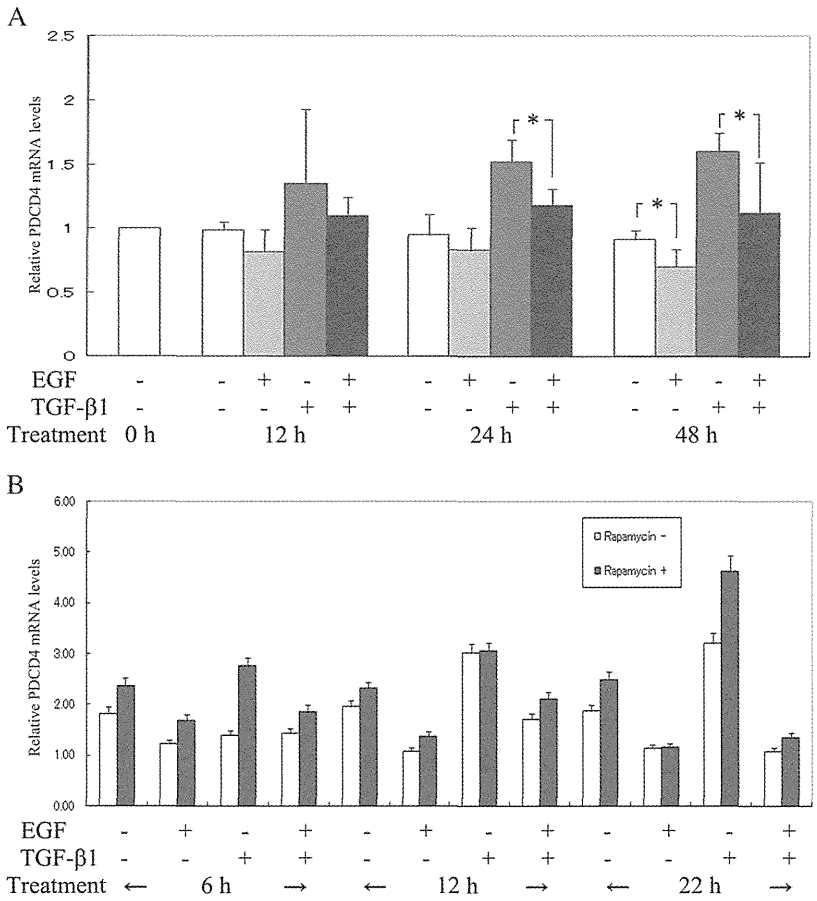

Fig. 7. The EGF–PI3K–Akt–mTOR signaling pathway regulates PDCD4 mRNA levels. (A) EGF down-regulates PDCD4 mRNA levels. Huh 7 cells were cultured for the times indicated in the figure in the presence or absence of EGF (20 ng/ml) and/or TGF-β1 (5 ng/ml). The mRNA levels of the cells were determined by real-time RT-PCR. The data are an average of 3 independent experiments. *, $P < 0.05$ as determined by a *t*-test comparison. (B) Rapamycin up-regulates PDCD4 mRNA levels. Huh 7 cells were cultured for the times indicated in the figure in the presence or absence of EGF (20 ng/ml) and/or TGF-β1 (5 ng/ml) with or without 0.1 nM rapamycin. The mRNA levels of the cells were determined by real-time RT-PCR. The data are the average of 3 independent experiments.

[4]. Also, TGF-β1 may inhibit the degradation of PDCD4 blocking the activation of the Akt–mTOR–S6K1 signaling pathway [23]. Therefore, the functions of TGF-β1 and EGF in Huh7 hepatoma cells are reciprocal: The expression of PDCD4 mRNA is stimulated by TGF-β1 and suppressed by EGF, and the degradation of PDCD4-protein is suppressed by TGF-β1 and stimulated by EGF.

It has been demonstrated that rapamycin can activate the Smad system to stimulate the transcription of target genes [24,25]. In rat renal mesangial cells, rapamycin stimulated the phosphorylation of Smad2/3 through a ROS-mediated mechanism to increase the level of active TGF-β1 [24]. Another research group demonstrated that rapamycin induced osteogenic differentiation in human embryonic stem cells by stimulating the BMP/Smad signaling system [25]. However, we did not observe the induction of Smad 2/3 phosphorylation in our experiment, indicating that rapamycin's stimulation of PDCD4 transcription is due to a different mechanism than those mentioned above.

Previous studies have shown that TPA has no effect on PDCD4 mRNA levels, although it down-regulates PDCD4-protein levels [23]. TPA may at least partly mediate the phosphorylation of S71 and S76 in the binding motif of ubiquitin ligase through a member of the PKC-δ and/or PKC-ε mediated signaling pathway because the knockdown of either PKC-δ or PKC-ε but not PKC-α was found to up-regulate PDCD4-protein levels in Huh7 cells [23]. The TPA mediation of the phosphorylation at S71 and S76 sites must occur independently from the phosphorylation of S67, as the expression of the PDCD4 mutant replaced serine with alanine at S67 was shown to be suppressed by TPA. It was shown previously that PKD1 was triggered when PKC-δ or PKC-ε activated NF-κB in Huh7 cells [26]. The knockdown of PKD1, however, did not affect the level of the PDCD4-protein (data not shown).

Schmid et al. reported that a MEK-1 inhibitor significantly increased PDCD4 expression, and they proposed that the MAP kinase signaling pathway also contributes to regulating the PDCD4 levels in the human embryonic kidney cell line HEK293 [22]. This regulation may be mediated by miR-21 which is regulated by AP-1 [27] and targets PDCD4 mRNA through its 3′-UTR [28,29]. In Huh7 hepatoma cells, the contribution of the MAP kinase pathway in controlling PDCD4 may be minor; the MEK-1 inhibitor, as well as P38 and JNK inhibitors, only had a slight effect on PDCD4 expression.

EGF and TPA are both known to be potent promoters of carcinogenesis. Both mitogens increase the degradation of tumor suppressor PDCD4, thereby stimulating protein synthesis, cell growth and consequently, carcinogenesis. Elucidating the mechanisms of PDCD4 degradation is necessary to generate PDCD4-targeted cancer prevention treatments.

In conclusion, EGF antagonizes the function of TGF-β1 suppressing the TGF-β1-induced apoptosis of Huh7 cells by inhibiting the synthesis

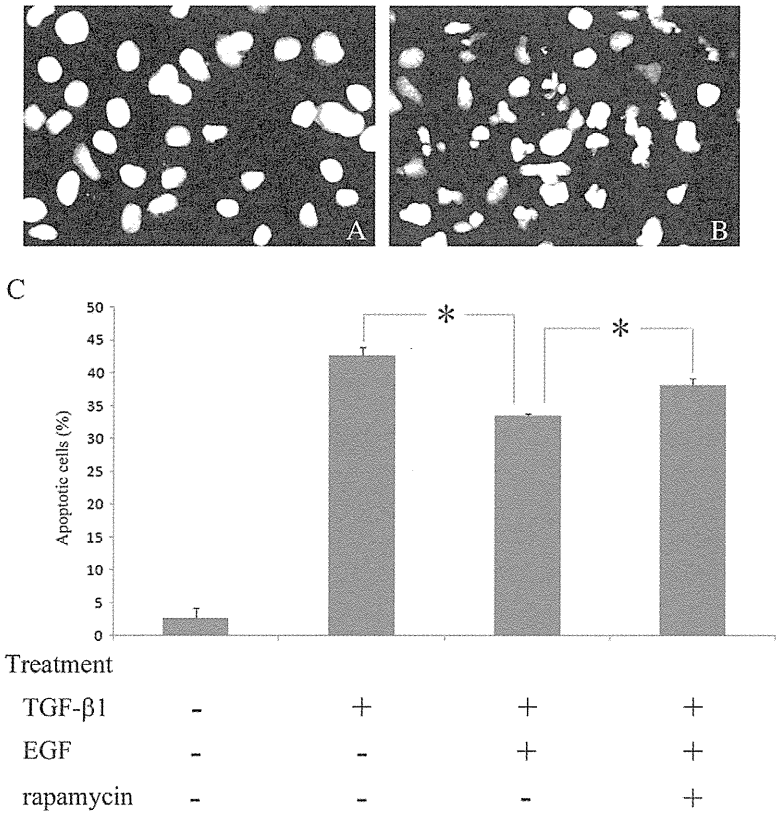

Fig. 8. Rapamycin inhibited the recovery of TGF-β1-induced apoptosis by EGF. Huh7 cells were cultured with or without TGF-β1 (5 ng/ml), EGF (20 ng/ml) and rapamycin (0.1 nM) for 24 h as indicated in the figure. The cells were stained with Hoechst 33342. (A) Cell nuclei with no treatment. (B) Cell nuclei with TGF-β1 treatment. (C) Irregular apoptotic cell nuclei were counted under microscopy. The data are an average of 3 independent experiments. More than 1000 cell nuclei from each sample were counted. *, $P < 0.01$ as determined by a *t*-test comparison.

of PDCD4 mRNA and increasing the degradation of the PDCD4-protein which causes apoptosis when overexpressed. TPA also stimulates the degradation of the PDCD4-protein by phosphorylating S71 and S76 which are located in the β-TRCP binding motif, via a mechanism that is independent of S67 phosphorylation. The PDCD4 mutants with the replacement of either serine 67, 71 or 76 with aspartic acid are degraded in the proteasome system.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Whole-body Insulin Resistance is Associated with Elevated Serum α-fetoprotein Levels in Patients with Chronic Hepatitis C

Yasunori Kawaguchi[1], Toshihiko Mizuta[1], Yuichiro Eguchi[1], Eiichi Sakurai[2], Yoichi Motomura[2], Hiroshi Isoda[1], Takuya Kuwashiro[1], Satoshi Oeda[1], Shinji Iwane[1], Hirokazu Takahashi[1], Keizo Anzai[1] and Iwata Ozaki[1]

Abstract

Objective Little is known about the relationship between elevated serum α-fetoprotein (AFP) levels and insulin resistance, which adversely influence the clinical course of chronic hepatitis C (CHC). Therefore, we investigated the association between serum AFP and insulin resistance in patients with CHC.

Methods We retrospectively investigated 300 patients with CHC without hepatoma who underwent liver biopsies and oral glucose tolerance tests. Patients taking antidiabetic drugs were excluded. We analyzed factors associated with elevated AFP levels (≥10.0 ng/mL) in 265 eligible patients. Twenty patients with a homeostasis model assessment for insulin resistance value of ≥2.0 and a whole-body insulin sensitivity index of <5.0 received prospective lifestyle intervention.

Results A univariate analysis showed that the body mass index, platelet count, levels of albumin, aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transpeptidase, glucose metabolism, hepatic inflammation, fibrosis and steatosis were associated with elevated AFP levels. In a multivariate analysis, a platelet count of $<15\times10^4$/μL, aspartate aminotransferase level of ≥50 IU/L, γ-glutamyl transpeptidase level of ≥35 IU/L, whole-body insulin sensitivity index of <5.0 and stage 3-4 fibrosis were independently associated with an elevated AFP level. A Bayesian Network analysis showed that the aspartate aminotransferase level, whole-body insulin sensitivity index and hepatic fibrosis were directly associated with an elevated AFP level. The lifestyle intervention significantly improved the serum AFP level, homeostasis model assessment for insulin resistance and whole-body insulin sensitivity index.

