Associations Between Serum Lipids and Hepatitis C Antiviral Treatment Efficacy

Darmendra Ramcharran,[1]* Abdus S. Wahed,[2] Hari S. Conjeevaram,[3] Rhobert W. Evans,[1] Tianyi Wang,[4] Steven H. Belle,[1,2] and Leland J. Yee,[1,5] for the Virahep-C Study Group

Approximately one half of patients who undergo antiviral therapy for chronic hepatitis C virus (HCV) genotype 1 infection do not respond to treatment. African Americans (AAs) are less responsive to treatment than Caucasian Americans (CAs), but the reasons for this disparity are largely unknown. Recent studies suggest that serum lipids may be associated with treatment response. The aims of this study were to evaluate baseline and changes in serum lipids during therapy, determine whether serum lipids are associated with virological response, and assess whether these measures explain the racial difference in efficacy. The study participants were from Virahep-C, a prospective study of treatment-naïve patients with genotype 1 HCV infection who received peginterferon (PEG-IN) alfa-2a plus ribavirin therapy for up to 48 weeks. Fasting serum lipids were analyzed at baseline and during and after therapy in 160 AAs and 170 CAs. A relative risk (RR) model was employed to evaluate characteristics associated with sustained virological response (SVR). Antiviral therapy was associated with changes in serum lipids during and after antiviral therapy, with the changes differing by race and the amount of PEG-IFN taken. Baseline lipid measures independently associated with higher rates of SVR were lower triglyceride and higher low-density lipoprotein cholesterol, with an interaction between high-density lipoprotein cholesterol (HDLc) and gender. Lipid measures did not contribute significantly to an explanation of the racial difference in SVR. *Conclusion:* Serum lipids are associated with SVR, although these paramaters did not explain the racial difference in treatment response. The results of this study are compatible with proposed biological mechanisms of HCV entry, replication, and secretion, and may underscore new potential therapeutic targets for HCV eradication. (HEPATOLOGY **2010;52:854-863**)

In the United States, chronic hepatitis C virus (HCV) infection is a major public health problem afflicting 3.6 million people with direct health care costs, including liver transplantation, exceeding $1 billion annually.[1,2] The current standard of treatment of combination peginterferon (PEG-IFN) and ribavirin is

Abbreviations: AA, African American; AUROC, area under the receiver operating curve; CA, Caucasian American; HCV, hepatitis C virus; HDLc, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; LDLc, low-density lipoprotein cholesterol; PEG-IFN, peginterferon; RR, relative risk; SVR, sustained virological response; TC, total cholesterol; TG, triglyceride.

From the [1]Departments of Epidemiology, [2]Biostatistics, and [4]Infectious Disease and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; the [3]Division of Gastroenterology, School of Medicine, University of Michigan, Ann Arbor, MI; and the [5]Division of Infectious Diseases, School of Medicine, University of Pittsburgh, Pittsburgh, PA.

Received March 22, 2010; accepted June 1, 2010.

The Virahep-C study was conducted as a cooperative agreement funded by the National Institute of Diabetes and Digestive and Kidney Diseases with cofunding from the National Center on Minority Health and Health Disparities and a Cooperative Research and Development Agreement with Roche Laboratories. The specific grant numbers and membership of the Virahep-C Study Group are provided in the primary publication of the study (reference 4). Funding for additional lipid profile analyses using stored serum samples was obtained for an ancillary study of the pathogenesis of steatosis and insulin resistance in chronic hepatitis C virus infection (KL2 RR024154-02 to L. J. Y. from the National Institutes of Health).

**D.R. is currently affilitated with Via Research, LLC, Princeton Junction, NJ*

Address reprint requests to: Darmendra Ramcharran, Ph.D., M.P.H., 24 Doylston Drive, Cranston, RI 02905. E-mail: dramcharran@gmail.com.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.23796

Potential conflict of interest: Dr. Yee owns stock in Pfizer.

Additional Supporting Information may be found in the online version of this article.

not completely effective in patients with hepatitis C genotype 1, the predominant viral type in the United States; approximately 46% people on combination therapy achieve sustained virological response (SVR).[3] Moreover, there is a racial difference in response: African Americans (AAs) have a significantly lower response to combination treatment compared with Caucasian Americans (CAs).[4-6] The factors that explain this racial disparity in efficacy are largely unknown.[4]

Changes in serum lipid levels during interferon therapy have been reported, although the results are inconsistent and differ by HCV genotype. Interferon therapy has been associated with increases in total cholesterol (TC) and triglyceride (TG) levels, with TC levels remaining significantly higher and TG levels returning to pretreatment levels after stopping therapy.[7] Other work has found significant increases in TG levels, and no significant change in TC levels.[8] Compared with pretreatment, significant increases in TC have been reported in a subgroup with HCV genotype 3, but not genotype 1 during therapy,[9] whereas another study reported higher TG levels during therapy in a group with genotype 1, but not in non–genotype 1.[8]

Recent studies further suggest that pretreatment serum lipid measures may be important predictors of treatment response. Several studies indicate that high pretreatment low-density lipoprotein cholesterol (LDLc) and TC levels are associated with higher rates of SVR in multivariable analyses.[10-14] In addition, higher pretreatment TG levels have also been reported among virological responders compared with nonresponders.[7] These studies further suggest that associations between lipid measures and virological response may be specific to HCV genotype 1 and possibly genotype 2. Little is known about the association between changes in lipid measures while on therapy and treatment response.

Observations from *in vitro* studies suggest relationships between lipoproteins and HCV that are important for mechanisms of viral entry into hepatocytes, viral replication, and secretion. Several studies suggest that HCV may combine with lipoproteins in the serum, possibly obscuring the virus from the host immune response, which may in turn help in viral entry into the hepatocytes.[15-18] Various receptors involved in lipoprotein-viral particle entry into hepatocytes are posited, including the scavenger receptor B1 (SR-B1) and LDL receptor.[19-22] Direct entry of free HCV (i.e., not associated with lipoproteins) is also proposed to occur through binding of the HCV envelope glycoprotein E2 with SR-B1 or its human analogue CD81.[23-25] Within the hepatocyte endoplasmic reticulum, studies indicate that HCV replication may be reliant on cholesterol metabolism and a secretion process consisting of HCV and very low-density lipoprotein conglomerate particles.[26-30] Recent work suggests that interferon therapy leads to down-regulation of SR-B1 expression.[31] This supports the notion that decreased lipoprotein expression may in turn impact serum lipoprotein and lipid profile measures. Therefore, associations between the serum lipids and treatment response are supported by biologically plausible mechanisms.

