

Steatosis in Chronic Hepatitis C: Relative Contributions of Obesity, Diabetes Mellitus, and Alcohol

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Steatosis has emerged as a histologic finding of importance to the progression of hepatitis C virus (HCV)-associated liver disease. However, most studies of HCV-associated steatosis have excluded alcohol drinkers and individuals with diabetes and thus have not addressed the relative contribution of known causes of steatosis to liver injury in HCV-associated disease. To address this issue, we studied 297 consecutive patients with HCV who met inclusion criteria. Alcohol consumption, demographics, and serologic tests were correlated with degrees of steatosis and fibrosis on liver biopsy. Liver biopsy specimens were also examined for evidence of significant alcohol or nonalcoholic steatohepatitis (NASH) injury. In univariate analysis, steatosis correlated with type 2 diabetes mellitus ($P = .005$) and body mass index (BMI) ($P = .0001$) but not with the intensity of alcohol intake (in grams per day). In multivariate analysis, BMI ($P = .0002$) and genotype 3a infection ($P = .02$) were independent predictors of steatosis. When patients with risk factors for NASH were excluded, genotype 3a infection was the only independent predictor of steatosis. Steatosis ($P = .04$) and inflammation ($P < .0001$) scores on liver biopsy were the only independent predictors of fibrosis. Significant alcohol or NASH injury was found in only 6% of biopsy specimens. In conclusion, steatosis in HCV infection is associated with risk factors for NASH, particularly obesity, rather than alcohol consumption. (HEPATOLOGY 2002;36:729-736.)

Hepatitis C virus (HCV) now predominates as a cause of chronic liver disease in Western countries. A striking feature of HCV infection is its association with fat accumulation within hepatocytes (steatosis).¹ Previous work has elucidated some aspects of the relationship between HCV and steatosis; HCV-infected patients with steatosis are more likely to have risk factors for nonalcoholic steatohepatitis (NASH), particularly higher body mass index (BMI) and serum triglyceride levels.² There is a significant association between steatosis and inflammation and fibrosis scores on liver biopsy,^{3,4} and genotype 3a has been linked to steatosis more strongly than other genotypes.^{4,5}

Hepatic steatosis in general has often been attributed to alcohol intake. The feeding of alcohol to mice⁶ and humans⁷ leads predictably to steatosis. This early mechanistic work on alcohol initially dominated our understanding of liver diseases characterized by fat accumulation. Indeed, nondrinking diabetic patients and obese patients were often suspected of covert alcohol use before the characterization of NASH, a fatty liver disease defined by the absence of alcohol.⁸ Understanding whether steatosis is independent of alcohol consumption is therefore an important step in the evaluation of any liver disease characterized by steatosis, including HCV-associated liver disease.

The primary aim of our study was to investigate the role of alcohol in HCV-related steatosis. This has been inadequately studied to date. Hourigan et al.³ were the first to examine this relationship and did not find any association between alcohol intake and steatosis. They also did not find a relationship between alcohol intake and fibrosis, however, which conflicts with the generally accepted view that alcohol intake contributes to fibrosis.⁹ Another recent study¹⁰ had similar findings to those of Hourigan et al., namely that estimated alcohol intake did not correlate with steatosis or fibrosis. Both of these stud-

Abbreviations: HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; BMI, body mass index.

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Received December 12, 2001; accepted May 30, 2002.

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doi:10.1053/jhep.2002.35064

ies used fairly simple estimates of alcohol intake, which may have been inadequate. However, other studies of HCV-associated steatosis have either excluded patients who drink alcohol at all^{2,5} or those who drink more than 30 to 40 g/d.^{4,11} Because both alcohol and HCV can produce steatosis independently, we hypothesized that the presence of both risk factors might lead to increased steatosis. Moreover, we hypothesized that steatosis may represent a mechanism of the increased fibrosis seen in patients with chronic hepatitis C infection who drink excessively.

