Effect of Treatment With Peginterferon or Interferon Alfa-2b and Ribavirin on Steatosis in Patients Infected With Hepatitis C

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It has been suggested that hepatitis C virus (HCV) and especially genotype 3 is associated with steatosis. We assess the effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis. We analyzed 1,428 naïve patients included in a randomized trial. A single pathologist scored steatosis at baseline and 24 weeks after the treatment. At baseline, steatosis was present in 935 of 1,428 patients (65%), including 175 (83%) of 210 patients with genotype 3 versus 760 (62%) of 1,218 with other genotypes ($P < .001$). The variables associated with steatosis in logistic regression were genotype 3 ($P < .001$), triglycerides greater than 1.7 mmol/L ($P < .001$), body mass index greater than 27 ($P < .04$), age greater than 40 years ($P < .001$), and septal fibrosis ($P = .007$). In genotype 3-infected patients, steatosis was associated with high viral load and with lower serum cholesterol. Steatosis was associated with lower sustained response rate, even after taking into account other factors ($P < .001$). Among virologic responders, steatosis was much improved in genotype 3, improvement of at least 1 grade in 77%, and disappearance in 46% compared with other genotypes, 46% and 29%, respectively ($P < .001$ both comparisons). In genotype 3 responders, the baseline low serum cholesterol was corrected by treatment ($P < .001$). Steatosis was associated with HCV genotype 3, triglycerides, high body mass index, age, fibrosis stage, and lower virologic response to treatment. In conclusion, sustained disappearance of the virus is associated with reduction of steatosis in genotype 3 as well as a correction of baseline low serum cholesterol. (HEPATOLOGY 2003;38:75-85.)

Liver steatosis is observed in approximately 50% of patients infected with hepatitis C virus (HCV).[1-11] Even after exclusion of the usual causes of steatosis, obesity, diabetes, alcohol, and drugs, the prevalence of steatosis is still around 30% to 40%.[3-4] It has been suggested that HCV genotype 3 is directly responsible for hepatocyte steatosis.[2-4] Steatosis could be the morphologic expression of a cytopathic effect of HCV genotype 3.[3-4] HCV genotype 3 core protein could inhibit very-low-density lipoprotein (VLDL) liver secretion and induce liver steatosis together with hypobeta-lipoproteinemia.[5]

One strong argument for a direct effect of HCV virus is the disappearance of steatosis with the disappearance of the virus. In previous studies, the disappearance of steatosis has been observed in a small number of patients infected by HCV genotype 3 and successfully treated with interferon or with interferon and ribavirin combination: 12 nonimmunodepressed patients[3,4] and 2 transplanted patients.[3] Additionally, hypobeta-lipoproteinemia was corrected (cholesterol and apolipoprotein B) in 7 sustained responders but without significant change in liver steatosis.[5]

The aim of this study was to assess the impact of sustained virologic response on liver steatosis using a large database of patients with paired biopsies recently included in a multicenter randomized trial of pegylated interferon

Abbreviations: HCV, hepatitis C virus; VLDL, very-low-density lipoprotein; BMI, body mass index.

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(peginterferon alfa-2b) and ribavirin combination.[7] Paired biopsies before and after an effective treatment permitted us to identify patients with treatable steatosis and nontreatable steatosis. Metabolic factors including body mass index (BMI), serum glucose, cholesterol, and triglycerides were also assessed to understand their relationship with steatosis. The *a priori* hypothesis was that there was a viral steatosis (inducing a decrease in serum cholesterol and treatable by effective antiviral treatment) and a nonviral steatosis (nontreatable) mainly related to metabolic factors. The specific aims were (1) to assess the prevalence of steatosis, (2) to assess factors associated with steatosis including infection with genotype 3, (3) to assess the impact of steatosis on treatment response, and (4) to assess the impact of treatment on steatosis.

Patients and Methods

The individual data from a large, international, multicenter, randomized trial of peginterferon alfa-2b (PEG-Intron; Schering Plough Corp, Kenilworth, NJ) or interferon alfa-2b (Intron A; Schering Plough Corp) in combination with ribavirin (Rebetol; Schering Plough Corp) described in detail elsewhere were obtained.[12] The patients in this study were previously untreated adults with HCV-RNA detectable in the serum by polymerase chain reaction (PCR) and alanine aminotransferase (ALT) above the upper limit of normal with a liver biopsy within 1 year prior to entry consistent with chronic hepatitis C. Patients were excluded if they had decompensated liver disease, hepatitis B virus (HBV) or human immunodeficiency virus (HIV) infection, daily alcohol consumption greater than 50 grams, or liver disease because of other causes (hemochromatosis, autoimmune, α-1-antitrypsin, steato-hepatitis).

For this particular study, patients were included if they had both pre- and posttreatment liver biopsies. A database was created that contained the following: gender, age at first biopsy, age at infection, body mass index, presumed mode of infection (parenteral, usually IV drug use, transfusion, other, or unknown), type of treatment (PEG-interferon ribavirin, interferon-ribavirin), steatosis grade, METAVIR fibrosis stage and activity grade at first and second biopsy, time elapsed between the 2 biopsies in months, quantification of viral load before treatment, at the end of treatment, at the end of follow-up (6 months after the end of treatment), and HCV genotype. All patients had fasting cholesterol and triglycerides obtained fasting prospectively at entry; weeks 12, 24, 36, and 48 of treatment; and weeks 12 and 24 posttreatment.

Patients were randomized to 1 of 3 treatment arms: standard interferon (interferon alfa-2b, 3 million units 3 times a week subcutaneously) plus ribavirin 1,000 to 1,200 mg/d for 48 weeks; peginterferon alfa-2b 1.5 μg per kg per week for the first 4 weeks, followed by 0.5 μg/kg per week for the next 44 weeks plus ribavirin 1,000 to 1,200 mg/d; peginterferon alfa-2b 1.5 μg/kg per week plus ribavirin 800 mg/d. For patients in the groups receiving ribavirin 1,000 to 1,200 mg/d, the dose was adjusted for body weight (1,000 mg for $<$75 kg and 1,200 mg for $\geq$75 kg).

