

## Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States

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**Background:** Hepatic steatosis has been associated with chronic hepatitis C (CHC), but its prevalence, risk factors, and clinical significance remain to be determined.

**Aims:** The present study determined the frequency of, and risk factors for hepatic steatosis and its association with activity and progression of CHC in a large cohort of U.S. patients.

**Methods:** This is a retrospective study that utilized systematic chart review and statistical analyzes to investigate 324 U.S. patients with CHC from a university medical center and a regional VA medical center.

**Results:** The frequency of hepatic steatosis was 66.0%. We demonstrated that not only being obese, but also overweight (i.e. body mass index  $\geq 25$  kg/m<sup>2</sup>) was independently associated with hepatic steatosis. In our cohort of patients with CHC, hepatic steatosis, especially grade II/III steatosis, was significantly associated with elevated aspartate aminotransferase at entry, persistently elevated alanine aminotransferase, and stage III/IV fibrosis. Grade II/III steatosis, was significantly associated with a higher histology activity index as well. Multivariate analysis indicated that steatosis, especially grade II/III steatosis, was independently associated with stage III/IV fibrosis.

**Conclusions:** Being overweight/obese serves as an independent risk factor for hepatic steatosis in U.S. patients with CHC. Steatosis accelerates activity and progression of CHC, and is independently associated with stage III/IV hepatic fibrosis in these patients.

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**Keywords:** Hepatitis C virus; Chronic hepatitis C; Hepatic steatosis; Obesity; Body mass index; Hepatic fibrosis

### 1. Introduction

Chronic hepatitis C virus (HCV) infection affects 170 million people worldwide, including 2.7 million of U.S.

individuals [1–3]. Although a great deal of progress has been made [3–7], factors affecting disease progression and development of cirrhosis in these patients remain incompletely defined.

Hepatic steatosis is a well-documented histological feature during HCV infection [8–18]. Most studies [8,10–15,19] have suggested that obesity is associated with steatosis in patients with chronic hepatitis C (CHC). However, a general threshold of body mass index (BMI) for steatosis and association of BMI with grade of steatosis remain to be determined.

Besides obesity, type 2 diabetes mellitus (DM) and hypertriglyceridemia have also been associated with hepatic steatosis in patients with non-alcoholic fatty liver disease

Received 1 April 2003; received in revised form 22 August 2003; accepted 2 September 2003

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**Abbreviations:** ALT, alanine aminotransferase; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; BMI, body mass index; CHC, chronic hepatitis C; DM, diabetes mellitus; HCV, hepatitis C virus; HAI, histology activity index; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; PCR, polymerase chain reaction.

[20–24]. Increased frequency of type 2 DM has been reported in patients with CHC [25,26]. A recent study included 7.7% patients with DM, but could not directly associate DM with hepatic steatosis [19]. In addition, the effect of hypertriglyceridemia on hepatic steatosis in HCV-infected patients has not been systematically examined. Therefore, it is important to determine whether DM and/or hypertriglyceridemia alone, or in combination with obesity, contribute to development of hepatic steatosis in these patients.

Expression of HCV proteins has been associated with hepatic steatosis in transgenic mice [27,28]. These results were further supported by findings that HCV genotype 3a (HCV-3a) is associated with both higher prevalence and grade of hepatic steatosis in patients with CHC [9,14,15,19, 29–31]. However, this association remains to be further defined [16].

Several studies have reported an association of steatosis with advanced hepatic fibrosis in patients with CHC [9–19]. However, it remains unknown whether a higher grade of steatosis is more associated with a higher stage of hepatic fibrosis. In addition, it remains controversial whether BMI affects progression of fibrosis directly [8]. A few studies have reported that hepatic steatosis is associated with higher levels of alanine aminotransferase (ALT) and histological activity index (HAI) [8,9,19], but these findings were not supported by other studies [11,12,16]. Thus, it is necessary to re-examine these issues in a large cohort of HCV-infected patients.

The aims of this retrospective study were to determine the prevalence of and the risk factors for hepatic steatosis and to assess the association of being overweight/obese and steatosis with activity and progression of CHC in U.S. patients.

## 2. Patients and methods

### 2.1. Patient population

The present study retrospectively included 324 consecutive patients from two Liver Clinics at Loma Linda VA Medical Center ( $n = 112$ ) and Loma Linda University Medical Center ( $n = 212$ ) between July 1999 and April 2002. The study protocol was approved and informed consent was exempted by the institutional review boards from both medical centers. Inclusion criteria were: (1) positive HCV RNA reverse transcription polymerase chain reaction (RT-PCR) for at least 6 months; (2) a liver biopsy to stage CHC; (3) abstinence from alcohol use for  $> 6$  months for those with daily alcohol use of  $> 30$  g; (4) negative serology for hepatitis B surface antigen and anti-HIV; (5) absence of other causes of chronic liver disease, history of prior anti-HCV treatment or recent use of steatosis-inducing agents; and (6) no history of hepatic decompensation defined as the presence of ascites, jaundice (total bilirubin  $> 3$  mg/dL or  $51 \mu\text{mol/l}$ ), variceal bleeding, or hepatic encephalopathy [6].