The second aim of our study was to investigate the potential role of another variable known to cause steatosis, type 2 diabetes mellitus, on HCV-related steatosis. Type 2 diabetes mellitus is a principal contributor to NASH,¹² one of the most common causes of liver disease in Western countries.¹³ However, little is known about the role of diabetes in the development of steatosis in chronic HCV disease because diabetic patients, like alcohol drinkers, were either excluded^{4,5,11} or included in insufficient numbers for adequate study^{2,3} in most of the epidemiologic studies of HCV-associated steatosis performed to date. The one recent study that included diabetic patients found no association between diabetes and steatosis.¹⁰ Hepatitis C has also been found to be an independent risk factor for diabetes,¹⁴ although this relationship is controversial.¹⁵ Because both HCV and diabetes are linked to steatosis, we believed that clarifying the contribution of each to hepatic steatosis and/or fibrosis would be important.

The third aim of our study was to clarify the histologic pattern of liver injury in patients with HCV-associated steatosis and to determine whether this pattern was altered by subjects' alcohol intake or type 2 diabetes mellitus. Alcohol injury is nearly indistinguishable histologically from that caused by NASH.¹⁶ However, hepatitis C infection and alcoholic liver injury can be readily distinguished from each other when each is studied separately.¹⁷⁻¹⁹ When NASH or alcohol-related injury is superimposed on HCV-related injury, a situation commonly encountered in the obese, diabetic, or alcoholic patient with HCV, histologic changes have not been well described. Given our study population, which included diabetic patients and alcohol drinkers, we were able to address histologic manifestations of these multiple potential causes of liver injury.

Patients and Methods

Patients. Patients were recruited from those undergoing liver biopsy for staging of HCV disease before consideration of therapy against HCV at the Veterans Affairs

and UCSF Medical Centers in San Francisco, CA. Thus, patients with clinically decompensated cirrhosis or contraindications to liver biopsy were not included in the study group. Consecutive patients evaluated (by T.L.W. and A.M.) between July 1997 and May 2000 were asked to participate in the study, which was approved by the local institutional review board. Following written, informed consent, all patients completed questionnaires at the time of liver biopsy. A total of 181 patients were recruited through the Veterans Affairs Medical Center and 116 from UCSF Medical Center, comprising a study group of 297 patients. Patients excluded were those who did not consent to enrollment (n = 2), had incomplete alcohol data (n = 81), had previously received treatment for chronic HCV infection (n = 37), were coinfecting with human immunodeficiency virus (n = 12), had undergone solid organ transplantation (n = 6), had other coexisting liver disease (n = 9), or were taking medications that can cause steatosis¹² (n = 4).

Questionnaire. Consenting patients were asked to complete a detailed questionnaire. This included demographic information and risk factors for acquisition of HCV. Complete histories of patients' injection drug use and blood transfusions were obtained. Other potential exposures to HCV, such as needle-sticks, combat injuries, tattoos, acupuncture, and sexual contacts were also recorded.

Alcohol Quantification. Alcohol consumption was assessed in detail. Beer, wine, and liquor consumption were quantified individually based on patients' typical quantity, frequency, and duration of use. The estimated number of drinks consumed over a patient's lifetime was multiplied by the alcohol content (each drink containing the equivalent of 10 g of pure ethanol), yielding an estimate of lifetime alcohol consumption in grams of ethanol. To attempt to address age bias (older patients having more years to drink and thus a higher lifetime intake), lifetime alcohol was then divided by the length of time each respondent had consumed any alcohol, yielding an average consumption over the span of drinking (in grams per day). This average consumption is subsequently referred to as "alcohol intensity."

Patients were also asked the CAGE questions, the most commonly used alcohol abuse screening questions.²⁰ The screen is considered positive (i.e., possibly indicative of alcohol abuse) if 2 or more of the 4 questions are answered in the affirmative.²¹

Diagnosis of HCV Infection. All patients tested positive for specific HCV antibodies (second-generation enzyme immunoassay; Abbott Laboratories, Chicago, IL), had detectable serum HCV RNA levels by polymerase chain reaction-based methodology, and had liver histol-

ogy compatible with chronic hepatitis C disease. Quantitative viral load and genotype were available in most of the patients. HCV RNA was quantified by branched DNA assay (Quantiplex version 2; Chiron Corp., Emeryville, CA) and by reverse-transcription polymerase chain reaction (AMPLICOR; Roche Diagnostic Systems, Branchburg, NJ). To allow comparison of different quantitative assays, we separated viremia into classes as described previously²²: class 1, low viremia (less than the 33.3rd percentile of the data); class 2, intermediate viremia (between the 33.3rd and 66.6th percentiles); class 3, high viremia (greater than the 66.6th percentile). HCV genotyping was performed by standard methodology (INNO-LiPA, second generation; Innogenetics, Zwijnaarde, Belgium).

