Steatosis in Hepatitis C: What Does It Mean?

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Hepatitis C and nonalcoholic fatty liver disease (NAFLD) are both common causes of liver disease. Thus, it is not surprising that they can coexist in the same individual. The prevalence of steatosis in patients with chronic hepatitis C differs between studies, probably reflecting population differences in known risk factors for steatosis, namely overweight, diabetes, and dyslipidemia. The pathogenic significance of steatosis likely differs according to its origin, metabolic (NAFLD or nonalcoholic steatohepatitis) or virus related (due to hepatitis C virus genotype 3). Whether or not steatosis determines fibrosis progression is not yet unproven.

Introduction

The hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide infecting approximately 170 million people. The severity of the disease varies widely, from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma. Liver lesions are thought to be mainly related to immune-mediated mechanisms. Factors influencing the outcome of chronic hepatitis C, including age, gender, and alcohol consumption, are poorly understood.

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of hepatic pathology, with simple steatosis without any evidence of necrosis or inflammation at one end, and severe inflammation with extensive fibrosis or cirrhosis at the other. Fatty liver alone probably has a good prognosis; in contrast, nonalcoholic steatohepatitis (NASH) can progress to cirrhosis in a significant proportion of cases. It is important to note that all the data available have been retrospectively collected.

Hepatic steatosis is a common histologic feature of chronic hepatitis C. This finding is also associated with other risk factors, including obesity, high alcohol consumption, type 2 diabetes, or hyperlipidemia. These factors may contribute to steatosis in patients with chronic hepatitis C. In addition, virologic factors may also play an important role. In this article we focus on the meaning of steatosis in patients with chronic hepatitis C.

Steatosis

Mechanisms of steatosis

Hepatic steatosis develops in the setting of multiple clinical conditions, including obesity, diabetes mellitus, alcohol abuse, protein malnutrition, total parenteral nutrition, acute starvation, drug therapy (*eg*, corticosteroid, amiodarone, perhexiline, estrogens, methotrexate), and carbohydrate overload [1–4,5••].

In the fed state, dietary triglycerides are processed by the enterocyte into chylomicrons, which are secreted into the lymph. The chylomicrons are hydrolyzed into fatty acids by lipoprotein lipase. These free fatty acids are transported to the liver, stored in adipose tissue, or used as energy sources by muscles. Free fatty acids are also supplied to the liver in the form of chylomicron remnants, which are then hydrolyzed by hepatic triglyceride lipase. During fasting, the fatty acids supplied to the liver are derived from hydrolysis (mediated by a hormone-sensitive lipase) of triglycerides stored in the adipose tissue. In the liver, the free fatty acids from all these sources are oxidized by mitochondria, used for triglyceride synthesis, or used to form phospholipids and cholesterol esters.

There are several mechanisms that can lead to hepatic steatosis (Fig. 1). Hepatic triglyceride accumulation occurs when the amount of fatty acid supplied to the liver from the intestine or adipose tissue exceeds the amount needed for mitochondrial oxidation, synthesis of phospholipids, and synthesis of cholesterol esters. This is the presumed mechanisms for steatosis in the setting of obesity, diabetes mellitus, and excessive dietary intake of fats or carbohydrates. Insulin resistance is found in obesity, type 2 diabetes, and cirrhosis. Patients with NAFLD demonstrate markedly increased insulin resistance compared with control subjects. Insulin resistance could contribute to hepatic steatosis by favoring peripheral lipolysis and hepatic uptake of fatty acids. It may also be the reason for increased expression of CYP2E1, thereby contributing to the production of pro-oxidants in a fatty liver. Decreased fatty acid oxidation with subsequent fatty infiltration of the liver may also contribute to the genesis of steatosis, particularly in the setting of hyperinsulinemia. Triglycerides can also accumulate in the liver because of decreased synthesis of lipoprotein and decreased export of lipids from the liver.

NAFLD and NASH

Nonalcoholic fatty liver disease represents a spectrum of conditions characterized histologically by macrovesicular

