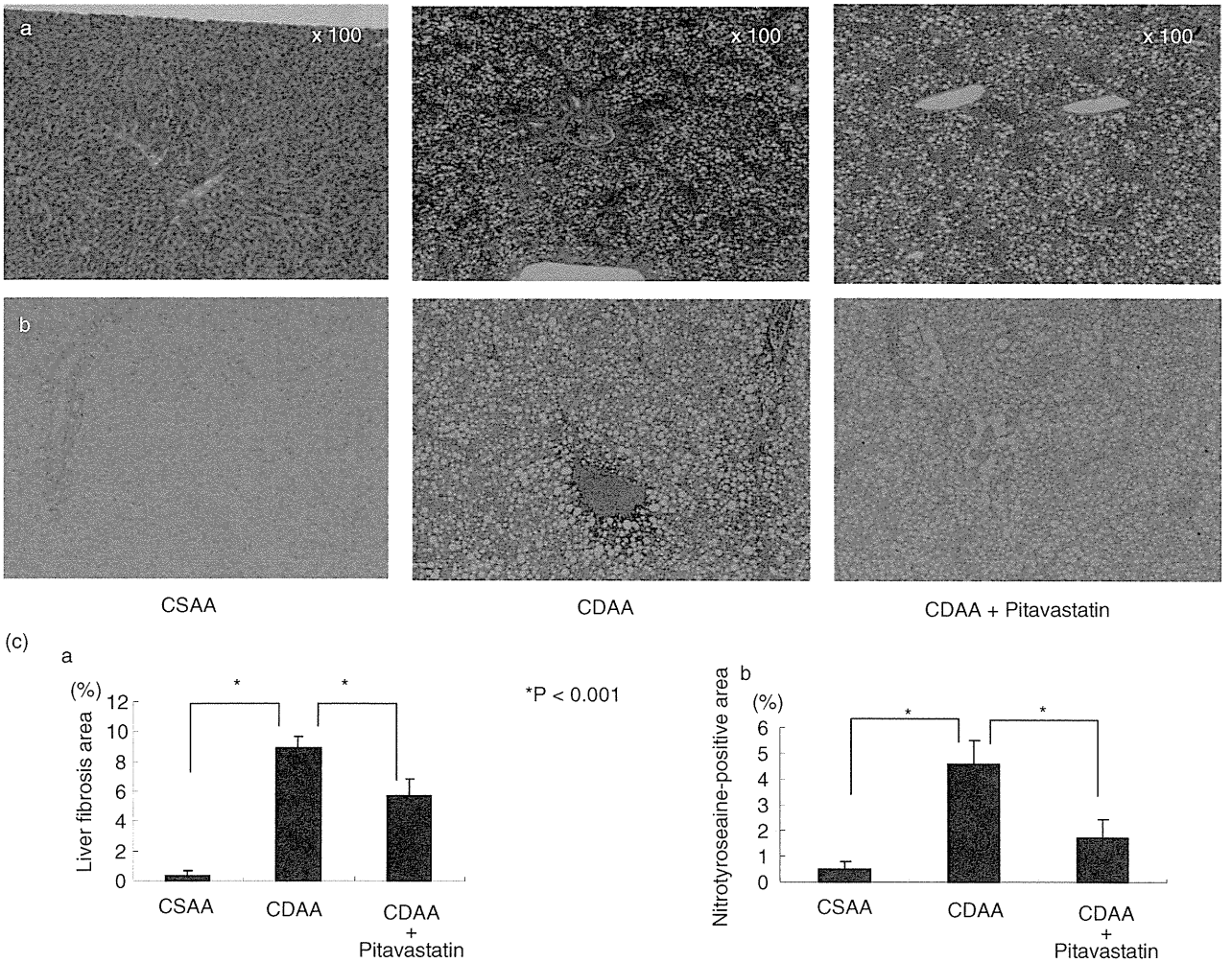

Figure 2 (a) Photomicrographs of liver sections stained with Masson trichrome staining. Choline-supplemented L-amino acid-defined (CSAA)-fed rat, choline-deficient L-amino acid-defined (CDAA)-fed rat and CDAA-fed rat with pitavastatin for 10 weeks. Pitavastatin markedly inhibited liver fibrosis (original magnification ×100). The degree of development of liver fibrosis was quantified using a computerized image analysis system. The liver fibrosis area is shown as a percentage of the microscopic field (ca). Liver histology of CDAA-fed rats showed severe fibrosis. The administration of pitavastatin significantly attenuated the development of fibrosis in CDAA-fed rats. Data are shown as the means ± standard deviations. (b) Photomicrographs of liver sections stained with nitrotyrosine in a CSAA-fed rat, CDAA-fed rat and CDAA-fed rat with pitavastatin for 10 weeks. Pitavastatin markedly inhibited oxidative stress (magnification ×100). The degree of oxidative stress in the liver was quantified using a computerized image analysis system (cb). Nitrotyrosine-positive cells in CDAA-fed rats increased significantly more than in CSAA-fed rats. The administration of pitavastatin significantly attenuated nitrotyrosine-positive cells in CDAA-fed rats. Data are shown as the means ± standard deviations.

in the serum of CDAA-fed rats were significantly higher than in CSAA-fed rats, and pitavastatin significantly attenuated its progression (Fig. 5a). The same effects were observed in the liver tissue. The mRNA expression of TGF-β1 for CDAA-fed rats was higher than that for CSAA-fed rats, and pitavastatin reduced the expression of TGF-β1 (Fig. 5b). As a marker of HSC activation, α-SMA expression was examined in the rat liver. In CDAA-fed rats, α-SMA expression was significantly higher than in CSAA-fed rats, and pitavastatin significantly attenuated its expression (Fig. 5c,d).

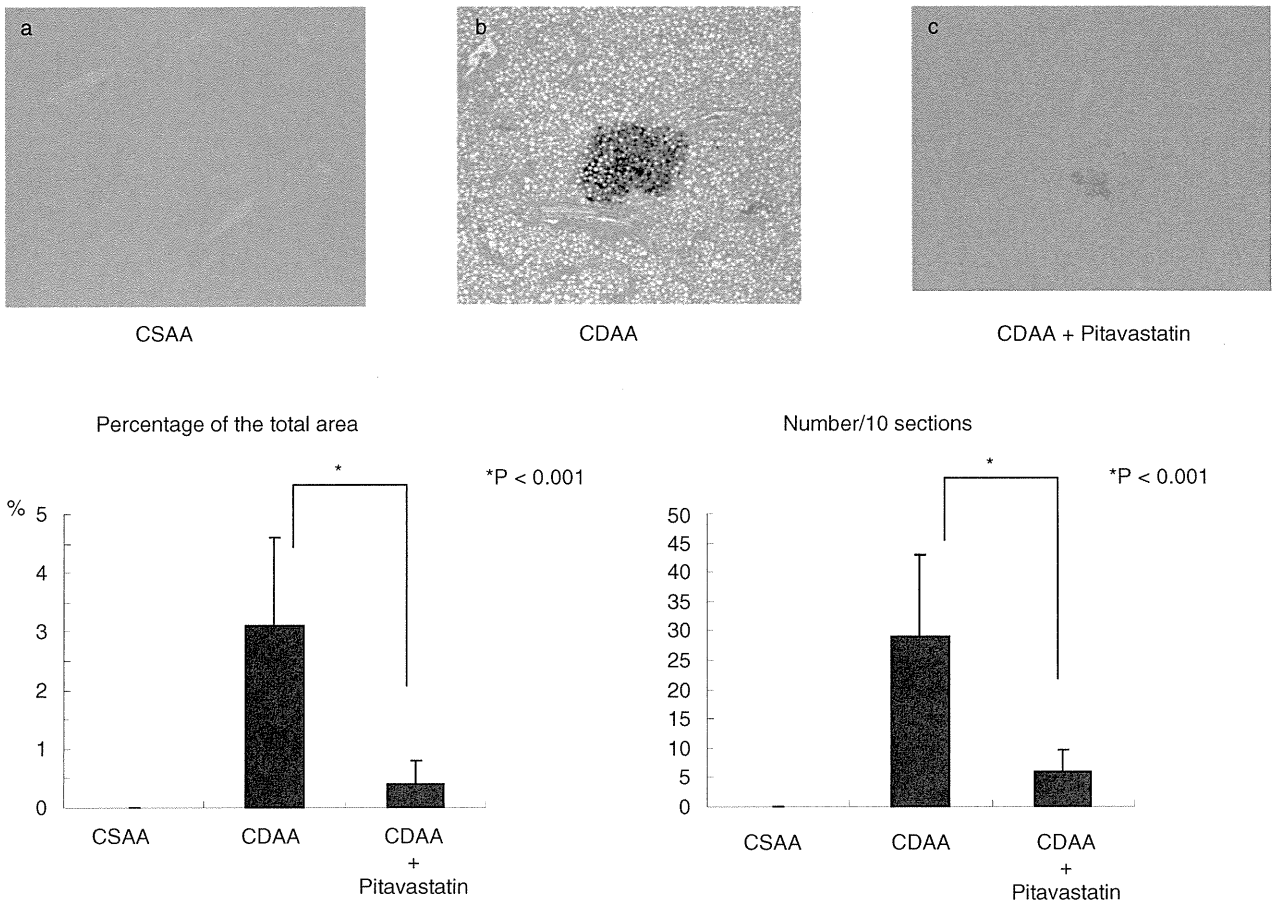

Figure 3 Photomicrographs of liver sections with stained glutathione S-transferase placental form (GST-P)-positive lesions in a choline-supplemented L-amino acid-defined (CSAA)-fed rat (a), choline-deficient L-amino acid-defined (CDAA)-fed rat (b) and CDAA-fed rat with pitavastatin (c) for 10 weeks (magnification ×100). The GST-P-positive percentage of the total area and numbers in 10 sections were measured and quantified using a computerized image analysis system. The GST-P-positive lesions in CDAA-fed rats increased, and the administration of pitavastatin significantly attenuated both the percentage of the total area and number in the 10 sections. Data are shown as the means ± standard deviations.