Histology. Histologic manifestations of hepatitis C include the following: (1) portal-based lymphoid infiltrates, often in follicles, (2) bile duct damage, (3) focal steatosis, and (4) portal-based fibrosis.^{1,18} Histologic manifestations of alcohol-induced hepatic injury include the following: (1) steatosis distributed variably through the hepatic lobule, (2) perivenular and pericellular fibrosis in zone 3 of the acinus, and (3) Mallory bodies.¹⁹ Liver histology in this study was assessed by staff pathologists at each institution. The Batts-Ludwig scoring system for chronic hepatitis C, with single inflammation (0-4) and fibrosis (0-4) scores,²³ was applied. Steatosis was scored by a single pathologist blinded to clinical data (J.A.) according to an accepted scoring system.²⁴ The scoring system was as follows: 0, no steatosis; 1, less than 33% of hepatocytes with steatosis; 2, 33% to 66% of hepatocytes affected; 3, more than 66% of hepatocytes affected. The pathologist also examined the centrilobular zone of the acinus in detail, noting the presence of perivenular fibrosis and/or pericellular inflammation centered in this region. When distinct centrilobular injury was seen, this was denoted as alcohol or NASH injury.

Serum Assays. Fasting serum lipid levels (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides), serum alanine aminotransferase levels, and HCV genotype and viral load were obtained when available within 6 months of questionnaire completion.

Variables Examined. Demographic characteristics analyzed included age, sex, race, military veteran status, and obesity as estimated by BMI, which was calculated by the following formula: weight in kilograms/(height in meters)². Ranges of BMI used were those outlined by expert consensus guidelines²⁵: a BMI greater than 25 kg/m² was defined as overweight and a BMI greater than 30 kg/m² as obese. Disease-associated variables included risk factors for acquisition (injection drug use, blood transfusion, both, or other), estimated age at infection and duration of infection, genotype, and viral load. Age at

infection and duration of infection were estimated from first exposure to injection drug use or blood transfusion. Year of first injection drug use was used if both risk factors were present; if neither was present, duration of infection and age at infection were not estimated. Alcohol-associated variables included alcohol intensity (in grams per day), time since consumption of last alcoholic beverage, and CAGE score. Demographic, histologic, alcohol, and HCV-associated data were gathered prospectively; BMI and serum lipid values were obtained prospectively in most and in the remainder by chart review.

Statistical Analysis. Demographic and histologic values and serum assays were compared across levels of steatosis and fibrosis. Data are expressed as percentages for categorical variables (e.g., sex, diabetes) and means and SDs for continuous variables (e.g., BMI, serum cholesterol). Before fitting a continuous predictor variable as a linear term into a model, it was divided into quartiles, and its relationship to the outcome variable was examined to ensure that its modeling as a linear term in the model was appropriate.

Spearman rank correlations were used to assess the significance of associations between ordinal or continuous predictor variables and steatosis and fibrosis where possible. Nonparametric Mann-Whitney tests for dichotomous predictor variables and Kruskal-Wallis tests for multicategory predictors were also used where appropriate (see Tables 3 and 5). The independent effect of each variable significantly associated with steatosis and fibrosis in univariate analysis (significance level $P < .05$, except as noted) was assessed by multivariate proportional odds models.

Results

Study Population. A total of 297 patients were studied. Characteristics of the group are shown in Table 1. BMI was available in 250 of 297 patients (84%); mean value was 28.5 kg/m² and falls in the overweight range for the U.S. population as a whole.²⁵ The distribution of BMI was as follows: less than 25 kg/m², 78 (31%); 25 to 27 kg/m², 36 (14%); 27 to 30 kg/m², 57 (23%); greater than 30 kg/m², 79 (32%). Alanine aminotransferase values were available in 288 patients and were abnormal in 209 (73%). Quantitative viral load was available in 252 patients, and HCV genotype was available in 279.