This study assessed the changes in serum lipids among patients undergoing combination therapy for chronic hepatitis C, the relationship between serum lipids (pretreatment levels and changes during treatment) and virological response, and whether serum lipids might explain the racial disparity in treatment efficacy.

Patients and Methods

Study Population. The participants in this study were from the Virahep-C study, an investigation of resistance to antiviral therapy for genotype 1 chronic HCV infection, which has been described.[4] In brief, the Virahep-C study evaluated clinical, immunological, virological, and host genetic factors that contribute to the lack of virological response to antiviral treatment and, in particular, the racial difference in efficacy. The study enrolled approximately equal numbers of Caucasian Americans (CAs) (n = 205) and African Americans (AAs) (n = 196), all of whom underwent combination PEG-IFN and ribavirin therapy for up to 48 weeks. At 24 weeks of therapy, patients were evaluated for the presence of HCV RNA; those with detectable levels of HCV RNA were labeled as nonresponders and discontinued therapy, and the remaining patients continued therapy for an additional 24 weeks. All patients were followed for an additional 24 weeks after completion of therapy. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the local institutional review board.

Of the 401 participants enrolled in Virahep-C, lipid profile analyses were performed among participants who granted genetic consent (n = 374) approved by the local institutional review board and had stored fasting serum samples at baseline (n = 335). Five participants who reported use of lipid-lowering medications were excluded from this evaluation, resulting in a final analysis sample of 330 participants (160 AAs and 170 CAs). During treatment (24 weeks after starting

therapy) and after treatment (24 weeks after stopping therapy), lipid profile data were additionally available for 253 and 245 of the participants, respectively.

Study Measures. The primary outcome for Virahep-C was SVR, defined as undetectable serum HCV RNA 24 weeks after the end of therapy. Serum lipid measures, TG, LDLc, high-density lipoprotein cholesterol (HDLc), and TC were obtained through analysis of stored fasting serum samples at the Heinz Nutrition Laboratory in the Department of Epidemiology, University of Pittsburgh. For serum samples with TG levels <400 mg/dL, the Friedewald formula was used to calculate LDLc indirectly (TC − HDLc − 0.20 × TG).[32] For samples with TG levels of at least 400 mg/dL, LDLc was assessed directly. Dyslipidemia was defined using the cutoffs from the National Cholesterol Education Program Adult Treatment Panel III recommendations as any of the following: LDLc ≥130 mg/dL, HDLc <40 mg/dL, TC ≥200 mg/dL, or TG ≥150 mg/dL.[33] A homeostasis model assessment (HOMA) variable, HOMA2, was calculated using fasting insulin and glucose measures with a Microsoft Excel HOMA2 calculator and insulin resistance was defined as a score ≥2.[34] Hepatic inflammation and fibrosis were assessed using the criteria of the histological activity index by a single hepatopathologist.[35,36] The amount of PEG-IFN and ribavirin taken by participants was estimated using data from the Medication Event Management System (Aardex, Zug, Switzerland).[37]

Statistical Analysis. Categorical measures were summarized as frequencies and percents with differences across nominally classified groups (race, gender, health insurance status, employment status, smoking status, alcohol consumption of at least 2 drinks per week, history of diabetes and hypertension, HCV genotype, and severe fibrosis) assessed using a Pearson's chi-square test or the exact equivalent. Differences in categorical measures across ordinal groups (i.e., educational attainment and iron scores) were assessed using a Jonckeere-Terpstra test, or the exact equivalent. Continuous measures were summarized as medians and interquartile ranges with differences in group distributions assessed using a Wilcoxon rank sum test (for comparison of two groups) or a Kruskal-Wallis test (for comparison of more than two groups). To assess whether changes in lipid profile measures significantly differed from baseline, a Wilcoxon sign rank test was used. Associations between the proportion of PEG-IFN and ribavirin taken with changes in serum lipids were assessed using Spearman's correlation analyses. For all statistical tests, $P < 0.05$ was considered statistically significant.

To evaluate factors associated with SVR, a relative risk model was employed with a robust variance estimator.[38] In regression models, TG, HDLc, and TC were transformed to the natural logarithm scale to achieve normality. All continuous predictors were centered. The relationships between baseline and 24-week changes during treatment in lipid profile measures and the probability of SVR were graphically assessed using smoothing spline plots. Due to different patterns observed by gender, smoothing spline plots for HDLc were examined separately for males and females. Two types of multivariable models of SVR were constructed using a stepwise approach. One type of multivariable model (models 1 and 2) allowed pretreatment characteristics and the amount of PEG-IFN taken during the first 24 weeks as eligible predictors. Model 2 allowed as additional eligible predictors the baseline lipid profile measures. A second type of multivariable model (model 3) also adjusted for body weight changes and allowed for the inclusion of variables representing baseline and changes in lipid profile measures during the first 24 weeks of therapy as eligible predictors. To compare the prediction of multivariable models, differences in area under the receiver operating curves (AUROCs) were assessed using a nonparametric method.[39]

Results

Baseline characteristics of the 330 participants are shown in Table 1. AAs did not significantly differ from CAs by age, gender, employment status, health risk behaviors (smoking status and weekly alcohol consumption), viral level, aspartate aminotransferase, international normalized ratio, white blood cell count, platelet count, percent iron/total iron-binding capacity, Ishak fibrosis, total histological activity index score, steatosis, TG, HDLc, or TC. Compared with CAs, a larger percentage of AAs had health insurance coverage (87% versus 78%, $P = 0.04$), public health insurance (31% versus 18%, $P = 0.006$), less education ($P = 0.008$), history of diabetes (15% versus 4%, $P < 0.001$), insulin resistance (46% versus 33%, $p = 0.02$), history of hypertension (40% versus 19%, $P < 0.001$), and higher prevalence of HCV subgenotype 1b (44% versus 28%, $P = 0.002$). As a group, AAs had higher body mass index (median 29.3 versus 27.4 kg/m^2, $P < 0.001$), higher HOMA2 scores (median 1.9 versus 1.5, $P < 0.001$), higher alkaline phosphatase levels (median 83 versus 78 U/L, $P = 0.043$), higher ferritin levels (median 246 versus 149 ng/mL, $P < 0.001$), lower alanine aminotransferase levels (median 60 versus 74.5 U/L, $P < 0.001$), lower total bilirubin levels (median 0.06