Effect of pitavastatin on the expression of α-SMA and PPAR-γ in HSC

To confirm the effect of pitavastatin on HSC, we measured the expression of α-SMA and PPAR-γ, an important inducer of TNF-α production, using human HSC lines (LX-2) (Fig. 6a) and isolated HSC from normal rats (Fig. 6b). α-SMA expression was decreased by adding pitavastatin dose-dependently and PPAR-γ expressions increased gradually in a dose-dependent manner. The same experiment was performed using freshly isolated HSC from normal rats. Three days after adding pitavastatin, α-SMA and PPAR-γ protein expressions were examined by western blotting. α-SMA decreased and PPAR-γ increased gradually by adding pitavastatin in a dose-dependent manner.

DISCUSSION

RECENTLY, VARIOUS CLINICAL statin treatments for NAFLD and NASH have been reported.[14–16,25–27] Almost all of these reports, except Nelson *et al.*,[27] describe the reduction of serum aminotransferase or reduction of steatosis of the liver. Nelson *et al.* reported that there was no statistically significant improvement in serum aminotransferase or hepatic steatosis, and the serum low-density lipoprotein of their patients receiving

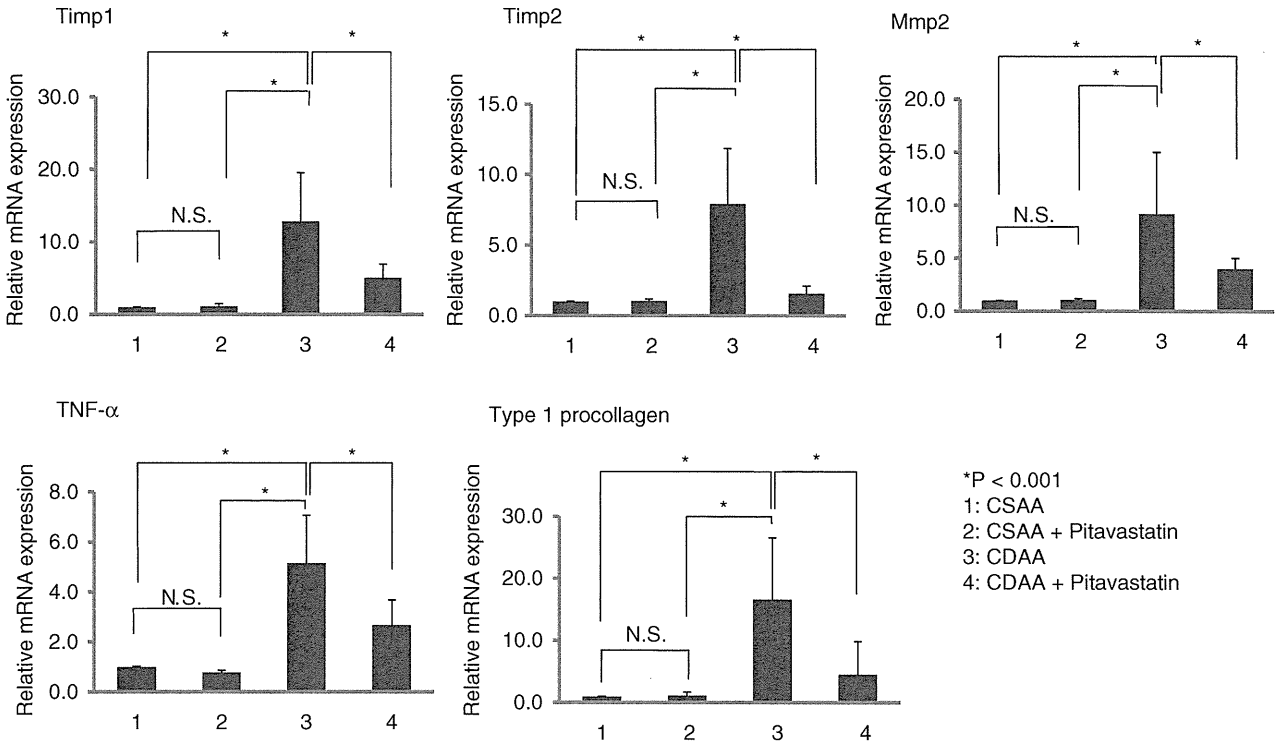

Figure 4 mRNA expressions of tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, matrix metalloproteinase (MMP)-2, tumor necrosis factor (TNF)-α, and type I procollagen. All gene expressions increased in choline-deficient L-amino acid-defined (CDAA)-fed rats, and were significantly attenuated with pitavastatin. Pitavastatin itself had no effect on these expressions in choline-supplemented L-amino acid-defined (CSAA)-fed rats. Data are shown as the means ± standard deviations. N.S., not significant.

simvastatin treatment was not reduced significantly, so one explanation for the different results is the insufficient efficacy of statins. It has been revealed that statins reduce TG in the liver by increasing PPAR-α expression and FA β-oxidation.[12,13] In this study, the serum aminotransferase, steatosis and TG in the liver were significantly reduced by pitavastatin in CDAA-diet rats. These findings are in agreement with the results of numerous clinical and experimental studies. On the other hand, it is important to prevent secondary factors when treating NASH to inhibit liver fibrosis. In clinical trials, the efficacy of statins for liver fibrosis is controversial. Hyogo's data reported that atorvastatin did not reduce liver fibrosis in some cases,[14] whereas Ekstedt *et al.* reported that statins prevented the progression of liver fibrosis despite a high-risk profile and they recommended prescribing statins for patients with elevated liver enzymes because of NAFLD.[26] There have been no studies of the long-term effects of statins, so more clinical research, such as controlled trials, is needed.

Statins have been shown to exhibit pleiotropic effects, including anti-inflammatory, plaque-stabilizing, antithrombotic, antifibrotic and antiproliferative properties,[17,28] and they are also considered to be effective for anti-steatosis and anti-tumorigenesis. Many investigations have reported that statins inhibit fibrosis in various organs[18,29,30] but there are no reports about the efficacy of statins for fibrosis, including liver steatosis and carcinogenesis in NASH, using animal models. Although the CDAA-diet rat does not wholly reflect the human form of this disease, it is a well-established, widely recognized and accepted animal model of NASH.[9,31] At the cellular and molecular levels, liver fibrosis is mainly characterized by cellular activation of HSC and is highly associated with the expression of collagen gene expression, mediators such as TGF-β1, TNF-α, PPAR-γ and oxidative stress.[32–35] In the present experiment, nitrotyrosine was used as an indicator of oxidative stress[22] and after 10-week CDAA diets, the expressions of TGF-β1, TNF-α and oxidative stress were increased, and pitavastatin significantly reduced their expressions and then reduced TIMP-1 and -2 mRNA, which play important roles in the progression of liver fibrosis.[35]

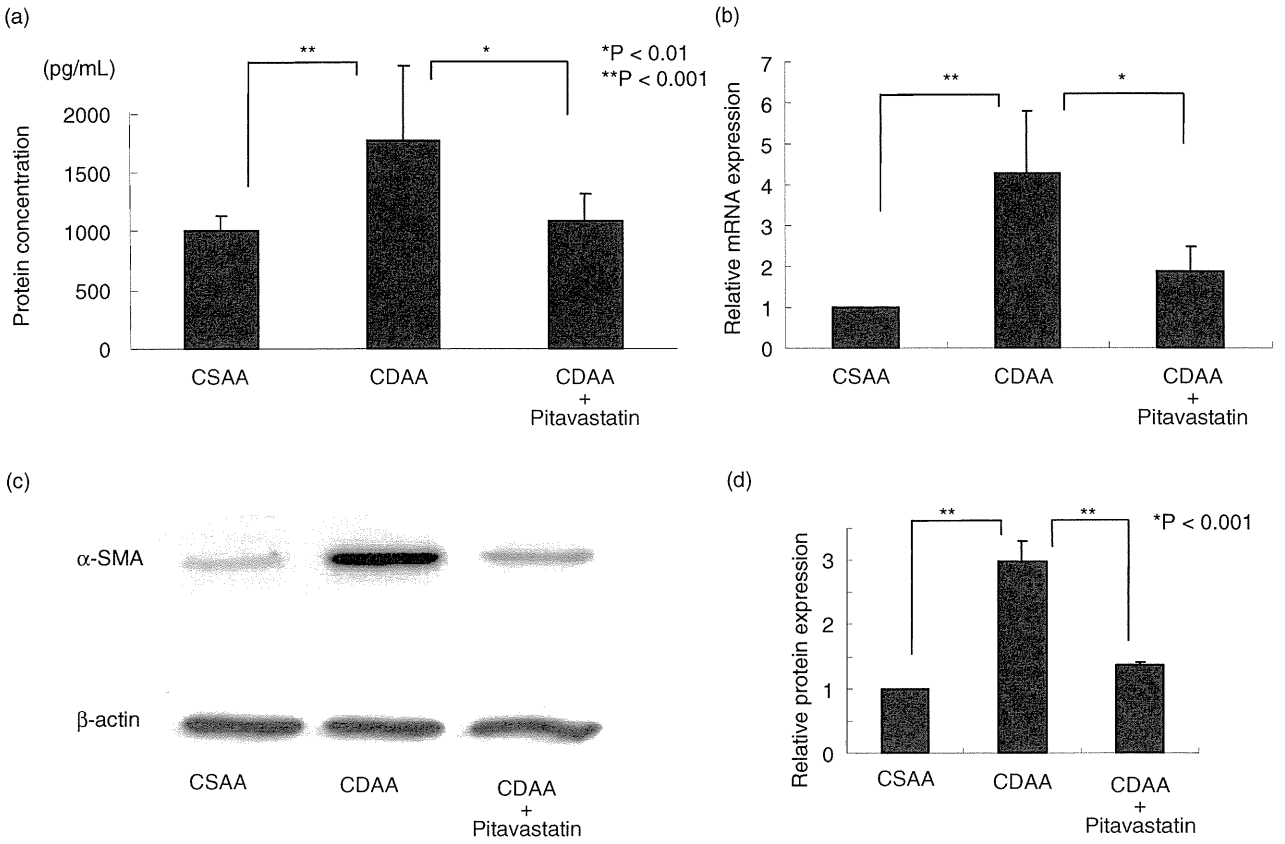

Figure 5 (a) Transforming growth factor (TGF)-β1 protein expression in serum was measured by enzyme-linked immunosorbent assay. TGF-β1 expression increased in choline-deficient L-amino acid-defined (CDAA)-fed rats, and was significantly attenuated by pitavastatin. Data are shown as the means ± standard deviations. (b) TGF-β1 mRNA expression in the liver was increased in CDAA-fed rats and significantly attenuated by pitavastatin. Data are shown as the means ± standard deviations. (c) Western blotting of α-smooth muscle actin (α-SMA) expressions in the liver of rats. (d) Quantitative analysis relative to choline-supplemented L-amino acid-defined (CSAA)-fed rats. α-SMA protein expressions increased in CDAA-fed rats, and pitavastatin administration significantly attenuated them. Data are shown as the means ± standard deviations.