Histologic Findings. The distribution of histologic scores is shown in Table 2. Most important, 280 of 297 biopsy specimens (94%) only had histologic evidence of hepatitis C. The rest, 17 of 297 (6%), had evidence of hepatitis C and additional evidence of either NASH or

Table 1. Characteristics of the Study Population

	n	Mean (SD) or Proportion
Demographics		
Age (yr)	297	49 (7)
Male sex	250	84.2%
U.S. military veteran	181	60.9%
BMI (kg/m ²)	250	28.5 (5.6)
Type 2 diabetes mellitus	23	7.7%
Alcohol intensity (g/d)	297	54.3 (66.3)
Ethnicity		
Black	49	16.6%
Asian	12	4.0%
White	190	64.0%
Latino-American	23	7.7%
Other/Declines	23	7.7%
Chemistries		
ALT	288	97.0%
Cholesterol (mg/dL)	196	169.3 (38.6)
Triglycerides (mg/dL)	166	125.1 (59.6)
HDL (mg/dL)	154	42.4 (14.0)
LDL (mg/dL)	154	104.8 (34.8)
Risk for HCV infection		
IDU	160	53.9%
BT	35	11.8%
Both IDU and BT	28	9.4%
Neither IDU nor BT	74	24.9%
HCV disease		
Age at infection (yr)*	223	24.9 (8.2)
Duration of HCV (yr)*	223	24.1 (7.6)
Genotype 1	194	69.5%
Genotype 2	42	15.1%
Genotype 3a	39	14.0%
Genotype 4	2	0.7%
Mixed	2	0.7%
Viral load		
Lowest tertile	82	32.5%
Middle tertile	74	29.4%
Upper tertile	96	38.1%

Abbreviations: ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IDU, injection drug use; BT, blood transfusion.

*Subjects with a history of injection drug use or a blood transfusion.

alcohol injury. None displayed only histologic evidence of NASH or alcohol-related injury.

Alcohol Consumption. Mean intensity of alcohol intake was 54.3 g/d (approximately 5½ alcoholic beverages per day), as shown in Table 1. Ninety-four patients (32%) had consumed more than 60 g/d over their years of drinking. Alcohol intake was not normally distributed, however, with a median of 28 g/d. Each successive quartile of alcohol intake was associated with a stepwise increase in the odds ratio for a positive CAGE screen, taken as 2 or more of 4 positive responses to the CAGE questions. The second quartile (½-3 drinks per day) had an odds ratio of 5.3 for a positive CAGE screen compared with the first quartile; the third (3-9 drinks per day) and fourth (>9 drinks per day) quartiles had odds ratios of 16.6 and 171.1, respectively, compared with the first quartile.

Table 2. Liver Histology

Finding	Score	n (Total 297)	Frequency (%)
Inflammation (mean, 1.7; SD, 0.7)	0	11	3.7
	1	82	27.6
	2	179	60.3
	3	24	8.1
	4	1	0.3
Fibrosis (mean, 1.6; SD, 1.2)	0	63	21.2
	1	83	27.9
	2	83	27.9
	3	40	13.5
	4	28	9.5
Steatosis (mean, 0.7; SD, 0.7)	0	126	42.4
	1	146	49.2
	2	18	6.1
	3	7	2.3
	Histologic pattern		
	HCV alone	280	94.3
	HCV + alcohol/NASH	17	5.7

Variables Associated With the Grade of Steatosis.

Univariate correlations between predictor variables and steatosis are shown in Table 3. Alcohol intensity, either overall or when dichotomized into greater than 60 g/d or 60 g or less per day, did not correlate with steatosis. Even subjects in the top 10% of alcohol intensity or those who drank alcohol within 1 week of undergoing liver biopsy

Table 3. Univariate Correlations With Steatosis

	Spearman Rank Correlation (Lower to Upper 95% CI)	P
Demographics		
Age (yr)	0.03 (-0.10 to 0.15)	.67
Male sex	NA	.06*
BMI (kg/m ²)	0.31 (0.19 to 0.43)	.0001
Type 2 diabetes mellitus	NA	.005*
Alcohol use		
Alcohol intensity (g/d)	-0.08 (-0.20 to 0.06)	.22
Alcohol > 60 g/d	NA	.77*
Chemistries		
Elevated ALT	NA	<.0001*
Cholesterol (mg/dL)	<0.01 (-0.15 to 0.14)	.90
Triglycerides (mg/dL)	0.15 (-0.01 to 0.30)	.05
HDL (mg/dL)	-0.13 (-0.29 to 0.04)	.11
LDL (mg/dL)	0.05 (-0.12 to 0.22)	.86
HCV disease		
Duration of HCV (yr)†	0.11 (-0.03 to 0.25)	.13
Genotype 3a‡	NA	.002*
Liver biopsy		
Inflammation (0-4)	0.15 (0.07 to 0.27)	.02
Fibrosis (0-4)	0.17 (0.05 to 0.29)	.007
Alcohol/NASH injury	NA	.40*
Cirrhosis	NA	.47*

Abbreviations: ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*Mann-Whitney P value.

