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Down-regulation of hepatic stearoyl-CoA desaturase 1 expression by angiotensin II receptor blocker in the obese *fa/fa* Zucker rat: possible role in amelioration of insulin resistance and hepatic steatosis

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Abstract
Background It has been reported that angiotensin II type 1 receptor blocker (ARB) can ameliorate hepatic steatosis and insulin resistance. Stearoyl-CoA desaturase 1 (SCD-1), which catalyzes the cellular synthesis of monounsaturated fatty acids, affects lipid metabolism. In this study, we investigated whether SCD-1 gene expression is affected by ARB treatment.
Methods Obese *fa/fa* Zucker rats fed a high-fat diet were treated with a potent ARB and olmesartan, and the resulting changes in the components of serum and liver were studied. Gene expression of hepatic SCD-1 was assayed using real-time PCR.
Results The serum glucose and insulin levels and hepatic TG content of the obese Zucker rats fed a high-fat diet were reduced after olmesartan administration, while the serum adiponectin level was increased. Real-time PCR revealed an increase of SCD-1 gene expression in the liver of these rats, followed by a reduction after olmesartan administration. The ratio of stearic acid (C18:0) to oleic acid (C18:1) in the liver was increased by olmesartan, indicating a reduction in the in vivo activity of SCD-1.
Conclusions ARB ameliorates hepatic steatosis and insulin resistance in obese *fa/fa* Zucker rats fed a high-fat diet. Gene expression of SCD-1 is decreased by olmesartan, suggesting that the beneficial effect is due partly to suppression of the key enzyme for hepatic lipid metabolism by ARB.

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Introduction

Metabolic syndrome is a cluster of metabolic alterations whose landmarks include visceral obesity, hyperlipidemia, hepatic steatosis, and insulin resistance [1]. A diet with a high carbohydrate and fat content is considered to be a causative factor in the development of insulin resistance in animals and humans [2–6].

Several lines of evidence have suggested that the renin–angiotensin system (RAS) participates in insulin resistance. Adipocytes are known to secrete angiotensinogen and angiotensin II (Ang II) as adipocytokines [7, 8]. Ang II induces insulin resistance via suppression of intracellular signal transduction of insulin and dysregulation of adipocytokines, including TNF-α and adiponectin [9–12].

Recently, blockade of the Ang II signal by Ang II type 1 receptor blocker (ARB) was reported to ameliorate insulin sensitivity in experimental animals and hypertensive patients [13–15], thereby possibly suppressing TNF-α production by skeletal muscle. Also, ARB can ameliorate insulin resistance in patients with essential hypertension by increasing the level of serum adiponectin [16]. On the other hand, large-scale randomized control studies have shown that ARB can prevent the development of diabetes mellitus in patients with essential hypertension [17–19].

Hepatic steatosis is associated with visceral obesity and insulin resistance, and may progress to nonalcoholic steatohepatitis (NASH) under certain circumstances. A low level of serum adiponectin and decreased sensitivity to

leptin are common in hepatic steatosis and NASH. Recently, it was reported that ARB is able to suppress hepatic fibrosis by suppressing the activation of stellate cells, which play a major role in production of the extracellular matrix [20]. However, little is known about how Ang II participates in insulin resistance and hepatic steatosis and how ARB is able to ameliorate these conditions.

Stearoyl-CoA desaturase-1 (SCD-1) is an enzyme that desaturates palmitate, the saturated end-product of de novo fatty acid synthesis. SCD-1 expression and monounsaturated fatty acid levels are markedly increased in livers of leptin-deficient *ob/ob* mice [21] and leptin receptor-deficient (*fa/fa*) Zucker diabetic fatty (ZDF) rats [22]. Leptin treatment reduces expression of the SCD-1 gene in these animals, indicating that it has a regulatory role in SCD-1 gene expression. While elucidation of leptin's role has permitted a detailed view of the biology underlying energy homeostasis, most obese individuals are leptin-resistant [23]. The *ob/ob* mice lacking SCD-1 are significantly less obese than *ob/ob* controls and have histologically normal liver with a significant reduction of both triglyceride (TG) storage and production of very low density lipoprotein (VLDL) [21]. Pharmacologic manipulation of SCD-1 may be of benefit in the treatment of obesity, diabetes, hepatic steatosis, and other components of metabolic syndrome. However, no study has yet investigated whether Ang II can regulate SCD-1 gene expression.