Table 1. Cohort Characteristics

Feature	Total (N = 330)	AA (n = 160)	CA (n = 170)	*P* Value
Demographics				
Age (years)	48 (43-52)	48 (45-52)	47 (42-52)	0.07
Male	216 (65.5)	106 (66.3)	110 (64.7)	0.77
Health insurance (m = 6)*				0.008
Uninsured	58 (17.9)	21 (13.4)	37 (22.2)	0.04
Public	79 (24.4)	49 (31.2)	30 (18.0)	0.006
Private	187 (57.7)	87 (55.4)	100 (59.9)	0.42
Education (m = 8)†				0.008
Less than high school	61 (18.9)	36 (23.1)	25 (15.1)	0.07
High school degree	78 (24.2)	41 (26.3)	37 (22.3)	0.16
Some college	105 (32.6)	50 (32.1)	55 (33.1)	0.16
College degree or more	78 (24.2)	29 (18.6)	49 (29.5)	–
Health risk behaviors				
Current smoker (m = 6)	128 (39.5)	65 (41.7)	63 (37.5)	0.44
Consumes ≥2 alcoholic drinks/week (m = 7)	66 (20.4)	38 (24.2)	28 (16.9)	0.10
General clinical features				
BMI (m = 5)	28.4 (25.2-32.4)	29.3 (26.6-33.9)	27.4 (24.4-31.4)	0.0002
History of diabetes	31 (9.4)	24 (15.0)	7 (4.1)	0.0007
HOMA2 (m = 22)	1.7 (1.0-2.7)	1.9 (1.3-3.2)	1.5 (0.9-2.3)	0.0003
Insulin-resistant (m = 22)	120 (39.0)	68 (45.6)	52 (32.7)	0.02
History of hypertension	97 (29.4)	64 (40.0)	33 (19.4)	<0.0001
Viral characteristics				
Log_{10} HCV level (m = 1)	6.5 (5.6-6.7)	6.4 (5.6-6.7)	6.5 (5.6-6.8)	0.25
HCV genotype‡				0.002
1, not otherwise specified	25 (7.6)	10 (6.3)	15 (8.8)	0.38
1a	177 (53.6)	79 (49.4)	98 (57.7)	0.13
1a/b	11 (3.3)	1 (0.6)	10 (5.9)	0.01
1b	117 (35.5)	70 (43.8)	47 (27.7)	0.002
Liver disease indicators				
ALT (IU/L)	69 (45-108)	60 (40-88)	74.5 (52-139)	<0.0001
AST (IU/L)	52 (37-79)	51.5 (35.5-71.5)	53 (38-87)	0.08
Alkaline phosphatase (IU/L)	79 (62-103)	83 (62-108)	78 (63-96)	0.043
Total bilirubin (mg/dL)	0.6 (0.5-0.8)	0.6 (0.4-0.8)	0.7 (0.5-0.9)	0.004
INR (m = 2)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	0.95
WBC count (10^3/mL) (m = 3)	6.0 (4.7-7.3)	5.7 (4.6-7.3)	6.25 (4.9-7.4)	0.055
Platelet count (10^3/mL) (m = 4)	207.5 (161-257)	212 (159-268)	207 (161-242)	0.33
Ferritin (ng/mL) (m = 2)	204 (96.8-366)	246 (122-422)	149 (78-287)	0.0001
Albumin (g/dL) (m = 2)	4.2 (4.0-4.4)	4.2 (3.9-4.4)	4.2 (4.0-4.5)	0.004
Iron/TIBC (m = 8)	34.1 (26.2-44.0)	33.9 (25.7-41.9)	34.3 (26.4-47.7)	0.18
Ishak fibrosis score (m = 1)	2 (1-3)	2 (1-3)	2 (1-3)	0.85
Ishak fibrosis score ≥3 (m = 1)	123 (37.4)	58 (36.5)	65 (38.2)	0.74
Fat score (m = 1)	0 (0-1)	0 (0-1)	0 (0-1)	0.19
Steatosis (>5 present) (m = 1)	209 (63.5)	97 (61.0)	112 (65.9)	0.36
Total HAI inflammation (m = 1)	8 (6-10)	8 (7-10)	9 (6-11)	0.58
Iron score (m = 35)				0.09
0	157 (53.2)	66 (47.5)	91 (58.3)	
1	115 (39.0)	62 (44.6)	53 (34.0)	
2	23 (7.8)	11 (7.9)	12 (7.7)	
Serum lipid measures				
TG (mg/dL)	102.5 (75-146)	105.5 (74.5-151)	98.5 (76-137)	0.21
LDLc (mg/dL)	115.1 (88.1-137.3)	106.4 (83.4-133.4)	118.7 (95.8-141.5)	0.009
HDLc (mg/dL)	41.8 (33.7-53.8)	42.3 (32.9-54.6)	41.3 (33.8-52.0)	0.66
TC (mg/dL)	185 (157-207)	179 (153.5-204.5)	187 (161-209)	0.10
Dyslipidemia	232 (70.3)	110 (68.8)	122 (71.8)	0.55

Data for each categorical variable are presented as n (%) with *P* values determined using Pearson's chi-square test (nominal variables) or the Jonckheere-Terpstra test (ordinal variables) or exact equivalents, where appropriate. Data for each continuous variable are presented as the median (interquartile range) with *P* values determined using a Wilcoxon rank sum test. Where the global *P* value is <0.05, *P* values were determined using Pearson's chi-square test with comparisons as follows.

*Each health insurance status category compared with other categories combined.

†Less than high school versus high school degree or more; high school degree versus more than high school degree; some college versus more than some college.

‡Each genotype compared with other categories combined.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HAI, histological activity index; INR, international normalized ratio; m, number with missing data; TIBC, total iron-binding capacity; WBC, white blood cell.

