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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C

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Abstract
Background Several epidemiological studies have reported that diabetes mellitus is a risk factor for hepatocellular carcinoma (HCC) in hepatitis C virus (HCV)-positive patients. However, it is unclear whether or not post-challenge hyperglycemia is a risk factor. The purpose of this study was to determine the association between post-challenge hyperglycemia and hepatocarcinogenesis in HCV-positive patients.
Methods A total of 203 HCV-RNA-positive subjects (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years; genotype 1b/2a/2b/3a: 152/38/12/1) who underwent liver biopsy and a 75-g oral glucose tolerance test, and who were treated with interferon (IFN) were enrolled in this study. None of the subjects had been treated with antidiabetic drugs. The subjects underwent ultrasonography and/or computed tomography every 6 months after the end of the IFN therapy.
Results Thirteen patients, including one patient who achieved a sustained viral response (SVR) with IFN, developed HCC. On multivariate analysis, male sex, age >65 years, excessive alcohol consumption, non-SVR, liver steatosis area >5% in liver specimens, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC. After matching subjects for sex, age, alcohol intake, and response to the IFN therapy, advanced fibrosis stages [hazard ratio (HR) 2.8], liver steatosis (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9) were significant risk factors for the development of HCC. Furthermore, after matching for the fibrosis stage, liver steatosis (HR 5.7) and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors for HCC development.
Conclusion Post-challenge hyperglycemia is an independent risk factor for HCC in HCV-positive patients.

Keywords Hyperglycemia · Oral glucose tolerance test · Hepatocellular carcinoma · Hepatitis C

Abbreviations

HCV	Hepatitis C virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
DM	Diabetes mellitus
HR	Hazard ratio
BMI	Body mass index
ALT	Alanine aminotransferase
HbA1c	Hemoglobin A1c
IFN	Interferon
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
HOMA-IR	Homeostasis model assessment for insulin resistance
SVR	Sustained viral response

Introduction

Chronic hepatitis C virus (HCV) infection is a disease that can progress to cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Several factors associated with HCC

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development in chronic HCV have been reported, including male sex, older age at infection, excessive alcohol consumption, coinfection with hepatitis B virus (HBV), and variations in HCV itself [3–6]. Recent epidemiological studies have shown that diabetes mellitus (DM) is also a risk factor for HCC in patients with chronic hepatitis C [7, 8], although studies in Taiwan revealed no association between DM and HCC [9, 10]. Therefore, it is still unclear whether or not DM is a significant risk factor for HCC.

Furthermore, despite the findings of these epidemiological studies, several issues remain unresolved. First, not all of the subjects in these studies underwent glucose tolerance tests, and DM was defined based on inconsistent criteria, with some studies defining diabetes based on the use of antidiabetic drugs such as insulin, the presence of fasting hyperglycemia, and/or abnormal levels of hemoglobin A1c (HbA1c). Accordingly, it is not clear whether specific components of DM, particularly post-prandial hyperglycemia, are risk factors for HCC. Second, no study has evaluated whether insulin resistance or hyperinsulinemia, which might develop in advance of hyperglycemia, is associated with the development of HCC. Third, it is unclear whether DM remains a risk factor for HCC after accounting for pathological liver findings such as fibrosis, inflammation, and steatosis, which are acknowledged risk factors for HCC [11–14]. Fourth, because many HCV-positive patients receive interferon (IFN) therapy, it is essential to consider the response to IFN therapies in such studies.

Therefore, considering these limitations of earlier studies, and the unanswered questions, we conducted a prospective cohort study of subjects with chronic hepatitis C, who underwent a 75-g oral glucose tolerance test (OGTT), liver biopsy, and IFN therapy.