### 2.2. Methods and data collection

Data were collected through chart review. These included age, gender, ethnicity, risk factors, and mode of HCV transmission, BMI, history of hyperlipidemia, and type 2 DM. History of alcohol including duration, intensity, and last consumption of alcohol beverage, and CAGE

score was taken. Patients who had a history of daily alcohol consumption of  $> 30$  g prior to study entry were considered to have alcohol use [24, 32]. According to National Institute of Health criteria, patients with a BMI between 25 and  $29.9 \text{ kg/m}^2$  were categorized as overweight, and patients with a BMI  $\geq 30 \text{ kg/m}^2$  were categorized as obese [33]. Hypertriglyceridemia were defined as  $\geq 200 \text{ g/l}$ . Patients were considered to have type 2 DM if using insulin or oral hypoglycemic agents in adulthood or if they had a fasting plasma glucose level of  $> 126 \text{ mg/dL}$  (i.e.  $7.0 \text{ mmol/l}$ ) at the time of enrollment [34].

Quantitative HCV RNA PCR was performed in 290 patients, and HCV genotyping was carried out with INNO LiPa HCV assay (Innogenetics, Zwijnaarde, Belgium) in 237 patients. Routine laboratory data included complete blood counts, albumin, ALT, aspartate aminotransferase (AST), alpha-fetoprotein (AFP), and fasting blood glucose, triglycerides, and ferritin. All patients with AFP  $\geq 20 \mu\text{g/l}$  underwent either abdominal computed tomography or magnetic resonance imaging and ruled out hepatocellular carcinoma. Three hundred and seventeen patients had also been regularly followed for  $> 12$  months with at least three follow-up ALT tests in 6-month intervals. Based on these ALT results, the patients were classified into three ALT patterns: persistently normal ALT, persistently elevated ALT, and fluctuating elevated ALT [35]. In 306 patients, ultrasonography was used to measure spleen size; a maximal spleen dimension  $> 13 \text{ cm}$  indicated splenomegaly.

### 2.3. Hepatic histology

Hematoxylin-eosin and trichrome stainings were used for histology assessment, and Perls' staining was used for iron grading. Knodell's scoring system was used to stage hepatic fibrosis [36]. While stage 0 represented the absence of hepatic fibrosis, stage III/IV represented advanced hepatic fibrosis. HAI (i.e. total Knodell score – fibrotic score) was used to grade activity of CHC [36]. In the present study, an HAI  $\geq 7$  was considered active liver injury. Steatosis was graded on a scale of 0–3: 0 = absence of steatosis; grade 1 =  $< 30\%$  of hepatocytes affected; grade 2 =  $30 \sim 70\%$  of hepatocytes affected; grade 3 =  $> 70\%$  of hepatocytes affected [8,14,19,37].

### 2.4. Statistical analysis

The descriptive data were presented as percentage or mean with standard deviation or range. Either Chi-square test, Student's  $t$ -test, or analysis of variance test was used to compare frequencies or means, respectively. Both univariate and multivariate analyzes were used and the odds ratios were calculated by using Logistic regression model. Multiple univariate analyzes were carried out to determine the risk factors for and association of CHC activity with steatosis (Tables 2 and 4). After Bonferroni's adjustment, a  $P$  value of  $< 0.004$  was considered as statistically significant for univariate analyzes. For all other analyzes, a  $P$  value of  $< 0.05$  was considered as statistically significant. The statistical software used included Statistical Analysis Software and Statistical Package for Social Science.

## 3. Results

### 3.1. Baseline demographic findings

The baseline demographic data are summarized in Table 1. Of the 307 patients with recorded BMI, the mean BMI was  $28.5 \pm 5.3$  ( $17\text{--}47$ )  $\text{kg/m}^2$ . Overweight was seen in 122/307 (39.7%) cases, and obesity was seen in 116/307 (37.8%) patients. Overall, 238/307 (77.5%) patients were overweight or obese. In 250 patients with documented information of drinking, 69.6% reported a past history of alcohol use. In 189 patients with recorded duration of sobriety, 32.8% have been sobered for 6–12 months and 67.2% have been sobered for  $> 12$  months.