Liver biopsy specimens were processed using standard techniques and evaluated for stage of fibrosis and grade of activity according to the METAVIR scoring system, for which the reproducibility has previously been established.[13,14] Fibrosis was staged on a scale of 0 to 4: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. Activity (the intensity of necroinflammatory activity mostly based on necrosis) was scored as follows: A0 = no histologic activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity. Steatosis was scored or graded as follows: grade 0 = no steatosis, grade 1 = between 1% and 5% of hepatocytes contained visible macrovesicular steatosis, grade 2 = between 6% and 32%, and grade 3 = more than 33%. Only 16 patients had steatosis greater than 66%. One pathologist (Z.G.) reviewed the biopsy specimens at the time these individual studies were undertaken without any information concerning the clinical, biologic, or treatment characteristics during the conduct of each study.

Serum HCV RNA level was determined by a single central laboratory (NGI, Los Angeles, CA). A quantitative reverse-transcription multicycle polymerase chain reaction was used, with a lower limit of detection of 50 IU/mL (100 copies/mL).[15] The median observed 3.5 million copies/mL, which is equivalent to 1.3 million IU/mL.[16] HCV genotyping was performed as previously described.[17] Serum HCV RNA level was expressed in copies/mL when expressed in log.

Classification of Steatosis. A retrospective analysis allowed patients to be separated into 4 groups on the basis of virologic response (sustained vs. not sustained), impact of treatment on steatosis (improvement vs. no improvement/worse), and metabolic risk factors (present vs. absent; improved or not during follow-up). Group 1, "viral steatosis": patients with a sustained virologic response in whom steatosis disappeared after treatment. Group 2, "mixed viral-metabolic steatosis": patients with a sustained virologic response with a decrease in steatosis of at least 1 grade and no improvement in metabolic risk factors. Group 3, "metabolic steatosis": patients who had at least 1 metabolic factor at baseline or at follow-up (BMI $\geq$27 or had serum fasting glucose $\geq$6 mmol/L or had serum triglycerides $\geq$1.7 mmol/L) without steatosis dis-

appearance or decrease in virologic responders. Group 4, "unclassified steatosis": patients not classified in other groups, *i.e.*, patients with no improvement in steatosis and no metabolic risk factor.

Statistical Methods. Univariate comparisons used Student's *t* test, Mann Whitney test, and Kruskal-Wallis test on ranks for quantitative factors with nonequal variance (Bonferroni method for multiple comparisons); Fisher exact test for qualitative factors; and Wilcoxon signed rank test for difference in medians. *P* values were 2-sided. Multivariate analysis used logistic regression analysis.[18] Independent factors associated with the percentage of patients without steatosis or with at least 1 steatosis stage improvement at the posttreatment biopsy were assessed by logistic regression model. The following factors were assessed in the model: age at first biopsy (younger than 40 years), gender, genotype (3 or other), baseline viral load (lower than 1.3 million IU/mL or higher, which was the median value), fibrosis stage (F0/F1 or F2/F3/F4) and activity grade (A0/A1 or A2/A3) at first biopsy, sustained response (yes or no), and the body mass index (lower than 27 or not, which was the median value). The regression analyses using quantitative value of factors gave the same results (data not shown). A sustained response was defined as a negative serum HCV RNA (less than 50 IU/mL) at the end of 24 weeks follow-up after the end of treatment.

Results

A total of 1,530 patients were randomized and treated, including 1,428 with a baseline biopsy and 1,034 patients with a biopsy pre- and posttreatment. Characteristics of patients with paired biopsies were similar to the included population (Table 1).

Prevalence of Steatosis Prior to Therapy. On the pretreatment biopsy, steatosis was present in 935 of 1,428 patients (65%), including 175 (83%) of 210 patients with genotype 3 versus 760 (62%) of 1,218 other genotypes ($P < .001$). Among the 1,034 patients with paired biopsies, steatosis was present in 670 patients (65%) at baseline and in 571 patients at the end of follow-up (55%) (Table 2).

Factors Associated With Baseline Steatosis. Patients with baseline steatosis in comparison with patients without steatosis were older; more often male, with higher body mass index, higher blood glucose, higher serum triglycerides; and more often genotype 3, with higher fibrosis stage and higher activity grades (Table 2).

The variables independently associated with baseline steatosis in logistic regression were genotype 3 (OR = 6.5, $P < .001$), triglycerides greater than 1.7 mmol/L (OR = 3.3, $P < .001$), BMI greater than 27 (OR = 2.5, $P = .04$), age greater than 40 years (OR = 2.1, $P < .001$), and fibrosis stage (OR = 1.6, $P = .007$) (Table 3A). Serum glucose was significantly associated with steatosis when BMI and fibrosis stage were not en-

Table 1. Characteristics of Patients Randomized and of Patients With Paired Biopsies Included in the Present Analysis

Baseline Characteristics	All n = 1,530 (%)	Paired Biopsies n = 1,034 (%)
Treatment received		
IFN + ribavirin 48 weeks	505	334
PEG 0.5-ribavirin 48 weeks	514	361
PEG 1.5-ribavirin 48 weeks	511	339
Age: Mean (y)	43	43
Gender: Male	1,010 (66)	672 (65)
Glucose (mmol/L)	5.3 ± 0.02	5.3 ± 0.03
≥6.0 mmol/L	209 (13)	133 (13)
Triglycerides (mmol/L)	1.3 ± 0.02	1.3 ± 0.02
≥1.7 mmol/L	301 (20)	193 (19)
Body Mass Index (kg/m^2)	27.4 ± 0.1	27.5 ± 0.2
≥27	732 (48)	507 (49)
Metabolic factors*		
None	608 (41)	413 (41)
1	568 (38)	393 (39)
2	266 (18)	172 (17)
3	45 (3)	29 (3)
Cholesterol (mmol/L)	4.5 ± 0.02	4.5 ± 0.03
Source of infection, %		
Transfusion	21	22
Intravenous drug	64	64
Other or unknown source	15	14
Duration between biopsies		
Mean month	21	21
Histology at first biopsy	1,428	1,034
METAVIR fibrosis stage		
No fibrosis (F0) %	1	1
Portal fibrosis (F1)	70	68
Few septa (F2)	17	17
Many septa (F3)	6	7
Cirrhosis (F4)	6	7
METAVIR activity grade, %		
No activity (A0)	1	1
Mild (A1)	16	16
Moderate (A2)	38	36
Severe (A3)	45	47
Steatosis grade		
0%	495 (35)	364 (35)
1%-5%	548 (38)	392 (38)
6%-32%	288 (20)	210 (20)
33%-66%	78 (5)	52 (5)
67%-100%	25 (2)	16 (2)
Genotype determination		
1a	581 (38)	400 (39)
1b	459 (30)	321 (31)
2	229 (15)	154 (15)
3	219 (14)	134 (13)
Other	44 (3)	25 (2)
Initial serum HCV RNA		
Median millions IU/mL, meq IU/ml^{-1}	1.4, 3.6	1.4 (3.6)