Patients and methods

Patients

Overall, 203 HCV-positive subjects who underwent liver biopsy and a 75-g OGTT between 2002 and 2007 and who were treated with IFN were enrolled in this study (Table 1). All of the subjects were positive for serum HCV-RNA detected by polymerase chain reaction (PCR). Criteria for inclusion in the study were: hemoglobin $\geq$12 g/dl, leukocyte count $\geq$3,000/mm^3, platelet count $\geq$90,000/μl, and serum creatinine levels within the normal range. Patients were excluded if they had decompensated liver disease; were hepatitis B surface antigen-positive; or had a history of liver transplantation, neoplastic disease (including HCC), severe cardiac or chronic pulmonary disease, autoimmune disease, a psychiatric disorder, or severe retinopathy; or were planning on becoming pregnant. In this study, subjects who met the criteria of both fasting glucose level $\geq$126 mg/dl and HbA1c $\geq$6.5%, were diagnosed as having overt DM and excluded because they should be treated for DM prior to IFN therapy. Subjects who were treated with antidiabetic drugs or subcutaneous insulin infusion were excluded because it was difficult to perform the 75-g OGTT and analyze its results, and because it is unclear whether antidiabetic drugs affect HCC occurrence.

Because the duration of IFN therapy differed among the subjects, the end of the IFN regimen was defined as the start of the study observation period. The endpoint of this study was HCC occurrence. The protocol was approved by the Local Review Board in accordance with the ethical guidelines of the Declaration of Helsinki (1975, as revised in 1983). Written informed consent was obtained from all patients.

Physical examination, serum biochemistry, and OGTT

Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). Venous blood samples were taken from all patients at around 0800 hours after a 12-h overnight fast, to determine blood cell count and blood chemistry. Serum HCV-RNA levels were analyzed by reverse-transcriptase PCR (nested PCR or Amplicor; Roche Diagnostic Systems, CA, USA) and HCV genotypes were determined by reverse-transcriptase PCR (Roche Diagnostic Systems, CA, USA).

Insulin resistance was evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR), using the following equation [15]: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mg/dl)/405. The reference values for fasting glucose level and fasting insulin level in our clinical laboratory are 70–110 mg/dl and 4–24 μU/ml, respectively. However, we considered subjects with HOMA-IR of >2.5 as showing insulin resistance, according to a previous report [16].

All subjects underwent a 75-g OGTT. Samples were collected at baseline and every 30 min after glucose ingestion for 120 min to measure glucose and insulin levels. All examinations were performed up to 3 months before starting IFN therapy.

Liver histology

Liver needle biopsies were performed percutaneously with a 16-G needle (Super-Core^{TM} semi-automatic biopsy instrument; InterV Clinical Products, Dartmouth, MA, USA) up to 3 months before starting IFN therapy. All subjects enrolled this study underwent liver biopsy. The

Table 1 Clinical characteristics of the patients

	Total number of patients ($n = 203$)	Patients with HCC ($n = 13$)
Age (years)[a]	55.4 ± 10.6	63.1 ± 6.5
Female % (M/F, *n*)	46.8 (108/95)	7.7 (11/1)
Alcohol consumption		
Excessive/daily/social or none, *n*	21/30/152	4/0/9
BMI[a]	23.5 ± 3.0	23.7 ± 2.7
IFN therapy history (naïve/>2), *n*	132/71	6/7
ALT (IU/l)[a]	71.3 ± 55.0	76.3 ± 44.8
Platelets ($\times 10^4$/μl)[a]	16.3 ± 6.2	12.1 ± 3.2
AFP (ng/ml)[a]	15.0 ± 37.8	14.3 ± 9.6
Viral load ($\times 10^6$ IU/ml)[a]	1.8 ± 1.5	1.5 ± 1.1
Genotype (1b/2a/2b/3a)[a]	152/38/12/1	12/0/1/0
Fasting glucose (mg/dl)[a]	86.7 ± 9.3	89.9 ± 14.4
Fasting insulin (μU/ml)[a]	9.4 ± 5.5	10.9 ± 7.8
HOMA-IR[a]	2.0 ± 1.3	2.7 ± 1.9
Liver histology		
A0/A1/A2/A3, *n*	1/71/106/25	0/3/8/2
F0/F1/F2/F3/F4, *n*	2/91/63/37/10	0/1/6/5/1
Steatosis <5/5–9/>10%, *n*	175/15/13	7/3/3
Response to IFN therapy; SVR, *n* (%)	89 (44.3)	1 (7.7)

HCC hepatocellular carcinoma, *BMI* body mass index, *IFN* interferon, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance, *SVR* sustained viral response

[a] Data are expressed as means ± SD

liver biopsy specimen was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin and Azan for histological evaluation. Pathological liver fibrosis and inflammation activity were evaluated according to the METAVIR scoring system (stages 0–4 for fibrosis and grades 0–4 for inflammatory activity) [17]. The area of steatosis in the liver specimen was calculated using Image J 1.42 (http://rsb.info.nih.gov/ij/). All liver biopsy specimens were evaluated by three experienced pathologists who were unaware of the clinical conditions of the patients.