**Table 1**  
**Baseline demographics of 324 enrolled patients**

Variables	Cases	Mean $\pm$ SD or percentage	Median (range)
Mean age at entry	322	48.24 $\pm$ 8.39	48 (25–79) years
Male:female	324	229:95 (70.70%:29.30%)	
Ethnicity	321		
African American	32	10.00%	
Asian	8	2.50%	
Caucasian	203	63.20%	
Hispanic	73	22.70%	
Other	5	1.60%	
History of alcohol use	250		
Never	76	30.40%	
$\leq 10$ years	34	13.60%	
$> 10$ years	140	56.00%	
Alcohol sobriety	189		
6–12 months	62	32.80%	
$> 12$ months	127	67.20%	
BMI	307	28.44 $\pm$ 5.29	28 (17–47) Kg/m <sup>2</sup>
Fibrosis staging:	324		
0	44	13.60%	
1	110	34.00%	
2	32	9.90%	
3	74	22.80%	
4	64	19.80%	
Histology activity index (HAI)	226		4 (0–12)
$\geq 7$	52	23.01%	
$< 7$	174	76.99%	
Hepatic steatosis	324		
Grade 0	110	34.00%	
Grade 1	117	36.10%	
Grade 2	65	20.10%	
Grade 3	32	9.90%	
HCV genotyping	237		
1	165	69.60%	
2	33	13.90%	
3 (3a = 23)	24	10.10%	
4	2	0.80%	
6	1	0.40%	
Mix	12	5.10%	
Biochemistry			
ALT	318	101.30 $\pm$ 73.57	83 (11–451) U/l
AST	322	79.21 $\pm$ 63.73	59 (6–497) U/l
Platelets	321	194.43 $\pm$ 77.89	194 (35–465) $\times 10^9/l$
Albumin	308	39.10 $\pm$ 5.70	40 (14–62) g/l
AFP	257	10.55 $\pm$ 16.65	5 (0–117) $\mu$ g/l
Triglycerides	211	141.22 $\pm$ 86.66	117 (33–497) g/l

Biochemically, 267 (83.9%) had elevated ALT. Longitudinally, 40/317 (12.6%) had persistently normal ALT, 216/317 (68.1%) had persistently elevated ALT, and 61/317 (19.2%) had fluctuating elevated ALT levels. Two hundred thirty-six (73.3%) had elevated AST. Forty-seven (15.3%) had hypoalbuminemia ( $< 35.0$  g/l) and 70 (21.8%) had platelet counts  $< 130 \times 10^9/l$ . In 257 cases who received an AFP assay, 29 (11.3%) had AFP  $\geq 20$   $\mu$ g/l, but negative abdominal CT for liver mass lesions.

Histologically, 186 (57.4%) had CHC and stage 0–2 fibrosis, while 74 (22.8%) and 64 (19.8%) had stage III and IV fibrosis, respectively. In the present study, patients with stage III and IV fibrosis were combined as a group with advanced fibrosis. Of the 226 patients with recorded HAI, the medium score was 4 with range of 0–12. Fifty-two (23.0%) had HAI  $\geq 7$  and 174 (76.1%) had HAI  $< 7$ . Of the 324 patients, 214 (66.0%) showed histological evidence of hepatic steatosis including 117 (36.1%) with grade I, 65 (20.1%) with grade II, and 32 (9.9%) with grade III steatosis. Because of the relatively small number of patients with grade II and III steatosis, these patients were combined for statistical analysis. Positive hepatic iron staining was seen in 46 (16.0%) patients.

### 3.2. Risk factors for hepatic steatosis

Table 2 summarizes results of univariate analysis of ten clinical, virological, and biochemical parameters as possible risk factors for steatosis. Although the frequency of steatosis was significantly associated with a BMI  $\geq 25$  kg/m<sup>2</sup> (Table 2, panel 1), it was more significantly associated with a BMI  $\geq 30$  kg/m<sup>2</sup> as indicated by a higher odds ratio (OR). In addition, when these patients were further divided based on BMI, the frequency of steatosis was significantly higher in individuals with a BMI  $\geq 30$  kg/m<sup>2</sup> (83.6%,  $n = 116$ ) than in those with a BMI  $\geq 25$  and  $< 30$  kg/m<sup>2</sup> (60.9%,  $n = 122$ ,  $P = 0.0009$ ). Thus, hepatic steatosis was more significantly associated with being obese than overweight. Multivariate analysis revealed that after adjusting for age, ethnicity, history of alcohol use, DM, hypertriglyceridemia, and HCV-3a infection, being overweight/obese (i.e. BMI  $\geq 25$  kg/m<sup>2</sup>) remained an independent risk for presence of and grade II/III hepatic steatosis (Table 3).

In our cohort of patients, a history of DM or hypertriglyceridemia was not statistically associated with steatosis. Both univariate (Table 2) and multivariate (Table 3) analyses did not reveal association of past history of alcohol use and duration of sobriety with hepatic steatosis in these patients. Although HCV-3a infection was more frequently seen in patients with hepatic steatosis, this difference was not statistically significant by both univariate and multivariate analyses (Tables 2 and 3). Steatosis was not associated with age at entry, gender, ethnicity, or high HCV load (Table 2).