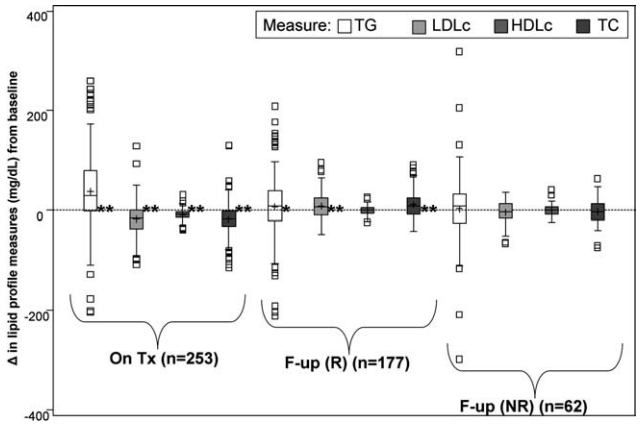

Fig. 1. Serum lipid profile changes during and after antiviral therapy. *$P < 0.05$, **$P < 0.0001$ (Wilcoxon signed rank test for differences from zero). Boxplots exclude extreme outliers for changes in TG levels: −768, −422, 477, 477, 562, 573, 629, and 1,678 mg/dL on treatment; 434 mg/dL during follow-up among 6-month virological responders. The median levels at baseline were: TG, 100 mg/dL; LDLc, 115.5 mg/dL; HDLc, 41.1; and TC, 183. F-up, assessment 24 weeks after stopping therapy; NR, treatment week 24 virological nonresponders (24-week course of therapy) at week 48; R, treatment week 24 virological responders (48-week course of therapy) at week 72.Tx, assessment at treatment week 24.

versus 0.07 mg/dL, $P = 0.004$), lower albumin levels (median 4.2 versus 4.2 g/dL, $P = 0.004$), and lower LDLc levels (median 106.4 versus 118.7 mg/dL, $P = 0.009$) than CAs. The prevalence of dyslipidemia was 70% overall and did not significantly differ by race.

Compared with pretreatment, there were significant changes in serum lipids during therapy and after completion of therapy (Fig. 1). During the initial 24 weeks of therapy, TG levels increased significantly (median +30 mg/dL), in contrast to significant declines in LDLc (−14.8 mg/dL), HDLc (median −8 mg/dL), and TC (median −17 mg/dL) ($P < 0.0001$ for all). After therapy, statistically significant changes in lipid measures were limited only to the 24-week virological responders. Among 177 participants who underwent a 48-week course of therapy (24-week virological responders), posttreatment TG levels remained significantly higher than pretreatment levels (median +8 mg/dL, $P = 0.03$), as did posttreatment LDLc (median +7.2 mg/dL, $P < 0.0001$) and TC levels (median +9 mg/dL, $P < 0.0001$), whereas HDLc levels did not significantly change (median +0.8 mg/dL, $P = 0.47$). Among 62 participants who underwent a 24-week course of therapy before stopping therapy (24-week virological nonresponders), there were no significant changes in posttreatment serum lipids compared with pretreatment levels (TG, median +9 mg/dL, $P = 0.41$; LDLc, median −3.2 mg/dL, $P = 0.36$; TC, median −3 mg/dL, $P = 0.50$; HDLc, +0.3 mg/dL, $P = 0.99$).

The proportion of PEG-IFN taken was significantly and directly associated with declines in LDLc ($r = -0.22$, $P = 0.005$) and TC levels ($r = -0.17$, $P = 0.008$) during the initial 24 weeks of therapy. The proportion of ribavirin taken was not significantly associated with any changes in serum lipid levels ($P > 0.05$ for all). Race was significantly associated with changes in serum lipids during the first 24 weeks of therapy. Compared with CAs, AAs had significantly greater increases in TG and declines in LDLc levels ($P = 0.003$ and $P < 0.0001$, respectively) (Fig. 2). The patterns of decreases in TC levels by race were similar to LDLc changes, although the differences were not statistically significant ($P = 0.054$).

Baseline characteristics associated with SVR are summarized in Table 2. CA race was significantly associated with higher SVR in unadjusted analyses (RR = 1.92, $P < 0.001$, AA reference). Features inversely related to SVR included education beyond high school (RR = 0.64, $P = 0.002$, less than high school degree reference), body weight (RR = 0.95 per 5 kg increase, $P = 0.01$), insulin resistance (RR = 0.63, $P = 0.003$, not insulin-resistant reference), the natural log of HOMA2 (RR = 0.77, $P < 0.001$), baseline $\log_{10}$ HCV level (RR = 0.77, $P < 0.001$), and more disease severity as measured by fibrosis (Ishak) score (RR = 0.90, $P = 0.02$). Additionally, platelet count (RR = 1.25 per 10^3 cells/mm^3 increase, $P = 0.01$) and amounts of PEG-IFN (RR = 1.41 per 10% increase, $P < 0.001$) and ribavirin (RR = 1.25 per 10% increase, $P < 0.001$) taken during the first 24 weeks of therapy were directly related to the rate of SVR. With regard to lipid levels, baseline TG (natural log scale) was inversely related (RR = 0.65, $P = 0.002$) and LDLc was directly related (RR = 1.05 per 10

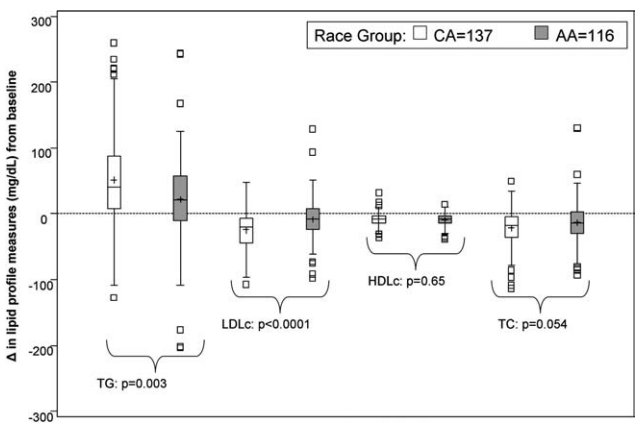

Fig. 2. Race and serum lipid profile changes during therapy. *P* values correspond to Wilcoxon rank sum tests for racial differences. Difference in lipid profile measures at treatment week 24 minus baseline.

Table 2. Univariate Models of SVR and Selected Predictors in Serum Lipid Assessment Subset