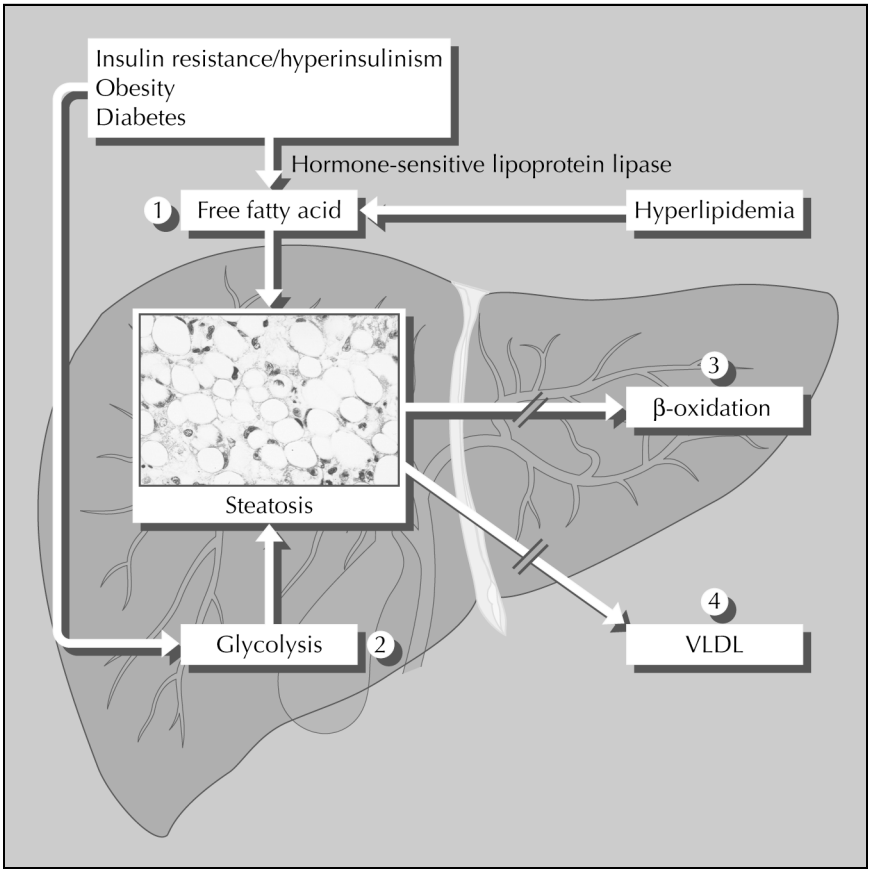

Figure 1. Pathophysiology of steatosis. Accumulation of fatty acids in the liver results mainly from four causes: 1) increased hepatic uptake of fatty acids; fatty acids are mobilized from peripheral adipocytes under the action of hormone-sensitive lipoprotein lipase; 2) increased glycolysis; 3) decreased removal of fatty acids due to impaired mitochondrial β oxidation; and 4) decreased export of fatty acids secreted from the hepatocyte bound to apolipoprotein B as very low density lipoprotein (VLDL).

steatosis occurring in patients who do not consume alcohol in amounts generally considered harmful to the liver [1,2]. There are two histologic patterns of NAFLD: fatty liver alone and steatohepatitis. The term NASH was introduced by Ludwig *et al.* in 1980 to describe the biopsy findings in patients with steatohepatitis in the absence of significant alcohol consumption.

Estimates based on imaging and autopsy studies suggest that approximately 20% to 30% of adults in the United States and other western countries have excess fat accumulation in the liver [2]. About 10% of these individuals, or fully 2% to 3% of adults, are estimated to have NASH.

Risk factors for NAFLD include obesity, diabetes, hypertriglyceridemia, and specific syndromes associated with insulin resistance [1,2]. The body mass index (BMI) is calculated by the following formula: weight in kilograms/height in meters^2 (kg/m^2). Overweight is defined by a BMI greater than 25 kg/m^2 and obesity by a BMI greater than 30 kg/m^2. The prevalence of NAFLD increases with increasing body weight. Wanless and Lentz [6] found steatosis in 70% of obese and 35% of lean patients in a consecutive autopsy study. The prevalence of steatosis is variable among studies, likely as a result of population differences in obesity, type 2 diabetes, and dyslipidemias.

The presence of NAFLD should be considered in individuals with persistent elevation of serum alanine aminotransaminase for which another cause cannot be found. Liver biopsies have been performed in very few studies. In one of these studies, the prevalence of steatosis in patients with abnormal liver tests (in whom known causes of liver disease were ruled out) was 64%, 32% with pure fatty liver and 32% with steatohepatitis [7].

Diagnosis and grading of steatosis

In an individual with abnormal liver tests, particularly elevation of aminotransferase and γ-glutamyl transferase activities, the presence of NAFLD and NASH may be suspected on clinical grounds (with associated factors such as obesity, diabetes, and hyperlipidemia); however, these are nonspecific findings. In addition, although ultrasonography, computed tomographic scan, and magnetic resonance imaging can demonstrate hepatic fat accumulation, they cannot diagnose or grade necroinflammation or fibrosis [1–4]. Thus, a liver biopsy is the only accurate method for the diagnosis and grading of necroinflammation and fibrosis [1–4]. Arguments against liver biopsy for the diagnosis of NAFLD include the generally good prognosis of most patients, the lack of an established effective therapy, and the risk and costs associated with the biopsy.

