ASPECTS OF THE LIPID PROFILE IN A COHORT WITH CHRONIC HEPATITIS C INFECTION

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BACKGROUND: In the United States, chronic hepatitis C virus (HCV) infection afflicts approximately 3.2 million persons and is the leading indication of liver transplantation. Therapies for chronic HCV are not completely effective and African Americans are significantly less responsive to therapy than Caucasian Americans. Studies suggest that lipoproteins and cholesterol metabolism play a role in biological mechanisms of the HCV life cycle. This dissertation characterizes the serum lipid profile in a cohort with genotype 1 chronic HCV infection, the predominant HCV genotype in the United States. **STUDY POPULATION:** Participants for this study were from the Virahep-C study, a prospective study of resistance to antiviral therapy involving 401 treatment naïve people with chronic hepatitis C (genotype 1) infection who underwent combination pegylated interferon alfa-2a + ribavirin therapy for up to 48 weeks. Included in this dissertation analysis were 330 participants who had serum lipid profile data before starting therapy. **RESULTS:** Before treatment, HCV viral level was directly associated with triglyceride levels, liver fat was directly and inversely related to triglycerides and high density lipoprotein cholesterol, respectively, and severe fibrosis was associated with lower of total and high density lipoprotein cholesterol levels. Over the course of therapy, all lipid profile measures changed during 6 months of treatment, and post-treatment, changes were limited to 6 month virological responders. For some lipid profile measures, changes during 6 months of treatment differed by race and were related to the amount of interferon taken. Lastly,

components of the lipid profile were significant predictors of sustained virological response in univariable and multivariable analyses. **CONCLUSIONS:** This dissertation highlights the importance of the lipid profile in relation to aspects of liver disease, potential mechanisms of HCV eradication attributed to antiviral therapy, and virological response to therapy. The findings are of public health significance as they may highlight opportunities for new therapeutic targets and intervention studies to improve virological response, as well as elucidate factors involved in the racial disparity in treatment efficacy.

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PREFACE

This dissertation project afforded me the unique opportunity to examine the relevance of the lipid profile to chronic HCV infection, a new area of focus in HCV research. The implementation of the three projects has been a rewarding experience and has stimulated more questions than answers, which may guide further studies. To my committee, thank you for your guidance and mentoring throughout this process. I have learned a great deal about epidemiology and am grateful for the outstanding training that I have received here in the Department of Epidemiology. To my family, friends, and especially my wife, without your support none of this would have been possible.

1.0   GENERAL BACKGROUND

1.1   THE HEPATITIS C VIRUS

1.1.1   Epidemiology

The hepatitis C virus (HCV) is a viral capsid-enclosed RNA virus of the *Flaviviridae* family in the *Hepacivirus* genus.[1] Six genotypes comprise HCV with varying predominance according to geography: genotype 1 predominates in the United States (US) and Northern Europe; genotype 2 in the Mediterranean region and the Far East; genotype 3 in Europe; genotypes 4a and 5a in South Africa; and genotype 6 in Hong Kong, Vietnam, and Australia.[2] The US prevalence of HCV in the non-institutionalized population is an estimated 1.6% (4.1 million persons) for HCV exposure, and 1.3% (3.2 million persons) for chronic infection.[3] A report from US correctional facilities suggests that the prevalence of HCV infection may be as high as 29% to 43% in institutionalized inmate populations.[4]

In the US, the primary mode of HCV transmission is parenteral and the main risk factors include injection drug use or receipt of a blood transfusion prior to 1991, the year when HCV screening of the blood supply was implemented. Although sexual intercourse and vertical transmission are other means of transmission, HCV transmission by these modes is believed to

be rare. Other risk factors of infection include exposure to contaminated blood products, needle-stick among health care workers, and tattooing.[5]

After initial exposure to HCV, the disease course varies and 55% to 85% develop chronic infection.[1] Factors associated with higher rates of HCV chronicity include younger age at infection, male gender, African American (AA) race, lack of a jaundice reaction early in the infection, and immune deficiency. During the early course of infection, levels of the liver enzyme alanine aminotransferase (ALT) initially increase, then decline and fluctuate thereafter.[6] Among chronically active HCV infected people, the disease course may progress with liver damage leading to cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC).[5] Aside from irregular fatigue, the majority of people with chronic hepatitis C infection are asymptomatic.[7]

1.1.2   Treatment

The current standard antiviral regimen for chronic HCV consists of combination pegylated interferon (PEG-IFN) alfa-2a and ribavirin for up to 48 weeks.[8] Antiviral therapy has several common side-effects, including anemia, neutropenia, fatigue, headache, pyrexia, myalgia, rigors, insomnia, nausea, alopecia, irritability, arthralgia, anorexia, dermatitis, and depression.[9] Management strategies for side-effects include dose reductions of ribavirin and PEG-IFN and in some cases treatment discontinuation is necessary.[9]

The efficacy of therapy differs by genotype. Rates of sustained viral response (SVR), defined as undetectable levels of HCV RNA 24 weeks after treatment cessation, are higher for genotypes 2, 3, and 4 (76% to 77%) than genotype 1 (46%).[8] A racial disparity in treatment efficacy exists in genotype 1 infected populations with AAs having lower rates of SVR (19% to

28%) compared to Caucasian Americans (CAs) (39% to 52%).[10-12] Factors that explain the racial difference in efficacy are unknown.[10]

1.1.3   Chronic HCV infection and liver disease

Chronic HCV infection is involved in the progression of specific forms of liver disease by the host immune response activation against hepatocellular tissue.[6] Although highly variable, the general sequelae of liver damage is hepatic inflammation, followed by either scar tissue formation (referred to as hepatic fibrosis) or lipid droplet accumulation (referred to as fatty liver disease, steatosis, or hepatosteatosis) which may lead to liver cirrhosis, end-stage liver disease, and an increased risk of HCC. Host etiological factors unrelated to HCV infection may be involved in liver disease and include poor nutrient intake, medication, alcohol consumption, and diabetes. Fibrosis and steatosis may precede chronic HCV infection, suggesting liver disease progression due to host etiology, or occur with chronic infection, indicating the involvement of HCV as a contributing or causative agent of liver disease. Hepatic iron deposition and iron overload also occur among individuals with chronic HCV infection have been associated with poor prognosis for treatment efficacy and liver disease progression; however, the biological role of serum iron and iron deposition remains unclear in the context of HCV infection.[13-15]

1.1.4   Liver disease measures

Noninvasive non-biopsy methods of assessing fibrosis and steatosis using ultrasound imaging techniques are available and are generally adequate to detect fibrosis and moderate to severe steatosis.[16-18] However, to quantify the extent of liver damage, percutaneous liver biopsy

is considered to be the gold standard. The Histological Activity Index (HAI) scoring system, also known as the Knodell score, was proposed in 1981 to provide a consistent and clinically validated measure with high intraobserver and interobserver agreement.[19] Since its inception, the HAI scoring system has been modified to include of four aspects of liver disease: periportal bridging; intralobular degeneration and focal necrosis; portal inflammation; and fibrosis. Compared to the original HAI, the Modified HAI system includes additional scoring criteria within each aspect of liver disease. Excluding the fibrosis score, the modified HAI scores are summed to create an overall measure of hepatic inflammation, also known as the Ishak necroinflammatory score.[20] Another scoring system is the METAVIR scale, which assesses the same aspects of liver disease as the Modified HAI system, though using different criteria.[21] In both the Modified HAI and METAVIR scales, cirrhosis is defined by higher scores within the fibrosis assessment. Within individuals with cirrhosis, hepatic function is graded by the Child-Pugh score (or the Child-Pugh-Turcotte modification) comprised of five non-biopsy measures, including total bilirubin, serum albumin, coagulopathy, ascites, and hepatic encephalopathy.[22] The percent fat involvement of hepatocytes assessed by liver biopsy interpretation is used to define steatosis and assign a fat score. Finally, an iron score ranging from 0 to 4 is assigned according to the amount of iron deposition in hepatocellular tissue.[22]

1.1.5 Public health implications of chronic HCV

In the US, chronic HCV infection-mediated end-stage liver disease has been implicated in the majority of adult liver transplants.[23] The estimated annual direct health care cost of HCV, including liver transplantation, is an estimated $1.8 billion and a 4-fold increase in the number of

persons with chronic HCV infections is projected to occur between 1990 and 2015.[24, 25] These statistics underscore the significant burden to the US health care system that is associated with chronic HCV infection.

Treatments to successfully cure the disease may not only halt or reverse the progression of liver disease caused by chronic HCV infection, but also reduce the potential for transmission.[5] From a public health perspective, primary prevention strategies to reduce the spread of infection include maintaining an adequately screened blood supply, using sterile percutaneous instruments for medical procedures or tattooing, and needle exchange programs for injection drug user populations. In addition, provision of substance abuse treatment for those with addiction may be another primary prevention strategy to reduce new exposures to HCV through injection drug use. For those with undiagnosed chronic HCV infection, targeted screening of at risk populations is a secondary prevention strategy. Screening programs may target high risk populations for HCV, such as inmate populations, which have been shown to have rates of HCV exposure varying between 29% to 43%.[4] Tertiary prevention strategies for chronic HCV currently include combination antiviral therapy, the efficacy of which varies depending on genotype, and demographic characteristics, such as gender and race.[8, 10-12] In HCV genotype 1, lower rates of virological response to treatment among AAs compared to CAs may also impact tertiary prevention approaches. Given the lower chance of treatment response, infected AA individuals may be less willing to undergo antiviral therapy than CAs.

More effective treatment approaches to chronic HCV may not only reduce the health care costs associated with antiviral therapy, allowing resources to be refocused on other public health problems, but also eradicate this chronic infectious disease from the human blood reservoir, reducing the potential for transmission. Moreover, effective treatment carries the potential to

improve the health-related quality of life for those with chronic HCV infection, which has been shown to improve among those who respond to treatment.[26] Thus, the public health implications of chronic HCV are significant and research to improve treatment approaches, as well as reduce transmission of infection is warranted.

1.2   THE LIPID PROFILE

1.2.1   Components and metabolic pathways

Serum lipoproteins are classified according to size, density, and apolipoprotein composition. By increasing size and decreasing density, lipoprotein classes consist of high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and chylomicrons. The density of each lipoprotein is primarily dictated by the amount of triglyceride (TG), a high energy store. Whereas HDL is associated with the structural proteins apolipoprotein A-I and A-II, apolipoprotein B-100 comprises VLDL, IDL, LDL, and apolipoprotein B-48 is the construct of chylomicrons. Cardiovascular disease (CVD) risk is inversely associated with serum concentrations of HDL-cholesterol (HDLc), and directly related to VLDL-cholesterol (VLDLc), LDL-cholesterol (LDLc), total cholesterol (TC), and TG.[27]

Lipoprotein metabolism includes both exogenous and endogenous pathways. In the exogenous pathway, dietary fat enters the serum from the small intestine in the form of chylomicrons comprised of large amounts of TG. Lipoprotein lipase removes TG for energy or storage and chylomicron remnants are removed from the serum by the liver. Cholesterol from

chylomicron remnants is extracted for further metabolism, incorporation into cellular membranes, or excretion in bile into the intestinal tract. The endogenous pathway involves hepatic TG secretion from the liver into the serum as TG rich VLDL particles. Similar to the exogenous pathway, the lipoprotein lipase enzyme removes TG for energy metabolism, modifying VLDL particles to IDL and LDL. Cholesterol is then either returned to the liver via LDL binding to the LDL receptor or is brought to peripheral tissue for incorporation into cellular membranes. In the serum, LDL may bind and deposit cholesterol, including within arterial walls, promoting atherosclerosis through the development of plaque.[6] In a reverse process, HDL removes cholesterol from the periphery and is taken up by the liver via scavenger receptor-mediated entry. Excess cholesterol may be excreted from the liver into the digestive tract as bile.[27]

1.2.2   Methods of assessment

Although there are numerous methods to assess lipid profile concentrations, the standard approach is to estimate TC, TG, and HDLc by direct methods and to calculate LDLc indirectly using the Friedewald equation when TG is less than 400 mg/dL (LDLc = TC – VLDLc – HDLc) where VLDLc = TG / 5.[27, 28]   When TG is at least 400 mg/dL, LDLc is estimated by direct methods. Reference samples (e.g., from the Centers for Disease Control and Prevention (CDC)) are used in laboratories that conduct routine lipid profile analyses for quality assessment and control purposes.[27]

2.0   INTRODUCTION

2.1   BIOLOGICAL MECHANISMS OF THE HCV LIFE CYCLE

Two major biological mechanism themes are apparent from *in vitro* and animal model studies: a mechanism of receptor mediated HCV entry from the serum into hepatocytes and the reliance on cholesterol and lipoprotein metabolic pathways for HCV replication and secretion. However, findings from non-epidemiologic data are varied and often contradictory, which may be attributed to the numerous HCV experimental particles and tissue systems used to simulate HCV infectivity.[29]

2.1.1   HCV receptor-mediated entry into hepatocytes

Studies suggest an association of HCV with lipoproteins in the serum in the form of lipoprotein-virus conglomerate (LVC) particles consisting of chylomicrons, VLDL, LDL, TG, and apolipoproteins B and E.[30, 31] Electron microscopy work further indicates that HCV capsids reside within the core of the LVC particle, a possible means of being masked from the host immune response.[30] Other work suggests that HCV may bind in a more specific manner with lipoproteins to form lipoprotein-viral particles with LDL and VLDL, LDL, or HDL.[32, 33] Various receptors involved in lipoprotein-viral particle entry into hepatocytes have been proposed, including the scavenger receptor B1 (SR-B1) and LDL receptor.[34-37] The LDL receptor

mechanism of entry, however, is controversial.[38, 39] Direct entry of free HCV (not associated with lipoproteins) may also occur through binding with SR-B1 or CD81.[40-42]

2.1.2 HCV replication and secretion

Based on *in vitro* work, HCV replication has been proposed to occur at the hepatocyte endoplasmic reticulum (ER), dependent on cholesterol metabolism.[29, 43-46] HCV secretion from the hepatocyte into the serum may also involve the metabolic pathway of VLDL resulting in the secretion of VLDL-HCV complexed particles. In contrast, other work using transgenic mouse models suggests that VLDL secretion may be disrupted by an interaction of HCV core with microsomal triglyceride transfer protein inhibiting viral secretion and resulting in intrahepatic TG accumulation, a possible mechanism of HCV-induced steatosis.[46] Reasons for different proposed mechanisms of HCV secretion from *in vitro* and animal studies are unclear.

2.2 LITERATURE REVIEW OF EPIDEMIOLOGIC STUDIES: CHRONIC HCV INFECTION, LIVER DISEASE, AND THE LIPID PROFILE

Compared to non-HCV infected control participants, studies have shown that those with chronic HCV infection tend to have lower TC, LDLc, HDLc, or TG levels [47-50], although one study found higher TG levels.[51] These findings suggest that there may be an association between chronic HCV infection and CVD risk. Comparing lipid profile measures, Marzouk et al. suggested that a "favorable" CVD risk profile is associated with chronic HCV infection based on findings of

significantly lower LDLc and TG levels in chronically infected people versus a non-HCV exposed group.[48]

The impact of antiviral HCV therapy on the lipid profile has also been investigated. A study of people treated with non-PEG-IFN found increasing TC and TG during antiviral therapy.[52] Post-therapy, TC levels continued to increase whereas TG returned to pre-treatment levels. Another study found that during mono- or combination therapy of either non-PEG-IFN or PEG-IFN, TG levels increased, though TC levels remained the same.[53] Changes in the lipid profile during antiviral therapy may differ according to HCV genotype. One study found significant increases in TC during and after therapy compared to baseline in a subgroup with HCV genotype 3, but not genotype 1, with post-treatment increases in TC only observed among genotype 3 sustained virological responders.[54] TG levels have been reported to increase during therapy in an exclusively genotype 1 infected cohort.[53]

The lipid profile in both HCV infected and non-infected cohorts has been associated with liver disease measures including steatosis and fibrosis.[55-58] In a non-HCV exposed group of people, TG and HDLc were directly and inversely related to steatosis, respectively.[55] TC and LDLc have been reported to be inversely related to steatosis and fibrosis when assessed in groups with chronic HCV infection.[56, 57] TG levels have also been associated with steatosis, although inversely in one report among genotype 3 and non-genotype 1 groups [57], and directly in a genotype 1 cohort [58]. The genotype specific manner of the relationship between TG and steatosis is consistent with the notion that genotype 3 steatosis is a viral-mediated process, whereas genotype 1 is an indirect consequence of metabolic changes, such as insulin resistance, that occur due to infection.[59, 60]

Lastly, a growing body of recent evidence suggests that baseline serum lipid profile measures may be important prognostic factors of treatment efficacy of combination antiviral therapy. Several studies suggest that high pretreatment LDLc and TC levels are associated with higher rates of treatment response in multivariable analyses.[61-65] Other work revealed higher baseline TG levels among sustained viral responders compared to non-responders, though baseline TC did not differ significantly between the two response groups.[52]

3.0   THREE PROPOSED DISSERTATION PROJECTS

3.1.1   Specific aims: project 1

Assess whether demographic, clinical, and liver disease severity (histological, biochemical, and virological) measures are associated with the lipid profile among treatment naïve participants with chronic HCV infection, genotype 1.

3.1.2   Specific aims: project 2

Evaluate changes between baseline and on- and post-treatment lipid profile measures. Assess whether baseline demographic, clinical, and liver disease measures (histological, biochemical, and virological) are associated with changes in lipid profile measures over the course of antiviral therapy.

3.1.3   Specific aims: project 3

Assess whether baseline measures of lipid profile and changes in lipid fractions during therapy are predictors of treatment efficacy. If lipid profile measures are significant predictors of treatment response, determine if multivariable models including lipid profile measures are more predictive of treatment efficacy compared to other multivariable assessments not including lipid

profile measures. Assess if lipid fraction measures explain any of the racial disparity in treatment efficacy.

4.0   THE VIRAHEP-C STUDY

The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) was a National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) funded project which enrolled participants between September 2002 and January 2004. Details of the Virahep-C study are described elsewhere.[10] In brief, the Virahep-C study was a multicenter treatment trial which included 196 AA and 205 CA participants to investigate the clinical, immunological, virologic, and host genetic factors that contribute to antiviral treatment efficacy, particularly racial differences. Treatment naïve participants between the ages of 18 and 70 years with chronic infection of hepatitis C virus (genotype 1) received combination therapy consisting of peginterferon alfa-2a and ribavirin for up to 48 weeks. Participants were to have undergone liver biopsy evaluations within 18 months prior to the screening evaluation and all biopsies were scored by a single hepatopathologist. There were several exclusion criteria including positive HIV or HBV status, consumption of more than 2 alcoholic drinks per day, evidence of alcohol or drug abuse, indication of anemia, decompensated liver disease, other chronic liver diseases, and inability or unwillingness to grant informed consent.

Funding for lipid profile analyses utilizing stored fasting serum samples was obtained for an ancillary study examining the relationships between host genetic polymorphisms, the lipid profile, and steatosis (KL2 RR024154-02 to LJY). Of the 401 participants enrolled in Virahep-C,

lipid profile analyses were conducted among participants who provided genetic consent (n=374) and had available stored fasting serum samples at baseline (n=335). Five participants who reported use of lipid lowering agents at the screening evaluation were excluded from statistical analyses. Thus, the sample for this dissertation analysis included 330 participants (160 AA and 170 CA).

5.0 PROJECT 1: THE SERUM LIPID PROFILE AND ASSOCIATIONS WITH LIVER DISEASE AND VIRAL LEVELS IN A TREATMENT NAÏVE CHRONIC HEPATITIS C INFECTED COHORT

5.1 ABSTRACT

BACKGROUND: Findings from *in vitro* and transgenic animal studies suggest mechanisms of hepatitis C virus (HCV) entry into hepatocytes via lipoprotein receptors, viral replication related to cholesterol metabolism, secretion from hepatocytes into the serum as HCV complexed with very low density lipoprotein (VLDL), and HCV-induced steatosis. Epidemiological studies of chronic HCV infection have shown that steatosis and fibrosis are inversely associated with total cholesterol (TC) and low density lipoprotein cholesterol (LDLc). Steatosis and fibrosis have also been associated with triglyceride (TG) levels, though the direction of the relationship is inconsistent across studies. **AIM:** To assess whether HCV viral level and histological factors are associated with the serum lipid profile in a treatment naïve cohort with chronic HCV genotype 1 infection. **METHODS:** Participants were from the Study of Viral Resistance to Antiviral Therapy (Virahep-C), a prospective study of treatment naïve participants who received combination therapy of PEG-IFN alfa-2a + ribavirin for up to 48 weeks. Pretreatment fasting lipid profiles, processed at a central lab, were analyzed for 160 African Americans and 170 Caucasian Americans. Liver biopsies were assessed for steatosis, histological inflammation, fibrosis, and iron deposition. Linear regression was used to evaluate associations of each lipid profile measure with serum HCV level and liver disease measures. Variables were transformed as appropriate to meet model assumptions. **RESULTS:** Adjusting for race and age, there was a significant direct relationship between TG and serum HCV level. Participants with steatosis had higher TG levels than those without steatosis. High density lipoprotein cholesterol (HDLc), LDLc, and TC levels were significantly lower for participants with severe fibrosis than those without this histological feature. In contrast to HDLc, LDLc, and TC, which were inversely related to serum ferritin, TG levels were directly related to this iron status measure. Iron status

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and saturation measures were also associated biopsy-assessed liver disease. **CONCLUSIONS:** In participants with HCV genotype 1 infection, more severe liver disease was associated with lower lipid profile levels, with the exception of TG levels which were directly related to steatosis. The direct relationship between HCV RNA level and TG levels is consistent with proposed mechanisms of VLDL/HCV particle secretion. In contrast, the direct relationship between TG level and steatosis is inconsistent with posited mechanisms of HCV-induced steatosis, a possible reflection of HCV genotype 1 infection and a metabolic etiology of steatosis. Although serum ferritin was associated with the lipid profile and liver disease, its biological relevance is unclear. Further investigation is warranted to clarify the role of the lipid profile in the context of chronic HCV infection.

5.2   INTRODUCTION

In the United States, approximately 4.1 million non-institutionalized people are infected with HCV, 3.2 million of whom are chronically infected.[3] A potential 4-fold increase in number of persons with chronic HCV infection is projected to occur between 1990 and 2015.[24] The estimated direct health care cost of HCV in 1997, including liver transplantation for which chronic HCV ranks as the leading indication in the US, was an estimated $1.8 billion.[23, 25] These statistics underscore the significant public health burden of chronic HCV infection.

Recent reports suggest that the serum lipid profile may be relevant to HCV and its life cycle. Compared to groups of people without HCV infection, several studies have associated chronic HCV with lower total cholesterol (TC), low density lipoprotein cholesterol (LDLc), and high density lipoprotein cholesterol (HDLc) levels.[47-50] Triglyceride (TG) levels have also been shown to be associated with HCV infection with reports of both lower [47-50] and higher levels[51], the direction of the relationship possibly reflecting different contributions of host factors to TG levels.

The lipid profile in both non-infected and HCV-infected cohorts have been shown to be associated with liver disease measures, including steatosis and fibrosis.[55-58] In a non-HCV exposed population, TG and HDLc were directly and inversely related to steatosis, respectively, and no significant association was found with LDLc.[55] Among chronic HCV infected cohorts, inverse relationships with TC and LDLc with steatosis and fibrosis have been reported.[56, 57] Triglyceride levels have also been shown to be associated with steatosis, although inversely in

one report [57], and directly in another[58], with the direction of the relationship differing by HCV genotype 3 and 1, respectively.