Conclusion Whole-body insulin resistance is associated with an elevated serum AFP level in patients with CHC. Lifestyle interventions targeting insulin resistance can reduce the serum AFP level and may ameliorate the clinical course of CHC.

Key words: α-fetoprotein, hepatitis C, whole-body insulin resistance, Bayesian Network analysis, lifestyle intervention

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Introduction

Approximately 170 million people worldwide have persistent hepatitis C virus (HCV) infection (1), the leading cause of liver cirrhosis and hepatocellular carcinoma [HCC (2, 3)]. The serum α-fetoprotein (AFP) level is an important predictor of the clinical course in patients with chronic hepatitis C (CHC), as elevated serum AFP levels are associated with a low viral response rate to interferon [IFN (4)], advanced fibrosis (5-7) and a high frequency of HCC (6, 8-10). Although IFN can lead to biochemical improvement and eradi-

[1]Department of Internal Medicine, Saga Medical School, Japan and [2]National Institute of Advanced Industrial Science and Technology, Japan
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Correspondence to Dr. Toshihiko Mizuta, mizutat@cc.saga-u.ac.jp

cation of HCV, which reduces the risk of HCC (11), the clinical impact of HCV eradication on HCC prevention is less significant in older patients than in younger patients (8). Nevertheless, prolonged IFN therapy can decrease the serum AFP levels and thus prevent hepatocarcinogenesis, even in elderly patients (10).

Recent studies have shown that insulin resistance (IR) in HCV-infected patients is associated with the response to antiviral therapy (12-15), progression of fibrosis (12, 16-18) and development of HCC (19). Although HCV itself can evoke hepatic IR (16) and systemic IR (20), we previously reported that visceral fat accumulation is more strongly associated with IR in patients with CHC than in patients with non-alcoholic fatty liver disease (21). Therefore, it is likely that reducing visceral fat via lifestyle modification can improve IR in HCV-infected patients.

These findings indicate that the serum AFP levels and IR indices, both of which are noninvasively assessed, are significantly associated with the clinical course in HCV-infected patients. However, little is known about the relationship between the serum AFP levels and IR in patients with CHC. Therefore, we conducted a retrospective study to identify clinical factors, including glucose metabolism and histologic findings, associated with high elevated serum AFP levels in HCV-infected patients with no evidence of HCC. We also conducted a pilot study to determine whether lifestyle modification can improve IR or other clinical factors, including the serum AFP levels, in patients with CHC.

Materials and Methods

Patients

We conducted a retrospective study of 300 HCV-infected patients with no evidence of HCC who visited Saga Medical School Hospital between January 2004 and March 2010. Patients who underwent a liver biopsy and a 75-g oral glucose tolerance test (OGTT) were included in the analysis, while patients taking antidiabetic drugs were excluded. We set the cutoff value for AFP as 10.0 ng/mL because an even slightly elevated AFP level is a risk factor for HCC (6, 9). We analyzed factors associated with elevated AFP levels (≥10.0 ng/mL) in 265 patients who met these criteria, including 137 men and 128 women, with a median age of 58 years (range: 24-75 years).

Between June 2007 and March 2009, we conducted a pilot study to investigate whether lifestyle intervention can improve IR or other clinical factors, including the serum AFP levels, in patients with CHC. Only patients with no evidence of HCC whose homeostasis model assessment for insulin resistance (HOMA-IR) value was ≥2.0 (15) were enrolled. There were 11 men and nine women, with a median age of 60 years (range: 37-71 years). The whole-body insulin sensitivity index (WBISI) was <5.0 in all 20 patients. The study protocol was approved by the Institutional Review Board of Saga Medical School Hospital in accordance with the ethical guidelines of the Declaration of Helsinki (1975, as revised in 1983), and written informed consent was obtained from all patients.

Clinical and laboratory assessments

All demographic and laboratory data were collected at the time of the liver biopsy. In the pilot study, some data were also collected after the intervention. The demographic data included sex, age, body mass index (BMI; kg/m^2), alcohol consumption and history of IFN therapy. Alcohol consumption was classified into three groups: none, occasionally (<140 g/week) or regularly (≥140 g/week). Venous blood samples were obtained after a 12-hour overnight fast for hematology and blood chemistry examinations. The serum AFP level (ng/mL) was measured using a chemiluminescent immunoassay kit (Abbott Japan, Tokyo, Japan). For the OGTT, the patients ingested a solution containing 75 g of glucose, and venous blood samples were collected at 0, 30, 60, 90 and 120 minutes to measure the plasma glucose (PG; mg/dL) and serum insulin (SI; μU/mL) levels. The PG levels were determined using the glucokinase method and the SI levels were measured using a chemiluminescent immunoassay kit (Abbott Japan). Glucose tolerance was evaluated according to the World Health Organization criteria (22). Briefly, normal glucose tolerance (NGT) was defined as a fasting PG (FPG) level of <110 mg/dL and a 2-hour PG level of <140 mg/dL. Impaired fasting glycemia (IFG) was defined as an FPG level of 110-126 mg/dL and a 2-hour PG level of <140 mg/dL. Impaired glucose tolerance (IGT) was defined as an FPG level of <126 mg/dL and a 2-hour PG level of 140-200 mg/dL. Diabetes mellitus (DM) was defined as an FPG level of ≥126 mg/dL or a 2-hour PG level of ≥200 mg/dL. The indices of basal insulin secretion and insulin sensitivity were evaluated using the homeostasis model assessment (HOMA) method (23), as follows:

β cell function (HOMA-β) = fasting SI (FSI) ×360/[FPG-63]

Insulin resistance (HOMA-IR) = FPG × FSI/405

The WBISI (24) was calculated as $10,000/(\text{FPG} \times \text{FSI} \times \text{mean PG 0-120} \times \text{mean SI 0-120})^{0.5}$.

In the pilot study, we also measured the serum adiponectin (μg/mL), leptin (ng/mL) and soluble tumor necrosis factor receptor 2 (sTNFR2; pg/mL) levels before and after the lifestyle intervention using a Human Adiponectin ELISA Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), a Human Leptin RIA Kit (Millipore Corporation, Billerica, MA, USA) and a Quantikine Human sTNFRII/TNFRSF1B Immunoassay (R&D SYSTEMS Inc., Minneapolis, MN, USA).

Among the patients who underwent abdominal computed tomography, the visceral fat area (VFA; cm^2) was measured at the umbilical level and calculated using the Fat Scan software program [N2 Systems, Osaka, Japan (25)].

Liver histology

A percutaneous liver biopsy was performed using a Super-Core™ Biopsy Instrument (Medical Device Technolo-

gies, Inc., Gainesville, FL, USA) under ultrasound guidance. In each patient, a 15-mm-long liver biopsy specimen was fixed in 10% formalin, embedded in paraffin, sectioned and stained with Hematoxylin and Eosin staining and Azan for a histologic evaluation. The degree of histologic hepatic fibrosis and inflammation was scored using the METAVIR scoring system (26). Based on the degree of lymphocyte infiltration and hepatocyte necrosis, the level of inflammation was classified from A0 to A3, with a higher score indicating more severe inflammation. Fibrosis was graded from F0 to F4 as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Steatosis was quantified as the percentage of hepatocytes that contained fat droplets classified into three groups: <5%, 5-30% and ≥30%.

Lifestyle intervention

The ideal body weight (kg) was calculated as 22× [height (m)]^2. A dietitian instructed each patient to maintain a total calorie intake of 25-35 kcal/ideal body weight/day according to the level of daily activity. The exercise intervention was based on the 'Exercise and Physical Activity Guide for Health Promotion 2006' published by the Ministry of Health, Labour and Welfare of Japan (27). Briefly, the patients were recommended to walk a minimum of 8,000 steps every day on flat terrain while wearing a pedometer. The patients were also instructed to record their diet and exercise activities in a diary. The lifestyle intervention was continued for >3 months with the goal of reducing the HOMA-IR to <2.0. In patients with limited improvements in IR, the intervention was discontinued at the discretion of the attending physician.

Statistical analysis

Continuous variables are presented as the median (range). Comparisons between groups were made using the Mann-Whitney *U* test for continuous variables and the χ^2 test or Fisher's exact probability test for categorical data. A multiple logistic regression analysis was used to identify factors independently associated with an elevated serum AFP level. Wilcoxon's signed-rank test was performed to analyze the paired samples. Values of $p<0.05$ were considered to be statistically significant.

A Bayesian Network (28, 29) is a directed acyclic graph that represents a joint probability distribution for a set of variables. Each node on the graph represents a variable, and a link between two nodes indicates a direct dependency between the variables. In the retrospective study, we used a Bayesian Network analysis to identify factors directly associated with an elevated serum AFP level.

Results

Retrospective study

The prevalence of an elevated AFP level (≥10 ng/mL) was 22.3% (59/265). Table 1 shows the characteristics of the patients stratified according to the serum AFP level (<10 vs. ≥10 ng/mL). A univariate analysis showed that BMI, the platelet count, the aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), triglyceride, high-density lipoprotein cholesterol (HDL-C) and albumin levels, HOMA-IR, WBISI, visceral obesity, hepatic inflammation, fibrosis and steatosis were associated with an elevated AFP level (Table 1). Variables with $p<0.01$ were used in the multiple logistic regression analysis. In terms of glucose metabolic factors, we included WBISI, as this factor was more strongly associated with the serum AFP level than the other indices. The VFA was excluded from this analysis because data were missing for a number of patients. The multiple logistic regression analysis showed that a platelet count of $<15\times10^4/\mu L$ (odds ratio [OR]: 2.74, 95% confidence interval [CI]: 1.27-5.91, p=0.01), an AST level of ≥50 IU/L (OR: 3.46, 95% CI: 1.24-9.65, p=0.018), a γ-GTP level of ≥35 IU/L (OR: 2.43, 95% CI: 1.03-5.71, p=0.042), a WBISI of <5.0 (OR: 3.55, 95% CI: 1.56-8.09, p=0.003) and stage 3-4 fibrosis (OR: 3.71, 95% CI: 1.43-9.58, p=0.007) were independently associated with an elevated AFP level (Table 2). The prevalence of an elevated AFP level according to the WBISI and fibrosis was 8.3% (11/133) and 36.4% (48/132) for a WBISI of ≥5.0 and <5.0, respectively, and 14.7% (33/224) and 63.4% (26/41) for fibrosis stage 0-2 and 3-4, respectively (Fig. 1). For the Bayesian Network analysis, we selected BMI, which affects metabolic factors, as well as the variables included in the multiple logistic regression analysis. The Bayesian Network analysis revealed that AST, WBISI and hepatic fibrosis were directly associated with an elevated AFP level (Fig. 2). Under conditions of AST ≥50 IU/L, WBISI <5.0 and fibrosis stage 3-4, 85% of the patients were presumed to have an elevated serum AFP level. On the other hand, when AST was ≥50 IU/L and fibrosis was stage 3-4, 71% of the patients with WBISI ≥5.0 were presumed to have a low serum AFP level (Table 3).