Limitations of studies

Histologic criteria used for fatty liver disease are different among studies. For instance, the grading and scores of steatosis are different from one study to another. Even the amount of steatosis considered to be the cut-off between normal and pathologic is not consistently defined. In

Table 1. Frequency and distribution of steatosis in patients with chronic hepatitis C

Study	Patients, *n*	Steatosis, *n* (%)			Distribution of grade of steatosis, *n* (%)	
		Overall	Genotype 3	Others	Mild (< 30%)	Moderate to marked (> 30%)
Mihm *et al.* [13]	85	73 (86)	ND	ND	60 (82)	13 (18)
Czaja *et al.* [14]	60	31 (52)	ND	ND	31(100)	0 (0)
Hourigan *et al.* [15]	148	91 (61)	14/17 (78)	14/23 (61)	61 (67)	30 (33)
Rubbia-Brandt *et al.* [16]	70	28 (40)	16/24 (67)	12/46 (26)	18 (65)	7 (25)
Adinolfi *et al.* [17]	180	86 (48)	20/26 (77)	66/154 (43)	44 (51)	42 (49)
Serfaty *et al.* [18]	100	ND	ND	ND	88 (88)	12 (12)
Monto *et al.* [19••]	297	171 (58)	ND	ND	146 (85)	25 (15)
Westin *et al.* [20]	98	41 (42)	22/25 (88)	11/45 (24)	25 (61)	16 (39)
Poynard *et al.* [21••]	1428	935 (65)	175/210 (83)	760/1218 (62)	836 (89)	103 (11)
Castéra *et al.* [22]	96	51 (54)	15/20 (75)	36/76 (47)	42 (82)	9 (18)
Asselah *et al.* [23••]	290	135 (46)	36/58 (63)	97/232 (42)	91 (68)	44 (32)
Rubbia-Brandt *et al.* [24••]	755	315 (42)	109/178 (61)	206/577 (36)	206 (65)	109 (35)
Total	3607	1957 (54)	407/558 (73)	1202/2371(51)	1648 (80)	410 (20)

ND—not determined.

addition, variations in the methods used to assess and quantify steatosis likely contribute to the heterogeneity in clinical studies. Thus, for future studies on the natural history of steatosis or steatohepatitis, it is extremely important to standardize the methodology of assessment of the microscopic lesions (fatty liver). For instance, standard histologic coloration is not appropriate for an accurate assessment of steatosis and oil red coloration is needed.

The data on the natural history of NAFLD and NASH are extremely limited and commonly based on retrospective studies. It is generally believed that there are several distinct histologic states in the natural history of these disorders that indicate progression of the lesions: fatty liver alone, steatohepatitis, steatohepatitis with fibrosis, and eventually cirrhosis [1–4]. It is also currently believed that although fatty liver alone has a good prognosis, NASH can progress to cirrhosis in a significant proportion of cases.

Evidence that HCV Can Induce Steatosis

In vitro and animal models

Both in vitro studies and the transgenic mouse model have suggested that the HCV core protein is possibly responsible for lipid accumulation. In cell culture at least two HCV proteins, core and NS5A, have been credited with the ability to alter lipid metabolism [8,9]. These proteins bind to the apolipoproteins A1 and A2, which are likely involved in triglyceride accumulation and storage in liver cells. Both the core and NS5A proteins are localized on the surface of lipid droplets. Overexpression of core protein further stimulates the formation of lipid droplets.

In transgenic mice, it has recently been reported that HCV core protein can inhibit microsomal triglyceride transfer protein (MTP) activity and very low density lipoprotein secretion, leading to steatosis [10]. Although a direct interaction with the MTP is unlikely because it would require the secretion of the core protein into the endoplasmic reticulum lumen, which has not been reported to date, the MTP inhibition may still be mediated by unknown factors. However, mice made transgenic with the HCV core protein have normal apolipoprotein B levels. In another work, the expression of both structural and nonstructural (core and NS5A) proteins in transgenic mice was confirmed to lead to steatosis [11]. Finally, it has also been shown that the core protein causes mitochondrial injury leading to oxidative stress which, in turn, disturbs lipid metabolism, thus contributing to steatosis [12].

Thus, there is evidence that HCV proteins can cause hepatic steatosis in the absence of immune response, at least under certain experimental conditions. It should be kept in mind though, that all models have used constructs derived from genotype 1 isolates. Because HCV genotype 3 has been more frequently associated with steatosis than HCV genotype 1 in humans, further studies using genotype 3 isolates are needed.

