Evolution of Hepatic Steatosis in Patients with Advanced Hepatitis C: Results from the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial

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Hepatic steatosis is a common histologic feature in patients with chronic hepatitis C (CHC) but there are no large longitudinal studies describing the progression of steatosis in CHC. We examined changes in steatosis on serial biopsies among CHC patients participating in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial. All 1050 patients in the trial had advanced fibrosis at baseline biopsy and were documented not to have had a sustained virological response to peginterferon and ribavirin. Most (94%) patients had genotype 1 infection. At least one protocol follow-up biopsy was read on 892 patients, and 699 had the last biopsy performed 3.5 years after randomization. At enrollment, 39% had cirrhosis and 61% had bridging fibrosis; 18%, 41%, 31%, and 10% had steatosis scores of 0, 1, 2, and 3 or 4, respectively. The mean steatosis score decreased in the follow-up biopsies in both the interferon-treated patients and controls with no effect of treatment assignment ($P = 0.66$). A decrease in steatosis score by ≥1 point was observed in 30% of patients and was associated with both progression to cirrhosis and continued presence of cirrhosis ($P = 0.02$). Compared to patients without a decrease in steatosis, those with a decrease in steatosis had worse metabolic parameters at enrollment, and were more likely to have a decrease in alcohol intake, improvement in metabolic parameters, and worsening liver disease (cirrhosis, esophageal varices, and deterioration in liver function). *Conclusion:* Serial biopsies demonstrated that in patients with CHC, steatosis recedes during progression from advanced fibrosis to cirrhosis. Decreased alcohol intake and improved metabolic parameters are associated with a decline in steatosis and may modulate hepatitis C progression. (HEPATOLOGY 2009;49:1828-1837.)

Hepatic steatosis is a common histologic feature in patients with chronic hepatitis C (CHC) and has been reported to be associated with fibrosis in cross-sectional studies.[1-3] However, very few studies have examined paired biopsies to determine the relationship between steatosis and fibrosis progression and to characterize the evolution of steatosis over time.

Baseline data from the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial, which included predominantly patients with genotype 1

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHC, chronic hepatitis C; HALT-C, hepatitis C antiviral long-term treatment against cirrhosis; HCV, hepatitis C virus; HOMA, homeostasis model assessment; INR, international normalized ratio; SVR, sustained virologic response.

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hepatitis C virus (HCV) infection, show that steatosis (defined as the presence of fat in >5% of hepatocytes) was present in 39% of patients and correlated strongly with metabolic factors associated with nonalcoholic fatty liver.[4] Furthermore, increasing severity of steatosis correlated with increasing stages of fibrosis up to but not including cirrhosis. We hypothesize that steatosis plays a role in fibrosis progression during the early stages of liver disease but steatosis regresses during the transition from advanced fibrosis to cirrhosis. Thus, steatosis may have greater use as a predictive tool for fibrosis progression in patients with mild fibrosis on initial biopsy and the role of steatosis in cirrhosis development may have been underestimated. However, direct evidence supporting our hypothesis is lacking because prior longitudinal studies of hepatic steatosis in patients with CHC included very few patients that progressed from fibrosis to cirrhosis.

The HALT-C Trial, which enrolled more than 1000 hepatitis C patients with bridging fibrosis or cirrhosis who underwent three liver biopsies over a 4-year period, provided an opportunity to evaluate changes in hepatic steatosis over time and their impact on progression to cirrhosis. The aims of this study were to (1) characterize changes in steatosis on serial biopsies, (2) determine the factors associated with changes in steatosis, and (3) examine the impact of changes in steatosis on progression to cirrhosis.

Patients and Methods

Patient Population. The design of the HALT-C Trial has been described.[5,6] Briefly, patients with detectable HCV RNA at 10 clinical centers had to meet the following criteria for enrollment: failure to have achieved a sustained virologic response (SVR) after previous interferon treatment with or without ribavirin, the presence of advanced hepatic fibrosis on liver biopsy (Ishak fibrosis score ≥3), no history of hepatic decompensation or hepatocellular carcinoma, and the absence of coexistent causes of liver disease or contraindications to the use of interferon or ribavirin. Patients with steatosis alone or mild to moderate steatohepatitis were enrolled but those with severe steatohepatitis, defined as the presence of marked steatosis, many Mallory bodies, and extensive zone 3 pericellular fibrosis were excluded. Patients with poorly controlled diabetes and those with active alcohol abuse within the past 12 months were also excluded.

Clinical and Laboratory Evaluation. Baseline evaluation included a complete history and physical examination (including height, weight, and waist circumference), and fasting blood tests (complete blood count, liver panel, basic metabolic panel, prothrombin time / international normalized ratio [INR], glucose, triglyceride, insulin, alpha-fetoprotein [AFP], HCV genotype, and HCV RNA). Standardized questionnaires were used to quantify lifetime alcohol consumption prior to enrollment[7] and to assess continued alcohol consumption (defined as ≥1 drink/week) at enrollment and every 6 months thereafter. Assays for HCV genotype and HCV RNA were performed at a single laboratory (University of Washington, Seattle, WA), as described. Insulin levels were tested using a radioimmunoassay in a single laboratory (Michigan Diabetes Research and Training Center, University of Michigan, Ann Arbor, MI). All other blood tests were performed at the hospital laboratories of the participating clinical centers.