Pilot study

The median duration of the intervention was 182 days (range: 91-380 days). The baseline characteristics of the patients included in the prospective study and the changes in parameters after the lifestyle intervention are presented in Table 4. The VFA (p=0.001) and BMI (p<0.001) decreased significantly after the lifestyle intervention. The leptin/adiponectin ratio decreased (p=0.028) along with the reduction of visceral fat. In terms of hematology and biochemical data, the platelet count (p=0.026) and levels of γ-GTP (p=0.04), total cholesterol (p=0.042), triglycerides (p=0.008), creatinine (p=0.025), total protein (p=0.006) and albumin (p=0.004) decreased. Among the markers of glucose metabolism, FPG (p<0.001), FSI (p=0.001) and HOMA-IR (p<0.001) decreased after the intervention, while WBISI increased [p<0.001 (Fig. 3)]. The serum AFP level also decreased significantly after the intervention [p=0.002 (Fig. 3)].

Table 1. Characteristics of Patients according to the Serum α-fetoprotein Levels

	AFP < 10 ng/mL n = 206	AFP ≥ 10 ng/mL n = 59	p value
Males/females	105/101	32/27	0.768
Age (years)	58 (24–75)	59 (39–74)	0.170
BMI (kg/m^2)	22.9 (15.7–33.8)	23.7 (17.9–31.1)	0.033
Alcohol intake, none/occasionally/regularly/unknown	73/71/54/8	27/15/14/3	0.437
History of IFN therapy, yes/no/unknown	54/148/4	21/37/1	0.370
Platelet count (× 10^4/μL)	16.7 (5.5–42.2)	12.8 (5.7–25.2)	< 0.001
AST (IU/L)	40 (17–163)	68 (15–233)	< 0.001
ALT (IU/L)	43 (9–395)	75 (19–243)	< 0.001
γ-GTP (IU/L)	30 (3–509)	60 (13–323)	< 0.001
Total cholesterol (mg/dL)	176 (94–314)	169 (83–242)	0.093
Triglyceride (mg/dL)	87 (36–607)	104 (49–245)	0.002
HDL-C (mg/dL)	49 (23–108)[a]	43 (24–79)[b]	0.016
Uric acid (mg/dL)	5.4 (2.4–10.5)[c]	5.8 (2.1–8.1)[d]	0.194
Creatinine (mg/dL)	0.7 (0.5–1.9)	0.7 (0.3–1.4)	0.348
Total protein (g/dL)	7.2 (5.9–9.9)	7.2 (5.7–8.6)	0.530
Albumin (g/dL)	4.2 (3.2–5.2)	4.0 (2.7–4.7)	< 0.001
FPG (mg/dL)	87 (67–133)	88 (75–118)	0.867
2-h glucose (mg/dL)	117 (57–338)	136 (85–305)	< 0.001
FSI (μU/mL)	7 (2–24)	11 (3–34)	< 0.001
2-h insulin (μU/mL)	47 (6–500)	93 (20–294)	< 0.001
HOMA-IR	1.4 (0.3–7.7)	2.4 (0.6–8.0)	< 0.001
WBISI	5.6 (1.2–23.3)	2.9 (1.0–12.3)	< 0.001
HOMA-β	99 (24–630)	148 (41–666)	< 0.001
Glucose tolerance: NGT/non-NGT	149/57	30/29	0.003
VFA (cm^2)	60 (10–221)[e]	94 (12–163)[f]	< 0.001
Liver histology			
Inflammation: A0/A1/A2/A3	8/132/63/3	0/19/31/9	< 0.001
Fibrosis: F0/F1/F2/F3/F4	10/125/56/14/1	0/12/21/23/3	< 0.001
Steatosis (%): < 5/5–30/≥ 30	159/42/5	36/15/8	0.012

Values are median (range) or number of patients. BMI: body mass index, IFN: interferon, AFP: α-fetoprotein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyl transpeptidase, HDL-C: high-density lipoprotein cholesterol, FPG: fasting plasma glucose, FSI: fasting serum insulin, HOMA-IR: homeostasis model assessment for insulin resistance, WBISI: whole-body insulin sensitivity index, HOMA-β: homeostasis model assessment for β cell function, NGT: normal glucose tolerance, VFA: visceral fat area. [a]n = 195, [b]n = 56, [c]n = 204, [d]n = 58, [e]n = 119, [f]n = 40.

Table 2. Factors Associated with Elevated Serum α-fetoprotein Level (Multiple Logistic Regression Analysis)

Variables	OR	95% CI	p value
Platelet < 15 × 10^4/μL	2.74	1.27–5.91	0.010
AST ≥ 50 IU/L	3.46	1.24–9.65	0.018
ALT ≥ 50 IU/L	0.68	0.24–1.98	0.482
γ-GTP ≥ 35 IU/L	2.43	1.03–5.71	0.042
Triglyceride ≥ 90 mg/dL	1.18	0.55–2.55	0.670
Albumin < 4 g/dL	0.86	0.37–1.98	0.724
WBISI < 5.0	3.55	1.56–8.09	0.003
Hepatic inflammation A2–A3	1.84	0.85–4.00	0.124
Hepatic fibrosis F3–F4	3.71	1.43–9.58	0.007

AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyl transpeptidase, WBISI: whole-body insulin sensitivity index

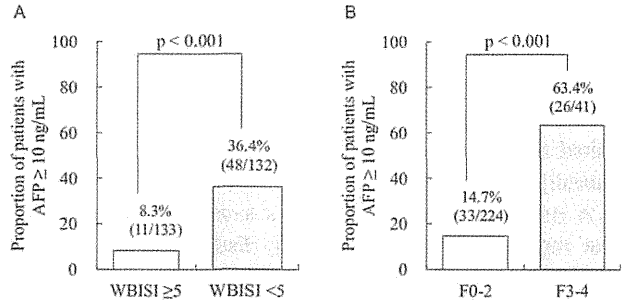

Figure 1. Prevalence of an elevated α-fetoprotein level (≥10 ng/mL) according to the (A) whole-body insulin sensitivity index and (B) hepatic fibrosis. AFP: α-fetoprotein, WBISI: whole-body insulin sensitivity index

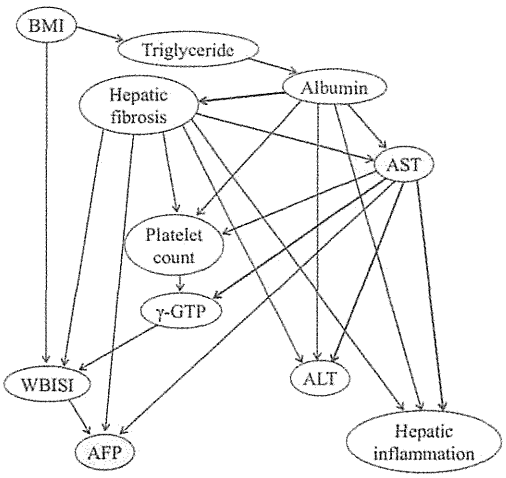

Figure 2. Bayesian Network analysis of the associations between an elevated serum α-fetoprotein level and the clinical factors. AFP: α-fetoprotein, WBISI: whole-body insulin sensitivity index, BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyl transpeptidase

Table 3. Bayesian Network Analysis of the Incidence of Elevated Serum α-fetoprotein

Parameters			Probability
WBISI ≥ 5	AST < 50	F0–2	0.042
WBISI ≥ 5	AST < 50	F3–4	0.333
WBISI ≥ 5	AST ≥ 50	F0–2	0.219
WBISI ≥ 5	AST ≥ 50	F3–4	0.286
WBISI < 5	AST < 50	F0–2	0.169
WBISI < 5	AST < 50	F3–4	0.400
WBISI < 5	AST ≥ 50	F0–2	0.356
WBISI < 5	AST ≥ 50	F3–4	0.846

WBISI: whole-body insulin sensitivity index, AST: aspartate aminotransferase

Discussion

In the present series of studies, we first demonstrated that systemic IR directly influences the elevation of the AFP level in patients with chronic HCV infection based on the results of multivariate and causal-relationship analyses.

The multiple logistic regression analysis showed that a decreased platelet count, increased serum AST and γ-GTP levels, whole-body IR and advanced hepatic fibrosis were independently associated with an elevated AFP level. Several studies have shown that an elevated AFP level is associated with a decreased platelet count, an increased AST level and advanced fibrosis in CHC patients without HCC (5-7), consistent with our findings. Furthermore, the Bayesian Network analysis, which has the ability to assess causal relationships based on conditional probabilities, revealed that an elevated AST level, whole-body IR and advanced fibrosis were directly associated with an elevated AFP level.

In terms of markers of glucose metabolism, WBISI, an index of whole-body IR, was selected as a feasible marker for AFP elevation, whereas HOMA-IR, an index of hepatic IR, was not selected. These results suggest that IR associated with an increased AFP level may be induced by an HCV-infected liver as well as obesity or other metabolic conditions, as systemic IR develops simultaneously in multiple organs, including the liver, skeletal muscle and adipose tissue (30). In fact, the Bayesian Network analysis did not reveal a relationship between an elevated AFP level and the HOMA-IR (data not shown).

Several researchers have reported that IR in HCV-infected patients is closely associated with hepatic fibrosis (12, 16-18). Our Bayesian Network analysis also identified a direct relationship between whole-body IR and hepatic fibrosis. However, both systemic IR and advanced fibrosis were independently and directly associated with an elevated AFP level. These results suggest that it may be possible to decrease the serum AFP level by improving IR, even in patients with advanced hepatic fibrosis.

Although no reports have described an association between the serum AFP and γ-GTP levels, it is well known that γ-GTP plays important roles in the generation of oxidative stress (31) and is correlated with IR (32). The Bayesian Network analysis also showed that the γ-GTP level influences the serum AFP level via the effects of whole-body IR.

In the second part of this study, we showed that prospective lifestyle modification can improve metabolic factors, including systemic IR and the serum AFP level. We found that the leptin/adiponectin ratio, a useful marker of metabolic syndrome in the general population that is correlated with IR in individuals with or without diabetes (33-35), decreased after the intervention. Therefore, we presumed that the reduction in visceral fat achieved with the lifestyle intervention caused a decrease in the leptin/adiponectin ratio, which then improved IR. However, we found no changes in the sTNFR2 levels, a marker for tumor necrosis factor, a key cytokine involved in HCV-associated IR and obesity-associated IR (20, 35-38), despite the reduction in body weight observed in this study. We previously reported that eradication of HCV by IFN decreases the serum sTNFR2 level and improves whole-body IR (20). Therefore, these results suggest that IR and elevated AFP levels in HCV-infected patients may be inhibited by the eradication of HCV with antiviral therapy.