Feature	RR (95% CI)	*P* Value
CA race	1.92 (1.45-2.56)	<0.001
Male gender	0.79 (0.61-1.02)	0.07
Age*	0.96 (0.89-1.04)	0.31
Years infected	1.005 (0.99-1.02)	0.54
High school education or less	0.64 (0.48-0.85)	0.002
Weight (kg)*	0.95 (0.91-0.99)	0.01
BMI (kg/m^2)	0.98 (0.96-1.01)	0.23
History of diabetes	0.52 (0.27-1.01)	0.054
HOMA2†	0.77 (0.65-0.90)	<0.001
Insulin-resistant	0.63 (0.46-0.86)	0.003
History of hypertension	0.76 (0.56-1.04)	0.09
Current alcohol use	0.92 (0.66-1.27)	0.60
Current smoker	1.10 (0.85-1.43)	0.48
ALT (IU/L)‡	1.09 (0.94-1.25)	0.26
ALT (IU/L)†	1.08 (0.90-1.31)	0.40
AST (IU/L)‡	0.83 (0.61-1.10)	0.18
AST (IU/L)†	0.83 (0.66-1.04)	0.11
INR	1.43 (0.48-4.26)	0.52
Hemoglobin (g/dL)	0.95 (0.86-1.05)	0.30
WBC count (per 10^3 cells/mm^3)	1.05 (0.99-1.11)	0.12
Platelet count (per 10^5 cells/mm^3)	1.25 (1.06-1.49)	0.01
Genotype 1a versus non–genotype 1a	0.88 (0.68-1.13)	0.32
Baseline viral level (IU)§	0.77 (0.66-0.89)	<0.001
Ishak fibrosis score	0.90 (0.82-0.98)	0.02
Ishak fibrosis score ≥3	0.82 (0.62-1.08)	0.16
Cirrhosis (fibrosis score 5-6)	0.65 (0.34-1.24)	0.20
Steatosis score	0.85 (0.67-1.07)	0.17
Steatosis (>5% present)	0.866 (0.66-1.11)	0.24
HAI inflammation score	0.995 (0.95-1.05)	0.85
Proportion of PEG-IFN taken‖	1.41 (1.18-1.68)	<0.001
Proportion of ribavirin taken‖	1.25 (1.15-1.35)	<0.001
Lipid parameters¶		
TG† (mg/dL)	0.65 (0.49-0.86)	0.002
LDLc# (mg/dL)	1.05 (1.02-1.09)	0.002
HDLc§ (mg/dL)	0.96 (0.64-1.44)	0.84
TC§ (mg/dL)	1.59 (0.84-3.01)	0.15

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HAI, histological activity index; INR, international normalized ratio; WBC, white blood cell.

*Per 5-unit increase.

†Natural log transformed.

‡Per 100-unit increase.

§Log10 transformed.

‖Per 10% increase in dose.

¶Eligible for entry in multivariable model 2.

#Per 10-unit increase.

mg/dL increase, $P = 0.002$) to the rate of SVR. Baseline HDLc and TC levels were not significantly associated with SVR.

The relationships between the probability of SVR and baseline and changes in TG, LDLc, HDLc, and TC and during 24 weeks of therapy are shown in Supporting Information Fig. 1. Although the probability of SVR was negatively associated with baseline TG, it was positively related to increases in TG during therapy. On the other hand, the probability of SVR was positively associated with baseline LDLc, but negatively associated with increases in LDLc from baseline during 24 weeks of therapy. Among males, HDLc appeared to be inversely related to SVR rates (Supporting Information Fig. 1C), whereas in females the relationship was opposite (Supporting Information Fig. 1D). The probability of SVR based on baseline and on-treatment changes in TC levels revealed similar patterns as LDLc.

In crude and race-adjusted regression models, the relationships between variables representing the changes in lipid profile measures (both during and after therapy) and the rate of SVR are summarized in Table 3. Adjusting for race, SVR rates were directly and significantly associated with increases in TG (natural log scale; RR = 1.29, $P = 0.02$) and declines in LDLc (RR = 0.97, $P = 0.02$, per 5 mg/dL increase) during 24 weeks of therapy, compared with pretreatment. Posttreatment changes from pretreatment values in both LDLc (RR = 1.04, $P = 0.001$, per 5 mg/dL increase) and TC (natural log scale; RR = 4.10, $P < 0.001$) were directly and significantly related to the rate of SVR.

The multivariable model reported by Conjeevaram et al.[4] based on 400 participants was fit for the 329 participants for whom serum lipid and covariate data were available (Table 4). In model 1, factors significantly associated with SVR included male gender (RR = 0.80, $P = 0.049$), Ishak fibrosis score (RR = 0.90, $P = 0.009$), and the amount of PEG-IFN taken during the first 24 weeks (RR = 1.38, $P < 0.001$ per 10% dose increase). In addition, there was a significant interaction between race and baseline viral level ($P = 0.005$), indicating that the magnitude of the inverse relationship between viral level and the rate of SVR differed by race, which was documented and described graphically in a previous report based on the Virahep-C cohort.[4] Using the same eligible predictors as model 1 and also allowing the baseline lipid profile variables to be eligible for entry, model 2 was created. In model 2, a significant interaction between HDLc and gender ($P = 0.02$) was found. Assessed graphically in Fig. 3A for an AA male and female adjusting for other parameters in the model, whereas HDLc (natural log scale) was inversely related to the rate of SVR for males, the relationship was direct among females. Additional multivariable analyses on a sample of 307 patients with available insulin resistance data did not indicate a significant relationship between insulin resistance measures and SVR, accounting for other parameters in model 2 (data not shown). The prediction of SVR did not significantly differ between models 1 and 2 (AUROC = 0.801 versus 0.811, respectively, $P = 0.42$) (Fig. 4A).

Table 3. Evaluation of Lipid Profile Measure Changes During and After Therapy as Predictors of SVR

	SVR			
	Unadjusted		Race-Adjusted	
Lipid Profile Measure	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value
Δ: On-treatment – baseline (n = 253)				
TG* (mg/dL)	1.43 (1.15-1.78)	0.001	1.29 (1.05-1.59)	0.02
LDLc† (mg/dL)	0.96 (0.92-0.98)	<0.001	0.97 (0.95-0.995)	0.02
HDLc* (mg/dL)	1.13 (0.63-2.02)	0.68	1.13 (0.65-1.96)	0.66
TC* (mg/dL)	0.49 (0.23-1.08)	0.08	0.64 (0.30-1.35)	0.24
Δ: Follow-up – baseline (n = 245)				
TG* (mg/dL)	1.29 (0.98-1.70)	0.07	1.24 (0.97-1.61)	0.09
LDLc† (mg/dL)	1.04 (1.02-1.06)	0.001	1.04 (1.01-1.06)	0.001
HDLc* (mg/dL)	0.94 (0.54-1.66)	0.84	0.92 (0.54-1.58)	0.78
TC* (mg/dL)	4.64 (2.46-8.76)	<0.001	4.10 (2.14-7.85)	<0.001

Abbreviation: CI, confidence interval.
*Natural log transformed.
†Per 5 mg/dL change.

