Table 2 Univariate analysis of prognostic factors in patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol

	n	Median survival (years)	2-year survival (%)	Hazard ratio	*p* value
Host-related variables					
Age (years)					
≥60	67	2.5	65		
<60	27	2.6	54	0.98 (0.60–1.59)	0.93
Gender					
Female	32	2.7	66		
Male	62	2.4	60	0.99 (0.61–1.56)	0.97
Blood transfusion					
Present	28	2.5	60		
Absent	66	2.7	63	0.77 (0.48–1.24)	0.28
Alcohol abuse[a]					
Present	11	2.0	55		
Absent	83	2.6	63	0.63 (0.33–1.20)	0.16
Smoking habit[b]					
Absent	63	2.5	59		
Present	31	3.4	69	0.79 (0.50–1.27)	0.33
HBs Ag					
Negative	80	2.5	64		
Positive	14	1.8	46	0.77 (0.40–1.49)	0.45
HCV Ab					
Negative	18	1.9	47		
Positive	76	2.5	65	0.93 (0.53–1.64)	0.81
Ascites					
Present	14	1.4	21		
Absent	80	2.8	69	0.29 (0.16–0.53)	**<0.01**
WBC ($\times 10^4$/mm^3)					
≤4.0	51	2.5	61		
>4.0	43	2.5	64	0.76 (0.49–1.19)	0.23
Hemoglobin (g/dL)					
<10	17	2.4	59		
≥10	77	2.6	63	0.69 (0.40–1.19)	0.18
Platelet ($\times 10^4$/mm^3)					
<7.5	36	2.5	67		
≥7.5	58	2.5	59	0.89 (0.57–1.37)	0.59
Total bilirubin (mg/dL)					
≥2.0	13	1.8	46		
<2.0	81	2.7	65	0.59 (0.32–1.09)	0.09
Albumin (g/dL)					
<3.0	33	1.6	35		
≥3.0	61	4.0	76	0.29 (0.18–0.47)	**<0.01**
AST (U/L)					
≥85	24	2.4	58		
<85	70	2.8	63	0.63 (0.38–1.04)	0.07
ALT (U/L)					
≥92	21	2.4	57		
<92	73	2.7	63	0.74 (0.44–1.24)	0.25
LDH (U/L)					
≥500	9	1.8	44		
<500	85	2.5	64	0.76 (0.36–1.58)	0.46
Prothrombin time (%)					
<70	41	2.4	58		
≥70	53	2.7	65	0.93 (0.60–1.45)	0.76
ICG R15 (%)					
≥30	46	2.2	52		
<30	43	3.4	71	0.68 (0.43–1.07)	0.09
Tumor-related variables					
Number of tumors					
Multiple	53	2.0	51		
Single	41	2.8	76	0.63 (0.41–0.98)	**<0.05**
Tumor distribution					
Bilateral	24	1.1	27		
Unilateral	70	2.8	73	0.39 (0.24–0.65)	**<0.01**
Maximum tumor size (cm)					
>3.0	40	1.6	42		
≤3.0	54	3.2	76	0.41 (0.26–0.66)	**<0.01**
Portal vein invasion					
Present	7	1.0	17		
Absent	87	2.6	65	0.36 (0.15–0.84)	**<0.05**
Alpha-fetoprotein (ng/mL)					
≥100	46	2.4	57		
<100	48	2.6	67	0.66 (0.42–1.02)	0.06
PIVKA II (mAU/mL)					
≥100	14	1.1	34		
<100	80	2.7	67	0.53 (0.29–0.97)	**<0.05**

p values lesser than 0.05 are given in bold

HBs Ag hepatitis B surface antigen, *HCV Ab* hepatitis C antibody, *WBC* white blood cell count, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactic dehydrogenase, *ICG* indocyanine green test, *PIVKA II* protein induced by vitamin K absence or antagonist-II

[a] Ethanol intake ≥80 g/day for ≥5 years

[b] >20 cigarettes/day for >10 years

patients. Neither severe toxicity including renal dysfunction or thrombocytopenia, nor complication or treatment related death were seen in the present study.