Pitavastatin also inhibited the expression of MMP-2 in this study. Because MMP-2 has been reported to increase when HSC are activated,[36] MMP-2 depression may reveal the inhibition of stellate cell activation by pitavastatin. PPAR-γ is reported as the key molecule of stellate cell activity[37] and several ligands of PPAR-γ prevent the activation of stellate cells and fibrogenesis.[38,39] In this study, the expression of PPAR-γ in HSC decreased and HSC were activated after culture for several days. Pitavastatin increased PPAR-γ and prevented fibrogenesis by inhibiting the activation of HSC, like other PPAR-γ ligands.[9] In our experimental study, pitavastatin significantly improved these liver functions and reduced fibrosis in rat liver.

Because the CDAA-fed rats used in the present study are predisposed to HCC, they were also used for the study of carcinogenesis inhibition. In the present experiment, pitavastatin significantly inhibited the development of GST-P-positive lesions, a precancerous state followed by possibility of inhibiting HCC. Statins reduced the risk of several kinds of cancers[40] and the effects of statins, such as the inhibition of cell proliferation, promotion of apoptosis, and inhibition of angiogenesis and metastasis, have been reported.[41] Our data suggest that statins are capable of inhibiting carcinogenesis in NASH patients.

Thus, the present study confirmed the diverse effects of statins, such as anti-inflammatory, antifibrotic and anticarcinogenic, in a rat model of NASH; therefore, not only currently recognized therapeutic agents, such as vitamin E, thiazolidinedione and ARB, but also statins may be useful preventive agents for NASH.

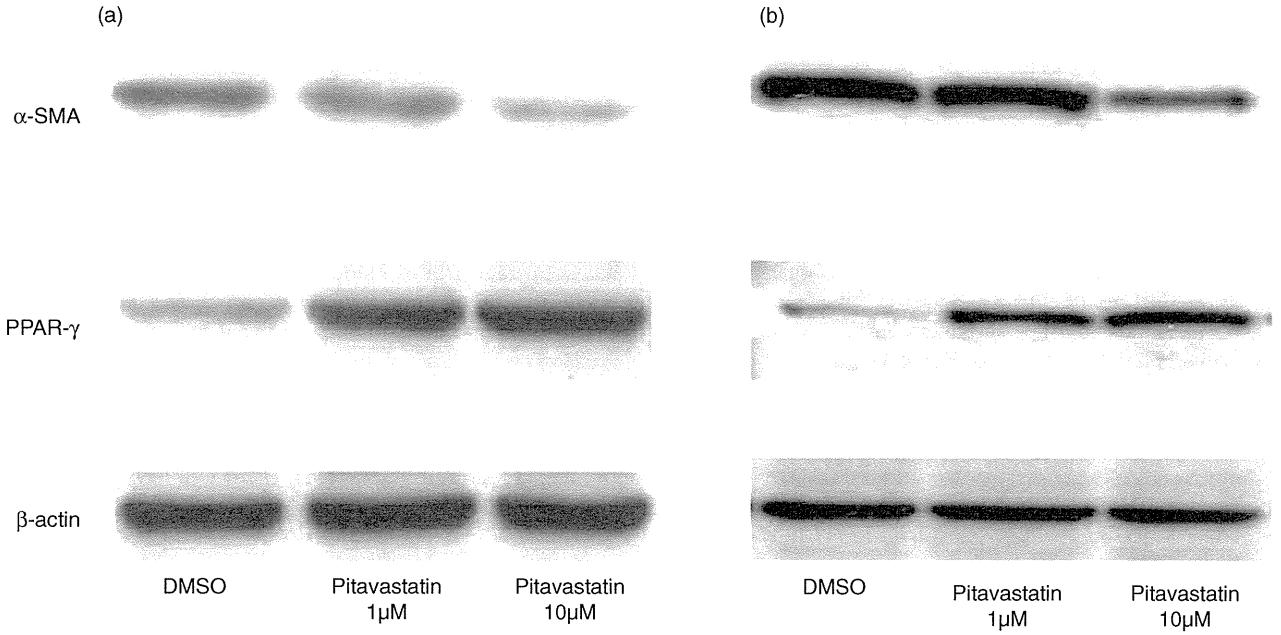

Figure 6 α-Smooth muscle actin (α-SMA) and peroxisome proliferator-activated receptor-γ (PPAR-γ) protein expression on stellate cells. (a) Human hepatic stellate cell (HSC) line LX-2 was incubated with Dulbecco's modified Eagle's medium (DMEM) for 24 h and medium with various concentrations of pitavastatin, and harvested after 24 h. (b) HSC were isolated from the rats and incubated with DMEM for 4 h, and then the culture medium was replaced with medium containing pitavastatin or the same concentration of dimethylsulfoxide (DMSO) as a control, and were harvested after 3 days. In both experiments, α-SMA decreased and PPAR-γ increased gradually after adding pitavastatin in a dose-dependent manner.

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Splenic Elasticity Measured with Real-time Tissue Elastography Is a Marker of Portal Hypertension[1]

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Purpose: To prospectively correlate spleen elasticity and degree of portal hypertension estimated with the hepatic venous pressure gradient (HVPG) and to evaluate splenic elasticity as a predictor of gastroesophageal varices.

Materials and Methods: The institutional review board approved this study, and patients provided written informed consent. In a pilot study of 60 patients with chronic liver damage, the authors measured liver and spleen elasticity with real-time tissue elastography (RTE), obtained serum markers related to fibrosis, examined hepatic and splenic blood flow with duplex Doppler ultrasonography, estimated HVPG, and performed upper gastrointestinal endoscopy. Then, with use of thresholds determined in the pilot study, the authors conducted a validation trial with another 210 patients, performing all studies except the measurement of HPVG. The relationship between HVPG and the other parameters was analyzed. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in the diagnosis of gastroesophageal varices were calculated by using cutoff values obtained from receiver operating characteristic curves.

Results: Among the parameters associated with HVPG, correlation was closest with splenic elasticity ($R = 0.854$, $P < .0001$). When 8.24 was selected as the cutoff of splenic elasticity for predicting HVPG of more than 10 mm Hg, the accuracy of diagnosing gastroesophageal varix was 90% (sensitivity, 96%; specificity, 85%; PPV, 83%; NPV, 97%). The results of the validation trial showed that the 8.24 cutoff for splenic elasticity was associated with a diagnostic accuracy of 94.8% (sensitivity, 98%; specificity, 93.8%; PPV, 82.1%; NPV, 99.4%) for gastroesophageal varices.

Conclusion: Splenic elasticity determined with RTE is the most closely associated parameter for evaluating HVPG and is useful as a clinical marker of portal hypertension and a predictive marker of gastroesophageal varices.

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The architectural distortion of liver cirrhosis leads to increased intrahepatic resistance, which in turn elevates portal venous pressure (1). Elevated portal venous pressure is related not only to the occurrence of an esophageal varix but also to liver failure (2), ascites (3), and bacterial translocation—including spontaneous bacterial peritonitis (4). The accurate identification of portal venous pressure is clinically important for estimating patient status and prognosis.

However, portal venous pressure can only be directly measured by using invasive techniques such as direct portal venography or direct splenic puncture. The hepatic venous pressure gradient (HVPG) is the currently accepted reference standard with which to evaluate portal hypertension (5). However, this is limited in clinical application because it is invasive. Moreover, HVPG cannot provide an accurate estimate of pressure in the portal vein. Thus, a parameter that can substitute for HVPG is desirable (6). Some investigators have indicated that measurement of hepatic elasticity could be a noninvasive method for determining portal venous pressure and may be useful for screening patients for conventional examinations including upper gastrointestinal endoscopy and hemodynamic studies (7). Moreover, the ability to noninvasively measure organ elasticity with ultrasonographic (US) or magnetic resonance (MR) elastography has provided the opportunity to investigate whether splenic elasticity is related to portal pressure (8). Recent preliminary results from a few healthy volunteers and patients with chronic liver disease have shown that splenic stiffness might be a better indicator of portal pressure than of hepatic elasticity (8). Elevated portal venous pressure causes histologic changes in the spleen (9–12). Doppler US (13) and transient elastography (14,15) might help predict the presence of clinically important portal hypertension, but these modalities cannot help accurately predict HVPG. The value of spleen elasticity determined with MR elastography in the prediction of esophageal varices has been reported (8). Spleen elasticity should be closely related to portal venous pressure because histologic changes in the spleen would be directly caused by portal hypertension. US-based real-time tissue elastography (RTE) can also help measure spleen elasticity. Measurements of spleen stiffness are not comparable among transient elastography, MR elastography, and RTE because RTE is a qualitative method of imaging elasticity and MR elastography and tissue elastography are quantitative. RTE, however, is an easier, more economical, and faster approach that does not require a specific dedicated system.

We established that RTE is useful for assessing liver fibrosis in patients with chronic hepatitis C (16). If splenic elasticity closely correlates with HVPG, it could serve as a predictive marker of a gastroesophageal varix before gastrointestinal endoscopy is performed.

We aimed to prospectively correlate spleen elasticity with the degree of portal hypertension estimated with the HVPG and to evaluate splenic elasticity as a predictor of gastroesophageal varices.

Advances in Knowledge

- The correlation between splenic elasticity evaluated with real-time tissue elastography and the degree of portal hypertension estimated with the hepatic venous pressure gradient (HVPG) is high ($R = 0.855$; 95% confidence interval: 0.767, 0.911; $P < .0001$).
- When 8.24 was used as the cutoff of splenic elasticity for predicting HVPG of more than 10 mm Hg, the accuracy of diagnosing gastroesophageal varix was 90%; sensitivity, 96%; specificity, 85%; positive predictive value, 83%; and negative predictive value, 97%.

Implication for Patient Care

- Splenic elasticity can be used as an accurate noninvasive predictor of the severity of portal hypertension and varices; in addition, it may help identify patients who are at risk for gastrointestinal bleeding.