Unexpectedly, the platelet count, which was negatively correlated with an elevated AFP level in the retrospective study, decreased after the lifestyle intervention. We assume that the reduction of the platelet count reflects an improvement in systemic inflammation, a key feature of obesity and DM (39). The adiponectin levels, which are inversely related to adiposity, decreased slightly after the lifestyle intervention, although the changes were not statistically significant. Because the serum adiponectin levels are affected by hepatic fibrosis, regardless of the cause of liver disease (40), the effects of lifestyle intervention on the serum adiponectin levels in HCV-infected patients may differ from those observed

Table 4. Patient Characteristics and Effects of the Lifestyle Intervention on Clinical Characteristics

	Baseline	After	p value
Males/females	11/9	–	–
Age (years)	60 (37–71)	–	–
BMI (kg/m^2)	25.9 (18.9–30.5)	25.0 (17.8–29.2)	< 0.001
Alcohol intake, none/occasionally/regularly	13/6/1	–	–
History of IFN, yes/no	9/11	–	–
Platelet count (× 10^4/μL)	15.1 (10.5–23.7)	14.3 (8.1–20.6)	0.026
AST (IU/L)	45 (20–155)	42 (18–202)	0.251
ALT (IU/L)	54 (18–227)	44 (15–266)	0.173
γ-GTP (IU/L)	43 (11–137)	35 (11–110)	0.040
Total cholesterol (mg/dL)	172 (121–221)	163 (117–209)	0.042
Triglyceride (mg/dL)	108 (48–230)	87 (33–238)	0.008
HDL-C (mg/dL)	42 (24–73)	40 (29–75)	0.419
Uric acid (mg/dL)	5.8 (2.9–8.9)	5.8 (3.0–8.5)	0.337
Creatinine (mg/dL)	0.77 (0.46–0.98)	0.75 (0.40–0.93)	0.025
Total protein (g/dL)	7.5 (6.8–8.6)	7.3 (6.3–8.8)	0.006
Albumin (g/dL)	4.2 (3.5–4.9)	4.1 (3.2–4.5)	0.004
FPG (mg/dL)	101 (85–110)	89 (75–107)	< 0.001
2-h glucose (mg/dL)	140 (89–305)	120 (78–202)	0.130
FSI (μU/mL)	13 (9–18)	9 (6–21)	0.001
2-h insulin (μU/mL)	88 (31–227)	66 (18–189)	0.057
HOMA-IR	2.8 (2.3–4.7)	1.9 (1.2–4.8)	< 0.001
WBISI	3.0 (1.5–4.2)	4.2 (1.4–7.8)	< 0.001
HOMA-β	135 (75–256)	127 (58–299)	0.737
Glucose tolerance, NGT/IGT/DM	10/8/2	15/4/1	0.153
AFP (ng/mL)	7.5 (3.0–47.0)	7.0 (2.0–30.5)	0.002
Adiponectin (μg/mL)	9.6 (1.9–20.7)[a]	8.6 (2.3–25.1)[b]	0.463
Leptin (ng/mL)	9.0 (2.1–16.2)[a]	5.2 (1.3–14.6)[b]	0.039
Leptin/adiponectin ratio	1.1 (0.2–4.9)[a]	1.5 (0.4–12.0)[b]	0.028
sTNFR2 (pg/mL)	3170 (2010–5000)[a]	3050 (1600–5000)[b]	0.938
VFA (cm^2)	96 (31–220)	68 (12–159)	0.001
Liver histology			
Inflammation: A0/A1/A2/A3	0/12/7/1	–	–
Fibrosis: F0/F1/F2/F3/F4	0/8/9/3/0	–	–
Steatosis (%): < 5/5–30/≥ 30	10/8/2	–	–

Values are medians (range) or number of patients. BMI: body mass index, IFN: interferon, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyl transpeptidase, HDL-C: high-density lipoprotein cholesterol, FPG: fasting plasma glucose, FSI: fasting serum insulin, HOMA-IR: homeostasis model assessment for insulin resistance, WBISI: whole-body insulin sensitivity index, HOMA-β: homeostasis model assessment for β cell function, NGT: normal glucose tolerance, IGT: impaired glucose tolerance, DM: diabetes mellitus, AFP: α-fetoprotein, sTNFR2: soluble tumor necrosis factor receptor 2, VFA: visceral fat area. [a]n=19, [b]n=18.

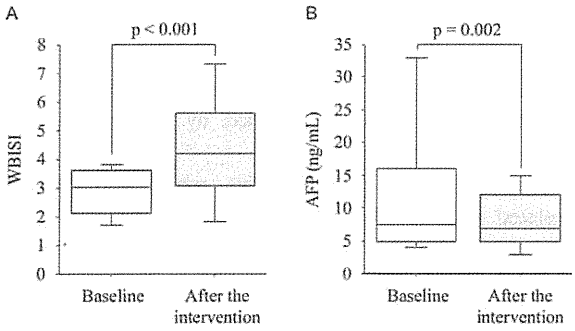

Figure 3. Effects of the lifestyle intervention on the (A) whole-body insulin sensitivity index and (B) serum α-fetoprotein level. AFP: α-fetoprotein, WBISI: whole-body insulin sensitivity index

in healthy subjects.

Several reports have shown that reductions in the serum AFP levels following IFN treatment in patients with CHC can help to prevent the development of HCC, irrespective of viral eradication (10, 11, 41). However, a large, randomized controlled trial recently showed that long-term maintenance peg-IFN therapy in patients with advanced CHC does not prevent liver-related deaths and actually increases the overall mortality, primarily due to non-liver-related causes (42). Therefore, the long-term administration of IFN to prevent HCC is not recommended in patients with advanced hepatic fibrosis. In HCV-infected patients, we previously reported that an increased BMI is associated with an increased risk of HCC at a younger age (43) and that the occurrence of hyperglycemia after a glucose load is a significant risk factor for the development of HCC (44). Taken together, it is likely that improvements in systemic IR and/or glucose me-

tabolism via appropriate lifestyle modification can help to safely prevent hepatocarcinogenesis, even in patients with advanced CHC.

One limitation of our study is that we did not measure the fucosylated fraction of AFP (AFP-L3), an accepted specific marker for HCC (45). Therefore, future studies should determine which fraction of AFP is decreased by lifestyle interventions. Another limitation is that we did not evaluate the changes in alcohol intake after the lifestyle intervention. Changes in alcohol intake may affect IR and the serum AFP level.

In conclusion, this study showed that whole-body IR, an elevated AST level and advanced fibrosis are independently and directly correlated with an elevated AFP level in patients with CHC. We also found that lifestyle modification can reduce the AFP level and whole-body IR. To our knowledge, this is the first report to examine the relationship between the serum AFP level and systemic IR and to show that lifestyle modification can reduce the serum AFP level. Further prospective studies are needed to confirm whether the reduction in the serum AFP level achieved via lifestyle modification can prevent hepatocarcinogenesis in HCV-infected patients.

The authors state that they have no Conflict of Interest (COI).

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HEPATOLOGY

Severity of non-alcoholic steatohepatitis is associated with substitution of adipose tissue in skeletal muscle

Yoichiro Kitajima,*,¶ Hideyuki Hyogo,‡ Yoshio Sumida,§ Yuichiro Eguchi,† Naofumi Ono,¶ Takuya Kuwashiro,* Kenichi Tanaka,* Hirokazu Takahashi,* Toshihiko Mizuta,* Iwata Ozaki,* Takahisa Eguchi,¶ Yuki Kimura,† Kazuma Fujimoto* and Keizo Anzai*; for the Japan Nonalcoholic Fatty Liver Disease Study Group (JSG-NAFLD)

*Department of Internal Medicine and †Division of Hepatology, Saga Medical School, Saga, ‡Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, §Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, and ¶Department of Clinical Gastroenterology, Eguchi Hospital, Ogi, Japan

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Correspondence
Professor Yuichiro Eguchi, Division of Hepatology, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan. Email: eguchiyu@cc.saga-u.ac.jp

Abstract

Background and Aims: The pathogenesis of non-alcoholic fatty liver disease (NAFLD) is now focusing on its organ cross-talk with not only adipose tissue but also systemic skeletal muscle. Cross-sectional and longitudinal studies were conducted to determine the role of intramuscular adipose tissue content (IMAC) measured by computed tomography on the severity of NAFLD/non-alcoholic steatohepatitis (NASH).

Methods: Two hundred eight Japanese patients with NAFLD/NASH diagnosed by liver biopsy were enrolled into a cross-sectional study. Twenty-one patients were enrolled in a longitudinal study and received a programmed diet and exercise intervention, in some cases the combination of pharmacotherapy. We measured IMAC in the multifidus muscle and biochemical parameters, and conducted liver histology to assess NAFLD/NASH status.

Results: Histopathological stage in terms of simple steatosis and Brunt's classification was significantly correlated with IMAC ($P < 0.01$). Multivariate logistic regression analysis indicated that risk factors associated with the severity of NASH were IMAC and aging (IMAC: odds ratio = 2.444, $P < 0.05$; Age: odds ratio = 2.355, $P < 0.05$). The interventions improved histopathological changes in 11 patients with NASH as well as IMAC.

Conclusion: These results suggest that skeletal muscle fat accumulation may have been linked to the pathogenesis and severity of NASH.

Introduction

The National Cholesterol Education Program Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) and International Diabetes Federation have proposed diagnostic criteria for metabolic syndrome[1,2] that are now widely used in clinical research. Generally, studies of metabolic syndrome have shown that the prevalence of obesity has progressively increased in Japan in association with increasing adoption of Westernized lifestyles.

Visceral fat accumulation is considered to be one of the main risk factors for metabolic syndrome.[3–5] Abdominal computed tomography (CT) is commonly used to determine hepatic lipid deposition as well as visceral fat accumulation. We previously reported that visceral fat accumulation is correlated with the grade of lipid deposition in hepatocytes in patients with chronic hepatitis C and non-alcoholic fatty liver disease (NAFLD).[6–10] We have also developed a quantitative method to evaluate steatosis (intramuscular adipose tissue content [IMAC]) in the lumber multifidus muscle by abdominal CT as follows: IMAC = region of interest (ROI) of the multifidus muscle (Hounsfield units)/ROI of subcutaneous fat (Hounsfield units). Using this method, we investigated the association between visceral fat and skeletal muscle, and liver of NAFLD.[10]

We have reported that patients with NAFLD showed greater increases in IMAC compared with healthy individuals. We also found that IMAC improved significantly following improvements of insulin resistance, visceral fat accumulation, and hepatic lipid deposition achieved by diet and exercise interventions.[10]

The multifidus muscle was chosen in the present study. The reasons why this study focused on this muscle were as follows: (i) this muscle supported the trunk during extension, folding, and rotation of the upper body; (ii) this muscle was shown on CT at the umbilical level, which is used to quantify visceral fat accumulation; and (iii) it was possible to estimate the effects of exercise therapy.

Recently, it is well known that visceral fat accumulation induce peripheral organ such as skeletal muscle, liver, and myocardium as

"ectopic fat."[11–13] And, several reports have focused on the relationship between skeletal muscle fat accumulation and NAFLD/non-alcoholic steatohepatitis (NASH).[14–16] Therefore, the aims of the present study were to: (i) measure IMAC in the multifidus muscle on abdominal CT in patients with NAFLD or NASH; (ii) evaluate the relationship between IMAC and other markers, pathological severity of NASH; and (iii) determine whether therapeutic interventions (diet and exercise, or combination of medication) could improve IMAC of the multifidus muscle.

