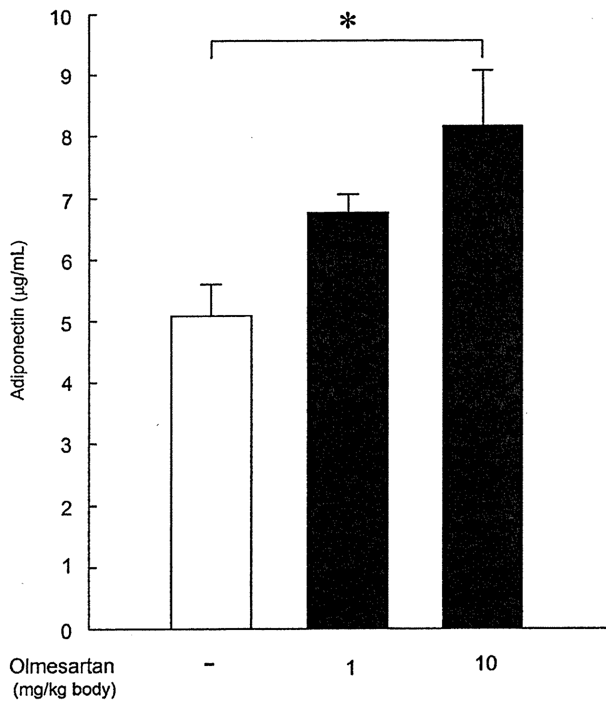

Fig. 3 Effect of olmesartan on serum adiponectin levels in obese Zucker rats fed a high-fat diet. Serum adiponectin levels are shown in groups of high-fat diet-fed obese rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control. High-fat diet was fed to rats for 8 weeks from 6 to 14 weeks of age. Values are mean ± SEM ($n = 5$) for these separate experiments. *$P < 0.05$

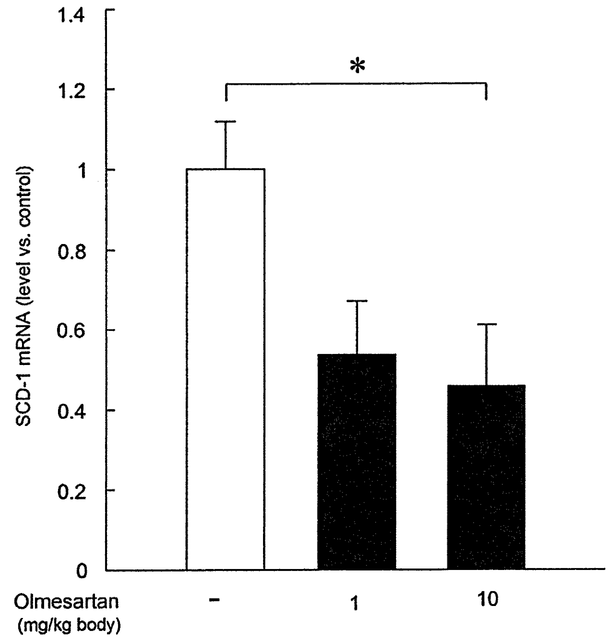

Fig. 4 Effect of olmesartan on SCD-1 mRNA level in liver of obese Zucker rats fed a high-fat diet. Expression levels of SCD-1 in liver were determined by real-time PCR and expressed as a ratio relative to that of GAPDH mRNA as an internal control. Comparisons of SCD-1 mRNA expression in liver samples are shown for groups of high-fat diet-fed obese Zucker rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control. A high-fat diet was fed to rats for 8 weeks from 6 to 14 weeks of age. Values are mean ± SEM ($n = 5$) for these separate experiments, and that for a vehicle-treated control obese rat is set as 1.0. *$P < 0.05$

metabolism. The serum adiponectin level in obese Zucker rats fed a standard diet was 7.9 ± 0.6 ng/ml.

Down-regulation of SCD-1 gene expression by ARB administration

All data were expressed as the SCD-1/GAPDH mRNA ratio in the same samples taken from obese Zucker rats fed a high-fat diet, and that of vehicle-treated control obese rats was set as 1.00 (Fig. 4). Olmesartan administration at a dose of 10 mg/kg/day decreased the level of SCD-1 mRNA by 46% compared with that observed in the vehicle-treated control rats fed a high-fat diet (1.00 ± 0.12 vs. 0.46 ± 0.15, $P < 0.05$; Fig. 4). The level of SCD-1 mRNA in obese Zucker rats fed a standard diet was 0.55 ± 0.09.

To confirm the decrease of SCD-1 gene expression induced by ARB in vivo, we examined the ratio of stearic acid (C18:0) to oleic acid (C18:1) in the liver of obese Zucker rats fed a high-fat diet. The ratio in vehicle-treated control obese rats was set as 1.0 (Fig. 5). Olmesartan administration at a dose of 10 mg/kg/day increased the ratio 1.4-fold relative to that observed in vehicle-treated control rats fed a high-fat diet ($P < 0.01$, Fig. 5), suggesting a decrease of SCD-1 activity in the liver. The ratio of C18:0 to C18:1 in the liver of obese Zucker rats fed a standard diet was 2.64 ± 0.39.

Discussion

Obese *fa/fa* Zucker rats fed a high-fat diet showed more severe hepatic steatosis and insulin resistance than obese rats fed a standard diet, suggesting that obese rats fed a high-fat diet are a good model for examining whether ARB administration can ameliorate hepatic steatosis and insulin resistance. In this study, olmesartan, a potent ARB, markedly decreased fasting blood levels of glucose and insulin, as well as the hepatic TG content, in obese Zucker rats fed a high-fat diet. These observations are also consistent with a previous study [28] of obese Zucker rats fed a standard diet. Our present data indicate that olmesartan ameliorates insulin resistance and hepatic steatosis, suggesting that the Ang II signal induces insulin resistance and hepatic steatosis, as described previously for other ARB agents [13–15].

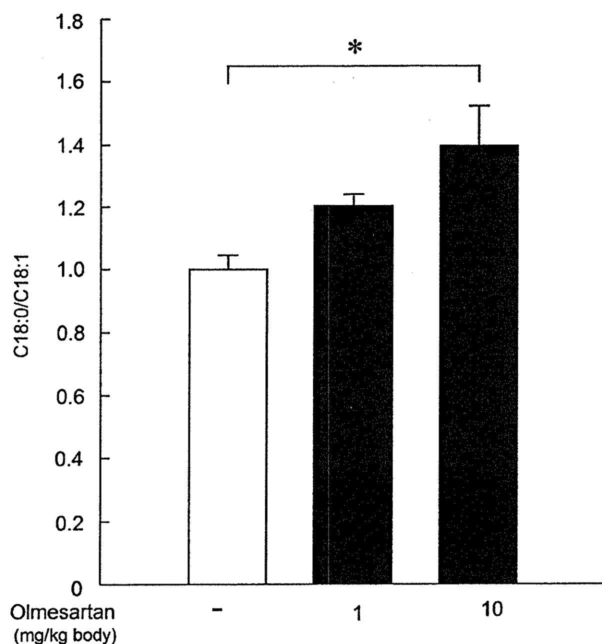

Fig. 5 Effect of olmesartan on fatty acid desaturation in liver of obese Zucker rats fed a high-fat diet. Values are expressed as the ratio of the levels of stearic acid (C18:0, saturated fatty acid) to oleic acid (C18:1, monounsaturated fatty acid) in liver. Comparisons of fatty acid ratio are shown in groups of high-fat diet-fed obese rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control. High-fat diet was fed to rats for 8 weeks from 6 to 14 weeks of age. Values are mean ± SEM ($n = 5$) for these separate experiments, and that for a standard diet-fed obese rats is set as 1.0. $*P < 0.05$

Indeed, it is known that Ang II stimulates serine-phosphorylation of the insulin receptor, insulin receptor substrate 1 (IRS-1), and phosphatidylinositol (PI) 3-kinase via the angiotensin II type 1 (AT1) receptor in insulin signal transduction [10]. As a result, the inhibition of insulin signaling induces insulin resistance. Therefore, our data suggest that inhibition of Ang II signaling via the AT1 receptor by ARB results in recovery of insulin signal transduction, thereby ameliorating insulin resistance.

Adiponectin, a hormone secreted by adipocytes, acts as a major antidiabetic and atherogenic adipocytokine [29]. Plasma adiponectin levels are decreased in obesity, insulin resistance, and type 2 diabetes [29]. Decreased adiponectin is implicated in the development of insulin resistance in obesity, which is reversed by replenishment of adiponectin [30–32]. This insulin-sensitizing effect of adiponectin seems to be mediated by inhibition of gluconeogenesis and stimulation of fatty acid oxidation via activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR)-α [33–35]. In this study, olmesartan administration increased the serum level of adiponectin, an action that could partly explain the amelioration of insulin resistance [16].

To investigate whether SCD-1 gene expression is affected by ARB via blockade of the AT1 receptor signal, we used a real-time PCR assay. We noticed that expression of the SCD-1 gene was significantly increased in the liver of obese rats fed a high-fat diet in comparison with that in the liver of obese rats fed a standard diet. Real-time PCR demonstrated that after olmesartan administration for 4 weeks at a dose of 10 mg/kg body/day, SCD-1 gene expression in obese rats fed a high-fat diet was restored to the level observed in obese rats fed a standard diet.