Therapy and follow-up protocol

Between 2002 and 2003, all of the treatment-naïve (hereafter, 'naïve') patients with genotype 1b/high viral load (>100 KIU/ml) and patients refractory to prior IFN therapy were treated with either IFNα2a or IFNβ plus oral ribavirin at body weight-dependent doses (total dose: 600 mg for patients <60 kg; 800 mg for patients weighing 60–80 kg; 1,000 mg for patients weighing ≥80 kg). Between 2004 and 2007, all of the naïve patients with genotype 1b/high viral load and patients refractory to prior IFN therapy were treated with either pegylated (Peg)-IFNα2a (180 μg/week subcutaneously) or Peg-IFNα2b (1.5 μg/kg/week subcutaneously) plus oral ribavirin in body weight-dependent doses. Patients with genotype 1b were treated for 48 weeks, while all other patients were treated for 24 weeks. Between 2002 and 2003, non-genotype 1b and low viral load (<100 KIU/ml) naïve patients were treated with IFNα2a or IFNβ for 24 weeks, and between 2004 and 2007, such patients were treated with Peg-IFNα2a (180 μg/week subcutaneously) monotherapy for 24 weeks. Patients were not randomized to therapy and the selection of the therapeutic protocol was at the study physicians' discretion.

Ultrasonography and/or computed tomography were performed every 6 months in all patients. At 6 months after the end of treatment, patients with a negative qualitative HCV-RNA test were considered to have a sustained viral response (SVR). Patients with a negative qualitative HCV-RNA test at the end of therapy and a positive HCV RNA text after therapy were considered to show relapse. Patients who never achieved viral clearance during therapy were considered non-responders.

Statistical analysis

Comparisons between groups were made using the Mann–Whitney *U* test for continuous variables and the χ^2 test for categorical data. Changes in biological parameters in each group were assessed using paired *t* tests. Continuous variables are summarized as means ± SD. Differences were considered significant at $P < 0.05$. The Cox proportional hazard regression model was used for univariate and multivariate analyses to determine the risk of HCC occurrence. Significant variables on univariate analyses were included in the multivariate analyses. In the multivariate analyses, up to three subjects without HCC

occurrence were randomly selected for each patient with HCC, and were matched by sex, age, alcohol intake, response to IFN therapy, and fibrosis stage. Statistical analyses were performed with SPSS II (SPSS Japan, Tokyo, Japan).

Results

Subject characteristics

The clinical characteristics of the 203 patients (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years) enrolled in this study are summarized in Table 1. The average observation time was 52.0 ± 19.5 months. Twenty-one (10.3%) patients were classified as having excessive alcohol intake (>50 g ethanol per day). Using the METAVIR scoring system, fibrosis was staged as F0 in two patients (1%), F1 in 91 (44.8%), F2 in 63 (31%), F3 in 37 (18.2%), and F4 in 10 (4.9%). All liver biopsy specimens taken before therapy showed typical features of chronic HCV infection, including infiltration of lymphocytes in Glisson's capsule, piecemeal necrosis, and periportal fibrosis. The average area of steatosis in the liver specimens was 2.6 ± 3.1%. During the observation period, 13 patients, including one patient who achieved SVR with IFN therapy, developed HCC (12 males and 1 female, mean age 62.8 ± 6.7 years).

OGTT results

The serum glucose and insulin levels during the 75-g OGTT are shown in Fig. 1a, b. In patients who developed HCC (HCC group), the glucose levels at 30 ($P = 0.002$), 90 ($P = 0.033$), and 120 ($P = 0.001$) min, and the insulin levels at 30 min ($P = 0.017$) were significantly higher than those in patients without HCC (non-HCC group). There were no significant differences in fasting glucose or insulin levels between the HCC group and the non-HCC group.