Methods

Patients. A total of 208 consecutive Japanese patients were attended Eguchi Hospital, Saga Medical School, Hiroshima University Hospital, or Nara City Hospital between January 2004 and April 2010 for the treatment of NAFLD were enrolled into the present studies. All of the patients had undergone biopsies at centers for digestive and liver diseases at each hospital. In this study, patients with evidence of excessive alcohol intake (> 20 g/day), other causes of liver diseases (e.g. viral hepatitis, autoimmune liver disease, biliary disease, liver cirrhosis, and hepatocellular carcinoma), or being treated with antihypertensive or antidiabetic agents were excluded. All of the patients underwent abdominal CT to measure IMAC as a marker for muscle steatosis.[10] All of the patients provided written informed consent, and the study protocol was approved by institutional review boards at each hospital.

Physical examination and serum biochemistry. Bodyweight and height were measured in all subjects. Body mass index (BMI) was calculated as bodyweight in kilograms divided by the square of the height in meters. Venous blood samples were taken from all subjects at around 09:00 h after a 12-h overnight fast. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), total cholesterol, triglyceride, albumin, fasting plasma glucose (FPG), and plasma insulin concentrations were determined by enzyme immunoassays.

Estimate of insulin resistance was calculated the homeostasis model of assessment-insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI). HOMA-IR reflects hepatic insulin resistance, whereas QUICKI reflects skeletal muscle insulin resistance.[17,18] The HOMA-IR was calculated using the formula: fasting plasma insulin × FPG/405, and the QUICKI was calculated using the formula: 1/(log fasting plasma insulin + log FPG). Based on a review of the literature, the following scores were calculated for each patient: FIB4 index,[19,20] NAFIC score.[21]

Liver histology. Liver biopsy specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin and Azan for histological evaluation. All liver biopsy specimens were reviewed by experienced hepatologists (Y.E and Y.S.) who were blinded to the patient's clinical status. Adequate liver biopsy samples were defined as samples > 1 cm long and/or ≥ 6 portal tracts. NASH was defined as steatosis with lobular inflammation and ballooning degeneration with or without Mallory–Denk bodies or fibrosis.[22,23] The histological findings of NASH were interpreted and scored by activity grade and fibrotic stage according to the classification system proposed by Brunt *et al.*[24] Hepatitis disease activity (i.e. necroinflammatory grade) was determined from the composite NAFLD activity score (NAS) as described by Kleiner *et al.*[25] NAS is the unweighted sum of the scores for steatosis, lobular inflammation, and hepatocellular ballooning, and ranges from 0 to 8. NAS < 3 is defined as "simple steatosis," NAS 3 or 4 is defined as "borderline NASH," and NAS ≥ 5 is defined as "definite NASH." If liver histology was too atypical to make a judgment, cases with an NAS of ≥ 5 were considered to have NASH. The severity of hepatic fibrosis (i.e. stage) was defined as follows: stage 1, zone 3 perisinusoidal fibrosis; stage 2, zone 3 perisinusoidal fibrosis with portal fibrosis; stage 3, zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis; and stage 4, cirrhosis.

Abdominal CT protocol and assessment. Unenhanced spiral acquisition of the liver was obtained during a breathhold at 5.0 mm collimation, 15.0 mm/rotation table speed (HQ mode, pitch 1:3), 120 kV (p), and auto mA (Bright Speed ELITE SD; GE Healthcare, Waukesha, WI, USA). Images were reconstructed at 10-mm intervals. All patients underwent abdominal CT in the morning after a 12-h overnight fast. On CT, ROIs of 40 mm^2 were placed along the periphery of the liver and the spleen, away from major vessels, at five points in each organ. The mean values of the five ROIs (Hounsfield units) were used to determine the liver-spleen (L/S) ratio as an index of hepatic fat accumulation.[26,27] Subcutaneous fat area (SFA; cm^2) and visceral fat area (VFA; cm^2) were measured at the umbilical level and were calculated using Fat Scan software (N2 System Co., Osaka, Japan).[28] Visceral obesity was defined as VFA ≥ 100 cm^2.[29]

CT analysis of the multifidus muscle. Subfascial muscular tissue in the multifidus muscle in an umbilical-level CT cross-sectional image was precisely traced, and CT values (in Hounsfield units) and area (cm^2) were measured using Advantage Workstation 4.1 software (GE Healthcare). CT values were measured for five 60 mm^2 ROIs on subcutaneous fat away from major vessels, and the mean values were used to determine the multifidus muscle/fat attenuation ratio.[10]

Longitudinal assessment of IMAC, histopathological changing, and other parameters. Patients with NASH diagnosed by liver biopsy received lifestyle intervention. The target of NAFLD/NASH treatment dietary energy intake was defined as standard bodyweight × 25–30 kcal, and exercise therapy was performed to achieve a target of 23 metabolic equivalent tasks (METs) × h/week (physical activity) + 4 METs × h/week (exercise).[9] After intervention, patients were divided into two groups according to whether or not IMAC was improved by diet and exercise therapy. To evaluate whether improvements in IMAC affected the histological findings, histopathological changes determined by Matteoni's and Brunt's classifications were compared between a group with the improvements in IMAC and a group with the non-improvements in IMAC.[22,24]

Clinical cases. The multifidus muscle of a 45-year-old healthy man is shown in Figure 1a. This individual showed no muscle steatosis (IMAC = –0.44). Figure 1b shows the multifidus muscle of a 45-year-old man with simple steatosis (Matteoni's classification = 2). The muscle in this individual shows increased muscle fat storage (IMAC = –0.26). Figure 1c shows the multifidus muscle of a 45-year-old man with NASH (Matteoni's classification = 3, Brunt's staging = 3), with extensive muscle fat storage (IMAC = –0.08).

Statistical analysis. Descriptive statistics (means and standard deviations) were calculated for all continuous variables, and frequencies were calculated for categorical variables. Differences between two groups were compared by the Mann–Whitney *U*-test. Pearson's correlation coefficient analysis and Spearman's correlation by rank analysis were used to compare IMAC and FIB4 index or NAFIC score. Differences and correlations among five groups were performed by Kruskal–Wallis analysis of variance followed by the Scheffe and Tukey–Kramer post-hoc test. Comparisons between before and after NASH therapy were tested using the Wilcoxon signed-rank test.

Effects of improvements in IMAC were evaluated by changes in the variable (Δ = variable after intervention – variable before intervention). Spearman's rank correlation was used to compare the Δ IMAC and each Δ parameters.

Multivariate logistic regression analysis was used to identify independent factors associated with the severity of NAFLD and NASH stage. Differences were considered significant at $P < 0.05$. All analyses were carried out using IBM SPSS (Version 19.0; SPSS, Inc., Tokyo, Japan).

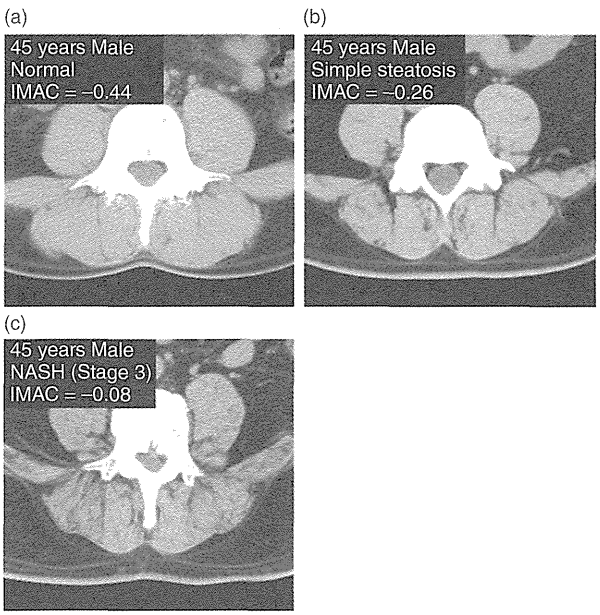

Figure 1 Cross-sectional computed tomographic images of the subfascial muscular tissue in the multifidus muscle taken at the umbilical level in a normal individual (a), a patients with simple steatosis (b), and a patient with non-alcoholic steatohepatitis (c).

Results

Characteristics of the patients with NAFLD. The clinical and biochemical characteristics of the patients enrolled in this study are summarized in Table 1. There were 208 patients (95 women and 113 men) with a mean age of 51 years; the women were significantly older than men. Bodyweight, VFA, ALT, γ-GTP, and serum albumin were significantly higher in men than in women, whereas ALP, L/S ratio, and IMAC were significantly higher in women than in men.

Characteristics of patients with NAFLD defined according to Matteoni's classification. BMI, AST, ALT, FPG, insulin, HOMA-IR, and IMAC were significantly higher in patients with types 3–4 NAFLD than in patients with types 1–2 NAFLD. By contrast, γ-GTP, QUICKI, and platelet count were significantly higher in patients with types 1–2 NAFLD than in those with types 3–4 NAFLD. There were no significant differences in the other characteristics between the two groups of patients (Table 2). As indicated in Table 3, multivariate logistic regression analysis indicated that risk factors associated with the severity of NAFLD were IMAC, aging, ALT, and HOMA-IR

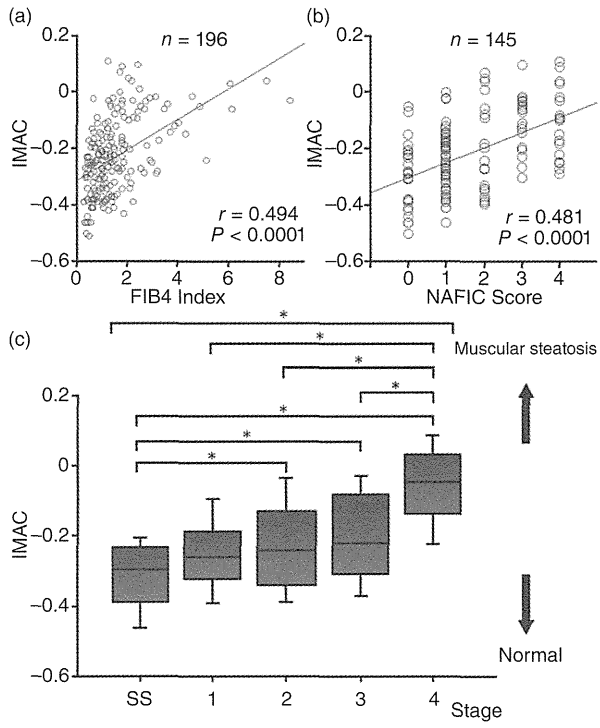

Figure 2 Intramuscular adipose tissue content (IMAC) was correlated with FIB4 index (a: $r = 0.494$, $P < 0.01$) and NAFIC score (b: $r = 0.481$, $P < 0.01$). (c) Comparison between IMAC and histopathological stage determined using Matteoni's and Brunt's classification. Histopathological stage determined by both classifications was significantly correlated with IMAC (*$P < 0.01$).