Frequency of steatosis in patients with chronic hepatitis C

The overall prevalence of steatosis in patients with chronic hepatitis C is 54%. Because steatosis directly increases with increasing BMI, its prevalence in patients with chronic hepatitis C largely depends on the population evaluated, with percentages that range from 40% to 86% (Table 1) [13–18,19••,20,21••,22,23••,24••]. The majority of patients with chronic hepatitis C and steatosis (80%) have a mild degree of steatosis affecting less than 30% of hepatocytes (Table 1). Therefore, steatosis is more frequent in patients with chronic hepatitis C than in the general population, where steatosis is

Table 2. Characteristics associated with the presence of steatosis in patients with chronic hepatitis C

Study	Patients, *n*	Characteristics associated with steatosis
Mihm *et al.* [13]	85	Genotype 3
Czaja *et al.* [14]	60	BMI, cholesterol, triglycerides
Hourigan *et al.* [15]	148	BMI
Rubbia-Brandt *et al.* [16]	70	Genotype 3, intrahepatic HCV RNA in genotype 3 patients
Adinolfi *et al.* [17]	180	BMI (only in genotype 1 patients), genotype 3
Serfaty *et al.* [18]	100	Hypobetalipoproteinemia, genotype 3
Monto *et al.* [19••]	297	BMI, genotype 3
Westin *et al.* [20]	98	BMI, alcohol consumption, genotype 3
Poynard *et al.* [21••]	96	BMI, genotype 3
Castéra *et al.* [22]	1428	Genotype 3, triglycerides, BMI, age > 40 y
Asselah *et al.* [23••]	290	BMI, genotype 3
Rubbia-Brandt *et al.* [24••]	755	Genotype 3, BMI, age, alcohol

BMI—body mass index; HCV—hepatitis C virus; RNA—ribonucleic acid.

estimated (based on imaging and autopsy studies) to affect 20% to 30% of adults [2]. In contrast, steatosis is less frequent in patients with chronic hepatitis C than in patients with abnormal alanine aminotransaminase levels, in whom major causes of chronic liver disease and hepatitis C have been ruled out. In these patients, steatosis is the more frequent finding. For instance, in a study from Italy the prevalence of steatosis in patients with abnormal liver tests was 64% [7]. In addition, steatosis is highly frequent in obese patients, occurring in 70% of cases in an autopsy study [6].

Factors associated with steatosis in patients with chronic hepatitis C

Host factors

Hepatitis C and NAFLD are both common causes of liver disease; therefore, it is not surprising that they can coexist in the same individual. The same factors (*eg,* obesity, alcohol, diabetes, and hyperlipidemia) associated with NAFLD have been found to be associated with steatosis in patients with chronic hepatitis C.

In almost all studies a link between steatosis and high BMI has been reported (Table 2) [13–18,19••,20,21••, 22,23••,24••]. Adinolfi *et al.* [17] reported that although steatosis was not significantly associated with BMI in the overall cohort of HCV-infected patients, the association became significant in those infected with HCV genotype 1 patients (and not in HCV genotype 3). In fact, visceral fat distribution rather than BMI was associated with steatosis. This led to the idea that in patients with HCV infection there is a "metabolic fat" (especially in patients with HCV genotype 1 infection) and a "viral fat" (especially in those infected with HCV genotype 3).

Alcohol intake is a known factor inducing steatosis. However, several studies have not found a significant association between the presence of steatosis and alcohol consumption. One potential explanation for this lack of association may be the exclusion of those with heavy alcohol intake in most studies.

The association between HCV infection and type 2 diabetes has been shown by cross-sectional studies that included prevalent cases of diabetes. Although most of the evidence supports that HCV infection antedates type 2 diabetes, it is also possible that persons with diabetes are at increased risk for acquiring HCV infection because of frequent hospital interventions and daily use of syringes [25]. Because the prevalence of other metabolic disorders, including hyperlipidemia and diabetes, is low in most populations with HCV chronic infection, their impact on steatosis cannot be correctly evaluated in cross-sectional studies.

Steatosis and HCV genotype 3

Several studies have found a significant association between HCV genotype 3 infection and the presence of steatosis (Table 2). Recently, we conducted a study to determine the characteristics (epidemiologic, biological, and histologic) associated with steatosis in patients with chronic hepatitis C [23••]. From November 2000 to July 2001, all consecutive adults with chronic hepatitis C admitted for liver biopsy were included in this study. The day of the liver biopsy a questionnaire for risk factors was completed prospectively, and a blood sample was taken for laboratory analysis. Our study included 290 patients (143 men, 147 women). The mean BMI was 24.1 ± 3.8. Proportions of genotype 1 and 3 were 54% and 20%, respectively. Steatosis was present in 135 patients (46.6%), in 63% of patients with genotype 3, and 39% of those with genotype 1 ($P = 0.03$). In multivariate analysis, liver steatosis was associated with HCV genotype 3 infection, high BMI, and high grade of necroinflammation. Thus, in patients without associated factors for steatosis, steatosis is more frequent in those with HCV genotype 3 than in those with HCV genotype 1. And based on the data available from published studies (Table 1), steatosis is present in 73% of patients infected with HCV genotype 3 and in 51% of patients infected with HCV genotype other than 3 (Table 1). The mechanisms underlying this steatosis genotype-specific association are unknown. We have recently found that HCV genotype 3 is associated with higher quasispecies heterogeneity than HCV genotype 1 [26].