Univariate and multivariate analyses of risk factors for HCC

On univariate analyses, male sex [hazard ratio (HR) 10.5], age >65 years (HR 5.3), excessive alcohol consumption (HR 4.6), non-SVR after IFN therapy (HR 9.5), advanced liver fibrosis (HR 2.9), α-fetoprotein >10 ng/ml (HR 4.6), liver steatosis area >5% (HR 5.7), and 120-min post-challenge hyperglycemia (>200 mg/dl; HR 6.3) were significant risk factors for the development of HCC (Table 2). BMI, fasting glucose, fasting insulin, insulin levels during the 75-g OGTT, HOMA-IR, cholesterol, and triglyceride were not associated with the development of HCC. Furthermore, viral load and genotype, and IFN therapy protocols were not associated with the development of HCC.

On multivariate analyses, male sex, age >65 years, excessive alcohol consumption, non-SVR, liver steatosis area >5%, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC (Table 3). When we limited the analyses to the HCC group ($n = 13$) and non-HCC patients ($n = 30$) matched for sex (male; $n = 27$), age (>65 years; $n = 8$), alcohol intake (excessive alcohol intake; $n = 8$), and response to IFN therapy (SVR; $n = 3$), advanced fibrosis stage (HR 2.8), liver steatosis area >5% (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9) were significant risk factors for the development of HCC. When we matched patients for fibrosis stage (advanced fibrosis stage; $n = 10$) as well as the above factors (male; $n = 27$, age >65 years; $n = 9$, excessive alcohol intake; $n = 8$, SVR; $n = 3$), liver steatosis area >5% (HR 5.7), and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors associated with the development of HCC.

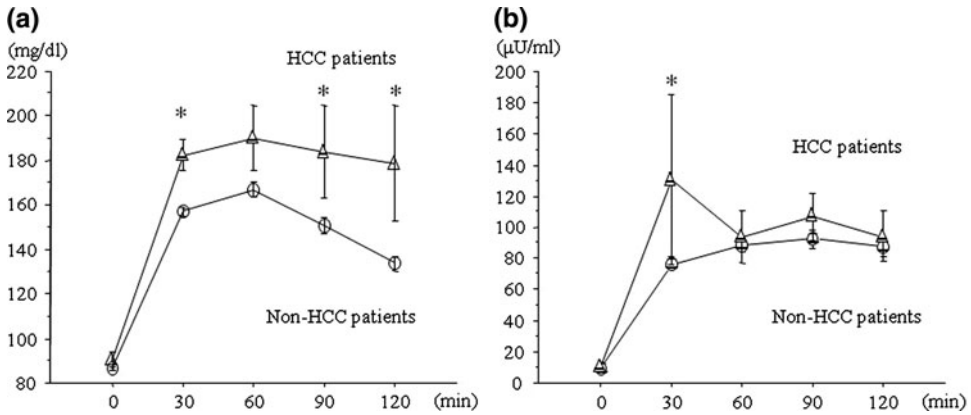

Fig. 1 **a** Serum glucose levels and **b** insulin levels on 75-g oral glucose tolerance test (OGTT). *Open triangles* patients who developed hepatocellular carcinoma (*HCC*). *Open circles* patients without HCC. *$P < 0.05$ by Mann–Whitney *U* test. *Error bar* ± standard deviation

Table 2 Univariate analyses: comparison of the risk factors for HCC between 13 patients with HCC and non-HCC patients

Variable	HCC ($n = 13$)	Non-HCC ($n = 190$)	HR (95% CI)
Male	12 (92.3)	96 (50.5)	10.5 (1.4–81.0)*
Age >65 years	6 (46.2)	29 (15.3)	5.3 (1.8–16.0)*
Excessive alcohol consumption[a]	4 (30.8)	17 (8.9)	4.6 (1.4–15.0)*
Response to IFN therapy; non-SVR	12 (92.3)	101 (53.2)	9.5 (1.2–73.2)*
Fibrosis stage; F3 and F4	6 (46.2)	41 (21.6)	2.9 (1.1–8.7)*
BMI >25	5 (38.5)	53 (27.9)	1.5 (0.5–4.7)
AFP >10 ng/ml	8 (61.5)	50 (26.3)	4.6 (1.4–15.2)*
Steatosis >5%	6 (46.2)	22 (11.6)	5.7 (1.9–17.1)*
Fasting glucose ≥126 mg/dl	1 (7.7)	2 (1.1)	6.4 (0.8–50.0)
Fasting insulin ≥15 μU/ml	2 (15.4)	29 (15.3)	0.9 (0.2–4.0)
HOMA-IR ≥3	2 (15.4)	33 (17.4)	0.7 (0.2–3.4)
120-min post-challenge hyperglycemia[b]	5 (38.5)	15 (7.9)	6.3 (2.0–19.1)*

