum ALT correlates with that in liver fat (19).

In our multivariate analysis, serum GGT and ALT were mutually independent in predicting incident diabetes. It is known that serum GGT is not only a marker of liver fat amount but also a marker of oxidative stress (20–22). GGT presenting at the outer side of the cell membrane is thought to maintain cellular glutathione levels, which are the major intracellular defense against free radicals (23). Increased oxidative stress impairs insulin secretion from the islets of Langerhans and insulin action in target tissues by damaging DNA, membranes, enzymes, etc. (24). Decreased insulin secretion and insulin sensitivity are major features of the pathophysiology of type 2 diabetes (25). This may be the reason why GGT and ALT has a highly predictive value for the development of diabetes. On the other hand, several epidemiologic studies examined which of these enzymes was the best marker for incident diabetes. Lee et al. (3,4) reported the dose–response relationship between GGT levels and incidence of diabetes in both Korean male workers and young black and white Americans with ALT or AST levels within the reference interval. Furthermore, in their other study, GGT levels within normal range predicted incidence of chronic elevation of ALT (26). These findings indicate the possibility that GGT is a more powerful predictor of incident diabetes than other liver enzymes. However, we showed in the ROC analysis that ALT and GGT but not AST have equally predictive value for the development of diabetes. These findings should be confirmed in other populations, having different BMI levels and lifestyles.

Some experimental studies have shown that selective deletion of the insulin receptor from muscle results in a slight increase in serum free fatty acid and triglycerides but not in glucose intolerance or diabetes (27), whereas a similar maneuver in the liver leads to marked glucose tolerance (28), suggesting that maintaining normal glucose concentrations is related to the liver rather than to peripheral tissue. Our stratified analysis showed that the associations of both GGT and ALT levels with the occurrence of diabetes were independent of markers of systemic insulin resistance, such as fasting insulin, BMI, waist-to-hip ratio, and HS-CRP. In our subjects, the areas under the ROC curve of GGT and ALT were also significantly larger than that of fasting insulin, waist-to-hip ratio, or HS-CRP. Insulin resistance in the liver through fat accumulation may offer a better explanation of the cause of diabetes than peripheral insulin resistance or systemic inflammation.

Alcohol intake causes fatty change of the liver. In alcoholic fatty liver, serum ALT tends to be depressed relative to serum AST, and serum GGT has the specificity to detect alcohol abuse (29), whereas liver fat accumulation caused by overeating predominantly increases ALT but not AST or GGT (19). However, these findings indicated that both GGT and ALT predict future diabetes, independent of current drinking habits. Additionally, in our stratified analyses, the associations of both GGT and ALT with diabetes were unrelated to alcohol intake levels. These observations suggest that elevated serum levels of GGT and ALT, irrespective of the causes of fatty liver, are associated with incident diabetes.

Viral hepatitis infection often increases liver enzyme levels without hepatic fat accumulation, and several clinical studies have shown that chronic hepatitis C virus infection is linked to type 2 diabetes (30,31). In our study, however, the distribution of hepatitis B and C virus positive markers

did not differ between subjects who developed diabetes and those who did not, indicating that viral hepatitis infection did not affect our findings.

A limitation of our study is that a diagnosis of diabetes was not based on a 75-gram oral glucose tolerance test, but on a single reading of fasting glucose level, as has been the case in other epidemiologic studies (3–5,9). Thus, subjects with diabetes having normal fasting glucose levels were misdiagnosed in our study. In addition, some of the participants who were classified as having worsening fasting glucose status may not have been so categorized after repeated testing. These misclassifications may have weakened the associations found in this study, and the true associations may, in fact, be stronger than those shown in our data.

In conclusion, we have shown that elevated serum GGT and ALT levels, even in the normal range, are better predictors of diabetes than the known risk factors except for BMI in a general Japanese population. The association between these enzymes and diabetes was found to be independent of insulin resistance, inflammation markers, and alcohol consumption levels. These results support the hypothesis that the liver is more important than previously thought in the pathogenesis of type 2 diabetes.

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