Findings from *in vitro* work support a mechanism involving HCV/lipoprotein complexes and LDL receptor or scavenger receptor-B1 (SR-B1) meditated HCV entry into hepatocytes.[34-37] However, the LDL receptor mediated mechanism is controversial.[38, 39] Entry of free HCV (not in association with lipoproteins) may also occur through binding with SR-B1 or CD81.[40-42] Other work posits a process of HCV viral particle assembly and secretion related to VLDL metabolic pathways.[29, 43-46] In addition, the interaction of the HCV core with microsomal triglyceride transfer protein (MTP) has been shown to disrupt VLDL secretion resulting in intrahepatic TG accumulation, a possible mechanism of HCV-induced steatosis.[46]

The lipid profile and liver disease may also be related to serum iron measures. In a non-HCV exposed group of people, elevated serum ferritin was associated with significantly lower HDLc and higher TG levels.[66] In addition, in an assessment of an exclusively HCV infected cohort, serum ferritin was found to be directly related to liver fat.[14] Chronic HCV infection has been associated with elevated ferritin levels compared to non-exposed groups, a possible reflection of HCV infection or HCV-induced liver disease.[67-69] These findings suggest that a relationship may exist between serum iron measures, liver disease, the lipid profile, and possibly HCV infection, although the biological mechanism is unclear.

The modifications the lipid profile associated with HCV infection may influence cardiovascular disease (CVD) risk, although several other factors may play a role. Compared to non-HCV exposed groups, results across the few studies that have assessed CVD risk are inconsistent.[48, 49, 51] Whereas one study suggests that CVD risk may be lower in association with chronic HCV based on comparisons of lipid profile measures,[48] other work suggests no

difference in risk based on arteriolsclerotic measures,[49] and yet another found higher atherosclerotic risk.[51] The different study findings may be attributed to the different study populations, measures of CVD used, or a combination of these study characteristics.

The relationship between the serum lipid profile, liver disease, and possible HCV entry, replication, and secretion processes is complex. This study characterizes factors associated with the serum lipid profile in a treatment naïve cohort with chronic HCV infection, genotype 1. Within an epidemiologic context, these findings reflect reports from *in vitro* and animal studies of potential pathogenic mechanisms of HCV-induced liver damage, as well as mechanisms of HCV entry into hepatocytes from the serum, replication, and secretion. In addition, this study also examines the associations between serum iron measures, liver disease, and lipid profile measures, and assesses factors associated with dyslipidemia.

5.3   METHODS

5.3.1   Study population

Participants were drawn from The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) cohort, which is described elsewhere.[10] In brief, the Virahep-C study included 196 AA and 205 CA participants to investigate the clinical, immunological, virologic, and host genetic factors that contribute to the racial disparity in antiviral treatment efficacy. Participants between the ages of 18 and 70 years with chronic HCV (genotype 1) infection were enrolled and underwent combination therapy consisting of PEG-IFN alfa-2a and ribavirin for up to 48 weeks. Funding for additional lipid profile analyses utilizing stored serum samples was

obtained for an ancillary study of the pathogenesis of steatosis and insulin resistance in chronic HCV infection (KL2 RR024154-02 to LJY). Lipid profile analyses were conducted among participants who provided genetic consent (n=374) and had available stored fasting serum samples at baseline (n=335). Five participants who reported use of lipid lowering agents at the screening evaluation were excluded from the statistical analysis. The final sample included in this analysis consisted of 330 participants (160 AA and 170 CA).

5.3.2 Study measures

Estimates of the lipid profile fractions, TG, LDLc, 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. If TG levels were less than 400 mg/dL, the Friedewald formula was used to calculate LDLc indirectly (LDLc = TC − HDLc − 0.20 X TG).[28] Samples for which TG levels were at least 400 mg/dL (n=3) had LDLc assessed directly. Dyslipidemia was defined using the cutoffs from the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recommendations as any of the following: LDLc greater than or equal to 130 mg/dL, HDLc less than 40 mg/dL, TC greater than or equal to 200 mg/dL, or TG greater than or equal to 150 mg/dL.[70]

Biopsy-assessed liver disease measures included fibrosis, fat score, inflammation, and iron score. Inflammation and fibrosis were assessed using the criteria of the Histological Activity Index (HAI) by a single hepatopathologist.[19, 20] An Ishak fibrosis score of at least 3 was classified as severe fibrosis and steatosis was defined as a fat score of greater than zero representing at least 5% fat involvement of hepatic tissue. An iron score of 0, 1, or 2 was

assigned according to the amount of iron deposits in hepatic tissue. Summing non-Ishak fibrosis scores from the HAI, a total HAI inflammation score was calculated. HAI inflammation scores between 0 and 6 were classified as mild, 7-11 as moderate, and 12 or greater as severe inflammation. Total HAI inflammation, Ishak fibrosis, and fat scores were also analyzed. Other non-biopsy serologic liver disease indicators included alanine and aspartate aminotransferase (ALT and AST, respectively), alkaline phosphatase, total bilirubin, the international normalized ratio (INR), white blood cell count (WBC), platelet count, serum ferritin (an iron status measure), and the percent iron/total iron binding capacity (TIBC) (an iron saturation measure).

5.3.3 Statistical analysis

Categorical measures were summarized as frequency and percent with differences across nominally classified groups (i.e., 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 the trend of distributions in lipid profile measures across ordinal fat score categories, test of trend was assessed using linear regression with lipid profile measures as the dependent variables. For all statistical tests, a p-value of <0.05 was considered statistically significant.

Since the dichotomous outcome of dyslipidemia was common, a relative risk model with a robust variance estimator was used.[71] Linear regression was employed to model each lipid profile measure as a continuous outcome. In regression models, TG, HDLc, and TC were transformed to the natural logarithm (ln) scale to achieve normality. Predictors were transformed to make associations with lipid profile measures linear and also centered about their means. In multivariable analyses, a stepwise selection approach was used for variable reduction. All analyses were conducted using Statistical Analysis Software (SAS) Version 9 (Carey, NC) or STATA Version 9 (College Station, TX).

5.4   RESULTS

Characteristics of the study cohort are summarized by race. (Tables 5-1 and 5-2) AAs did not significantly differ from CAs by age, gender, employment status, health risk behaviors (smoking status and weekly alcohol consumption), HCV level, AST, INR, WBC count, platelet count, percent Iron/TIBC, Ishak fibrosis, total HAI score, steatosis, TG, HDLc, and TC (p>0.05 for all). Compared to CAs, a larger percentage of AAs had any form of health insurance coverage, public health insurance, less education, a history of diabetes or hypertension, and HCV sub-genotype 1b (p<0.05 for all). As a group, AAs had higher body mass indices (BMIs), higher alkaline phosphatase, and ferritin levels, and lower ALT, total bilirubin, albumin, and LDLc levels than CAs (p<0.05 for all). Several significant racial differences were noted among females, but not males, with AA females having higher BMIs, waist to hip ratios, and lower LDLc and TC levels compared to CA females (p<0.05 for all).

Liver disease severity was associated with lipid profile measures. (Figures 5-1 and 5-2) Participants with severe fibrosis tended to have lower HDLc and TC levels compared to those with non-severe fibrosis (Figure 5-1: p=0.002 and 0.004, respectively), whereas participants with higher fat scores tended to have higher TG and lower HDLc levels (Figure 5-2: p<0.0001 and p=0.01 for trend, respectively). In regression models, serum ferritin and the percent iron/TIBC were associated with lipid profile measures in age and race-adjusted, and multivariable models (p<0.05). (Tables 5-3, 5-4, 5-6) The iron measures also differed significantly by steatosis, fibrosis, and hepatic iron score (p<0.05) (Table 5-7). Furthermore, in the multivariable model of LDLc (Table 5-6), a significant interaction (p<0.0001) was found between race and serum ferritin. Interpreted from the model as race-specific ferritin coefficients, the decline in LDLc per 100 ng/mL increase in ferritin was significantly larger in CAs compared to AAs (CA: β=-3.4, p<0.0001; AA: β=-1.7, p=0.0001).

Significant direct relationships were found between the natural log of TG and $\log_{10}$ HCV RNA levels in unadjusted (β=0.11, p=0.003), age and race-adjusted (β=0.10, p=0.004), and multivariable regression models (β=0.09, p=0.01). (Figure 5-3, Table 5-3 and 5-6) Adjusting for age, LDLc concentrations were a mean 10.5 mg/dL lower among AAs than CAs. Adjusting for age and race, males had lower HDLc and TC levels, and higher TG levels, than females (on natural log scale β=-0.24, p<0.001, β=-0.07, p=0.002, β=0.14, p=0.14, respectively). (Tables 5-3 and 5-4) Other factors associated with lipid profile measures in multivariable models (Table 5-6) were aspects of liver disease, including HAI inflammation which was inversely related to LDLc and the natural log of TC, severe Ishak fibrosis associated with lower natural log transformed HDLc levels (β=-0.10, p=0.006)), and fat score (directly related to the natural log of TG (β=0.17, p<0.001)). Among those using ACE inhibitors (n=31) to treat hypertension, levels were

significantly lower for LDLc (β=-21.2, p<0.001), and the natural logs of HDLc (β=-0.15, p=0.03) and TC (β=-0.09, p=0.02) compared to non-users in multivariable analyses.

Using the NCEP ATP III definition, the prevalence of dyslipidemia was 70 percent in this cohort. (Table 5-4) Of those classified as dyslipidemic, low HDLc was the most common criteria of dyslipidemia (64%), followed by high TC (45%), high LDLc (43%), and high TG (32%). (Table 5-8) Adjusting for age and race, the risk of dyslipidemia was significantly greater among males (30% higher risk compared to females, p=0.001), and was directly related to both BMI (10% increased risk per 5 unit increase in BMI, p=0.02) and waist to hip ratio. (Table 5-5) In multivariable regression models, a greater risk of dyslipidemia was associated with older age (10% excess risk per 10 year increase, p=0.03), male gender (30% higher risk compared to females, p=0.01), larger waist to hip ratio, and public health insurance (20% excess risk compared to private insurance, p=0.03), a possible surrogate of socioeconomic status. (Table 5-6)

5.5   DISCUSSION

In this cohort of people with chronic HCV genotype 1 infection there was significant direct association between HCV and TG levels, the latter of which is related to VLDLc. This is relevant to proposed biological mechanisms of HCV replication and secretion from hepatocytes. Potentially important in the context of HCV-induced liver damage, steatosis was also associated with higher TG levels. In contrast to TG levels, other lipid profile measures were lower among those with advanced liver disease as measured by hepatic fibrosis, fat involvement, and inflammation.

The significant direct relationship between HCV and TG levels is consistent with findings from *in vitro* work suggesting that HCV is secreted from hepatocytes into the serum complexed with VLDL.[29, 43-46] However, this observation is not compatible with findings of an inverse relationship between apolipoprotein B (the apolipoprotein corresponding to VLDL, TG, and LDL) and HCV levels in an epidemiologic study in a non-genotype 1 group.[56] The inconsistency between *in vitro* and epidemiological studies may reflect unaccounted host factors *in vitro* experiments and genotype differences in *in vivo* experiments.

In transgenic mouse models, inhibition of the microsomal triglyceride transfer protein by exposure to HCV core protein has been proposed as a mechanism of HCV-induced steatosis, a plausible basis for associations between hypobetalipoproteinemia and chronic HCV infection.[46] This mechanism is supported by two epidemiologic studies, which found inverse relationships between steatosis and TC, TG, and apolipoprotein B levels in a genotype 3 infected group, but not in genotype 1.[56, 57] In contrast, there was a direct relationship between TG and liver fat score in the present study and in the larger Virahep-C cohort.[58] The inconsistent relationships between steatosis and TG (or apolipoprotein B levels) may be attributed to the Virahep-C study being an exclusively genotype 1 infected group and that the etiology of steatosis may differ by HCV genotype.[59, 60] Whereas steatosis is posited to occur due to metabolic changes that occur with genotype 1 infection, steatosis associated with genotype 3 infection may occur by a different direct viral-induced process.

The current study found associations between steatosis and severe fibrosis with lower HDLc and severe fibrosis with lower TC levels. These findings are consistent with other findings from both non-HCV infected[55] and chronically HCV infected groups[56, 57] that have associated liver disease with lower levels of HDLc and TC. The inverse relationship between liver disease

and non-TG lipid profile measures may reflect the disruption of lipid and lipoprotein metabolism due to liver damage, albeit by host factors (e.g., poor nutrient intake, alcohol consumption, or excess body weight) or attributed to chronic HCV infection.[6]

Despite lacking a defined biological role, serum iron measures (ferritin and the percent iron/TIBC) were related to the lipid profile (TG directly, other lipid profile measures indirectly). In addition, the iron measures were significantly higher for those with severe fibrosis, steatosis, or hepatic iron deposition. These findings are consistent with a report from a non-HCV exposed cohort, which found significantly lower HDLc and higher TG levels in a group with high ferritin levels of at least 100 ng/mL ferritin levels, compared to those below this cutpoint.[66] Findings are also consistent with another study of exclusively HCV infected people, which found a direct relationship between ferritin and liver fat.[14] Other work using National Health and Nutrition Examination Survey (NHANES) data has reported associations between elevated ferritin and iron saturation in HCV infected, compared to a non-exposed group of people.[67-69] These studies suggest a relationship between serum iron measures, liver disease, and the lipid profile, which may be impacted by chronic HCV infection; however, biological mechanisms are unclear. Further investigation is warranted given the strong associations observed in this cohort.

The current study estimated the prevalence of dyslipidemia, a risk factor for CVD,[27] to be 70 percent. Little is known about dyslipidemia or cardiovascular disease (CVD) risk in people with chronic HCV infection and the ability to compare findings across studies is limited due to different study population characteristics. In work based on a rural Egyptian study population, Marzouk et al. suggests that chronic HCV infection may correspond to a "favorable" CVD lipid profile based on observations of significantly lower TG and LDLc levels associated with chronic HCV infection (genotype not specified).[48] In a Japanese cohort of chronically HCV infected

people (genotype not specified), CVD risk, as assessed by arteriosclerotic assessments with pulse wave velocity measures, did not differ significantly compared to a non-infected group, despite associations of infection with lower serum TG and TC levels.[49] In contrast, chronic HCV infection has been associated with greater early atherosclerosis risk, as inferred through carotid intima-media thickness measurements in a cohort predominately infected with HCV genotype 1, compared to a non-infected group.[51] Thus, despite the different lipid profile patterns associated with chronic HCV infection compared to non-infection, the relationship between chronic HCV infection and CVD risk is unclear.

Given the cross-sectional nature of this analysis, this study is not without limitation, including the inability to infer causation or temporality of relationships between lipid profile and liver disease measures. In addition, the stepwise variable reduction approach used in multivariable modeling may also have influenced the results (refer to section 8.1 for details of stepwise modeling). It is possible that alternative modeling approaches, such as manually selecting variables and constructing multivariable models based on biological and clinical relevance, may have yielded different findings. For example, health insurance status was found to be a feature associated with dyslipidemia in multivariable analyses. However, this insurance status may serve as a proxy for other factors associated with socioeconomic status, such as race, educational attainment, and employment status, which may have entered the multivariable model if another modeling method was used.

Despite these limitations, this study has a number of strengths in that it assessed factors associated with lipid profile measures, which may have implications to proposed mechanisms of HCV entry into hepatocytes, replication, and secretion from hepatocytes into the serum. In addition, the Virahep-C cohort, which by design enrolled and treated approximately equal

numbers of AAs and CAs, afforded a unique opportunity to assess if factors associated with the lipid profile differed by race. This study also characterized the prevalence and associated factors of dyslipidemia using a standard definition.

In conclusion, the current study found associations between lipid profile measures and both HCV level and liver disease measures. In addition, this study found that dyslipidemia was a common occurrence, which may have implications for CVD risk. These findings may guide further investigations of the impact of antiviral therapy on the lipid profile, as well as the evaluation of lipid profile measures as predictors of treatment efficacy.

5.6   TABLES

Table 5-1. Cohort characteristics (1 of 2)

Feature*	Overall (n=330)	AA (n=160)	CA (n=170)	*p*
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)[a]				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)[b]				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)	--
Employed (m=4)	204 (62.6)	101 (63.9)	103 (61.3)	0.63
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
Male	28.5 (25.9,31.5)	28.5 (26.2,32.3)	28.4 (25.8,31.2)	0.35
Female	28.4 (24.0,34.1)	32.4 (27.2,36.0)	25.0 (22.3,31.7)	<0.0001
Waist to hip ratio (m=23)	0.9 (0.9,1.0)	0.9 (0.8,1.0)	0.9 (0.8,1.0)	0.44
Male	0.9 (0.9,1.0)	0.9 (0.9,1.0)	0.9 (0.9,1.0)	0.22
Female	0.8 (0.8,0.9)	0.9 (0.8,0.9)	0.8 (0.8,0.9)	0.008
Diabetic	31 (9.4)	24 (15.0)	7 (4.1)	0.0007
Hypertensive	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[c]				0.002
1, NOS	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

m=number with missing data

*Each categorical variable is summarized as n (%) with p-values corresponding to a Pearson's chi-square test (nominal variables) or the Jonckheere-Terpstra test (ordinal variables) or exact equivalents, where appropriate; each continuous variable is summarized as a median (interquartile range) with a p-value corresponding to a Wilcoxon rank sum test.

For features with two or more categories, the global p-value is listed in the first row of the feature.

Where the global p-value is <0.05, p-values correspond to Pearson's chi-square with comparisons as follows:
[a]Each health insurance status category compared to other categories combined; [b]Less than high school vs. high school degree or more; high school degree vs. more than high school degree; some college vs. more than some college; [c]Each genotype compared to other categories combined

Table 5-2. Cohort characteristics (2 of 2)

Feature*	Overall (n=330)	AA (n=160)	CA (n=170)	*p*
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
Alk 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 $\geq$ 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
Male	106.5 (76,161)	112.5 (76,170)	105 (76,149)	0.23
Female	93.5 (74,134)	95.5 (72,139)	92.5 (76.5,122)	0.71
LDLc (mg/dL)	115.1 (88.1,137.3)	106.4 (83.4,133.4)	118.7 (95.8,141.5)	0.009
Male	114.8 (87.2,134.2)	110.3 (86.4,131.3)	117.8 (89.8,137.6)	0.18
Female	116.6 (90.6,143.9)	104.9 (76.8,138.1)	122.5 (100.7,156.7)	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
Male	38.3 (32.4,46.9)	39.3 (31.8,49.8)	37.2 (32.8,45.5)	0.38
Female	51.4 (39.4,61.4)	51.5 (38.0,51.5)	51.4 (41.2,59.5)	0.87
TC (mg/dL)	185 (157,207)	179 (153.5,204.5)	187 (161,209)	0.10
Male	179 (155,204)	177.5 (152,204)	179.5 (155,205)	0.74
Female	189 (164,216)	184 (155,208)	192.5 (172.5,224.5)	0.02

m=number with missing data

*Each categorical variable is summarized as n (%) with p-values corresponding to a Pearson's chi-square test (nominal variables) or the Jonckheere-Terpstra test (ordinal variables) or exact equivalents, where appropriate; each continuous variable is summarized as a median (interquartile range) with a p-value corresponding to a Wilcoxon rank sum test.

For features with two or more categories, the global p-value is listed in the first row of the feature.

Table 5-3. Adjusted* regression models of lipid profile measures

Feature	ln(TG) β* (95%CI)	p	LDLc β* (95%CI)	p	ln(HDLc) β* (95%CI)	p	ln(TC) β* (95%CI)	p
Demographics								
AA[a]	0.1 (-0.04,0.2)	0.19	-10.5 (-18.3,-2.7)	0.008	0.02 (-0.05,0.09)	0.57	-0.04 (-0.08,0.01)	0.09
Age (years)[b]**	0.03 (-0.04,0.1)	0.43	2.8 (-1.8,7.4)	0.23	-0.01 (-0.05,0.04)	0.74	0.02 (-0.02,0.04)	0.30
Male	0.14 (0.03,0.25)	0.02	-6.6 (-15.0,1.9)	0.13	-0.24 (-0.31,-0.18)	<0.001	-0.07 (-0.11,-0.03)	0.002
Health risk behaviors								
Current smoker	0.13 (0.01,0.25)	0.03	5.2 (-3.2,13.5)	0.23	-0.05 (-0.13,0.02)	0.14	0.03 (-0.02,0.08)	0.21
≥2 Alcoholic drinks/week	-0.03 (-0.15,0.10)	0.68	-4.9 (-14.4,4.6)	0.31	0.07 (-0.02,0.15)	0.13	-0.02 (-0.07,0.04)	0.53
General clinical features								
BMI***	0.07 (0.01,0.12)	0.01	1.6 (-1.7,4.8)	0.35	-0.05 (-0.08,-0.02)	0.002	0.004 (-0.01,0.02)	0.69
Waist to hip ratio^	0.45 (0.06,0.84)	0.03	2.1 (-29.5,33.7)	0.90	-0.74 (-1.04,-0.44)	<0.001	-0.12 (-0.31,0.07)	0.21
Diabetic	0.15 (-0.02,0.32)	0.08	-6.5 (-18.1,5.2)	0.28	-0.14 (-0.25,-0.02)	0.02	-0.05 (-0.12,0.02)	0.13
Hypertensive	0.15 (0.02,0.29)	0.03	-11.7 (-20.4,-3.0)	0.008	-0.09 (-0.17,-0.01)	0.03	-0.06 (-0.11,-0.003)	0.04
Viral characteristics								
Log₁₀ HCV level	0.10 (0.03,0.18)	0.004	-0.2 (-5.1,4.7)	0.94	-0.03 (-0.08,0.01)	0.17	0.003 (-0.02,0.03)	0.83
Liver disease indicators								
ALT (IU/L)`	0.02 (-0.06,0.11)	0.58	-4.4 (-10.2,1.4)	0.14	-0.07 (-0.12,-0.02)	0.01	-0.04 (-0.08,-0.003)	0.03
AST (IU/L)`	0.08 (-0.01,0.18)	0.09	-7.2 (-13.9,-0.6)	0.03	-0.06 (-0.12,0.0004)	0.052	-0.04 (-0.08,-0.004)	0.03
Alk phosphatase (IU/L)`	0.40 (0.26,0.55)	<0.001	-9.2 (-19.9,1.5)	0.09	-0.10 (-0.20,0.01)	0.07	-0.02 (-0.09,0.04)	0.51
Total bilirubin (mg/dL)^	-0.03 (0.23,0.02)	0.23	0.04 (-3.7,3.8)	0.98	-0.01 (-0.03,0.02)	0.72	-0.01 (-0.03,0.02)	0.63
INR	-0.31 (-0.78,0.15)	0.19	-8.2 (-43.6,27.3)	0.65	-0.26 (-0.53,0.001)	0.051	-0.18 (-0.39,0.04)	0.10
WBC count (10³/mL)	0.03 (0.01,0.06)	0.02	0.9 (-1.0,2.8)	0.36	-0.03 (-0.05,-0.02)	<0.001	0.002 (-0.01,0.01)	0.73
Platelet count (10³/mL)****	-0.03 (-0.11,0.05)	0.43	9.2 (3.8,14.6)	0.001	0.05 (-0.002,0.1)	0.06	0.06 (0.03-0.09)	<0.001
Ferritin (ng/mL)****	0.03 (0.02,0.05)	<0.001	-1.9 (-3.1,-0.8)	0.001	-0.02 (-0.03,-0.005)	0.005	-0.01 (-0.02,-0.003)	0.004
%Iron/TIBC**	-0.0001 (-0.02,0.01)	0.91	-0.9 (-2.5,0.6)	0.24	-0.01 (-0.02,-0.002)	0.02	-0.01 (-0.02,0.002)	0.12
Total HAI inflammation^	0.39 (-0.71,1.48)	0.49	-106.8 (-175.1,-38.5)	0.002	-0.36 (-0.94,0.21)	0.22	-0.62 (-0.98,-0.26)	0.001

NOTE: Transformations as noted: ` = natural log; ^ = 1 – reciprocal

*Age and race adjusted unless otherwise specified as follows:

    [a]Age-adjusted only; [b]Race-adjusted only

Per 10 unit increase;*Per 5 unit increase;****Per 100 unit increase

Table 5-4. Cohort characteristics by dyslipidemic status

Feature	Dyslipidemia	
	Yes (n=232)	No (n=98)
Demographics		
AA	110 (68.8)	
CA	122 (71.8)	
Age (years)	48 (44,52.5)	46.5 (42,51)
Male	166 (76.9)	
Female	66 (57.9)	
Health insurance		
Uninsured	44 (75.9)	
Public	58 (73.4)	
Private	124 (66.3)	
Educational attainment		
<High school	47 (77.1)	
High school degree	57 (73.1)	
Some college	65 (61.9)	
≥College degree	56 (71.8)	
Employed	142 (69.6)	
Unemployed	86 (70.5)	
Health risk behaviors		
Current smoker	95 (74.2)	
Non-smoker	131 (66.8)	
Alcohol consumption		
≥2 drinks/week	42 (63.6)	
<2 drinks/week	185 (72.0)	
General clinical features		
BMI	29.0 (26.0,32.6)	27.3 (24.0,31.0)
Male	28.8 (26.2,32.0)	27.7 (24.7,30.0)
Female	30.3 (25.0,34.9)	26.9 (23.3,33.8)
Waist to hip ratio	0.9 (0.9,1.0)	0.9 (0.8,0.9)
Male	0.9 (0.9,1.0)	0.9 (0.9,1.0)
Female	0.9 (0.8,0.9)	0.8 (0.8,0.9)
Diabetic	24 (77.4)	
Non-diabetic	208 (69.6)	
Hypertensive	68 (70.1)	
Non-hypertensive	164 (70.4)	
Viral characteristics		
Log_{10} HCV level	6.5 (5.7,6.8)	6.3 (5.6,6.7)

*Each categorical variable is summarized as n (%) and each continuous variable is summarized as a median (interquartile range).