Data are expressed as numbers (%)

HR hazard ratio, *CI* confidence interval, *BMI* body mass index, *IFN* interferon, *SVR* sustained viral response, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance

* $P < 0.05$

[a] More than 50 g ethanol/day

[b] Serum glucose level was more than 200 mg/dl at 120 min on 75-g oral glucose tolerance test (OGTT)

Table 3 Multivariate analyses: comparison of the risk factors for HCC between 13 patients with HCC and non-HCC patients

Variable	HCC	Non-matched		Sex, age, alcohol intake, response to IFN matched		Sex, age, alcohol intake, response to IFN, fibrosis stage matched	
	$n = 13$	$n = 190$	HR (95% CI)	$n = 30$	HR (95% CI)	$n = 30$	HR (95% CI)
Male	12 (92.3)	96 (50.5)	18.8 (2.2–161.4)*	Matched	–	matched	–
Age >65 years	6 (46.2)	29 (15.3)	9.9 (2.5–39.9)*	Matched	–	Matched	–
Excessive alcohol consumption[a]	4 (30.8)	17 (8.9)	7.2 (1.4–37.5)*	Matched	–	Matched	–
Response to IFN therapy; non-SVR	12 (92.3)	101 (53.2)	20.4 (2.1–200.9)*	Matched	–	Matched	–
Fibrosis stage; F3 and F4	6 (46.2)	41 (21.6)	6.3 (1.2–33.3)*	8 (26.7)	2.8 (1.0–11.3)*	Matched	–
AFP >10 ng/ml	8 (61.5)	50 (26.3)	1.3 (0.4–1.8)	15 (50)	0.5 (0.7–3.1)	15 (50)	0.4 (0.1–2.2)
Steatosis >5%	6 (46.2)	22 (11.6)	5.6 (1.4–22.6)*	3 (10)	5.4 (1.1–27.3)*	2 (3.3)	5.7 (1.2–27.1)*
120-min post-challenge hyperglycemia[b]	5 (38.5)	15 (7.9)	19.5 (3.7–104.1)**	4 (13.3)	4.9 (1.3–18.9)*	2 (6.7)	6.9 (1.7–28.4)*

Data are expressed as numbers (%)

HR hazard ratio, *CI* confidence interval, *IFN* interferon, *SVR* sustained viral response, *AFP* alpha-fetoprotein

* $P < 0.05$, ** $P < 0.001$

[a] More than 50 g ethanol/day

[b] Serum glucose level was more than 200 mg/dl at 120 min on 75-g OGTT

Clinical characteristics of patients with post-challenge hyperglycemia

The clinical characteristics of 20 patients with 120-min post-challenge hyperglycemia on the 75-g OGTT and the remaining 183 patients are summarized and compared in Table 4. Fasting glucose levels and the HCC occurrence rate were significantly higher in patients with post-challenge hyperglycemia. On the other hand, the SVR rates were not significantly different, being 40% in patients with post-challenge hyperglycemia and 44.8% in patients without post-challenge hyperglycemia. The rate of patients with advanced liver fibrosis was higher in patients with post-challenge hyperglycemia than in the other patients, although the difference was not statistically significant.

Table 4 Clinical characteristics of the patients with/without 120-min post-challenge hyperglycemia