In multivariable modeling, model 3 was constructed using 250 participants who had covariate, baseline lipid profile, and treatment week 24 lipid profile data (Table 3). Model 3 evaluated the relationships between SVR and lipid profile changes during therapy along with those variables eligible for entry into model 2. Variables in models 2 and 3 were similar, though the baseline TG (natural log scale) and LDLc levels were not significantly associated with SVR in model 3, whereas the change in LDLc during the first 24 weeks was retained. Similar to model 2, there was a significant interaction ($P = 0.009$) in model 3 between HDLc (natural log scale) and gender, a relationship that was inverse among males and direct among females (Fig. 3B). The AUROC for model 3 was not significantly different than that of model 1 fit to the same 250 participants (AUROC = 0.799 versus 0.779, $P = 0.19$) (Fig. 4B). In separate multivariable assessments based on a patient subsample with available insulin resistance data (n = 231) and accounting for parameters in model 3, insulin-resistant status as a dichotomous measure, but not HOMA2 as a continuous measure, was significantly associated with SVR (data not shown). In all three multivariable models, race remained a significant predictor of SVR, and the magnitude of the association changed little with the addition of serum lipid measures.

Discussion

This evaluation of serum lipids and virological response showed a relationship between baseline lipid

Table 4. Multivariate Models of SVR and Selected Predictors

	Model 1		Model 2		Model 3	
Feature	RR (95% CI)	*P* Value	RR (95%CI)	*P* Value	RR (95%CI)	*P* Value
CA race	1.98 (1.47-2.67)	<0.001	1.81 (1.33-2.45)	<0.001	2.28 (1.58-3.30)	<0.001
Male gender	0.80 (0.64-0.999)	0.049	0.81 (0.62-1.07)	0.14	0.96 (0.72-1.27)	0.76
Baseline viral level (IU)* and CA race interaction	1.51 (1.13-2.00)	0.005	1.49 (1.12-1.97)	0.006	1.72 (1.23-2.41)	0.001
Ishak fibrosis score	0.90 (0.83-0.97)	0.009	0.91 (0.83-0.98)	0.02	0.90 (0.83-0.98)	0.02
Amount of PEG-IFN taken†	1.38 (1.18-1.62)	<0.001	1.37 (1.18-1.60)	<0.001	0.98 (0.85-1.12)	0.77
Lipid parameters						
TG‡ (mg/dL)			0.69 (0.52-0.91)	0.009		
HDLc‡ (mg/dL) and male gender interaction			0.40 (0.18-0.87)	0.02	0.36 (0.16-0.77)	0.009
LDLc§ (mg/dL)			1.03 (1.001-1.07)	0.046		
ΔLDLc§ (mg/dL)					0.97 (0.95-0.996)	0.02

Model 1 is a replication of the Conjeevaram et al.[4] model on a subset of participants with available baseline serum lipid data (n = 330). Model 2 includes baseline serum lipid variables as eligible for entry. Model 3 is a multivariate model with baseline and on-treatment changes from baseline in serum lipid variables as eligible for entry (n = 253). This model also adjusts for changes in body weight (data not shown).
Abbreviation: CI, confidence interval.
*Log10 transformed.
†Per 10% increase in dose.
‡Natural log transformed.
§Per 5-unit increase.

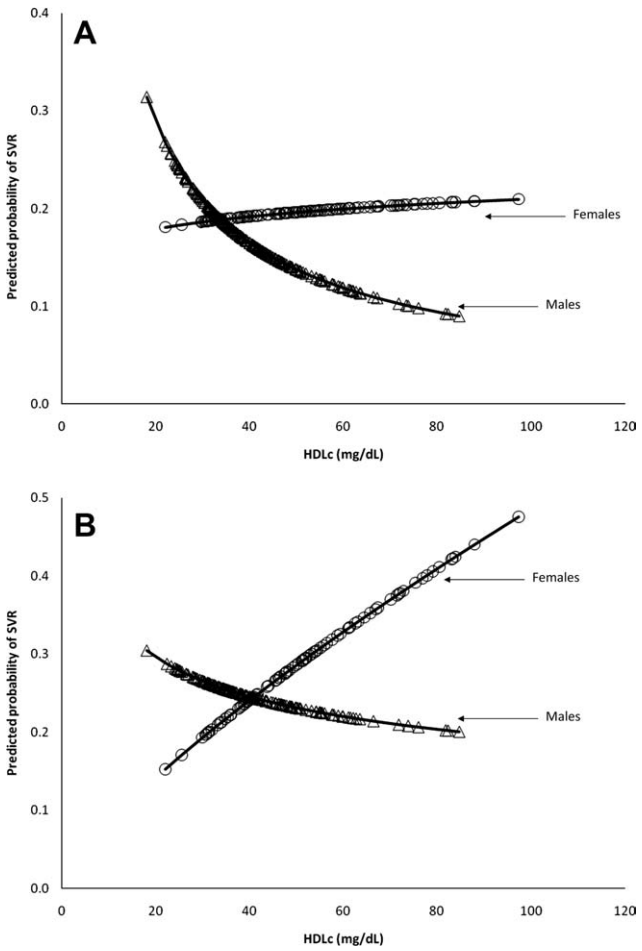

Fig. 3. (A) Predicted probabilities of SVR and HDLc by gender from model 2. Predicted probabilities were calculated adjusting for other parameters in the model for an individual with the following characteristics: AA, Ishak fibrosis score of 2, took 80% of the prescribed PEG-IFN during 24 weeks of therapy, and a mean baseline viral level of 6.3 $\log_{10}$ IU/mL, natural log of TG of 4.7 mg/dL, and LDLc level of 116.2 mg/dL. (B) Predicted probabilities of SVR and HDLc by gender from model 3. Predicted probabilities were calculated adjusting for other parameters in the model for an individual with the following characteristics: AA, Ishak fibrosis score of 2, took 80% of the prescribed PEG-IFN during 24 weeks of therapy, mean baseline viral level of 6.3 $\log_{10}$ IU/mL, and on-treatment change in LDLc level from pretreatment of −16.8 mg/dL.

measurements and on-treatment changes in lipids with antiviral response to therapy. Pretreatment TG and LDLc levels were inversely and directly related to the SVR rate, respectively. Baseline LDLc was significantly higher in CAs compared with AAs. Furthermore, changes in these two parameters during the first 24 weeks of therapy were associated with virological response and differed significantly by race. In regression models, several lipid profile parameters at baseline (TG and LDLc, HDLc [with an interaction with gender]) and on-treatment changes (TG and LDLc) were significant predictors of SVR. However, including serum lipid measures did not significantly improve the prediction of SVR compared with models without these measures, nor did serum lipid measures account for the racial difference in treatment efficacy between CAs and AAs.