Table 5-5. Relative risk of dyslipidemia

Feature	Dyslipidemia	
	RR* (95%CI)	*p*
Demographics		
AA[a]	0.9 (0.8,1.1)	0.44
Age (years)[b]**	1.1 (0.98,1.2)	0.14
Male	1.3 (1.1,1.6)	0.001
Health insurance[c]		0.16
Public	0.9 (0.8,1.2)	0.56
Private	0.8 (0.7,1.02)	0.08
Educational attainment[d]		0.12
High school degree	0.9 (0.8,1.1)	0.55
Some college	0.8 (0.7,0.96)	0.02
$\geq$College degree	0.9 (0.7,1.1)	0.26
Employed	1.003 (0.9,1.2)	0.97
Health risk behaviors		
Current smoker	1.1 (0.98,1.3)	0.09
$\geq$2 Alcoholic drinks/week	0.9 (0.7,1.1)	0.30
General clinical features		
BMI***	1.1 (1.01,1.2)	0.02
Waist to hip ratio^	2.8 (1.5,5.1)	0.001
Diabetic	1.1 (0.9,1.4)	0.29
Hypertensive	0.97 (0.8,1.1)	0.70
Viral characteristics		
Log_{10} HCV level	1.1 (0.97,1.2)	0.16

Transformations as follows:` = natural log; ^ = 1 – reciprocal

*Age and race adjusted unless otherwise specified as follows: [a]Age-adjusted only; [b]Race-adjusted only

Reference group for regression models as follows: [c]Uninsured; [d]Less than high school degree;

**Per 10 unit increase

***Per 5 unit increase

Table 5-6. Multivariable regression models of dyslipidemia and lipid profile measures

Feature	Dyslipidemia RR (95%CI)	p	ln(TG) β (95%CI)	p	LDLc β (95%CI)	p	ln(HDLc) β (95%CI)	p	ln(TC) β (95%CI)	p
AA Race~	0.9 (0.8,1.1)	0.21	0.05 (-0.06,0.15)	0.37	-8.0 (-15.6,-0.3)	0.04	0.01 (-0.05,0.08)	0.68	-0.04 (-0.08,0.004)	0.08
Age (years)~*	1.1 (1.01,1.2)	0.03	-0.001 (-0.06,0.06)	0.96	5.1 (0.4,9.7)	0.03	-0.002 (-0.05,0.04)	0.93	0.04 (0.01,0.06)	0.02
Male	1.3 (1.1,1.5)	0.01							-0.06 (-0.10,-0.01)	0.01
Health insurance[a]		0.04								
Public	0.99 (0.8,1.2)	0.93								
Private	0.8 (0.7,1.01)	0.06								
Waist to hip ratio^	2.1 (1.1,4.1)	0.02					-0.61 (-0.91,-0.31)	<0.001		
Hypertensive treatment										
ACE inhibitors					-21.2 (-32.3,-10.0)	<0.001	-0.15 (-0.28,-0.01)	0.03	-0.09 (-0.17,-0.01)	0.02
Diet									-0.15 (-0.28,-0.03)	0.02
Log_{10} HCV level			0.09 (0.02,0.16)	0.01						
Alk phosphatase (IU/L)`			0.34 (0.20,0.48)	<0.001						
WBC count (10^3/mL)							-0.03 (-0.04,-0.01)	0.001		
Platelet count (10^3/mL)$									0.04 (0.01,0.07)	0.01
Ferritin (ng/mL)$			0.02 (0.01,0.03)	0.003	-3.4 (-4.8,-1.9)	<0.001				
Ferritin$ X Race						0.001				
AA					-1.7 (-2.8,-0.7)	0.001				
CA					-3.4 (-4.8,-1.9)	<0.001				
%Iron/TIBC*							-0.01 (-0.02,-0.005)	0.001		
Total HAI inflammation^					-86.3 (-154.2,-18.4)	0.01			-0.43 (-0.80,-0.06)	0.02
Ishak fibrosis score ≥ 3							-0.10 (-0.17,-0.03)	0.006		
Fat score			0.17 (0.09,0.25)	<0.001						

Transformed scale as noted: ` = natural log; ^ = 1 – reciprocal

~Forced into the multivariable model

[a]Uninsured reference group; *Per 10 unit increase; $Per 100 unit increase

Table 5-7. Iron status and liver biopsy measures by race

Iron saturation and liver biopsy measure	AA			CA			Overall		
	Median (IQR)	p*		Median (IQR)	p*		Median (IQR)	p*	
Ferritin (ng/mL)									
Ishak fibrosis score ≥ 3	295 (176,545)	0.06		245 (127,411)	<0.0001		263 (139,472)	<0.0001	
<3	223.5 (105,404.5)			125.5 (53.5,245)			167.5 (77.5,302.5)		
Steatosis (≥5% present)	264 (128,516)	0.06		203 (114,337)	0.004		239 (120,414)	0.0005	
No steatosis	214 (94,357)			109.5 (44,196)			160 (75,291)		
HAI scores		0.95			0.01			0.07	
0–6 (mild)	266 (82.7,422)			97.5 (33.9,265.5)			132 (60,339)		
7–11 (moderate)	241 (125,478)			191 (113,300)			218 (116,374)		
≥12 (severe)	264 (143,388)			168 (77,379)			192 (91.8,379)		
Iron score		<0.0001			<0.0001			<0.0001	
0	127 (76,233)			107 (38,187)			115 (53.5,198.5)		
1	373 (242,545)			268 (173,470)			331.5 (218,514)		
2	613 (474, 955)			359 (216,558.5)			527 (285,663)		
%Iron/TIBC									
Ishak fibrosis score ≥ 3	36.9 (27.7,44.1)	0.02		36.2 (27.7,51.8)	0.17		36.5 (27.7,46.6)	0.01	
<3	31.2 (23.8,40.2)			33.3 (26.2,45.8)			32.4 (25.4,41.9)		
Steatosis (≥5% present)	34.1 (26.9,41.9)	0.41		35.6 (27.6,49.3)	0.27		34.8 (27.3,45.1)	0.17	
No steatosis	33.5 (22.6,40.8)			33.8 (25.9,41.9)			33.7 (24.4,41.9)		
HAI scores		0.63			0.35			0.34	
0–6 (mild)	29.3 (23.8,41.3)			31.2 (25.7,45.7)			32.2 (24.6,42.1)		
7–11 (moderate)	34.5 (26.3,42.5)			37.1 (28.4,49.3)			35.7 (27.1,44.6)		
≥12 (severe)	34.6 (28.0,39.8)			31.9 (26.7,44.4)			33.5 (26.9,39.8)		
Iron score		<0.0001			<0.0001			<0.0001	
0	27.4 (22.1,37.3)			33.2 (24.4,42.1)			31.2 (23.5,39.3)		
1	37.4 (30.4,43.3)			36.5 (27.7,46.6)			37.3 (29.0,44.8)		
2	51.4 (28.5,67.0)			53.4 (36.2,58.6)			51.9 (32.2,61.2)		

*p-values correspond to Wilcoxon rank sum or Kruskal-Wallis tests for differences in iron saturation across liver biopsy measures

Table 5-8. Criteria for dyslipidemia

High TC	High LDLc	High TG	Low HDLc	n (%)
			X	69 (29.7)
X	X			48 (20.7)
		X	X	33 (14.2)
X	X	X	X	18 (7.8)
X	X		X	14 (6.0)
X				12 (5.1)
		X		11 (4.7)
	X		X	9 (3.9)
	X			6 (2.6)
X		X	X	5 (2.2)
X	X	X		4 (1.7)
X		X		3 (1.3)
104	99	74	148	232

Table 5-9. Supplement 1: Dyslipidemia by additional features

Feature	Dyslipidemia	
	Yes (n=232)	No (n=98)
Viral characteristics		
HCV genotype		
1, NOS	18 (72.0)	
1a	126 (71.2)	
1b	9 (81.8)	
1a/1b	79 (67.5)	
Liver disease indicators		
ALT (IU/L)	71 (47,110)	63.5 (42,104)
AST (IU/L)	54 (38,79)	49 (33,76)
Alk phosphatase (IU/L	79 (63.5,103)	79.5 (61,102)
Total bilirubin (mg/dL)	0.6 (0.5,0.8)	0.6 (0.4,0.8)
INR	1.0 (0.9,1.1)	1.0 (0.9,1.1)
WBC count (10^3/mL)	6.1 (4.8,7.5)	5.5 (4.5,7.0)
Platelet count (10^3/mL)	210 (161,257)	206 (163,257)
Ferritin (ng/mL)	204 (94,365)	203 (102,367)
Albumin (mg/dL)	4.2 (4.0,4.4)	4.2 (4.0,4.4)
Iron/TIBC	33.2 (25.9,42.9)	35.9 (27.4,44.2)
Ishak score	2 (1,3)	2 (1,3)
Ishak fibrosis score $\geq$ 3	88 (71.5)	
<3	143 (69.4)	
Fat score	0 (0,1)	0 (0,1)
Steatosis (>5 present)	153 (73.2)	
No steatosis	78 (65.0)	
Total HAI inflammation	9 (6, 11)	8 (6, 10)
Iron score		
0	105(66.9)	
1	88 (76.5)	
2	15 (65.2)	

*Each categorical variable is summarized as n (%) and each continuous variable is summarized as a median (interquartile range).

Table 5-10. Supplement 2: Relative risk of dyslipidemia (additional features)

Feature	Dyslipidemia	
	RR* (95%CI)	*p*
Viral characteristics		
HCV genotype[a]		
1, NOS	0.997 (0.8,1.3)	0.98
1b	1.1 (0.8,1.6)	0.39
1a/1b	0.9 (0.8,1.1)	0.38
Liver disease indicators		
ALT (IU/L)`	1.1 (0.96,1.2)	0.24
AST (IU/L)`	1.1 (0.9,1.2)	0.37
Alk phosphatase (IU/L)`	1.1 (0.9,1.3)	0.36
Total bilirubin (mg/dL)^	1.02 (0.9,1.1)	0.63
INR	1.4 (0.8,2.5)	0.21
WBC count (10^3/mL)	1.04 (1.003,1.1)	0.03
Platelet count (10^3/mL)****	0.997 (0.9,1.1)	0.96
Ferritin (ng/mL)****	1.002 (0.97,1.02)	0.86
Albumin (g/dL)	1.1 (0.9,1.3)	0.41
%Iron/TIBC**	1.003 (0.99,1.01)	0.67
Ishak score	1.02 (0.97,1.1)	0.45
Ishak score $\geq$ 3	1.003 (0.9,1.2)	0.97
Fat score	1.1 (0.997,1.2)	0.06
Steatosis (>5% present)	1.1 (0.95,1.3)	0.17
Total HAI inflammation^	1.2 (0.4,4.0)	0.75
Iron score[b]		0.18
1	1.1 (0.98,1.3)	0.08
2	0.97 (0.7,1.3)	0.87

NOTE: Transformations as noted: ` = natural log; ^ = 1 – reciprocal
[a]HCV genotype 1a reference; [b]0 Iron score reference
**Per 10 unit increase; ****Per 100 unit increase

Table 5-11. Supplement 3. Lipid profile measures in subgroups

Feature	LDLc Median (IQR)	p*	HDLc Median (IQR)	p*	TC Median (IQR)	p*	TG Median (IQR)	p*
Gender		0.33		<0.001		0.008		0.02
Male	114.8 (87.2,134.2)		38.3 (32.4,46.9)		179 (155,204)		106.5 (76,161)	
Female	116.6 (90.6,143.9)		51.4 (39.4,61.4)		189 (164,216)		93.5 (74,134)	
Health insurance		0.46		0.96		0.83		0.07
Uninsured	116.7 (87.0,134.6)		41.1 (33.5,57.6)		185.5 (151,208)		89 (69,126)	
Public	104.5 (83.5,138.1)		40.0 (34.4,56.5)		185 (154,205)		110 (86,149)	
Private	116.5 (91.0,137.2)		42.5 (33.4,52.0)		183 (160,206)		100 (74,147)	
Educational attainment		0.41		0.20		0.33		0.81
<High school	121.8 (86.9,140.4)		40.9 (32.6,54.7)		188 (159,207)		114 (76,149)	
High school degree	115.6 (83.5,137.3)		38.7 (32.7,49.9)		179 (152,205)		97 (73,129)	
Some college	111.5 (85.0,132.4)		45.4 (34.7,56.0)		183 (150,206)		100 (77,141)	
≥College degree	115.2 (94.5,143.4)		44.3 (34.7,54.1)		185 (165,209)		99 (76,144)	
Employed	114.8 (89.6,137.1)	0.68	42.2 (33.4,51.9)	0.32	181 (158.5,206.5)	0.9995	100 (74,146)	0.94
Unemployed	115.2 (85.0,137.6)		41.8 (34.7,57.7)		186 (154,206)		101 (75,146)	
Current smoker	113.1 (85.8,142.4)	0.66	40.6 (33.3,52.6)	0.17	185.5 (157,210)	0.52	108 (80,156.5)	0.07
Non-smoker	115.1 (88.7,132.8)		43.6 (33.8,54.3)		182.5 (157,205)		98 (73.5,138)	
Alcohol consumption		0.40		0.12		0.70		0.68
≥2 drinks/week	112.0 (81.7,136.7)		45.9 (35.7,53.8)		187 (150,205)		99 (74,144)	
<2 drinks/week	115.5 (89.8,137.6)		40.8 (33.4,54.1)		183 (159,207)		105 (76,146)	
Diabetic	104.5 (86.4,126.8)	0.17	37.9 (30.6,50.7)	0.045	162 (150,196)	0.14	123 (96,165)	0.02
Non-diabetic	116.2 (89.2,139.9)		42.3 (33.8,54.4)		158 (139,185)		99 (74,143)	
Hypertensive	105.6 (80.8,129.9)	0.02	39.4 (31.5,52.0)	0.07	180 (149,201)	0.06	108 (79,171)	0.04
Non-hypertensive	117.8 (91.6,141.3)		42.3 (34.4,55.2)		185 (160,209)		99 (74,138)	
HCV genotype		0.77		0.68		0.57		0.26
1, NOS	114.0 (90.6,155.8)		46.1 (34.8,53.2)		195 (150,219)		115 (80,164)	
1a	115.4 (88.1,137.3)		41.1 (33.7,52.1)		180 (160,205)		96 (73,138)	
1b	114.6 (87.6,134.2)		42.1 (33.5,54.2)		188 (154,206)		104 (78,146)	
1a/1b	118.7 (94.5,135.2)		37.1 (28.9,45.0)		176 (163,195)		108 (83,183)	

*p-value corresponds to the Wilcoxon rank sum or Kruskal-Wallis test

Table 5-12. Supplement 4: Adjusted regression models of lipid fraction (additional features)

Feature	ln(TG) β* (95%CI)	p	LDLc β* (95%CI)	p	ln(HDLc) β* (95%CI)	p	ln(TC) β* (95%CI)	p
Demographics								
Health insurance[a]		0.27		0.87		0.91		0.97
Public	0.11 (-0.06,0.28)	0.21	-0.2 (-13.3,12.9)	0.98	-0.0009 (-0.11,0.11)	0.99	0.01 (-0.07,0.08)	0.83
Private	0.01 (-0.14,0.17)	0.88	2.1 (-9.0,13.3)	0.71	-0.02 (-0.11,0.08)	0.73	0.01 (-0.06,0.07)	0.80
Educational attainment[b]		0.92		0.48		0.16		0.28
High school degree	-0.06 (-0.24,0.13)	0.54	-5.4 (-16.4,5.7)	0.25	-0.04 (-0.14,0.06)	0.44	-0.05 (-0.12,0.01)	0.13
Some college	-0.06 (-0.22,0.11)	0.51	-6.5 (-17.6,4.6)	0.25	0.06 (-0.05,0.16)	0.30	-0.04 (-0.10,0.03)	0.24
≥College degree	-0.04 (-0.21,0.13)	0.65	0.4 (-11.1,11.9)	0.95	0.05 (-0.07,0.16)	0.43	-0.001 (-0.07,0.06)	0.97
Employed	-0.02 (-0.13,0.10)	0.79	0.8 (-7.5,9.0)	0.85	-0.04 (-0.11,0.03)	0.28	-0.01 (-0.05,0.04)	0.82
Health risk behaviors								
Current smoker	0.13 (0.01,0.25)	0.03	5.2 (-3.2,13.5)	0.23	-0.05 (-0.13,0.02)	0.14	0.03 (-0.02,0.08)	0.21
≥2 Alcoholic drinks/week	-0.03 (-0.15,0.10)	0.68	-4.9 (-14.4,4.6)	0.31	0.07 (-0.02,0.15)	0.13	-0.02 (-0.07,0.04)	0.53
General clinical features								
BMI: Males***	0.09 (0.01,0.18)	0.03	2.1 (-1.9,6.2)	0.30	-0.07 (-0.10,-0.03)	0.001	0.01 (-0.02,0.04)	0.42
BMI: Female***	0.05 (-0.01,0.10)	0.10	1.8 (-3.4,7.1)	0.49	-0.04 (-0.08,-0.004)	0.03	-0.001 (0.03,0.02)	0.95
Waist to hip ratio: Male^	0.10 (-0.54,0.75)	0.75	22.6 (-21.7,67.0)	0.32	-0.27 (-0.68,0.14)	0.19	0.09 (-0.18,0.36)	0.52
Waist to hip ratio: Female^	0.53 (0.02,1.05)	0.04	9.3 (-43.2,61.9)	0.73	-0.68 (-1.07,-0.30)	0.001	-0.12 (-0.40,0.16)	0.40
Viral characteristics								
HCV genotype[c]		0.14		0.68		0.75		0.48
1, NOS	0.15 (-0.06,0.37)	0.16	9.6 (-9.1,28.2)	0.32	0.03 (-0.10,0.16)	0.63	0.07 (-0.02,0.17)	0.14
1b	0.17 (-0.06,0.40)	0.15	3.0 (-23.1,17.1)	0.77	-0.11 (-0.34,0.13)	0.38	-0.01 (-0.12,0.10)	0.80
1a/1b	0.11 (-0.02,0.23)	0.09	-2.1 (-10.3,6.1)	0.61	0.01 (0.06,0.09)	0.70	0.01 (-0.03,0.06)	0.56
Liver disease indicators								
Ishak fibrosis score	0.02 (-0.02,0.06)	0.28	-3.3 (-6.0,-0.5)	0.02	-0.04 (-0.06,-0.01)	0.003	-0.03 (-0.04,-0.01)	0.001
Ishak fibrosis score ≥ 3	0.05 (-0.07,0.16)	0.43	-9.1 (-17.2,-1.01)	0.03	-0.11 (-0.18,-0.04)	0.001	-0.08 (-0.12,-0.03)	0.001
Fat score	0.22 (0.14,0.30)	<0.001	-4.4 (-10.5,1.7)	0.15	-0.07 (-0.13,-0.01)	0.02	-0.01 (-0.04,0.03)	0.61
Steatosis (>5% present)	0.21 (0.11,0.32)	<0.001	-7.3 (-15.5,1.0)	0.08	-0.09 (-0.16,-0.02)	0.01	-0.03 (-0.07,0.02)	0.27
Iron score[d]		0.21		0.64		0.01		0.43
1	0.11 (-0.01,0.23)	0.08	-4.0 (-12.6,4.5)	0.36	-0.11 (-0.19,-0.04)	0.004	-0.03 (-0.08,0.02)	0.19
2	0.02 (-0.23,0.27)	0.86	-0.5 (-16.5,15.6)	0.96	-0.08 (-0.22,0.05)	0.24	-0.01 (-0.10,0.07)	0.78

Transformation as noted: ` = natural log; ^ = 1 – reciprocal

*Age and race adjusted

Reference group for regression models as follows: [a]Uninsured; [b]Less than high school degree; [c]1a genotype; [d]0 Iron score

**Per 10 unit increase; ***Per 5 unit increase; ****Per 100 unit increase

Table 5-13. Supplement 5: Adjusted* regression models of dyslipidemia and lipid profile measures (hypertension treatments)

	Dyslipidemia		ln(TG)		LDLc		ln(HDLc)		ln(TC)	
Treatment	RR (95%CI)	p	β (95%CI)	p	β (95%CI)	p	β (95%CI)	p	β (95%CI)	p
Hypertension Tx[a]										
Calcium channel blockers (n=24)	0.9 (0.7,1.3)	0.58	0.12 (-0.13 – 0.37)	0.36	-1.0 (-16.3,14.3)	0.90	0.07 (-0.05,0.20)	0.23	0.04 (-0.04,0.12)	0.36
Diet (n=8)	1.02 (0.7,1.5)	0.91	-0.13 (-0.47 – 0.22)	0.48	-17.9 (-41.9,6.0)	0.14	-0.17 (-0.36,0.02)	0.08	-0.16 (-0.29,-0.03)	0.02
Diuretic (n=27)	1.2 (0.9,1.1)	0.15	0.31 (0.05 –0.57)	0.02	-5.4 (-19.0,8.2)	0.43	-0.13 (-0.26,-0.01)	0.04	0.001 (-0.08,0.08)	0.97
ACE inhibitors (n=25)	1.1 (0.9,1.4)	0.28	0.24 (-0.04 – 0.52)	0.09	-21.9 (-33.6,-10.2)	<0.001	-0.16 (-0.30,-0.02)	0.02	-0.11 (-0.19,-0.03)	0.007
Beta blockers (n=21)	0.9 (0.7,1.3)	0.71	0.28 (0.07 – 0.49)	0.009	-10.7 (-23.7,2.4)	0.11	-0.10 (-0.26,0.06)	0.24	-0.03 (-0.11,0.04)	0.39