### 3.3. Association of hepatic steatosis with the parameters of activity and stage of CHC

To test the association of steatosis with parameters related to activity and stage of CHC, univariate analysis was used to assess 12 clinical and biochemical parameters (Table 4). The presence of steatosis was significantly associated with elevated level of AST at entry and longitudinally persistent elevation of ALT, which are biochemical surrogates indicating active CHC [35,38].

**Table 2****Univariate analysis of the association of steatosis with clinical and virologic parameters in patients with CHC<sup>a</sup>**

Variables	Steatosis grade I–III vs. grade 0			Steatosis grade II/III vs. grade 0		
	Cases <sup>b</sup>	OR (95% CI) <sup>c</sup>	<i>P</i> value	Cases <sup>d</sup>	OR (95% CI) <sup>c</sup>	<i>P</i> value
Age at entry						
< 50 years	122/187			40/85		
≥ 50 years	90/135	1.07 (0.668–1.701)	0.7904	56/121	1.03 (0.592–1.799)	0.9123
Gender						
Male	61/95			75/151		
Female	153/229	0.89 (0.540–1.472)	0.6526	22/56	0.66 (0.351–1.224)	0.1849
Ethnicity						
Caucasian	137/203			66/132		
African Am	19/32	0.71 (0.332–1.521)	0.3875	6/19	0.48 (0.171–1.324)	0.1547
Hispanic	48/73	0.93 (0.532–1.637)	0.8089	22/47	0.91 (0.466–1.761)	0.7715
History of alcohol use						
Never use	43/76			16/49		
≤ 10 years	24/34	1.84 (0.775–4.379)	0.1668	11/21	2.26 (0.799–6.443)	0.1241
> 10 years	99/140	1.85 (1.036–3.315)	0.0376	47/88	2.36 (1.140–4.902)	0.0208
Alcohol sobriety						
6–12 months	44/62			23/41		
> 12 months	89/127	0.96 (0.492–1.867)	0.9003	45/83	0.93 (0.437–1.968)	0.8433
BMI						
< 25	33/69			14/50		
≥ 25	171/238	2.78 (1.606–4.827)	0.0003	79/146	3.03 (1.509–6.093)	0.0018
BMI						
< 30	107/191			45/129		
≥ 30	97/116	4.00 (2.269–7.078)	<0.0001	48/67	4.71 (2.479–8.969)	<0.0001
H/O diabetes						
No	176/266			73/163		
Yes	37/54	1.11 (0.594–2.085)	0.7383	23/40	1.67 (0.829–3.355)	0.1513
Triglycerides (≥ 200 g/l)						
No	111/171			54/114		
Yes	31/40	1.86 (0.832–4.168)	0.1306	17/26	2.09 (0.864–5.099)	0.1017
HCV > 1,000,000 copies/ml						
No	124/196			56/128		
Yes	66/94	1.37 (0.806–2.323)	0.2448	27/57	1.33 (0.712–2.490)	0.3697
HCV genotype 3a						
No	139/214			61/136		
Yes	17/23	1.53 (0.578–4.041)	0.3923	11/17	2.25 (0.788–6.445)	0.1295

<sup>a</sup> After Bonferroni's adjustment, a *P* value < 0.004 was considered as statistically significant.<sup>b</sup> Cases: ratio of patients with grade I–III steatosis to total patients in that group.<sup>c</sup> OR, odds ratio; and CI, confidence interval.<sup>d</sup> Cases: ratio of patients with grade II/III steatosis to total patients in that group.**Table 3****Multivariate analysis of the association of steatosis with clinical and virologic parameters in patients with CHC**

Variables	Steatosis grade I–III vs. steatosis grade 0		Steatosis grade II/III vs. steatosis grade 0	
	OR (95% CI) <sup>a</sup>	<i>P</i> value	OR (95% CI) <sup>a</sup>	<i>P</i> value
Age (≥ 50 years)	0.580 (0.237–1.422)	0.2339	0.402 (0.129–1.255)	0.1167
Ethnicity	0.806 (0.506–1.284)	0.3647	0.702 (0.387–1.274)	0.2447
BMI (≥ 25 kg/m <sup>2</sup> )	4.180 (1.597–10.939)	0.0036	9.738 (2.309–41.072)	0.0019
H/O diabetes	2.289 (0.610–8.591)	0.2196	2.722 (0.586–12.647)	0.2014
Triglycerides (≥ 200 g/l)	2.493 (0.720–8.639)	0.1496	4.146 (0.928–18.516)	0.0626
Genotype-3a	2.099 (0.378–11.662)	0.3967	3.905 (0.477–31.946)	0.2040
Past H/O alcohol use	1.194 (0.747–1.909)	0.4592	1.339 (0.745–2.407)	0.3297

<sup>a</sup> OR, odds ratio; and CI, confidence interval.