Univariate and multivariate analysis

The median survival times, two-year survival, hazard ratios and *p* values of the survival time for univariate analysis are shown in Table 2. Among the host-related factors, absence of ascites and a serum albumin level of >3.0 g/dL were

significantly associated with a longer survival time. Among the tumor-related factors, single nodule, unilateral distribution of tumors, maximum tumor size <3.0 cm, absence of portal vein invasion, and PIVKA II level <100 mAU/mL were significantly associated with a longer survival time. The results of multivariate analysis using the Cox proportional hazard model are shown in Table 3. In the multivariate analyses, only those variables identified as significant by the univariate analysis were entered. Serum albumin ≥3.0 g/dL, maximum tumor size <3.0 cm, absence of ascites, and unilateral distribution of the tumors were significantly associated with favorable survival.

Risk groups based on the regression model

For the clinical application of these findings, a prognostic index was calculated based on the regression coefficients derived from the four variables identified by multivariate analysis (Table 3), as follows: prognostic index = score for albumin (0 for ≥3.0, 1 for <3.0 g/dL) + score for ascites (0 for absence, 1 for presence) + score for maximum tumor size (0 for ≤3.0, 1 for >3.0 cm) + score for tumor distribution (0 for unilateral, 1 for bilateral). The index values ranged from 0 to 4. The patients were then classified into three groups according to the prognostic index, as follows: good prognosis group (Group A: prognostic index = 0, $n = 31$ patients) (equivalent to patients with none of the four prognostic factors); intermediate prognosis group (Group B: prognostic index = 1, $n = 28$ patients) (equivalent to patients with one of the four prognostic factors); poor prognosis group (Group C: prognostic index ≥ 2, $n = 35$ patients) (equivalent to patients with two or more of the four prognostic factors). The survival curves for the three groups are shown in Fig. 2. The median survival times in the good, intermediate, and poor prognosis groups were 4.3, 2.7, and 1.1 years, respectively. There were significant differences in the survival time among the three groups ($p < 0.01$).

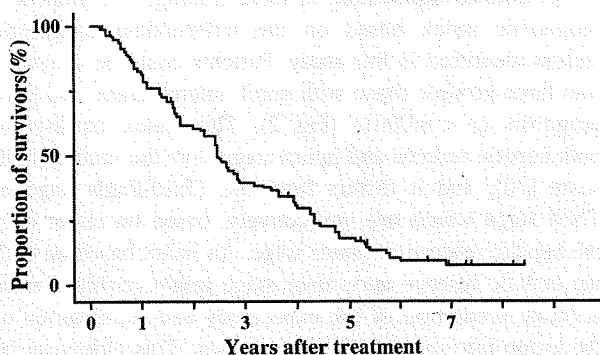

Fig. 1 Overall survival curve for all patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol. *Tick marks* indicate censored cases

Discussion

TAE has been widely used for cases with unresectable HCC and is currently the mainstay of non-surgical treatment for HCC, because it has been shown to exert a marked antitumor effect against HCC and can be administered for any type of HCC, regardless of the size, location or number of tumors [1]. In addition, the survival benefit of this treatment modality has been verified by two meta-analyses [2, 3] of seven randomized controlled trials [4–10]. However, TAE has deleterious effects on liver functions, thereby impairing the baseline prognosis. On the other hand, TAI has milder hepatotoxicity, but also shows a lower antitumor efficacy against advanced HCC than TAE. However, in a randomized controlled trial of TAE versus TAI with zinostatin-stimalamer and lipiodol, TAI and TAE were reported to yield comparable survival [16]. Moreover, the result of our retrospective analysis of TAE versus TAI using cisplatin–lipiodol suspension indicated similar outcomes for the two modalities [17]. From the results of these two studies, we could not conclude that additional embolization is not necessary for the treatment of advanced HCC, but there may be a subset of patients of advanced HCC in which TAI alone may yield sufficient treatment efficacy and survival. Therefore, this analysis of prognostic factors was carried out to enable identification of appropriate candidates for TAI using cisplatin–lipiodol suspension among HCC patients with no prior treatment. This single-institution study was undertaken using a unified method for tumor staging and identical procedures for treatment, follow-up, and supportive care throughout the duration of the study, to enable us to obtain reliable results for confirming important