Patients were randomized to receive pegylated interferon alpha-2a 90 μg weekly for 3.5 years or no treatment. The patients were seen every 3 months. At each visit, patients were evaluated clinically and blood tests were

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performed. Fasting glucose and triglyceride were tested 1.5 and 3.5 years after randomization and fasting insulin was tested 1.5 years after randomization. Endoscopy to evaluate for esophageal varices was performed at the time of randomization and again 3.5 years after randomization.

Body mass index (BMI) was calculated using the formula: weight in kg / height in meters.[2] Patients were considered to be overweight if their BMI was between 25 and 29, and obese if their BMI was 30 or higher. Patients were considered diabetic if they had a current diagnosis of diabetes or if their fasting blood glucose exceeded 126 mg/dL. Truncal obesity was defined as waist circumference >102 cm in men and >88 cm in women. Hypertriglyceridemia was defined as serum triglyceride >150 mg/dL. Insulin resistance was estimated by the homeostasis model assessment (HOMA 2).[8]

Interpretation of Liver Histology. All patients had a liver biopsy performed prior to enrollment. Liver biopsies were repeated 1.5 and 3.5 years after randomization. All biopsies were reviewed in conference by a panel of 12 hepatic pathologists, who used the Ishak scoring system to grade inflammation (0-18) and to stage fibrosis (0-6).[9] The panel of pathologists met and had discussion and practice sessions prior to actual scoring of the biopsies. In general, only biopsies longer than 1.5 cm were scored. A few biopsies that were smaller were included if the panel of hepatic pathologists agreed that scores can be confidently assigned. Discrepancies were resolved around a multiheaded microscope by consensus. In the rare case where a consensus could not be reached, scores were determined by the majority vote, which meant the median score was used.

Hepatic steatosis was graded as 0 (<1%), 1 (1%-5%), 2 (5%-33%), 3 (33%-67%), and 4 (>67%) according to the percentage of hepatocytes with fat. Only 1% of patients had a steatosis score of 4 on baseline biopsy; therefore, patients with a steatosis score 3 or 4 were combined as one group. Mallory bodies were graded as present or absent. Zone 3 pericellular fibrosis was scored as absent, and mild or moderate when present. A change in steatosis score was defined as an increase or decrease by ≥1 point. Progression to cirrhosis was defined as an increase in Ishak fibrosis score from ≤4 to ≥5.

Statistical Analyses. Patients who had at least one follow-up biopsy were analyzed, for patients who had two follow-up biopsies, the latter one performed 3.5 years after randomization was used to maximize the interval between the baseline and follow-up biopsies. Changes in steatosis score between baseline and follow-up biopsies were computed. Changes in clinical, laboratory, and histologic features were computed using the same time-points. Chi-square and *t* tests were used to determine categorical and continuous variables, respectively, that were significantly different between patients with at least one follow-up biopsy versus those without follow-up biopsy, and patients with a decrease versus those with an increase in steatosis score. Logistic regression was used to test for associations between clinical, laboratory, and histologic features and change in steatosis score. For multivariate analysis of factors associated with a decrease in steatosis, only patients with steatosis on baseline biopsy were included. Comparison was made between patients with a decrease in steatosis score by ≥1 point from baseline to follow-up biopsies and those with no decrease (increase or no change) in steatosis score. Analysis of variance (ANOVA) was used to compare changes in insulin, glucose, and HOMA 2 among five levels of changes in steatosis score from baseline to follow-up biopsies obtained 1.5 years after randomization. In all analyses a *P* value of 0.05 was considered statistically significant. All analyses were conducted using SAS version 9.1.3 (SAS Inc., Cary, NC).

Results

Among 1050 patients randomized, 892 (85%) had at least one follow-up biopsy; of these, 699 had the last biopsy performed 3.5 years after randomization. Follow-up biopsies were not available on the remaining 158 patients for the following reasons: withdrew from study (n = 66), developed clinical outcome (n = 64), marked thrombocytopenia (n = 11), refused biopsy (n = 7), and miscellaneous reasons (n = 10).

Baseline Characteristics. Baseline characteristics of the 892 patients included in this study are shown in Table 1. The mean age of the patients was 50.3 years, 71% were men, and 19% were black. At enrollment, 40% of the patients were overweight and 43% were obese, 24% were diabetic, 44% men and 66% women had truncal obesity, and 31% had hypertriglyceridemia. The vast majority (94%) had genotype 1, 3% had genotype 3, and the remainder had genotypes 2 (1.9%), 4 (1.0%), 6 or mixed (0.1%) HCV infection. Cirrhosis was present in 39% of patients and 18%, 41%, 31%, and 10% of patients had steatosis score of 0, 1, 2, and 3 or 4, respectively. Of the 158 patients who did not have follow-up biopsies, 75 (47.5%) had a clinical outcome or marked thrombocytopenia. Reflecting the high proportion who had progression of liver disease, these patients had more advanced liver disease based on laboratory tests and Ishak fibrosis score than the patients included in this study. They also had greater steatosis and more Mallory bodies but did not differ in demographics or metabolic risk factors.