Steatosis and HCV replication

Rubbia-Brandt *et al.* [16] found a significant correlation between steatosis score and titer of intrahepatic HCV RNA in patients infected with genotype 3, indicating that steatosis could result from a virus-related cytopathic effect in these patients. In this study, when only the 18 liver biopsies from patients with HCV genotype 3 and steatosis were considered, a significant correlation was found between high steatosis score and high intrahepatic HCV RNA titer (for both strands of HCV RNA genomic and minus). Two other studies, also based on patients infected with HCV genotype 3, found an association between degree of steatosis and serum HCV RNA levels [17,21••].

Additional studies, however, have not confirmed these findings. This may be due, in part, to the fact that HCV RNA viral load was not included in the initial evaluation. Interestingly, intrahepatic HCV RNA levels were not correlated with the degree of liver injury, including steatosis, necroinflammation, and fibrosis in 47 patients with chronic hepatitis C (25 with genotype 1, 20 with genotype 3, and two with genotype 2) [27]. Therefore, in genotype 3 infection, the relationship between high level of HCV replication and high grade of steatosis is controversial and needs confirmation. If confirmed, it would strongly suggest the existence of a direct viral cytopathic effect hypothesis, which sharply differs from the generally accepted idea of immune-mediated liver cell damage [28]. Furthermore, HCV is a noncytolytic virus in various expression systems (cell line and transgenic mice).

HCV-related steatosis and antiviral therapy

Rubbia-Brandt *et al.* [29,30] reported the case of a patient with chronic HCV genotype 3 infection and steatosis, in whom steatosis disappeared when HCV replication was inhibited by treatment, and recurred when the viral replication (and hepatitis) relapsed after treatment withdrawal. This observation supports the direct relationship between HCV genotype 3 infection and steatosis.

In a recent study, Kumar *et al.* [31] tested the hypothesis of whether antiviral treatment altered hepatic steatosis in chronic hepatitis C. In 28 patients with HCV genotype 1 and 34 patients with HCV genotype 3, they determined the severity of steatosis in pre- and post-treatment liver biopsies using computer-assisted morphometric image analysis as well as conventional semiquantitative scoring. Before treatment, hepatic steatosis was present in 16 (57%) patients infected with HCV genotype 1 and 21 (62%) of those with HCV genotype 3. Whereas in the former group there was no change in steatosis after treatment, regardless of the treatment response, a sustained virologic response was significantly associated with reduction of steatosis ($P < 0.001$) in the latter. Interestingly, in this group of genotype 3 infected patients, there was no change in steatosis among those without sustained virologic response. By logistic regression analysis, sustained virologic response was the only variable predictive of improvement in hepatic steatosis ($P = 0.007$).

In another study, Poynard *et al.* [21••] assessed the effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis. They analyzed 1428 naive patients included in a randomized trial. Among virologic responders, steatosis was markedly improved in patients with HCV genotype 3. An improvement of at least one grade occurred in 77% of these patients, compared with 46% of those infected with other genotypes. Likewise, a disappearance of steatosis was observed in 46% and 29%, respectively ($P < 0.001$ for both comparisons). In genotype 3 responders, the baseline low serum cholesterol was also corrected by treatment ($P < 0.001$). In conclusion, these data provide strong support for a direct causal association between HCV genotype 3 infection and hepatic steatosis.

Steatosis in Hepatitis C: What Does It Mean?

Limitations of studies

Published data on the influence of steatosis on the natural history of chronic hepatitis C have several limitations that may explain discrepancies between studies.

Variations in the methodology

Only limited studies are available that in addition are generally cross-sectional or retrospective. Furthermore, different histologic criteria have been used in the various studies of fatty liver disease. Moreover, all factors affecting steatosis are not always collected (*eg,* alcohol consumption, BMI, and so forth) and multivariate analysis is infrequently done.

Variations in the populations and the HCV genotype distribution

Populations studied have different risk factors for steatosis (*eg,* obesity is more frequent in the United States). In a study from the United States, the mean BMI was 28.5 kg/m^2 with the following distribution: 31% less than 25 kg/m^2, 37% from 25 kg/m^2 to 30 kg/m^2, and 32% greater than 30 kg/m^2 [19••], meaning that 69% of the patients were overweight and 32% were obese. In our study including 290 patients with chronic hepatitis C, the mean BMI was 24 kg/m^2 with the following distribution: 62% less than 25 kg/m^2, 30% from 25 kg/m^2 to 30 kg/m^2, and 8% greater than 30 kg/m^2 [23••]. The setting of the study gives an idea of the population studied (recruitment shift): internal medicine (more metabolic disorders) and transplantation center (more patients with advanced liver disease).