*Race and age adjusted models

Reference group for regression models as follows: [a]No treatment for hypertension
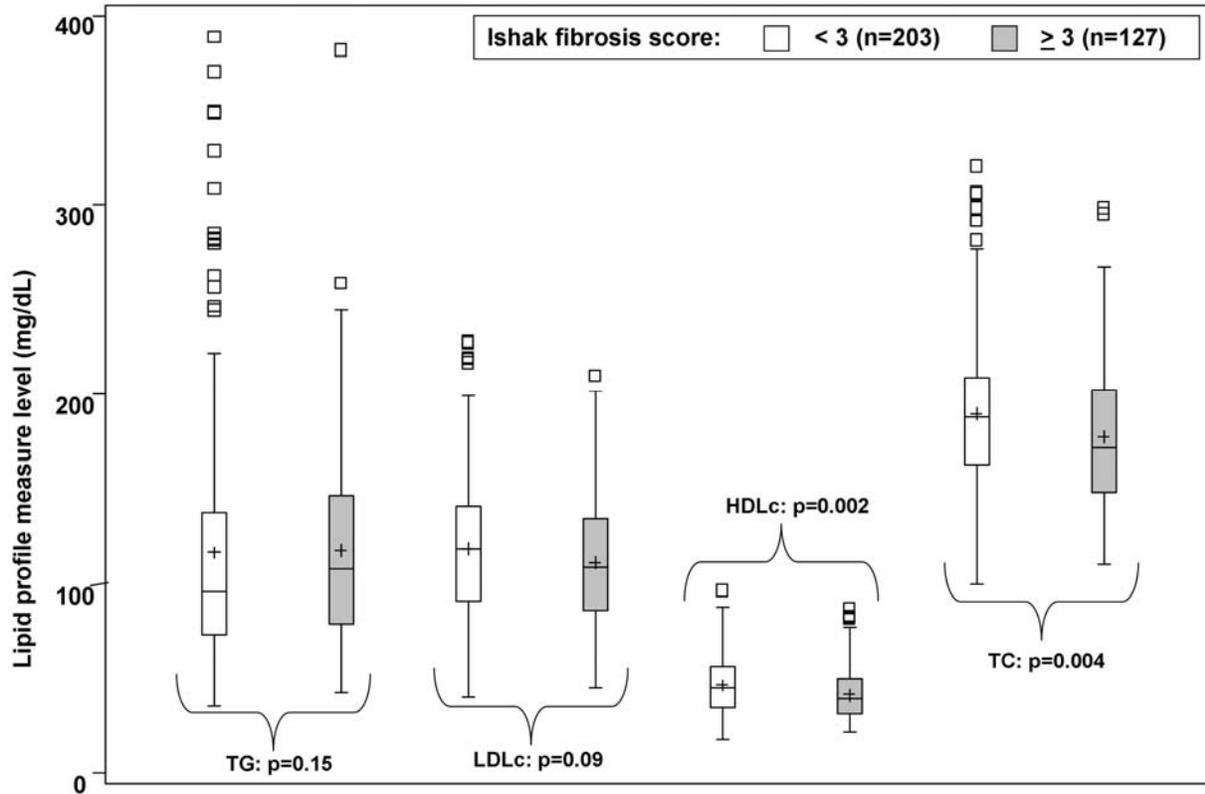
Figure 5-1. Lipid profile measures by Ishak fibrosis score
p-values correspond to Wilcoxon rank sum tests of differences by fibrosis score (Ishak fibrosis score ≥ 3 vs Ishak fibrosis score <3)
NOTE: Boxplots exclude three extreme TG level outliers: 600 and 980 mg/dL (non-severe fibrosis); 481 mg/dL (severe fibrosis).
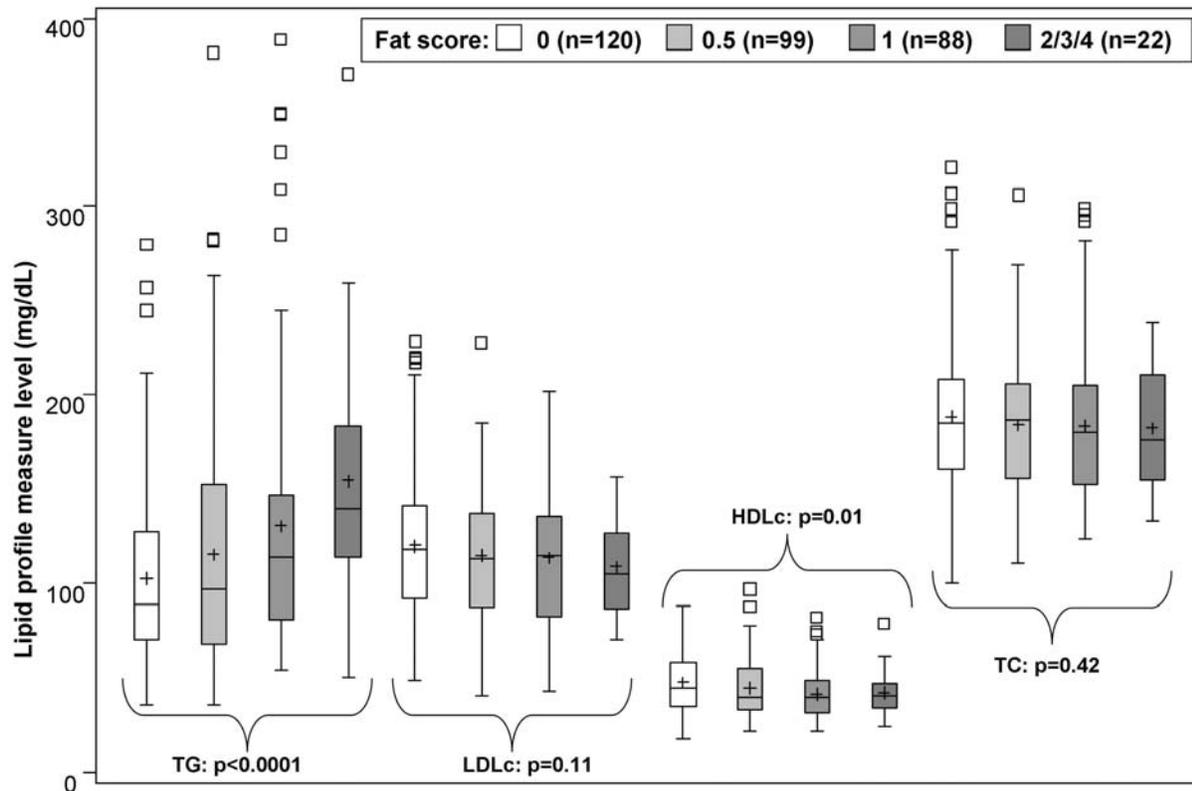
Figure 5-2. Lipid measures by liver fat score
p-values correspond to a test of trend with fat score. (Trend test for TG, HDLc, and TC on the natural log scale)
NOTE: Boxplots exclude three extreme TG level outliers: 980 mg/dL (fat score=0.5); 481 and 600 mg/dL (fatscore=1).
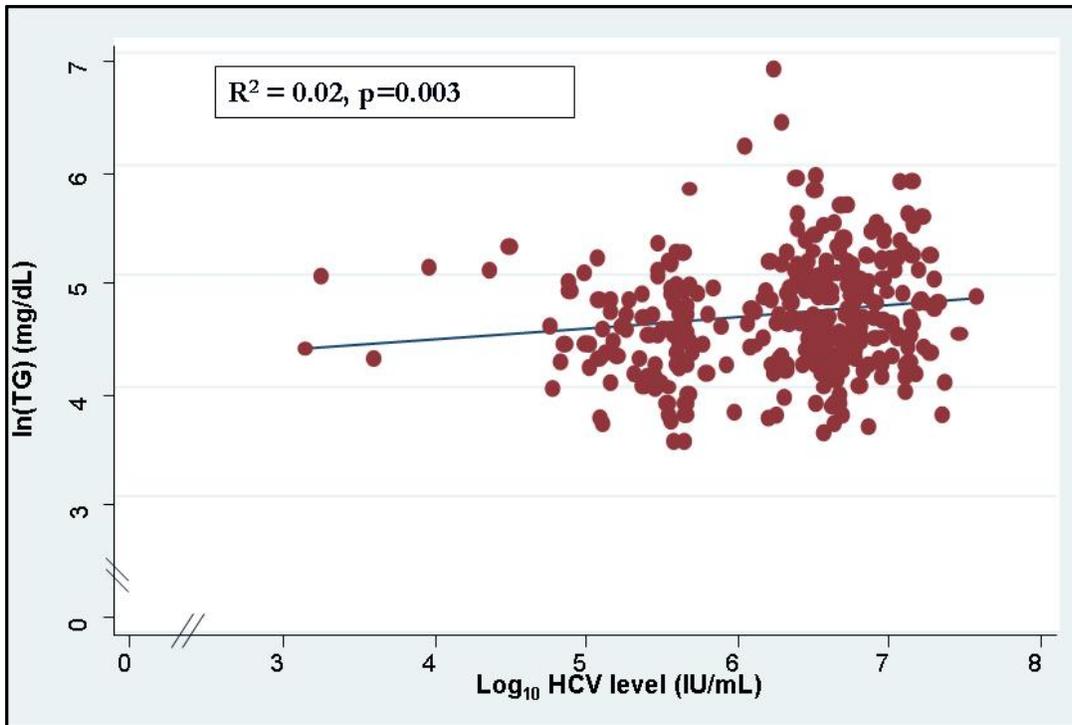
Figure 5-3. Relationship between TG and HCV RNA level
ln=natural log transformation
Back-transformed, one $\log_{10}$ increase in HCV level corresponds to an 11.4 mg/dL TG increase on the raw scale.

6.0   PROJECT 2: RACE AND CHANGES IN THE SERUM LIPID PROFILE DURING ANTIVIRAL THERAPY FOR CHRONIC HEPATITIS C INFECTION

6.1   ABSTRACT

BACKGROUND: Few studies have assessed the impact of antiviral therapy on the lipid profile in HCV infection and findings are inconsistent. **AIM:** To characterize changes in the lipid profile in a chronic HCV genotype 1 infected cohort undergoing combination PEG-IFN alfa-2a + ribavirin therapy. **METHODS:** Data were drawn from the Virahep-C study, an investigation of resistance to therapy among 401 participants who underwent up to 48 weeks of therapy. This analysis is based on 288 participants (135 African American (AA) and 153 Caucasian American (CA)) who had at least 1 post-baseline fasting serum sample. The on-treatment lipid profile assessment was conducted using stored serum collected after 24 weeks of therapy and the post-therapy assessment 24 weeks after the cessation of therapy. Generalized estimating equation (GEE) models were used to assess changes in the lipid profile during therapy and to evaluate racial differences. **RESULTS:** After 24 weeks of therapy triglyceride (TG) levels were significantly greater than pretreatment and low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc), and total cholesterol (TC) levels were significantly lower. Declines during the first 24 weeks of therapy in LDLc and TC were significantly associated with the amount of PEG-IFN taken. Post-therapy, significant changes in the lipid profile from baseline were found only among participants who underwent a 48-week course of therapy. In this group, TG, LDLc, and TC levels were significantly higher than pretreatment levels. The relationship between race and changes from pretreatment to 24 weeks of treatment in TG, LDLc, and TC differed with CAs having larger increases in TG and larger declines in LDLc and TC than AAs. **CONCLUSIONS:** Antiviral therapy is associated with changes in the lipid profile during and after antiviral therapy, with the changes differing by race and the amount of PEG-IFN taken. This suggests that the lipid profile may be involved in mechanisms of antiviral

therapy and HCV eradication. Further investigations are warranted to determine if lipid profile measures predict treatment efficacy and if the difference in lipid profile between AAs and CAs explain the racial disparity response to treatment.

6.2   INTRODUCTION

Chronic HCV infection is a major public health problem in the United States (US) afflicting at least 3.2 million persons (1.3%) with an estimated direct health care cost of $1.8 billion annually.[3, 25] The current standard treatment, combination PEG-IFN and ribavirin, is not completely effective and virological response for genotype 1, the predominant form of HCV in the US, differs by race with African Americans (AAs) having lower rates sustained virological response (SVR) (19–28%) than Caucasian Americans (CAs) (39–52%).[10-12] Reasons for the racial disparity in efficacy are unknown.[10]

Recent *in vitro* work suggests that serum lipoproteins, lipoprotein receptors, and cholesterol metabolism may mask HCV from the host immune response, provide a means of HCV entry into hepatocytes, and play a central role in HCV replication and secretion from hepatocytes into the serum.[29-37, 40-46] Furthermore, interferon exposure has been shown to down-regulate scavenger receptor B-1 expression, potentially relevant to mechanisms of interferon-induced HCV eradication.[72] The down-regulation of scavenger receptor B-1, a receptor involved in the uptake of high density lipoprotein into hepatocytes from the serum,[27] suggests that interferon therapy may also influence lipoprotein levels.

In trials evaluating factors associated with virological response to treatment, low density lipoprotein cholesterol (LDLc) and total cholesterol (TC) levels have been directly related to virological response.[61-65] In addition, comparing sustained virological responders to non-responders, another study found that responders tended to have significantly higher pretreatment

triglyceride (TG) levels, whereas TC levels did not significantly differ.[52] These findings suggest that lipid profile measures may be important predictors of virological response.

The lipid profile has also been reported to change over the course of interferon therapy, although the results are inconsistent and differ by HCV genotype. Compared to pretreatment, interferon mono-therapy has been associated with increases in TC and TG levels, with TC levels remaining significantly higher and TG levels returning to pretreatment levels after stopping therapy.[52] Other work found significant increases in TG levels, and no significant change in TC levels.[53] Compared to pretreatment, significant increases in TC have been reported in a subgroup with HCV genotype 3, but not genotype 1 during therapy[54], whereas another study reported higher TG levels during therapy in a group with genotype 1, but not in non-genotype 1.[53]

Findings from *in vitro* and epidemiological studies suggest the involvement of the serum lipid profile in mechanisms of antiviral therapy and treatment response. This study characterizes changes in the serum lipid profile over the course of combination antiviral therapy in an HCV genotype 1 infected cohort. In addition, this study assesses pretreatment patient and disease characteristics that predict changes in the lipid profile during and after therapy.

6.3   METHODS

6.3.1   Study population

Participants were drawn from the Virahep-C study, an investigation of resistance to antiviral therapy that has been described elsewhere.[10] In brief, Virahep-C enrolled and treated approximately equal numbers of CA (n=205) and AA (n=196) participants who underwent a

combination antiviral regimen of PEG-IFN alfa-2a and ribavirin for up to 48 weeks for chronic

HCV infection, genotype 1. The primary aim of the Virahep-C study was to investigate clinical,

immunological, virological, and host genetic factors involved in the resistance to antiviral

treatment, and in particular, racial differences in virological response. Funding for lipid profile

analyses was obtained for participants in an ancillary study to Virahep-C examining the

relationship between host genetic polymorphisms, the lipid profile, and steatosis (KL2

RR024154-02 to LJY). Therefore, only those who granted genetic consent (n=374) were eligible

for this investigation. Stored fasting serum samples were available for 330 of the participants

before treatment, for 253 of these 24 weeks after beginning therapy, and for 239 participants 24

weeks after stopping therapy. Among the 239 post-treatment samples, 177 were from

participants who were 6 month virological responders who had undergone a 48 week course of

therapy, and 62 were from 6 month virological non-responders who had only a 24 week course

of therapy. The final sample for this analysis consisted of 288 participants (135 AA and 153 CA)

who had a pretreatment and at least 1 available post-baseline stored fasting serum sample.

6.3.2   Study measures

Estimates of the lipid profile fractions, TG, LDLc, HDLc, and TC, were obtained through

analyzing stored fasting serum samples at the Heinz Nutrition Laboratory in the Department of

Epidemiology, University of Pittsburgh. If TG levels were less than 400 mg/dL, the Friedewald

formula was used to calculate LDLc indirectly (LDLc = TC − HDLc − 0.20 X TG).[28] For

samples with TG levels of 400 mg/dL or greater, LDLc was assessed directly. Dyslipidemia was

defined using the cutoffs from the National Cholesterol Education Program (NCEP) Adult

Treatment Panel (ATP) III recommendations as any of the following: LDLc greater than or equal

to 130 mg/dL, HDLc less than 40 mg/dL, TC greater than or equal to 200 mg/dL, or TG greater than or equal to 150 mg/dL.[70] An Ishak fibrosis score of at least 3 was classified as severe fibrosis and steatosis was defined as a fat score of greater than zero representing at least 5% fat involvement of hepatic tissue. Total HAI inflammation, Ishak fibrosis, and fat scores were also analyzed as continuous parameters. Inflammation and fibrosis were assessed using the criteria of the Histological Activity Index (HAI) by a single hepatopathologist.[19, 20] The amount of PEG-IFN and ribavirin taken by participants was estimated using data from the Medication Event Management System (MEMS) (Aardex, Zug, Switzerland).[73]

6.3.3   Statistical analysis

Categorical measures were summarized as frequency and percent with differences across nominally classified groups (i.e., 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 if changes in lipid profile measures significantly differed from baseline, a Wilcoxon sign rank test was used. Linear regression was conducted to compare the trend in the changes in lipid profile measures distributions across categories of the amount of PEG-IFN taken. Compared to baseline,

changes in dyslipidemic status during and after treatment were assessed using the McNemar's test of homogeneity. For all statistical tests, a p-value of <0.05 was considered to be statistically significant. Generalized estimating equation (GEE) models were employed with each lipid profile measure as the outcome over time reflecting changes during and after treatment, compared to its baseline value. To account for extreme outliers of dependent variables, TG, HDLc, and TC levels were transformed using the natural logarithm (ln).

Baseline characteristics were included in GEE models with three time-dependent or post-baseline covariates: participant body weight, the amount of PEG-IFN taken, and 6 month virological response. Variable reduction was achieved using a stepwise approach. As needed, continuous covariates were transformed in models to improve linearity of associations, and predictors were centered about their means. All analyses were conducted using Statistical Analysis Software (SAS) Version 9 (Carey, NC) or STATA Version 9 (College Station, TX).

6.4   RESULTS

Characteristics of the study cohort are summarized by race in Table 6-1. AAs tended to be older, have higher BMIs and lower ALT levels than CAs (p<0.05 for all). In addition, a higher proportion of AAs were diabetic, hypertensive, and infected with genotype 1b compared to CAs (p<0.05 for all). No significant racial differences (p>0.05 for all) in histological measures were found. Among lipid profile measures, racial differences at baseline were only found for LDLc, which was significantly lower in AAs than CAs (109 versus 119 mg/dL, p=0.02).

Compared to pretreatment, there were significant changes in the serum lipid profile during therapy and after therapy significant changes were limited to 6 month virological

responders. (Figure 6-1) During the first 24 weeks of therapy, TG levels increased significantly, in contrast to LDLc, HDLc, and TC, which declined (p<0.0001 for all). During follow up and among participants who underwent a 48 week course of therapy (6 month virological responders), TG levels remained significantly higher than pretreatment (p<0.05), as did LDLc (p<0.0001) and TC levels (p<0.0001). During follow up among 6 month non-responders, there were no significant changes in the lipid profile compared to pretreatment, although the lack of statistical significance may be related to the smaller sample size of this group (n=62) than 6 month virological responders (n=177). During 24 weeks of treatment, 61% of participants who were not dyslipidemic at baseline became newly classified as dyslipidemic, a significant change which occurred within both races. (Table 6-2) Post-treatment, there was no significant change in the percentage of the cohort classified as dyslipidemic compared to baseline.

Though 84% of participants took at least 90% of the maximum dose of PEG-IFN for the initial 24 weeks of therapy, as the percentage of the maximum dose of PEG-IFN taken (<90%, 90% to 99.9%, and 100%) increased, so did the declines in LDLc and TC from baseline (p for trend=0.004 and 0.02, respectively). (Figure 6-2) Changes in the serum lipid profile differed significantly by race during the first 6 months of therapy. (Figure 6-3) In particular, increases in TG and the decreases in LDLc levels were greater among CAs than AAs (p=0.003 and p<0.0001, respectively). The patterns of decreases in TC levels by race were similar to LDLc changes, although the differences were not statistically significant (p=0.054). In GEE models evaluating the relationship between race and lipid profile measures during 24 weeks of therapy, the interaction between race and time was statistically significant for TG, LDLc, and TC (p<0.05 for all), adjusting for the proportion of PEG-IFN taken and body weight changes. (Table 6-3) Interpreted as race-specific coefficients, compared to AAs, CAs had significantly larger

increases in the natural log of TG (β_{AA}=0.22, p<0.0001; β_{CA}=0.44, p<0.001) and larger decreases in LDLc (β_{AA}=-13.9, p=0.005; β_{CA}=-24.7, p<0.0001) and TC (β_{AA}=-0.08, p<0.0001; β_{CA}=-0.13, p<0.0001) from baseline.

In multivariable assessments adjusting for the amount of PEG-IFN taken, weight change, 6 month virological response, and race, interaction between race and time remained statistically significant for LDLc and TC (p<0.001 for both) indicating that the change in these lipid profile measures differed significantly by race. (Table 6-4) The declines in LDLc and TC were significantly different from zero in both race groups (p<0.01 for all), with the declines being greater among CAs than AAs (LDLc: β_{CA}=-24.1 and β_{AA}=-8.4; natural log of TC: β_{CA}=-0.13 and β_{AA}=-0.07). Compared to females, males had a greater increases TG (β=0.12, p=0.02) and declines HDLc (β=-0.21, p<0.001) and TC (β=-0.05, p=0.03) on the natural log scale. Histological measures of liver disease were also significantly associated with the natural logs of TG and HDLc (fat score β=0.20, p=0.004 and β=-0.07, p=0.01, respectively), LDLc (HAI inflammation score), and TC (Ishak fibrosis score β=-0.02, p=0.03).

6.5   DISCUSSION

During 24 weeks of combination PEG-IFN and ribavirin therapy there were significant changes in TG, LDLc, HDLc, and TC from baseline. Compared to pre-treatment levels, there were also post-treatment changes, but they were limited to 6 month virological responders who underwent a 48 week course of treatment. During the first 24 weeks of therapy, greater amounts of PEG-IFN taken were associated with significantly larger declines in LDLc and TC from pretreatment levels. Lastly, the significant increases in TG and declines in LDLc and TC during the first 24

weeks of treatment significantly differed by race and remained statistically significant after accounting for the amount of PEG-IFN taken and body weight changes.

Few studies have assessed changes in the serum lipid profile during and after therapy for chronic HCV and findings are inconsistent. The significant increase in TG levels during therapy found here is consistent with other study findings.[52, 53] However, the increase in TC post-therapy compared to pretreatment is consistent with one study,[52] but not another.[53] Note, thought, that these studies reported increases in TC during therapy, in contrast to the current study which found significant declines in TC during treatment. In another study, TC levels did not significantly change during or after therapy compared to pretreatment.[54] The different findings across studies suggest the need for further investigation but may be due to the disparate treatment regimens, HCV genotypes with which participants were infected, and other participant characteristics.

The current study is the first epidemiologic study to report an association between declines in LDLc and TC during 24 weeks of therapy and the amount of PEG-IFN taken. Recent *in vitro* work found that the expression scavenger receptor B-1, a lipoprotein receptor for high density lipoprotein, decreased with interferon exposure.[72] These findings suggest that antiviral therapy may change lipoprotein receptor expression, which may in turn impact circulating lipoprotein levels and the serum lipid profile. HCV eradication may likewise result in resolution of liver disease and subsequent changes in lipid and lipoprotein metabolism. The significant racial differences in the changes in TG, LDLc, and TC during the first 6 months of therapy is another novel finding and warrants further evaluation given the racial disparity in virological response between AAs and CAs found in other studies.[10-12]

This study also assessed dyslipidemia, a risk factor for cardiovascular disease (CVD) for which incident cases arose during treatment. However, the prevalence post-treatment did not differ significantly from baseline suggesting that although the CVD risk profile may increase in association with antiviral therapy, the increased risk may be transient. However, CVD risk based on other measures not accounted for in this study, such as inflammatory, atherosclerotic, and arteriolsclerotic assessments, may yield more information to better characterize the impact of antiviral therapy to CVD. Prospective studies utilizing these measures are needed.

This study has limitations, which may influence the results. For instance, numerous side-effects are associated with PEG-IFN therapy including gastrointestinal disturbances, such as nausea, weight loss, and anorexia.[9] Although body weight changes during and after treatment were controlled for in the analysis, it is possible that modifications to dietary intake may have occurred, resulting in significant changes in the serum lipid profile measures. Food intake assessments were not included in the Virahep-C study design. Another limitation was the use of stepwise modeling for variable selection (refer sections 5.5 and 8.1 for discussion of limitations).

This study demonstrates that combination therapy is associated with significant changes in the serum lipid profile and these findings are potentially relevant to antiviral mechanisms of PEG-IFN and ribavirin for the treatment of chronic HCV infection. This study also found that during 24 weeks of therapy, the changes in the serum lipid profile differed by race. Further evaluation of the relationship between serum lipid profile measures and treatment efficacy is warranted to determine if the lipid profile explains any of the racial difference in treatment response between AAs and CAs.