Changes in Hepatic Steatosis. Mean steatosis score

Table 1. Baseline Characteristics of Patients

Characteristics	Patients with at Least 1 Follow-up Biopsy (n = 892)	Patients with No Follow-up Biopsy (n = 158)	*P* Value
Demographics			
Age, years	50.3 (7.1)	49.2 (7.5)	0.074
Gender			
Male	632 (70.9)	113 (71.5)	0.86
Female	260 (29.1)	45 (28.5)	
Race			
White	641 (71.9)	111 (70.2)	0.09
Black	168 (18.8)	23 (14.6)	
Hispanic	64 (7.2)	20 (12.7)	
Others	19 (2.1)	4 (2.5)	
Metabolic factors			
Diabetes	213 (23.9)	45 (28.5)	0.22
BMI, kg/m^2	29.8 (5.3)	30.2 (6.2)	0.53
BMI >30	380 (42.6)	68 (43.0)	
Waist circumference, cm			
Male	100.9 (11.7)	100.7 (15.0)	0.90
Female	96.5 (16.0)	95.0 (18.8)	0.65
Hypertension	590 (66.1)	115 (72.8)	0.10
Triglyceride, mg/dL	142.2 (118.3)	142.7 (95.7)	0.95
Glucose, mg/dL	110.4 (43.4)	110.3 (39.8)	0.98
Insulin*, uU/mL	50.7 (49.2)	55.6 (60.1)	0.42
HOMA 2†	5.7 (4.9)	5.9 (5.0)	0.61
Viral factors			
HCV RNA, log_{10} IU/mL	6.5 (0.5)	6.3 (0.6)	0.0008
HCV genotype 3	26 (2.9)	6 (3.9)	0.61
Lab values			
WBC $\times 1000/mm^3$	5.8 (1.9)	5.7 (2.1)	0.59
Hemoglobin, g/dL	15.0 (1.4)	14.8 (1.6)	0.08
Platelets $\times 1000/mm^3$	169 (64)	140 (71)	<0.0001
Prothrombin time,INR	1.0 (0.1)	1.1 (0.1)	0.0002
ALT, U/L	108 (79)	104 (66)	0.51
AST, U/L	87 (59)	94 (55)	0.14
Alkaline phosphatase, U/L	98 (45)	111 (46)	0.002
Total bilirubin, mg/dL	0.78 (0.40)	0.85 (0.42)	0.04
Albumin, g/dL	3.9 (0.4)	3.7 (0.5)	<0.0001
AST/ALT ratio	0.9 (0.3)	1.0 (0.3)	0.0006
Liver Histology			
Ishak inflammation score	7.5 (2.0)	7.8 (2.2)	0.18
Hepatocellular iron grade	0.5 (0.7)	0.5 (0.7)	0.99
Ishak fibrosis score	4.0 (1.3)	4.5 (1.3)	0.0001
3 or 4	547 (61.4)	75 (47.5)	
5 or 6	345 (38.6)	83 (52.5)	
Steatosis score	1.3 (0.9)	1.5 (0.9)	0.01
0	162 (18.2)	16 (10.1)	
1	368 (41.3)	67 (42.4)	
2	274 (30.7)	56 (35.4)	
3 or 4	88 (9.9)	19 (12.0)	
Mallory bodies			
Absent	767 (86.0)	125 (79.1)	0.03
Present	125 (14.0)	33 (20.9)	
Zone 3 pericellular fibrosis			
Absent	542 (65.0)	91 (59.1)	0.19
Mild	243 (29.1)	56 (36.4)	
Moderate	49 (5.9)	7 (4.6)	

*Data available on 698 patients.

†Data available on 677 patients.

Data expressed as number (%) or mean ± SD.

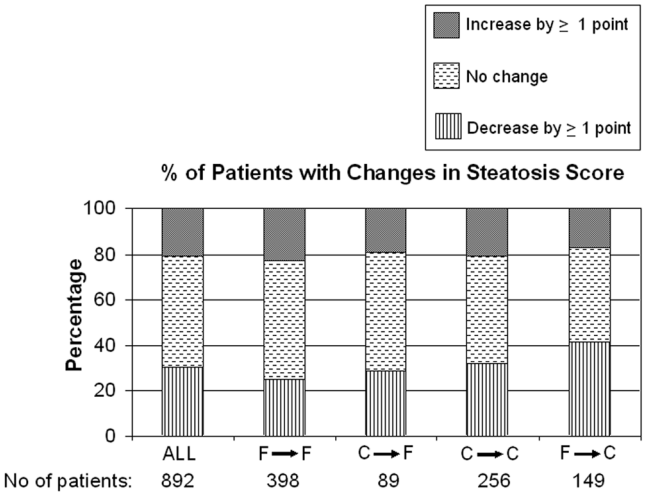

Fig. 1. Changes in steatosis scores in patients with fibrosis versus cirrhosis. Stacked columns show percent of patients with decrease in steatosis score by ≥1 points, no change, and increase in steatosis score by ≥1 points among all 892 patients (ALL), 256 patients who had bridging fibrosis on baseline and last biopsies (F → F), 89 patients who had cirrhosis on baseline biopsy and bridging fibrosis on last biopsy (C → F), 398 patients who had cirrhosis on both biopsies (C → C), and 187 patients who had bridging fibrosis on baseline biopsy and cirrhosis on last biopsy (F → C). Decrease in steatosis score by ≥1 points was observed in 30.4% of ALL patients, being most common in those who progressed from fibrosis to cirrhosis: 41.6% of F → C versus 25.1% of F → F patients, 29.2% of C → F patients, 32.4% of C → C patients.