In this study, we investigated whether a potent ARB, olmesartan, is able to ameliorate hepatic steatosis and insulin resistance in obese *fa/fa* Zucker rats, which have a defect in the leptin receptor, fed a high-fat diet, and whether expression of the SCD-1 gene in the liver is affected by olmesartan. The SCD-1 gene was found to be overexpressed in the liver of obese rats fed a high-fat diet relative to the level in obese rats fed a standard diet and showed reduced expression following exposure to olmesartan for 4 weeks.

Materials and methods

Animals

Five-week-old male obese (*fa/fa*) Zucker rats were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). All rats were housed in a temperature-controlled room (20–23°C) with a 12-h light/dark cycle (light on 0600–1800 hours), and had free access to a laboratory standard diet and water. All animal studies were done according to a protocol approved by the Animal Experimentation Committee of Yamagata University Faculty of Medicine, Japan.

Experimental design

At 6 weeks of age, the rats were divided into two groups: obese rats fed a standard diet ($n = 5$) and obese rats fed a high-fat diet ($n = 15$). These diets had the following compositions (as a percentage of total calories): standard diet (CRF-1, Charles River Laboratories Japan Inc., 10% fat, 20% protein, and 70% carbohydrate); high-fat diet [no. D12450B, Research Diets Inc., New Brunswick, NJ, 45% fat (predominantly from lard), 20% protein, and 35% carbohydrate]. All animals were fed standard or high-fat diets for 8 weeks until the end of the experiment. After 4 weeks on the diets, the high-fat diet-fed obese rats ($n = 15$) were further divided into three experimental groups ($n = 5$ rats per group) treated with olmesartan at 1 or 10 mg/kg body/day and treated with vehicle (0.5% carboxymethyl cellulose) alone as the control. Olmesartan (CS-866), a potent ARB, was kindly provided by Daiichi-Sankyo Co. Ltd. (Tokyo, Japan). Standard diet-fed obese rats ($n = 5$ rats per group) were treated with vehicle alone. The drug was administered once daily by oral gavage for 4 weeks.

Blood and liver tissue sampling

After the end of drug treatment, all 14-week-old rats were fasted overnight (13–15 h, food removed at 1800 h) and then killed under ether anesthesia. Blood was rapidly collected from the inferior vena cava, and serum was prepared by centrifugation (3,000 rpm, 10 min, 4°C) of the blood and stored at −20°C until further analysis. The liver tissue was immediately removed and snap-frozen in liquid nitrogen, and stored at −80°C until further study.

Biochemical assay of serum components

Serum glucose (Glu), triglyceride (TG), and free fatty acid (FFA) were measured using enzymatic assay kits (Shino-Test Co., Tokyo; Wako Pure Chemical Industries Ltd., Osaka; Eiken Chemical Co. Ltd., Tokyo, Japan, respectively) on a Hitachi Autoanalyzer 7181 (Hitachi High-Technologies Inc., Tokyo, Japan). Serum levels of insulin and adiponectin were respectively measured using a rat insulin ELISA kit (Shibayagi Co. Ltd., Gunma, Japan) and a rat adiponectin ELISA kit (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan).

Measurement of triglyceride in liver

Lipid extraction was performed by a modified version of the method described previously by Folch [24]. Liver tissues were homogenized with methanol/chloroform (1/2 v/v, 20 ml/g tissue), and aliquots of the organic phase were evaporated under nitrogen gas. The dried lipid extracts

were dissolved in isopropyl alcohol. TG content within the lipid extracts was measured using an enzymatic assay kit (Wako Pure Chemical Industries) on a Hitachi Autoanalyzer 7181 (Hitachi High-Technologies).