In addition, HCV genotype distribution differs from one region to another in the world. Genotype 3 is more frequent in Europe than in the United States. In a study from the United States with 297 patients with chronic hepatitis C, HCV genotype 3 was present in only 39 patients (14% of cases), making subanalysis in this subgroup difficult [19••]. In contrast, in a recent study from Europe, 178 of 755 patients (24%) had an HCV genotype 3 infection [24••].

Table 3. Relationship between the presence of steatosis and histologic grade and stage of chronic hepatitis C

Study	*n*	Histology score	Analysis	Inflammation	Fibrosis
Mihm *et al.* [13]	85	Knodell	Univariate	Yes (ND)	Yes (ND)
Czaja *et al.* [14]	60	Knodell, Scheuer	Univariate	No (ND)	No (ND)
Hourigan *et al.* [15]	148	Scheuer	Multivariate	Yes (ND)	Yes ($P < 0.03$)
Rubbia-Brandt *et al.* [16]	70	Knodell	Multivariate	Yes ($P < 0.001$)	Yes ($P = 0.02$)
Adinolfi *et al.* [17]	180	Knodell, Scheuer	Multivariate	Yes ($P < 0.007$)	Yes ($P < 0.001$)
Serfaty *et al.* [18]	100	Knodell	Multivariate	No (ND)	Yes ($P = 0.008$)
Monto *et al.* [19••]	297	Batts-Ludwig	Multivariate	No ($P = 0.52$)	No ($P = 0.26$)
Westin *et al.* [20]	98	Ishak	Multivariate	Yes (ND)	Yes (ND)
Poynard *et al.* [21••]	96	Metavir	Multivariate	No ($P = 0.09$)	Yes ($P = 0.007$)
Castéra *et al.* [22]	1428	Metavir	Multivariate	No (ND)	Yes ($P = 0.03$)
Asselah *et al.* [23••]	290	Metavir	Multivariate	Yes ($P < 0.001$)	No ($P = 0.35$)
Rubbia-Brandt *et al.* [24••]	755	Metavir	Multivariate	No	Yes ($P < 0.001$)

ND—not determined.

Steatosis and fibrosis

Factors influencing the outcome of chronic hepatitis C are poorly understood [32]. There is some controversy with regard to the influence of steatosis on the progression of fibrosis. Among 11 studies listed (Table 3), eight found an association between the presence of steatosis and high stage of fibrosis. However, in four of these studies, this association reached a low statistical degree of significance. The discrepancies observed between studies may be due to several reasons, such as the differences in the histologic scores used, the lack of multivariate analysis, and the differences in metabolic risk factors for steatosis.

The fact that obesity has been related to the severity of hepatic fibrosis and risk of cirrhosis is an argument for a role of steatosis in the progression of fibrosis. In several studies on alcoholics and patients with HCV chronic infection, obesity has been shown to favor hepatic fibrosis [33–37]. Liver fibrosis can develop in overweight patients who are free of any other cause of liver disease [38]. However, in overweight patients, the grade of steatosis is not associated with septal fibrosis, underlying the fact that a high BMI may be associated with fibrosis through other mechanisms different from steatosis. In the leptin-deficient ob/ob mouse, which has profound insulin resistance and glucose intolerance, marked hepatic steatosis is present without steatohepatitis or fibrosis.

In a retrospective study with two paired liver biopsies, aggravation of steatosis was found to be associated with fibrosis progression [22]. In this study, which analyzed steatosis on two liver biopsies, the number of patients was relatively small ($n = 91$), and the association between increased steatosis and increased fibrosis does not demonstrate the causal relationship.

In a recent study by Rubbia-Brandt *et al.* [24••], a multivariate analysis was carried out in 755 chronic hepatitis C patients consecutively admitted at three referral hospitals. Steatosis was independently associated with fibrosis. When multivariate analysis was repeated on patients divided according to viral genotype (*ie,* 3 vs non-3) to look for type-specific risk factors, steatosis was associated with fibrosis only in type 3 infected patients. The findings from this study suggest that "nonviral steatosis" may be as benign as NAFLD, whereas viral steatosis (genotype 3–induced steatosis) may accelerate fibrosis progression.

Thus, although some studies have found a positive association between steatosis and fibrosis [19••], not all studies have been able to confirm these findings. In our recent study including 290 patients with chronic hepatitis C, we found by univariate, but not multivariate, analysis an association between steatosis and high stage of fibrosis. Discrepancies between these results and those of recent studies showing an association between steatosis and high stage of fibrosis may be explained by the fact that risk factors for nonviral steatosis were more common in the latter studies. In fact, some patients may have NASH due to obesity and metabolic disorders, in addition to having chronic hepatitis C.