SCD-1 is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids, introducing a single double bond into its substrates, palmitic (16:0) and stearic (18:0) acids, to generate palmitoleic (16:1) and oleic (18:1) acids as products [36, 37]. The enzyme is located predominantly in the endoplasmic reticulum, where it undergoes rapid turnover in response to a variety of nutritional and hormonal signals [38]. The gene is also transcriptionally regulated by a number of factors including sterol regulatory element-binding protein 1 (SREBP-1) and polyunsaturated fatty acid (PUFA) [39, 40].

Regulation of SCD-1 by leptin seems to be relatively specific [22], although the precise mechanism by which the hormone represses the enzyme is currently unknown. Recent studies of SCD-1 have yielded many new insights into the biology of lipid metabolism and have demonstrated that mice lacking SCD-1 (SCD-$1^{-/-}$ mice) are resistant to high-fat diet-induced obesity and glucose intolerance [41]. A consequence of SCD-1 deficiency is activation of lipid oxidation in addition to reduced TG synthesis and storage. Furthermore, SCD-$1^{-/-}$ mice exhibit increased thermogenesis and insulin signaling in skeletal muscle and brown adipose tissue [42–44]. These lines of evidence have revealed that SCD-1 is an important metabolic control point in lipid metabolism and a promising drug target for the treatment of metabolic syndrome.

In vivo antisense oligonucleotide (ASO) reduction of target genes is a powerful tool for identifying novel metabolic drug targets and elucidating the role of various genes in cellular metabolic pathways. Two recent studies have shown that an ASO-mediated approach can prevent the development of high-fat diet-induced obesity, hepatic steatosis, and insulin resistance [45, 46]. To examine whether SCD-1 activity is inhibited by olmesartan in vivo, we analyzed the ratio of stearic acid (C18:0) to oleic acid (C18:1) in the liver of obese Zucker rats fed a high-fat diet. The ratio was significantly increased by olmesartan, suggesting that SCD-1 activity was suppressed in the liver.

This study showed that ARB can improve insulin resistance and hepatic steatosis in obese rats fed a high-fat diet. This improvement may be partly explained by an increase of adiponectin, as reported previously [33–35]. In

addition, the present data suggest that the ARB-induced decrease of SCD-1 gene expression in the liver participates in the improvement of insulin resistance and hepatic steatosis independently of leptin signaling. However, it is still unknown whether changes in SCD-1 occur as a direct result of ARB on liver cells or as a consequence of systemic changes or changes in body composition, and whether SCD-1 is a direct target of Ang II or the AT1 receptor.

In addition, it has recently been reported that ARB can reduce SREBP-1c gene expression [47]. Accordingly, it would also be expected that SCD-1 gene expression may be partly decreased via suppression of SREBP-1 gene expression by ARB. This issue should be clarified by in vitro experiments using primary hepatocytes or hepatoma cells to examine whether they show direct regulation of SCD-1 gene expression by ARB, and this is currently underway in our laboratory. In this study, ARB treatment caused an increase in the serum adiponectin level and suppressed hepatic SCD-1 expression in obese Zucker rat fed a high-fat diet. However, no previous report has indicated that adiponectin is related to the regulation of SCD-1 gene expression. Therefore, further investigation is needed to clarify whether adiponectin signaling suppresses SCD-1 gene expression.

Previous studies demonstrated that Ang II stimulation via the AT1 receptor increases the gene expression and secretion of leptin in human or rat adipocytes [48, 49] and that administration of ARB suppresses leptin production by inhibition of Ang II signaling [50]. In this study we showed that the serum insulin level and hepatic TG content of obese Zucker rats fed a high-fat diet were significantly increased approximately two- and four-fold relative to those fed a standard diet, respectively. Additionally, in lean Zucker rats fed a high-fat diet, the serum insulin level and hepatic TG content were also significantly increased approximately two- and five-fold, respectively (data not shown). After olmesartan administration, the serum insulin level and hepatic TG content of obese Zucker rats fed a high-fat diet were both decreased to 60% of the values in the vehicle-treated control. On the other hand, in lean Zucker rats, the serum insulin level and hepatic TG content were decreased to approximately 40 and 27% (data not shown). These observations suggest that the effects of ARB on insulin resistance and hepatic steatosis were greater in lean Zucker rats than in obese Zucker rats. The differences in efficacy of ARB between these two models may be partly due to the differences in leptin action. In the case of the normal leptin receptor, leptin signaling may also partly contribute to the effects of ARB on insulin resistance and hepatic steatosis, thereby increasing the effects of ARB in comparison with leptin receptor deficiency.

In conclusion, our present study has shown that obese *fa/fa* Zucker rats, which have a deficiency of the leptin receptor, develop serious insulin resistance and hepatic steatosis when fed a high-fat diet. Moreover, the mRNA level of SCD-1, a key enzyme in hepatic lipogenesis, is evidently increased in the liver. A potent ARB, olmesartan, was able to ameliorate insulin resistance and hepatic steatosis and to suppress the gene expression of hepatic SCD-1. These data suggest that olmesartan-induced down-regulation of SCD-1 gene expression is partly involved in the amelioration of insulin resistance and hepatic steatosis.

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Impact of metabolic syndrome on elevated serum alanine aminotransferase levels in the Japanese population

Takafumi Saito[a,*], Yuko Nishise[a], Naohiko Makino[a], Hiroaki Haga[a], Rika Ishii[a], Kazuo Okumoto[a], Jun-itsu Ito[a], Hisayoshi Watanabe[a], Koji Saito[a], Hiroaki Takeda[a], Hitoshi Togashi[b], Isao Kubota[c], Makoto Daimon[d], Takeo Kato[d], Sumio Kawata[a]

[a]*Department of Gastroenterology, Yamagata University School of Medicine, Yamagata 990-9585, Japan*
[b]*Health Administrative Center, Yamagata University, Yamagata 990-8560, Japan*
[c]*Department of Cardiology, Pulmonology and Nephrology, Yamagata University School of Medicine, Yamagata 990-9585, Japan*
[d]*Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology, Yamagata University School of Medicine, Yamagata 990-9585, Japan*

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Abstract

Measurement of the serum alanine aminotransferase (ALT) level is used as an initial test for detection of liver diseases, and recent studies have also highlighted its potential value as a measure of overall health and survival as a marker of an increased risk of metabolic disorder. This study was designed to clarify the prevalence of elevated ALT levels in the Japanese population and to assess factors associated with ALT elevation. The subjects were 2165 individuals aged 40 to 85 years who participated in a Japanese community-based study referred to as the *Takahata Study*. Serum ALT levels and factors associated with ALT elevation were investigated. Among 2087 subjects who were negative for hepatitis B and C, the rates of elevated ALT greater than 30 U/L in men and greater than 25 U/L in women were 217 (22.7%) of 957 and 239 (21.2%) of 1130, respectively. These ALT cutoff levels had a specificity of more than 80% for exclusion of subjects with none or 1 of 3 metabolic risk factors: hypertension, lipid metabolism abnormality, and hyperglycemia. Multivariate analysis revealed 5 factors with a significant association with ALT elevation in men (n = 957): high γ-glutamyltranspeptidase, low adiponectin, high low-density lipoprotein cholesterol, high body mass index, and high homeostasis model assessment insulin resistance index. Similarly, 4 factors were significantly associated with ALT elevation in women (n = 1130): high γ-glutamyltranspeptidase, low adiponectin, high body mass index, and high homeostasis model assessment insulin resistance index. These results suggest that elevated ALT levels in the Japanese population older than 40 years have a strong association with metabolic syndrome–related features including obesity and insulin resistance.
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1. Introduction

Metabolic syndrome due to visceral fat obesity and increased insulin resistance has a risk for progression to a broad spectrum of metabolic syndrome–related diseases, including type 2 diabetes mellitus, hypertension, cardiovascular disease, and nonalcoholic fatty liver disease (NAFLD) [1,2], as well as to systemic cancer development [3]. There has been a worldwide increase in the number of obese individuals at risk of metabolic syndrome–related diseases, and determination of risk factors for metabolic syndrome is required to prevent further spread of these diseases through proper intervention in the general population.

Elevation of serum alanine aminotransferase (ALT) is a sign of possible underlying liver disease, but an unexplained prevalence of ALT elevation in the general population and a strong association of elevated ALT with NAFLD have also been reported in Western countries [4-8]. In addition, several studies have shown that elevated serum ALT levels have a positive association with metabolic syndrome–related diseases such as type 2 diabetes mellitus [9] and cardiovascular diseases [10]; and several prospective studies suggest that elevated ALT levels predict the development of metabolic syndrome [11,12]. A close relationship between elevated ALT and mortality has also been found in community residents [13]. These reports suggest that the ALT level is a good indicator of overall health, particularly in the context of

* Corresponding author. Tel.: +81 23 628 5308; fax: +81 23 628 5311.
E-mail address: tasaitoh@med.id.yamagata-u.ac.jp (T. Saito).

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lifestyle-related diseases in association with metabolic syndrome [14]. Thus, measurement of ALT may identify people in the general population with a risk of these diseases. However, to date, there have been few comprehensive studies of elevated ALT in association with many metabolic factors including an insulin-sensitive adipocytokine in a large population sample.