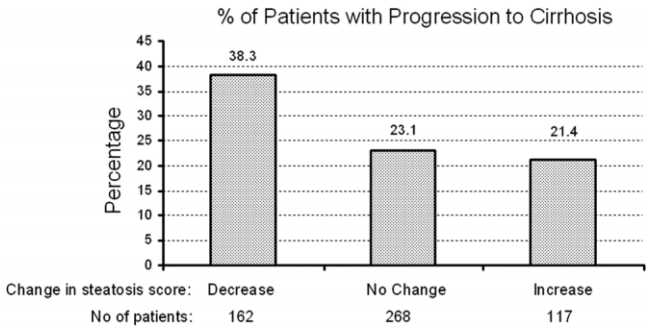

Fig. 2. Correlation between change in steatosis score and progression to cirrhosis on follow-up biopsy: 38.3% of patients with a decrease in steatosis score by ≥1 point progressed to cirrhosis compared to 23.1% of those with no change and 21.4% of those with increase in steatosis score ($P = 0.0008$).

decreased in the follow-up biopsies in both the interferon-treated patients (1.34 versus 1.23, $P = 0.013$) and controls (1.31 versus 1.18, $P = 0.002$), with no effect of treatment assignment ($P = 0.66$). Consequently, both groups of patients were combined for all subsequent analyses.

Approximately half (434, 49%) of the patients had no change in steatosis score, whereas 271 (30%) had a decrease in steatosis score by ≥1 point and 187 (21%) had an increase in steatosis score by ≥1 point. Steatosis was approximately twice as likely to decrease as to increase (odds ratio 2.1, 95% confidence interval [CI] = 1.66-2.82). A net decrease in steatosis score was more likely in patients who had cirrhosis on the second biopsy (either progressed from bridging fibrosis to cirrhosis or had cirrhosis on both biopsies): odds ratio 2.86 (95% CI 1.94-4.20) than those who had bridging fibrosis on the second biopsy: odds ratio 1.62 (95% CI 1.12-2.35). A test for interaction was statistically significant ($P = 0.04$), meaning that the decline in steatosis was greater in patients with progression or maintenance of cirrhosis. Figure 1 shows that a decrease in steatosis score was observed in 42% of patients who progressed from fibrosis to cirrhosis compared to 25%, 29%, and 32% of patients who had fibrosis on both biopsies, cirrhosis on baseline biopsy but fibrosis on follow-up biopsy, and cirrhosis on both biopsies, respectively ($P = 0.02$).

Correlation Between Change in Steatosis and Progression to Cirrhosis. Among 547 patients who had Ishak fibrosis score ≤4 on baseline biopsies, 149 (27%) had progressed to Ishak 5 or 6 (cirrhosis) on the follow-up biopsies. Patients with a decrease in steatosis score of at least 1 point between biopsies were more likely to progress to cirrhosis than those with unchanged or an increase in steatosis score: 38.3% versus 23.1%, and 21.4%, respectively ($P = 0.0008$) (Fig. 2). Figure 3 shows serial biopsies of a patient who pro-

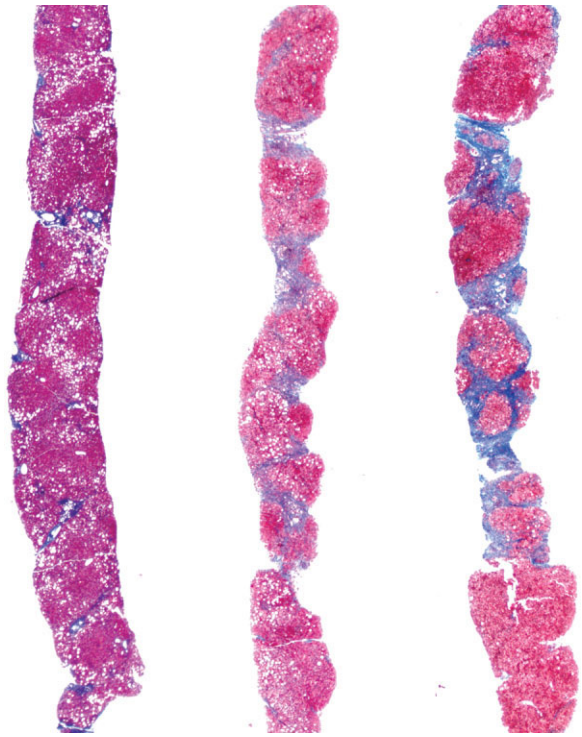

Fig. 3. Serial biopsies of a patient obtained at baseline, Year 1.5, and Year 3.5 showing progression from Ishak fibrosis score of 3 to 6 with concomitant decrease in steatosis score from 3 to 2.

gressed from Ishak fibrosis score of 3 on the baseline biopsy to a score of 5 on the Year 1.5 biopsy and a score of 6 on the Year 3.5 biopsy, with concomitant decrease in steatosis score from 3 to 2 and 2, respectively.

To confirm the relationship between change in steatosis and progression to cirrhosis (and portal hypertension), change in steatosis score between the baseline and Year 3.5 biopsies in relation to the presence of esophageal varices and the interval development of esophageal varices was evaluated. Both the presence of esophageal varices (31%) at the end of the study and the development of varices (21%) by the end of the study were associated with a decrement in steatosis at the last biopsy ($P = 0.017$ and $P = 0.015$, respectively).