Table 6-1. Cohort characteristics

Feature*	AA (n=135)	CA (n=153)	Overall (n=288)	*p*
Demographics				
Age (years)	49 (46,52)	47 (42,51)	48 (44,52)	0.03
% Male	89 (65.9)	100 (65.4)	189 (65.5)	0.92
Clinical features				
BMI (m=4)	28.8 (26.4,33.4)	27.9 (24.9,31.5)	28.4 (25.3,32.3)	0.007
%Diabetic	21 (15.6)	5 (3.3)	26 (9.0)	0.0003
%Hypertensive	57 (42.2)	30 (19.6)	87 (30.2)	<0.0001
Viral characteristics				
Log_{10} HCV level (m=1)	6.5 (5.6,6.7)	6.5 (5.7,6.8)	6.5 (5.6,6.8)	0.35
HCV genotype[a]				0.004
%1, NOS	6 (4.4)	14 (9.2)	20 (6.9)	0.12
%1a	68 (50.4)	86 (56.2)	154 (53.5)	0.32
%1a/b	1 (0.7)	9 (5.9)	10 (3.5)	0.02
%1b	60 (44.4)	44 (28.8)	104 (36.1)	0.006
Liver disease indicators				
ALT (IU/L)	63 (41,90)	76 (52,140)	71 (46.5,110)	0.0002
AST (IU/L)	54 (36,73)	53 (38,89)	54 (37,80.5)	0.11
Histological measures				
Ishak fibrosis score	2 (1,3)	2 (1,3)	2 (1,3)	0.98
%Ishak fibrosis score ≥ 3	52 (38.5)	60 (39.2)	112 (38.9)	0.90
Fat score	0.5 (0,1)	0.5 (0,1)	0.5 (0,1)	0.16
%Steatosis (>5% present)	80 (59.3)	101 (66.0)	181 (62.9)	0.24
Total HAI inflammation	8 (7,10)	9 (6,11)	8 (6,11)	0.90
Iron score (m=28)				0.10
0	57 (47.5)	81 (57.9)	138 (53.1)	
1	52 (43.3)	49 (35.0)	101 (38.9)	
2	11 (9.2)	10 (7.1)	21 (8.1)	
Baseline serum lipid measures				
TG (mg/dL)	105 (74,165)	97 (74,137)	100 (74,146.5)	0.13
LDLc (mg/dL)	108.8 (83.3,133.1)	118.7 (93.1,141.9)	115.5 (88.1,137.5)	0.02
HDLc (mg/dL)	42.7 (32.6,54.2)	40.7 (33.7,50.7)	41.0 (33.5,52.1)	0.34
TC (mg/dL)	183 (155,204)	185 (160,209)	185 (156,206)	0.22

m=missing

*Each categorical variable is summarized as n (%) with p-values corresponding to a Pearson's chi-square test (nominal variables) or the Jonckheere-Terpstra test (ordinal variables) or exact equivalents, where appropriate; each continuous variable is summarized as a median (interquartile range) with a p-value corresponding to a Wilcoxon rank sum test.

For features with two or more categories, the global p-value is listed in the first row of the feature. Where the global p-value is <0.05, p-values correspond to Pearson's chi-square with comparisons as follows:

[a]Each genotype compared to other categories combined

Table 6-2. Dyslipidemic status during and after therapy compared to baseline

Baseline status	Tx week 24 status		F-up week 24 status (R)		F-up week 24 status (NR)	
	+	−	+	−	+	−
Both races						
+	167 (66.0)	11 (4.4)	113 (63.8)	19 (10.7)	31 (50.0)	10 (16.1)
−	46 (18.2)	29 (11.5)	19 (10.7)	26 (14.7)	5 (8.1)	16 (25.8)
p-value		*<0.0001*		*1.0*		*0.20*
CAs						
+	94 (68.6)	6 (4.4)	76 (65.5)	12 (10.3)	7 (46.7)	1 (6.7)
−	25 (18.3)	12 (8.8)	13 (11.2)	15 (12.9)	1 (6.7)	6 (40.0)
p-value		*0.006*		*0.84*		*1.0*
AAs						
+	73 (62.9)	5 (4.3)	37 (60.7)	7 (11.5)	24 (51.1)	9 (19.2)
−	21 (18.1)	17 (14.7)	6 (9.8)	11 (18.0)	4 (8.5)	10 (21.3)
p-value		*0.002*		*0.78*		*0.17*

Tx=dyslipidemic status after 24 weeks of treatment

F-up=dyslipidemic status 24 weeks after stopping treatment

R=treatment 24 virological responders (underwent 48 weeks of treatment)

NR=treatment week 24 virological non-responders (underwent only 24 weeks of treatment)

p=McNemar's test for homogeneity ($p < 0.05$ reflects significant change in dyslipidemic status)

Table 6-3. Adjusted* GEE models

Predictors	ln(TG) β (95%CI)	p	LDLc β (95%CI)	p	ln(HDLc) β (95%CI)	p	ln(TC) β (95%CI)	p
	All participants: baseline through treatment week 24(week 24)							
Week 24`	NA	NA	NA	NA	-0.22 (-0.25,-0.19)	<0.0001	NA	NA
AA**	0.10 (-0.03,0.22)	0.12	-7.3 (-15.6,1.0)	0.08	0.05 (-0.02,0.11)	0.16	-0.01 (-0.06,0.03)	0.63
Race X time⁻		0.01		<0.0001		0.71		0.02
CA: week 24`	0.40 (0.31,0.49)	<0.0001	-24.7 (-29.5,-20.0)	<0.0001			-0.13 (-0.16,0.10)	<0.0001
AA: week 24`	0.22 (0.13,0.32)	<0.0001	-8.4 (-14.2,-2.5)	0.005			-0.08 (-0.11,-0.05)	<0.0001
	Treatment week 24 virological responders only: baseline through follow-up week 24 (week 72)							
Global time^		<0.0001		<0.0001		<0.0001		<0.0001
Week 24`	0.39 (0.31,0.47)	<0.0001	NA	NA	-0.22 (-0.25,-0.19)	<0.0001	NA	NA
Week 72~	0.08 (0.02,0.15)	0.02	NA	NA	0.01 (-0.02,0.04)	0.55	NA	NA
AA**	-0.10 (-0.24,0.03)	0.14	0.09 (-11.1,11.3)	0.99	0.07 (-0.003,0.15)	0.06	0.01 (-0.05,0.07)	0.79
Race X time⁻		0.27		0.02		0.71		0.03
CA: week 24`			-26.4 (-31.8,-21.0)	<0.0001			-0.13 (-0.17,-0.10)	<0.0001
AA: week 24`			-13.9 (-22.7,-5.1)	0.002			-0.09 (-0.13,-0.04)	<0.0001
CA: week 72~			10.2 (5.7,14.7)	<0.0001			0.07 (0.04,0.09)	<0.0001
AA: week 72~			6.4 (-0.6,13.5)	0.07			0.04 (0.004,0.07)	0.03
	Treatment week 24 virological non-responders only: baseline through follow-up week 24 (week 48)							
Global time^		0.01		0.22		<0.0001		0.0006
Week 24`	0.19 (0.06,0.32)	0.004	-4.7 (-10.3,0.8)	0.10	-0.22 (-0.28,-0.16)	<0.0001	-0.07 (-0.10,-0.03)	<0.0001
Week 48~	0.03 (-0.08,0.13)	0.62	-2.1 (-7.8,3.5)	0.46	0.02 (-0.04,0.07)	0.50	-0.01 (-0.04,0.03)	0.65
AA**	0.11 (-0.10,0.31)	0.31	3.0 (-11.7,17.6)	0.69	0.01 (-0.12,0.14)	0.91	0.04 (-0.05,0.13)	0.42
Race X time⁻		0.81		0.44		0.24		0.81

*Adjusted for the proportion of PEG-IFN taken, body weight change (not shown), and race (regardless of statistical significance)

**CA reference

^p-value indicates overall test for time only

`Indicates treatment week 24 assessment (on-treatment change from baseline)

~Indicates follow-up week 24 assessment (post-treatment change from baseline)

⁻ Non-statistically significant race/time interactions (p>0.05) were not included in models

NA=Not applicable due to statistically significant race/time interaction (refer to race and time specific coefficients)

Table 6-4. Multivariable GEE models

Baseline characteristic	ln(TG) β (95%CI)	p	LDLc β (95%CI)	p	ln(HDLc) β (95%CI)	p	ln(TC) β (95%CI)	p
AA race*	0.01 (-0.11,0.12)	0.92	-0.5 (-9.3,8.3)	0.91	0.02 (-0.04,0.09)	0.48	0.01 (-0.04,0.06)	0.75
Global test of time		<0.001		0.007		<0.001		<0.001
Tx week 24	0.34 (0.27,0.41)	<0.001	NA	NA	-0.22 (-0.25,-0.19)	<0.001	NA	NA
F-up week 24	0.07 (0.01,0.13)	0.02	NA	NA	0.01 (-0.01,0.04)	0.36	NA	NA
Race X time⁻		0.10		<0.001		0.96		<0.001
CA: Tx week 24			-24.1 (-29.0,-19.2)	<0.001			-0.13 (-0.15,-0.10)	<0.001
AA: Tx week 24			-8.4 (-14.1,-2.6)	0.004			-0.07 (-0.11,-0.04)	<0.001
CA: F-up week 24			9.3 (5.0,13.5)	<0.001			0.06 (0.04,0.09)	<0.001
AA: F-up week 24			2.1 (-2.8,7.0)	0.40			0.02 (-0.01,0.04)	0.23
Male gender**	0.12 (0.02,0.22)	0.02			-0.21 (-0.28,-0.14)	<0.001	-0.05 (-0.10,-0.004)	0.03
Ishak fibrosis score							-0.02 (-0.03,-0.002)	0.03
Fat score	0.20 (0.12,0.27)	0.004			-0.07 (-0.13,-0.02)	0.01		
Total HAI inflammation^			-55.0 (-108.7,-1.3)	0.045				

NOTE: Models were adjusted for the proportion of PEG taken, body weight change, 6 month responder status (not shown), and race regardless of statistical significance.

NA=Not applicable due to statistically significant race/time interaction (refer to race and time specific coefficients)

Transformations as noted: ` = natural log; ^ = 1 – reciprocal.

-Non-statistically significant race/time interactions (p>0.05) were not included in models

*CA reference

**Female reference

Table 6-5. Supplement 1a: Changes in TG during and after therapy by categorical features

Feature	n	Median (IQR)	p*	n	Median (IQR)	p*	n	Median (IQR)	p*
		Tx – BL (n=253)			**F-up – BL (R) (n=177)**			**F-up – BL (NR) (n=62)**	
Demographics									
Race			0.005			0.50			0.97
AA race	116	21 (-10.5,60.5)		61	2 (-19,33)		47	9 (-28,40)	
CA race	137	40 (8,91)		116	10 (-19,42)		15	9 (-25,30)	
Gender						0.96			0.43
Male	167	24 (-12,87)	0.16	113	7 (-25,43)		45	10 (-11,33)	
Female	86	39 (8,83)		64	9 (-13,28.5)		17	-10 (-32,30)	
Health insurance			0.002			0.18			0.68
Uninsured	45	12 (-21,49)		30	8.5 (-31,42)		8	20.5 (-12,36)	
Public	54	21.5 (-23,56)		37	-9 (-21,33)		11	9 (-11,40)	
Private	149	41 (5,91)		106	13 (-9,40)		43	1 (-31,32)	
Educational attainment			0.06			0.67			0.28
<High school	43	17 (-15,59)		25	7 (-17,33)		12	9.5 (-30.5,43)	
High school degree	61	37 (-9,85)		39	8 (-28,22)		15	21 (-24,68)	
Some college	83	28 (-7,85)		58	10 (-17,44)		20	-1 (-16.5,31.5)	
≥College degree	59	53 (14,88)		49	15 (-16,40)		14	-4.5 (-35,11)	
Employed	164	40 (3,90.5)	0.02	114	8 (-13,37)	0.34	44	6 (-29.5,32.5)	0.75
Unemployed	86	22.5 (-15,54)		60	8.5 (-31,44.5)		18	10 (-11,40)	
Health risk behaviors									
Current smoker	94	25 (-15,82)	0.29	69	5 (-19,33)	0.14	22	20.5 (-11,42)	0.09
Non-smoker	154	32.5 (4,86)		104	12.5 (-16,42)		40	-1 (-29.5,29.0)	
≥2 alcoholic drinks/week	48	23 (-7.5,68.5)	0.35	33	2 (-25,32)	0.39	15	20 (-47,32)	0.83
<2 alcoholic drinks/week	199	35 (1,86)		139	12 (-16,41)		47	9 (-24,40)	
General clinical features									
Diabetic	23	17 (-12,98)	0.79	11	2 (-53,58)	0.93	11	10 (-11,19)	0.85
Non-diabetic	230	30.5 (1,83)		166	8 (-19,38)		51	3 (-28,40)	
Hypertensive	73	25 (-7,66)	0.13	44	-2 (-25.5,33.5)	0.36	28	3.5 (-47.5,20.5)	0.10
Non-hypertensive	180	33 (0.5,87.5)		133	9 (-17,42)		34	10 (-11,40)	
Viral characteristics									
HCV genotype			0.45			0.19			0.16
1, NOS	16	49.5 (-10.5,95.5)		15	-3 (-43,17)		3	-97 (-110,-25)	
1a	135	28 (1,73)		93	9 (-12,44)		34	9.5 (-24,34)	
1b	92	26.5 (-7,82.5)		61	4 (-28,33)		24	19 (8.5,35.5)	
1a/1b	10	87.5 (-24,158)		8	33.5 (-35,69.5)		1	9 (NA,NA)	
Liver disease indicators			0.046			0.90			0.96
Ishak fibrosis score ≥ 3	102	21.5 (-7,54)		67	7 (-16,38)		30	10.5 (-28,33)	
<3	151	38 (3,91)		110	9 (26,40)		32	2 (-23.5,36)	
Steatosis (>5% present)	160	32.5 (-2,85)	0.77	110	8 (-19,40)	0.89	39	-5 (-47,31)	0.045
No steatosis	93	26 (3,79)		67	9 (-21,38)		23	10 (-4,49)	
Iron score			0.0006			0.49			0.33
0	123	40 (5,88)		90	10 (-17,40)		25	3 (-22,27)	
1	86	12 (-24,43)		56	5.5 (-18,41)		26	3.5 (-25,40)	
2	19	49 (-21,119)		14	-0.5 (-57,17)		5	31 (31,32)	

Tx=treatment week 24 (during treatment) ; BL=baseline (pre-treatment); F-up=Follow-up week 24 (post-treatment)

*p-value corresponds to the Wilcoxon rank sum or Kruskal-Wallis test.

R=6 month virological responders

NR=6 month nonresponders

Table 6-6. Supplement 1b: Changes in LDLc during and after therapy by categorical features

Feature	Tx – BL (n=253)			F-up – BL (R) (n=177)			F-up – BL (NR) (n=62)		
	n	Median (IQR)	p*	n	Median (IQR)	p*	n	Median (IQR)	p*
Demographics									
Race			<0.001			0.33			0.51
AA race	116	-7.4 (-24.1,-7.6)		61	4.5 (12.2,22.2)		47	-3.9 (-17.4,13)	
CA race	137	-20.4 (-44.5,-6.5)		116	9.4 (-7.7,28.3)		15	1.7 (-9.5,17.9)	
Gender			0.11			0.56			0.40
Male	167	-17.2 (-38.4,-2.1)		113	7.2 (-9.7,24.5)		45	-4.1 (-17.4,7.8)	
Female	86	-11.5 (-32.0,5.0)		64	7.7 (-7.0,27.0)		17	1.1 (-9.5,16.7)	
Health insurance			0.18			0.61			0.20
Uninsured	45	-7.5 (-28.0,8.9)		30	12.9 (-2.9,24.0)		8	-7.7 (-23.0,2.2)	
Public	54	-17.3 (-35.0,0.0)		37	7.5 (-9.2,36.1)		11	-6.1 (-24.7,7.8)	
Private	149	-15.9 (-41.2,-2.1)		106	5.0 (-9.4,24.5)		43	1.1 (-13.8,17.9)	
Educational attainment			0.74			0.83			0.10
<High school	43	-20.4 (-38.4,-7.4)		25	8.4 (-9.4,19.5)		12	2.3 (-26.1,14.3)	
High school degree	61	-12.2 (-33.4,1.4)		39	6.8 (-9.7,23.3)		15	-12.5 (-27.3,-4.5)	
Some college	83	-15.0 (-37.0,2.1)		58	6.9 (-7.5,23.0)		20	1.2 (-11.9,19.1)	
≥College degree	59	-13.3 (-41.2,6.0)		49	9.8 (-8.4,35.0)		14	1.6 (-3.9,13.0)	
Employed	164	-14.2 (-35.1,2.3)	0.36	114	7.6 (-7.1,28.3)	0.46	44	-1 (-16.7,17.3)	0.57
Unemployed	86	-18.1 (-42.8,-2.1)		60	5.5 (-10.7,23.2)		18	-5.2 (-15.1,7.8)	
Health risk behaviors									
Current smoker	94	-16.9 (-37.5,-3.8)	0.25	69	9.7 (-7.5,36.1)	0.22	22	-5.7 (-27.4,13.0)	0.29
Non-smoker	154	-14.2 (-37.0, 3.4)		104	5.3 (-9.0,24.0)		40	-0.8 (-9.8,11.6)	
≥2 alcoholic drinks/week	48	-12.8 (-27.3,5.6)	0.22	33	3.7 (-9.7,23.3)	0.61	15	-4.6 (-26.7,17.7)	0.57
<2 alcoholic drinks/week	199	-15.9 (-40.9,1.1)		139	7.6 (-9.0,28.3)		47	-2.7 (-15.1,13.0)	
General clinical features									
Diabetic	23	-8.5 (-22.5,2.3)	0.15	11	16.8 (-1.7,47.2)	0.23	11	0.7 (-5.3,20.7)	0.35
Non-diabetic	230	-15.1 (-38.4,1.1)		166	6.7 (-9.2,24.5)		51	-4.1 (-17.4,13.0)	
Hypertensive	73	-8.5 (-26.1, 2.3)	0.07	44	16.0 (4.0,33.0)	0.02	28	-1.8 (-18.0,8.5)	0.83
Non-hypertensive	180	-17.6 (-42.1, 0.1)		133	3.6 (-10.5,24.2)		34	-3.2 (-15.1,16.9)	
Viral characteristics									
HCV genotype			0.06			0.42			0.18
1, NOS	16	-18.1 (-59,-9.4)		15	17.1 (-3.5,22.3)		3	17.9 (5.5,31.4)	
1a	135	-15.9 (-36.9,-0.3)		93	3.7 (-10.5,28.3)		34	-0.4 (-12.5,16.9)	
1b	92	-13.0 (-29.0, 4.6)		61	8.5 (-2.4,24.5)		24	-4.9 (-18.0,1.6)	
1a/1b	10	-41.7 (-60.8,-7.7)		8	22.3 (-0.6,35.1)		1	-9.5 (NA,NA)	
Liver disease indicators									
Ishak fibrosis score ≥ 3	102	-14.3 (-29.4,-4.1)	0.70	67	10.4 (-1.1,33.9)	0.07	30	-2.8 (-18.8,7.8)	0.82
<3	151	-17.2 (-42.1,3.7)		110	2.1 (-9.4,24.0)		32	-3.3 (-13.2,17.3)	
Steatosis (>5% present)	160	-17.4 (-37.0,-2.3)	0.25	110	7.6 (-8.4,25.3)	0.81	39	0.3 (-18.5,17.7)	0.65
No steatosis	93	-9.5 (-38.1,5.3)		67	5.4 (-9.4,28.3)		23	-4.5 (-15.1,7.8)	
Iron score			0.87			0.46			0.84
0	123	-16.5 (-41.2,2.1)		90	7.1 (-9.4,24.5)		25	-2.7 (-13.8,9.2)	
1	86	-14.6 (-38.8,0.0)		56	9.8 (-5.5,29.8)		26	0.9 (-17.4,17.9)	
2	19	-14.6 (-29.9,-5.6)		14	-1.8 (-7.1,17.1)		5	-4.1 (-15.1,3.9)	

Tx=treatment week 24 (during treatment) ; BL=baseline (pre-treatment); F-up=Follow-up week 24 (post-treatment)
*p-value corresponds to the Wilcoxon rank sum or Kruskal-Wallis test.
R=6 month virological responders
NR=6 month nonresponders

Table 6-7. Supplement 1c: Changes in HDLc during and after therapy by categorical features

Feature	Tx – BL (n=253)			F-up – BL (R) (n=177)			F-up – BL (NR) (n=62)		
	n	Median (IQR)	p*	n	Median (IQR)	p*	n	Median (IQR)	p*
Demographics									
Race			0.65			0.70			0.35
AA race	116	-8.3 (-14.2,-2.7)		61	-0.1 (-3.9,5.2)		47	0.8 (-7.6,6.9)	
CA race	137	-8.0 (-13.9,-3.5)		116	1.1 (-5.6,6.9)		15	-3.1 (-7.1,3.4)	
Gender			0.004			0.16			0.15
Male	167	-7.2 (-12.8,-2.8)		113	-1.5 (-3.9,5.6)		45	-1.0 (-7.1,3.4)	
Female	86	-10.9 (-19.6,-4.3)		64	-1.7 (-6.9,6.1)		17	3.9 (-5.0,12.6)	
Health insurance			0.66			0.94			0.43
Uninsured	45	-8.4 (-16.2,-4.5)		30	-0.2 (-5.0,8.2)		8	3.0 (-1.5,9.2)	
Public	54	-8.1 (-12.9,-2.4)		37	2.7 (-5.2,8.2)		11	-1.7 (-8.9,1.9)	
Private	149	-7.8 (-14.2,-3.2)		106	0.5 (-5.6,5.1)		43	-0.9 (-7.6,6.9)	
Educational attainment			0.04			0.50			0.46
<High school	43	-5.8 (-12.1,-1.8)		25	0.7 (-2.9,4.0)		12	1.2 (-3.4,6.1)	
High school degree	61	-7.6 (-12.1,-0.3)		39	2.7 (-3.8,9.5)		15	-5.0 (-8.9,3.2)	
Some college	83	-10.4 (-16.3,-5.3)		58	-0.5 (-6.7,5.3)		20	0.9 (-1.6,10.8)	
≥College degree	59	-8.8 (-14.2,-4.2)		49	0.6 (-5.2,5.1)		14	0 (-7.2,6.4)	
Employed	164	-8.1 (-13.7,-3.3)	0.80	114	0.8 (-5.2,6.3)	0.71	44	-0.1 (-7.4,7.3)	0.86
Unemployed	86	-8.1 (-15.4,-2.8)		60	1.1 (-6.5,5.1)		18	0.3 (-3.6,1.9)	
Health risk behaviors									
Current smoker	94	-7.3 (-14.1,-1.2)	0.0501	69	2.8 (-2.9,8.2)	0.006	22	0.6 (-7.1,6.2)	0.96
Non-smoker	154	-9.1 (-14.6,-4.3)		104	-1.7 (-6.8,4.7)		40	-1.0 (-6.8,6.7)	
≥2 alcoholic drinks/week	48	-8.8 (-17.5,-4.8)	0.30	33	2.0 (-3.7,7.4)	0.33	15	3.2 (-4.9,6.9)	0.42
<2 alcoholic drinks/week	199	-7.8 (-14.1,-3.0)		139	0.1 (-5.9,5.2)		47	-0.9 (-7.9,6.4)	
General clinical features									
Diabetic	23	-6.3 (-12.8,-1.6)	0.31	11	1.5 (-8.3,8.0)	0.83	11	0.9 (-7.9,5.9)	0.98
Non-diabetic	230	-8.1 (-14.2,-3.3)		166	0.8 (-5.5,5.6)		51	-0.9 (-7.1,6.9)	
Hypertensive	73	-8.4 (-13.9,-3.6)	0.81	44	0.0 (-5.1,6.3)	0.87	28	1.3 (-2.5,7.1)	0.13
Non-hypertensive	180	-8.0 (-14.2,-2.8)		133	0.9 (-5.9,5.9)		34	-1.9 (-7.6,3.9)	
Viral characteristics									
HCV genotype			0.35			0.12			0.25
1, NOS	16	-7.7 (-10.5,-3.8)		15	1.4 (-5.1,6.3)		3	-1.4 (-4.9,0.9)	
1a	135	-7.7 (-12.9,-3.0)		93	1.8 (-5.1,7.8)		34	-0.4 (-8.0,6.9)	
1b	92	-9.4 (-14.8,-3.5)		61	-1.9 (-7.7,4.2)		24	1.0 (-4.5,6.7)	
1a/1b	10	-4.7 (-15.0,3.7)		8	3.2 (-1.4,7.3)		1	-23.0 (NA,NA)	
Liver disease indicators									
Ishak fibrosis score ≥ 3	102	-7.8 (-14.2,-2.8)	0.43	67	2.4 (-6.3,7.4)	0.72	30	1.4 (-3.6,10.4)	0.15
<3	151	-8.5 (-14.2,-3.3)		110	0.3 (-5.1,4.9)		32	-1.2 (-7.4,3.7)	
Steatosis (>5% present)	160	-8.1 (-13.7,-3.4)	0.996	110	-0.1 (-6.0,5.1)	0.23	39	0.9 (-4.9,6.9)	0.19
No steatosis	93	-7.8 (-16.1,-2.4)		67	2.1 (-5.1,8.2)		23	-2.1 (-8.9,3.4)	
Iron score			0.06			0.38			0.60
0	123	-8.8 (-14.2,-4.3)		90	-0.9 (-6.1,5.1)		25	0.8 (-5.9,3.4)	
1	86	-7.0 (-13.9,-0.5)		56	2.8 (-5.2,8.2)		26	-1.0 (-8.0,7.7)	
2	19	-6.8 (-11.1,-2.0)		14	0.1 (-3.9,3.9)		5	-1.3 (-8.9,-0.9)	

Tx=treatment week 24 (during treatment) ; BL=baseline (pre-treatment); F-up=Follow-up week 24 (post-treatment)

*p-value corresponds to the Wilcoxon rank sum or Kruskal-Wallis test.