Table 1 Baseline characteristics of the patients with NAFLD subjects

Variables	All patients ($n = 208$)	Males ($n = 113$)	Females ($n = 95$)	P value
Age (years)	51.0 ± 15.2	44.7 ± 14.2	58.6 ± 12.7	< 0.0001
Weight (kg)	74.1 ± 16.9	81.8 ± 16.7	64.9 ± 11.7	< 0.0001
BMI (kg/m^2)	28.3 ± 4.6	28.9 ± 4.9	27.7 ± 4.2	0.1432
VFA (cm^2)	149.0 ± 55.3	159.6 ± 58.4	136.5 ± 48.6	< 0.01
AST (IU/L)	49.4 ± 34.5	48.3 ± 36.3	50.7 ± 32.3	0.2762
ALT (IU/L)	77.0 ± 60.0	84.3 ± 64.3	68.4 ± 53.5	< 0.05
ALP (IU/L)	263.0 ± 137.1	256.0 ± 169.8	271.3 ± 82.8	< 0.01
γ-GTP (IU/L)	89.2 ± 96.0	105.0 ± 115.2	70.4 ± 60.6	< 0.01
TC (mg/dL)	209.4 ± 43.3	207.5 ± 45.8	211.5 ± 40.3	0.2093
TG (mg/dL)	180.8 ± 135.9	191.3 ± 156.4	168.1 ± 105.4	0.0704
FPG (mg/dL)	113.8 ± 35.1	109.5 ± 31.2	118.9 ± 38.9	0.0913
Insulin (μg/mL)	15.6 ± 10.3	17.0 ± 12.5	13.8 ± 6.0	0.4907
Insulin resistance				
HOMA-IR	4.4 ± 3.3	4.6 ± 3.7	4.1 ± 2.5	0.9278
QUICKI	0.32 ± 0.04	0.32 ± 0.04	0.32 ± 0.03	0.9187
Platelet count (× 10^4/μL) ($n = 196$)	22.9 ± 6.9	22.5 ± 6.3	23.4 ± 7.5	0.5768
Serum albumin (g/dL)	4.6 ± 0.4	4.7 ± 0.4	4.4 ± 0.4	< 0.01
L/S ratio	0.83 ± 0.31	0.76 ± 0.28	0.92 ± 0.31	< 0.0001
IMAC	−0.23 ± 0.13	−0.31 ± 0.10	−0.14 ± 0.11	< 0.0001

Data are means ± standard deviation.

Statistical analysis was performed using the Mann–Whitney *U*-test. Differences were considered significant at $P < 0.05$.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

(IMAC: OR = 3.671, $P < 0.05$; Age: OR = 2.895, $P < 0.05$; ALT = 6.222, $P < 0.01$; HOMA-IR = 3.035, $P < 0.05$).

Characteristics of patients with NASH defined according to Brunt's classification. Age and AST were significantly higher in patients with stages 3–4 NASH than in those with stages 1–2 NASH. By contrast, IMAC was significantly greater in patients with stages 3–4 than in those with stages 1–2 NASH. There were no significant differences in the other factors between the two groups of patients (Table 2). As shown in Table 4, multivariate logistic regression analysis indicated that risk factors associated with the severity of NASH were IMAC and aging (IMAC: OR = 2.444, $P < 0.05$; Age: OR = 2.355, $P < 0.05$).

Comparing the IMAC to severity model of NAFLD/NASH and histopathological stage. IMAC and severity model of NAFLD/NASH in each groups of patients are compared in Figure 2a,b. IMAC was correlated with FIB4 index ($r = 0.494$, $P < 0.01$) and NAFIC score ($r = 0.481$, $P < 0.01$). IMAC and histopathological stage in both groups of patients are compared in Figure 2c. Histopathological activity determined by simple steatosis and Brunt's classification was significantly correlated with IMAC ($P < 0.01$).

Effects of lifestyle interventions on IMAC in patients with NASH. The effects of the prescribed treatment on the clinical characteristics of 21 patients with NASH (13 women and 8 men) are shown in Table 5. The mean intervention period at 24.0 months, in some cases, was administering pharmacotherapy with a sulfonylurea, α-glucosidase inhibitor, or hypocholesterolemic drug. Overall, 11 patients showed improvements in IMAC during the treatment period. These patients also showed significant decreases in bodyweight, BMI, SFA, ALT, γ-GTP, total cholesterol, triglyceride, FPG, 180-PG, Insulin, and HOMA-IR following treatment, while the QUICKI and L/S ratio increased significantly. The histopathological changes determined by Matteoni's and Brunt's classifications and NAS have been improved in comparison with pretreatment biopsy. By contrast, among patients with no improvement in IMAC, improvement of total cholesterol was observed, but platelet count decreased significantly, and no significant changes were found in the other factors. The histopathological changes and NAS were not improved in comparison with pretreatment biopsy.

Effects improvements in IMAC on other parameters. As shown in Table 6, the ΔIMAC was significantly correlated with Δ weight ($\rho = 0.507$, $P < 0.05$), Δ BMI ($\rho = 0.512$, $P < 0.05$), Δ SFA ($\rho = 0.653$, $P < 0.01$), Δ triglyceride ($\rho = 0.458$, $P < 0.05$), Δ FPG ($\rho = 0.678$, $P < 0.01$), Δ Insulin ($\rho = 0.466$, $P < 0.05$), Δ HOMA-IR ($\rho = 0.493$, $P < 0.05$), Δ QUICKI ($\rho = -0.571$, $P < 0.01$), Δ L/S ratio ($\rho = -0.714$, $P < 0.01$), histopathological assessments (Δ steatosis, $\rho = 0.800$; Δ lobular inflammation, $\rho = 0.686$; Δ ballooning, $\rho = 0.769$; $P < 0.01$ for each), and Δ NAS ($\rho = 0.800$, $P < 0.01$). The Δ IMAC evaluation did not indicate a correlation between Δ VFA, Δ ALT, Δ total cholesterol, Δ platelet count, and Δ serum albumin.

Table 2 Comparison of biochemical parameters according to stage of NAFLD/NASH determined by Matteoni's and Brunt's classifications

Variables	Simple steatosis ($n = 38$)	NASH ($n = 170$)	*P* value*	Stage 1–2 ($n = 104$)	Stage 3–4 ($n = 66$)	*P* value**
Sex (male/female)	24/14	89/81	0.2278	58/46	31/35	0.2656
Age (years)	47.2 ± 15.6	51.9 ± 15.0	0.0829	49.2 ± 14.5	56.2 ± 14.8	< 0.01
Weight (kg)	72.8 ± 15.6	74.3 ± 17.2	0.7317	75.4 ± 17.4	72.7 ± 16.9	0.4204
BMI (kg/m^2)	26.9 ± 4.1	28.7 ± 4.7	< 0.05	28.8 ± 4.9	28.4 ± 4.4	0.7993
VFA (cm^2)	134.2 ± 40.4	149.4 ± 54.0	0.6732	149.4 ± 54.0	157.0 ± 63.2	0.6732
AST (IU/L)	39.3 ± 26.0	51.7 ± 39.8	< 0.01	49.7 ± 39.8	54.9 ± 28.3	< 0.01
ALT (IU/L)	61.6 ± 50.8	80.4 ± 69.8	< 0.01	83.2 ± 69.8	76.1 ± 45.7	0.7363
ALP (IU/L)	236.6 ± 62.2	268.8 ± 148.0	0.1754	251.1 ± 77.1	296.4 ± 214.6	0.0964
γ-GTP (IU/L)	93.5 ± 136.2	88.3 ± 85.2	< 0.05	92.5 ± 99.5	81.8 ± 56.2	0.9025
TC (mg/dL)	221.2 ± 40.1	206.7 ± 43.6	0.0548	210.8 ± 42.9	200.6 ± 44.3	0.0892
TG (mg/dL)	156.8 ± 72.6	186.3 ± 146.1	0.5567	189.7 ± 166.8	180.8 ± 107.0	0.8761
FPG (mg/dL)	103.0 ± 35.1	116.2 ± 34.8	< 0.01	114.3 ± 34.4	119.3 ± 35.4	0.2988
Insulin (μg/mL)	10.8 ± 7.8	16.7 ± 10.5	< 0.0001	17.0 ± 11.8	16.2 ± 7.7	0.6029
Insulin resistance						
HOMA-IR	2.8 ± 2.5	4.8 ± 3.3	< 0.0001	4.8 ± 3.5	4.7 ± 2.9	0.7002
QUICKI	0.35 ± 0.05	0.32 ± 0.03	< 0.0001	0.32 ± 0.03	0.32 ± 0.03	0.7260
Platelet count (× 10^4/μL) ($n = 196$)	24.9 ± 6.5	22.4 ± 6.9	< 0.05	23.3 ± 6.9	21.0 ± 6.8	0.0894
Serum albumin (g/dL)	4.5 ± 0.4	4.6 ± 0.4	0.2087	4.6 ± 0.4	4.5 ± 0.5	0.1806
L/S ratio	0.89 ± 0.34	0.82 ± 0.30	0.2056	0.79 ± 0.30	0.86 ± 0.29	0.1392
IMAC	−0.31 ± 0.11	−0.22 ± 0.13	< 0.01	−0.24 ± 0.12	−0.18 ± 0.14	< 0.05
Histopathological assessment						
Fibrosis (0/1/2/3/4)	(38/0/0/0/0)	(0/36/68/58/8)		(0/36/68/0/0)	(0/0/0/58/8)	

*P values are shown for comparisons between simple steatosis and NASH; **P values are shown for comparisons between stages 1–2 and 3–4. Data are means ± standard deviation.

Statistical analysis was performed using the Mann–Whitney U-test. Differences were considered significant at $P < 0.05$.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

Table 3 Multivariate analyses of clinical factors associated with the severity of NAFLD (SS *vs* NASH) in Matteoni's and Brunt's classifications

Variables	OR	95% CI	*P* value
IMAC (SS *vs* NASH)	3.671	1.084–11.722	< 0.05
Age (males > 45 years, females > 50 years)	2.895	1.045–8.021	< 0.05
Sex (male *vs* female)	1.683	0.604–4.687	0.3192
ALT (≥ 50)	6.224	2.009–19.277	< 0.01
HOMA-IR (≥ 2.2)	3.035	1.084–8.493	< 0.05
L/S ratio (< 0.9)	0.715	0.239–2.144	0.5498
VFA (≥ 100 mm^2)	0.671	0.201–2.234	0.5154

ALT, alanine aminotransferase;CI, confidence interval; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; SS, simple steatosis; VFA, visceral fat area.

Table 4 Multivariate analyses of clinical factors associated with the severity of NASH (stage1–2 *vs* 3–4) in Matteoni's and Brunt's classifications

Variables	OR	95% CI	*P* value
IMAC (stage 1–2 *vs* 3–4)	2.444	1.118–5.341	< 0.05
Age (males > 45 years, females > 55 years)	2.355	1.042–5.321	< 0.05
Sex (male *vs* female)	1.577	0.719–3.457	0.2554
ALT (≥ 50)	1.864	0.766–4.536	0.1699
HOMA-IR (≥ 2.2)	0.847	0.314–2.287	0.7438
L/S ratio (< 0.9)	1.187	0.501–2.810	0.6964
VFA (≥ 100 mm^2)	1.160	0.575–4.457	0.3675

ALT, alanine aminotransferase; CI, confidence interval; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NASH, non-alcoholic steatohepatitis; OR, odds ratio; VFA, visceral fat area.