Analysis of liver fatty acid

The procedure used for lipid extraction was the same as that for liver TG measurement [24]. Fatty acids in lipids were analyzed using a modification of the method described previously [25]. The dried lipid extracts were treated with 5% KOH–ethanol/water (9/1 v/v) solution. The hydrolyzed lipids were then mixed with *n*-hexane and water, and extracted into the aqueous phase. Pentadecane acid as an internal control was then added to the aqueous phase. The aqueous phase was homogenized with 6 M HCl and *n*-hexane, and the fatty acids were extracted into *n*-hexane, then dried under nitrogen gas and transmethylated with BF_3–methanol/benzene/methanol (7/6/7 v/v/v) solvent at 100°C. Fatty acid methyl esters were extracted into *n*-pentane and analyzed on a gas chromatograph (HP6890 series; Agilent Technologies Japan Ltd., Tokyo, Japan) equipped with a capillary column (DB-WAX; 30 m × 0.32 mm, 0.25 μm film, Agilent Technologies, Japan). Fatty acid methyl esters were identified by comparison with the internal control. SCD-1 activity index was calculated from the precursor-to-product ratio as stearic acid to oleic acid (C18:0/C18:1).

Expression analysis of SCD-1 mRNA in liver

Liver mRNA levels of SCD-1 were measured by real-time PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. Two-step real-time PCR was performed as described previously [26, 27]. Total RNAs were isolated from liver tissues using an RNeasy Plus Mini Kit (QIAGEN Inc., Hilden, Germany). A cDNA was synthesized from 1 μg of total RNA using a random primer (Takara Bio Inc., Mie, Japan) and SuperScript® II RNase H^- Reverse Transcriptase (Invitrogen) in accordance with the manufacturer's instructions. Real-time PCR reactions were performed using a Fast Start DNA Master SYBR I Kit (Roche Diagnostic AG, Basel, Switzerland) on a LightCycler® 2.0 System (Roche Diagnostic). Specific primers were designed using a Perfect Real Time Support System (Takara Bio) and were as follows: SCD-1 (NM1 39192), GCTTGTGGAGCCACAGGACTTAC (forward), ATCCCGGGCCCATTCATATAC (reverse); GAPDH (NM017008), GACAACTTTGGCATCGTGGA (forward), ATGCAGGGATGATGTTCTGG (reverse). PCR reactions for all samples were run in triplicate. Data were analyzed using the LightCycler Software program version 3.5 (Roche Diagnostic). The amounts of all mRNAs were calculated using a standard curve constructed using serial dilutions of a concentrated cDNA sample. The expression level of SCD-1 was normalized with that of GAPDH.

Data analysis and statistics

All data in figures are expressed as mean ± standard error of the mean (SEM). For comparisons between two groups, statistical analysis was performed using unpaired Student's or Welch's *t* test. Mann–Whitney *U* test was used when appropriate. For comparisons among three groups, data were analyzed by one-way ANOVA with the Tukey–Kramer multiple comparisons test. Differences were considered significant at $P < 0.05$.

Results

Amelioration of hyperglycemia, hyperinsulinemia, and insulin resistance by ARB administration

Obese *fa/fa* Zucker rats fed a high-fat diet showed severe hyperglycemia and hyperinsulinemia. Administration of olmesartan at a dose of 10 mg/kg/day for 4 weeks ameliorated the hyperglycemia and hyperinsulinemia in comparison with vehicle treatment in obese rats fed a high-fat diet (glucose: 291.0 ± 20.6 vs. 434.8 ± 52.2 mg/dl, $P < 0.05$; insulin: 29.0 ± 5.3 vs. 49.0 ± 5.6 ng/ml, $P < 0.05$; Fig. 1a, b). These data suggested that ARB is able to ameliorate insulin resistance in obese Zucker rats fed a high-fat diet. The serum concentrations of glucose and insulin in vehicle-treated obese Zucker rats fed a standard diet were 283.8 ± 19.4 mg/dl and 21.3 ± 4.1 ng/ml, respectively.