R=6 month virological responders

NR=6 month nonresponders

Table 6-8. Supplement 1d: Changes in TC during and after therapy by categorical features

Feature	n	Tx – BL (n=253) Median (IQR)	p*	n	F-up – BL (R) (n=177) Median (IQR)	p*	n	F-up – BL (NR) (n=62) Median (IQR)	p*
Demographics									
Race			0.054			0.33			0.60
AA race	116	-13.5 (-30,2.5)		61	7 (-7,19)		47	-6 (-19,16)	
CA race	137	-18 (-36,-4)		116	9.5 (-7,30.5)		15	5 (-20,13)	
Gender			0.23			0.94			0.10
Male	167	-18 (-34,-3)		113	9 (-6,23)		45	-8 (-19,8)	
Female	86	-14 (-30.0,7)		64	6 (-8.5,28.5)		17	7 (-1,13)	
Health insurance			0.80			0.94			0.67
Uninsured	45	-20 (-36,-2)		30	9 (0,21)		8	-1 (-25,6)	
Public	54	-20.5 (-38,-5)		37	3 (-7,33)		11	-6 (-26,28)	
Private	149	-17 (-30,-1)		106	9 (-8,25)		43	-5 (-18,17)	
Educational attainment			0.16			0.90			0.43
<High school	43	-21 (-35,-11)		25	8 (-6,15)		12	0 (-25,15.5)	
High school degree	61	-14 (-27,2)		39	7 (-2,24)		15	-16 (-26,13)	
Some college	83	-21 (-38,-2)		58	8.5 (-4,21)		20	0 (-13,21.5)	
≥College degree	59	-15 (-27,7)		49	13 (-9,37)		14	2.5 (-15,8)	
Employed	164	-14 (-28.5,2)	0.008	114	11 (0,30)	0.04	44	-7 (-19,18)	0.89
Unemployed	86	-25.5 (-43,-5)		60	2.5 (-12.5,22)		18	2.5 (-19,11)	
Health risk behaviors									
Current smoker	94	-19 (-32,-4)	0.36	69	13 (-2,33)	0.24	22	-6 (-22,13)	0.58
Non-smoker	154	-15 (-33,2)		104	7.5 (-8.5,23.7)		40	-0.5 (-15.5,14.5)	
≥2 alcoholic drinks/week	48	-19 (-29,-7)	0.95	33	5 (-7,24)	0.69	15	-5 (-28,8)	0.44
<2 alcoholic drinks/week	199	-16 (-35,0)		139	9 (-7,30)		47	0 (-19,17)	
General clinical features									
Diabetic	23	-13 (-25,10)	0.09	11	3 (0,53)	0.54	11	6 (-13,19)	0.37
Non-diabetic	230	-17 (-35,-2)		166	9 (-8,25)		51	-7 (-19,13)	
Hypertensive	73	-17 (-30,2)	0.67	44	13 (1.5,34.5)	0.15	28	-3 (-18,12.5)	0.72
Non-hypertensive	180	-15 (-35,-1)		133	7 (-9,24)		34	-3 (-19,13)	
Viral characteristics									
HCV genotype			0.39			0.57			0.40
1, NOS	16	-20 (-50.5,-7.5)		15	7 (-6,20)		3	8 (-13,8)	
1a	135	-14 (-32,1)		93	9 (-11,32)		34	3 (-19,17)	
1b	92	-17.5 (-33.5,2)		61	7 (-3,20)		24	19 (-8.5,35.5)	
1a/1b	10	-25 (-46,-16)		8	23.5 (8.5,44.5)		1	31 (NA, NA)	
Liver disease indicators			0.98			0.055			0.99
Ishak fibrosis score ≥ 3	102	-17 (-32,-1)		67	14 (2,37)		30	-1 (-20,13)	
<3	151	-17 (-36,2)		110	5 (-9,23)		32	-5.5 (-17,14.5)	
Steatosis (>5% present)	160	-19 (-35,-1.5)	0.18	110	8.5 (-7,25)	0.999	39	-1 (-20,11)	0.35
No steatosis	93	-14 (-29,0)		67	9 (-8,30)		23	-6 (-16,24)	
Iron score			0.86			0.27			0.95
0	123	-19 (-30,-2)		90	9 (-7,24)		25	-1 (-15,13)	
1	86	-16 (-36,0)		56	9 (-3.5,33.5)		26	0.5 (-22,19)	
2	19	-11 (-34,0)		14	-3.5 (-12,17)		5	-11 (-16,5)	

Tx=treatment week 24 (during treatment) ; BL=baseline (pre-treatment); F-up=Follow-up week 24 (post-treatment)

*p-value corresponds to the Wilcoxon rank sum or Kruskal-Wallis test.

R=6 month virological responders

NR=6 month nonresponders

Table 6-9. Supplement 2a: Relative risk of dyslipidemia by selected predictors (1 of 2)

Feature	RR* (95%CI)	p
Demographics		
AA	0.96 (0.86,1.07)	0.50
AA X time[−]		0.63
Age (years)**	1.05 (0.97,1.13)	0.22
Age X time[−]		0.48
Male	1.23 (1.09,1.39)	0.001
Male X time[−]		0.58
Health insurance[a]		0.97
Uninsured	1.01 (0.89,1.15)	0.86
Public	0.99 (0.87,1.13)	0.92
Insurance X time[−]		0.10
Educational attainment[b]		0.09
High school degree	0.91 (0.78,1.06)	0.22
Some college	0.89 (0.77,1.04)	0.14
$\geq$College degree	1.02 (0.89,1.18)	0.74
Education X time[−]		0.15
Employed	1.05 (0.94,1.17)	0.37
Employed X time[−]		0.12
Health risk behaviors		
Current smoker	1.09 (0.99,1.20)	0.09
Smoking X time[−]		0.26
$\geq$2 Alcoholic drinks/week	0.88 (0.77,1.003)	0.06
Alcohol intake X time[−]		0.31
General clinical features		
BMI***	1.07 (1.02,1.12)	0.003
BMI X time[−]		0.92
Waist to hip ratio^	2.22 (1.41,3.48)	0.001
Waist to hip ratio X time[−]		0.56
Diabetic	1.22 (1.09,1.37)	<0.001
Diabetic X time[−]		0.66
Hypertensive	1.03 (0.92,1.16)	0.57
Hypertensive X time[−]		0.96

Transformations as noted: ^ = 1 – reciprocal

*Adjusting for race, the proportion of PEG-IFN taken between time points (time dependent covariate), time, and 6 month responder status.

Reference group for regression models as follows: [a]Private insurance; [b]Less than high school degree

**Per 10 unit increase; ***Per 5 unit increase

–Not statistically significant (p>0.05) interaction terms not included in models.

Table 6-10. Supplement 2b: Relative risk of dyslipidemia by selected predictors (2 of 2)

Feature	RR* (95%CI)	p
Viral characteristics		
Log$_{10}$ HCV level	1.11 (1.03,1.19)	0.007
Viral level X time$^-$		0.38
HCV genotype[a]		0.02
1, NOS	1.02 (0.84,1.24)	0.86
1b	1.002 (0.90,1.12)	0.97
1a/b	1.20 (1.06,1.36)	0.003
HCV Genotype X time$^-$		0.72
Liver disease indicators		
ALT (IU/L)`	1.03 (0.95,1.11)	0.51
ALT X time$^-$		0.67
AST (IU/L)`	1.02 (0.92,1.12)	0.74
AST X time$^-$		0.34
Alk phosphatase (IU/L)`	1.11 (0.96,1.29)	0.15
Alk phosphatase X time$^-$		0.46
Total bilirubin (mg/dL)^	1.03 (0.96,1.10)	0.45
Total bilirubin X time$^-$		0.76
INR		
INR X time$^-$		0.02
WBC count (10^3/mL)	1.03 (1.01,1.05)	0.002
WBC X time$^-$		0.72
Platelet count (10^3/mL)***	1.05 (0.98,1.13)	0.14
Platelet count X time$^-$		0.11
Ferritin (ng/mL)***	1.001 (0.98,1.02)	0.89
Ferritin X time$^-$		0.95
Albumin (g/dL)	1.01 (0.87,1.18)	0.88
Albumin X time$^-$		0.88
%Iron/TIBC**	1.01 (0.99,1.02)	0.34
%Iron/TIBC X time$^-$		0.88
Ishak score	1.01 (0.98,1.04)	0.65
Ishak score X time$^-$		0.11
Ishak score $\geq$ 3	1.03 (0.93,1.13)	0.63
Ishak score $\geq$ 3Xtime$^-$		0.88
Fat score	1.08 (1.01,1.16)	0.02
Fat score X time$^-$		0.37
Steatosis (>5% present)	1.09 (0.98,1.22)	0.12
Steatosis X time$^-$		0.63
Total HAI inflammation^	0.59 (0.34,1.03)	0.06
HAI inflammation X time$^-$		0.08
Iron score[b]		0.24
1	1.09 (0.98,1.21)	0.12
2	0.98 (0.80,1.19)	0.83
Iron score X time$^-$		0.16

Transformations as noted: ` = natural log; ^ = 1 – reciprocal

*Adjusting for race, the proportion of PEG-IFN taken between time points (time dependent covariate), time, and 6 month responder status.

Reference group for regression models as follows: [a]1a genotype; [b]0 Iron score

**Per 10 unit increase; ***Per 100 unit increase

–Not statistically significant (p>0.05) interaction terms not included in models.

Table 6-11. Supplement 3a: GEE models of each lipid profile measure by selected predictors (1 of 4)

Feature	ln(TG) β* (95%CI)	p	LDLc β* (95%CI)	p	ln(HDLc) β* (95%CI)	p	ln(TC) β* (95%CI)	p
Demographics								
AA	-0.02 (-0.13,0.08)	0.67			0.04 (-0.02,0.11)	0.21		
AA X time⁻		0.07		<0.001		0.97		<0.001
Age (years)**	0.001 (-0.06,0.06)	0.99	2.1 (-2.5,6.8)	0.37	-0.01 (-0.06,0.03)	0.57		
Age X time⁻		0.45		0.23		0.67		0.04
Male	0.12 (0.02,0.22)	0.02	-6.6 (-14.7,1.5)	0.11	-0.22 (-0.29,0.15)	<0.001	-0.07 (-0.11,-0.02)	0.007
Male X time⁻		0.37		0.58		0.31		0.84
Health insuranceᵃ				0.41		0.78		0.25
Uninsured			-3.7 (-14.8,7.4)	0.52	0.02 (-0.07,0.11)	0.68	-0.04 (-0.11,0.03)	0.25
Public			-6.0 (-15.4,3.4)	0.21	-0.02 (-0.10,0.06)	0.64	-0.04 (-0.09,0.02)	0.17
Insurance X time⁻		0.007		0.72		0.68		0.85
Educational attainmentᵇ		0.59		0.10		0.30		0.08
High school degree	-0.04 (-0.21,0.13)	0.63	-4.7 (-15.5,6.2)	0.40	0.02 (-0.08,0.12)	0.63	-0.02 (-0.09,0.05)	0.51
Some college	-0.001 (-0.16,0.16)	0.99	-4.8 (-15.4,5.8)	0.38	0.08 (-0.02,0.18)	0.12	-0.01 (-0.07,0.06)	0.78
≥College degree	0.05 (-0.10,0.21)	0.50	6.9 (-5.0,18.9)	0.25	0.07 (-0.03,0.18)	0.16	0.06 (-0.01,0.13)	0.12
Education X time⁻		0.052		0.72		0.63		0.33
Employed			3.7 (-4.2,11.6)	0.36	0.01 (-0.06,0.08)	0.79		
Employed X time⁻		0.03		0.43		0.94		0.01
Health risk behaviors								
Current smoker	0.11 (0.0002,0.21)	0.0496	1.3 (-6.7,9.3)	0.75	-0.05 (-0.12,0.01)	0.11	0.01 (-0.04,0.06)	0.70
Smoking X time⁻		0.18		0.22		0.06		0.25
≥2 Alcoholic drinks/week	-0.08 (-0.20,0.03)	0.15	-0.6 (-9.5,8.2)	0.89	0.09 (0.01,0.17)	0.03	0.001 (-0.05,0.05)	0.97
Alcohol intake X time⁻		0.59		0.15		0.23		0.59

*Adjusting for race, the proportion of PEG-IFN taken between time points (time dependent covariate), time, and 6 month responder status.

Reference group for regression models as follows: ᵃPrivate insurance; ᵇLess than high school degree

**Per 10 unit increase

–Not statistically significant (p>0.05) interaction terms not included in models.

Table 6-12. Supplement 3b: GEE models of each lipid profile measure by selected predictors (2 of 4)

Feature	ln(TG) β* (95%CI)	p	LDLc β* (95%CI)	p	ln(HDLc) β* (95%CI)	p	ln(TC) β* (95%CI)	p
General clinical features								
BMI**			0.8 (-2.2,3.8)	0.60	-0.05 (-0.08,-0.02)	<0.001	-0.001 (-0.02,0.02)	0.92
BMI X time⁻		0.03		0.43		0.84		0.45
Waist to hip ratio (WHR)^			-7.8 (-38.7,23.0)	0.62	-0.65 (-0.93,-0.37)	<0.001		
WHR X time⁻		0.01		0.26		0.95		0.003
Diabetic	0.12 (-0.05,0.29)	0.18	-2.0 (-15.2,11.1)	0.77	-0.16 (-0.26,-0.06)	0.002	-0.03 (-0.11,0.05)	0.40
Diabetic X time⁻		0.98		0.09		0.90		0.09
Hypertensive			-3.1 (-11.0,4.9)	0.45	-0.10 (-0.17,0.03)	0.004	-0.03 (-0.08,0.02)	0.26
Hypertensive X time⁻		0.049		0.06		0.49		0.89
Viral characteristics								
Log₁₀ HCV level	0.08 (0.01,0.15)	0.02	2.5 (-2.6,7.7)	0.34	-0.02 (-0.06,0.02)	0.34	0.02 (-0.01,0.05)	0.21
Viral level X time⁻		0.77		0.30		0.88		0.22
HCV genotype[a]						0.69		0.44
1, NOS					0.01 (-0.12,0.15)	0.84	0.07 (-0.03,0.17)	0.20
1a/1b					-0.10 (-0.27,0.07)	0.24	-0.04 (-0.14,0.05)	0.39
1b					-0.01 (-0.08,0.06)	0.85	0.01 (-0.04,0.06)	0.74
HCV Genotype X time⁻		0.0004		0.02		0.72		0.37

NOTE: β coefficients represent unit difference for each continuous variable on the raw scale or on a transformed scale as noted:

^ = 1 – reciprocal

*Adjusting for race, the proportion of PEG-IFN taken between time points (time dependent covariate), time, and 6 month responder status.

**Per 5 unit increase

[a]1a genotype reference group

–Not statistically significant (p>0.05) interaction terms not included in models.

Table 6-13. Supplement 3c: GEE models of each lipid profile measure by selected predictors (3 of 4)

Feature	ln(TG) β* (95%CI)	p	LDLc β* (95%CI)	p	ln(HDLc) β* (95%CI)	p	ln(TC) β* (95%CI)	p
Liver disease indicators								
ALT (IU/L)`	0.02 (-0.06,0.10)	0.63	-1.5 (-7.0,3.9)	0.59	-0.04 (-0.09,0.01)	0.15	-0.02 (-0.05,0.01)	0.27
ALT X time⁻		0.40		0.88		0.89		0.90
AST (IU/L)`	0.06 (-0.03,0.14)	0.23	-1.9 (-8.3,4.4)	0.55	-0.02 (-0.09,0.04)	0.45	-0.01 (-0.05,0.03)	0.51
AST X time⁻		0.22		0.88		0.92		0.93
Alk phosphatase (IU/L)`			-2.6 (-13.4,8.2)	0.64	-0.10 (-0.21,0.0003)	0.051	-0.002 (-0.07,0.07)	0.95
Alk phosphatase X time⁻		0.003		0.73		0.57		0.53
Total bilirubin (mg/dL)^	-0.02 (-0.06,0.03)	0.50	0.2 (-2.8,3.2)	0.88	-0.01 (-0.03,0.02)	0.48	-0.004 (-0.02,0.02)	0.73
Total bilirubin X time⁻		0.18		0.06		0.39		0.052
INR	-0.52 (-0.94,-0.11)	0.01	-2.1 (-35.7,31.5)	0.90	-0.05 (-0.31,0.20)	0.69		
INR X time⁻		0.42		0.052		0.89		0.03
WBC count (10³/mL)	0.03 (0.01,0.06)	0.10			-0.03 (-0.05,-0.02)	<0.001		
WBC X time⁻		0.56		0.02		0.18		0.03
Platelet count (10³/mL)***	-0.01 (-0.08,0.06)	0.74	6.9 (1.6,12.2)	0.01	0.03 (-0.02,0.08)	0.21	0.05 (0.02,0.08)	0.003
Platelet count X time⁻		0.80		0.69		0.72		0.58
Ferritin (ng/mL)***			-1.3 (-2.4,-0.2)	0.02	-0.01 (-0.02,0.003)	0.13	-0.01 (-0.01,0.0001)	0.052
Ferritin X time⁻		0.02		0.07		0.39		0.52
Albumin (g/dL)	0.08 (-0.07,0.23)	0.31			-0.01 (-0.11,0.08)	0.78	0.01 (-0.06,0.08)	0.72
Albumin X time⁻		0.11		0.02		0.59		0.12
%Iron/TIBC**	0.003 (-0.01,0.02)	0.65	-0.7 (-2.0,0.5)	0.25	-0.01 (-0.02,-0.01)	0.001	-0.01 (-0.01,0.002)	0.14
%Iron/TIBC X time⁻		0.69		0.23		0.16		0.44

Transformations as noted: ` = natural log; ^ = 1 – reciprocal

*Adjusting for race, the proportion of PEG-IFN taken between time points (time dependent covariate), time, and 6 month responder status.

**Per 10 unit increase

***Per 100 unit increase

–Not statistically significant (p>0.05) interaction terms not included in models.

Table 6-14. Supplement 3d: GEE models of each lipid profile measure by selected predictors (4 of 4)

Feature	ln(TG) β* (95%CI)	p	LDLc β* (95%CI)	p	ln(HDLc) β* (95%CI)	p	ln(TC) β* (95%CI)	p
Liver biopsy measures								
Ishak score			-1.7 (-4.1,0.7)	0.17	-0.03 (-0.05,-0.01)	0.02	-0.02 (-0.03,-0.005)	0.009
Ishak score X time⁻		0.02		0.86		0.26		0.59
Ishak score ≥ 3	-0.03 (-0.13,0.07)	0.52	-3.9 (-11.3,3.6)	0.31	-0.08 (-0.15,-0.01)	0.02	-0.05 (-0.09,-0.004)	0.03
Ishak score ≥ 3Xtime⁻		0.10		0.60		0.76		0.60
Fat score	0.18 (0.10,0.26)	<0.001	-3.7 (-9.0,1.6)	0.18	-0.07 (-0.13,-0.01)	0.02	-0.01 (-0.04,0.03)	0.64
Fat scoreXtime⁻		0.14		0.78		0.90		0.60
Steatosis (>5% present)	0.21 (0.11,0.31)	<0.001	-7.9 (-15.6,-0.1)	0.046	-0.10 (-0.17,-0.03)	0.003	-0.03 (-0.08,0.01)	0.16
SteatosisXtime⁻		0.22		0.75		0.82		0.32
Total HAI inflammation^	0.16 (-0.79,1.12)	0.74	-68.6 (-126.7,-10.4)	0.02	-0.23 (-0.72,0.27)	0.37	-0.43 (-0.76,-0.09)	0.01
HAI inflammation X time⁻		0.47		0.37		0.39		0.45
Iron score*				0.60		0.19		0.50
1			-1.4 (-9.8,6.9)	0.74	-0.07 (-0.14,0.01)	0.07	-0.03 (-0.08,0.02)	0.30
2			-5.7 (-20.6,9.1)	0.45	-0.05 (-0.17,0.08)	0.45	-0.03 (-0.12,0.05)	0.41
Iron score X time⁻		0.002		0.53		0.08		0.28

^ = 1 – reciprocal transformation

*Adjusting for race, the proportion of PEG-IFN taken between time points (time dependent covariate), time, and 6 month responder status.