Discussion

In this study, we objectively evaluated skeletal muscle steatosis by measuring IMAC and found that skeletal muscle steatosis increased significantly with increasing stage of NASH. Multivariate analysis also suggested that there was a relationship between the stage of NASH and IMAC. These results suggest the presence of a physiological link between the liver and skeletal muscle in patients with NASH.

IMAC is significantly different between female and male. We hypothesized that gender differences in the IMAC may be affected by type of muscle fibers. Muscle fibers are classified into type I, type IIa, and type IIb fibers. Previous studies have reported that the

Table 5 Characteristics of all patients with NASH at baseline, and of patients with/without improvements in IMAC before and after the intervention

Variables	All patients ($n = 21$)	Patients with improvements in IMAC ($n = 11$)			Patients with no improvement in IMAC ($n = 10$)		
	Baseline	Before	After	*P* value	Before	After	*P* value
Sex (male/female)	8/13	4/7	4/7		4/6	4/6	
Age (years)	52.4 ± 16.9	48.9 ± 13.5	50.2 ± 13.7	< 0.01	56.2 ± 19.9	59.3 ± 20.6	< 0.01
Weight (kg)	73.5 ± 16.8	77.5 ± 14.8	72.3 ± 15.6	< 0.05	69.0 ± 18.6	68.0 ± 17.7	0.4838
BMI (kg/m^2)	28.7 ± 4.1	30.1 ± 4.1	28.1 ± 4.7	< 0.05	27.3 ± 3.7	26.9 ± 3.4	0.4833
VFA (cm^2)	144.6 ± 47.7	136.3 ± 33.2	113.8 ± 54.2	0.0754	153.6 ± 60.5	144.8 ± 51.5	0.9594
SFA (cm^2)	254.3 ± 98.3	298.7 ± 100.0	261.7 ± 116.9	< 0.05	205.4 ± 72.8	205.7 ± 84.3	0.9594
ALT (IU/L)	104.1 ± 97.0	90.5 ± 63.5	31.4 ± 14.0	< 0.01	119.0 ± 126.4	70.8 ± 50.0	0.1392
γ-GTP (IU/L)	91.6 ± 78.0	81.1 ± 40.5	34.5 ± 18.5	< 0.01	103.1 ± 106.9	88.9 ± 112.2	0.2839
TC (mg/dL)	233.8 ± 43.9	249.7 ± 37.9	198.9 ± 43.5	< 0.01	216.2 ± 45.1	176.7 ± 28.2	< 0.05
TG (mg/dL)	187.2 ± 123.3	215.6 ± 159.0	102.9 ± 35.9	< 0.01	155.9 ± 60.1	155.9 ± 90.5	0.8785
FPG (mg/dL)	117.6 ± 37.1	129.0 ± 44.3	113.6 ± 43.1	< 0.05	105.0 ± 23.4	110.2 ± 17.8	0.0926
60-PG (mg/dL)	230.1 ± 65.5	234.9 ± 68.6	163.6 ± 61.0	0.0910	225.2 ± 66.0	209.0 ± 48.7	0.4618
180-PG (mg/dL)	155.7 ± 55.4	160.5 ± 47.3	106.0 ± 31.0	< 0.05	150.1 ± 67.1	156.8 ± 58.9	0.7150
Insulin (μg/mL)	16.8 ± 6.9	18.2 ± 7.0	11.1 ± 6.1	< 0.05	15.3 ± 6.3	18.3 ± 11.7	0.3329
Insulin resistance							
HOMA-IR	5.1 ± 3.0	6.0 ± 3.3	3.3 ± 2.3	< 0.05	4.1 ± 2.4	4.9 ± 2.9	0.2411
QUICKI	0.31 ± 0.03	0.30 ± 0.03	0.33 ± 0.04	< 0.05	0.32 ± 0.04	0.31 ± 0.03	0.0926
Platelet count (× 10^4/μL)	22.7 ± 5.7	24.8 ± 5.3	24.8 ± 7.3	0.7211	20.4 ± 5.5	18.9 ± 5.7	< 0.05
Serum albumin (g/dL)	4.7 ± 0.4	4.6 ± 0.5	4.4 ± 0.2	0.5606	4.8 ± 0.4	4.7 ± 0.4	0.6771
L/S ratio	0.78 ± 0.32	0.61 ± 0.27	1.07 ± 0.12	< 0.01	0.97 ± 0.25	0.93 ± 0.25	0.1846
IMAC	−0.21 ± 0.15	−0.20 ± 0.16	−0.25 ± 0.18	< 0.01	−0.21 ± 0.14	−0.17 ± 0.15	< 0.01
Histopathological assessment							
Fibrosis (0/1/2/3/4)	(0/5/6/10/0)	(0/2/4/5/0)	(1/3/6/1/0)	0.0702	(0/3/2/5/0)	(0/2/4/3/1)	0.5637
Steatosis (0/1/2/3)	(0/9/5/7)	(0/1/5/5)	(1/7/2/1)	< 0.05	(0/8/0/2)	(0/6/2/2)	0.1573
Lobular inflammation (0/1/2/3)	(0/10/8/3)	(0/2/7/2)	(2/8/0/1)	< 0.01	(0/8/1/1)	(0/6/3/1)	0.3173
Ballooning (0/1/2)	(0/9/12)	(0/1/10)	(1/8/2)	< 0.01	(0/8/2)	(0/3/7)	< 0.05
NAS	5.1 ± 1.7	6.7 ± 0.5	3.0 ± 1.0	< 0.01	3.3 ± 0.7	4.3 ± 0.9	< 0.05

Data are means ± standard deviation. *P* values are for comparisons between types 1–2 and 3–4. Statistical analysis was performed by Wilcoxon signed-rank test, and differences were considered significant at $P < 0.05$. 60-PG, 60 min post-challenge plasma glucose; 180-PG, 180 min post-challenge plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; QUICKI, quantitative insulin sensitivity check index; SFA, subcutaneous fat area; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

a type IIb fibers in obese women had increased than in a type I fiber, and exercise therapy transformed muscle fibers from type IIb to type IIa.[30–32] However, the distribution of fiber types is unclear in a gender; in addition, our study did not perform histopathological evaluation. Further studies are needed to the distribution of fiber types related to the IMAC.

A previous study demonstrated that the intramuscular lipid content in the femoral biceps determined by ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) was associated with aging and obesity.[33]

Previous other studies have demonstrated a relationship between liver and skeletal muscle steatosis determined by ^{1}H-MRS in patients with NAFLD or type 2 diabetes and discusses the significance of diet and exercise therapy.[34–36] Our results using IMAC determined by CT are agreement with these earlier reports phenomenologically, although there was no comparative study between ^{1}H-MRS and CT for evaluation of skeletal muscle steatosis.

Several other reports have demonstrated a relationship between NAFLD and the risk of cardiac diseases, as well as physiological links between visceral fat accumulation, the liver, and muscle steatosis in metabolic syndrome, suggesting that these factors are associated with the risk of cardiac diseases.[15,37] However, a relationship between the severity of fibrosis in NASH and skeletal muscle has not yet been demonstrated.

Previous studies have reported that the composition of muscle tissue and the physiological activity of cells are influenced by exercise and weight control in patients with type 2 diabetes mellitus and NAFLD.[14,34,35,38] It was also reported that muscle atrophy is caused by an age-associated decrease in exercise, coupled with a subsequent increase in interstitial adipose tissue and the deposition of lipids, primarily neutral fat, in skeletal muscle cells.[10,39] Studies using mice have reported that skeletal muscle glucose absorption is associated with glucose tolerance and insulin resistance.[40,41] Our study showed that IMAC has improved in patients with a significant amelioration in glucose metabolism in the post-challenge 180-min plasma glucose with the amelioration of insulin resistance and histopathological changes. In patients with liver cirrhosis, abnormal glucose tolerance and insulin resistance were reported to be related to abnormal glucose transporter type 4 (GLUT4) expression in skeletal muscle.[42] GLUT4 is an insulin-regulated glucose transporter expressed in adipose tissue and skeletal muscle, and is primarily responsible for insulin-stimulated glucose uptake into cells.

Table 6 Relationships between Δ IMAC and Δ parameters in the intervention patients

Variables	All patients ($n = 21$)	
	ρ	P
Δ Weight (kg)	0.507	< 0.05
Δ BMI (kg/m^2)	0.512	< 0.05
Δ VFA (cm^2)	0.307	0.1739
Δ SFA (cm^2)	0.653	< 0.01
Δ ALT (IU/L)	0.227	0.3194
Δ γ-GTP (IU/L)	0.420	0.0625
Δ TC (mg/dL)	−0.013	0.9325
Δ TG (mg/dL)	0.458	< 0.05
Δ FPG (mg/dL)	0.678	< 0.01
Δ Insulin (μg/mL)	0.466	< 0.05
Insulin resistance		
Δ HOMA-IR	0.493	< 0.05
Δ QUICKI	−0.571	< 0.01
Δ Platelet count (× 10^4/μL)	−0.162	0.4514
Δ Serum albumin (g/dL)	−0.070	0.7077
Δ L/S ratio	−0.714	< 0.001
Histopathological assessment		
Δ Fibrosis (0/1/2/3/4)	0.419	0.0870
Δ Steatosis (0/1/2/3)	0.800	< 0.001
Δ Lobular inflammation (0/1/2/3)	0.686	< 0.01
Δ Ballooning (0/1/2)	0.769	< 0.001
Δ NAS	0.800	< 0.001

Data are means ± standard deviation. Δ = variable after intervention − variable before intervention. Spearman's rank correlation was used to correlate continuous or discrete variables $P < 0.05$.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; IMAC, intramuscular adipose tissue content; L/S ratio, liver-spleen attenuation ratio; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; QUICKI, quantitative insulin sensitivity check index; SFA, subcutaneous fat area; TC, total cholesterol; TG, triglyceride; VFA, visceral fat area; γ-GTP, γ-glutamyl transpeptidase.

Recently, it was proposed that physiologically active materials derived from skeletal muscle, such as interleukin-6 (IL-6), should be referred to as myokines. IL-6 released from skeletal muscle in response to exercise was reported to enhance glucose absorption via GLUT4 by increasing phosphatidylinositol 3-kinese expression in the muscle. Additionally, IL-6 was also reported to enhance 5′ adenosine monophosphate-activated protein kinase-regulated lipid oxidation by increasing the expression of signal transducer and activator of transcription 3 (STAT3), and increase hepatic glucose absorption.[43,44] Several studies demonstrated that exercise therapy improved not only liver function and insulin resistance but also IL-6 in NAFLD/NASH patients.[32,45] However, IL-6 concentrations are chronically increased in obese and insulin-resistant patients and may induce insulin resistance.[46] Recently, IL-6 was reported to increase the expression of insulin receptor substrate 2 and enhance insulin sensitivity by activating STAT3.[47] According to that study, if IL-6 is continuously increased, STAT3 is not activated because of negative feedback exerted by suppressor of cytokine signaling 3, for example, a signaling pathway that can even induce insulin resistance.[47]

Our present results are generally in agreement with these earlier reports. Dietary and exercise therapy increases fatty acid oxidation and glucose absorption in skeletal muscle, and alleviates skeletal muscle steatosis and insulin resistance. We previously reported that visceral fat accumulation and skeletal muscle steatosis are independent factors for the severity of fatty liver.[10] We believe that controlling visceral fat accumulation and muscle steatosis by diet and exercise therapy enhances hepatic insulin sensitivity, attenuates fatty liver, and reverses liver fibrosis.

