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

Yasunori Kawaguchi¹, Toshihiko Mizuta¹, Yuichiro Eguchi¹, Eiichi Sakurai², Yoichi Motomura², Hiroshi Isoda¹, Takuya Kuwashiro¹, Satoshi Oeda¹, Shinji Iwane¹, Hirokazu Takahashi¹, Keizo Anzai¹ and Iwata Ozaki¹

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 $< 15 \times 10^4/\mu\text{L}$, 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-

¹Department of Internal Medicine, Saga Medical School, Japan and ²National 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; $\mu\text{U/mL}$) 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:

$$\beta \text{ cell function (HOMA-}\beta\text{)} = \text{fasting SI (FSI)} \times 360 / [\text{FPG} - 63]$$

$$\text{Insulin resistance (HOMA-IR)} = \text{FPG} \times \text{FSI} / 405$$

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

In the pilot study, we also measured the serum adiponectin ($\mu\text{g/mL}$), 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 sTNFR2/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 $22 \times [\text{height (m)}]^2$. 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 $<15 \times 10^4/\mu\text{L}$ (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 $\geq$ 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 ($\times 10^4/\mu\text{L}$)	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 ($\mu\text{U/mL}$)	7 (2–24)	11 (3–34)	< 0.001
2-h insulin ($\mu\text{U/mL}$)	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/ $\geq$ 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. ^an = 195, ^bn = 56, ^cn = 204, ^dn = 58, ^en = 119, ^fn = 40.

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

Variables	OR	95% CI	p value
Platelet < $15 \times 10^4/\mu\text{L}$	2.74	1.27–5.91	0.010
AST $\geq$ 50 IU/L	3.46	1.24–9.65	0.018
ALT $\geq$ 50 IU/L	0.68	0.24–1.98	0.482
γ -GTP $\geq$ 35 IU/L	2.43	1.03–5.71	0.042
Triglyceride $\geq$ 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

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 identi-

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

fied 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 ($\times 10^4/\mu\text{L}$)	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 ($\mu\text{U/mL}$)	13 (9–18)	9 (6–21)	0.001
2-h insulin ($\mu\text{U/mL}$)	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 ($\mu\text{g/mL}$)	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/ $\geq$ 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. ^an=19, ^bn=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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