*Metabolic factors (BMI ≥27, glucose ≥6 mmol/L, triglycerides ≥1.7 mmol/L) were missing at baseline in 43 patients randomized and in 27 patients with paired biopsies.

Table 2. Characteristics of 1,034 Patients With Paired Biopsies According to Presence of Steatosis at Baseline and at End of Follow-up

	Steatosis at Baseline, n = 670					No Steatosis at Baseline, n = 364		
Characteristics of Steatosis at End of Follow-up	All n = 670 (%)	Improved n = 341 (%)	Not improved n = 329 (%)	Disappeared n = 198 (%)	Not Disappeared n = 472 (%)	All n = 364 (%)	Never Steatosis n = 265 (%)	Steatosis Occurrence n = 99 (%)
Treatment received								
IFN + ribavirin 48 weeks	206 (31)	114 (33)	92 (28)	74 (37)	132 (28)	128 (35)	97 (37)	31 (31)
PEG 0.5-ribavirin 48 weeks	254 (38)	124 (36)	130 (40)	63 (32)	191 (40)	129 (35)	74 (28)	33 (33)
PEG 1.5-ribavirin 48 weeks	2510 (31)	103 (30)	107 (33)	61 (31)	149 (32)	107 (30)	94 (35)	35 (35)
Age: Mean (y)	44.4 ± 0.3*	44.2 ± 0.4	44.5 ± 0.4	43.8 ± 0.4	44.6 ± 0.4	41.8 ± 0.4*	41.7 ± 0.5	42.1 ± 0.8
Gender: Male	459 (69)*	223 (65)	236 (72)	128 (65)	331 (70)	216 (59)*	143 (54)	73 (74)
Serum glucose (mmol/L)	5.4 ± 0.1*	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.1 ± 0.1*	5.1 ± 0.1	5.2 ± 0.1
>6.0 mmol/L	101 (15)	49 (14)	52 (16)	21 (16)	80 (17)	32 (9)	21 (8)	11 (11)
Body mass index (kg/m^2)	28.6 ± 0.2*	28.3 ± 0.3	28.9 ± 0.3	27.2 ± 0.4	29.2 ± 0.2	25.4 ± 0.2*	24.7 ± 0.3	27.4 ± 0.5
Source of infection								
Transfusion	147 (22)	74 (22)	73 (22)	40 (20)	107 (23)	78 (21)	62 (23)	16 (16)
Intravenous drug	420 (63)	211 (62)	209 (64)	127 (64)	293 (62)	240 (66)	170 (64)	70 (71)
Duration between biopsies								
Mean month	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.3	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.4
Histology at first biopsy								
METAVIR fibrosis stage								
No fibrosis (F0) or portal fibrosis (F1)	429 (64)	218 (64)	211 (64)	133 (67)	296 (63)	291 (80)	218 (82)	73 (74)
Few (F1), many septa (F3), cirrhosis (F4)	241 (36)*	123 (36)	118 (36)	65 (33)	176 (27)	73 (20)*	47 (18)	26 (26)
METAVIR activity grade								
No activity (A0) or mild (A1)	93 (14)	39 (11)	54 (6)	28 (14)	65 (14)	83 (23)	54 (21)	29 (30)
Moderate (A2) or severe (A3)	577 (86)*	302 (89)	275 (84)	170 (86)	407 (86)	281 (77)*	211 (79)	70 (70)
Steatosis								
0%	0 (0)	0	0	0 (0)	0 (0)	364 (100)	265 (100)	99 (100)
1%-5%	392 (59)	150 (44)	242 (74)	150 (76)	242 (51)	0	0	0
6%-32%	210 (31)	133 (39)	77 (23)	36 (18)	174 (37)	0	0	0
33%-100%	68 (10)	58 (17)	10 (3)	12 (6)	56 (12)	0	0	0
Genotype determination								
1a	250 (37)	117 (34)	133 (40)	68 (34)	182 (39)	150 (41)	112 (28)	38 (38)
1b	204 (31)	97 (28)	107 (33)	57 (29)	147 (31)	117 (32)	81 (25)	36 (36)
2	84 (13)	38 (11)	46 (14)	21 (11)	63 (13)	70 (19)	51 (19)	19 (19)
3	117 (17)*	82 (24)	35 (11)*	47 (24)	70 (15)*	17 (5)	11 (4)	6 (6)
Other	15 (2)	7 (2)	8 (2)	5 (3)	10 (2)	10 (2)	10 (4)	0 (0)
Initial serum HCV RNA								
Median millions IU/mL, meq IU/mL	1.4, 3.8	1.4, 3.7	1.4, 3.8	1.5, 3.9	1.4, 3.7	1.4, 3.8	1.4, 3.6	1.7, 4.2
Lipids assessments								
Cholesterol	4.5 ± 0.04	4.4 ± 0.08	4.6 ± 0.08	4.4 ± 0.08	4.5 ± 0.07	4.6 ± 0.04*	4.6 ± 0.08	4.4 ± 0.1
Triglycerides	1.4 ± 0.04*	1.3 ± 0.06	1.6 ± 0.1	1.2 ± 0.06	1.6 ± 0.07	1.1 ± 0.04*	1.1 ± 0.06	1.2 ± 0.09

*There was a significant difference between patients with and without steatosis ($P < .02$ for cholesterol, $P < .001$ for triglycerides, glucose, BMI, age, fibrosis stage, activity grade, and genotype 3).

tered into the model (OR = 1.7, $P = .005$). Serum cholesterol was not associated with steatosis.