†Estimated in subjects with a history of injection drug use or a blood transfusion.

‡Compared with other genotypes.

Table 4. Multivariate Proportional Odds Model for Steatosis

	Odds Ratio	Lower to Upper 95% CI	P
Male sex	2.31	0.98-5.45	.06
BMI	1.10	1.05-1.16	.0002
Diabetes	1.10	0.38-3.20	.86
Alcohol intensity	1.00	0.99-1.00	.10
Genotype 3a*	2.47	1.12-5.43	.02
Inflammation	1.15	0.75-1.78	.52
Fibrosis	1.14	0.91-1.44	.26

NOTE. n = 219.

*Compared with other genotypes.

did not have more steatosis than those with less alcohol intake (Mann-Whitney *P* values of .88 and .65, respectively). Patient ethnicity (Kruskal-Wallis *P* value of .38), HCV viral load (Mann-Whitney *P* value of .39), age, and duration of infection also did not correlate with steatosis.

The clinical significance of independent correlation between an abnormal alanine aminotransferase value and steatosis or fibrosis was unclear, so this variable was not included in the multivariate models. Although alcohol intensity did not correlate with steatosis in univariate analysis, it was included in multivariate analysis to further clarify this relationship. In addition, although triglycerides correlated with steatosis in univariate analysis (*P* = .05), it was not included in multivariate analysis because values were only available in 166 patients. Multivariate analysis of steatosis in 219 patients is shown in Table 4. BMI and genotype 3a infection correlated independently with steatosis. The effect of diabetes mellitus on steatosis was not independent of the effect of BMI.

To determine whether HCV has a direct effect on steatosis, we performed a subanalysis in patients without clinical risk factors for steatosis. Subjects with BMI greater than 27 kg/m², alcohol intake greater than 60 g/day, or type 2 diabetes mellitus were excluded, and the remaining 67 patients were reanalyzed using a multivariate proportional odds model. Genotype 3a was by far the strongest predictor of steatosis in this multivariate analysis (odds ratio, 12.01; *P* = .004) and was the only independent predictor of steatosis.

Variables Associated With Fibrosis Stage. Univariate correlations between various predictor variables and fibrosis are shown in Table 5. One of the strongest associations was between diabetes and fibrosis. To investigate this further, the proportion of diabetic patients at each fibrosis level was examined: fibrosis stage 0 (n = 63), 0 diabetic patients (0%); stage 1 (n = 83), 3 patients (4%); stage 2 (n = 83), 7 patients (8%); stage 3 (n = 40), 6 patients (15%); stage 4 (n = 28), 7 patients (25%).

A multivariate model of fibrosis is shown in Table 6. Age and duration of infection were highly correlated and

Table 5. Univariate Correlations With Fibrosis

	Spearman Rank Correlation (Lower to Upper 95% CI)	P
Demographics		
Age (yr)	0.14 (0.01 to 0.26)	.04
Male sex	NA	.01*
BMI (kg/m ²)	0.21 (-0.03 to 0.34)	.002
Type 2 diabetes mellitus	NA	<.0001*
Alcohol use		
Alcohol intensity (g/d)	0.10 (-0.03 to 0.22)	.13
Alcohol intensity > 60 g/d	NA	.004*
Chemistries		
Elevated ALT	NA	.002*
HCV disease		
Duration of HCV (yr)†	0.13 (-0.01 to 0.27)	.07
Age at infection (yr)†	-0.01 (-0.15 to 0.14)	.92
Genotype 3a‡	NA	.04*
Viral load§	NA	.38
Liver biopsy		
Inflammation (0-4)	0.4 (0.29 to 0.50)	<.0001
Steatosis (0-3)	0.17 (0.05 to 0.29)	.007
Alcohol/NASH injury	NA	.28*

Abbreviation: ALT, alanine aminotransferase.