Decrease in serum FFA level, serum TG level, and hepatic TG content by ARB administration

Olmesartan administration at a dose of 10 mg/kg/day decreased the serum level of FFA in comparison with vehicle treatment in obese rats fed a high-fat diet (697.2 ± 47.5 vs. 1,004.0 ± 215.4 μEQ/l; Fig. 2a), although the difference was not statistically significant. On the other hand, olmesartan administration did not change the serum level of TG (vehicle: 154.6 ± 16.0 vs. olmesartan: 189.6 ± 28.3 and 213.4 ± 31.7 mg/dl; Fig. 2b). Histologically, the hepatocytes of obese rats fed a high-fat diet contained fat droplets in three zones of all hepatic lobules (data not shown). The hepatic TG content was decreased dose-dependently by olmesartan administration at 1 and 10 mg/kg/day (vehicle: 362.9 ± 27.8 vs. olmesartan: 252.8 ± 25.3 and 215.1 ± 21.2 mg/g liver, $P < 0.05$ and $P < 0.005$, respectively; Fig. 2c), suggesting that ARB worked to ameliorate fatty

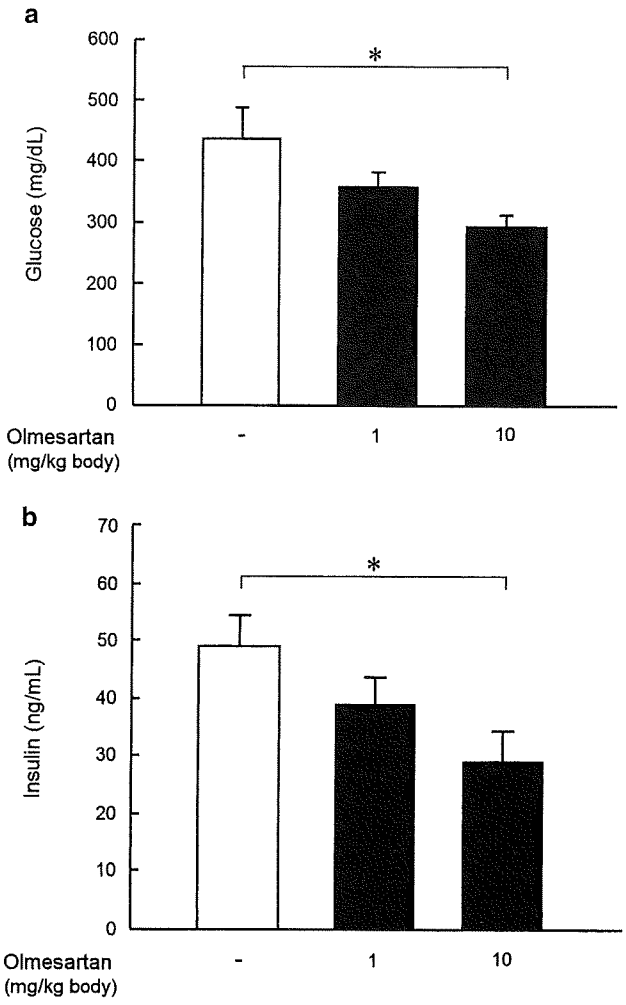

Fig. 1 Effect of olmesartan on serum levels of glucose and insulin in obese Zucker rats fed a high-fat diet. Serum levels of (**a**) glucose and (**b**) insulin in high-fat diet-fed obese Zucker rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control. 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. *$P < 0.05$