Table 3 Significant prognostic factors determined by multivariate analysis with the Cox proportional hazard model

Variable	Coefficient	Hazard ratio (95% confidence intervals)	*p* value
Albumin ≥3.0 g/dL	0.94	0.39 (0.23–0.66)	<0.001
Maximum tumor size ≤3.0 cm	1.01	0.37 (0.19–0.69)	0.001
Absence of ascites	0.81	0.45 (0.11–0.40)	0.002
Unilateral tumor distribution	0.77	0.46 (0.27–0.79)	0.004

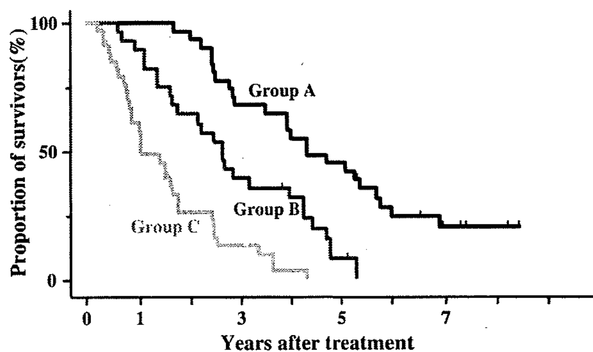

Fig. 2 Survival curves for the three groups determined by a prognostic index. *Group A* good prognosis (31 patients), *Group B* intermediate prognosis (28 patients), *Group C* poor prognosis (35 patients). *Tick marks* indicate censored cases

prognostic factors, predicting life expectancy and designing future clinical trials of TAI for HCC.

In this study, cisplatin was administered as the anticancer agent for TAI. Cisplatin has been reported to exert its actions by binding to the DNA in cancer cells, inhibiting DNA synthesis and subsequent cellular division. It is one of the key drugs for advanced HCC, that constituted a component of the combined chemotherapeutic regimen used in three of the seven randomized controlled trials of TAE reported until date [6, 7, 9]. In Japan, a favorable tumor response (33.8%) was reported in a clinical study of intra-arterial administration of cisplatin for advanced HCC [21], and the treatment has been approved for the treatment of HCC by the Ministry of Health, Labour and Welfare of Japan. Lipiodol has been used as a carrier for anticancer agents in targeting chemotherapy [13–15], and a suspension of cisplatin powder in lipiodol was used in this study. It has been reported that stronger antitumor effect is obtained by hepatic arterial administration of a combination of lipiodol and an anticancer agent than by that of an anticancer agent alone [26]. Recently, a lipophilic cisplatin derivative that can be suspended in lipiodol, SM-11355, was reported to show promising tumor efficacy (CR rate: 56%) in a phase II trial, and further trial is ongoing [27]. Therefore, combined therapy with cisplatin and lipiodol has been expected to become established as a valid option for the treatment of HCC. The response rate (51%: 95% confidence interval, 41–61%) at one month obtained in this study was more favorable than that in a clinical study of cisplatin alone, because TAI with an emulsion of an anticancer agent and lipiodol could be expected to exert more potent effects than an anticancer agent alone. However, follow-up at one month might be insufficient for evaluation of the rate/pattern of recurrence of HCC.

The median survival time and survival rates at two years in the current study were 2.5 years and 65.2%, respectively. These results were comparable or superior to those of TAE reported from the aforementioned seven randomized controlled trials [4–10]. Although the study was based on a retrospective cohort design, the treatment efficacy of TAI with cisplatin–lipiodol suspension was promising and comparable to that of TAE for HCC.