High viral load was associated with steatosis only among genotype 3 patients ranging from 5.9 log copies in patients without steatosis to 6.6 in patients with severe steatosis ($P < .001$) (Fig. 1A). There was a significant decrease in serum cholesterol according to steatosis grade ($P < .001$) in patients infected with genotype 3 but not in patients infected with genotypes other than genotype 3 (Fig. 1C). At baseline, the cholesterol value was lower in genotype 3 (4.0 ± 0.1) versus genotypes non-3 (4.6 ± 0.06, $P = .002$).

There was a significant increase of serum triglycerides according to steatosis grade ($P < .0001$) in patients infected by genotypes other than 3 with a significant difference between grade 2 versus grades 0 and 1 (both $P < .05$). There was no significant association in patients infected by genotypes other than 3 (Fig. 1E). The mean triglycerides value was lower in patients infected by genotype 3 (1.0 ± 0.12) versus patients infected by non-3 genotypes (1.4 ± 0.05, $P = .002$). There was a significant increase of serum glucose ($P < .001$) (Fig. 1G) and of BMI (Fig. 1I) ($P < .001$) according to steatosis grades whatever the genotypes.

Table 3. Factors Associated With Steatosis in Multivariate Analysis

Factor	Odds Ratio Exp (B)	Lower 95% CI	Upper 95% CI	Significance
A: Factors associated with the presence of steatosis at baseline*				
Genotype 3	6.5	3.7	11.6	$P < .001$
Triglycerides ≥1.7 mmol/L	3.3	2.1	5.0	$P < .001$
Body mass index ≥27	2.5	1.8	3.3	$P = .04$
Age ≥40 years	2.1	1.6	2.9	$P < .001$
Male	1.3	0.9	1.7	$P = .13$
Serum glucose ≥6 mmol/L†	1.5	1.0	2.2	$P = .07$
Fibrosis stage F2 or F3 or F4 at baseline	1.6	1.1	2.2	$P = .007$
Activity grade A2 or A3 at baseline	1.4	0.9	2.0	$P = .09$
B: Factors associated with the absence of steatosis at the end of follow-up after treatment (logistic regression model) in patients with paired biopsies‡				
Baseline steatosis grade S01/S234	0.18	0.12	0.27	$P < .0001$
Body mass index <27	0.49	0.37	0.65	$P < .0001$
Sustained viral response	0.48	0.36	0.66	$P < .0001$
Female gender	0.61	0.46	0.82	$P < .0001$
Baseline viral load ≥3.5 millions	0.72	0.54	0.96	$P = .02$
Genotype 3	0.57	0.33	0.98	$P = .04$

*A total of 1,007 patients had all the data, and 653 patients had steatosis at baseline (65%).

†Serum glucose was significantly associated with steatosis when BMI and fibrosis stage were not entered into the model (odds ratio = 1.7; $P = .005$).

‡A total of 1,007 patients had all data, and 555 patients had steatosis at the second biopsy (55%).

Impact of Steatosis on Treatment Response. Absence of baseline steatosis, as well as end of follow-up steatosis, was associated with higher sustained response, except in patients with genotype 3 (Table 4). This strong association persisted after adjustment with known factors of response, genotype 1, 4, 5, and 6, viral load, fibrosis, age, and gender. This significant association also persisted after taking into account BMI, glucose and triglycerides, and before or after treatment (Table 4). The worst group in term of virologic response (21%) was patients infected by genotype 1, 4, 5, or 6, with baseline viral load greater than 3.5 million copies per milliliter, extensive fibrosis, and steatosis (Table 4). At the other extreme, the sustained response rate of patients with genotype 2 and no extensive fibrosis without steatosis was 98% (51 of 52) versus 89% in patients with genotype 2, no extensive fibrosis but with steatosis (47 of 53, $P = .05$).

Impact of Treatment on Steatosis. Among the 670 patients with baseline steatosis, an improvement of at least 1 grade was observed at the end of follow-up in 341 (51%) patients, with a disappearance in 198 (30%) patients (Tables 2 and 5). There were fewer patients with steatosis among sustained responders (270 of 574, 47%) than in nonresponders (301 of 460, 65%, $P < .001$).

Steatosis was more significantly reduced by treatment among patients with genotype 3 than in other genotypes. Among virologic-sustained responders, baseline steatosis improved in at least 1 stage in 77 (77%) and disappeared in 46 of 100 (46%) patients infected by genotype 3 versus 107 (46%) and 69 (29%) of 235 patients infected by non-3 genotypes, respectively ($P < .001$ both comparisons).

In univariate analysis, the baseline characteristics associated with steatosis improvement or disappearance at the end of follow-up (Table 2) were genotype 3 (improvement, $P < .001$; disappearance, $P = .006$), steatosis grade lower than 5% ($P < .001$, $P < .001$, respectively), BMI lower than 27 ($P = .14$, $P < .001$, respectively), and sustained virologic response ($P = .04$, $P = .007$, respectively). Among patients with steatosis prior to therapy, the variables independently associated with improvement of steatosis by logistic regression were genotype 3 (OR = 5.1, 95% CI 3.4-7.7, $P < .001$), BMI greater than 27 (OR = 1.4, 95% CI 1.1-1.9, $P = .005$), age greater than 40 years (OR = 1.6, 95% CI 1.2-2.3), and sustained virologic response (OR = 1.4, 95% CI 1.1-1.8, $P = .01$). BMI and age were not significantly associated with steatosis improvement in univariate analysis (Table 2). Two characteristics, significantly associated with disappearance of steatosis at the end of follow-up in multivariate analysis (Table 3B), were not significant in univariate analysis: female gender ($P = .08$) and viral load ($P = .37$) (Table 2).