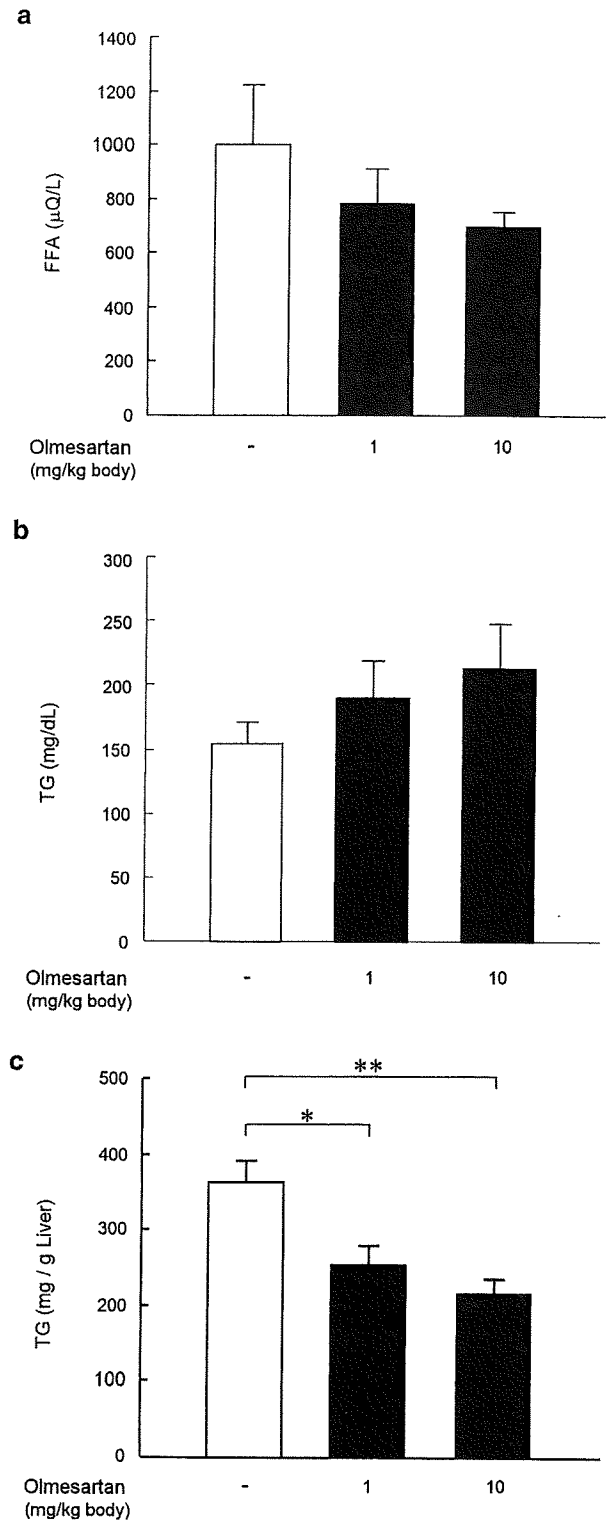

Fig. 2 Effect of olmesartan on serum triglyceride, serum free fatty acid, and liver triglyceride in obese Zucker rats fed a high-fat diet. (**a**) Serum free fatty acid (FFA) level and triglyceride (TG) level in serum (**b**) and liver (**c**) are shown, respectively, in groups of high-fat diet-fed obese 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. *$P < 0.05$, **$P < 0.01$

liver in obese rats fed a high-fat diet. The serum concentrations of FFA and TG, and the hepatic TG content in obese Zucker rats fed a standard diet were 650.4 ± 80.7 μEQ/l, 554.2 ± 43.8 mg/dl, and 95.1 ± 15.9 mg/g liver, respectively.

Increase of serum adiponectin level by ARB administration

Olmesartan administration at a dose of 10 mg/kg/day increased the serum adiponectin level in comparison with vehicle treatment in obese rats fed a high-fat diet (8.2 ± 0.9 vs. 5.1 ± 0.5 μg/ml, $P < 0.05$; Fig. 3), suggesting a mechanism for improvement of glucose and fat