Correlation Between Changes in Steatosis and Alcohol Consumption. Changes in steatosis may be related to changes in alcohol consumption. Alcohol consumption was acknowledged in 149/892 (16.7%) patients at enrollment and in 192/880 (21.8%) patients at the time of the last follow-up biopsy. Patients who had steatosis on the last follow-up biopsy (scores of 1-4) were more likely to admit to alcohol consumption during the 6 months prior to that biopsy than those who denied alcohol consumption: 23% (175/762) versus 14.4% (17/118), ($P = 0.04$). Of the 719 patients who had steatosis (score of ≥ 1) on the baseline biopsy and in whom alcohol history at the time of the follow-up biopsy was available, steatosis decreased in 28.6% (44/154) of those who continued to drink versus 38.9% (220/565) of those who denied drinking at the time of the follow-up biopsy ($P = 0.02$).

Factors Associated with Change in Steatosis Score from Enrollment to Last Follow-Up Biopsy. Features at enrollment were examined as predictors of changes in steatosis score. Compared to patients with an increase in steatosis score, patients with a decrease in steatosis score were more likely to be Hispanic, had worse metabolic parameters (diabetes, BMI, waist circumference, triglyceride, and HOMA 2), more deranged liver chemistries (higher aspartate and alanine aminotransferase [AST, ALT], alkaline phosphatase, and lower albumin), and more steatosis, fibrosis, Mallory bodies as well as pericellular fibrosis (Table 2). Changes in baseline features between enrollment and the last biopsy were also examined in relation to changes in steatosis. Patients with a decrease in steatosis score were more likely to have an improvement in metabolic parameters (BMI and triglyceride) and a worsening of liver chemistries (AST, ALT, alkaline phosphatase, and INR) and hepatic fibrosis compared to those who had an increase in steatosis score (Table 2).

Because there was an overall trend toward a decrease in steatosis and an association between a decrease in steatosis score and progression to cirrhosis, multivariate analysis was performed to determine the factors associated with a decrease in steatosis score. For this analysis, 162 patients with baseline steatosis score of 0 were excluded, and 271 patients with a decrease in steatosis score of ≥ 1 point were compared to 459 patients with no change or an increase in steatosis score by ≥ 1 point. Of greatest interest was the association of change in factors that might be related to steatosis. The results of a multivariate model that controlled for baseline factors (BMI, truncal obesity, triglyceride concentration, HOMA 2, HCV RNA, genotype, AST/ALT ratio, INR, total bilirubin, inflammation and fibrosis scores, and presence of Mallory bodies and zone 3 fibrosis) and alcohol consumption at the time of the follow-up biopsy found that an increased AST/ALT ratio, decreased triglycerides, and decreased hepatic inflammation score were associated with a decrease in steatosis score (Table 3).

Correlation Between Changes in Steatosis and Changes in HOMA 2. Insulin resistance plays an important role in the pathogenesis of hepatic steatosis. Follow-up insulin testing was performed at 1.5 but not 3.5 years after randomization. Therefore, changes in steatosis score between baseline and follow-up biopsies obtained 1.5 years after randomization and plasma glucose, insulin, and HOMA 2 at these same timepoints were analyzed to determine if there was an association between changes in steatosis score and changes in HOMA 2. A decrease in steatosis score was accompanied by a decrease in plasma glucose, insulin, and HOMA 2, whereas an increase in steatosis score was accompanied by no change or increase in plasma glucose, insulin, and HOMA 2 (Table 4).

Discussion

Steatosis is a well-documented feature of hepatitis C, but its stability and evolution over time have not been well characterized. Inferences have been drawn from cross-sectional studies, including baseline data of HALT-C and from relatively small studies with paired biopsies. Using the carefully documented histological information on sequential biopsies, robust prospective data collection, and large cohort of patients, the current study revealed several new findings regarding steatosis in patients with hepatitis C. Steatosis varied considerably between the first and last biopsies, but overall tended to decrease despite an increase in the number of patients who admitted to actively drinking alcohol just prior to the last biopsy. Whereas few baseline features were predictors of reduction in steatosis, decrease in triglyceride concentrations, and HOMA 2 were associated with a decrement in steatosis.

Few studies with paired biopsies have examined the evolution of steatosis in patients with hepatitis C and the impact of steatosis on fibrosis progression.[10-14] These studies typically included small numbers of patients (total

Table 2. Characteristics of Patients with and without Changes in Steatosis Score on Follow-up Biopsies