Recently, the number of people having metabolic syndrome has rapidly increased in many countries. In particular, Asian individuals have been observed to have a high prevalence of visceral fat accumulation [15]. To estimate the spread of metabolic risk for the occurrence of metabolic syndrome–related diseases in the population and to define preventive strategies, investigation of the prevalence of elevated ALT and determination of factors associated with elevated ALT are required in a large population sample. Therefore, we conducted a large-scale cross-sectional study of ALT levels and factors associated with elevated ALT in Japanese adult subjects representative of the general population.

2. Materials and methods

2.1. Subjects

This study was performed as a community-based survey and consisted of a self-administered questionnaire on lifestyle, measurement of physical status, and collection of blood samples from participants. The subjects were the general population aged 40 to 85 years in the town of Takahata, which is located in Yamagata Prefecture, approximately 350 km north of Tokyo. From June 2004 to November 2005, 2401 individuals (1055 men and 1346 women) took part in the research program. Of these people, 236 for whom data were incomplete were excluded from further analysis, leaving 2165 subjects (991 men and 1174 women) aged 40 to 85 years. We examined the prevalence of elevated ALT in a large sample population and determined the factors currently associated with elevated ALT in Japan. The study was approved by the institutional ethics committee, and written informed consent was obtained from all subjects.

2.2. Measurements

The subjects used a self-reported questionnaire to document medical history, current medication, family history, and clinical symptoms. The presence of a smoking habit (current smoker, nonsmoker, or past smoker) and alcohol intake (current drinker, nondrinker, or past drinker) were determined through an interview. Systolic and diastolic blood pressures were determined using a mercury manometer in a sitting position after resting for at least 5 minutes. These measurements were performed twice, and the mean was used for statistical analysis. Body mass index (BMI) was calculated from weight (in kilograms) divided by the height squared (in square meters), and *obesity* was defined as BMI of at least 25 kg/m^2. Blood samples were collected in the morning and shipped to a central laboratory to be assayed. Ordinary biochemical tests for serum levels of ALT, albumin, fasting blood glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, γ-glutamyl transpeptidase (γ-GTP), and cholinesterase were performed. Fasting insulin was measured using a chemiluminescent immunoassay kit (Kyowa Medics, Tokyo, Japan). Insulin resistance was calculated based on the homeostatic metabolic assessment method (HOMA-IR), as follows: HOMA-IR = fasting plasma insulin × fasting plasma glucose/405, where insulin is expressed in microunits per milliliter and glucose in milligrams per deciliter [16]. Insulin resistance was considered to have changed when HOMA-IR was greater than 2, as previously recommended [17]. Adiponectin was measured using an enzyme immunoassay kit (Human Adiponectin ELISA; Otsuka, Tokyo, Japan). Anti–hepatitis C virus (HCV) antibody, hepatitis B surface antigen, and antinuclear antibody were detected with a latex hemagglutination kit (Ortho HCVAb LPIA III; Ortho Clinical Diagnostics, Tokyo, Japan), a chemiluminescent immunoassay kit (Architect HBsAg QT; Abbott, Tokyo, Japan), and an enzyme immunoassay kit (MESACUP ANA Test; MBL, Tokyo, Japan), respectively.

2.3. Metabolic risk factors

According to the National Cholesterol Education Program Adult Treatment Panel III criteria [18] and the Japanese diagnostic criteria for metabolic syndrome published in April 2005 [19], we defined the metabolic risk for the occurrence of metabolic syndrome–related diseases as the presence of 2 or 3 of the following abnormalities: triglycerides of at least 150 mg/dL and/or HDL cholesterol less than 40 mg/dL, systolic blood pressure of at least 130 mm Hg and/or diastolic blood pressure of at least 85 mm Hg, and fasting glucose of at least 110 mg/dL.

2.4. Statistical analysis

Alanine aminotransferase levels were analyzed as the primary data to determine the prevalence of elevated ALT in the subjects. Analysis of the following 17 factors was performed to assess a potential association with elevated ALT levels in 2087 subjects (957 men and 1130 women) who were negative for viral markers for hepatitis B or hepatitis C: age, serum albumin, antinuclear antibody, γ-GTP, cholinesterase, adiponectin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, BMI, fasting glucose, fasting insulin, HOMA-IR, blood pressure, smoking habit, and drinking habit. The relationship of each factor with elevated ALT was assessed by univariate analysis with a χ^2 test or Fisher exact test for categorical variables, Mann-Whitney test for ordinal data, and unpaired t test for continuous variables. The factors of age and univariate predictors with P less than .10 were included in a multiple

Table 1
ALT levels and seroprevalence of viral hepatitis markers in the study population

	Male (n = 991)		Female (n = 1174)		Total (n = 2165)		*P* value	Test[a]
	n	(%)	n	(%)	n	(%)		
Age group								
40-49	93	(9.4)	128	(10.9)	221	(10.2)	.034	M
50-59	220	(22.2)	294	(25.0)	514	(23.7)		
60-69	338	(34.1)	383	(32.6)	721	(33.3)		
70-79	306	(30.9)	338	(28.8)	644	(29.7)		
>80	34	(3.4)	31	(2.6)	65	(3.0)		
Mean ± SD	64.1 ± 10.2		63.0 ± 10.1		63.5 ± 10.1		.011	T
Seroprevalence of hepatitis B and C								
Both negative	957	(96.6)	1130	(96.3)	2087	(96.4)	.217	F
Positive for HCVAb	12	(1.2)	24	(2.0)	36	(1.7)		
Positive for HBsAg	22	(2.2)	19	(1.6)	41	(1.9)		
Both positive	0	(0.0)	1	(0.1)	1	(0.0)		
ALT (U/L)								
Mean ± SD	24.9 ± 13.8		20.8 ± 11.0		22.7 ± 12.5		<.001	T
Median	21		18		19			
Minimum	6		4		4			
Maximum	122		115		122			

M indicates Mann-Whitney test; F, Fisher exact test; T, *t* test; HCVAb, hepatitis C virus antibody; HBsAg, hepatitis B surface antigen.
[a] Comparison of male with female subjects.

logistic regression model to identify factors associated with elevated ALT levels. We estimated 95% confidence intervals (CIs) with maximum likelihood procedure. A backward-elimination procedure was adopted to remove the most insignificant variable in the regression model at each step until the *P* values for the variables that remained in the working model were all less than .10. The appropriateness of the logistic regression models was confirmed by the Hosmer-Lemeshow test. A 2-tailed *P* value less than .05 was considered statistically significant. Analyses were performed using SAS version 8.2 software (SAS Institute, Cary, NC) or SPSS version 15.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. ALT levels and seroprevalence of viral hepatitis markers in the study population

The characteristics of the subjects and ALT levels are shown in Table 1. Anti-HCV antibody and hepatitis B

Table 2
Association between the number of metabolic risk factors and ALT levels

No. of risk	Male			Female		
ALT (U/L)	2 or 3 n = 253 Sensitivity	0 or 1 n = 704 Specificity	Accuracy	2 or 3 n = 188 Sensitivity	0 or 1 n = 942 Specificity	Accuracy
≥17	83	28	43	73	42	47
≥18	78	36	47	69	49	53
≥19	72	41	49	63	56	57
≥20	66	47	52	58	62	61
≥21	61	52	54	52	67	64
≥22	56	58	57	48	71	67
≥23	53	62	60	43	75	70
≥24	50	66	62	37	78	72
≥25	46	69	63	34	81	74
≥26	43	72	64	30	84	75
≥27	38	75	65	28	86	76
≥28	36	77	66	25	87	77
≥29	34	79	67	23	88	77
≥30	32	81	68	20	89	78
≥31	29	83	68	19	90	78
≥32	28	85	70	17	92	79
≥33	27	86	70	16	93	80
≥34	26	88	71	15	94	80
≥35	24	88	71	13	94	81
≥36	24	89	72	11	95	81

Table 3
Prevalence of elevated ALT levels in the study population

	ALT ≧30		ALT ≧25				P value[a]
	Male (n = 957)		Female (n = 1130)		Total (N = 2087)		
	n	%	n	%	n	%	
Age groups							
40-49	29	31.5	18	14.4	47	21.7	.004
50-59	68	32.2	67	23.4	135	27.2	.032
60-69	72	22.3	94	25.5	166	24.0	.328
70-79	47	15.8	57	17.8	104	16.8	.591
≥80	1	2.9	3	10.0	4	6.3	.333
All ages	217	22.7	239	21.2	456	21.8	.281

[a] Fisher exact test for each age group and age-adjusted Cochran-Mantel-Haenszel χ^2 test for all ages.

surface antigen were positive in 36 (1.7%) and 41 (1.9%) of 2165 subjects, respectively; and 1 subject (1/2165, 0.0005%) was positive for both. The prevalence of anti-HCV antibody and that of hepatitis B surface antigen did not differ between men and women. The mean ALT levels in men and women were (mean ± SD) 24.9 ± 13.8 and 20.8 ± 11.0 U/L, respectively; and ALT was significantly higher in men than in women ($P < .001$).

3.2. Determination of normal ALT levels in subjects with a low potential risk for liver injury

Normal ALT levels were determined in subjects with a low potential risk of liver disease. These subjects met the following criteria: normal BMI, normal LDL cholesterol, and normal triglycerides, as described by van der Poorten et al [20]. Subjects with high systolic blood pressure, excessive alcohol consumption, and hepatitis B and C infection were excluded, as defined by Prati et al [21]. For the 120 men and 215 women in the study population who met these criteria, the mean ALT levels were 20.2 ± 7.4 U/L (median, 19) and 17.5 ± 7.7 U/L (median, 16), respectively; and the level was significantly higher in men than in women ($P < .001$).