In summary, it seems that there is an association between steatosis and fibrosis. Whether this association reflects causality is at present unknown. In addition, the mechanisms underlying this relationship remain unknown. In fact, steatosis could be a marker but not a cause of disease progression. The frequent association between the presence of steatosis and the grade of necroinflammation may suggest that steatosis is a marker of necroinflammation that, in turn, is a marker of fibrosis progression. The question then becomes the following: "Because genotype 3 is associated with steatosis and steatosis is associated with fibrosis progression, how can we explain the lack of association between genotype 3 infection and stage of fibrosis in several previous studies?"

Steatosis and necroinflammation

Several studies have found an association between necroinflammation and steatosis (Table 3). Necroinflammatory activity is a dynamic process in chronic hepatitis C and may fluctuate over time. The activity score reflects the severity of necrosis and inflammation at a given point. In most cross-sectional studies, the degree of necroinflam-

matory activity has been associated with the presence of steatosis. The mechanisms responsible for this association are unknown. In vitro studies have shown that the HCV core protein could lead to oxidative stress [12]. In addition, steatosis of any cause can be associated with the development of inflammatory changes in the setting of oxidative stress. Moreover, HCV infection is associated with increased production of cytokines [40] that enhances inflammation and leads to increased lipid peroxidation. Finally, one may hypothesize that steatosis is the consequence of more severe cell injury and necroinflammation in chronic hepatitis C rather than the direct cause of worsening of fibrosis.

How do we reconciliate all these data?

Hepatitis C and NAFLD are both common causes of liver disease. Therefore, it is not surprising that they can coexist in the same individual. The wide range of steatosis in patients with chronic hepatitis C probably reflects the existence of population differences in overweight, diabetes, and dyslipidemia. Steatosis may not have the same pathogenic significance, whether its origin is metabolic (NAFLD or NASH) or virus related (due to HCV genotype 3).

Conclusions

Approximately half of patients with chronic hepatitis C have steatosis, which is mild in the majority of cases. Steatosis can be a reflection of necroinflammation rather than a cause of progression of fibrosis. Whether or not steatosis determines fibrosis progression is yet to be proven. In clinical practice, risk factors for steatosis always have to be assessed in patients with chronic hepatitis C. If possible, and given their association with pathologic conditions such as obesity, diabetes, and hyperlipidemia, these conditions should be treated.

References and Recommended Reading

Papers of particular interest, published recently,
have been highlighted as:

- • Of importance
- •• Of major importance

1. Sanyal AJ: **AGA technical review on nonalcoholic fatty liver disease.** *Gastroenterology* 2002, **123:**1705–1725.
2. Clark JM, Brancati FL, Diehl AM: **Nonalcoholic fatty liver disease.** *Gastroenterology* 2002, **122:**1649–1657.
3. Neuschwander Tetri BA, Caldwell SH: **Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference.** *Hepatology* 2003, **37:**1202–1219.
4. Chitturi S, Farrell GC: **Etiopathogenesis of nonalcoholic steatohepatitis.** *Semin Liver Dis* 2001, **21:**27–41.

5.•• Brunt EM, Tiniakos DG: **Steatosis, steatohepatitis: review of effects on chronic hepatitis C.** *Curr Hepat Rep* 2002, **1:**38–44.
Provides insights into steatosis, steatohepatitis, and chronic hepatitis C.

6. Wanless IR, Lentz JS: **Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors.** *Hepatology* 1990, **12:**1106–1110.
7. Skelly MM, James PD, Ryder SD: **Findings on liver biopsy to investigate abnormal liver function tests in the absence of diagnostic serology.** *J Hepatol* 2001, **35:**195–199.
8. Barba G, Harper F, Harada T, *et al.*: **Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets.** *Proc Natl Acad Sci U S A* 1997, **94:**1200–1205.
9. Shi ST, Polyak SJ, Tu H, *et al.*: **Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins.** *Virology* 2002, **292:**198–210.
10. Perlemuter G, Sabile A, Letteron P, *et al.*: **Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis.** *FASEB J* 2002, **16:**185–194.
11. Lerat H, Honda M, Beard MR, *et al.*: **Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus.** *Gastroenterology* 2002, **122:**352–365.
12. Okuda M, Li K, Beard MR, *et al.*: **Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein.** *Gastroenterology* 2002, **122:**366–375.
13. Mihm S, Fayyazi A, Hartmann H, Ramadori G: **Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype.** *Hepatology* 1997, **25:**735–739.
14. 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.
15. Hourigan LF, Macdonald GA, Purdie D, *et al.*: **Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis.** *Hepatology* 1999, **29:**1215–1219.
16. Rubbia-Brandt L, Quadri R, Abid K, *et al.*: **Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3.** *J Hepatol* 2000, **33:**106–115.
17. Adinolfi LE, Gambardella M, Andreana A, *et al.*: **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.
18. Serfaty L, Andreani T, Giral P, *et al.*: **Virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C.** *J Hepatol* 2001, **34:**428–434.