Characteristic	Decrease (n = 271)	No Change (n = 434)	Increase (n = 187)	*P* Value‡
Treatment assignment				
Treatment	130 (48.0)	220 (50.7)	94 (50.3)	0.63
Control	141 (52.0)	214 (49.3)	93 (49.7)	
Baseline features				
Demographics				
Age, years	50.0 (6.6)	50.6 (7.4)	50.1 (7.1)	0.79
Gender, N (%)				
Male	194 (71.6)	300 (69.1)	138 (73.8)	0.60
Female	77 (28.4)	134 (30.9)	49 (26.2)	
Race, N (%)				
White	196 (72.3)	308 (71.0)	137 (73.3)	0.02
Black	39 (14.4)	89 (20.5)	40 (21.4)	
Hispanic	27 (10.0)	30 (6.9)	7 (3.7)	
Others	9 (3.3)	7 (1.6)	3 (1.6)	
Metabolic factors				
Diabetes	65 (24.0)	112 (25.8)	36 (19.3)	0.23
Hypertension	184 (67.9)	281 (64.8)	125 (66.8)	0.81
BMI, kg/m^2	30.4 (5.5)	29.9 (5.4)	28.8 (4.6)	0.0008
BMI >30	130 (48)	182 (42)	68 (36.6)	
Waist circumference, cm	101.1 (13.0)	99.5 (13.7)	97.7 (12.2)	0.006
Triglyceride, mg/dL	156.4 (124.0)	140.6 (121.2)	125.3 (99.7)	0.003
Glucose, mg/dL	116 (54)	109 (39)	105 (35)	0.01
Insulin, μU/mL*	56.7 (50.9)	52.0 (53.8)	38.3 (29.3)	<0.0001
HOMA 2†	6.6 (6.7)	5.5 (3.9)	4.7 (3.3)	0.0004
Viral factors				
HCV RNA, $\log_{10}$ IU/mL	6.47 (0.43)	6.42 (0.53)	6.53 (0.50)	0.20
HCV genotype 3	11 (4.1)	11 (2.5)	4 (2.2)	0.26
Lab values				
Platelets × 1000/mm^3	166 (66)	171 (65)	171 (58)	0.35
Prothrombin time, INR	1.03 (0.10)	1.03 (0.11)	1.03 (0.10)	0.74
ALT, U/L	124 (87)	101 (78)	98 (67)	0.0004
AST, U/L	99 (70)	83 (55)	78 (51)	0.0003
Alkaline phosphatase, U/L	101 (48)	99 (47)	91 (36)	0.007
Total bilirubin, mg/dL	0.81 (0.41)	0.76 (0.37)	0.77 (0.44)	0.32
Albumin, g/dL	3.86 (0.36)	3.90 (0.38)	3.94 (0.38)	0.04
AST/ALT ratio	0.84 (0.28)	0.88 (0.28)	0.87 (0.31)	0.38
Liver histology				
Ishak inflammation score	7.86 (1.87)	7.35 (2.10)	7.35 (2.08)	0.008
Hepatocellular iron grade	0.48 (0.69)	0.46 (0.66)	0.58 (0.75)	0.14
Ishak fibrosis score	4.11 (1.24)	4.04 (1.25)	3.94 (1.28)	0.15
Ishak fibrosis score, N (%)				
2	17 (6.3)	35 (8.1)	20 (10.7)	
3 or 4	145 (53.5)	233 (53.7)	97 (51.9)	
5 or 6	109 (40.2)	166 (38.2)	70 (37.4)	
Steatosis score	2.06 (0.68)	1.23 (0.70)	0.48 (0.63)	<0.0001
Steatosis score, N (%)				
0	0 (0.0)	50 (11.5)	112 (59.9)	
1	54 (19.9)	253 (58.3)	61 (32.6)	
2	146 (53.9)	114 (26.3)	14 (7.5)	
3-4	71 (26.2)	17 (3.9)	0 (0.0)	
Mallory bodies, N (%)				
Present	62 (22.9)	51 (11.8)	12 (6.4)	<0.0001
Zone 3 pericellular fibrosis, N (%)				
Absent	158 (61.5)	256 (63.8)	128 (72.7)	0.02
Mild	75 (29.2)	126 (31.4)	42 (23.9)	
Moderate	24 (9.3)	19 (4.7)	6 (3.4)	
Changes in features: FU - baseline				
Metabolic factors				
BMI	−0.51 (2.73)	0.05 (2.39)	0.52 (2.42)	<0.0001
Waist circumference, cm	−0.30 (9.9)	0.67 (9.1)	1.73 (8.2)	0.03
Triglyceride	−20 (127)	3 (97)	19 (86)	0.0001
Glucose	−2.58 (56.3)	2.24 (40.8)	4.04 (40.2)	0.15
Viral factors				
HCV RNA (FU-baseline), $\log_{10}$ IU/mL	−0.51 (1.18)	−0.30 (0.95)	−0.48 (1.25)	0.78
Lab values				
Platelets × 1000/mm^3	−26 (50)	−23 (45)	−20 (44)	0.16
Prothrombin time, INR	0.07 (0.16)	0.04 (0.12)	0.03 (0.10)	0.0005
ALT, U/L	−33 (79)	−10 (88)	−11 (69)	0.002
AST, U/L	−11 (71)	−0.4 (73)	5 (57)	0.01
Alkaline phosphatase, U/L	−2.6 (40)	−1.0 (35.6)	0.9 (32.3)	0.31
Total bilirubin, mg/dL	0.22 (0.48)	0.14 (0.47)	0.14 (0.46)	0.08
Albumin, g/dL	−0.13 (0.45)	−0.11 (0.41)	−0.07 (0.43)	0.22
AST/ALT ratio	0.20 (0.30)	0.11 (0.23)	0.16 (0.33)	0.19
Liver histology				
Ishak inflammation score	−0.84 (2.47)	−0.45 (2.75)	−0.47 (2.69)	0.12
Hepatocellular iron grade	−0.07 (0.71)	−0.06 (0.65)	−0.17 (0.68)	0.17
Ishak fibrosis score	0.30 (1.46)	−0.05 (1.33)	0.02 (1.37)	0.04

*Data available on 698 patients.