3.3. Association between the number of metabolic risk factors and ALT levels

The cutoff values of ALT levels for effective screening for metabolic syndrome were determined based on the association between the number of metabolic risk factors found in 2087 subjects who were negative for viral markers for hepatitis B or C and ALT levels, as shown in Table 2. To determine the cutoff required to identify people with a risk of metabolic syndrome, we defined the *upper limit* of ALT as that required to exclude subjects with none or 1 of the 3 metabolic risk factors (as described above) with a specificity of more than 80%. These cutoff levels were determined to be 30 and 25 U/L for men and women, respectively. Using these proposed upper limits, the sensitivities for identifying subjects with 2 or 3 risk factors were 32% and 34% in men and women, respectively.

3.4. Prevalence of elevated ALT levels in the study population without hepatitis B or C

The rates of elevated ALT higher than the upper limits (30 U/L in men and 25 U/L in women) were 217 (22.7%) of 957 men and 239 (21.2%) of 1130 women. The prevalence of elevated ALT in women increased from 14.4% at 40 to 49 years old to 23.4% at 50 to 59 years old and to 25.5% at 60 to 69 years old, whereas those in men did not vary as much with age, with a similar rate of more than 30% at both 40 to 49 and 50 to 59 years old. The rate of elevated ALT was significantly higher in men than in women in the age groups of 40 to 49 ($P < .01$) and 50 to 59 years ($P < .05$) (Table 3).

3.5. ALT levels in subjects classified by the number of metabolic risk factors

The number of subjects with 2 or 3 of the 3 metabolic risk factors were 441 (21.1%) of 2087 total subjects, 253 (26.4%)

Table 4
ALT levels in subjects classified by the number of metabolic risk factors

	Male			Female		
	0 or 1 risk (n = 704)	2 or 3 risk (n = 253)	P value[a]	0 or 1 risk (n = 942)	2 or 3 risk (n = 188)	P value[a]
ALT (U/L)						
Mean	23.1	29.2	<.001	20.0	24.1	<.001
SD	11.3	17.9		9.9	13.4	
Median	20	24		18	21	
Minimum	6	9		4	8	
Maximum	116	122		111	115	

[a] *t* test (log-transformed value).

Table 5
Factors associated with elevated ALT levels in male subjects (elevated ALT, ≥30)

	Normal ALT n = 740		Elevated ALT n = 217		Univariate test	*P* value	Multivariate test			
							OR[a]	95% CI		*P* value
	n	(%)	n	(%)				Upper	Lower	
Age group										
40-49	63	(8.5)	29	(13.4)	M	<.001				
50-59	143	(19.3)	68	(31.3)						
60-69	251	(33.9)	72	(33.2)						
≥70	283	(38.2)	48	(22.1)						
Albumin (g/dL)										
Low (<3.7)	2	(.3)	0	(.0)	F	1.000				
Middle (3.7-5.5)	738	(99.7)	217	(100.0)						
High (>5.5)	0	(.0)	0	(.0)						
Antinuclear antibody										
Negative	632	(85.4)	183	(84.3)	C	.696				
Positive	108	(14.6)	34	(15.7)						
γ-GTP (U/L)										
Low (<60)	654	(88.4)	118	(54.4)	C	<.001	1.00			
High (≥60)	86	(11.6)	99	(45.6)			5.57	3.80	8.16	<.001
Cholinesterase (U/L)										
Low (<3500)	26	(3.5)	4	(1.8)	M	.165				
Middle (3500-8000)	707	(95.5)	210	(96.8)						
High (>8000)	7	(.9)	3	(1.4)						
Adiponectin (μg/mL)										
Mean ± SD	8.2 ± 4.2		6.1 ± 3.7		T	<.001	0.93	0.88	0.98	.010
Total cholesterol (mg/dL)										
Low (<150)	51	(6.9)	11	(5.1)	M	.005				
Middle (150-219)	568	(76.8)	152	(70.0)						
High (>219)	121	(16.4)	54	(24.9)						
LDL cholesterol (mg/dL)										
Low (<70)	29	(3.9)	8	(3.7)	M	.015	0.79	0.32	1.95	.612
Middle (70-139)	565	(76.4)	148	(68.2)			1.00			
High (>139)	146	(19.7)	61	(28.1)			1.58	1.06	2.35	.024
HDL cholesterol (mg/dL)										
High (≥40)	667	(90.1)	189	(87.1)	C	.200				
Low (<40)	73	(9.9)	28	(12.9)						
Triglyceride (mg/dL)										
Low (≤149)	618	(83.5)	142	(65.4)	C	<.001				
High (≥150)	122	(16.5)	75	(34.6)						
BMI										
Normal (<25)	554	(74.9)	113	(52.1)	C	<.001	1.00			
Obese (≥25)	186	(25.1)	104	(47.9)			1.85	1.28	2.68	.001
Fasting blood glucose (mg/dL)										
Low (<110)	649	(87.7)	176	(81.1)	C	.013				
High (≥110)	91	(12.3)	41	(18.9)						
Insulin (μU/mL)										
Low (<3)	149	(20.1)	16	(7.4)	M	<.001				
Middle (3-18)	584	(78.9)	194	(89.4)						
High (>18)	7	(.9)	7	(3.2)						
HOMA-IR										
0-1.9	630	(85.1)	138	(63.6)	M	<.001	1.00			
2.0-3.9	94	(12.7)	63	(29.0)			1.93	1.25	2.98	.003
≥4	16	(2.2)	16	(7.4)			2.94	1.26	6.86	.013
Blood pressure										
Normal	189	(25.5)	61	(28.1)	C	.449				
Hypertension	551	(74.5)	156	(71.9)						
Smoking habit										
Never	286	(38.6)	87	(40.1)	C	.469				
Current	250	(33.8)	64	(29.5)						
Former	204	(27.6)	66	(30.4)						
Drinking habit										
Never or former	209	(28.2)	54	(24.9)	C	.333				
Current	531	(71.8)	163	(75.1)						

(continued on next page)

Table 5 (*continued*)

	Normal ALT n = 740		Elevated ALT n = 217		Univariate test	*P* value	Multivariate test			
							OR[a]	95% CI		*P* value
	n	(%)	n	(%)				Upper	Lower	
Current medication[b]										
No	719	(97.2)	215	(99.1)	F	.132				
Yes	21	(2.8)	2	(0.9)						

C indicates χ^2 test.

[a] Multiple logistic regression analysis. Age group and the variables with *P* less than .1 on univariate analysis were included in the model.

[b] Current medication for hypertension, lipid metabolism abnormality, and diabetes was excluded.

Table 6

Factors associated with elevated ALT levels in female subjects (elevated ALT, ≥25)

	Normal ALT n = 891		Elevated ALT n = 239		Univariate test	*P* value	Multivariate test			
							OR[a]	95% CI		*P* value
	n	%	n	%				Upper	Lower	
Age group										
40-49	107	(12.0)	18	(7.5)	M	.521	1.00			
50-59	219	(24.6)	67	(28.0)			1.51	0.81	2.81	.196
60-69	274	(30.8)	94	(39.3)			1.71	0.94	3.12	.081
≥70	291	(32.7)	60	(25.1)			1.11	0.59	2.08	.756
Albumin (g/dL)										
Low (<3.7)	0	(.0)	0	(.0)	F					
Middle (3.7-5.5)	891	(100.0)	239	(100.0)						
High (>5.5)	0	(.0)	0	(.0)						
Antinuclear antibody										
Negative	697	(78.2)	191	(79.9)	C	.572				
Positive	194	(21.8)	48	(20.1)						
γ-GTP (U/L)										
Low (<60)	875	(98.2)	198	(82.8)	C	<.001	1.00			
High (≥60)	16	(1.8)	41	(17.2)			11.54	6.12	21.75	<.001
Cholinesterase (U/L)										
Low (<3500)	19	(2.1)	2	(.8)	M	.488				
Middle (3500-8000)	848	(95.2)	231	(96.7)						
High (>8000)	24	(2.7)	6	(2.5)						
Adiponectin (μg/mL)										
Mean ± SD	11.5 ± 5.5		9.5 ± 5.5		T	<.001	0.97	0.93	1.00	.047
Total cholesterol (mg/dL)										
Low (<150)	23	(2.6)	0	(.0)	M	<.001				
Middle (150-219)	597	(67.0)	137	(57.3)						
High (>219)	271	(30.4)	102	(42.7)						
LDL cholesterol (mg/dL)										
Low (<70)	11	(1.2)	2	(.8)	M	<.001				
Middle (70-139)	611	(68.6)	136	(56.9)						
High (>139)	269	(30.2)	101	(42.3)						
HDL cholesterol (mg/dL)										
High (≥40)	857	(96.2)	225	(94.1)	C	.165				
Low (<40)	34	(3.8)	14	(5.9)						
Triglyceride (mg/dL)										
Low (≤149)	794	(89.1)	194	(81.2)	C	.001				
High (≥150)	97	(10.9)	45	(18.8)						
BMI										
Normal (<25)	662	(74.3)	118	(81.2)	C	<.001	1.00			
Obese (≥25)	229	(25.7)	121	(18.8)			2.02	1.43	2.84	<.001
Fasting blood glucose (mg/dL)										
Low (<110)	834	(93.6)	199	(83.3)	C	<.001				
High (≥110)	57	(6.4)	40	(16.7)						
Insulin (μU/mL)										
Low (<3)	84	(9.4)	11	(4.6)	M	.003				
Middle (3-18)	801	(89.9)	222	(92.9)						