In regard to the host-related factors, absence of ascites and a serum albumin level >3.0 g/dL were found to be favorable prognostic factors by multivariate analysis. Ascites and albumin are the most important factors to consider when evaluating the hepatic reserve, being included in both the Okuda staging system [28] and Child-Pugh classification [29], and have been shown to be prognostic factor in previous studies of patients with advanced HCC [19, 20, 22–24]. In regard to the tumor-related factors, a maximum tumor size ≤3.0 cm and unilateral distribution of the tumors were identified as being significantly associated with a longer survival time by multivariate analysis. Increased tumor size and bilateral distribution of tumors are the well-known unfavorable prognostic factors in HCC patients, and have been shown to be correlated with increased tumor volume and poorer differentiation of HCC, which reflect a more advanced stage and higher malignant potential of the tumors [22]. However, these prognostic factors for TAI with lipiodol in this study were similar to those identified for TAI without lipiodol [19–21] or TAE in previous reports [22–24], and no specific prognostic factors for TAI could be identified in this study.

For clinical application of these findings, we propose a prognostic index based on the independent prognostic factors identified in this study. Patients could be classified into three groups: those with good, intermediate, and poor prognosis ($p < 0.0001$) (Fig. 2). This index consists of both hepatic reserve and tumor stage, like the modified JIS score [30], and it differs from the Child-Pugh stage or TNM stage which are, respectively, based on either only the hepatic reserve or tumor stage. An index based on both the hepatic reserve and tumor stage might enable a more accurate prediction of life expectancy and stratification of the group into more distinct prognoses. This index can be easily calculated, because it is based on variables obtained during routine examinations before TAI. It can, therefore, be used to stratify patients with HCC before TAI according to the predicted survival. Accordingly, patients with good prognosis may obtain sufficient treatment efficacy and survival with TAI alone. In contrast, patients with a poor prognosis may be treated with supportive care only because of the extremely short median survival (1.1 years) expected, or may be treated other more aggressive treatments, such as more intensive chemotherapy. Recently, systemic chemotherapy for advanced HCC has become an important treatment modality, because sorafenib has been proven to confer a survival benefit and to show promise as a standard

treatment for patients with advanced HCC [31]. To improve the treatment efficacy, further chemotherapy regimens, such as the combination therapy comprising TAI with cisplatin suspended in lipiodol and sorafenib or other molecularly targeted agents, remain as challenges to be met following further detailed investigations. These findings may be helpful in predicting the life expectancy in HCC patients treated with TAI and provide more information to stratify patients in future TAI trials. It is also important to validate this prognostic index by applying it to other populations of HCC patients.

In conclusion, TAI with cisplatin suspended in lipiodol exhibited favorable tumor efficacy and survival in patients with HCC. Although no specific prognostic factors for TAI could be identified in this study, the results of the prognostic factors and the prognostic index may be helpful for predicting life expectancy, determining the most appropriate treatment strategies, and designing future clinical trials.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