Materials and Methods

Patients

Our institutional ethics committee approved all study protocols, and patients provided written informed consent. We initially enrolled 277 consecutive patients with chronic liver disease who underwent RTE between January 2009 and February 2010. Exclusion criteria were obesity (body mass index >25) that prevented splenic US measurements of elasticity ($n = 6$) and portal tumor thrombus associated with hepatocellular carcinoma that was not confirmed as being chronic ($n = 1$). The final number of participating patients was 270 (Fig 1). In the pilot portion of the study, liver and spleen elasticity were measured in 60 patients between January and May 2009. We also measured HVPG in these patients, and they agreed to undergo upper gastrointestinal

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Abbreviations:
ALT = alanine aminotransferase
APRI = aspartate aminotransferase–to–platelet ratio index
AST = aspartate aminotransferase
AUC = area under the ROC curve
CI = confidence interval
HVPG = hepatic venous pressure gradient
NPV = negative predictive value
PPV = positive predictive value
RHA = right hepatic artery
ROC = receiver operating characteristic
RPV = right portal vein
RTE = real-time tissue elastography

Author contributions:
Guarantors of integrity of entire study, M.H., H.O., Y. Koizumi, Y.H., M.O.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, M.H., H.O., Y. Koizumi, Y. Kisaka, Y.H.; clinical studies, all authors; statistical analysis, M.H., H.O., Y. Koizumi, Y.H.; and manuscript editing, M.H., Y.H., M.O.

Potential conflicts of interest are listed at the end of this article.

Figure 1

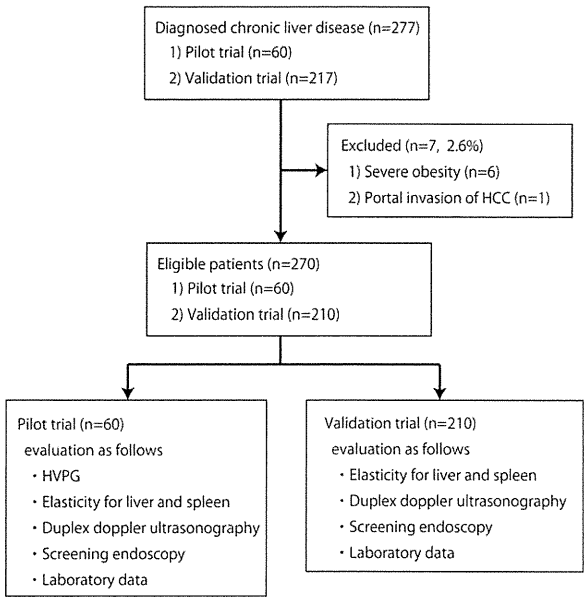

Figure 1: Flow chart of trial participants.

endoscopy. Subsequently, 210 patients participated in the validation trial, in which all studies included in the pilot study except for the measurement of HVPG were performed between June 2009 and February 2010.

Observers

Two hepatologists (M.H. and Y. Koizumi, with 13 and 6 years of experience, respectively) performed Doppler US and RTE. Both of these investigators had performed more than 100 liver and splenic stiffness evaluations before starting this study. Two other hepatologists (Y. Kisaka and H.O., with 10 and 4 years of experience, respectively) performed venography. They had previously measured HVPG in another 50 patients.

Measurements of Liver and Splenic Elasticity

Hepatic and splenic elasticity were measured by using RTE (EUB-7500; Hitachi Medical Systems, Tokyo, Japan) with a linear probe (central frequency, 5.5 MHz; EUP-L52, Hitachi Medical Systems) in B mode to visualize the liver and spleen and then in elastography mode (Fig 2). Regions of interest were simultaneously placed on small intrahepatic veins and hepatic parenchyma, and signals were evaluated. We then calculated the elastic ratio (proportion of small veins to that of hepatic or splenic parenchyma). The area of the regions of interest placed on both the liver and the spleen parenchyma was within 2 × 1 cm (long distance: mean ± standard deviation = 1.90 cm ± 0.12, range = 1.5–2 cm; short distance: mean = 0.90 cm ± 0.11, range = 0.6–1 cm). The size of the region of interest in the small vessels was usually within 0.3 × 0.5 cm (long distance: mean = 0.44 cm ± 0.07, range = 0.3–0.5 cm; short distance: mean = 0.28 cm ± 0.01, range = 0.2–0.3 cm). A higher elastic ratio is indicative of more hepatic and splenic elasticity. The means of five measurements were calculated as described (16).

Measurements from Doppler US

The patients fasted overnight and refrained from smoking cigarettes before undergoing five repeated Doppler US measurements with a 3.5-MHz probe (EUB-7500, Hitachi Medical Systems), as previously described (17). The Doppler sample gate was set at 4–5 mm for the portal vein and 2–3 mm from the arterial side. The angle was always less than 60°. The Doppler sampling cursor was placed in the middle of the portal vein with a width that was about half that of the lumen. Maximal blood velocity in the right portal vein (RPV) and right hepatic artery (RHA) were calculated from fast Fourier transformation values. The RHA/RPV ratio was calculated by dividing the maximal blood velocity of the RHA by that of the RPV (17). Maximal velocity and the diameter of the major portal trunk were also measured to calculate the congestion index of the portal vein by dividing its cross-sectional area by the blood flow velocity within it as described previously (18). Peak systolic velocity and end diastolic velocity were measured, and the splenic resistive index ([peak systolic velocity − end diastolic velocity]/peak systolic velocity) and pulsatility index ([peak systolic velocity − end diastolic velocity]/mean velocity) were determined. The resistive and pulsatility indexes of the splenic artery with the sampling cursor placed near the splenic hilum were measured by using the formulas described earlier.

Measurements of HVPG

The right hepatic vein was catheterized through the right femoral vein, and pressure in both the wedged and free position was measured by using a 5-F balloon-tipped catheter. The HVPG was calculated by subtracting the free hepatic venous pressure from the wedged venous pressure.

Clinical Data Collection

Basic demographic data, including age, sex, and cause of liver disease, were obtained from all patients. In addition, blood cell counts, biochemical data, coagulation profiles, and serologic data were obtained before performing US, upper gastrointestinal endoscopy, and measurement of HVPG. All examinations were performed within 1 week of each other. The functional grade of cirrhosis was determined with the Child-Pugh scoring system. Serum fibrosis markers, as well as the aspartate aminotransferase (AST)-to-alanine aminotransferase (ALT) ratio (19), the AST-to-platelet ratio index (APRI) (20), and the FIB-4

index (21) were calculated. The APRI was calculated by using the following formula: [(AST/reference AST) × 100]/platelet count. FIB-4 is a simple index for diagnosing hepatic fibrosis and is based on four parameters. The FIB-4 index was calculated as follows: [age (in years) × AST (in units per liter)]/[platelet count (in 10^{10}/L)] × [ALT (in units per liter)½]. Volumetric measurements of the spleen were automatically obtained from computed tomographic (CT) scans by using a workstation (Virtual Place Advance; AZE, Tokyo, Japan) (22). From the CT scans, we defined a huge shunt as one in which the diameter of the collateral vessels was greater than 10 mm.

Statistical Analysis

Results are expressed as means ± standard deviations. Data were analyzed by using the Student *t* test for unpaired data and the χ^2 test and Fisher exact test as appropriate. The relationship between HVPG and the other parameters was analyzed with the Pearson product-moment correlation coefficient. Receiver operating characteristic (ROC) curves were constructed, and the area under the ROC curve (AUC) was calculated by using the trapezoidal rule. Optimal cutoff values for liver stiffness were selected to maximize sensitivity, specificity, and diagnostic accuracy. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated by using cutoffs obtained from the ROC curves. The correlation between two variables was calculated by using the Pearson product-moment correlation coefficient. Univariate and multivariate logistic regression was performed with the Wald test. Multivariate logistic regression models included parameters that significantly differed in univariate analysis between an HVPG of less than or more than 10 mm Hg (splenic elasticity, hepatic elasticity, RHA/RPV ratio, platelet count) and an HVPG of less than or more than 12 mm Hg (splenic elasticity, hepatic elasticity, RHA/RPV ratio, platelet count, albumin level, prothrombin activity, FIB-4 index, and APRI). All data were analyzed with software (JMP, version 8; SAS Institute Japan, Tokyo, Japan).

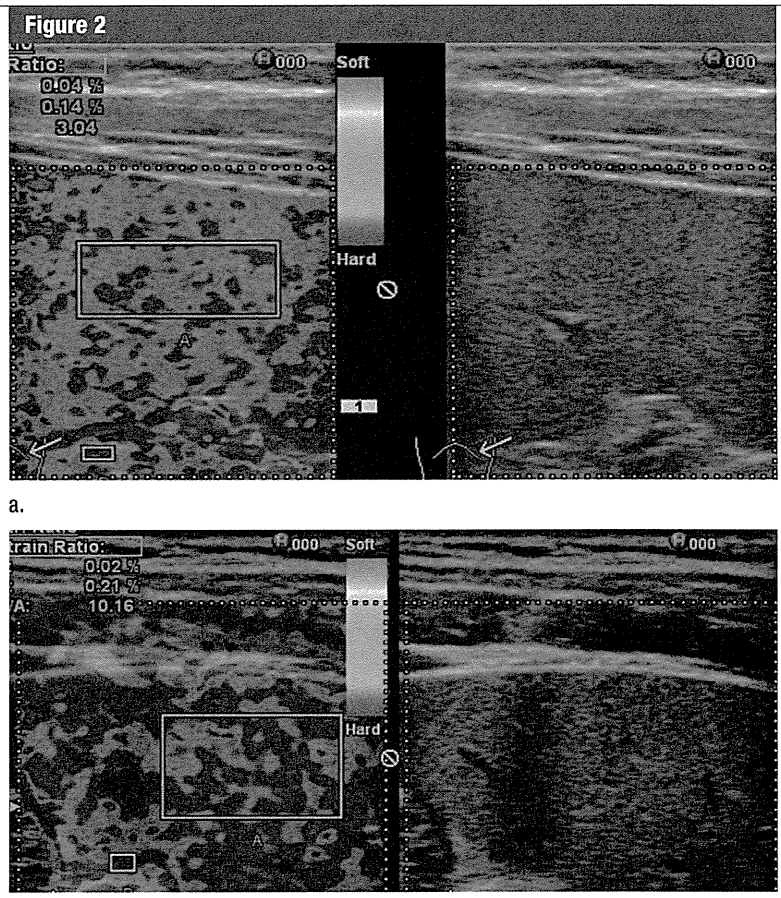

Figure 2: Splenic RTE images from patients with high and low HVPG. Image on right is B-mode image; image on left is color-coded elastography image overlaid on B-mode image. Rectangular boxes are regions of interest selected for splenic parenchyma (large box) and small vessel (small box). **(a)** Images in a 65-year-old man with hepatitis C–related liver cirrhosis (Child-Pugh class A) and without gastroesophageal varices. HVPG was low (6.76 mm Hg). Splenic and hepatic elastic ratios were 3.12 and 3.21, respectively. **(b)** Images in a 48-year-old man with hepatitis B–related liver cirrhosis (Child-Pugh class B) and gastroesophageal varices. HVPG was high (24.12 mm Hg). Splenic and hepatic elastic ratios were 10.10 and 3.41, respectively.