	Post-challenge hyperglycemia		*P* value
	With ($n = 20$)	Without ($n = 183$)	
Age (years)[a]	58.2 ± 8.3	55.1 ± 10.8	0.223
Female % (M/F, *n*)	35 (13/7)	48 (95/88)	0.265
Alcohol consumption			
Excessive or habitual/social or none, *n*	2/18	28/155	0.523
BMI[a]	23.9 ± 2.5	23.4 ± 3.0	0.477
ALT (IU/l)[a]	68.1 ± 35.1	71.7 ± 56.6	0.781
Platelets ($\times 10^4$/μl)[a]	16.0 ± 6.0	16.3 ± 6.2	0.830
AFP (ng/ml)[a]	21.0 ± 44.4	14.4 ± 37.0	0.458
Viral load ($\times 10^6$ IU/ml)[a]	1.4 ± 1.3	1.8 ± 1.6	0.205
Genotype (1b/non-1b), *n*	15/5	137/46	0.989
Fasting glucose (mg/dl)[a]	96.5 ± 15.9	85.8 ± 8.5	<0.001
Fasting insulin (μU/ml)[a]	9.4 ± 4.5	9.3 ± 5.6	0.978
HOMA-IR[a]	2.4 ± 1.4	2.0 ± 1.2	0.228
Liver histology			
A0–1/A2–3, *n*	4/16	68/115	0.128
F0–2/F3–4, *n*	12/8	144/39	0.060
Steatosis <5/5–9/>10%, *n*	17/0/3	158/15/10	0.122
Response to IFN therapy; SVR, *n* (%)	8 (40)	82 (44.8)	0.681
HCC occurrence, *n* (%)	5 (25)	8 (4.4)	<0.001

BMI body mass index, *IFN* interferon, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance, *SVR* sustained viral response

[a] Data are expressed as means ± SD

Cumulative HCC occurrence rate

The cumulative HCC occurrence rates in the patients with 120-min post-challenge glucose levels of ≥200 and those with levels of <200 mg/dl are shown in Fig. 2a. While the HCC occurrence rates at 3 and 5 years were 3.3 and 4.3% in patients with 120-min glucose <200 mg/dl, the corresponding rates were 15.0 and 28.1% in patients with 120-min glucose ≥200 mg/dl. There was a significant difference in the HCC occurrence rate between patients with 120-min glucose <200 versus those with ≥200 mg/dl ($P < 0.001$).

Figure 2b shows the cumulative HCC occurrence rates in patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5%. The rates at 3 and 5 years were 14.3 and 20.4% in patients with a liver steatosis area of >5% versus 2.9% and 4.7%, respectively, in patients with a liver steatosis area of ≤5%. There was a significant difference in the HCC occurrence rate between patients with a liver steatosis area of ≤5% versus those with a liver steatosis area of >5% ($P < 0.001$).

Comparison of 75-g OGTT results between patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5%

The serum glucose and insulin levels during the 75-g OGTT in patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5% are shown in Fig. 3a, b. There were no differences in glucose levels between the two groups. In contrast, fasting and 30-min insulin levels were significantly higher in patients with a liver steatosis area of >5% versus those with a liver steatosis area of ≤5% (fasting insulin: 11.4 ± 6.0 vs. 9.0 ± 5.3 μU/ml, $P = 0.035$; 30-min insulin: 118.9 ± 147.6 vs. 73.4 ± 51.8 μU/ml, $P = 0.003$).

Discussion

This study has revealed that post-glucose challenge hyperglycemia is an independent risk factor for the development of HCC in chronic hepatitis C patients without overt DM or those who are not being treated with antidiabetic drugs.

Although it is unclear why post-challenge hyperglycemia influences hepatic carcinogenesis, we assumed that the mechanism might involve oxidative stress associated with an acute increase in glucose levels. Four of the five patients with HCC and glucose levels of >200 mg/dl at 120-min after the glucose load had normal fasting glucose levels. A previous study showed that acute glucose fluctuations caused greater oxidative stress than sustained chronic hyperglycemia in patients with type 2 DM [18]. Moreover, the activation of oxidative stress as a result of hyperglycemia plays an important role in the pathogenesis of