†Data available on 677 patients.

‡*P*-values are for comparison between patients with decrease vs. those with increase in steatosis score.

of 96-136), most of whom had mild fibrosis on the initial biopsy. Three studies found that baseline steatosis was an independent predictor of fibrosis progression. One study included only patients with baseline Metavir F0-1 (absent or portal tract fibrosis),[14] another study had 26 patients with Metavir F3 (bridging fibrosis) but none had F4 (cirrhosis),[11] and the third study included 18 patients with Ishak fibrosis ≥3.[13] A fourth study observed that worsening of steatosis was independently associated with fibrosis progression.[12] In this fourth study, 13 patients had Metavir F2-3 (septal/bridging fibrosis) but none had F4. A fifth study that included 12% of patients with cirrhosis found that neither baseline steatosis nor a change in steatosis correlated with fibrosis progression.[10] Taken together, these studies support a role of steatosis in fibrosis progression in hepatitis C patients with mild fibrosis. However, the relationship between steatosis and fibrosis is more complicated in patients with advanced fibrosis.

A previous report of the baseline biopsies from patients entering the HALT-C Trial demonstrated an association between steatosis and fibrosis, up to but not including cirrhosis.[4] Studies of explant livers[15] from patients with decompensated HCV-related cirrhosis also observed a low prevalence of any steatosis, and severe steatosis was rare.[16,17] The inference is that steatosis decreases when hepatitis C patients progress to cirrhosis and steatosis may completely disappear in those who decompensate. The results of the current study, based on 892 paired biopsies from patients who had bridging fibrosis or cirrhosis at enrollment, provide the most direct evidence to date that steatosis declines as patients progress from advanced fibrosis to cirrhosis or remain cirrhotic. In this regard, we confirmed and significantly

Table 3. Multivariate Analysis of Factors Associated with Decrease in Steatosis Score

	Decrease vs. No Decrease (Increase + No change)	
Variables	**Adjusted OR**	**95% CI**
Metabolic factors		
BMI (baseline)	1.18	1.02–1.37*
BMI change (FU-baseline)	0.89	0.72–1.11
Truncal obesity (baseline)	0.68	0.50–0.93*
Truncal obesity (FU-baseline)	0.87	0.65–1.16
Triglyceride (baseline)	0.98	0.92–1.05
Triglyceride (FU-baseline)	0.91	0.85–0.98*
HOMA 2 (baseline)	1.001	0.997–1.01
Viral factors		
Log HCV RNA (baseline)	1.41	0.85–2.32
Log HCV RNA (FU-baseline)	0.90	0.72–1.12
HCV genotype 3		
Yes vs. no	2.04	0.62–6.70
Labs		
AST/ALT (baseline)	0.94	0.86–1.02
AST/ALT (FU-baseline)	1.17	1.06–1.29*
PT/INR (baseline)	1.14	0.88–1.49
PT/INR (FU-baseline)	1.25	0.99–1.57
Total bilirubin (baseline)	1.06	0.99–1.13
Total bilirubin (FU-baseline)	1.05	0.99–1.11
Liver histology		
Ishak inflammation score (baseline)	0.90	0.77–1.05
Ishak inflammation score (FU-baseline)	0.88	0.79–0.99*
Hepatocellular iron grade (baseline)	0.91	0.62–1.34
Hepatocellular iron grade (FU-baseline)	0.69	0.45–1.05
Fibrosis scores (baseline)	1.01	0.81–1.26
Fibrosis scores (FU-baseline)	1.20	0.99–1.44
Mallory bodies (baseline)		
Present vs. absent	1.63	0.89–3.00
Zone 3 pericellular fibrosis (baseline)		
Present (mild/moderate) vs. absent	1.14	0.70–1.84
Alcohol consumption		
Still drinking at time of last biopsy		
Yes vs. no	0.93	0.53–1.65

*Significant at 5% level.

162 patients with baseline steatosis score of 0 were excluded from this analysis.

An odds ratio greater than one indicates an association with decrease in steatosis.

Odds ratios for numeric variables represent unit increases with the exception of the following variables where unit increases were as stated: BMI, 2 kg/m^2; waist circumference, 10 cm; triglyceride, 25 mg/dL; HOMA 2, 0.1; AST/ALT ratio, 0.1; total bilirubin, 0.1 mg/dL; INR, 0.1.

extended the findings of other studies that have suggested this phenomenon.[15,18,19] Of the five studies with paired biopsies described earlier, three compared steatosis between the two biopsies. In two studies, there was a trend toward worsening steatosis. One study did not include any patient with cirrhosis, whereas the other study included 18 patients with Metavir ≥F3.[12,13] A third study that included 136 patients with paired biopsies, of whom 11 had cirrhosis at baseline and 15 progressed to cirrhosis during follow-up noted that 20% of patients had decrease in steatosis, whereas 13% had increase in steatosis.[10] These data support the notion that steatosis is more likely to worsen in patients with mild fibrosis and to regress in those with more advanced liver disease.