Results

Patient Characteristics

The characteristics of the patients in the training and validation sets did not significantly differ except for rates of varices, which were present in 26 of the 60 patients (43%) in the training set and 47 of the 210 (22%) in the validation set ($P = .0049$) (Table 1).

Parameters Associated with HVPG

Hepatic elasticity, splenic elasticity, RHA/RPV ratio, platelet count, FIB-4 index, and congestion index showed a significant correlation with HVPG (hepatic elasticity: $r = 0.510$, 95% confidence interval [CI] = 0.294, 0.676; splenic elasticity: $r = 0.854$, 95% CI = 0.767, 0.911; RHA/RPV ratio: $r = 0.401$, 95% CI = 0.164, 0.594; platelet count: $r = 0.446$, 95% CI = 0.217, 0.629; APRI: $r = 0.255$, 95% CI = 0.001, 0.478; FIB-4 index: $r = 0.335$, 95% CI = 0.089, 0.543; congestion index: $r = 0.594$,

Table 1

Patient Characteristics

Characteristic	Training Set	Validation Set	*P* Value
Age (y)*	68.0 ± 8.9 (35–89)	62.1 ± 12.7 (22–89)	.092
Men	67.4 ± 7.9 (54–89)	62.2 ± 12.6 (25–89)	.475†
Women	69.1 ± 10.5 (35–80)	62.0 ± 12.8 (22–84)	.920‡
M:F ratio	39:21	113:97	.138
Cause of liver damage§			
HBV	11	34	.079
HCV	43	131	
Alcohol	4	16	
NAFLD	1	17	
PBC	1	12	
Noncirrhosis	12		
Cirrhosis	48		
Child-Pugh class			
A (without cirrhosis)	46	161	.087
B	8	28	
C	6	21	
Esophageal varices			
Present	26	47	.0049
Absent	34	143	

Note—Except where indicated, data are numbers of patients.

* Data are means ± standard deviations, with ranges in parentheses.

† *P* value between men and women in the training set.

‡ *P* value between men and women in the validation set.

§ HBV = hepatitis B virus, HCV = hepatitis C virus, NAFLD = nonalcoholic fatty liver disease, PBC = primary biliary cirrhosis.

Table 2

Parameters Associated with HVPG in the Training Set

Parameter*	Mean ± SD†	*r* Value	95% CI
HVPG (mm Hg)	9.5 ± 6.1	NA‡	...
Hepatic elasticity	3.8 ± 0.77	0.510	0.294, 0.676
Splenic elasticity	7.6 ± 3.2	0.854	0.767, 0.911
RHA/RPV ratio	3.3 ± 1.0	0.401	0.164, 0.594
Spleen volume (cm^3)	299.5 ± 183.9	0.232	−0.023, 0.459
Platelet count ($\times 10^{10}$/L)	11.1 ± 6.4	0.446	0.217, 0.629
HOMA-IR	4.6 ± 3.0	0.097	−0.160, 0.343
AST/ALT ratio	1.5 ± 0.51	0.128	−0.13, 0.370
APRI	0.75 ± 0.95	0.255	0.001, 0.478
FIB-4 index	7.6 ± 7.3	0.335	0.089, 0.543
Congestion index	0.080 ± 0.061	0.594	0.401, 0.737
Splenic artery velocity (cm/sec)	55.9 ± 15.0	0.047	−0.209, 0.297
Splenic artery PI	1.48 ± 0.38	0.062	−0.195, 0.311
Splenic artery RI	0.68 ± 0.20	0.216	−0.040, 0.446

* HOMA-IR = homeostasis model assessment of insulin resistance, PI = pulsatility index, RI = resistive index.

† SD = standard deviation.

‡ NA = not applicable.

95% CI = 0.401, 0.737) (Table 2). None of the other measured parameters (ie, spleen volume, homeostasis model assessment of insulin resistance, AST/ALT ratio, peak velocity of the splenic artery, pulsatility index of the splenic artery, or resistive index of the splenic artery) showed significant correlation with HVPG. Although both splenic and hepatic elasticity were linearly correlated with HVPG, the *r* value was higher for splenic than for hepatic elasticity (Fig 3, Table 2). Only four patients in our cohort had vascular abnormalities of at least 10 mm nourished by collateral vessels and shunts, and these were located in the lower part of the correlation line (Fig 3a).

Diagnostic Accuracy for Predicting Elevated HVPG and the Presence of Gastroesophageal Varices

HVPG was elevated by more than 10 and 12 mm Hg in 28 and 19 patients, respectively. Hepatic elasticity (AUC, 0.832; 95% CI: 0.714, 0.917; $P = .0042$), splenic elasticity (AUC, 0.978; 95% CI: 0.902, 0.999; $P < .0001$), RHA/RPV ratio (AUC, 0.748; 95% CI: 0.619, 0.851; $P < .0001$), platelet count (AUC, 0.809; 95% CI: 0.688, 0.900; $P = .0040$), and congestion index (AUC, 0.740; 95% CI: 0.713, 0.916; $P = .0019$) were significant factors for predicting HVPG elevation of more than 10 mm Hg (Table 3). Among these parameters, splenic elasticity was the most accurate predictive factor for HVPG elevation (>10 mm Hg). Significantly predictive parameters in the univariate analysis (splenic elasticity, hepatic elasticity, RHA/RPV ratio, and platelet count) were entered into a multivariate logistic regression model to predict elevation of HVPG of more than 10 mm Hg (Table 4). Only splenic elasticity was selected as a significant factor (odds ratio, 9.070; 95% CI: 2.277, 134.290; $P = .020$).

The results for predicting an HVPG elevation of more than 12 mm Hg were similar. When analyzed with use of ROC curves, hepatic elasticity (AUC, 0.781; 95% CI: 0.656, 0.878; $P = .0004$), splenic elasticity (AUC, 0.948; 95% CI: 0.859, 0.989; $P < .0001$), platelet count (AUC, 0.798; 95% CI: 0.675, 0.891; $P = .0078$), and congestion index (AUC, 0.806; 95% CI: 0.684, 0.897; $P = .0001$) were also significantly better for predicting HVPG elevation of more than 12 mm Hg (Table 3). In the multivariate analysis of the parameters that

significantly differed in the univariate analysis between HVPG of more than or less than 12 mm Hg, splenic elasticity was the only significantly predictive parameter (odds ratio, 17.708; 95% CI: 2.591, 765.094; $P = .040$) (Table 5).

The diagnostic accuracy of cutoff values for splenic elasticity in predicting the presence of gastroesophageal varices was 90% (sensitivity, 96%; specificity, 85%; PPV, 83%; NPV, 97%) (Table 6) with a cutoff value of 8.24 for HVPG of more than 10 mm Hg selected from the ROC curves in the training set and 78% (sensitivity, 54%; specificity, 97%; PPV, 93%; NPV, 73%) (Table 6) with a cutoff value of 9.99 selected as the splenic elasticity required to predict an elevated HVPG of more than 12 mm Hg. We could not measure HVPG in all patients in the validation set. Thus, the diagnostic accuracy of gastroesophageal varices was analyzed by using the corresponding splenic elasticity for HVPG from the ROC curves (HVPG of 10 and 12 mm Hg corresponded to cutoff values for splenic elasticity of 8.24 and 9.99, respectively). The diagnostic accuracy of the same cutoff values in patients with HVPG of more than 10 and 12 mm Hg in the validation set was 94.8% (sensitivity, 98%; specificity, 93.8%; PPV, 82%; NPV, 99.4%) and 82.9% (sensitivity, 26%; specificity, 99.4%, PPV, 92%; NPV, 82.2%), respectively (Table 6). When splenic elasticity was set at the cutoff (8.24 for HVPG of 10 mm Hg; 9.99 for HVPG of 12 mm Hg), sensitivity, specificity, PPV, NPV, and diagnostic accuracy were high in both the training and validation sets. The validation set indicated that a cutoff value of 9.99 for splenic elasticity would be highly accurate in the prediction of a gastroesophageal varix.

Figure 3

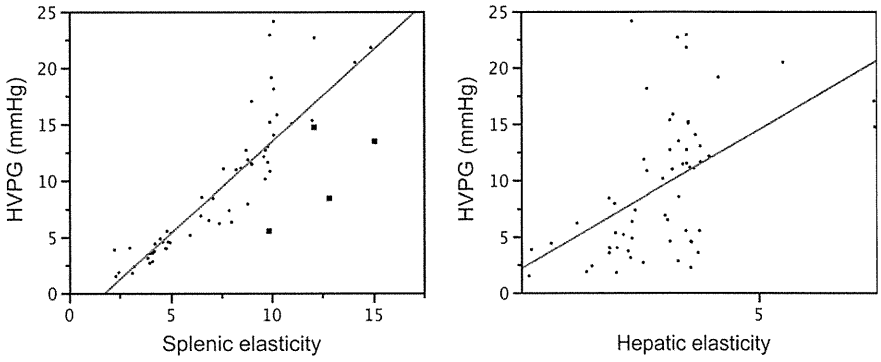

Figure 3: **(a)** Scatterplot shows correlation between splenic elastic ratio and HVPG among patients in training set. Splenic elasticity and HVPG show significant linear correlation ($R = 0.85$, $P < .0001$). Square data points indicate patients with vascular abnormalities of at least 10 mm nourished by collateral vessels and shunts. **(b)** Scatterplot shows correlation between hepatic elasticity and HVPG ($R = 0.51$, $P < .0001$).