*Mann-Whitney *P* value.

†Patients with a history of injection drug use or a blood transfusion.

‡Compared with other genotypes.

§In tertiles.

||Kruskal-Wallis *P* value.

confounded the effects of each other, so only age was included in the model. The multivariate proportional odds assumption for fibrosis was not met when age was included as a continuous variable but was met when age was expressed in quartiles. Among variables correlating with fibrosis in univariate analysis, inflammation and steatosis scores on liver biopsy retained a statistically significant correlation in multivariate analysis. Alcohol was not associated with fibrosis in the multivariate model.

Variables Associated With Histologic Evidence of Alcohol or NASH Injury. All 297 liver biopsy specimens had injury consistent with chronic hepatitis C infection.

Table 6. Multivariate Proportional Odds Model for Fibrosis

	Odds Ratio	Lower to Upper 95% CI	P
Age*			
Q2 vs. Q1	1.14	0.56-2.31	.73
Q3 vs. Q1	2.03	0.95-4.32	.07
Q4 vs. Q1	1.61	0.80-3.25	.18
Male sex	0.51	0.24-1.11	.09
BMI	1.04	1.00-1.09	.08
Diabetes	1.98	0.74-5.34	.18
Genotype 3a†	1.19	0.57-2.48	.63
Alcohol > 60 g/d	1.47	0.86-2.52	.16
Steatosis	1.47	1.02-2.13	.04
Inflammation	3.60	2.36-5.49	<.0001

NOTE. n = 219.

*Expressed in quartiles.

†Compared with other genotypes.

Seventeen patients (6%) also had histologic evidence of significant alcohol or NASH injury. A significant alcohol/NASH injury pattern did not correlate with average alcohol intake of greater than 60 g/d, a BMI in the top 25% of the study population, or a history of alcohol intake within 1 week of questionnaire completion (data not shown). Evidence of alcohol/NASH injury also did not correlate with the degree of steatosis ($P = .40$; Table 3) or fibrosis ($P = .28$; Table 5).

Discussion

The causes and significance of hepatic steatosis in chronic HCV infection continue to be elucidated. Because alcohol and type 2 diabetes mellitus are primary causes of steatosis in the general population, many investigators examining the relationship between HCV and steatosis have chosen to exclude patients from their studies who drink moderately or are diabetic. We included such patients in this study so that the relative contributions of various factors to HCV-associated steatosis could be assessed.

In contrast to the prevailing belief that alcohol is a primary cause of steatosis in many liver diseases, including chronic hepatitis C infection, intensity of alcohol use was found not to play a role in steatosis in our patients. Even patients in the top 10% of alcohol intake or those who had recently consumed alcohol did not have more steatosis than patients with less alcohol intake. We did find a univariate association between higher alcohol intake and hepatic fibrosis, as has been reported in several series,^{9,26} and our method of estimating alcohol intake (alcohol intensity) correlated well with patient responses to CAGE questions. These latter findings encourage confidence in our detailed method of estimating alcohol intake. Most of our patients had stopped drinking for more than 6 months before study entry, and alcoholic steatosis is known to resolve with abstinence.²⁷ This may be one reason why no relationship between alcohol intake and steatosis was found, although even the 8% of our patients who had consumed alcohol within 1 week before liver biopsy did not have more steatosis than those who had not done so. The impact on liver histology of such temporal changes in alcohol intake remains poorly understood.

BMI correlated independently with steatosis in our study. BMI was also found to correlate independently with steatosis by Hourigan et al.,³ whereas Adinolfi et al.⁴ only found an independent correlation with visceral obesity, as measured by the surrogate of waist circumference. Future metabolic studies should help clarify whether certain distributions of fat are more pertinent to steatosis than others. Published studies of HCV-associated steatosis have all found a link between obesity and steatosis²⁸;

this study is the first to our knowledge to show the relative impact of obesity on steatosis compared with the full range of other known predictors of steatosis. In multivariate analysis, obesity remained the most important cause of steatosis in patients with chronic HCV infection.