Why steatosis tends to increase with the progression of fibrosis in CHC, but then decreases with the development of cirrhosis is uncertain. Decrease in steatosis has also been observed in patients with nonalcoholic fatty liver progress to cirrhosis.[20] Insulin resistance has been suggested as the major factor associated with the presence of hepatocyte steatosis, but cirrhosis presents an even more insulin-resistant state. One possible explanation may be the reduced access of free fatty acids and triglycerides to hepatocytes as a consequence of portosystemic shunting or loss of sinusoidal fenestrations accompanying cirrhosis.[21,22] Alternatively or concomitantly, limited exposure of hepatocytes to circulating insulin with portosystemic shunting may decrease insulin's fat-storing signal.[23] In this study, we observed a correlation between decrease in steatosis and the presence as well as new development of esophageal varices, supporting a relationship between decrease in hepatic steatosis and portal hypertension.

It is possible that an apparent decrease in steatosis with the development of cirrhosis could be a visual artifact of the histological interpretation. With greater fibrosis, attention may be diverted from the fewer hepatocytes visible on biopsy. Sampling error and inter- and intraobserver variability may also contribute to the changes observed.[24-26] However, endoscopic and laboratory data support an association between a decrease in steatosis and progression of liver disease. Steatosis was more likely to diminish among patients who had esophageal varices at the last biopsy or developed varices between the first and last biopsy than those without varices. Steatosis was also more likely to decrease among patients who had changes in laboratory markers that indicate worsening liver disease. Thus, there was independent, nonhistological evidence of increased portal pressure and worsening liver function with decline in steatosis.

Besides histologic progression to cirrhosis, a number of metabolic, laboratory and histologic features were associated with changes in steatosis. In general, differences in these features between the baseline and last biopsy correlated better with changes in steatosis than baseline values. Indeed, some of the baseline features associated with a change in steatosis appeared to be counterintuitive. Thus, improvements in steatosis were seen with *higher* baseline BMI, waist circumference, triglyceride concentration, glucose and insulin concentrations, HOMA 2, AST and ALT activity, higher inflammatory score, and the presence of Mallory bodies (Table 2). These are usually considered features of fatty liver. We suggest that these unlikely associations with a decrease in steatosis may have been analytical artifacts of the strong tendency for steatosis to decrease among patients with more

Table 4. Changes in Insulin, Glucose, and HOMA 2 from Baseline to M24 (n = 859)

	Steatosis Change (Year 1.5-Baseline)				
	Decrease in 2 or More Points (N = 16)	Decrease in 1 Point (N = 187)	No Change (N = 457)	Increase in 1 Point (N = 176)	Increase in 2 or More Points (N = 23)
Change in insulin* (*P* Value: 0.07)	−7.79 (75.4)	−14.8 (54.7)	−12.8 (53.0)	−1.70 (45.9)	13.2 (64.2)
Change in glucose† (*P* Value: 0.01)§	−10.7 (27.3)	−3.05 (45.1)	−3.78 (38.9)	5.83 (31.8)	14.5 (27.6)
Change in HOMA 2‡ (*P* Value: 0.005)§	−3.17 (3.45)	−2.19 (7.43)	−1.13 (4.49)	0.02 (3.65)	−0.02 (3.13)

Values expressed as mean (SD).
*Change in insulin was available for 654 patients.
†Change in glucose was available for 842 patients.
‡Change in HOMA 2 was available for 624 patients.
§Significant at 5% level. ANOVA was used for comparison.

severe steatosis on the initial biopsy (i.e., regression toward the mean). As expected, a decrease in steatosis was more common in patients who were not drinking alcohol at the time of the last biopsy. Of more interest were the findings on univariate analysis of improvement in steatosis with weight loss, improved triglyceride concentration, and declines in ALT and AST activities. We did not observe a relationship of a change in viral level with a change in steatosis in this study of nonresponders. In multivariate analysis, declines in triglyceride concentration and inflammation score, as well as an increase in AST/ALT ratio, were associated with a decrease in steatosis (Table 3). In a separate analysis that was limited to the Year 2 biopsy for patients who had insulin and glucose measures at that time, there was an association between decrease in steatosis and improvement in HOMA 2. An association between weight loss and a decrease in steatosis had been reported in a prior study of 10 hepatitis C patients.[27] The current study with serial biopsies from roughly 900 patients provided strong evidence that steatosis declines with improvement in metabolic features in CHC, just as it does in nonalcoholic fatty liver.

In summary, this comprehensive study of the evolution of hepatic steatosis in a large cohort of patients with CHC demonstrated that steatosis recedes as patients progressed from advanced fibrosis to cirrhosis. The results suggest a complex relationship between steatosis and liver disease progression. Metabolic features associated with fatty liver are associated with steatosis in patients with hepatitis C and likely play a role in fibrosis progression during early stages of disease. Improvement in metabolic parameters is associated with a decrease in steatosis in patients with hepatitis C as it does in nonalcoholic fatty liver. However, a decrease in steatosis can also occur when liver disease progresses to cirrhosis. The two pathways by which hepatic steatosis diminish appear to operate independently, although additional research is needed to clarify whether decreased steatosis is a result of cirrhosis or a factor contributing to progression to cirrhosis. Regardless, alcohol abstinence and improvement in metabolic parameters can ameliorate hepatic steatosis and may modulate hepatitis C progression and should be encouraged particularly in patients who have failed current therapy.

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