Table 3

Comparison of AUCs for Predicting Elevated HVPG and the Presence of Gastroesophageal Varices in the Training Set

Parameter*	HVPG >10 mm Hg	HVPG >12 mm Hg	Varices
Hepatic elasticity	0.832 (0.714, 0.917)	0.781 (0.656, 0.878)	0.833 (0.714, 0.917)
Splenic elasticity	0.978 (0.902, 0.999)	0.948 (0.859, 0.989)	0.908 (0.873, 0.993)
RHA/RPV ratio	0.748 (0.619, 0.851)	0.743 (0.614, 0.847)	0.756 (0.628, 0.858)
Spleen volume	0.739 (0.609, 0.844)	0.681 (0.550, 0.797)	0.747 (0.619, 0.851)
Platelet count	0.809 (0.688, 0.900)	0.798 (0.675, 0.891)	0.811 (0.691, 0.801)
HOMA-IR	0.617 (0.568, 0.811)	0.613 (0.478, 0.736)	0.731 (0.601, 0.838)
AST/ALT ratio	0.573 (0.439, 0.700)	0.765 (0.498, 0.753)	0.545 (0.412, 0.674)
APRI	0.705 (0.621, 0.853)	0.746 (0.638, 0.865)	0.748 (0.620, 0.851)
FIB-4 index	0.778 (0.652, 0.875)	0.822 (0.702, 0.909)	0.766 (0.640, 0.866)
Congestion index	0.740 (0.713, 0.916)	0.806 (0.684, 0.897)	0.852 (0.738, 0.931)
Splenic artery velocity	0.474 (0.343, 0.607)	0.524 (0.390, 0.653)	0.477 (0.390, 0.653)
Splenic artery PI	0.575 (0.441, 0.703)	0.497 (0.370, 0.634)	0.611 (0.478, 0.736)
Splenic artery RI	0.629 (0.495, 0.751)	0.611 (0.476, 0.734)	0.635 (0.501, 0.756)

Note.—Data are AUCs. Numbers in parentheses are 95% CIs.

* HOMA-IR = homeostasis model assessment of insulin resistance, PI = pulsatility index, RI = resistive index.

Discussion

Splenic elasticity has a close linear relationship with HVPG and can help predict the presence of esophageal varices in patients with chronic liver disease.

There are some possible explanations for why splenic elasticity is more closely associated with HVPG. Portal hypertension would initially and directly induce hypersplenism, which would increase spleen elasticity and be reflected as histologic changes in the spleen. Splenic elasticity is the result of portal hypertension, which develops as a result of hepatic elasticity that increases not only because of hepatic fibrosis but also as a result of structural and biologic changes that are related to portal hypertension (23,24). These include major angioarchitectural modifications involving neoangiogenesis and the presence of cell types undergoing active contraction in response to the intrahepatic predominance of vasoconstrictor stimuli (25,26). Portal hypertension will evoke hyperplasia among splenic histiocytes (9), arterial terminal lengthening (10), increased white pulp volume (11), and even fibrosis between splenic trabeculae (12). These changes result in portal hypertension and, thus, increased splenic elasticity.

Another reason why the spleen is more elastic than the liver could be the anatomic shape of these organs. Hepatic

Table 4

Univariate and Multivariate Analysis of Clinical Parameters for Predicting HVPG Greater Than 10 mm Hg in the Training Set

	Univariate Analysis		Multivariate Analysis	
Parameter*	*P* Value	Odds Ratio	*P* Value	Odds Ratio
Sex	.515	0.836		
Age	.391	0.975		
Hepatic elasticity	.0001	5.901	.188	8.143
RHA/RPV ratio	.011	2.323	.869	0.901
Splenic elasticity	<.0001	6.869	.020	9.070
Spleen volume	.009	1.004	.973	1.000
AST level	.966	1.003		
ALT level	.987	1.000		
Platelet count	.005	0.738	.872	0.973
γ-GTP level	.621	1.001		
Albumin level	.003	0.252	.640	0.23
Prothrombin time	.006	0.933	.561	0.92
HOMA-IR	.126	1.143		
AST/ALT	.552	1.354		
APRI	.089	3.114		
FIB-4 index	.106	1.188		
Child-Pugh class	.178	0.987		
PI of splenic artery	.678	1.349		

* GTP = guanosine triphosphate, HOMA-IR = homeostasis model assessment of insulin resistance, PI = pulsatility index.

Table 5

Univariate and Multivariate Analysis of Clinical Parameters for Predicting HVPG Greater Than 12 mm Hg in the Training Set

	Univariate Analysis		Multivariate Analysis	
Parameter*	*P* Value	Odds Ratio	*P* Value	Odds Ratio
Sex	.529	0.831		
Age	.576	0.982		
Hepatic elasticity	.007	5.929	.354	6.171
RHA/RPV ratio	.045	1.934	.938	0.946
Splenic elasticity	.007	12.935	.040	17.708
Spleen volume	.154	1.002		
AST level	.302	1.007		
ALT level	.889	0.998		
Platelet count	.019	0.734		
γ-GTP level	.323	0.995		
Albumin level	.001	0.116		
Prothrombin time	.002	0.923		
HOMA-IR	.926	1.008		
AST/ALT	.239	1.935		
APRI	.023	4.284	.117	3.525
FIB-4 index	.009	1.221	.159	0.627
Child-Pugh class	.001	6.554	.594	1.768
PI of splenic artery	.823	0.852		

* GTP = guanosine triphosphate, HOMA-IR = homeostasis model assessment of insulin resistance, PI = pulsatility index.

elasticity is usually measured from the right lobe. Merriman et al (27) found that histologic findings frequently differed between the left and right lobes of patients with nonalcoholic fatty liver disease. This variability would induce a more distant relationship between hepatic elasticity and HVPG. Conversely, the spleen is more homogeneous than the liver and is less anatomically divided.

To our knowledge, this is the first study to measure splenic elasticity with US-based RTE. Splenic elasticity could also be noninvasively measured with MR elastography or transient elastography (FibroScan; Echosens, Paris, France). Measurements of spleen stiffness are not comparable among quantitative transient elastography, MR elastography, and qualitative RTE. However, RTE is easier, more economical, and faster and does not require a dedicated system. A trial of MR elastography (8) has revealed a close correlation between splenic and hepatic elasticity. However, the correlation between splenic elasticity and portal venous pressure measured with HVPG has not, to our knowledge, been investigated. Specific facilities are required for MR elastography, which takes more time. Thus, RTE is more practical than MR elastography in the identification of splenic elasticity.

From the practical aspects of obtaining US information about the spleen, we believe that measuring splenic elasticity with transient elastography might be very difficult because images are not visible during measurements. Thus, the images obtained with transient elastography are not seen in real time. Further studies should compare the accuracy of splenic elasticity measured with RTE to that measured with other modalities. Because we previously found that the elasticity of small vessels is suitable as a reference, we used that of the small hepatic and splenic veins as reference values in the present study (16). Moreover, such elasticity does not change over time and it does not undergo transformation with disease (eg, arteriosclerosis) (16).

Hepatic and splenic blood flow should reflect portal hypertension, and both have been assessed by using duplex

Table 6

Sensitivity, Specificity, PPV, NPV, and Diagnostic Accuracy of Cutoff Values for Splenic Elasticity in Predicting the Presence of Gastroesophageal Varices

Patient Group and Cutoff Value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic Accuracy (%)
Training set (n = 60)					
8.24	96 (25/26)	85 (29/34)	83 (25/30)	97 (29/30)	90 (54/60)
9.99	54 (14/26)	97 (33/34)	93 (14/15)	73 (33/45)	78 (47/60)
Validation set (n = 210)					
8.24	98 (46/47)	93.8 (153/163)	82 (46/56)	99.4 (153/154)	94.8 (199/210)
9.99	26 (12/47)	99.4 (162/163)	92 (12/13)	82.2 (162/197)	82.9 (174/210)

Note.—Numbers in parentheses are numbers of patients.

Doppler US (RHA/RPV ratio) (17), the congestion index (18), splenic artery velocity (28,29), and the pulsatility and resistive indexes of the splenic artery (28,29). We also measured these US factors, but they did not associate with HVPG as much as splenic elasticity determined with RTE. We postulated that duplex Doppler US would be unreliable because a shunt could decrease the resistance of splenic venous blood outflow. In fact, a large portosystemic shunt reduces portal venous pressure by changing blood flow (30). Conversely, serum markers (AST/ALT ratio [19], APRI [20], FIB-4 index [21], homeostatis model assessment of insulin resistance) of hepatic fibrosis and splenic volume (31) have all been described as predictors of portal hypertension. However, our results contradicted these findings.

Splenomegaly is caused by congestion due to portal hypertension (32). Hepatofugal and splanchnic venous blood is shunted via portosystemic collateral vessels to the systemic circulation during severe portal hypertension to avoid high pressure in the portal vein. We measured the volume of the spleen with CT, whereas Berzigotti et al (31) measured spleen volume with two-dimensional US. Although splenic volume was shown to correlate with an HVPG of more than 10 mm Hg at univariate analysis, we could not identify a correlation between HVPG and spleen size at multivariate analysis. This could be due to the relationship between spleen volume and splenic elasticity; however, there was no correlation between those parameters (r = 0.229).

We validated that splenic elasticity measured with RTE could be a good predictor of gastroesophageal varix. The 2005 Baveno Consensus Workshop (33) and the American Association for the Study of Liver Diseases 2007 Single Topic Symposium on Portal Hypertension (34) have both recommended endoscopic screening for esophageal varices, regardless of Child-Pugh class and cause. Patients with cirrhosis and gastroesophageal varices have an HVPG of at least 10–12 mm Hg. Moreover, variceal hemorrhage does not occur when the HVPG is reduced to 12 mm Hg. Thus, we investigated 10 and 12 mm Hg as HVPG cutoffs. Our results indicated that we could estimate the presence of gastroesophageal varices from splenic elasticity. This means that we could reduce the frequency of screening-detected gastroesophageal varices by using gastrointestinal endoscopy. Otherwise, high splenic elasticity would alert operators to the need for gastrointestinal endoscopy. Splenic elasticity could be a useful clinical marker with which to estimate the prognosis of such patients. This requires evaluation in a long-term follow-up study of patients with chronic liver damage.

This study has several limitations. We did not estimate inter- and intraobserver variability when measuring splenic elasticity. Moreover, we did not directly compare splenic and hepatic elasticity. We excluded extremely obese patients because we previously showed that elasticity measured with RTE is accurate up to a body mass index of 25 (moderately obese) (16). Moreover, splenic elasticity measured with RTE would be influenced by vascular abnormalities such as collateral vessels and shunts. Further study is needed to resolve these limitations, and MR elastography might be considered for extremely obese patients.