Among patients without steatosis prior to treatment, steatosis occurred among 6 of 17 patients infected by genotype 3 (35%) and among 93 of 347 patients infected by non-3 genotypes (27%; not significant). Factors independently associated with absence of steatosis occurrence by logistic regression were as follows: sustained virological response (OR = 0.40, 95% CI 0.27-0.74, $P = .002$), female gender (OR = 0.47, 95% CI 0.28-0.80, $P = .005$), and BMI lower than 27 (OR = 0.50, 95% CI 0.30-0.83, $P = .007$).

In genotype 3 sustained responders, the baseline low serum cholesterol was corrected by treatment ($P < .001$) (Fig. 2). After treatment, there was a significant increase of cholesterol levels among responders both in genotype 3 (0.84 mmol/L, $P < .0001$) and in genotype non-3 responders (0.34 mmol/L, $P < .0001$). There was no difference in nonresponders.

There was a significant increase of triglycerides levels after treatment among responders, both for genotype 3 (0.49 mmol/L, $P < .0001$) and for genotype non-3 responders (0.26 mmol/L, $P < .0001$). There was no difference in nonresponders.

Factors Associated With End of Follow-up Steatosis. Six variables were independently associated with absence of end of follow-up steatosis in logistic regression (Table 3): absence or minimal baseline steatosis stage, BMI lower than 27, a sustained virological response, female gender, high viral load, and genotype 3.

Among nonresponder patients, steatosis was still more frequent in genotype 3, 19 of 21 (90%) versus 282 of 439 (64%) non-3 genotype (Fisher exact test, $P = .02$). Viral load was associated with end of follow-up steatosis only among 21 nonresponder genotype 3 patients ranging from 5.7 log copies in patients without steatosis to 7.0 log copies in patients with severe steatosis ($P = .07$) (Fig. 1B).

In sustained responders, there were no significant differences in serum cholesterol at the end of follow-up whatever the genotype (Fig. 1D) (Table 5). In contrast, there was a significant increase according to steatosis grade