However, our study has several limitations that should be discussed. First, the severity of NASH and skeletal muscle steatosis must be evaluated serially. We must also determine the differences in skeletal muscle steatosis between patients with or without the severity of NASH must be clarified. The relationship between myokines that affect skeletal muscle steatosis, such as IL-6, and the severity of liver fibrosis must also be evaluated. In conclusion, our study suggested that substitution of adipose tissue in skeletal muscle was associated with the severity of NAFLD/NASH. From these results, we think that interventions aimed at improved hepatic status, as well as systemic metabolic disorders, should be considered when developing new therapeutic strategies for NASH/NAFLD.

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Original Article

Evaluation narcotic analgesic use and survival time in terminal stage liver diseases compared with lung cancer: a retrospective chart review

Shunya Nakashita,[1,3] Yuichiro Eguchi,[2,3,*] Toshihiko Mizuta,[1] Shigetaka Kuroki,[3] Naofumi Ono,[3] Takahisa Eguchi,[3] Keizo Anzai[1] and Kazuma Fujimoto[1]

[1]Department of Internal Medicine and [2]Department of Liver Disease Control and Assistance Task Force, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan
[3]Eguchi Hospital, 1054-2 Kanada, Mikatsuki-cho, Ogi, Saga 845-0032, Japan

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Hepatocellular carcinoma (HCC) and liver cirrhosis are fatal diseases. This study aimed to investigate survival time and palliative care in terminal HCC and/or liver cirrhosis compared with lung cancer. Between January 2004 and December 2010, we enrolled 116 patients with terminal cirrhosis and/or HCC or lung cancer admitted to a municipal hospital in Japan; 48 had liver cirrhosis, 35 HCC and 33 lung cancer. By retrospective chart review, we evaluated: (i) rate of usage of narcotic analgesics and (ii) survival time from onset of coma (Glasgow Coma Scale less than 8). Time between coma and death was significantly shorter in the liver disease patients (cirrhosis and/or HCC: 7.0 h) than in lung cancer (44.0 h, $p = 0.045$). Total bilirubin was higher in HCC compared with cirrhosis ($p<0.01$). Rate of usage of narcotic analgesics was higher in lung cancer (20/33: 60.6%) than in liver disease (17/83: 20.5%, $p<0.01$); analgesics were used more frequently in HCC than in liver cirrhosis ($p<0.01$). These results suggest that liver cirrhosis and HCC patients do not always require palliative care and that survival time from onset of coma due to liver disease was not prolonged compared with lung cancer.

Key Words: **hepatocellular carcinoma, liver cirrhosis, palliative care, coma, narcotic analgesics**

Hepatocellular carcinoma (HCC) is the sixth most common malignant disease and has the third highest mortality worldwide.[1] In Japan, more than 50% of cases of HCC are due to hepatitis C virus, approximately 45,000 patients are diagnosed with HCC each year and approximately 34,000 patients per year die with HCC.[2] Supportive care of HCC and/or liver cirrhosis, including nutritional support, has been improved recently, but it has not been clearly demonstrated whether the quality of life and prognosis of these patients have improved. Terminal care of HCC and/or liver cirrhosis is important; in Japan, terminal care is mainly provided in municipal hospitals rather than the hospital to which the patient was initially referred.

The questions most commonly asked by the patient and family during terminal care are "how long do I have?" and "how long does he/she have?", indicating that estimated survival time is an important issue in terminal care. The survival time between onset of coma and death might be shorter in HCC than in other malignant diseases and the usage rate of narcotic analgesics might be less, but these factors have not been investigated in previous studies.

This retrospective study in a municipal hospital evaluated: (i) the rate of usage of narcotic analgesics and (ii) the survival time from onset of coma due to HCC and/or liver cirrhosis. These factors were compared with lung cancer, which has a high mortality of approximately 70,000 per year in Japan.[3]

Materials and Methods

We enrolled patients with terminal cirrhosis, terminal HCC or terminal lung cancer with performance status (PS)[4] greater than 3 who were admitted to Eguchi Hospital between January, 2004 and December, 2010 because the family could no longer care for them. PS was determined as follows: 0 – fully active, able to perform all pre-disease activities without restriction; 1 – restricted in physically strenuous activities but ambulatory and able to perform light or sedentary tasks (e.g. light housework, office work); 2 – ambulatory and capable of all self-care but unable to work, active for more than 50% of waking hours; 3 – capable of only limited self-care, confined to bed or chair for more than 50% of waking hours; 4 – completely disabled and unable to perform self-care, confined to bed or chair; or 5 – dead. All patients gave informed consent that their treatment would be limited to terminal care and that resuscitation would not be attempted in an emergency. Narcotic analgesics were applied as required. We retrospectively evaluated the patients' main symptoms on admission, their consciousness level and the duration between onset of coma (Glasgow Coma Scale less than 8) and death.[5]

The data in Tables 1and 2 were evaluated by the chi-square test for independence, the Mann-Whitney U test or the Kruskal-Wallis test. The data in Fig. 1 and 2 were evaluated by the chi-square test. Statistical analysis was performed using IBM SPSS Statistics ver. 19. Differences were considered significant if the probability of the difference occurring by chance was less than 5 in 100 ($p<0.05$).

Results

The background characteristics of the patients are shown in Table 1. There were 48 cases of terminal liver cirrhosis, 35 cases of terminal HCC and 33 cases of terminal lung cancer. Patients in the lung cancer group were older (82.0 ± 11.0 years) than those in the two liver disease groups (69.5 ± 11.9 years and 72.0 ± 10.2 years, respectively, both $p<0.001$). Men predominated in both the lung cancer and the liver disease groups, but there was no significant difference in sex ratio among the groups. Duration of hospitalization did not differ among the three groups. Cause of

*To whom correspondence should be addressed.
E-mail: eguchiyu@cc.saga-u.ac.jp

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Table 1. Background characteristics of the liver disease and lung cancer patients

	Cirrhosis (n = 48)	HCC (n = 35)	Lung cancer (n = 33)	p value
Age (years)	69.5 ± 11.9	72.0 ± 10.2	82.0 ± 11.0	<0.001
Sex (Men/Women)	28/20	26/9	21/12	ns
Period of hospitalization (days)	42.5 ± 89.6	28.0 ± 99.3	33.0 ± 62.9	ns
Cause of cirrhosis (HBV/HCV/alcohol/others)	6/28/9/5	4/27/1/3	—	ns
Child-Pugh (A/B/C)	1/12/31	4/7/23	—	ns

HBV, hepatitis B virus; HBC, hepatitis C virus; ns, not significant.

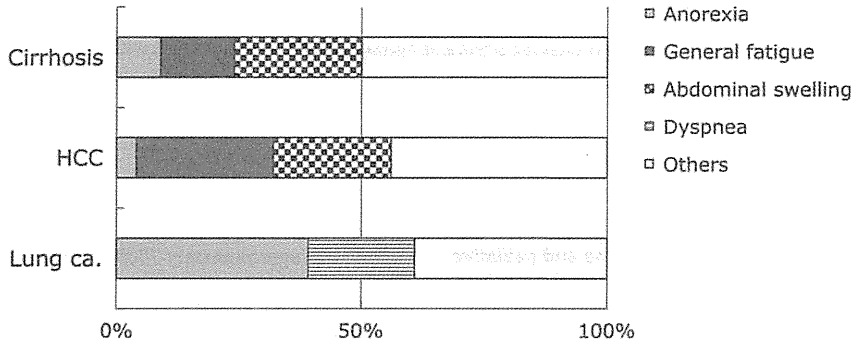

Fig. 1. Major complaint motivating hospitalization in liver cirrhosis, hepatocellular carcinoma (HCC) and lung cancer (Lung ca.).

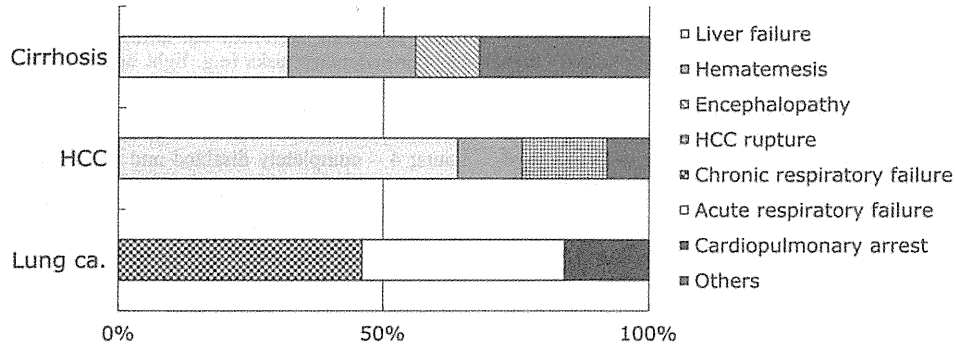

Fig. 2. Main cause of coma in liver cirrhosis, hepatocellular carcinoma (HCC) and lung cancer (Lung ca.).

liver cirrhosis and Child–Pugh class did not differ between the liver cirrhosis and HCC groups.

Fig. 1 shows the main complaints that led directly to hospitalization. In liver cirrhosis and HCC patients, these were general fatigue (15% and 28%, respectively) and abdominal swelling, mainly due to ascites (26% and 24%); the main complaints in the lung cancer patients were anorexia (38%) and dyspnea (21%).

Fig. 2 shows the direct cause of coma. In liver cirrhosis and HCC patients, the most common cause was liver failure (cirrhosis: 32%, HCC: 64%). Hematemesis and encephalopathy induced coma in the cirrhosis patients (24% and 12%, respectively) and hematemesis and rupture of HCC were risk factors in the HCC patients. Respiratory failure (chronic: 46%, acute: 38%) and cardiopulmonary arrest were the main causes of coma in the lung cancer patients.

Survival time from onset of coma and use of narcotic analgesics are shown in Table 2. Time between coma and death was significantly shorter in the liver disease patients (cirrhosis and/or HCC: 7.0 h) compared with the lung cancer patients (44.0 h, $p = 0.045$), with no significant difference between cirrhosis and HCC. Total bilirubin was higher in HCC compared with cirrhosis, but there was no difference in the rate of ascites. Rate of usage of narcotic analgesics was significantly higher in the lung cancer patients (20/33: 60.6%) than in the liver disease patients (17/83: 20.5%, $p<0.01$). Analgesics were used more frequently in HCC than in liver cirrhosis ($p<0.01$).

Discussion

The results of the present study indicate that: (i) the rate of usage of narcotic analgesics in the terminal stage was significantly lower in patients with liver cirrhosis and/or HCC (17/83: 20.5%) compared with lung cancer patients (20/33: 60.6%, $p<0.01$); and (ii) the time between onset of coma and death was significantly

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