*0 Iron score reference group

⁻Not statistically significant (p>0.05) interaction terms not included in models.
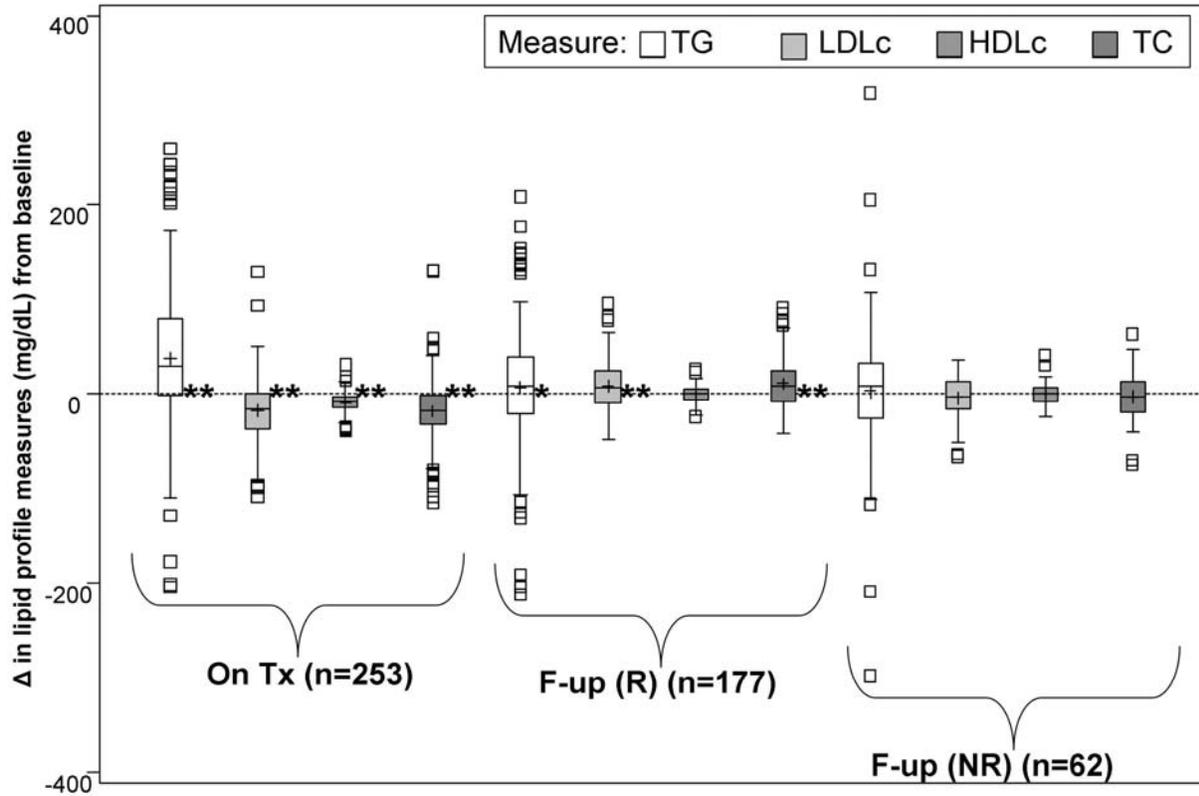
Figure 6-1. Serum lipid profile changes during and after antiviral therapy
* p<0.05;* * p<0.0001 (Wilcoxon signed rank test for differences from zero)
Tx=assessment at treatment week 24
F-up=assessment 24 weeks after stopping therapy
R=Among treatment week 24 virological responders (underwent a 48 week course of therapy) at week 72
NR=Among treatment week 24 virological nonresponders (underwent a 24 week course of therapy) at week 48
NOTE: Boxplots exclude extreme outliers for changes in TG levels: -768, -422, 477, 477, 562, 573, 629, and 1678 mg/dL on treatment; 434 mg/dL during follow-up among 6 month virological responders. Baseline median levels as follows: 100 mg/dL (TG); 115.5 mg/dL (LDLc); 41.1 (HLDc); and 183 (TC).
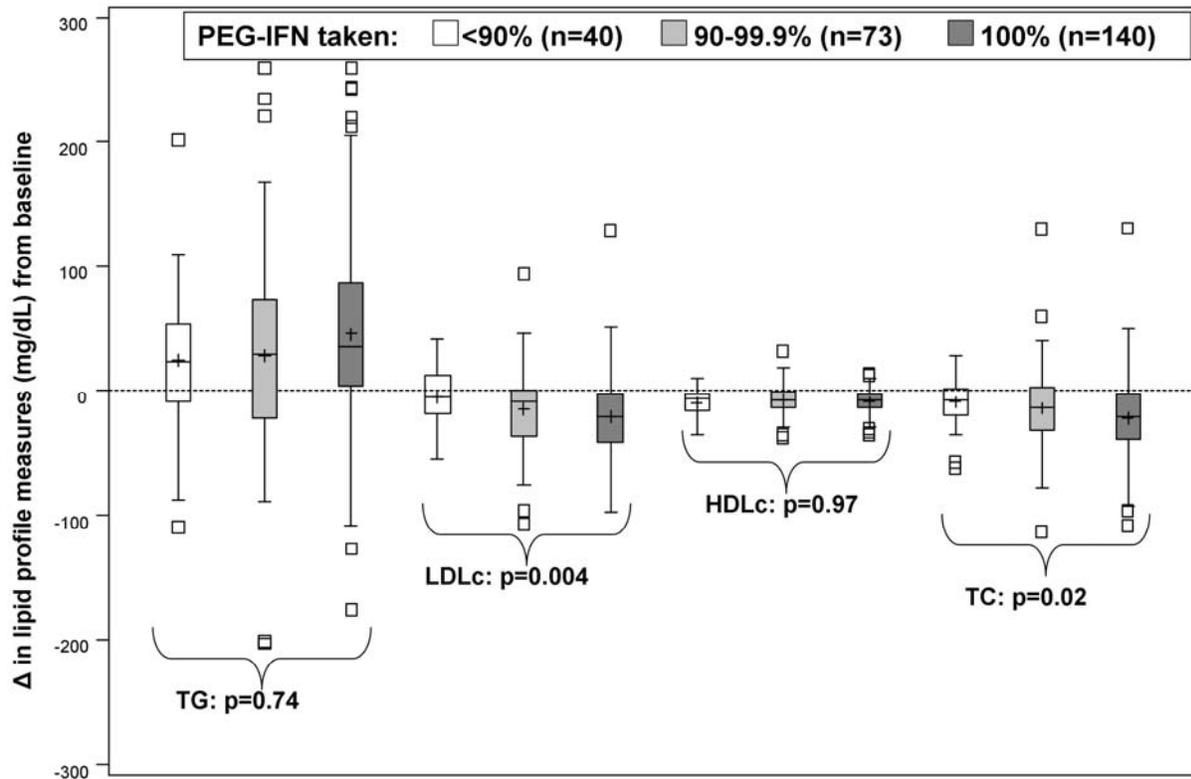
Figure 6-2. The amount of PEG-IFN taken and serum lipid profile changes during therapy
p corresponds to test of trend for lipid profile measures with the amount of PEG-IFN taken. Trend test for changes in TG, HDLc, and TC was on the natural log scale.
NOTE: Difference in lipid profile measures at treatment week 24 minus baseline
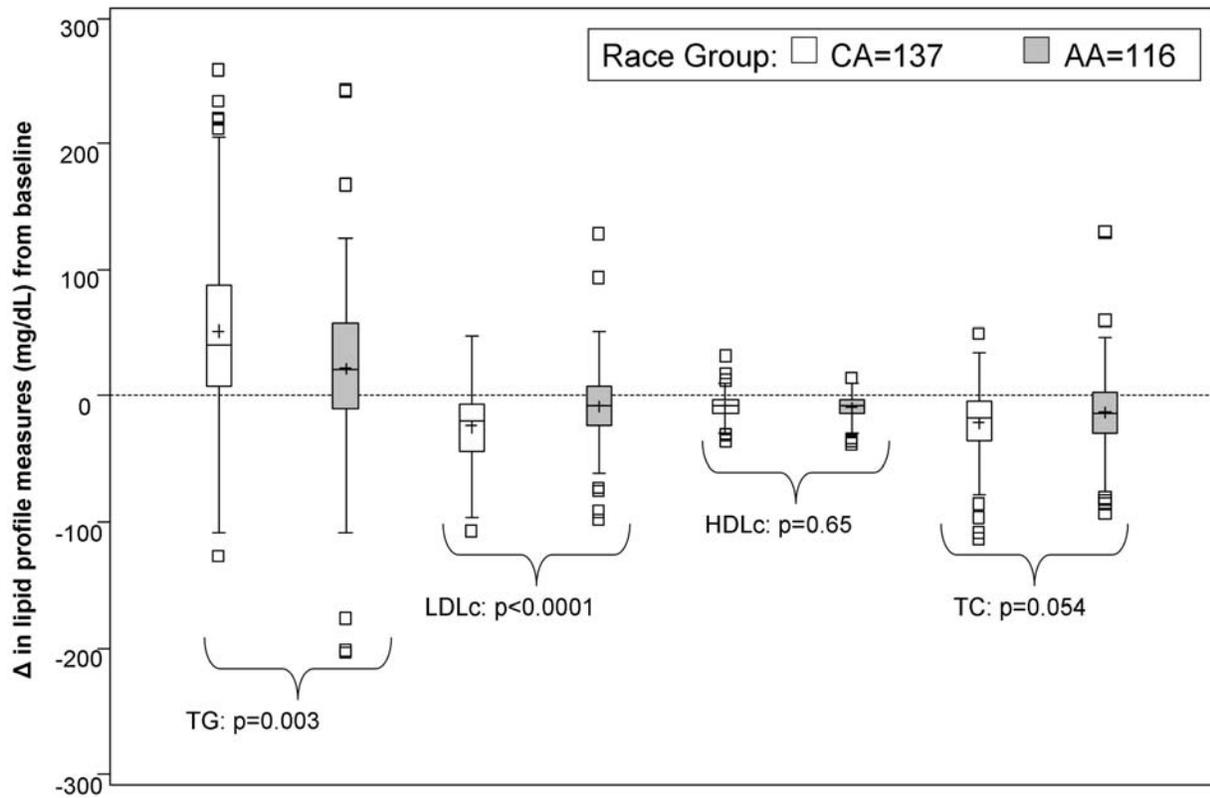
Figure 6-3. Race and serum lipid profile changes during therapy
*Wilcoxon test for racial differences
NOTE: Difference in lipid profile measures at treatment week 24 minus baseline

7.0 PROJECT 3: ASSOCIATIONS BETWEEN THE SERUM LIPID PROFILE AND HEPATITIS C ANTIVIRAL TREATMENT EFFICACY

7.1 ABSTRACT

BACKGROUND: Approximately one half of patients who undergo antiviral therapy for chronic HCV genotype 1 infection will not respond to treatment. In addition, African Americans (AAs) are less responsive to treatment than Caucasian Americans (CAs) and reasons for this disparity are unknown. Several recent studies suggest that pretreatment lipid profile measures are predictive of virological response to therapy. **AIMS:** To evaluate if lipid profile measures are predictive of virological response and to evaluate if these measures explain the racial difference in efficacy. **METHODS:** Participants were from Virahep-C, a prospective study of treatment naïve participants who received combination therapy of PEG-IFN alfa-2a + ribavirin for up to 48 weeks. Pretreatment fasting lipid profiles were analyzed for 160 AAs and 170 CAs. A relative risk (RR) model was employed to evaluate characteristics associated with sustained virological response (SVR) defined as undetectable HCV RNA 24 weeks after the cessation of therapy. Baseline patient histological, virological, demographic, and clinical variables were eligible for entry in multivariable modeling, in addition to the amount of PEG-IFN taken and body weight changes during the first 24 weeks of therapy. **RESULTS:** In univariable assessments, triglyceride (TG) and low density lipoprotein cholesterol (LDLc) were associated with SVR. In multivariable modeling, factors associated with a higher rate of SVR included CA race, the amount of PEG-IFN taken, and LDLc. In contrast, male gender, high HCV viral level, Ishak fibrosis score, and TG were associated with lower rates of SVR. Significant interactions were also detected between baseline viral level and race and between high density lipoprotein cholesterol (HDLc) and gender. The final area under the receiver operator curve was 0.811 and

did not significantly improve the prediction of SVR compared to a more parsimonious, previously published model (p=0.44). **CONCLUSIONS:** TG, LDLc, and the interaction of HDLc and gender were significantly associated with SVR in addition to other previously identified factors. The lipid parameters did not explain the racial difference in treatment response. These findings are compatible with proposed biological mechanisms of HCV entry, replication, and secretion, and underscore new potential therapeutic targets for HCV eradication.

7.2   INTRODUCTION

In the United States (US), chronic HCV infection is a major public health problem afflicting 3.6 million persons with direct health care costs, including liver transplantation, exceeding $1 billion dollars annually.[3, 74] The current standard treatment, combination PEG-IFN alfa-2a and ribavirin, is less effective in genotype 1 (the predominate viral type in the US) with approximately 46% people achieving sustained virological response (SVR) compared to other genotypes.[8] Moreover, in the US, genotype 1 virological response is characterized by a racial differences as African Americans (AAs) are significantly less responsive than Caucasian Americans (CAs).[10-12] Factors that explain the racial disparity in efficacy are unknown.[10]

Recent studies suggest that pretreatment lipid profile measures may be important predictors of treatment response. Several studies indicate that high pretreatment low density lipoprotein cholesterol (LDLc) and total cholesterol (TC) levels are associated with higher rates of SVR in multivariable analyses.[61-65] In addition, pretreatment triglyceride (TG) levels have been found to be higher among virological responders compared to non-responders.[52] These studies also suggest that associations between lipid profile measures and virological response may be specific to HCV genotype 1 and possibly genotype 2.

The observations from epidemiologic studies reflect work from *in vitro* evaluations that relate lipoproteins and HCV to mechanisms of viral entry into hepatocytes from the serum, replication, and secretion. Several studies suggest that HCV may complex with lipoproteins in the serum, possibly masking the virus from the host immune response.[30-33] Various receptors involved in lipoprotein-viral particle entry into hepatocytes are posited, including the scavenger

receptor B1 (SR-B1) and LDL receptor.[34-37] Direct entry of free HCV (i.e., not associated with lipoproteins) is also proposed to occur through binding with SR-B1 or CD81.[40-42] Within the hepatocyte endoplasmic reticulum, studies support HCV replication being reliant on cholesterol metabolism and a process of HCV secretion with very low density lipoprotein (VLDL) as complexed particles.[29, 43-46] Recent work suggests that SR-B1 may play a role in the mechanism of antiviral therapy with down-regulation of SR-B1 expression, the lipoprotein receptor which mediates removal of high density lipoprotein from the serum, following exposure to interferon alfa.[72] This supports the notion that down regulation may impact serum lipoprotein and lipid profile measures.

Associations between the serum lipid profile and treatment response are supported by biologically plausible mechanisms. This study assesses relationships between measures of the lipid profile, both pretreatment and on-treatment changes, and virological response in a cohort of participants with chronic HCV genotype 1 infection. In addition, this study evaluates if lipid profile measures account for any of the racial difference in treatment efficacy.

7.3   METHODS

7.3.1   Study population

Participants for this study were from the Virahep-C study, an investigation of resistance to antiviral therapy for chronic HCV infection, genotype 1. This study has been described elsewhere.[10] In brief, Virahep-C sought to evaluate 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 (CA) (n=205) and African Americans (AA) (n=196), all of whom underwent a combination PEG-IFN alfa-2a and ribavirin regimen for up to 48 weeks. At 24 weeks of therapy, participants were evaluated for the presence of HCV RNA and those with detectable levels discontinued therapy and entered follow-up, whereas the others continued therapy for an additional 24 weeks.

Funding for lipid profile analyses utilizing stored fasting serum samples was obtained for an ancillary study to examine relationships between host genetic polymorphisms, the lipid profile, and steatosis (KL2 RR024154-02 to LJY). Of the 401 participants enrolled in Virahep-C, lipid profile analyses were conducted among participants who granted genetic consent (n=374) and had stored fasting serum samples at baseline (n=335). The five participants who reported use of lipid lowering medications were excluded from this evaluation resulting in a final analysis sample of 330 participants (170 CA and 160 AA). During treatment (24 weeks after starting therapy) and post-treatment (24 weeks after stopping therapy) lipid profile data were available for 253 and 245 of the participants, respectively.

7.3.2   Study measures

The primary outcome for Virahep-C was sustained virological response (SVR), defined as undetectable serum HCV RNA 24 weeks after the end of therapy. The lipid profile 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 less than 400 mg/dL,

the Friedewald formula was used to calculate LDLc indirectly (LDLc = TC − HDLc − 0.20 X TG).[28] For samples with TG levels of at least 400 mg/dL, LDLc was assessed directly. Inflammation and fibrosis were assessed using the criteria of the Histological Activity Index (HAI) by a single hepatopathologist.[19, 20] The amount of PEG-IFN and ribavirin taken by participants was estimated using data from the Medication Event Management System (MEMS) (Aardex, Zug, Switzerland).[73]

7.3.3   Statistical analysis

To evaluate factors associated with SVR, a relative risk model was employed with a robust variance estimator.[71]. For all statistical tests, a p-value of <0.05 was considered statistically significant. 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. Two types of multivariable models of sustained virological response were constructed using a stepwise approach. One type of multivariable model (models 1 and 2) allowed pretreatment characteristics and the amount of PEG-IFN alfa-2a taken during the first 24 weeks as eligible predictors. Model 2 allowed as eligible predictors baseline lipid profile measures. A second type of multivariable model (model 3) also adjusted for body weight changes and allowed 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 the area under the receiver operating curves (AUROCs) were assessed using a non-parametric method.[75]

7.4 RESULTS

Characteristics of the 330 participants are shown in Tables 5-1 and 5-2 on pages 31–32 and described on page 24.

Characteristics associated with SVR are summarized in Table 7-1. Features significantly associated with higher SVR included CA race (RR=1.92, p<0.001, AA reference) and education beyond high school (RR=0.64, p=0.002, less than high school degree reference). Features inversely related to SVR included body weight (RR=0.95 per 5 kg increase, p=0.01), baseline $\log_{10}$ HCV level (RR=0.77, p<0.001), and fibrosis (Ishak) score (RR=0.90, p=0.02), whereas platelet count (RR=1.25 per 10^3 cells/mm^3 increase, p=0.01) and the 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. Baseline natural log of TG (RR=0.65, p=0.002) and LDLc (RR=1.05 per 10 mg/dL increase, p=0.002) were inversely and directly related to the rate of SVR, respectively. The natural logs of baseline HDLc and TC levels were not significantly associated with SVR.

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 7-2. SVR rates were directly associated with increases and declines in the natural log of TG (RR$_{crude}$=1.43, p=0.001; RR$_{adjusted}$=1.29, p=0.02) and LDLc (RR$_{crude}$=0.96, p<0.001; RR$_{adjusted}$=0.97, p=0.02, per 5 mg/dL increase) during 24 weeks of therapy, respectively, compared to pretreatment. Post-treatment values, and increases in both LDLc (RR$_{crude}$=1.04, p=0.001; RR$_{adjusted}$=1.04, p=0.001, per 5 mg/dL increase) and the natural log of TC (RR$_{crude}$=4.64, p<0.001; RR$_{adjusted}$=4.10, p<0.001) from baseline were directly related to the rate of SVR.

Assessed graphically in smoothing spline plots, relationships between the probability of SVR and baseline and changes in TG, LDLc, TC, and HDLc (by gender) during 24 weeks of therapy are displayed Figures 7-1 to 7-5. The natural logs of baseline TG and increases in TG during therapy were inversely and directly related to the probability of SVR, respectively. Baseline LDLc and declines in LDLc from baseline during 24 weeks of therapy were directly related to the probability of SVR. Although not statistically significant in regression models (Tables 7-1 and 7-2), baseline and on-treatment changes in the natural log of TC showed a similar relationship to SVR as LDLc. In males, the natural log of HDLc was inversely related to SVR rates, while in females the relationship was opposite.

The multivariable model reported by Conjeevaram et al. based on 400 participants was fit for the 329 participants for whom lipid profile and covariate data were available.[10] (Table 7-3: model 1) In model 1, factors significantly associated with SVR included CA race (RR=1.98, p<0.001), male gender (RR=0.80, p=0.049), baseline viral level (RR=0.57, p=<0.001 per $\log_{10}$ increase), 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, (RR=0.86, p=0.03 for CAs, RR=0.57, p<0.001 for AAs). Using the same eligible predictors as model 1 and 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) with SVR was found indicating that the inverse relationship between HDLc and the rate of SVR decreased 66% (p=0.006) per 1 unit natural log increase of HDLc among males, whereas no significant

relationship was found among females. The prediction of SVR did not significantly differ between the models 1 and 2 (AUROCs=0.801 vs 0.811, respectively, p=0.42). (Figure 7-6)

In multivariable modeling, model 3 was constructed using 250 participants who had covariate, baseline lipid profile, and treatment week 24 lipid profile data. (Table 7-3) Model 3 evaluated the relationships between lipid profile changes during therapy as predictors of SVR, in addition to other variables eligible for entry into models 1 and 2. Variables included in models 2 and 3 were similar, though the baseline natural log of TG and LDLc levels were not included in model 3, but the change in LDLc during the first 24 weeks was retained. The AUROC for model 3 was not significantly different than that of model 1 fit to the same 250 participants (AUROC=0.799 vs 0.779, p=0.19). (Figure 7-7) In contrast to model 2, in model 3 the significant interaction (p=0.009) between the natural log of HDLc and the rate of SVR indicated a direct relationship (RR=2.15, p=0.008) among females, but not males (p=0.35), a possible reflection of the smaller subset upon which model 3 was fit. In all three multivariable models, race remained a significant predictor of SVR and the strength of the association was little changed by the addition of the lipid profile measures.

7.5   DISCUSSION

This evaluation of the lipid profile and virological response showed that pretreatment TG and LDLc levels were inversely and directly related to the rate of SVR, respectively. Furthermore, changes in these two parameters during the first 24 weeks of therapy were associated with virologic response with larger increases in TG and larger declines in LDLc associated with higher rates of SVR. In multivariable modeling, several lipid profile parameters (baseline TG,

LDLc, HDLc, and TG, and LDLc changes during treatment) were significant predictors of SVR. However, including the lipid profile measures did not significantly improve the prediction of SVR compared to models without these measures, nor did lipid profile measures account for the racial difference in treatment efficacy between CAs and AAs.

The direct relationship between pretreatment LDLc levels and the rate of SVR is consistent with findings from several other studies.[61-65] Other work [52] has reported an association between higher pretreatment TG levels and virologic response, opposite of the relationship in the current study, possibly a reflection of the HCV genotype distributions. In this study only people infected with HCV genotype 1 were included, whereas the predominant genotype represented in the previously referenced study was genotype 2. In multivariable analyses, significant interactions between HDLc levels and gender in relation to virologic response were found, which have not been previously reported. These relationships warrant further investigation and validation in other cohorts to clarify if lipid profile measures are important predictors of treatment response. Post-therapy, 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 the replication and secretion of HCV, the significant relationships between both baseline and changes from baseline LDLc and TG levels and rates of SVR are biologically feasible.[29-37, 43-46] The direct relationship between LDLc and SVR may partially be explained by competition for LDL receptor sites preventing viral entry into hepatocytes, increasing exposure to the host immune response in the serum. These findings suggest that the lipid profile 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 is warranted. Treatment trials investigating statins and fibrates to improve virological response have yielded mixed results.[13, 76, 77]

This study suggests that lipid profile measures are predictors of SVR, but that these measures do not explain the racial disparity in treatment efficacy between CAs and AAs. Accounting for other predictors of virologic response, incorporating lipid profile variables did not yield a significant improvement in the prediction of SVR compared to another more parsimonious model. However, this study underscores the potential relevance of the serum lipid profile in virologic response and further research is warranted to investigate the relationships between other characterizations of the lipid profile, genetic determinates of lipid metabolism, and SVR.

7.6   TABLES

Table 7-1. Univariable models of SVR and selected predictors in the lipid profile assessment subset

Feature	RR (95%CI)	p
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
$\leq$ High school education	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
History of hypertension	0.76 (0.56,1.04)	0.09
Antidepressant drug use	0.60 (0.18,2.00)	0.40
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
White blood cells (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 vs. non-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 $\geq$ 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)	0.86 (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

Transfomations as noted: *Log$_{10}$ transformed; **Natural log transformed
Relative risk: †per 5 unit increase; ^per 10 unit increase; [#]per 100 unit increase; `per 10% increase in dose
***Eligible for entry in multivariable model 2

Table 7-2. Evaluation of lipid profile measure changes during and after therapy as predictors of SVR

Lipid profile measure	SVR Unadjusted RR (95%CI)	p	SVR Race-adjusted RR (95%CI)	p
Δ: On Tx – Baseline	n=253		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: F-up – Baseline	n=245		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

*Natural log transformed

†per 5 mg/dL change

Table 7-3. Multivariable models of SVR and selected predictors

Feature	Model 1 RR (95%CI)	Model 1 p	Model 2 RR (95%CI)	Model 2 p	Model 3 RR (95%CI)	Model 3 p
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)*	0.57 (0.44,0.73)	<0.001	0.58 (0.45,0.75)	<0.001	0.47 (0.34,0.63)	<0.001
Baseline viral level (IU)* and race interaction		0.005		0.006		0.001
CA	0.86 (0.75,0.98)	0.03	0.87 (0.75,0.997)	0.045	0.80 (0.69,0.93)	0.004
AA	0.57 (0.44,0.73)	<0.001	0.58 (0.45,0.75)	<0.001	0.47 (0.34,0.63)	<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)			NA	NA	NA	NA
HDLc** (mg/dL) and gender interaction				0.02		0.009
Male			0.44 (0.25,0.79)	0.006	0.76 (0.43,1.35)	0.35
Female			1.10 (0.60,2.02)	0.75	2.15 (1.22,3.78)	0.008
LDLc^ (mg/dL)			1.03 (1.001,1.07)	0.046		
ΔLDLc^ (mg/dL)					0.97 (0.95,0.996)	0.02

Model 1=Replication of Conjeevaram et al. model [9] on subset of participants with available baseline lipid profile data (n=330)

Model 2=Baseline lipid profile variables as eligible for entry

Model 3=Multivariable model with baseline and on treatment changes from baseline in lipid profile variables as eligible for entry (n=253). Model also adjusts for changes in body weight (not shown).