In conclusion, splenic elasticity measured with noninvasive RTE showed close correlation with HVPG. Splenic elasticity is a useful predictive marker of gastroesophageal varix and has the potential to serve as a marker with which to estimate the prognosis of patients with chronic liver diseases.

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Original Article

Aerobic exercise improves insulin resistance and decreases body fat and serum levels of leptin in patients with hepatitis C virus

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Aim: Hepatitis C virus infection often complicates glucose intolerance, which can be caused by insulin resistance. Aerobic exercise can improve insulin resistance and decrease body fat in patients with diabetes. The aim of the present study is to clarify whether aerobic exercise improves insulin resistance and decreases body fat in patients with chronic hepatitis C (CH-C).

Methods: Seventeen patients with CH-C received nutrition education at entry and every two months thereafter. The following were evaluated before and after 6 months of walking at least 8000 steps/day monitored using a pedometer that started 2 months after entry: body composition, fat and muscle weight, visceral and subcutaneous fat areas (VFA and SFA, respectively), liver function tests, the Homeostatic Model of Assessment of Insulin Resistance (HOMA-IR), serum tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, adiponectin, leptin and the Short Form-36.

Results: Fifteen of the 17 patients completed the study protocol. Bodyweight, body mass index, fat weight, VFA, SFA, alanine aminotransferase level and HOMA-IR were significantly decreased at the end of the study ($P = 0.004$, =0.004, =0.008, =0.041, =0.001, =0.023 and =0.002, respectively). Serum levels of TNF-α, IL-6 and adiponectin did not change, whereas those of leptin significantly decreased ($P = 0.002$).

Conclusion: Patients with CH-C could safely walk as aerobic exercise. Furthermore, walking improved insulin resistance and decreased body fat while lowering serum levels of leptin.

Key words: adiponectin, hepatocellular carcinoma, tumor necrosis factor-α, visceral fat, walking

INTRODUCTION

THE PREVALENCE OF worldwide chronic hepatitis C virus (HCV) infection is high, and this condition progresses to cirrhosis and hepatocellular carcinoma (HCC) over a period of 20–30 years.[1–3] Diabetes mellitus (DM) often accompanies HCV infection, especially in patients with liver cirrhosis.[4,5] Insulin resistance is reportedly induced by HCV core antigen and other cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6[6]. Clearance of HCV by pegylated interferon (PEG IFN) plus ribavirin (RBV), which is a standard therapy against HCV, improves insulin resistance.[7] This unfavorable factor for patients with chronic hepatitis C (CH-C) disrupts antiviral effects and causes progression to liver fibrosis.[8,9] Visceral obesity is also an unfavorable factor for patients with CH-C that causes insulin resistance, and it is often accompanied by liver steatosis.[10] Various therapies have targeted improving insulin resistance such as pioglitazone, an anti-diabetic agent,[11] and branched-chain amino acids (BCAA).[12,13]

Leptin and adiponectin are adipocytokines that are expressed and secreted by adipocytes.[14,15] Adiponectin has a negative relationship with insulin resistance in patients with HCV, and levels are decreased in obese patients.[16,17] In contrast, leptin has a positive relationship with insulin resistance and body mass index (BMI).[17,18] Aerobic exercise can reduce insulin resistance and serum leptin in obese adults without HCV infection.[19]

Aerobic exercise improves insulin sensitivity and reduces bodyweight in patients with type 2 diabetes.[20]

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Moreover, aerobic exercise improves the health-related quality of life (HRQOL) of patients with various diseases.[21,22] Walking is easy to start and it is free of charge, and patients with compensated liver cirrhosis are not restricted in terms of physical exercise.[23] Thus, we investigated whether walking combined with diet therapy improves insulin resistance and decreases body fat in patients with CH-C and examined the mechanisms of improved insulin resistance by measuring levels of pro-inflammatory cytokines and adipocytokines.

METHODS

Participants

WE PROSPECTIVELY SELECTED 17 patients with CH-C at Ehime University Hospital between May 2007 and April 2009 for enrollment in this study based on the following criteria: (i) positive for anti-HCV and positive for HCV RNA according to qualitative polymerase chain reaction (PCR); (ii) not under antiviral therapy at entry; (iii) positive for chronic hepatitis or compensated cirrhosis without HCC; (iv) not taking BCAA; (v) negative for hepatitis B surface (HBs) antigen; (vi) free of co-infection with HIV; (vii) not pregnant; (viii) unable to perform prescribed exercise; (ix) systolic blood pressure of less than 180 mmHg or diastolic blood pressure of less than 110 mmHg; (x) ischemic heart disease or severe arrhythmia; (xi) free of other types of hepatitis, including hemochromatosis, primary biliary cirrhosis, Wilson's, alcoholic and autoimmune liver diseases; (xii) fasting plasma glucose of less than 250 mg/dL, or negative for urine ketone bodies; and (xiii) willing to provide written informed consent to participate in a program that incorporated diet and walking. Three of 17 patients were diagnosed with diabetes mellitus. None of them was administrated antidiabetic agents. The other 14 patients did not have glucose intolerance according to fasting plasma glucose and hemoglobin A1c levels.

The Ethics Committee of Ehime University Hospital approved the study (approval ID #0704005), which proceeded according to the 1975 Declaration of Helsinki. Written informed consent to participate in this study was obtained from all of the patients before entry.

Laboratory investigations

A blood sample was taken after overnight (12-h) fasting for routine evaluations of total protein, serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), fasting plasma glucose (FPG), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides. Insulin resistance was evaluated using the Homeostatic Model of Assessment of Insulin Resistance (HOMA-IR), which was calculated as described.[24] The presence of HBs antigen and anti-HCV antibody was determined using enzyme immunoassay kits (Abbott Japan, Chiba, Japan). The HCV RNA titers were determined using the Cobas-HIGH-RANGE assay (Roche Molecular Diagnostics, Tokyo, Japan) and are expressed as KIU/mL. The HCV genotype was determined using a PCR-based method (HCV Core Genotype; SRL, Tokyo, Japan). Patients with FPG of 140 mg/dL or more were excluded from the HOMA-IR. Finally, data shown in Tables 1 and 2 were obtained from 15 and 13 patients, respectively.

Levels of TNF-α, IL-6, total adiponectin and leptin were determined in thawed serum samples after storage at −20°C, using Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA). The detection limits of the assay were 0.106 pg/mL for TNF-α, 0.70 pg/mL for IL-6, 0.246 μg/mL for adiponectin and 7.8 pg/mL for leptin.

Procedures

Bodyweight, height and fat distribution were measured and blood was sampled at the start of the program. BMI was calculated as weight (kg) divided by height (m) squared. Repeated octapolar bioimpedance of each patient was analyzed using the InBody 720 multifrequency analyzer (Biospace, Seoul, Korea). The body fat ratio (%) was computed using proprietary algorithms. The body constituent of each patient was determined every 2 months for 8 months. Areas of subcutaneous (SFA) and visceral (VFA) fat were measured using computed tomography. We defined the scanned area between the skin and muscle as SFA and that identified as fat density inside the peritoneum was defined as VFA. The single-slice method of scanning was applied at the umbilical level to assess abdominal fat masses.

All patients received standardized nutritional counseling regarding adequate caloric intake in sessions delivered by dieticians every 2 months for 8 months. The daily calorie intake was investigated by dieticians using a questionnaire regarding food intake for 3 days.

The target dietary energy intake was defined as standard bodyweight (kg) × 30 kcal containing 60% carbohydrate, 20% fat and 20% protein. Two months later, the patients were instructed to walk a minimum of 8000 steps/day for at least 3 days each week on a flat field while wearing an electronic pedometer (Kenz Lifecorder EX; Suzuken, Aichi, Japan) to evaluate the daily

4step count. Exercise intensity was prescribed according to a ratio (%) of age-predicted maximal heart rate and rating of perceived exertion. The patients were counseled to achieve 50% of their target heart rate calculated using the Karvonen formula: (maximum heart rate – resting heart rate) × 0.5 + resting heart rate. Maximal heart rate was estimated as 220 – age (years).

Evaluation of quality of life

The Short Form-36 (SF-36) is a scale that is used to evaluate HRQOL. We used the Japanese version of the SF-36 questionnaire that comprises eight multi-item domains. Physical functioning (PF), role – physical (RP) and bodily pain (BP) were correlated with physical health. Mental health (MH), role – emotional (RE) and social functioning (SF) were correlated with mental health, and general health (GH) and vitality (VT) scales were correlated with both physical and mental health. In addition, the eight domains are aggregated into two summary measures: physical (PCS) and mental (MCS) component summary scales. The calculation of PCS and MCS scale scores for Japanese were obtained from a local study.[25]

Statistical analysis

All data are expressed as median, minimal and maximal values. Distributions of continuous variables were analyzed using the Wilcoxon rank sum test. $P < 0.05$ was considered significant. Calculations were performed using SPSS for Windows, Release ver. 18.0J.

RESULTS

Baseline clinical characteristics and clinical courses of the patients

THE BASELINE CLINICAL characteristics of the patients are shown in Table 1 (median age, 61 years; range, 43–70 years; female, 65%). Three and 14 of them had clinical liver cirrhosis and chronic hepatitis, respectively. All were infected with HCV genotype 1b. Fifteen patients completed the study protocol. One patient each dropped out due to knee pain at 2 months and depression at 4 months. By the end of the study, 47% of the patients achieved an average of 8000 steps/day. Insulin resistance was significantly improved among patients who achieved an average of 8000 steps/day ($P = 0.018$), but was not found in those who did not achieve this step target ($P = 0.463$).

Changes in physical and clinical parameters after 6 months of exercise

The data in Figure 1(a) show that daily oral intake did not significantly differ between the start of the study and at 6 months thereafter ($P = 0.730$). The participants were given nutrition counseling by dieticians at the time of the entry in this study, and none of the patients had excessive oral intake before intervention. The data in Figure 1(b) show that the daily numbers of steps monitored using the pedometer had increased by 6 months compared with those at the start of the study ($P = 0.003$). The changes in physical and clinical parameters after 6 months of exercise are shown in Table 2.