Table 6 (*continued*)

	Normal ALT n = 891		Elevated ALT n = 239		Univariate test	*P* value	Multivariate test			
							OR[a]	95% CI		*P* value
	n	%	n	%				Upper	Lower	
High (>18)	6	(.7)	6	(2.5)						
HOMA-IR										
0-1.9	731	(82.0)	130	(54.4)	M	<.001	1.00			
2.0-3.9	148	(16.6)	94	(39.3)			2.44	1.68	3.55	<.001
≥4	12	(1.3)	15	(6.3)			4.93	2.14	11.33	<.001
Blood pressure										
Normal	336	(37.7)	67	(28.0)	C	.006				
Hypertension	555	(62.3)	172	(72.0)						
Smoking habit										
Never	821	(92.1)	221	(92.5)	C	.494				
Current	44	(4.9)	14	(5.9)						
Former	26	(2.9)	4	(1.7)						
Drinking habit										
Never or former	760	(85.3)	210	(87.9)	C	.312				
Current	131	(14.7)	29	(12.1)						
Current medication[b]										
No	879	(98.7)	237	(99.2)	F	.746				
Yes	12	(1.3)	2	(0.8)						

[a] Multiple logistic regression analysis. Age group and the variables with *P* less than .1 on univariate analysis were included in the model.
[b] Current medication for hypertension, lipid metabolism abnormality, and diabetes was excluded.

of 957 men, and 188 (16.6%) of 1130 women. The ALT levels in these subjects were 29.2 ± 17.9 U/L in men and 24.1 ± 13.4 U/L in women; and thus, the mean levels were close to the cutoff values determined in this study. These values were significantly higher than those for subjects who had 0 or 1 metabolic risk factor for both men and women ($P < .001$) (Table 4).

3.6. Factors associated with elevated ALT levels

Factors associated with elevated ALT higher than the upper limits were investigated in 2087 subjects who were negative for anti-HCV antibody and serum hepatitis B surface antigen. The results for 957 men and 1130 women are shown in Tables 5 and 6, respectively. In men, 10 factors with a significant association with elevated ALT were identified in univariate analysis: age group, high γ-GTP, low adiponectin, high total cholesterol, high LDL cholesterol, high triglycerides, high BMI, high fasting glucose, high fasting insulin, and high HOMA-IR. In women, 10 factors associated with elevated ALT were identified in univariate analysis: high γ-GTP, low adiponectin, high total cholesterol, high LDL cholesterol, high triglycerides, high BMI, high fasting glucose, high fasting insulin, high HOMA-IR, and hypertension. A current drinking habit was not associated with elevated ALT in either men or women in univariate analysis. Multivariate logistic regression models were constructed for men and women using variables with low *P* values in univariate analysis. This analysis revealed 5 factors in men (high γ-GTP: odds ratio [OR], 5.57; 95% CI, 3.80-8.16; $P < .001$; low adiponectin: OR, 0.93; 95% CI, 0.88-0.98; $P < .02$; high LDL cholesterol: OR, 1.58; 95% CI, 1.06-2.35; $P < .03$; high BMI: OR, 1.85; 95% CI, 1.28-2.68; $P < .01$; and high HOMA-IR [2.0-3.9]: OR, 1.94; 95% CI, 1.26-2.98; $P < .01$; [≥4]: OR, 2.94; 95% CI, 1.26-6.86; $P < .02$) and 4 factors in women (high γ-GTP: OR, 11.54; 95% CI, 6.12-21.75; $P < .001$; low adiponectin: OR, 0.97; 95% CI, 0.93-1.00; $P < .05$; high BMI: OR, 2.02; 95% CI, 1.43-2.84; $P < .001$; and high HOMA-IR [2-3.9]: OR, 2.44; 95% CI, 1.68-3.55; $P < .001$; [≥4]: OR, 4.93; 95% CI, 2.14-11.33; $P < .001$) with a significant association with elevated ALT levels.

4. Discussion

Elevated serum ALT levels in the general population are closely associated with NAFLD, which is a liver phenotype of metabolic syndrome [4-8]. Alanine aminotransferase activities have also been shown to be useful as an indicator of general health [14], and ALT is a predictor of mortality in community residents [13]. Mortality may be due to unrecognized liver diseases, but may also be due to other causes of ALT elevation, such as atherosclerosis, hypertension, and type 2 diabetes mellitus, which are linked to nonliver health risks. This suggests the importance of determining the association of ALT levels with metabolic factors influencing the occurrence of metabolic syndrome–related diseases in a large population sample. Our results clearly indicate that elevated ALT levels unrelated to hepatitis virus infection are closely associated with metabolic syndrome–related features in a study population that is representative of the general Japanese population older than 40 years old. This suggests that measurement of ALT levels is likely to be a useful primary screening test for metabolic syndrome in the population.

In this study, the seroprevalences of hepatitis B and C were 1.7% and 1.9%, respectively, similar to the standard rates in the Japanese population [22]. Because hepatitis B and C infection is associated with elevated ALT levels, subjects positive for hepatitis markers were excluded from further analysis. To date, the upper limits of ALT levels in screening tests for the general population have not been established clearly; and therefore, we reevaluated these limits for effective screening of metabolic syndrome in the Japanese adult population. Previous reports have shown that sex has a significant influence on ALT levels [23,24]; and therefore, we assessed ALT levels separately for men and women. The ALT cutoff levels for effective screening of individuals with metabolic syndrome for men and for women were proposed in this study on the basis of the relationship between ALT levels and the number of the 3 major metabolic risk factors. Upper limits of 30 U/L in men and 25 U/L in women gave a good specificity of more than 80% for exclusion of subjects with none or 1 of the 3 metabolic risk factors: hypertension, lipid metabolism abnormality, and hyperglycemia. Using these cutoff values, we demonstrated that approximately 20% of the male and female subjects older than 40 years had ALT elevation. A current drinking habit was identified in 694 (72.5%) of 957 men and 160 (14.3%) of 1130 women, but a drinking habit itself was not significantly associated with elevated ALT in univariate analyses in this population, although there is no doubt that excess intake of alcohol causes liver injury in each individual. Multivariate analysis clearly showed that metabolic syndrome–related features that reflect obesity and insulin resistance, including high BMI, high LDL cholesterol, high HOMA-IR, and lower adiponectinemia, were associated with elevated ALT in the study population.

Elevated serum γ-GTP also showed a significant association with elevated ALT in both male and female subjects. These results were replicable in subjects without a history of alcohol consumption (data not shown). Previous studies have documented that elevated serum γ-GTP has a risk for metabolic syndrome and type 2 diabetes mellitus in middle-aged Japanese male office workers [25] and may represent an early marker of subclinical inflammation and increased oxidative stress in healthy individuals [26,27]. Our results are consistent with these studies, and we also found that elevated γ-GTP was associated with obesity and insulin resistance in both men and women. Therefore, γ-GTP is a promising marker for metabolic syndrome and particularly for prediction of development of metabolic syndrome–related diseases; and this warrants a further prospective study.

Because high serum ALT levels often reflect hepatic fat accumulation and inflammation, they are well correlated with the prevalence of NAFLD in the population in cases of unexplained ALT elevation. The importance of ALT activity as an indicator of NAFLD has been demonstrated in association with metabolic abnormalities caused by central obesity and insulin resistance [28-30]. Nonalcoholic fatty liver disease is classified into 2 categories: simple fatty liver and nonalcoholic steatohepatitis (NASH), which is intractable and progressive. The population with elevated ALT levels includes those with NASH [7,8,31] as a phenotype of metabolic syndrome in the liver. Fat droplets in liver tissue are often depleted in the advanced stage of NASH, and such cases may be diagnosed as cryptogenic liver cirrhosis or liver cancer [32]. In fact, the prevalence of obesity, hypertriglyceridemia, or type 2 diabetes mellitus is significantly higher in cases of liver cancer that develop from cryptogenic cirrhosis compared with those caused by HCV infection or excess intake of alcohol [33]. Because a cohort study showed prospectively that individuals with NAFLD had a higher mortality due to liver disease–related deaths [34], people in the general population with high ALT levels are of particular concern because those with NASH have a risk for progression to cirrhosis or cancer.

Individuals with minor elevation of serum ALT levels that are close to the upper limits of the reference range are also of concern because elevated ALT itself is closely associated with insulin resistance, even in the absence of NAFLD and obesity [35,36]. Recent studies have shown that elevated ALT could be a prognostic marker for development of metabolic syndrome [11,12]. Because individuals with ALT elevation have a potential risk for development of various metabolic syndrome–related diseases, including type 2 diabetes mellitus [9], cardiovascular disease [10], atherothrombosis [37], and obstructive sleep apnea [38], it may be worthwhile to notify those with minor ALT elevation of the risk of such diseases. In fact, in this study, we found that mean ALT activities in subjects with 2 or 3 metabolic risk factors were not particularly high, tending only to be close to the upper limit. Thus, minor ALT elevation is also an important feature for effective screening of metabolic syndrome. Elevation of ALT beyond the cutoff levels determined in this study was strongly associated with a broad spectrum of metabolic syndrome–related features, including obesity and insulin resistance. A prospective study of the association between elevated ALT levels and the occurrence of metabolic syndrome–related diseases is now in progress in this Takahata cohort, which includes more than 4000 people and is representative of the Japanese adult population.