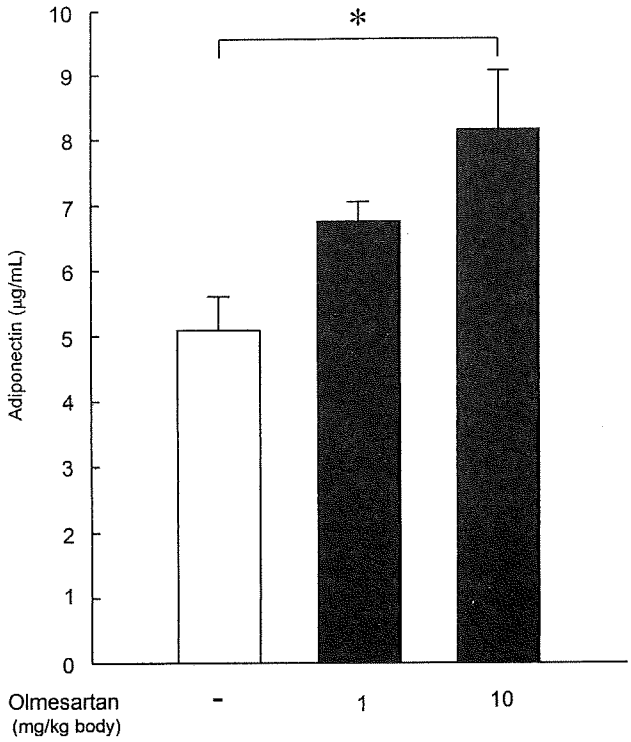

Fig. 3 Effect of olmesartan on serum adiponectin levels in obese Zucker rats fed a high-fat diet. Serum adiponectin levels are shown in groups of high-fat diet-fed obese rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control. High-fat diet was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

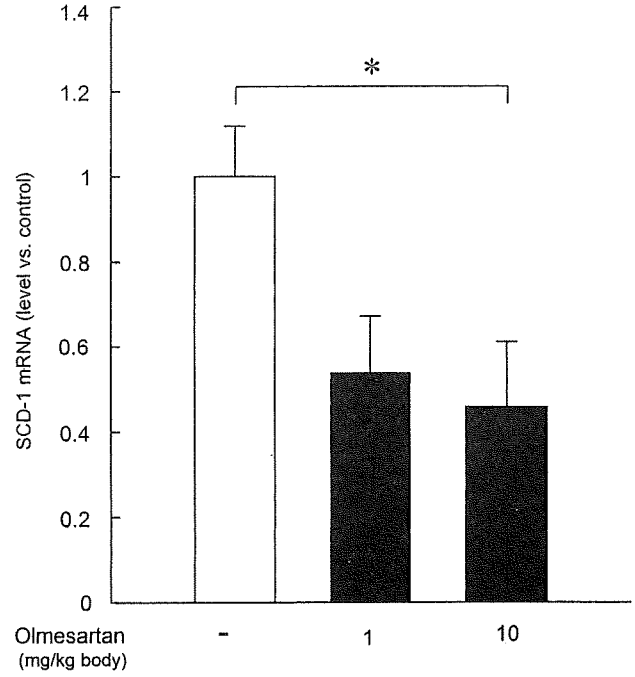

Fig. 4 Effect of olmesartan on SCD-1 mRNA level in liver of obese Zucker rats fed a high-fat diet. Expression levels of SCD-1 in liver were determined by real-time PCR and expressed as a ratio relative to that of GAPDH mRNA as an internal control. Comparisons of SCD-1 mRNA expression in liver samples 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].