**Table 4**  
**Univariate analysis of the association of steatosis with parameters of activity and stage of CHC<sup>a</sup>**

Variables	Steatosis grade I–III vs. grade 0			Steatosis grade II/III vs. grade 0		
	Cases <sup>b</sup>	OR (95% CI) <sup>c</sup>	<i>P</i> value	Cases <sup>d</sup>	OR (95% CI) <sup>c</sup>	<i>P</i> value
Age at first exposure						
< 40 years	153/233			67/157		
≥ 40 years	12/16	1.57 (0.490–5.019)	0.4484	5/9	1.49 (0.385–5.782)	0.5622
Stage III/IV fibrosis						
No	110/186			40/116		
Yes	104/138	2.11 (1.301–3.433)	0.0025	57/91	3.19 (1.798–5.642)	<0.0001
HAI						
< 7	123/174			50/101		
≥ 7	45/52	2.66 (1.127–6.303)	0.0256	27/34	3.93 (1.571–9.855)	0.0035
Hepatic iron staining						
Negative	167/241			76/150		
Positive	29/46	0.76 (0.391–1.460)	0.4047	13/30	0.75 (0.338–1.640)	0.4643
Elevated ALT (>40 U/l)						
No	25/51			9/35		
Yes	186/267	2.38 (1.300–4.380)	0.0050	87/168	3.10 (1.372–7.018)	0.0065
ALT pattern						
Normal	17/40			6/29		
High	158/216	3.71 (1.839–7.388)	0.0002	73/131	4.83 (1.843–12.630)	0.0013
Elevated AST (>40 U/l)						
No	42/86			16/60		
Yes	171/236	2.75 (1.654–4.590)	<0.0001	81/146	3.42 (1.773–6.622)	0.0002
Elevated AFP (≥20 µg/l)						
No	146/228			67/149		
Yes	26/29	4.86 (1.429–16.575)	0.0114	18/22	7.33 (2.074–25.976)	0.0020
Serum ferritin (>300 µg/l)						
No	105/155			77/171		
Yes	49/65	1.46 (0.756–2.813)	0.2606	20/36	1.53 (0.740–3.145)	0.2520
Hypoalbuminemia (<35 g/l)						
No	163/261			74/172		
Yes	38/47	2.53 (1.177–5.475)	0.0216	17/26	2.05 (1.056–5.926)	0.0372
Thrombocytopenia (<130 × 10 <sup>9</sup> /l)						
No	159/251			69/161		
Yes	53/70	1.80 (0.986–3.299)	0.0554	27/44	2.11 (1.070–4.190)	0.0312
Splenomegaly						
No	143/225			59/141		
Yes	60/81	1.64 (0.930–2.886)	0.0876	33/54	2.10 (1.150–4.147)	0.0170

<sup>a</sup> After Bonferroni's adjustment, a *P* value <0.004 was considered as statistically significant.<sup>b</sup> Cases: ratio of patients with grade I–III steatosis to total patients in that group.<sup>c</sup> OR, odds ratio; and CI, confidence interval.<sup>d</sup> Cases: ratio of patients with grade II/III steatosis to total patients in that group.

In addition, steatosis was significantly associated with stage III/IV fibrosis, the most important histological criteria for advanced CHC. However, steatosis was not associated with elevated serum ALT, AFP and ferritin at entry, hypoalbuminemia, and hepatic iron staining.

To further examine the pathogenic role of steatosis on CHC progression, the same univariate analysis was also performed to compare patients lacking steatosis to those with grade II/III steatosis (Table 4, panels 2). All three variables that had been associated with the presence of steatosis (panel 1) were even more associated with grade II–III steatosis as indicated by higher ORs (panel 2). Furthermore, grade II/III steatosis was also significantly associated with a higher HAI, a histological indicator of

active CHC, and elevated AFP, a reported surrogate of advanced hepatic fibrosis [38]. Grade II/III steatosis was not associated with age at the exposure, elevated ALT and ferritin at entry, hepatic iron staining, hypoalbuminemia, thrombocytopenia, and splenomegaly. A multivariate analysis revealed that after adjusting for ALT and AST levels, ALT pattern, hypoalbuminemia, thrombocytopenia, and HAI, the presence of steatosis, especially grade II/III steatosis, was independently associated stage III/IV fibrosis (Table 5). These data indicate that in patients with CHC, a higher grade of steatosis is more associated with an active and progressive CHC.