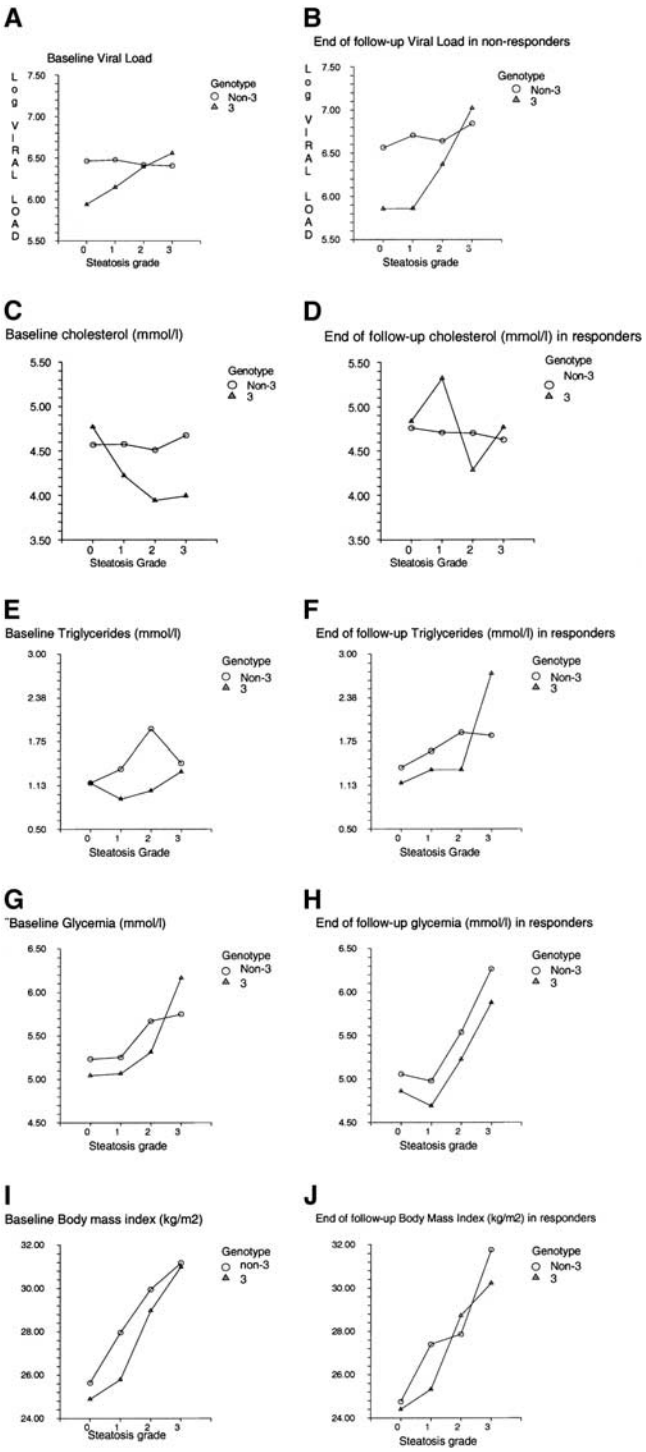

Fig. 1. Association between steatosis grade (baseline and end of follow-up) and genotypes (3 vs. non-3) and viral load (baseline **panel A**; end of follow-up among nonresponders, **panel B**), serum cholesterol (baseline, **panels C and D**), triglycerides (**panels E and F**), serum glucose (**panels G and H**), and BMI (**panels I and J**). Grade 0 is no steatosis, grade 1 between 1% and 5%, grade 2 between 6% and 32%, and grade 3 is 33% or more. (A) At baseline, there was a significant association in genotype 3 between viral load and steatosis grades ($P < .001$). Among grades, the differences were significant between grade 3 versus grade 1 ($P < .001$) and grade 0 ($P < .001$). There was no significant difference in non-3 genotypes. (B) At end of follow-up, there was a significant association in 21 genotype 3 patients still viremic between viral load and steatosis grades ($P = .07$). There was no significant difference in non-3 genotypes. (C) At baseline, among patients infected by genotype 3, there was a significant decrease of serum cholesterol according to steatosis grades ($P < .001$). There was no significant difference in non-3 genotypes. (D) In responders' patients, at the end of follow-up, there were no significant differences in serum cholesterol whatever the genotype. (E) At baseline, among patients infected by genotype 3, there was no significant association between triglycerides and steatosis. Among patients infected by non-3 genotype, there was a significant increase according to steatosis grade ($P < .0001$), with a significant difference between grade 2 versus grade 0 and 1 (both $P < .05$). When all patients with genotype 3 were compared with non-3, there was a significantly lower mean triglycerides value (1.06 ± 0.12) versus non-3 (1.38 ± 0.05; $P = .02$). (F) In responders' patients, at the end of follow-up, there was a significant increase of triglycerides according to steatosis grades whatever the genotypes ($P = .005$). (G) At baseline, there was a significant increase of serum glucose according to steatosis grades whatever the genotype ($P < .001$). (H) In responders' patients, at the end of follow-up, there was a significant increase of serum glucose according to steatosis grades whatever the genotypes ($P < .001$). (I) At baseline, there was a significant increase of BMI according to steatosis grades whatever the genotypes ($P < .001$). (J) In responders' patients, at the end of follow-up, there was a significant increase of BMI according to steatosis grades whatever the genotype ($P < .001$).

Table 4. Steatosis, Metabolic Factors, and Virologic-Sustained Response

Response Factors	Nonresponders n = 460 (%)	Sustained Responders n = 574 (%)	χ^2 *P* Value	Logistic Regression* *P* Value Odds Ratio (95% CI)
Treatment received			.07	**.72**
IFN + ribavirin 1,000-1,200	153 (46)	181 (54)		.92
PEG 1.5-ribavirin (<11 mg/kg)	97 (44)	125 (56)		(0.57-1.48)
PEG 1.5-ribavirin (≥11 mg/kg)	43 (37)	74 (63)		
PEG 0.5-ribavirin 1,000-1,200	167 (46)	194 (54)		
Genotype 2 or 3			<.001	**<.001**
No	424 (57)	322 (43)		.10
Yes	36 (13)	252 (88)		(0.07-0.14)
Viral load <3.5 million copies			<.001	**<.001**
No	289 (53)	254 (47)		.48
Yes	171 (35)	320 (65)		(0.36-0.64)
No fibrosis or limited to portal tract			<.001	**<.001**
No	172 (55)	142 (45)		.53
Yes	288 (40)	432 (60)		(0.39-0.74)
Age <40 years			.02	**.03**
No	353 (49)	366 (51)		.70
Yes	107 (34)	208 (66)		(0.51-0.94)
Female			.02	**.37**
No	318 (47)	357 (53)		.87
Yes	142 (40)	217 (60)		(0.64-1.18)
Baseline metabolic factors				
Body mass index <27†			.07	**.97**
No	237 (48)	259 (52)		1.00
Yes	215 (42)	296 (58)		(−0.29-0.30)
Glucose <6.0 mmol/L‡			.002	**.05**
No	81 (56)	63 (44)		.65
Yes	379 (43)	511 (57)		(0.43-0.99)
Triglycerides <1.7 mmol/L			<.001	**.07**
No	108 (56)	85 (44)		.71
Yes	352 (42)	489 (58)		(0.50-1.03)
Number of metabolic factor			<.001	**.36**
None	165 (40)	248 (60)		.87
1	171 (44)	222 (56)		(0.64-1.17)
2	97 (56)	75 (44)		
3	19 (66)	10 (34)		
Steatosis at first biopsy			<.001	**<.001**
0%	125 (34)	239 (66)		0.48
1%-5%	189 (48)	203 (52)		(0.35-0.66)
6%-32%	115 (55)	95 (45)		
33%-100%	31 (46)	37 (54)		
Steatosis, genotype, viral load, and fibrosis				
Genotype 3				
No steatosis	4 (24)	13 (76)	.34	
Steatosis	17 (15)	100 (85)		
Genotype 2				
No steatosis	3 (4)	67 (96)	.04	
Steatosis	12 (14)	72 (86)		
Genotype 1, 4, 5, 6				
No steatosis	118 (43)	159 (57)	<.001	
Steatosis	306 (65)	163 (35)		
High viral load (>3.5 million copies)				
No steatosis	78 (41)	114 (59)	<.001	
Steatosis	211 (60)	140 (40)		
Extensive fibrosis (stage 2, 3, 4)				
No steatosis	30 (41)	43 (59)	.007	
Steatosis	142 (59)	99 (41)		
Genotype 1, 4, 5, 6, and high viral load				
No steatosis	76 (49)	79 (51)	<.001	
Steatosis	196 (76)	62 (24)		
Genotype 1, 4, 5, 6, high viral load, fibrosis				
No steatosis	23 (61)	15 (39)	.03	
Steatosis	76 (79)	20 (21)		
End of follow-up metabolic factors§				
Body mass index <27			.02	**.84**
No	208 (49)	217 (51)		.97
Yes	215 (41)	305 (59)		(0.71-1.32)
Glucose <6.0 mmol/L			.005	**.07**
No	100 (53)	87 (47)		.71
Yes	355 (42)	485 (58)		(0.49-1.03)
Triglycerides <1.7 mmol/L			.32	**.14**
No	115 (42)	160 (58)		.79
Yes	342 (45)	413 (55)		(0.57-1.08)
Number of metabolic factor			.27	**.68**
None	162 (42)	225 (58)		1.07
1	138 (44)	174 (56)		(0.78-1.45)
2	91 (49)	96 (51)		
3	29 (53)	26 (47)		
Steatosis at second biopsy			<.001	**<.001**
0%	159 (34)	304 (66)		.44
1%-5%	202 (49)	207 (51)		(0.33-0.58)
6%-32%	81 (62)	49 (38)		
33%-100%	18 (56)	14 (44)		

NOTE. *P* values in last column are in bold.

*Eight variables were included in the logistic regressions: treatment received (PEG interferon and ribavirin at the approved dose versus the others), genotype (2 or 3 vs. the others), viral load, fibrosis stage, age, female gender, steatosis, and 1 by 1 metabolic factor (BMI, glucose, triglycerides at baseline and at end of follow-up). Each odds ratio was adjusted for the other 7 variables.