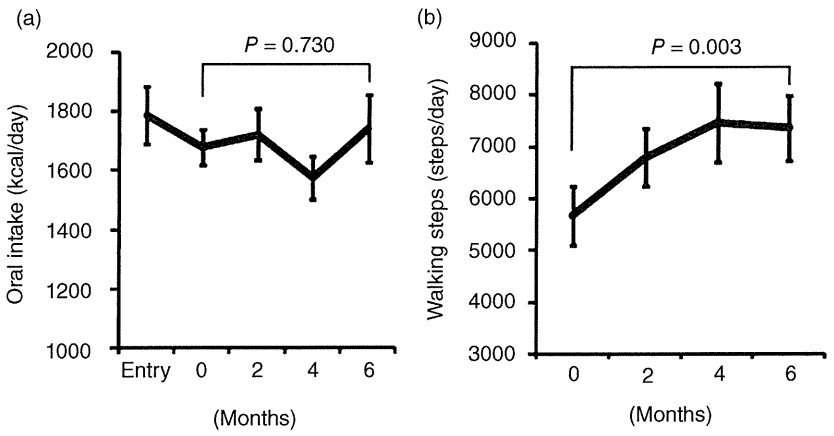

Figure 1 Changes in parameters after 6 months of exercise. (a) Oral intake did not significantly change during this study. (b) Number of steps significantly increased at 6 months compared with that at start of exercise ($P = 0.003$).

Table 1 Baseline characteristics of 17 patients with HCV

Parameters	All patients ($n = 17$)
Sex (M/F)	6/11
Age (years)	61 (43–70)
Body weight (kg)	62.7 (45–88)
Body mass index (kg/m^2)	25.6 (18.7–29.7)
Fat weight (kg)	23.0 (14.0–32.0)
Muscle weight (kg)	21.9 (16.4–33.0)
SFA (cm^2)	213.0 (126.5–350.1)
VFA (cm^2)	125.4 (43.9–211.6)
Total protein (g/dL)	7.5 (7.0–9.0)
Serum Albumin (g/dL)	4.0 (3.0–4.6)
AST (IU/L)	41 (22–61)
ALT (IU/L)	43 (19–95)
γ-GTP (IU/L)	30 (14–87)
FPG (mg/dL)	107 (88–185)
HOMA-IR	2.3 (1.0–9.2)
Total cholesterol (mg/dL)	176 (129–218)
LDL cholesterol (mg/dL)	97 (56–138)
HDL cholesterol (mg/dL)	53 (23–111)
Triglyceride (mg/dL)	89 (38–335)
HCV RNA titer (KIU/mL)	2300 (5->5100)

Data are expressed as median, minimal, and maximal values. The HOMA-IR was evaluated in 15 patients.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; FPG, fasting plasma glucose; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; γ-GTP, glutamyl transpeptidase; HOMA-IR, Homeostatic Model of Assessment of Insulin Resistance; SFA, subcutaneous fat areas; VFA, visceral fat areas.

Bodyweight, BMI, fat weight, VFA, SFA, serum level of ALT and HOMA-IR significantly decreased after 6 months of exercise ($P = 0.004$, =0.004, =0.008, =0.041, =0.001, =0.023 and =0.002, respectively). Muscle weight, total protein, serum albumin, total cholesterol, LDL cholesterol, HDL cholesterol, AST, γ-GTP, prothrombin time and HCV RNA titer did not change and none of the liver function parameters worsened in any participant during the study period.

The HOMA-IR, BMI, bodyweight, SFA and VFA were significantly reduced in patients who achieved their daily step targets ($P = 0.018$, =0.028, =0.028, =0.043, =0.043, respectively) after 6 months of exercise. On the contrary, only SFA was reduced in the patients who did not achieve their targets ($P = 0.018$).

Changes in cytokines after 6 months of exercise

We measured levels of two cytokines and two adipokines to clarify relationships among these parameters and insulin resistance and reduced fat volume. The changes in TNF-α, IL-6, adiponectin and leptin are shown in Figure 2. The median values of serum levels of TNF-α before and after 6 months of exercise were 1.8 (range 0.7–6.6) and 2.2 (range 1.2–3.5) pg/mL, respectively, which did not significantly differ. The median serum levels of IL-6 before and after exercise were 5.6 (range 1.9–20.7) and 3.8 (range 1.3–31.4) pg/mL, respectively, which tended to decrease, but did not reach significance ($P = 0.06$). The median serum levels of total adiponectin before and after exercise were 27 560 (range 7520–71 610) and 21 680 (range 6420–70 960) ng/mL, respectively, which did not significantly differ. However, the median serum levels of leptin significantly decreased after exercise from 19 000 (range 4710–45 260) to 9700 (range 3190–22 120) pg/mL ($P = 0.002$).

Changes in HRQOL parameters after 6 months of exercise

The changes in HRQOL parameters before and after 6 months of exercise are shown in Figure 3. The VT score on the SF-36 scale tended to improve after exercise ($P = 0.087$), whereas no other parameter changed between before and after exercise for 6 months.

DISCUSSION

OUR FINDINGS DEMONSTRATED that aerobic exercise improves insulin resistance and decreases body fat without decreasing muscle weight in patients infected with HCV. One of the mechanisms involved in improving insulin resistance is a decrease in pro-inflammatory cytokines, such as serum IL-6 and TNF-α, which plays a crucial role in insulin resistance. High levels of TNF-α contribute to HCV-related insulin resistance[6] and IL-6 induces insulin resistance[26] through the suppression of cytokine signaling.[27] However, our findings showed that serum levels of TNF-α did not change after exercise. Serum levels of IL-6 tended to decrease, but not significantly. Aerobic training reduces TNF-α and IL-6 gene expression in skeletal muscle but not in serum.[28] Because we did not examine cytokine levels in skeletal muscle, liver and adipose tissue, we cannot conclude that insulin resistance was not associated with pro-inflammatory cytokines such as TNF-α and IL-6 in our patients.

Leptin is an adipocytokine that is associated with hepatic steatosis.[29] The present study found that serum leptin levels decreased after exercise. In fact, serum levels of leptin decreased together with a decrease in fat volume induced by exercise in our patients. Leptin resis-

Table 2 Changes in physical and clinical parameters after 6 months of exercise

	Before	After 6 months	*P*-value
Bodyweight (kg)	62.7 (45.0–88.0)	61.5 (45.3–88.1)	$P = 0.004$
Body mass index (kg/m^2)	25.6 (18.7–28.5)	25.4 (18.6–28.6)	$P = 0.004$
Fat weight (kg)	23.2 (12.4–30.9)	21.0 (12.2–30.6)	$P = 0.008$
Muscle weight (kg)	22.5 (16.9–32.9)	22.8 (17.2–33.3)	$P = 0.955$
SFA (cm^2)	204.0 (126.5–350.1)	169.4 (93.5–347.2)	$P = 0.001$
VFA (cm^2)	127.4 (43.9–211.6)	116.0 (26.6–207.0)	$P = 0.041$
Total protein (g/dL)	7.8 (6.7–8.5)	7.7 (6.8–9.0)	$P = 0.887$
Serum albumin (g/dL)	4.4 (3.5–4.7)	4.3 (3.5–4.8)	$P = 0.917$
AST (IU/L)	32 (22–61)	32 (20–86)	$P = 0.410$
ALT (IU/L)	43 (19–95)	32 (16–93)	$P = 0.023$
γ-GTP (IU/L)	31 (14–121)	27 (13–105)	$P = 0.098$
FPG (mg/dL)	105 (88–185)	102 (88–155)	$P = 0.063$
HOMA-IR	2.5 (1.0–8.1)	1.9 (1.3–5.6)	$P = 0.023$
Total cholesterol (mg/dL)	178 (129–218)	187 (130–251)	$P = 0.234$
LDL cholesterol (mg/dL)	99 (56–138)	107 (46–165)	$P = 0.861$
HDL cholesterol (mg/dL)	50 (23–111)	57 (29–101)	$P = 0.077$
Triglyceride (mg/dL)	90 (38–335)	82 (39–301)	$P = 0.059$

Data are expressed as median, minimal and maximal values. "Before", before starting exercise; "after 6 months", after 6 months of exercise. The HOMA-IR was evaluated in 13 patients.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; FPG, fasting plasma glucose; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; γ-GTP, glutamyl transpeptidase; HOMA-IR, Homeostatic Model of Assessment of Insulin Resistance; SFA, subcutaneous fat areas; VFA, visceral fat areas.

tance is associated with insulin resistance.[30] Our results indicate that aerobic exercise decreases serum leptin by improving leptin resistance. Importantly, leptin promotes the proliferation, migration, invasiveness and angiogenesis of HCC.[31,32] Moreover, leptin plays a profibrogenic role by activating Kupffer cells and macrophages, and stimulating endothelial cells.[33] We postulate that a decrease in serum leptin could contribute to preventing the occurrence and progression of hepatocellular carcinoma and progression to liver cirrhosis. On the other hand, levels of total adiponectin did not change after the exercise intervention in this study. Weight reduction reportedly increases plasma levels of adiponectin.[34] One factor that might explain the discrepancy between the present study and that report is the smaller decrease in the bodyweight of our patients. We found that the amounts of VFA decreased in our patients. However, this would not be sufficient to increase serum levels of total adiponectin. A recent report has described that exercise does not affect serum levels of adiponectin despite significant improvements in insulin action.[35] Adiponectin levels increased only in patients who lost weight in this report. A key factor in adiponectin regulation is TNF-α, baseline levels of which are higher in patients with CH-C than in healthy controls.[17] Levels of TNF-α did not decrease in our patients after the intervention. These factors might explain why adiponectin levels were not altered by the decrease in leptin.

Some problems were associated with introducing the exercise, such as achieving the target number of steps and patient compliance. Only half of our patients achieved their step goals. To motivate people to habitually walk is challenging when they do not normally do so. Powerful evidence showing that aerobic exercise can lower serum levels of leptin, which is associated with HCC might motivate patients to comply. Moreover, appropriate instruction or a specialized program for the patients would support continued compliance with exercise. The other problem is the limitation of exercise for patients with advanced liver disease. One of our patients quit the study because of knee pain. Because patients with CH-C are becoming older, low- or no-impact alternatives to walking, such as swimming, should be considered.

Liver function did not worsen in any patient who participated in the study because we excluded those with advanced liver cirrhosis. However, physical exercise apparently decreases portal blood flow and increases portal pressure in patients with cirrhosis.[36] Whether or not physical exercise affects liver function in patients with decompensated liver cirrhosis should be clarified.