Independent of BMI, and particularly in patients without "fatty liver risk factors," HCV genotype 3a was the second independent cause of steatosis in this study. This suggests that viral variables may lead to altered hepatocyte lipid metabolism. Potential mechanisms for this include a direct effect of higher levels of virus on the hepatocyte⁵ or alterations in lipoprotein processing.¹¹ Steatosis, along with liver inflammation, seems to be the strongest predictor of fibrosis, and the fact that genotype 3a causes some of this steatosis likely explains why 3a infection correlated with fibrosis in our univariate analysis (Table 5).

Diabetes may play a role in HCV-associated steatosis, as has been proposed in NASH,¹² but we found its role to be less than that of obesity and not significant in multivariate analysis. Elevated BMI is a causal factor in type 2 diabetes mellitus; the link between the 2 may be so strong that a correlation between diabetes and steatosis independent of BMI cannot be shown. Given the interest in the relationship between HCV and diabetes, we explored our data further and found a stepwise relationship between fibrosis level and diabetes. This leads us to hypothesize that diabetes places individuals at risk for fibrosis and that in turn fibrosis can lead to diabetes. Both relationships are supported by the findings of other groups.^{14,15,29,30} Diabetes, perhaps through obesity, may cause steatosis and in turn fibrosis, and/or fibrosis itself may result in impaired glucose metabolism. Thus, diabetes and fibrosis can each play a role in the pathogenesis of the other; this may be one reason why conflicting findings on the relationship between hepatitis C and diabetes have been found.^{14,15} Precirrhotic versus postcirrhotic diabetes has been incompletely characterized.

Histologic characteristics, particularly inflammation but also steatosis, correlated with fibrosis independently of all other variables, including such accepted predictors as age and heavy alcohol use.^{9,26} Histologic inflammation has a well-established association with fibrosis,⁹ although exactly how and under what circumstances inflammation impacts fibrosis remains controversial.³¹ Steatosis has now been found in many studies^{2-5,10,11} to correlate with fibrosis, and our data support these results. The strength of the association between steatosis and fibrosis, greater than that between both age and alcohol intake and fibrosis in this study, suggests that obesity and through it steatosis are primary contributors to disease progression in chronic hepatitis C.

However, histologic evidence of significant alcohol or NASH injury is uncommon in patients infected with hepatitis C. Only 6% of our cohort displayed such histology (Table 2), similar to the 10% of patients found to have NASH in another recent study of steatosis in HCV infection.¹⁰ In both NASH and chronic HCV infection, the degree of intrahepatic fat accumulation has been found to be linked to both the inflammation and the scarring seen histologically.^{3,32}

Whether serum lipid levels are abnormal in patients with HCV, and if so whether this relates to steatosis, remains unknown. NASH may have some relationship to lipid abnormalities, most commonly hypertriglyceridemia, with approximately 20% of patients affected.³³ The few studies of lipids in chronic HCV infection found a correlation between higher serum cholesterol and triglyceride levels and steatosis in one study² and a stepwise decrease in all lipids with progression of liver disease in another.³⁴ Cirrhotic patients in our study had slightly lower total cholesterol and high-density lipoprotein levels and higher triglyceride levels, but neither of these was statistically significant (data not shown). Table 3 shows that, in the subset of patients for whom data were available, triglycerides but not the other classes of serum lipids (cholesterol, high-density lipoprotein, low-density lipoprotein) correlated with steatosis in univariate analysis, but none retained correlation in multivariate analysis. The diversity of our population, with many factors contributing independently to changes in serum lipids, may have masked a real relationship between triglycerides and steatosis. Steatosis may contribute to high triglyceride levels, or the reverse may be true. More study is required to clarify the role of serum lipids in the HCV-steatosis interaction.

With this study, we have attempted to find the causes of HCV-associated steatosis in a typical clinical cohort in which multiple risk factors for steatosis coexist. We, like other groups,^{3-5,10} find that steatosis correlates independently with fibrosis in multivariate analysis. The intensity of a patient's alcohol intake does not lead to more steatosis, but risk factors for NASH, particularly elevated BMI, do. Moreover, despite correlating with NASH risk factors, HCV-associated steatosis is not generally accompanied by the centrilobular fibrosis of advanced histologic NASH. We hope that this study will help to clarify the complex relationships between HCV and both host and environment in the generation of steatosis, which is increasingly seen to play a key role in disease progression.

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