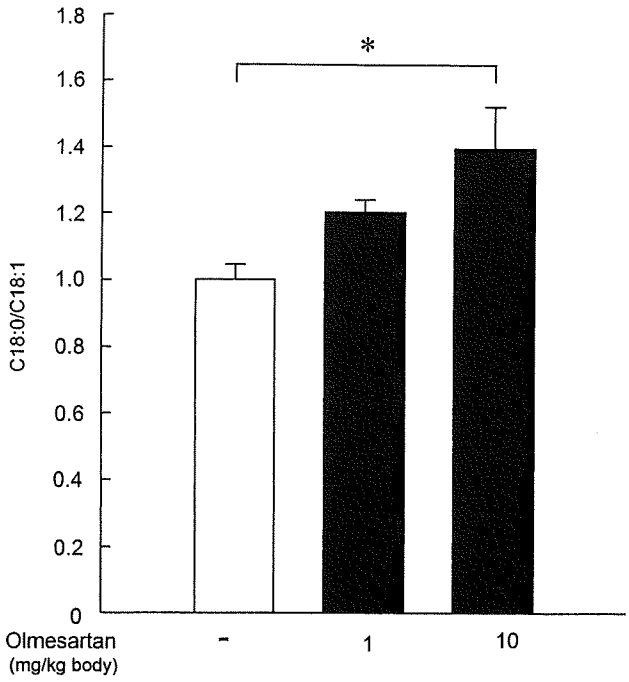

Fig. 5 Effect of olmesartan on fatty acid desaturation in liver of obese Zucker rats fed a high-fat diet. Values are expressed as the ratio of the levels of stearic acid (C18:0, saturated fatty acid) to oleic acid (C18:1, monounsaturated fatty acid) in liver. Comparisons of fatty acid ratio are shown 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

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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.

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

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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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Impacts of Changes in Obesity Parameters for the Prediction of Blood Pressure Change in Japanese Individuals

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Key Words
Waist circumference · Body mass index · Blood pressure · Health screening

Abstract
Aims and Methods: By analyzing data from 2,861 individuals who underwent general health screening 2 years running, we have investigated the impact of changes in waist circumference (WC) and body mass index (BMI) over a 1-year period on systolic blood pressure (BPs). We termed WC, BMI, and BPs at the first visit as WC1, BMI1, and BPs1, respectively, and those at the second visit as WC2, BMI2, and BPs2, respectively. The %dWC, %dBMI, and %dBPs was defined as (WC2 – WC1)/WC1 × 100, (BMI2 – BMI1)/BMI1 × 100, and (BPs2 – BPs1)/BPs1 × 100, respectively. ***Results:*** In multivariate regression analysis using age, BPs1, WC1, and %dWC as independent variables, %dWC was a significant predictor for %BPs only in men. %dBMI was a significant predictor for %BPs in both genders when age, BPs1, BMI1, and %dBMI were used as independent variables. Compared with individuals with both %dWC <0 and %dBMI <0, age-adjusted %dBPs was significantly greater in those with both %dWC <0 and %dBMI ≥0; however, it did not significantly differ in those with both %dWC ≥0 and %dBMI <0. ***Conclusion:*** Our data suggest that the impact of BMI change might be greater than WC change in terms of BPs change during this short period.

Introduction

Much evidence supports a positive association between obesity parameters and hypertension [1–4], although the strength of such an association may differ according to the parameter used [5]. In addition, a loss or gain in body weight may affect blood pressure levels [6, 7], even in relatively lean or non-obese individuals [8, 9]. Therefore, weight control may be an important target for better blood pressure control, leading to a reduction in mortality from heart and cerebrovascular disease [4]. Compared with weight, or body mass index (BMI), less information seems to be available on whether, or to what extent, a loss (or gain) in waist circumference (WC) would result in a change in blood pressure. We previously reported that a reduction or gain in obesity parameters may affect the status of chronic kidney disease in individuals who underwent general health screening [10]. To this end, here we investigated the mode of association be-

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tween changes in WC or BMI over a 1-year period and changes in blood pressure levels in Japanese individuals. We analyzed the data separately for each gender, because there may be gender differences in the strength of the association between various obesity parameters and blood pressure [11].