To further define the association of steatosis with fibrosis, two additional analyzes were carried out. First,



**Table 5**  
Multivariate analysis of the association of steatosis with parameters of activity and stage of CHC

Variables	Steatosis grade I–III vs. steatosis grade 0		Steatosis grade II–III vs. steatosis grade 0	
	OR (95% CI) <sup>a</sup>	P value	OR (95% CI) <sup>a</sup>	P value
Fibrosis (III/IV)	3.987 (1.531–10.385)	0.0046	5.599 (2.041–15.361)	0.0008
HAI ( $\geq 7$ )	1.392 (0.502–3.858)	0.5248	1.928 (0.612–6.077)	0.2624
Elevated ALT ( $> 40$ U/l)	1.746 (0.619–4.925)	0.2919	1.603 (0.435–5.902)	0.4779
ALT patterns	0.829 (0.457–1.504)	0.5367	1.127 (0.530–2.395)	0.7562
Elevated AST ( $> 40$ U/l)	2.136 (0.953–4.789)	0.0653	1.999 (0.739–5.408)	0.1725
Hypoalbuminemia ( $< 35$ g/l)	1.980 (0.490–8.005)	0.3381	1.606 (0.343–7.513)	0.5476
Thrombocytopenia $< 130 \times 10^9/l$	1.366 (0.387–4.828)	0.6279	2.204 (0.568–8.557)	0.2536

<sup>a</sup> OR, odds ratio; and CI, confidence interval.

the mean fibrosis score was  $1.7 \pm 1.3$  in patients without hepatic steatosis, which was increased to  $2.4 \pm 1.2$  ( $P = 0.001$ ) in patients with grade II/III steatosis. Second, in patients without hepatic steatosis, 30.9% had stage III/IV fibrosis. However, in patients with grade II/III steatosis, 58.8% ( $P = 0.0002$ ) had stage III/IV fibrosis.

#### 4. Discussion

The present study investigated a large cohort of US patients with CHC and demonstrated that the frequency of steatosis was as high as 66.0% in these patients. Hourigan et al. reported that 20.0% of Australian patients with CHC have grade II/III steatosis [6]. Using the same scoring system, grade II/III steatosis was seen in 30.0% of our patients with CHC. Therefore, hepatic steatosis, especially a higher grade of steatosis, is a very common presentation in U.S. patients with CHC.

Although it is well accepted that obesity is associated with hepatic steatosis in patients with CHC, the threshold BMI for hepatic steatosis has not been reported. In our cohort of patients, BMI  $\geq 25$  kg/m<sup>2</sup> has been significantly associated with hepatic steatosis. Thus, for the first time, our data demonstrated that even being overweight can contribute to hepatic steatosis in patients with CHC. Furthermore, multivariate analyzes confirmed that this association was independent of age, ethnicity, a past history of alcohol use, DM, hypertriglyceridemia, and HCV-3a infection. Although both being overweight and obese were associated with hepatic steatosis, this association was more significant in patients with obesity. Thus, a higher BMI carries higher risk for hepatic steatosis in patients with CHC. It should be noted that the mean BMI in our cohort of patients and the other U.S. studies (19 and Hu et al. unpublished data) is higher than that reported from other countries [8,9]. This may contribute to a relatively higher prevalence of steatosis in the U.S. patients with CHC. Interestingly, we also found that BMI  $\geq 25$  kg/m<sup>2</sup> was associated with any grade of steatosis, indicating that being overweight/obese may not affect grade of steatosis in these patients. Taken together,

our data indicate that being overweight/obesity plays an independent pathogenic role in the development, but not grade of hepatic steatosis in HCV-infected US patients.

Alcohol use can cause hepatic steatosis. In the present study, 69.6% reported a past history of alcohol use defined as  $> 30$  g/day, but all had achieved 6-month sobriety prior to enrollment. Our data did not reveal an association of past history of alcohol use with hepatic steatosis. In addition, the prevalence of steatosis was not different in patients who were sobered for 6–12 vs.  $> 12$  months. Although our data provided additional support to previous findings [8,18,19] that a history of alcohol use may not be associated with hepatic steatosis in HCV-infected patients, a larger prospective study will be needed to further address this issue.

In transgenic mice, expression of HCV proteins has been associated with hepatic steatosis [27,28]. Some studies have also revealed a higher frequency and grade of hepatic steatosis in patients with HCV-3a infection [9,19,29–31]. A recent study indicated that effective HCV treatment is associated with reduced steatosis in patients with HCV-3 infection [15]. However, this association remains controversial [16]. The present study involved a small group of such patients and did not reveal an association between HCV-3a infection and either presence or grade of hepatic steatosis. Furthermore, a recent study reported that a high prevalence of hepatic steatosis (40.1%) was seen in patients with chronic hepatitis B virus infection as well [39]. Clearly, additional studies, including utilization of HCV replication system, will be required to disclose the association of HCV-3 infection with steatosis.