Few studies have assessed in detail changes in serum lipids during and after therapy for chronic HCV

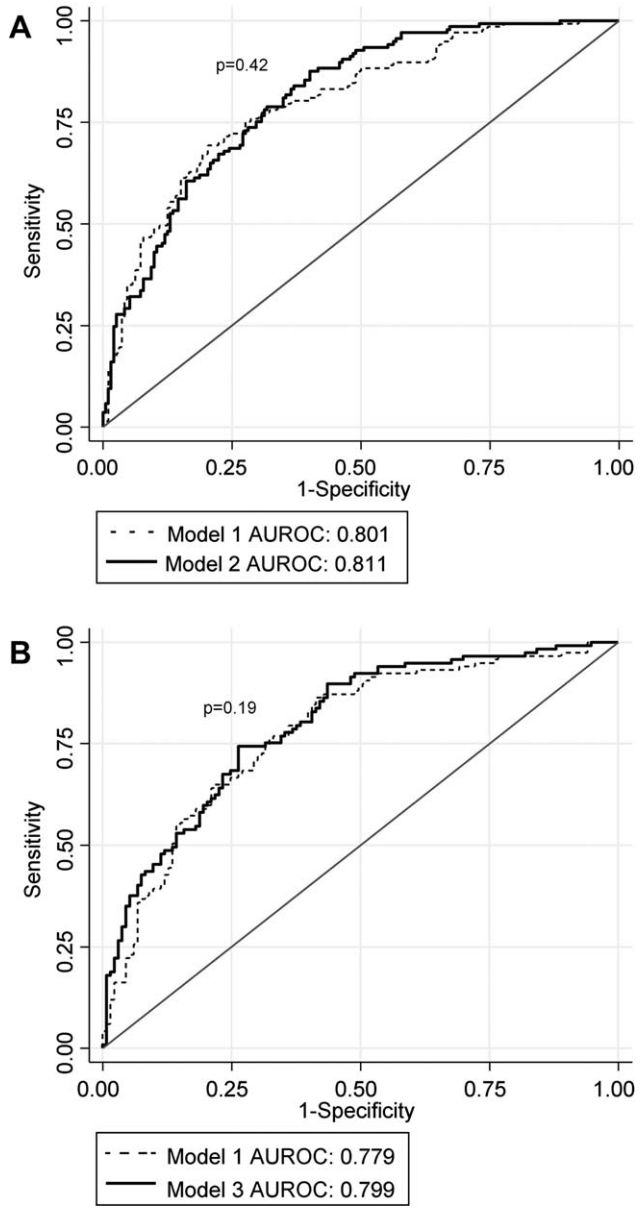

Fig. 4. (A) Receiver operator curves of multivariable models 1 and 2 (n = 329). Model 1 includes race, gender, baseline viral level, baseline viral level and race interaction, Ishak fibrosis score, and amount of PEG-IFN taken. Model 2 includes variables in model 1 plus baseline TG, baseline HDLc, baseline HDLc and gender interaction, and baseline LDLc. (B) Receiver operator curves of multivariable models 1 and 3. Model 1 includes race, gender, baseline viral level, baseline viral level and race interaction, Ishak fibrosis score, and amount of PEG-IFN taken. Model 3 includes variables in model 1, body weight change, baseline HDLc, baseline HDLc and gender interaction, and change in LDLc during 24 weeks of therapy.

infection. Compared with pretreatment, the significant increase in TG levels during therapy found here is consistent with findings in other studies that reported mean increases of 45 mg/dL and 60 mg/dL in TG levels.[8,9] We note that compared with pretreatment, these studies did not report significant changes in TC during or after therapy, whereas our study found significant declines in TC during treatment.[8,9] However, the significant increase in TC levels posttreatment compared with pretreatment is consistent with one study that reported an approximate 10.5 mg/dL significant mean increase,[7] whereas another study did not report significant changes.[8] The difference in findings across studies may be due to variable sample sizes, disparate treatment regimens, inclusion of patients with different HCV genotypes, and other participant characteristics. The direct relationship between pretreatment LDLc levels and SVR rate is consistent with findings from several other studies.[10-14]

Hamamoto et al.[7] reported an association between higher pretreatment TG levels and virological response, which is opposite of the relationship in our study, possibly a reflection of HCV genotype or host lipid receptor genetic differences. Whereas only individuals infected with HCV genotype 1 were included in Virahep-C, genotype 2 was the predominant genotype in the previously referenced study. Single-nucleotide polymorphisms in the receptors involved in the serum lipoprotein particle uptake into hepatocytes (SR-B1 and LDL receptors) may also account for the different relationships observed in the two study populations. In multivariable analyses, significant interactions between HDLc levels and gender in relation to virological response were found, which have not been previously reported. These relationships warrant further investigation and validation in other cohorts to clarify whether lipid profile measures are important predictors of treatment response. Posttreatment increases from baseline in LDLc and TC were found to be associated with SVR, which may correspond to HCV eradication and the subsequent resolution of HCV-induced liver damage.

With evidence from *in vitro* work supporting several possible mechanisms involving serum lipoproteins, cholesterol metabolism, lipoprotein receptors, and HCV entry, replication and secretion, the significant relationships between both baseline and changes from baseline LDLc and TG levels and rates of SVR are biologically feasible.[15-22,26-30] The direct relationship between LDLc and SVR may partially be explained by competition for LDL receptor sites preventing viral entry into hepatocytes, increasing exposure of HCV to the host immune response in the serum. These findings suggest that serum lipids may yield some prognostic value in determining the probability of treatment success and possibly highlight new therapeutic targets. Further prospective investigation of the impacts of dietary modification and lipid-lowering agents on virological response may be warranted. Treatment trials investigating statins and fibrates to improve virological response have yielded mixed results.[40-42] As documented in the Virahep-C cohort,[43] insulin resistance may also contribute to the relationship between serum lipids and SVR.

In conclusion, this study suggests that serum lipid measures are predictors of SVR, but that their predictive ability is ameliorated by race such that these measures do not explain the racial disparity in treatment efficacy between CAs and AAs. However, this study underscores the potential relevance of serum lipids to virological response. Future investigations may seek to assess relationships between SVR, other characterizations relevant to serum lipids, and genetic determinants of lipid metabolism.