In conclusion, the results of this study clearly show that elevated ALT levels in the Japanese population older than 40 years are associated with obesity and insulin resistance, which in turn are associated with metabolic syndrome. This suggests that, in addition to detection of liver disease, screening of serum ALT levels may contribute to identifying the potential risk of metabolic syndrome–related diseases in the general population.

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Relationship between Alcohol Consumption and Serum Adiponectin Levels: The Takahata Study—A Cross-Sectional Study of a Healthy Japanese Population

Yuko Nishise, Takafumi Saito, Naohiko Makino, Kazuo Okumoto, Jun-Itsu Ito, Hisayoshi Watanabe, Koji Saito, Hitoshi Togashi, Chisaki Ikeda, Isao Kubota, Makoto Daimon, Takeo Kato, Akira Fukao, and Sumio Kawata

Departments of Gastroenterology (Y.N., T.S., N.M., K.O., J.-I.I., H.W., K.S., C.I., S.K.); Cardiology, Pulmonology, and Nephrology (I.K.); Neurology, Hematology, Metabolism, Endocrinology, and Diabetology (M.D., T.K.); and Public Health (A.F.), Yamagata University School of Medicine, and Yamagata University Health Administration Center (H.T.), Yamagata 990-9585, Japan

Context: The relationship between alcohol consumption and serum adiponectin levels has not been fully explored in an Asian population.

Objective: Our goal was to determine whether alcohol consumption is associated with a change in adiponectin levels in a healthy Japanese population.

Design: This was a cross-sectional study.

Setting: Subjects were recruited from participants in a health check-up program.

Participants: This study included 2932 subjects (1306 men and 1626 women).

Main Outcome Measures: The effects of total weekly or daily volume of ethanol intake on serum adiponectin levels were evaluated. In addition, the correlation of clinical traits with serum adiponectin levels was examined. A multivariate regression model was used to control for possible confounding factors.

Results: Alcohol consumption was weakly correlated with decreased serum adiponectin levels in men [Spearman's ordered correlation coefficient (r_s) = -0.141; $P < 0.001$]; an even weaker correlation was seen in women ($r_s = -0.055$; $P = 0.025$). Multivariate analysis demonstrated that alcohol consumption was independently associated with hypoadiponectinemia.

Conclusion: In contrast to reports from the United States and Europe among White and Black subjects, our study demonstrated an inverse association between alcohol intake and serum adiponectin levels in Asian subjects, suggesting ethnic differences in the effects of alcohol consumption on serum adiponectin levels. ***(J Clin Endocrinol Metab* 95: 3828–3835, 2010)**

Adiponectin, predominantly synthesized in adipose tissue, is a major modulator of insulin action and resistance (1). It is also related to lipid metabolism, particularly higher levels of high-density lipoprotein cholesterol (HDL-C) and lower levels of triglycerides (2). Higher adiponectin levels are associated with a lower risk of coronary heart disease (3, 4) and type 2 diabetes (5).

Light to moderate alcohol intake is associated with lower risk for coronary heart disease, potentially by increasing HDL-C levels (6) or enhancing fibrinolysis (7).

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Abbreviations: ADH, Alcohol dehydrogenase; ALDH2, acetaldehyde dehydrogenase type 2; ALT, alanine aminotransferase; BMI, body mass index; FBG, fasting blood glucose; γ-GTP, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HMW, high molecular weight; LDL-C, low-density lipoprotein cholesterol; r_s, Spearman's ordered correlation coefficient.

Several previous studies performed in White and Black populations investigated the association between adiponectin concentrations and the risk of developing cardiovascular disease or type 2 diabetes and showed that alcohol intake was associated with elevated serum adiponectin levels (3). In contrast, recent studies in mice and rats have demonstrated that chronic ethanol feeding decreases circulating adiponectin concentrations (8, 9).

As previously described, there are ethnic differences both in serum adiponectin levels (10) and in the risk of type 2 diabetes and cardiovascular disease between Asian and White individuals that are not explained by conventional risk factors (11). In light of these findings, we hypothesized that alcohol consumption may have a different effect on modulation of adiponectin levels in individuals of Asian descent. This relationship has not been fully elucidated on a large scale because of the limited number of subjects. Given the sample size available to us, we chose to evaluate the relationship between alcohol consumption and serum adiponectin levels among a Japanese general population while adjusting for potential confounding factors.

Subjects and Methods

Study population

This study is a part of the Japanese prospective, population-based study held in an agricultural area located about 350 km north of Tokyo. The design and methods of these studies have been reported elsewhere (12–14). Briefly, the study was designed to evaluate the role of lifestyle, diet, and genetic factors in the subsequent development of many common diseases. The study cohort consists of subjects recruited from participants in the regular health check-up program for residents. Since 2004, the baseline survey and subsequent follow-up surveys have been conducted annually. The survey collects information on lifestyle and anthropometric measurements and collects blood and urine specimens from participants on the morning of the survey. The study protocols were approved by the ethics committee at Yamagata University.

Of 3826 participants in the health check-up program from June 1, 2004, through November 30, 2005, the present study population started with 3166 subjects aged 40 yr or older who agreed to participate (83%). Written informed consent was obtained from all subjects. For this analysis, we restricted subjects to those with available information on drinking status and adiponectin levels (n = 3130). We also excluded those who ate breakfast before blood was drawn or those with missing information regarding biomedical variables, anthropometrical variables, or blood pressure. Thus, data from 2932 subjects (1306 men and 1626 women) who met all eligibility criteria were analyzed.

Data collection and measurements

Height, weight, and blood pressure were measured with the subject in light clothes and without shoes, and the body mass index (BMI) (kilograms per square meter) was calculated. After blood samples were drawn, they were frozen in aliquots at −70 C within 4 h and stored frozen until measurements. Biochemical variables evaluated in this study included levels of total adiponectin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides, fasting blood glucose (FBG), fasting serum insulin, alanine aminotransferase (ALT), and γ-glutamyltransferase (γ-GTP). Plasma glucose, serum lipids, and liver enzymes were assayed by routine automated laboratory methods in a single laboratory (BML Inc., Tokyo, Japan). Serum insulin concentrations were measured using a chemiluminescent immunoassay kit (Kyowa Medics, Tokyo, Japan), with intra- and interassay coefficients of variation of 2.0–3.0 and 0.9–4.7%, respectively. Plasma total adiponectin levels were determined by a human adiponectin ELISA (Otsuka Pharmaceutical Co., Tokyo, Japan). Intra- and inter-assay coefficients of variation were 3.3–3.6 and 3.2–7.3%, respectively. All biochemical measurements were performed using plasma samples collected after an overnight fast. The estimate of insulin resistance was done using the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated from FBG and fasting insulin levels using the following formula: FBG (milligrams per deciliter) × fasting plasma insulin (microunits per milliliter)/405.

Assessment of alcohol consumption and smoking history

Information on alcohol consumption and smoking habits of each individual was obtained in face-to-face interviews. Alcohol consumption was calculated on the basis of ethanol volume, and each drinker's status was defined according to the total weekly volume of ethanol intake. The amounts of alcoholic beverages, including beer, wine, and whisky, were converted to an equivalent amount of sake (rice wine). One hundred eighty milliliters of sake contains 20 g ethanol; 180 ml sake equals 500 ml beer, 180 ml wine, or 60 ml whisky in alcohol content. Information on smoking habits was categorized as current use, past use, or never. To assess the reliability of the amount of alcohol consumption, we compared the volume of ethanol intake in the present study with the information on similar items in the survey conducted using a self-administered questionnaire during May 16 through May 29, 2005. Among 1457 subjects who completed the lifestyle questionnaire, Spearman's ordered correlation coefficient (r_s) between the two variables was 0.71.

Statistical analysis

Because alcohol habits are gender related (15), the analysis was conducted according to gender. Variables are given as means ± SD for variables with a normal distribution, median (25th–75th percentile) for skewed variables or n (percent) for numerical or categorized variables. The skewed variables (adiponectin, glucose, insulin, and triglyceride levels) were log transformed before statistical analysis.