A recent study revealed that both DM and hypertriglyceridemia may not directly associated with hepatic steatosis in HCV-infected patients [19]. Consistent with these results, we observed relatively higher, but not statistically different frequencies of DM and hypertriglyceridemia in HCV-infected patients with hepatic steatosis. The present study did not reveal an association of steatosis with age, gender, ethnicity, HCV-load, serum ferritin, and hepatic iron.

Several studies have indicated an association between steatosis and progression of hepatic fibrosis in different

ethnic patients with CHC [8,11,18]. However, data on this issue remain limited and controversial in U.S. patients [10,14,16,17,19]. More importantly, it remains unknown whether a higher grade of steatosis is more associated with a higher stage of hepatic fibrosis. The present study assessed a large cohort of U.S. patients and found that presence of steatosis, especially a higher grade of hepatic steatosis, was significantly associated with stage III/IV fibrosis. Patients with grade II/III steatosis had significantly higher mean fibrotic scores and percentage of stage III/IV fibrosis. In addition, patients with grade II/III steatosis had significantly higher frequencies of hypoalbuminemia, thrombocytopenia, and splenomegaly, the clinical evidence of portal hypertension and advanced hepatic fibrosis. After adjusting for serum levels of ALT and albumin, platelet count, ALT pattern, and HAI, multivariate analysis revealed that presence of grade II/III steatosis was independently associated with stage III/IV hepatic fibrosis. Taken together, our results indicate that the presence of grade II/III steatosis may accelerate progression of fibrosis in U.S. patients with CHC.

It remains controversial whether hepatic steatosis may accelerate fibrosis by stimulating activity of CHC. Some have reported a possible association of a higher grade of hepatic steatosis with higher levels of ALT and HAI [8,9,19], but others were unable to establish this association [11,16]. Through a thorough univariate analysis, we found that the grade II/III steatosis was significantly associated with a higher HAI, the most reliable histological evidence of active liver injury. In addition, the presence of steatosis, especially grade II/III steatosis, was associated with persistently elevated ALT and elevated AST and AFP at entry, a series of clinical parameters representing active liver injury. Thus, our data provide additional evidence that a higher grade of hepatic steatosis may promote HCV-mediated liver injury. This steatosis-related ongoing liver injury may accelerate progression of hepatic fibrosis and result in an increased incidence of advanced fibrosis in these patients. Consistent with our findings, an effective weight loss is associated with reduction in steatosis, ALT elevation, and fibrosis stage in patients with CHC [40].

In conclusion, this study reveals a high prevalence of hepatic steatosis in the U.S. patients with CHC, that is independently associated with being overweight/obese as defined by BMI  $\geq 25$  kg/m<sup>2</sup>. The present study also indicates that in patients with CHC, grade II/III steatosis may accelerate activity and progression of the disease, and is associated with a significantly higher frequency of stage III/IV fibrosis.

## Acknowledgements

The authors are indebted to Dr. Pramila N. Singh for his invaluable comments on statistical analysis. Part of this work was published as an abstract in *Hepatology* 2002; 36: 349A and presented in 53 AASLD annual meeting in

November 2002 in Boston. This work was supported by a grant from the Division of Gastroenterology and Hepatology, Loma Linda University School of Medicine (K.-Q. H.).

## References

- [1] Madhava V, Burgess C, Drucker E. Epidemiology of chronic hepatitis C infection in sub-Saharan Africa. *Lancet Infect Dis* 2002; 2:293–302.
- [2] Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, et al. Prevalence of hepatitis C virus infection in the United States. *N Engl J Med* 1999;341:556–562.
- [3] National Institute of Health Consensus Development Conference Statement, Management of Hepatitis C: 2002-June 10–12, 2002. *Hepatology* 2003;36:S3–20.
- [4] Seeff LB. Natural history of hepatitis C. *Hepatology* 1997;26: 21S–28S.
- [5] DiBisceglie AM. Natural history of hepatitis C: its impact on clinical management. *Hepatology* 2000;31:1014–1018. 3B. NIH censuses in hepatitis C 2002.
- [6] Hu KQ, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. *Hepatology* 1999;29: 1311–1316.
- [7] Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; 112:463–472.
- [8] Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999;29: 1215–1219.
- [9] Adinolfi LE, Gambardella M, Andreana A, Tripodi M-F, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001;33:1358–1364.
- [10] Czaja AJ, Carpenter HA, Santrach PJ, Moore SB. Host- and disease-specific factors affecting steatosis in chronic hepatitis C. *J Hepatol* 1998;29:198–206.
- [11] Hwang SJ, Luo JC, Chu CW, Lai CR, Lu CR, Tsay SH, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. *J Gastroenterol Hepatol* 2001;16:190–195.
- [12] Giannini E, Geppa P, Botta F, Fasoli A, Romagnoli P, Cresta E, et al. Steatosis and bile duct damage in chronic hepatitis C: distribution and relationships in a group of Northern Italian patients. *Liver* 1999;19: 432–437.
- [13] Clouston AD, Jonsson JR, Purdie DM, Macdonald GA, Pandeya N, Shorthouse C, et al. Steatosis and chronic hepatitis C: analysis of fibrosis and stellate cell activation. *J Hepatol* 2001;34:314–320.
- [14] Hu KQ, Esrailian E, Runyon B, Thompson K, Chase R, Abdelhalim F, et al. Clinical implication of hepatic steatosis in patients with chronic hepatitis C. *Hepatology* 2002;34:187A.
- [15] Kumar D, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic steatosis after sustained therapeutic response. *Hepatology* 2002;36:1266–1272.
- [16] Negro F. Hepatitis C virus and liver steatosis: is it the virus? Yes it is, but not always. *Hepatology* 2002;36:1050–1052.
- [17] Contos MJ, Garcia N, Sterling RK, Shiffman ML, Luketic V, Sargeant C. The impact of non-alcoholic fatty liver disease on the progression of hepatitis C. *Hepatology* 2001;34(Suppl.):1139. (Abstract).
- [18] Sumida Y, Nakashima T, Yoh T, Kakisaka Y, Ishikawa H, Mitruyoshi H, et al. Independent predictors of hepatic steatosis and fibrosis in