†BMI missing in 27 patients.

‡When steatosis was entered in the model, glucose had no more significant value.

§BMI missing in 89 patients, glucose in 7, triglycerides in 4, and at least 1 metabolic factor in 93 patients.

Table 5. Evolution of Metabolic Factors and Steatosis According to Genotype and Virologic Response

Characteristics	Genotype 3		Genotype non-3	
	Sustained Responders n = 113 (%)	Nonresponders n = 21 (%)	Sustained Responders n = 461 (%)	Nonresponders n = 439 (%)
Treatment received				
IFN + ribavirin	36 (32)	7 (33)	145 (31)	146 (33)
PEG 1.5 ribavirin	33 (29)	3 (14)	166 (36)	137 (31)
PEG 0.5 ribavirin	44 (39)	11 (52)	150 (35)	156 (36)
Age: Mean y	40.0 ± 0.7	43.1 ± 1.4	43.0 ± 0.4	45.0 ± 0.3
Gender: Male	74 (66)	16 (76)	283 (61)	302 (69)
Body mass index				
At baseline	27.0 ± 0.5	27.2 ± 1.0	27.1 ± 0.2	28.1 ± 0.3
At end of follow-up	26.5 ± 0.5	27.3 ± 1.0	26.7 ± 0.2	27.5 ± 0.2
Serum glucose				
At baseline (mmol/L)	5.2 ± 0.1	5.6 ± 0.4	5.2 ± 0.06	5.4 ± 0.05
>6.0 mmol/L	12 (11)	4 (19)	51 (11)	77 (18)
At end of follow-up (mmol/L)	5.4 ± 0.1	5.7 ± 0.4	5.3 ± 0.04	5.6 ± 0.08
>6.0 mmol/L	19 (17)	6 (29)	68 (15)	94 (22)
Cholesterol				
At baseline	4.0 ± 0.1	4.1 ± 0.3	4.7 ± 0.06	4.4 ± 0.04
At end of follow-up	4.9 ± 0.1	4.3 ± 0.2	5.1 ± 0.05	4.4 ± 0.04
Triglycerides				
At baseline	1.0 ± 0.05	1.1 ± 0.1	1.3 ± 0.04	1.5 ± 0.08
At end of follow-up	1.5 ± 0.1	1.3 ± 0.1	1.6 ± 0.05	1.6 ± 0.07
Steatosis at first biopsy				
0%	13 (11)	4 (19)	226 (49)	121 (28)
1%-5%	39 (35)	5 (24)	164 (36)	184 (42)
6%-32%	36 (32)	8 (39)	59 (13)	107 (24)
33%-100%	25 (22)	4 (19)	12 (3)	27 (6)
Steatosis at second biopsy				
0%	56 (50)	2 (10)	248 (54)	157 (36)
1%-5%	46 (41)	9 (43)	161 (35)	193 (44)
6%-32%	7 (6)	7 (33)	42 (9)	74 (17)
33%-100%	4 (3)	3 (14)	10 (2)	15 (3)

NOTE. At baseline, the cholesterol value was lower in genotype 3 (4.0 ± 0.1) versus genotype non-3 (4.6 ± 0.06, $P = .002$), as well as the triglycerides value (1.0 ± 0.1 vs. 1.4 ± 0.05, $P = .002$). After treatment, there was a significant increase of cholesterol levels among responders both in genotype 3 (0.84 mmol/L, $P < .0001$) and in genotype non-3 responders (0.34 mmol/L, $P < .0001$). There was no difference in nonresponders. There was a significant increase of triglycerides levels after treatment among responders both for genotype 3 (0.49 mmol/L, $P < .0001$) and for genotype non-3 responders (0.26 mmol/L, $P < .0001$). There was no difference in nonresponders. There was no significant difference for serum glucose and BMI comparisons.

of triglycerides ($P = .005$) (Fig. 1F), glucose ($P < .001$) (Fig. 1H), and BMI ($P < .001$) (Fig. 1J) whatever the genotype.

Retrospective Classification of Steatosis. A total of 613 patients (105 with genotype 3 and 508 non-3) had baseline steatosis and paired biopsies and estimates of metabolic factors (BMI, serum glucose, serum triglycerides) at baseline and follow-up.

Among the 105 patients with genotype 3 with baseline steatosis, 40 patients (38%) belonged to the group of "viral steatosis" (sustained virologic response and disappearance of steatosis); 25 patients (24%) belonged to the group of "mixed viral-metabolic steatosis" (sustained virologic response with decrease of initial steatosis without metabolic improvement if any at baseline); 32 patients (30%) belonged to the group of "metabolic steatosis" (metabolic factor at baseline or at follow-up, without steatosis improvement in sustained responders); and 8 patients (8%) belonged to the group of "unclassified steatosis" (steatosis not classified as viral, probable viral, or metabolic).

Among the 508 patients with genotype non-3 with baseline steatosis, 63 patients (12%) belonged to the group of "viral steatosis"; 32 patients (6%) belonged to the group of "mixed viral-metabolic steatosis"; 338 patients (67%) belonged to the group of "metabolic steatosis"; and 75 patients (15%) belonged to the group of "unclassified steatosis."

Discussion

This study confirms the high prevalence (65%) of steatosis among patients with chronic hepatitis C and the association with genotype 3, overweight, high fasting serum glucose, triglycerides, male gender, and age. For the first time, it has been clearly demonstrated that effective

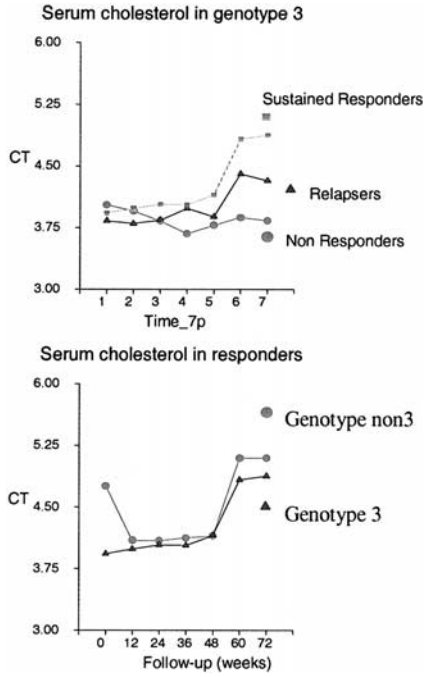

Fig. 2. Serum cholesterol according to response and genotype 3.

treatment of HCV leads to a reduction or disappearance of liver steatosis. The impact was particularly observed in patients with HCV genotype 3 infection.