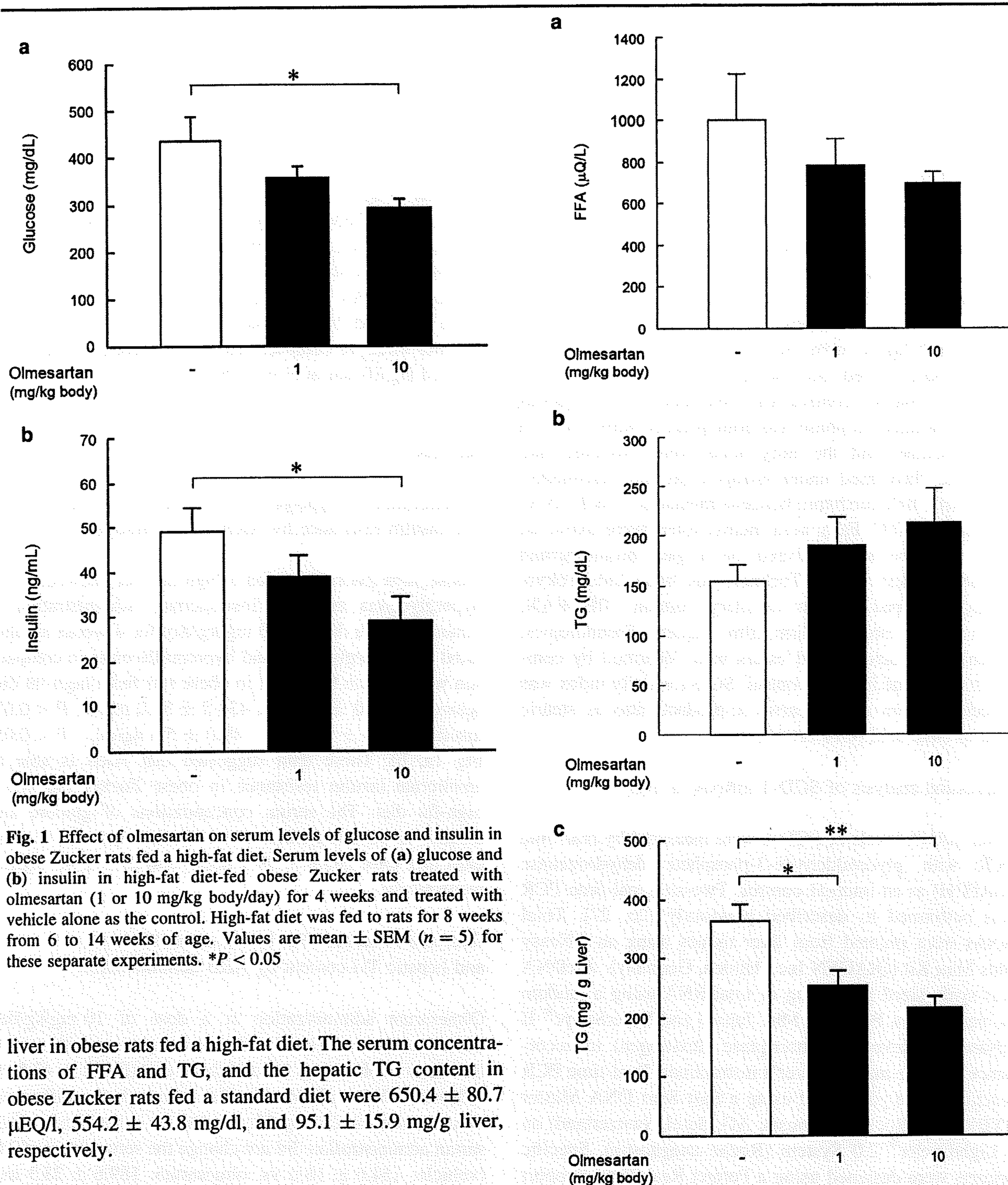

Fig. 1 Effect of olmesartan on serum levels of glucose and insulin in obese Zucker rats fed a high-fat diet. Serum levels of (**a**) glucose and (**b**) insulin in high-fat diet-fed obese Zucker rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control.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

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. 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. $^{*}P < 0.05$, $^{**}P < 0.01$