Subjects and Methods

Study Population

The study was approved by the Ethical Committees of University of Tokyo and Mitsui Memorial Hospital. Between October 2005 and October 2006, 3,312 (1,203 women, 2,109 men) individuals underwent general health screening (visit 1), and they visited our institute again in the following year (visit 2). Among these 3,312 individuals, 2,861 (1,114 women, 1,747 men) who reported not taking antihypertensive drugs at both visits were enrolled in the present study. After about 10 min of rest, systolic blood pressure (BPs) and diastolic blood pressure (BPd) were measured in the sitting position by automated sphygmomanometer, BP-203RVIII (Omron Colin, Tokyo, Japan). Blood pressure was measured twice and the mean of these data were taken. With the subject standing, WC was measured at the umbilical level to the nearest 1 cm by trained physicians and technicians [12]. After changing into a robe from our institute, height and weight were measured, and the weight of the robe was subtracted from the value indicated by the scales. Age, WC, BMI, and BPs at visit 1 were designated age1, WC1, BMI1, and BPs1, respectively. Similarly, WC, BMI, and BPs at visit 2 were designated WC2, BMI2, and BPs2, respectively. %dWC, %dBMI, and %dBPs were defined as (WC2 – WC1)/WC1 × 100, (BMI2 – BMI1)/BMI1 × 100, and (BPs2 – BPs1)/BPs1 × 100, respectively.

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol (TC), HDL cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, hemoglobin A_{1C} was determined using the latex agglutination immunoassay. Serum creatinine was measured by TBA-200FR (Toshiba Medical Systems, Tochigi, Japan) using commercially available kits, Accuras Auto CRE (Shino-test, Tokyo, Japan), according to the manufacturer's instructions. Accuracy control was performed every day by constructing X-bar and R charts using commercially available standards. Estimated glomerular filtration rate (eGFR) was calculated by the following equation: $eGFR = 194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287}$ (× 0.739 if female) [13]. Serum insulin was measured by enzyme immunoassay. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (μU/ml) × fasting plasma glucose (mg/dl)]/405 [14].

Statistical Analysis

Data are expressed as the mean ± SD unless stated otherwise. Analyses of variance with trend analysis, Tukey's post-hoc analysis and multiple regression analysis were conducted as appropriate to assess the statistical significance of differences between groups using computer software Dr. SPSS II (SPSS, Inc., Chicago, Ill., USA). A value of $p < 0.05$ was taken to be statistically significant.

Results

Baseline Characteristics

As described in the Methods section, among the 3,312 individuals who underwent general health screening visited our institute again in the following year; 2,861 (1,114 women, 1,747 men) who reported not taking antihypertensive drugs at both visits were enrolled in the current study (table 1). The mean ± SD of the interval between the two visits of the individuals enrolled was 355 ± 52 days. The mean ± SD age of the enrolled women (51.3 ± 9.9 years) and men (52.5 ± 10.1 years) was significantly smaller than that of the women (60.7 ± 8.3 years) and men (59.0 ± 8.5 years), respectively ($p < 0.001$), who were excluded because of the antihypertensive medication at either or both visits. Similarly, the mean BMI values of enrolled women (21.2 ± 2.9) and men (23.5 ± 2.7) were significantly smaller than those of the excluded women (22.5 ± 3.2) and men (25.0 ± 2.8), respectively ($p < 0.001$).

WC1 ranged between 51.8 and 118.5 cm, and a WC1 ≥90 cm was found in 71/1,114 women (6.4%), and a WC1 ≥85 cm was found in 183/1,114 men (16.4%). BMI1 ranged between 13.1 and 39.4. A BMI1 ≥25 was found in 110/1,114 women (9.9%) and 453/1,747 men (25.9%), and BMI1 ≥30 was found only in 12/1,114 (1.1%) women and 33/1,747 (1.9%) men. The correlation coefficients between %dWC, %dBMI, %dBPs, WC1, BMI1, and BPs1 are described in table 2. The correlation between %dWC and %dBMI was found to be moderate in men (r = 0.476), whereas it was weak in women (r = 0.241). The relationship between %dBMI and %dBPs was found to be statistically significant in the both genders. On the other hand, the relationship between %dWC and %dBPs was statistically significant only in men. Among the study subjects, it was reported that 60 subjects experienced a WC change of –10 cm or less, and 94 subjects experienced a WC change of +10 cm or more. After excluding these 154 individuals from the study population, the results obtained were not essentially changed (data not shown). It was calculated that a 10% weight gain (loss) over a 1-year period was associated with a 3.88 mm Hg BPs gain (loss) in women and a 9.86 mm Hg BPs gain (loss) in men.