The analysis of treatment impact on steatosis was not the primary or secondary end point of this randomized trial. However, because of the number of patients included, the blind evaluation of the histologic features including steatosis staging, and the clinical and biochemical details prospectively recorded, this ancillary study has allowed an increased understanding of steatosis in patients infected with HCV.

We found a prevalence of steatosis at baseline in 65% of patients with chronic hepatitis C overall. Previous reports have found a prevalence of steatosis that ranged from a low of 31% (7) to a high of 72% (9), but, because these all represent different cohorts of patients collected in different locations from different populations, no direct comparison can be made. Like others,[3,4,10] we found that genotype 3 was more strongly associated with steatosis (83% of 210 patients in the present cohort) than other genotypes.

Most previous studies that have graded the degree of steatosis have done so by one thirds, with 1+ being up to 30% or 33%, 2+ being 30% to 60% or 33% to 66%, and 3+ being greater than 60% or 66%. The consequence of such a system is that a biopsy specimen with only 2% fat, which most pathologists would consider insignificant, is placed in the same category as one with 30% fat, which is clearly significant. To address this, we chose to assign a grade of 1 to biopsy specimens with 5% or less fat, which accounted for 59% of baseline biopsy specimens with steatosis, and we were surprised to find that even this trivial amount of fat was associated with BMI (Fig. 1I and J) and higher serum triglycerides (Fig. 1E and F) than patients with absolutely no fat in the liver biopsy specimen.

We confirmed that genotype 3, BMI, age, and male gender were the most significant factors associated with steatosis. Serum glucose and serum triglycerides also had significant impact on steatosis, independent of BMI, suggesting that insulin resistance without being overweight may be associated with steatosis. Steatosis was also associated with fibrosis stage. There was an expected association between fibrosis and male gender, glucose, and BMI. Therefore, the inclusion of fibrosis in the multivariate model reduces the strength of the association between steatosis and male gender, BMI, and glucose.

Assessment of the treatment effect and the presence of metabolic factors allow for characterization of 3 main steatosis profiles: viral, metabolic, and nonclassified. Our data provide strong evidence for a causal relationship between the HCV virus genotype 3 and steatosis. Genotype 3 was mainly represented by genotype 3a, and the exclusion of a few cases of non-3a did not change the results (data not shown).

The first line of evidence is the significant association between viral load and grade of steatosis at baseline and among nonresponders at the end of follow-up. These associations were not observed for other genotypes (Fig. 1). There was no difference in other causes of steatosis.

The second line of evidence is the higher disappearance and improvement rates of steatosis in sustained virologic responders in comparison with nonresponders. Viral steatosis represented 54% of genotype 3 steatosis, which is 3 times more than in genotype non-3 (18%). This impact of the treatment persisted after adjustment for metabolic factors, gender, and age (Table 3). After disappearance of detectable serum HCV RNA in patients with genotype 3 infection, the same associations were observed with metabolite factors than among patients infected with non-3 genotypes (Fig. 1). From these results, all future studies on steatosis in patients infected with HCV must analyze separately patients infected with genotype 3.

The direct cytopathic effect with steatosis of HCV genotype 3 is now well established but not fully understood. One hypothesis, which could also explain the high prevalence of hypobeta-lipoproteinemia (low cholesterol, low apolipoprotein B), is a competition of this genotype core protein with the liver secretion of VLDL.[4,5,10,19,21] This hypothesis was strongly supported by the negative association between cholesterol and steatosis observed only in

genotype 3 (Fig. 1C). Furthermore, the lower serum cholesterol concentration observed at baseline in patients infected with genotype 3 in comparison with patients infected by other genotypes was corrected only in sustained responders (Fig. 2). This biologic phenomenon is not marginal because the baseline reduction of serum cholesterol was 20%.

This study, like previous publications,[20,21] also suggests that steatosis induces a mechanism of resistance to interferon and ribavirin combination treatment. This mechanism is unknown but seems independent of other known response factors (genotype 1, high viral load, extensive fibrosis) as well as independent of metabolic factors assessed in this study (BMI, serum glucose, and triglycerides). This mechanism seems also specific of metabolic steatosis because the viral steatosis observed in genotype 3 was not associated with lower sustained response. This was not due to the high response rate in genotype 3. A lower response rate was observed in patients with steatosis versus without steatosis in patients infected by genotype 2, which share the same good response rate as genotype 3 (Table 4).

One weakness of this study was the absence of objective assessment of alcohol consumption during follow-up. Because of that and because heavy drinkers were excluded from this trial, generalization of the results are difficult among a population of heavy drinkers. We cannot exclude that alcohol could be a factor explaining the few occurrences of steatosis during follow-up. The factors associated with an absence of steatosis occurrence were the previously known factors: low BMI and female gender as well as a sustained virologic response.

Interferon has been described as a possible factor of steatosis because it inhibits the transcription of mitochondrial DNA into mitochondrial messenger RNA.[22,23] In fact, the inverse has been demonstrated. Interferon combined with ribavirin was a very effective treatment of liver steatosis among genotype 3 patients. Furthermore, there was no difference in steatosis grade or occurrence among the different regimens using nonpegylated or pegylated interferon alfa-2b and according to the different doses of this trial.

In conclusion, the better understanding of factors related with steatosis will permit us to improve the management of patients. In patients infected with genotype 3 HCV, most of them will be sustained responders, and the steatosis will disappear in half of them. In contrast, in patients infected by non-3 genotype HCV, steatosis is mainly associated with metabolic factors. The management of overweight, diabetes, and hypertriglyceridemia must be particularly encouraged.

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