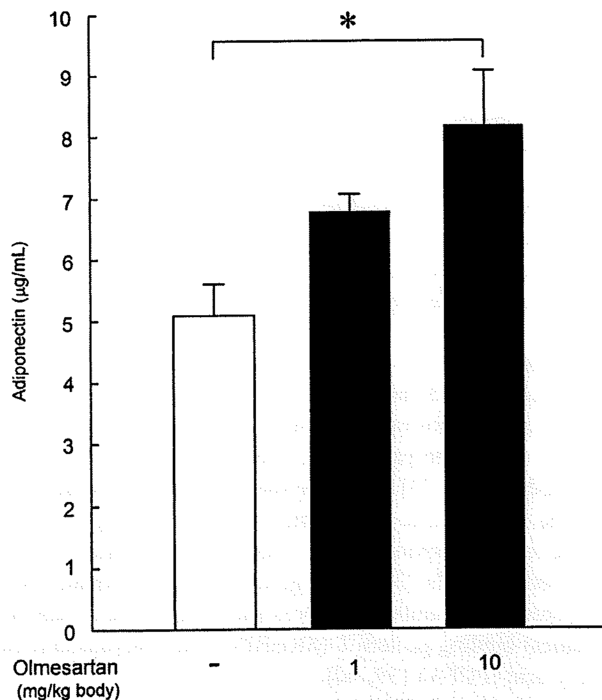

Fig. 3 Effect of olmesartan on serum adiponectin levels in obese Zucker rats fed a high-fat diet. Serum adiponectin levels are shown in groups of high-fat diet-fed obese rats treated with olmesartan (1 or 10 mg/kg body/day) for 4 weeks and treated with vehicle alone as the control. High-fat diet was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

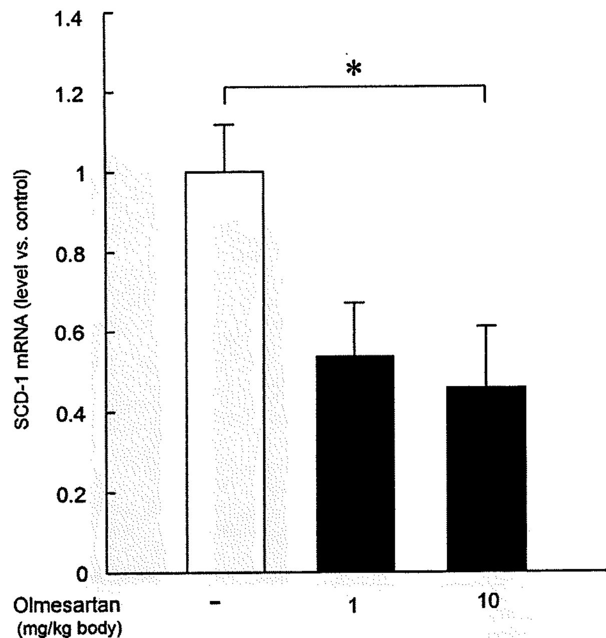

Fig. 4 Effect of olmesartan on SCD-1 mRNA level in liver of obese Zucker rats fed a high-fat diet. Expression levels of SCD-1 in liver were determined by real-time PCR and expressed as a ratio relative to that of GAPDH mRNA as an internal control. Comparisons of SCD-1 mRNA expression in liver samples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

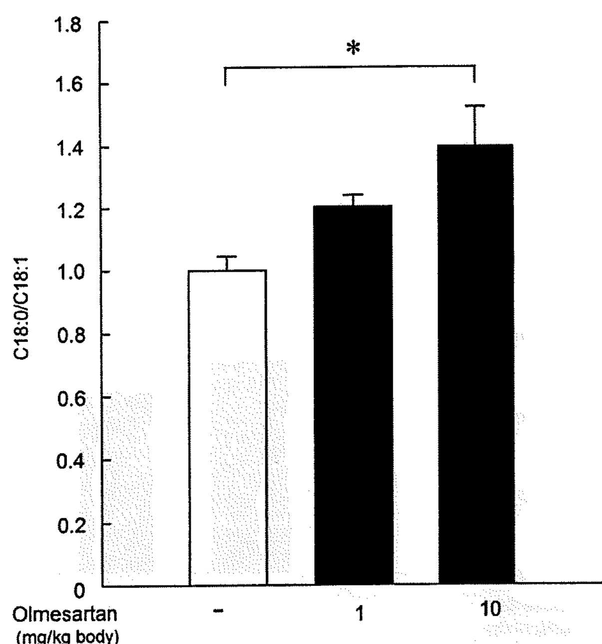

Fig. 5 Effect of olmesartan on fatty acid desaturation in liver of obese Zucker rats fed a high-fat diet. Values are expressed as the ratio of the levels of stearic acid (C18:0, saturated fatty acid) to oleic acid (C18:1, monounsaturated fatty acid) in liver. Comparisons of fatty acid ratio are shown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

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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

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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

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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).

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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)						

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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)						