19.•• Monto A, Alonzo J, Watson JJ, *et al.*: **Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol.** *Hepatology* 2002, **36:**729–736.
A total of 297 patients with chronic hepatitis C were analyzed for host and viral factors related to steatosis and fibrosis.

20. Westin J, Nordlinder H, Lagging M, *et al.*: **Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients.** *J Hepatol* 2002, **37:**837–842.

21.•• Poynard T, Ratziu V, McHutchison J, *et al.*: **Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C.** *Hepatology* 2003, **38:**75–85.
Assessed the effect of treatment on viral steatosis. In patients with HCV genotype 3 infection, sustained disappearance of the virus is associated with reduction of steatosis.

22. Castéra L, Hézode C, Roudot-Thoraval F, *et al.*: **Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies.** *Gut* 2003, **52:**288–292.

23.•• Asselah T, Boyer N, Guimont MC, *et al.*: **Liver fibrosis is not associated with steatosis but with necroinflammation in French patients with chronic hepatitis C.** *Gut* 2003, in press.
Controversy about the role of steatosis in fibrosis progression is reported.

24.•• Rubbia-Brandt L, Fabris P, Paganin S, *et al.*: **Steatosis affects chronic hepatitis C progression in a genotype-specific way.** *Gut* 2003, in press.
A total of 755 patients with chronic hepatitis C were studied and only viral steatosis (HCV genotype 3) was found to be associated with fibrosis progression.

25. Mehta SH, Brancati FL, Strathdee SA, *et al.*: **Hepatitis C virus infection and incident type 2 diabetes.** *Hepatology* 2003, **38:**50–56.
26. Asselah T, Martinot M, Cazals-Hatem D, *et al.*: **Hypervariable region 1 quasispecies in hepatitis C virus genotypes 1b and 3 infected patients with normal and abnormal alanine aminotransferase levels.** *J Viral Hepat* 2002, **9:**29–35.
27. McGuinness PH, Bishop GA, Painter DM, *et al.*: **Intrahepatic hepatitis C RNA levels do not correlate with degree of liver injury in patients with chronic hepatitis C.** *Hepatology* 1996, **23:**676–687.
28. Boyer N, Marcellin P: **Pathogenesis, diagnosis and management of hepatitis C.** *J Hepatol* 2000, **32:**98–112.
29. Rubbia-Brandt L, Giostra E, Mentha G, *et al.*: **Expression of liver steatosis in hepatitis C virus infection and pattern of response to alpha interferon.** *J Hepatol* 2001, **35:**307.
30. Rubbia-Brandt L, Leandro G, Spahr L, *et al.*: **Liver steatosis in chronic hepatitis C: a morphological sign suggesting infection with HCV genotype 3.** *Histopathology* 2001, **39:**119–124.
31. 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.
32. Marcellin P, Asselah T, Boyer N: **Fibrosis and disease progression in hepatitis C.** *Hepatology* 2002, **36:**47–56.
33. Ortiz V, Berenguer M, Rayón JM, *et al.*: **Contribution of obesity to hepatitis C-related fibrosis progression.** *Am J Gastroenterol* 1997, **9:**2408–2414.
34. Friedenberg F, Pungpapong S, Zaeri N, Braitman LE: **The impact of diabetes and obesity on liver histology in patients with hepatitis C.** *Diabetes Obes Metab* 2003, **5:**150–155.
35. Hickman IJ, Clouston AD, Macdonald GA, *et al.*: **Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C.** *Gut* 2002, **51:**89–94.
36. Teli MR, Day CP, Burt AD, *et al.*: **Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver.** *Lancet* 1995, **346:**987–990.
37. Iturriaga H, Bunout D, Hirsch S, Ugarte G: **Overweight as a risk factor or a predictive sign of histological liver damage in alcoholics.** *Am J Clin Nutr* 1988, **47:**235–238.
38. Ratziu V, Giral P, Charlotte F, *et al.*: **Liver fibrosis in overweight patients.** *Gastroenterology* 2000, **118:**1117–1123.
39. Ghany MG, Kleiner DE, Alter H, *et al.*: **Progression of fibrosis in chronic hepatitis C.** *Gastroenterology* 2003, **124:**97–104.
40. Neuman MG, Benhamou JP, Malkiewicz IM, *et al.*: **Kinetics of serum cytokines reflect changes in the severity of chronic hepatitis C presenting minimal fibrosis.** *J Viral Hepat* 2002, **9:**134–140.