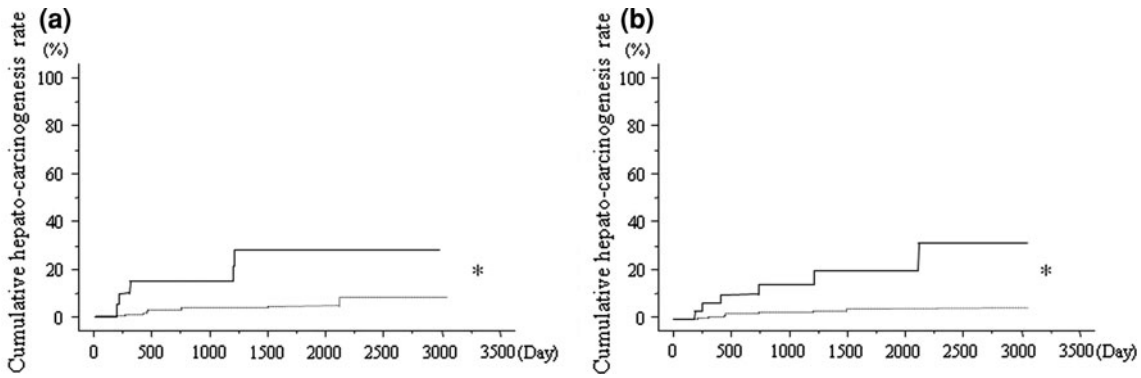

Fig. 2 **a** The cumulative HCC occurrence rates in patients with 120 min post-challenge hyperglycemia (serum glucose level more than 200 mg/dl at 120 min on 75-g GTT; *thick line*) and patients without hyperglycemia (serum glucose level less than 200 mg/dl at 120 min on 75-g GTT; *thin line*). **b** The cumulative HCC occurrence rates in patients with liver steatosis of more than 5% (*thick line*) and patients with liver steatosis of 5% or less (*thin line*). $*P < 0.001$ by log-rank test

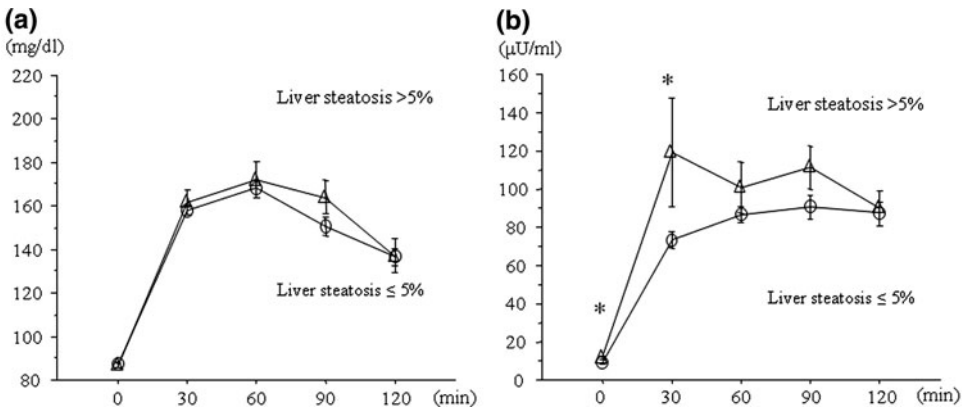

Fig. 3 **a** Serum glucose levels and **b** insulin levels on 75-g OGTT. *Open triangles* patients with liver steatosis of more than 5%. *Open circles* patients with liver steatosis of 5% or less. $*P < 0.05$ by Mann–Whitney *U* test. *Error bar* ± standard deviation

diabetic complications and carcinogenesis [19]. It was also reported that DNA damage caused by oxidative stress could be associated with hepatocarcinogenesis [20, 21]. Because it was previously demonstrated that the post-challenge glucose level was correlated with post-prandial glucose and HbA1c levels [22, 23], the patients with post-challenge hyperglycemia were assumed to have been exposed to daily fluctuations in glucose levels and oxidative stress. These findings might explain why the post-challenge glucose level, but not fasting glucose, was associated with HCC occurrence in the present study. However, further studies that include the assessment of oxidative stress are needed to elucidate the association between acute glucose fluctuations and hepatocarcinogenesis.

Hyperinsulinemia caused by insulin resistance is a well-known carcinogenic factor in several organs, including the liver [24, 25]. It has been shown that HCV itself, including its core protein, induces insulin resistance by impairing the insulin signaling pathway [26, 27]. It was also reported that insulin resistance was more severe in chronic HCV-infected patients than in patients with chronic hepatitis caused by another etiology [28, 29]. However, our study failed to show any associations between HOMA-IR or fasting insulin and the development of HCC. Instead, we found that hepatic steatosis was an independent risk factor for HCC. Moreover, we found significant differences in the fasting and 30-min insulin levels after a glucose load, but not in glucose levels at any time, between patients with and without steatosis, so that HOMA-IR and the area under the curve of insulin concentrations during the 75-g OGTT were higher in patients with liver steatosis (data was not shown). These findings suggest that hyperinsulinemia or insulin resistance might influence hepatic carcinogenesis via hepatic steatosis.