Alcohol consumption was treated both as a continuous variable and as a categorical variable: abstainer, less than 120 g/wk, 120–239 g/wk, and 240 g/wk or more. BMI (<22.0, 22.0–24.9, and ≥25.0) and HOMA-IR (<2.0, 2.0–3.9, and ≥4.0) were categorized before statistical analysis. One-way ANOVA was used for testing between multiple groups, and Dunnett's test was used for subsequent comparison of abstainers with other groups. An unpaired t test was used to compare continuous data, and the χ^2 test was used for the analysis of proportions between groups. Pearson's correlation coefficient or r_s was calculated to evaluate

TABLE 1. Characteristics of study participants

	Men (n = 1306)	Women (n = 1626)	*P* value[a]
Age (yr)			
40–49	142 (10.9)	188 (11.6)	0.351
50–59	312 (23.9)	426 (26.2)	
60–69	447 (34.2)	546 (33.6)	
≥70	405 (31.0)	466 (28.7)	
Adiponectin (μg/ml)	7.0 (5.1–9.9)	10.4 (7.4–14.9)	<0.001
BMI (kg/m²)			
<22.0	424 (32.5)	550 (33.8)	0.731
22.0–24.9	485 (37.1)	588 (36.2)	
≥25.0	397 (30.4)	488 (30.0)	
Blood pressure (mm Hg)			
Systolic	136.1 ± 15.7	133.1 ± 16.1	<0.001
Diastolic	81.9 ± 9.9	77.5 ± 9.8	<0.001
Serum lipids (mg/dl)			
Total cholesterol	193.4 ± 31.0	207.3 ± 0.9	<0.001
HDL-C	56.3 ± 14.4	61.6 ± 14.2	<0.001
LDL-C	119.1 ± 28.9	128.9 ± 29.6	<0.001
Triglycerides	95 (69–136)	88 (65–118)	<0.001
Glucose tolerance			
Glucose (mg/dl)	96.9 ± 19.5	92.3 ± 13.3	<0.001
Insulin (μU/ml)	4.2 (3.0–7.0)	5.0 (3.9–8.0)	<0.001
HOMA-IR			
<2.0	1084 (83.0)	1292 (79.5)	0.001
2.0–3.9	184 (14.1)	303 (18.6)	
≥4.0	38 (2.9)	31 (1.9)	
Liver enzymes			
ALT (IU)	21 (17–29)	18 (15–24)	<0.001
γ-GTP (IU)	32 (21–52)	19 (14–26)	<0.001
Alcohol consumption (g/wk)			
None	351 (26.9)	1384 (85.1)	<0.001
<120	366 (28.0)	207 (12.7)	
120–239	285 (21.8)	28 (1.7)	
≥240	304 (23.3)	7 (0.4)	
Smoking habit			
Never	506 (38.7)	1495 (91.9)	<0.001
Current	445 (34.1)	88 (5.4)	
Former	355 (27.2)	43 (2.6)	

χ^2 test, unpaired *t* test, or Mann-Whitney *U* test was used for analyses. Data are n (%) unless otherwise indicated: mean ± SD for blood pressure, total cholesterol, HDL-C, LDL-C, and glucose; median (25th–75th percentile) for adiponectin, triglycerides, insulin, ALT, and γ-GTP.

[a] Men *vs.* women.

the relationship between two continuous or ordered variables. Multiple regression analysis was used with covariance analyses, and log-transformed adiponectin was used as the independent variable. In multivariable analyses, the impact of the effect of 10 g/d alcohol consumption was assessed. The SPSS 15.0 program for Windows (SPSS Inc., Chicago, IL) was used for the statistical analyses. $P < 0.05$ (two sided) was considered statistically significant.

Results

Characteristics of the 2136 subjects are shown in Table 1. There were significant differences in adiponectin levels, lipid levels, glucose, insulin, HOMA-IR, and both systolic and diastolic blood pressure between men and women. Levels of all these variables, except for HDL-C and triglycerides, were significantly higher in women than in men. Only 15% of female subjects were drinkers compared with 73% of men ($P < 0.001$).

The relationship between adiponectin concentrations and potentially confounding factors and alcohol intake are shown in Table 2. Using correlation analysis, we found a small and significant negative correlation for adiponectin concentrations and alcohol consumption in men ($r_s = -0.141$; $P < 0.001$) and a weaker negative correlation in women ($r_s = -0.055$; $P = 0.025$). Significant negative correlations with adiponectin concentrations were observed in total cholesterol, LDL-C, triglyceride, BMI, blood glucose, insulin, HOMA-IR, ALT, γ-GTP, systolic and diastolic blood pressure, and smoking habits in both in men and women. A positive correlation was observed in HDL-C levels in both genders.

In the next analysis, we used categorized data on alcohol consumption to investigate the relationship between alcohol intake and serum adiponectin levels. As shown in Fig. 1, adiponectin levels significantly decreased in a dose-

TABLE 2. Relationship between serum adiponectin concentrations and other factors studied

	Men (n = 1306)		Women (n = 1626)	
	Adiponectin levels or correlation coefficient[a]	*P* value	Adiponectin levels or correlation coefficient[a]	*P* value
BMI (kg/m^2)				
<22.0	8.4 (6.2–12.1)	<0.001	12.9 (9.2–17.6)	<0.001
22.0–24.9	6.9 (5.1–9.4)		10.0 (7.3–14.4)	
≥25.0	6.0 (4.4–8.1)		9.0 (6.4–12.7)	
Blood pressure (mm Hg)				
Systolic	−0.009	0.749	−0.029	0.242
Diastolic	−0.100	<0.001	−0.027	0.275
Serum lipids (mg/dl)				
Total cholesterol	−0.113	<0.001	−0.029	0.245
HDL-C	0.329	<0.001	0.355	<0.001
LDL-C	−0.103	<0.001	−0.097	<0.001
Triglyceride	−0.390	<0.001	−0.307	<0.001
Glucose tolerance				
Glucose (mg/dl)	−0.091	0.001	−0.183	<0.001
Insulin (μU/ml)	−0.341	<0.001	−0.441	<0.001
HOMA-IR				
<2.0	7.6 (5.4–10.3)	<0.001	11.4 (8.3–15.9)	<0.001
2.0–3.9	5.3 (3.8–6.7)		7.5 (5.7–10.7)	
≥4.0	4.9 (3.4–7.0)		5.6 (4.3–7.7)	
Liver enzymes				
ALT (IU)	−0.264	<0.001	−0.185	<0.001
γ-GTP (IU)	−0.300	<0.001	−0.223	<0.001
Alcohol consumption (g/wk)	−0.141	<0.001	−0.055	0.025
Smoking habit				
Never	7.5 (5.4–10.4)	<0.001	10.5 (7.5–15.0)	0.002
Current	6.7 (4.7–9.3)		9.1 (5.9–13.9)	
Former	7.2 (5.0–10.0)		9.8 (6.6–14.7)	

ANOVA, Pearson's correlation coefficient, or Spearman's correlation coefficient was used for analyses.

[a] Data are median (25th–75th percentile) of serum adiponectin levels, Pearson's correlation coefficient, or Spearman's correlation coefficient.

dependent manner in men ($P < 0.001$). A similar trend was noted in women ($P = 0.029$), although the relationship was not as clear as that seen in men. In women, a borderline significant decrease of serum adiponectin levels was observed among drinkers who consumed less than 120 g/wk of ethanol compared with abstainers ($P = 0.053$). A decrease in serum adiponectin levels was not noted in those who consumed 120 g/wk or more of ethanol compared with abstainers.

We also examined the established relationship between alcohol consumption and HDL-C levels. Significant positive correlations were demonstrated ($r_s = 0.165$, $P < 0.001$ for men; and $r_s = 0.118$, $P < 0.001$ for women), indicating that these relationships were consistent with previous studies.

Subsequently, we conducted a multiple regression analysis to assess the effect of 10 g/d alcohol intake on adiponectin concentrations, controlling for potential confounding factors. We included age, sex, BMI, systolic blood pressure, LDL-C, HDL-C, triglycerides, glucose, HOMA-IR, ALT, and smoking habits as covariates. Alcohol consumption was independently associated with hypoadiponectinemia: 10 g/d ethanol intake was associated with a 0.028 (95% confidence interval = −0.040 to −0.016; $P < 0.001$) μg/ml decrease of log-transformed adiponectin concentrations (Table 3).

Discussion

In this population-based cross-sectional study, we found that alcohol intake and serum adiponectin levels were significantly inversely associated in men. A suggested inverse association was demonstrated in women who consumed less than 120 g/wk alcohol. The weak inverse association between alcohol consumption and serum adiponectin concentrations was found even after adjustment for possible confounding factors. These are contradictory observations when compared with several previous epidemiological and experimental reports performed in White and Black populations (4, 16), but they are consistent with experimental studies in animal models (8, 9). Recently, Kawamoto *et al.* (17) reported an inverse relationship between high molecular weight (HMW) adiponectin and alcohol consumption among healthy Japanese men in a cross-sectional study. HMW complex is the most active