- Japanese patients with chronic hepatitis C. *Gastroenterology* 2001;120:A381.
- [19] Monto A, Alonzo J, Watson JJ, Grunfeld G, Wright T. Steatosis in chronic hepatitis: relative contribution of obesity, diabetes mellitus, and alcohol. *Hepatology* 2002;36:729–736.
- [20] Angulo P. Non-alcoholic fatty liver disease. *N Engl J Med* 2002;346:1221–1231.
- [21] Younossi ZM, Diehl AM, Ong JP. Non-alcoholic fatty liver disease: an agenda for clinical research. *Hepatology* 2002;35:746–752.
- [22] Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with non-alcoholic steatohepatitis. *Hepatology* 1999;30:1356–1362.
- [23] Bacon B, Farahvash M, Janney C, Neuschwander-Tetri B. Non-alcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology* 1994;107:1103–1109.
- [24] Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000;132:112–117.
- [25] Mason AL, Lai JY, Hoang N, Qian KP, Alexander GJ, Xu L, et al. Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999;29:328–333.
- [26] Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000;133:592–599.
- [27] Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. *J Gen Virol* 1997;78:1527–1531.
- [28] Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, et al. Steatosis and liver cancer in transgenic mice expressing the structural and non-structural proteins of hepatitis C virus. *Gastroenterology* 2002;122:352–365.
- [29] Adinolfi LE, Utili R, Ruggiero G. Body composition and hepatic steatosis as precursors of fibrosis in chronic hepatitis C patients. *Hepatology* 1999;30:1530–1531.
- [30] Rubbia-Brandt L, Giostra E, Quadri R, Male P-J, Mentha G, Spahr L, et al. Evidence that liver steatosis is virally mediated in a subset of patients infected by hepatitis C virus genotype 3 [Abstract]. *J Hepatol* 1999;30(Suppl.):133A.
- [31] Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male P-J, Mentha G, et al. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000;33:106–115.
- [32] Serfaty L, Chazouilleres O, Poujol-Robert A, Morand-Joubert L, Dubois C, Chretien Y, et al. Risk factors for cirrhosis in patients with chronic hepatitis C virus infection: results of a case-control study. *Hepatology* 1997;26:776–779.
- [33] Donato KA. Executive summary of the clinical guidelines on the identification, evaluation, and treatment of obesity in adults. *Arch Intern Med* 1998;158:1855–1867.
- [34] Alberti KGMM, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional Report of a WHO Consultation. *Diabetic Medicine* 1998;15:539–553.
- [35] Hu KQ, Bonacini M, Lindsay KL, Redeker AG. The clinical profile of chronic hepatitis C in a major county medical center outpatient setting in the United States. *Journal of Tongji Medical University* 2003; in press.
- [36] Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1:431–435.
- [37] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Non-alcoholic steatohepatitis: a proposal for grading and staging the histologic lesions. *Am J Gastroenterol* 1999;94:2467–2474.
- [38] Hu K-Q, Elhazin B, Hillebrand DJ, Abdelhalim F, Kyulo N. Clinical significance of elevated alpha fetoprotein (AFP) in patients with chronic hepatitis C. *Hepatology* 2002;36:544A.
- [39] Soon DKW, Pan AX, Yeo SH, Ho LH, Wise SD. Fatty liver (FL) in chronic hepatitis B carriers may affect the interpretation of alanine aminotransferase (ALT) elevations. *J Hepatol* 2002;36(Suppl. 1):131.
- [40] Hickman IJ, Clouston AD, Macdonald GA, Purdie DM, Prins JB, Ash S, et al. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. *Gut* 2002;51:89–94.