References

1. Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006;144:705-714.
2. Kim WR. The burden of hepatitis C in the United States. HEPATOLOGY 2002;36:S30-S34.
3. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.
4. Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, et al. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 2006;131:470-477.
5. Jeffers LJ, Cassidy W, Howell CD, Hu S, Reddy KR. Peginterferon alfa-2a (40 kd) and ribavirin for black American patients with chronic HCV genotype 1. HEPATOLOGY 2004;39:1702-1708.
6. Muir AJ, Bornstein JD, Killenberg PG. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. N Engl J Med 2004;350:2265-2271.
7. Hamamoto S, Uchida Y, Wada T, Moritani M, Sato S, Hamamoto N, et al. Changes in serum lipid concentrations in patients with chronic hepatitis C virus positive hepatitis responsive or non-responsive to interferon therapy. J Gastroenterol Hepatol 2005;20:204-208.
8. Naeem M, Bacon BR, Mistry B, Britton RS, Di Bisceglie AM. Changes in serum lipoprotein profile during interferon therapy in chronic hepatitis C. Am J Gastroenterol 2001;96:2468-2472.
9. Fernandez-Rodriguez CM, Lopez-Serrano P, Alonso S, Gutierrez ML, Lledo JL, Perez-Calle JL, et al. Aliment Pharmacol Ther 2006;24: 507-512.
10. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403-410.
11. Backus LI, Boothroyd DB, Phillips BR, Mole LA. Predictors of response of US veterans to treatment for the hepatitis C virus. HEPATOLOGY 2007;46:37-47.

12. Economou M, Milionis H, Filis S, Baltayiannis G, Christou L, Elisaf M, et al. Baseline cholesterol is associated with the response to antiviral therapy in chronic hepatitis C. J Gastroenterol Hepatol 2008;23: 586-591.
13. Gopal K, Johnson TC, Gopal S, Walfish A, Bang CT, Suwandhi P, et al. Correlation between beta-lipoprotein levels and outcome of hepatitis C treatment. HEPATOLOGY 2006;44:335-340.
14. Martinez-Bauer E, Crespo J, Romero-Gomez M, Moreno-Otero R, Sola R, Tesei N, et al. Development and validation of two models for early prediction of response to therapy in genotype 1 chronic hepatitis C. HEPATOLOGY 2006;43:72-80.
15. Andre P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, et al. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol 2002;76:6919-6928.
16. Andre P, Perlemuter G, Budkowska A, Brechot C, Lotteau V. Hepatitis C virus particles and lipoprotein metabolism. Semin Liver Dis 2005; 25:93-104.
17. Dreux M, Cosset FL. HCV and lipoproteins: is oxLDL an Achilles' heel of the Trojan horse? HEPATOLOGY 2006;43:903-905.
18. Monazahian M, Kippenberger S, Muller A, Seitz H, Bohme I, Grethe S, et al. Binding of human lipoproteins (low, very low, high density lipoproteins) to recombinant envelope proteins of hepatitis C virus. Med Microbiol Immunol 2000;188:177-184.
19. Carriere M, Rosenberg AR, Conti F, Chouzenoux S, Terris B, Sogni P, et al. Low density lipoprotein receptor transcripts correlates with liver hepatitis C virus RNA in patients with alcohol consumption. J Viral Hepat 2006;13:633-642.
20. Molina S, Castet V, Fournier-Wirth C, Pichard-Garcia L, Avner R, Harats D, et al. The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. J Hepatol 2007;46:411-419.
21. Monazahian M, Bohme I, Bonk S, Koch A, Scholz C, Grethe S, et al. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999;57:223-229.
22. Petit JM, Minello A, Duvillard L, Jooste V, Monier S, Texier V, et al. Cell surface expression of LDL receptor in chronic hepatitis C: correlation with viral load. Am J Physiol Endocrinol Metab 2007;293: E416-E420.
23. Kapadia SB, Barth H, Baumert T, McKeating JA, Chisari FV. Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. J Virol 2007;81: 374-383.
24. Molina S, Castet V, Pichard-Garcia L, Wychowski C, Meurs E, Pascussi JM, et al. Serum-derived hepatitis C virus infection of primary human hepatocytes is tetraspanin CD81 dependent. J Virol 2008;82:569-574.
25. von Hahn T, Lindenbach BD, Boullier A, Quehenberger O, Paulson M, Rice CM, et al. Oxidized low-density lipoprotein inhibits hepatitis C virus cell entry in human hepatoma cells. HEPATOLOGY 2006;43:932-942.
26. Gastaminza P, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV. Cellular determinants of hepatitis C virus assembly, maturation, degradation and secretion. J Virol 2008;82:2120-2129.
27. Huang H, Sun F, Owen DM, Li W, Chen Y, Gale M Jr, et al. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. Proc Natl Acad Sci USA 2007;104:5848-5853.
28. Nielsen SU, Bassendine MF, Burt AD, Martin C, Pumeechockchai W, Toms GL. Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients. J Virol 2006;80:2418-2428.
29. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, 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.
30. Ye J. Reliance of host cholesterol metabolic pathways for the life cycle of hepatitis C virus. PLoS Pathog 2007;3:e108.
31. Murao K, Imachi H, Yu X, Cao WM, Nishiuchi T, Chen K, et al. Interferon alpha decreases expression of human scavenger receptor class BI, a possible HCV receptor in hepatocytes. Gut 2008;57:664-671.
32. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
33. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.
34. Diabetes Trials Unit. A software implementation of the HOMA2 model. Edition 2.2. Oxford: University of Oxford; 2004.
35. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. HEPATOLOGY 1981;1:431-435.
36. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22:696-699.
37. Kruse W, Rampmaier J, Ullrich G, Weber E. Patterns of drug compliance with medications to be taken once and twice daily assessed by continuous electronic monitoring in primary care. Int J Clin Pharmacol Ther 1994;32:452-457.
38. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702-706.
39. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837-845.
40. Bader T, Fazili J, Madhoun M, Aston C, Hughes D, Rizvi S, et al. Fluvastatin inhibits hepatitis C replication in humans. Am J Gastroenterol 2008;103:1383-1389.
41. Fujita N, Kaito M, Kai M, Sugimoto R, Tanaka H, Horiike S, et al. Effects of bezafibrate in patients with chronic hepatitis C virus infection: combination with interferon and ribavirin. J Viral Hepat 2006; 13:441-448.
42. O'Leary JG, Chan JL, McMahon CM, Chung RT. Atorvastatin does not exhibit antiviral activity against HCV at conventional doses: a pilot clinical trial. HEPATOLOGY 2007;45:895-898.
43. Conjeevaram HS, Kleiner DE, Everhart JE, Hoofnagle JH, Zacks S, Afdhal NH, et al. Race, insulin resistance and hepatic steatosis in chronic hepatitis C. HEPATOLOGY 2007;45:80-87.