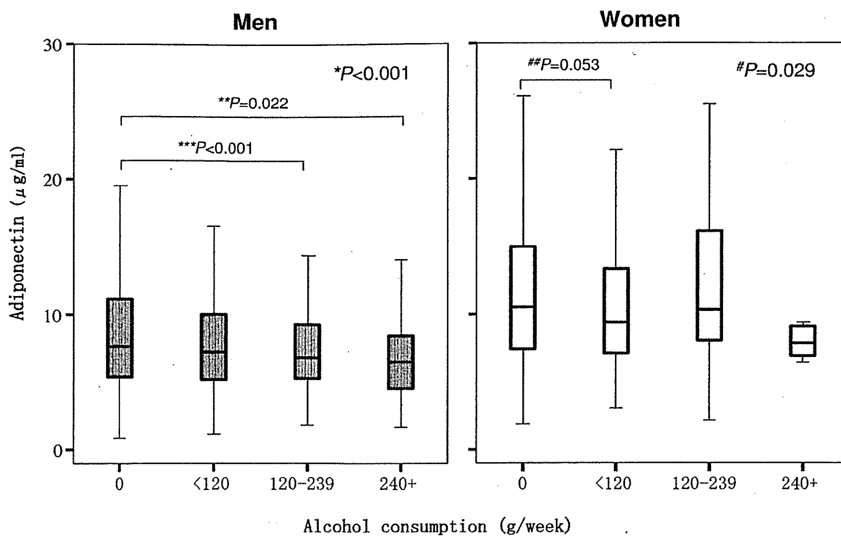

FIG. 1. Box plots illustrating serum plasma adiponectin concentrations for each level of alcohol consumption by gender. *Horizontal lines inside each box* represent medians, and the *top and bottom of the boxes* are the 25th and 75th quartiles, respectively. The *error bars* indicate 95% confidence intervals. *, $P < 0.001$ in men, and #, $P = 0.029$ in women for comparisons by ANOVA; **, $P = 0.022$, and ***, $P < 0.001$ in men, and ##, $P = 0.053$ in women for comparisons with abstainers in each group (Dunnett's test).

form of adiponectin and was closely associated with the type 2 diabetes when compared with total adiponectin (18). Moreover, it was shown that moderate alcohol consumption had different effects on HMW adiponectin, medium molecular weight adiponectin, and low molecular weight adiponectin (19). Further study is necessary to evaluate the effect of HMW on the association between serum adiponectin levels and alcohol consumption in a Japanese population.

Multiple regression analysis demonstrated that serum adiponectin levels were significantly related to sex, age, BMI, HDL-C, triglyceride, HOMA-IR, and ALT. All of the results are in good agreement with previous reports (3, 4, 10, 20, 21). Schulze *et al.* (4) observed an inverse relationship between plasma adiponectin levels and BMI and triglyceride but a positive relationship between plasma adiponectin levels and HDL-C and age in diabetic men. Ferris *et al.* (10) reported that serum adiponectin levels inversely correlated HOMA-IR in White subjects. A sex-based difference in plasma adiponectin levels was supported by previous studies (21, 22) and could be partly explained by differences in body fat distributions (22).

The consistent findings regarding the relationship between serum adiponectin levels and BMI, serum lipids, and insulin resistance and between alcohol consumption and HDL-C levels imply that factors related to ethnic differences, alcohol metabolism, and dietary intake may explain the discrepancies between our results and those of previous studies conducted in humans.

Alcohol is initially oxidized to acetaldehyde, mainly by the alcohol dehydrogenase (ADH) enzyme, and acetaldehyde is subsequently oxidized into acetate by the acetaldehyde dehydrogenase type 2 (ALDH2) enzyme (23). The gene that encodes these two representative alcohol-metabolizing enzymes displays polymorphisms that modulate individual differences in alcohol- and acetaldehyde-oxidizing capacity. Several ethnic differences in distribution of the ADH and ALDH2 genotypes, and in subsequent ethanol metabolism, have been demonstrated. First, the ADH class IV isozyme (σ-ADH), which is present predominantly in the upper gastrointestinal tract but not in the liver and which contributes to gastric ethanol oxidation, is absent or markedly decreased in 80% of Japanese people (24, 25). Second, about 85% of Japanese subjects are carriers of the ADH2*2 allele compared with only 5% or less of European and White American subjects (26). The ADH2*2 encodes an active enzyme and may be expected to generate more acetaldehyde because of this higher activity. Third, the ADH3*1 allele, coding for the rapidly acting ADH3, is more predominant (~95%) in Japanese subjects, whereas it is present in only 40–50% of White subjects (27). Finally, the ALDH2*2 allele, which encodes a catalytically inactive subunit, is present in about 45% of Japanese subjects, although it is extremely rare in White subjects (26). The latter three features indicate a failure to rapidly metabolize acetaldehyde, leading to excessive accumulation of acetaldehyde and higher susceptibility to acetaldehyde among a considerable number of Japanese subjects compared with White subjects. Ethanol and its metabolites, especially acetaldehyde, have been shown to have a toxic influence (23). Acetaldehyde is not only a highly toxic metabolite with extraordinary reactivity but was also shown to induce proinflammatory cytokines, TNF-α, and IL-1β in HepG2 cells (28), whereas TNF-α decreased the levels of adiponectin in human differentiated adipocytes (29). We assume that acetaldehyde and/or acetaldehyde adducts produced through oxidation of ethanol potentially modulate, in part, the association between alcohol intake and serum adiponectin concentrations in the Japanese population. Adjustments for polymorphisms in alcohol-metabolizing genes may explain the differences noted in ethnic groups.

Dietary factors play an important role in the development of type 2 diabetes and ischemic heart disease, because excess caloric intake contributes to the development of obesity, a major risk factor for both diseases. Studies on

TABLE 3. Multivariate-adjusted associations between serum adiponectin concentrations and alcohol consumption in 2932 subjects

Variables	Partial correlation coefficient	SE	Standardized partial correlation coefficient	95% confidence interval		P value
				Lower limit	Upper limit	
Sex (men,[a] women)	0.267	0.022	0.244	0.223	0.310	<0.001
Age (yr)	0.106	0.009	0.192	0.089	0.124	<0.001
BMI (<22,[a] 22–24.9, ≥25) (mm Hg)	−0.068	0.012	−0.099	−0.090	−0.045	<0.001
Systolic blood pressure (mm Hg)	0.000	0.001	−0.002	−0.001	0.001	0.902
LDL-C (mg/dl)	−0.001	0.000	−0.029	−0.001	0.000	0.058
HDL-C (mg/dl)	0.008	0.001	0.222	0.007	0.010	<0.001
Triglyceride (mg/dl)	−0.001	0.000	−0.081	−0.001	0.000	<0.001
Glucose (mg/dl)	−0.001	0.001	−0.025	−0.002	0.000	0.144
HOMA-IR (<2.0,[a] 2.1–3.9, ≥4.0)	−0.200	0.021	−0.170	−0.241	−0.158	<0.001
ALT (IU/liter)	−0.002	0.001	−0.060	−0.004	−0.001	<0.001
Smoking status (never,[a] current/former)	−0.031	0.022	−0.027	−0.074	0.011	0.147
Alcohol consumptions (10 g/d)	−0.028	0.006	−0.083	−0.040	−0.016	<0.001

Multiple regression analysis was used in covariance analyses for serum adiponectin concentrations after log transformation as independent variable.

[a] Reference category.

the dietary predictor of plasma adiponectin concentrations in animal models demonstrated that a high-fat diet is related to decreased serum adiponectin levels, just as it related to an increase in insulin resistance (30). Several controversial observations regarding fat intake have been reported when alcohol consumption accompanied this intake. High-fat, ethanol-containing food decreased serum adiponectin concentrations in mice (8) and rats (31). Decreases in serum adiponectin concentrations after ethanol feeding were dependent on the type of fat in the diet. Ethanol-containing diets high in unsaturated fats contributed to ethanol-induced decreases in adiponectin levels, whereas inclusion of saturated fats in the ethanol-feeding protocol prevented decreased adiponectin levels (9). A diet enriched in saturated fatty acids effectively reversed alcohol-induced necrosis, inflammation, and fibrosis despite continued alcohol consumption (32). The precise mechanism through which dietary fatty acids plus ethanol affect adiponectin expression and its secretion has yet to be determined. The protective action of saturated fatty acids is suggested to be partly caused by down-regulation of TNF-α (30, 33), which suppresses an adiponectin expression (29). In the Japanese population, both intake of total fat and that of saturated fats are lower than in the U.S. population (16, 34). The lower intake of saturated fat in the Japanese population may contribute to the different influence of alcohol consumption on adiponectin concentrations between Japanese and White subjects. However, it was not helpful to compare the effect of the intake of saturated fats with that of unsaturated fats in our study, because intake of these two fats was highly correlated ($r_s = 0.87$) among 1457 subjects who had completed the nutritional survey conducted in the same district using a self-administered questionnaire (unpublished data).

Carbohydrate intake may also be a factor that modulates the relationship between alcohol intake and adiponectin concentrations. In epidemiological studies, high glycemic loads, which were calculated by multiplying the carbohydrate content of each food by its glycemic index, were significantly associated with lower adiponectin concentrations in healthy men (16). For Japanese people, rice is the primary food that contributes to total carbohydrate and energy intake, which is seldom the case in Western populations. Data from the nutritional survey conducted in the same district (unpublished data) have shown that carbohydrate intake accounted for about 59% of total energy intake, and the mean glycemic load was about 206 among subjects aged 40 yr or over. Both parameters were higher than those of White adults (16). Although the effect of the dietary glycemic intake on the relationship between alcohol intake and adiponectin concentrations has not been fully elucidated, the higher intake of carbohydrate in the Japanese population may contribute to the different influence of alcohol consumption on adiponectin concentrations between Japanese and White subjects.

Our study demonstrated an inverse association between alcohol intake with serum adiponectin levels in men, with less clear findings in women. This discrepancy might be explained, in part, by the gender difference in ethanol metabolism. Women differ from men in several factors associated with alcohol metabolism (35), including 1) a lower gastric σ-ADH activity, which mediates the first-pass mechanism of ethanol in women, and 2) a decreased volume of ethanol distribution (body size and distribution space for alcohol, with water space being smaller