Konishi et al. [30] reported that post-challenge hyperglycemia, but not insulin resistance, was a risk factor for HCC occurrence in chronic hepatitis C patients. Although their results are similar to our own, there is a difference in terms of whether or not hepatic steatosis is a risk factor for HCC occurrence. The discrepancy between these two studies might be due to the methods used to measure liver steatosis. In our study, an image analyzer was used to precisely measure the fat-occupied area, thus allowing us to include the actual area of steatosis in the analyses.

Liver fibrosis can not only cause hepatic cancer, but it can also cause insulin resistance and glucose intolerance.

To overcome any potential bias due to liver fibrosis, we performed case-matched multivariate analyses. These analyses showed that post-challenge hyperglycemia and hepatic steatosis were associated with the development of HCC, independent of hepatic fibrosis.

DM is generally diagnosed based on pre- or post-prandial blood glucose, HbA1c, or glycoalbumin levels. However, it was previously reported that fasting glucose, HbA1c, and glycoalbumin were inadequate tests for the diagnosis of impaired glucose tolerance in patients with advanced liver fibrosis [31, 32]. Although the measurement of post-prandial blood glucose might be an easy method to determine post-challenge hyperglycemia, these values are likely to fluctuate according to the meal content or the length of time after the meal. Therefore, we believe that OGTTs are an indispensable and useful method to detect post-challenge hyperglycemia and to predict the risk of HCC in chronic HCV-infected patients.

It is still unclear what stage in the progression of glucose intolerance carries the greatest risk for HCC. This is because the earlier cohort studies that investigated possible associations between DM and HCC occurrence did not use consistent diagnostic criteria for "overt DM" [7–10]. Because the glucose levels at 120 min during an OGTT are more precise and sensitive parameters for the diagnosis of glucose intolerance than the evaluation of pre- and/or post-prandial hyperglycemia [33, 34], our data suggest that the stages of DM/glucose intolerance preceding "overt DM" may also be associated with HCC occurrence.

Our study revealed significant differences in glucose levels not only at 120 min, but also at 30 and 90 min during OGTTs, between the HCC and non-HCC patients. Furthermore, the 30- and 90-min glucose levels were significant risk factors for HCC on univariate analyses (30 min $>$ 175 mg/dl: HR 4.3, 95% CI 1.4–13.1; 90 min $>$ 175 mg/dl: HR 3.6, 95% CI 1.2–11.1). However, these HRs were smaller than the HR for 120-min and they were not significant on multivariate analysis. Interestingly, according to previous studies, such as DECODE and DECODA, 120-min post-challenge glucose levels were associated with increased risks for macrovascular events and heart disease-related death [35, 36]. Although the mechanisms underlying these associations are not yet fully understood, it seems that 120-min post-challenge hyperglycemia is an important factor involved in several events.

There is no doubt that the eradication of HCV with IFN is an effective approach to reduce the risk of HCC in chronic HCV-infected patients [37]. Our data indicate that the SVR achieved by IFN treatment is a significant factor that inhibits the development of HCC. Recently, it was reported that HCV infection per se downregulated the cell surface expression of the glucose transporter [38]. We have previously reported that the eradication of HCV contributes to improvements in insulin resistance and post-challenge hyperglycemia [39]. These findings suggest that the eradication of HCV by IFN therapy contributes to improvements in glucose intolerance. According to our present results, however, post-challenge hyperglycemia was independent of the IFN response, which means that patients with both glucose intolerance and sustained HCV infection are at increased risk for HCC. These results indicate that improvement of glucose intolerance should be considered as one of the strategies to prevent HCC in patients with chronic hepatitis C, particularly those in whom HCV cannot be eradicated.

A limitation of our study is that the severity of glucose intolerance might change following IFN therapy, because of HCV eradication or because of adverse effects of IFN such as anorexia and body weight loss. To confirm whether or not glucose intolerance is a true risk factor for HCC, future studies should include continued assessment of glucose tolerance following IFN therapy.

In conclusion, the assessment of post-challenge hyperglycemia using a 75-g OGTT is useful for estimating the risk of HCC in HCV-positive patients. Future studies are needed to elucidate the underlying mechanism and identify possible treatments to further reduce the risk of HCC.

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