Transformations as follows: *Log$_{10}$ transformed; **Natural log transformed

RR estimate adjustment as follows: ^per 5 unit increase; `per 10% increase in dose

NA=Not applicable due to significant interaction with gender (refer to gender specific coefficients)
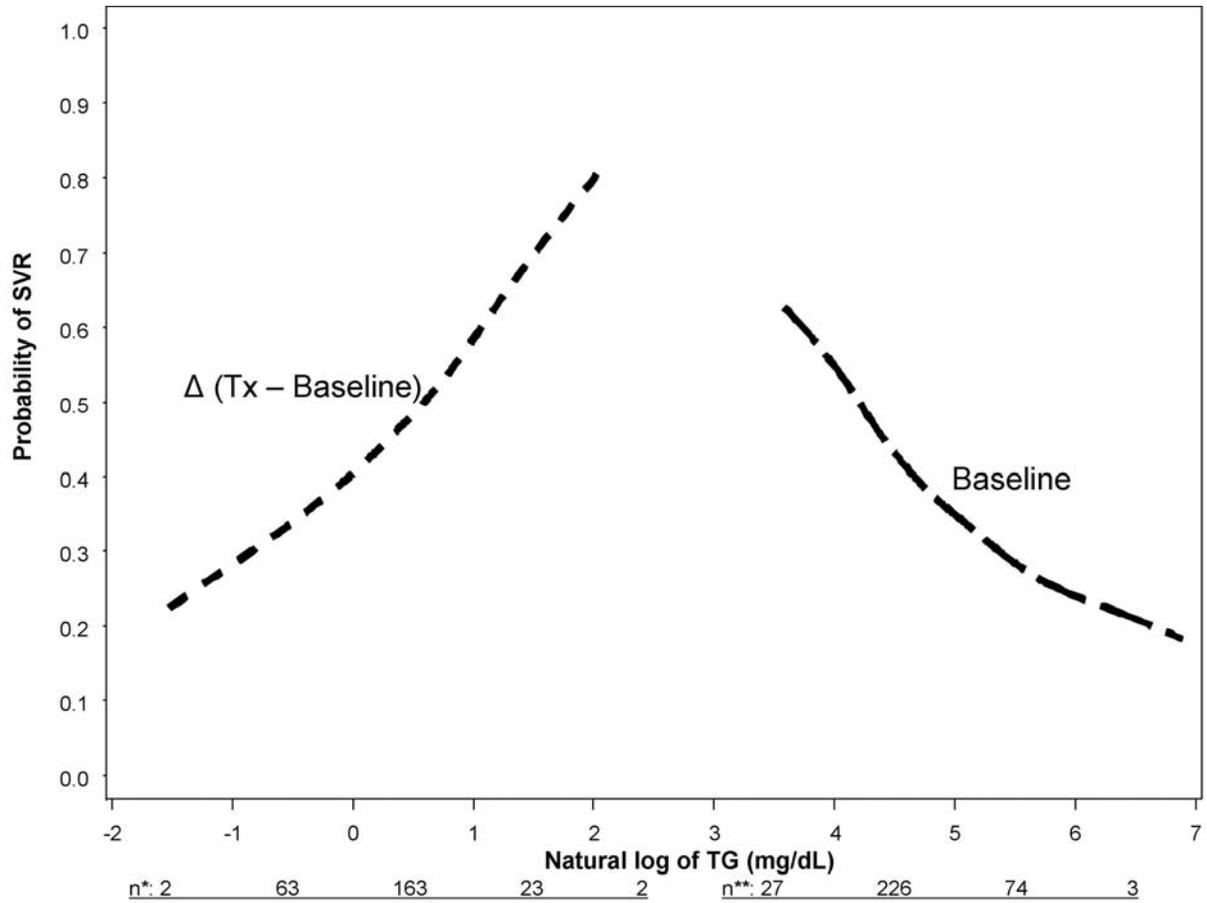
Figure 7-1. Baseline and changes in the natural log (ln) of TG during 24 weeks of therapy and the probability of SVR
*Number of participants within interval for changes in ln(TG) from baseline
**Number of participants within interval for baseline ln(TG)
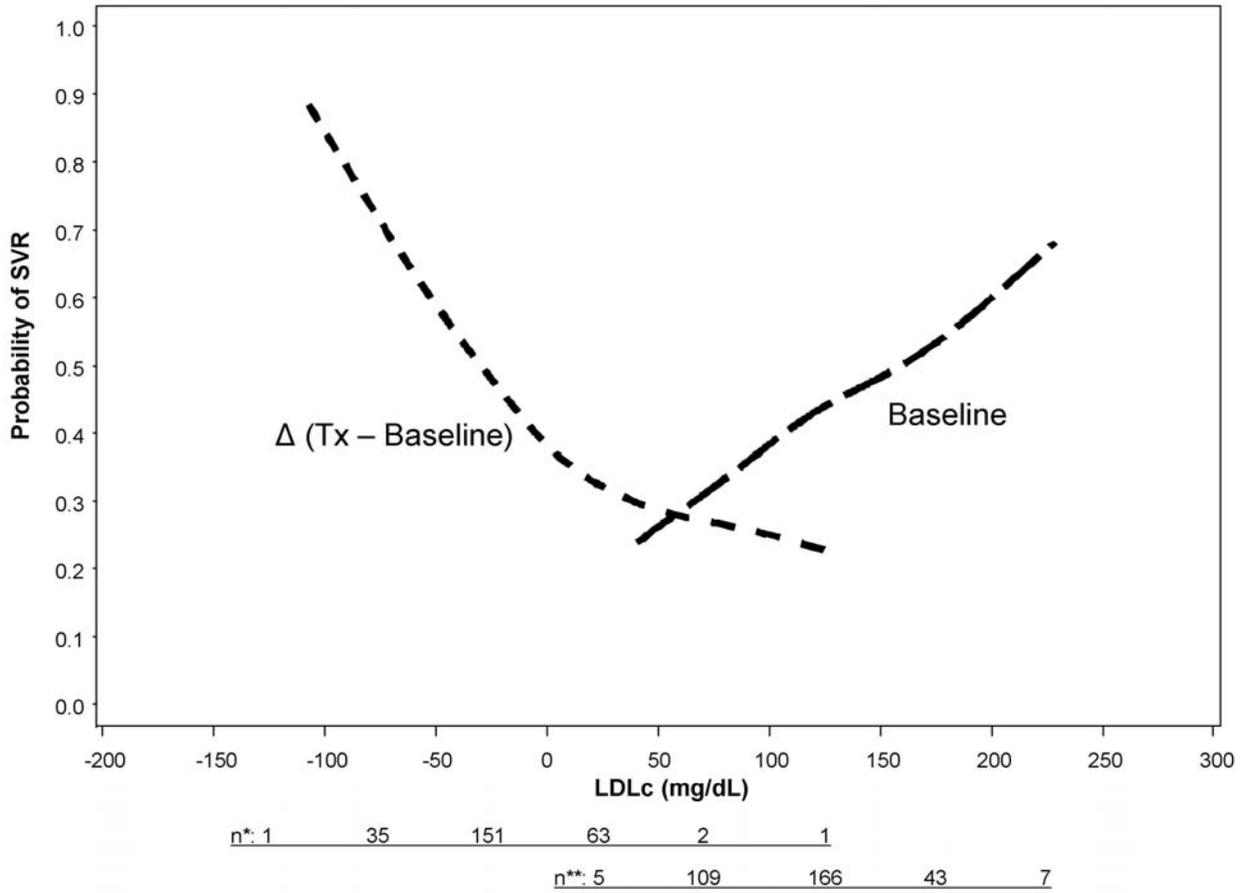
Figure 7-2. Baseline and changes in LDLc during 24 weeks of therapy and the probability of SVR
*Number of participants within interval for changes in LDLc from baseline
**Number of participants within interval for baseline LDLc
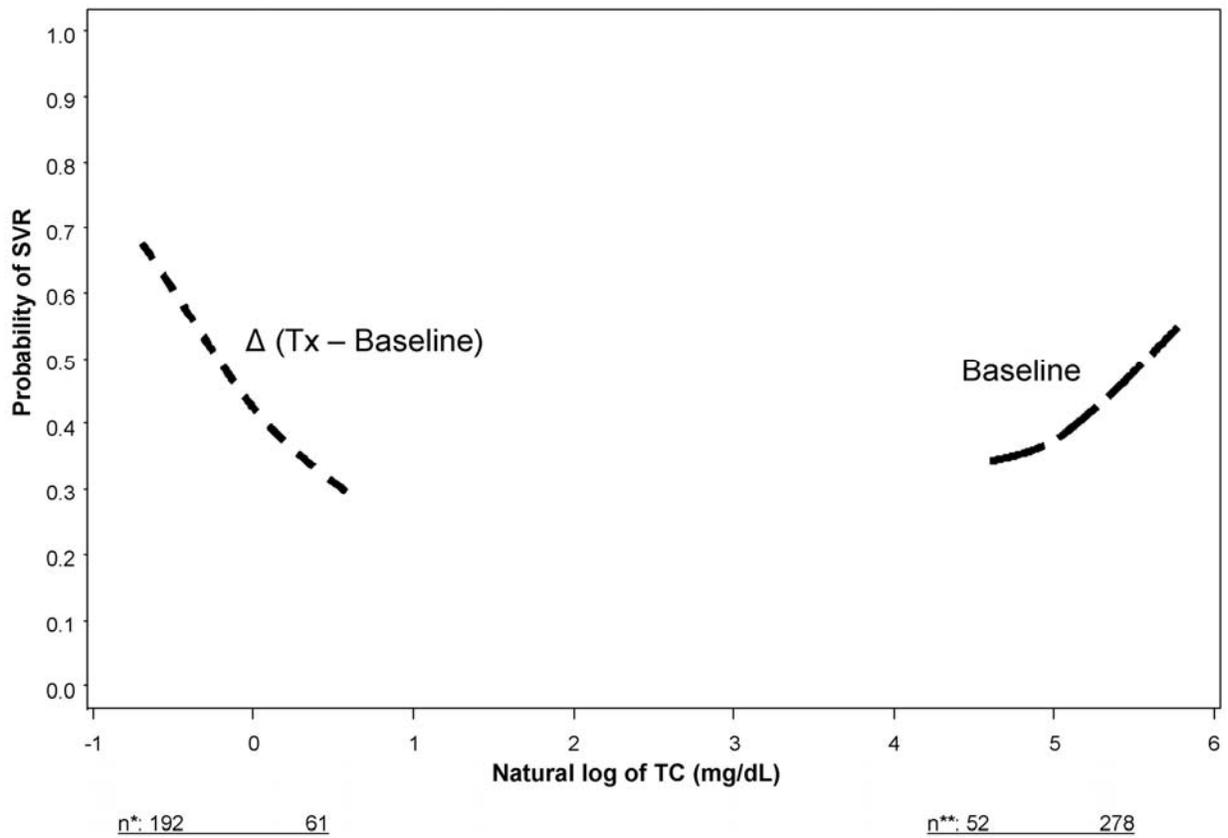
Figure 7-3. Baseline and changes in the natural log (ln) of TC during 24 weeks of therapy and the probability of SVR
*Number of participants within interval for changes in ln(TC) from baseline
**Number of participants within interval for baseline ln(TC)
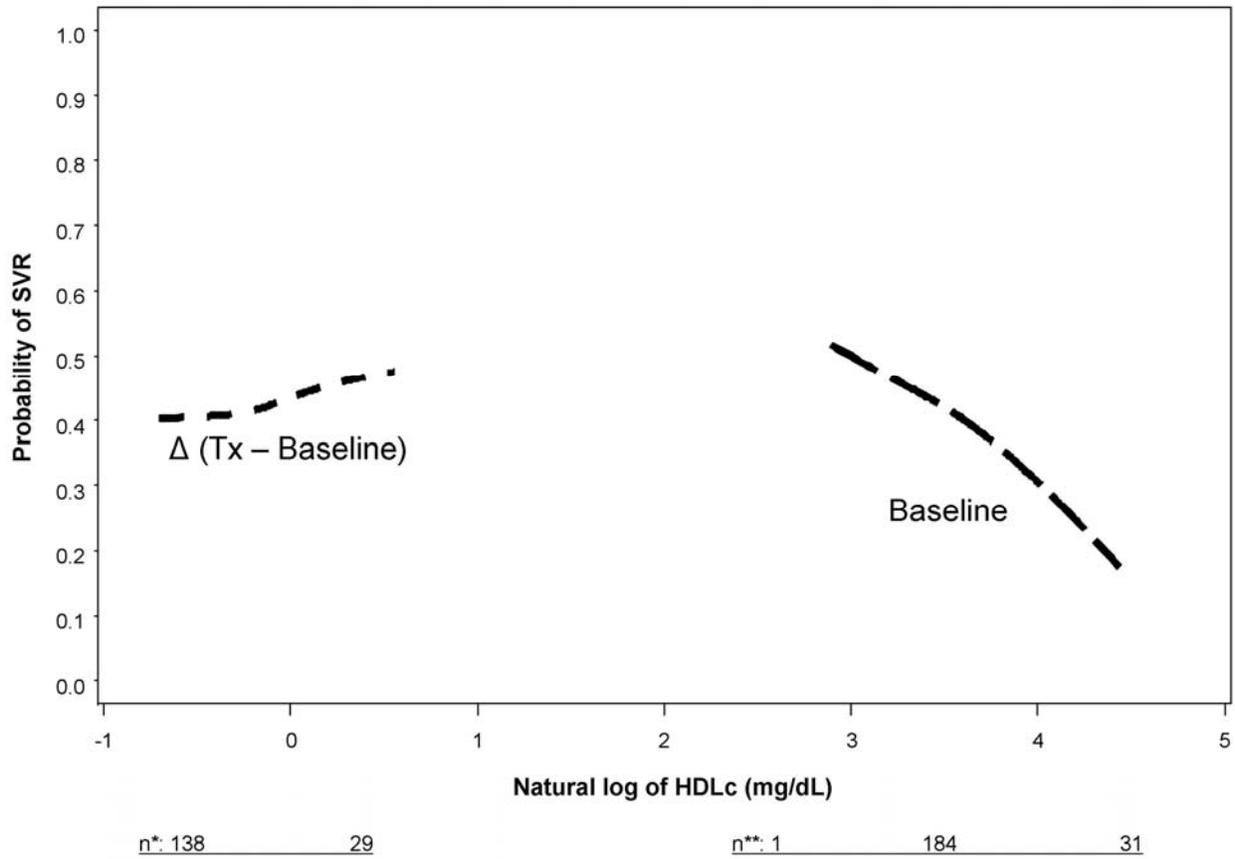
Figure 7-4. Baseline and changes in the natural log (ln) of HDLc during 24 weeks of therapy and the probability of SVR among males
*Number of males within interval for changes in ln(HDLc) from baseline
**Number of males within interval for baseline ln(HDLc)
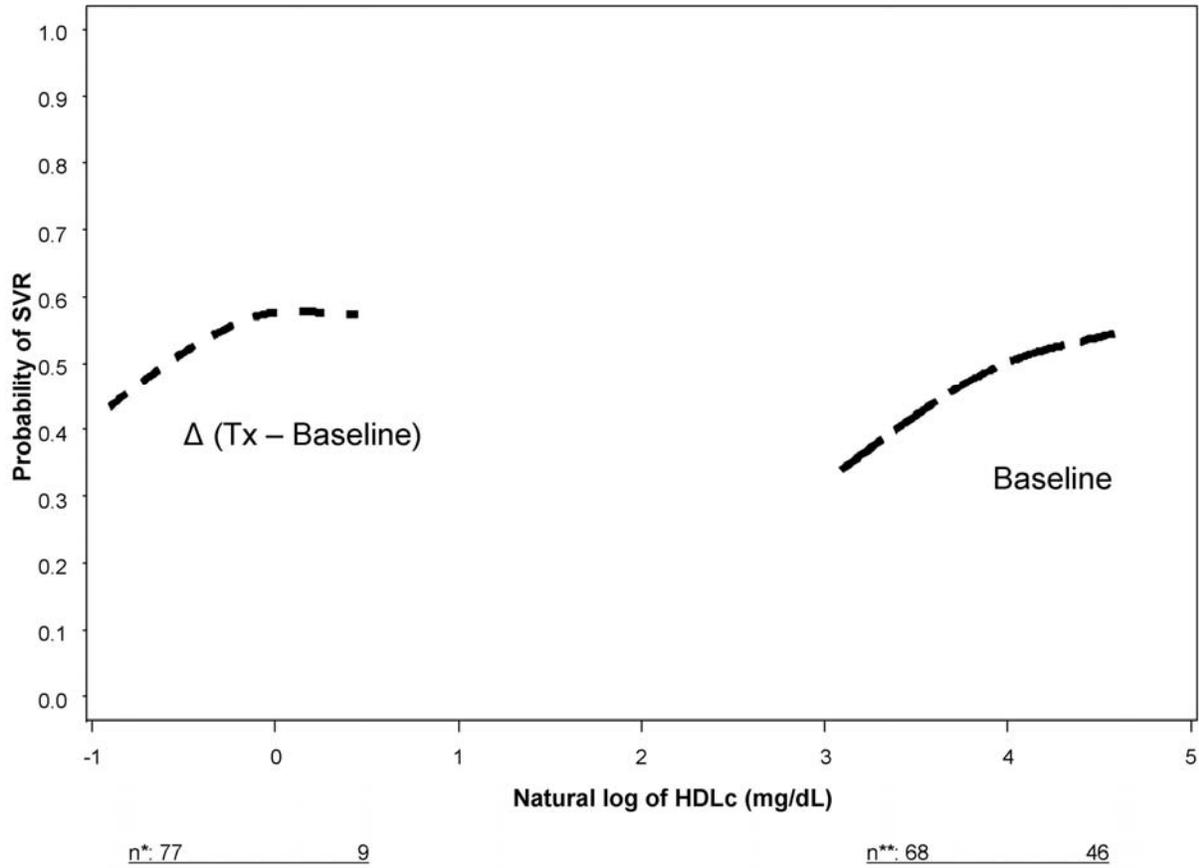
Figure 7-5. Baseline and changes in the natural log (ln) of HDLc during 24 weeks of therapy and the probability of SVR among females
*Number of females within interval for changes in ln(HDLc) from baseline
**Number of females within interval for baseline ln(HDLc)
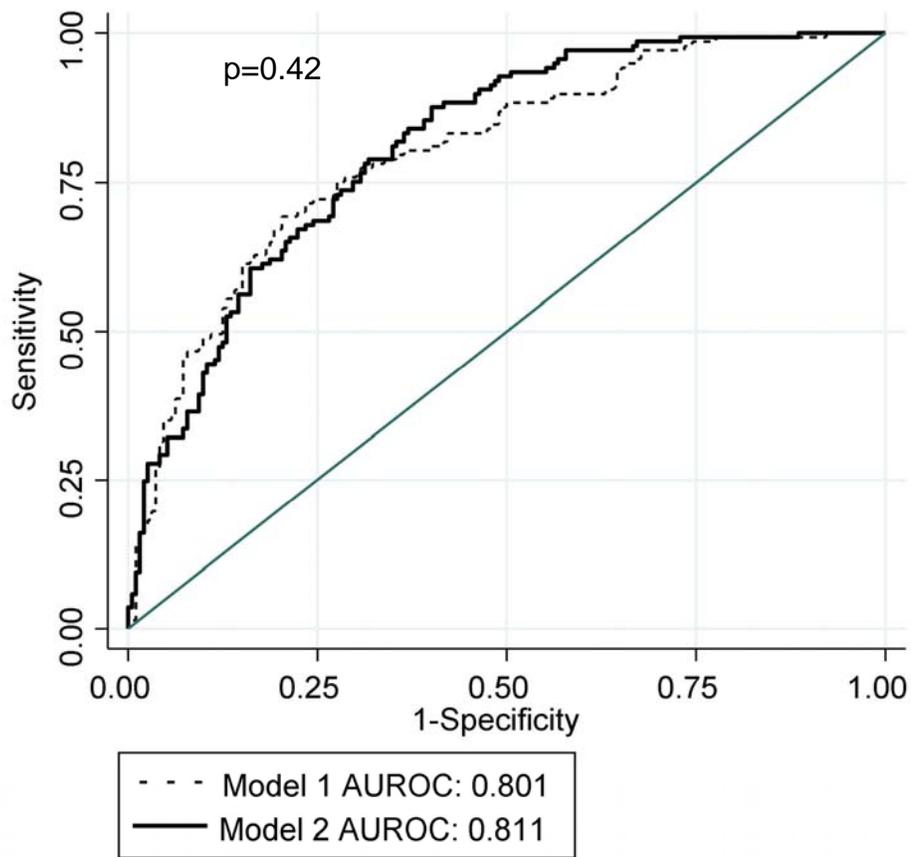
Figure 7-6. Receiver operator curves of multivariable models 1 and 2 (n=329)
AUROC=area under the receiver operator curve
Model 1=Includes race, gender, baseline viral level, baseline viral level and race interaction, Ishak fibrosis score, and the amount of PEG-IFN taken
Model 2=Includes variables in model 1, plus baseline TG, baseline HDLc, baseline HDLc and gender interaction, and baseline LDLc
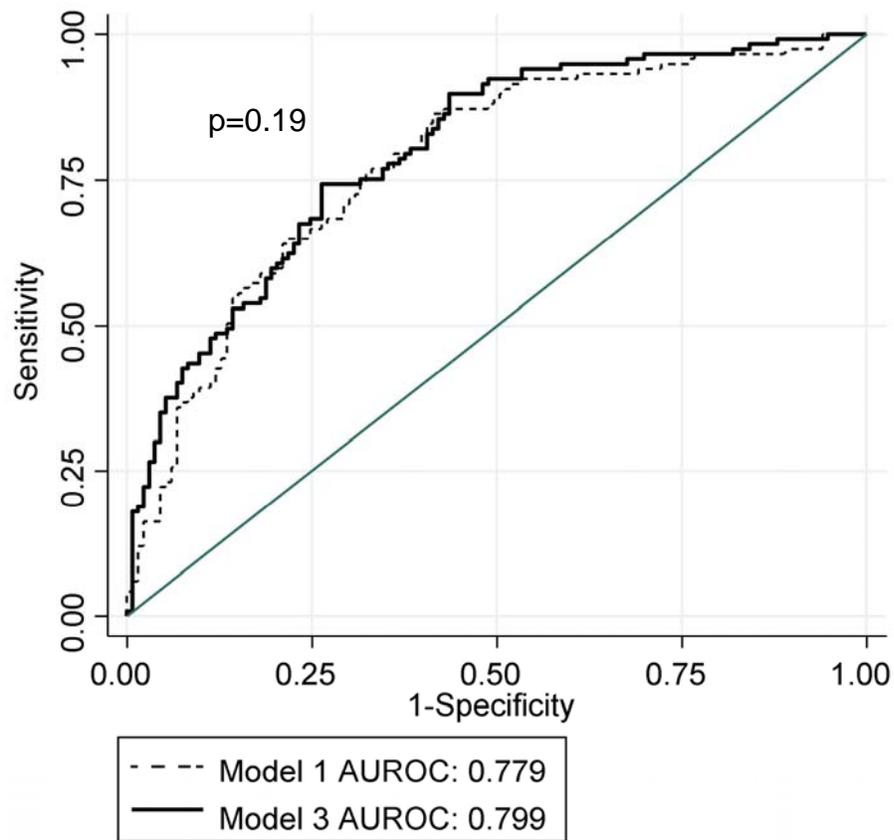
Figure 7-7. Receiver operator curves of multivariable Models 1 and 3
AUROC=area under the receiver operator curve
Model 1=Includes race, gender, baseline viral level, baseline viral level and race interaction, Ishak fibrosis score, and the amount of PEG-IFN taken
Model 2=Includes variables in model 1, body weight change, baseline HDLc, baseline HDLc and gender interaction, and the change in LDLc during 24 weeks of therapy

8.0   GENERAL DISCUSSION

The three epidemiologic projects of this dissertation investigated different aspects of the serum lipid profile in the context of chronic HCV genotype 1 infection. Sharing this theme, the studies evaluated associations between the lipid profile measures and liver disease and viral characteristics prior to the initiation of treatment, characterized changes in the lipid profile measures during and after therapy, and assessed the relationships between lipid profile measures and treatment efficacy. The three projects revealed several new findings which underscore the relevance of the serum lipid profile in HCV infection, treatment, and treatment efficacy. The first project found significant associations between the serum lipid profile and several aspects of liver histology and a direct relationship between TG and HCV viral levels. The second project revealed significant changes in all aspects of the lipid profile during the first 24 weeks of therapy. For some components of the lipid profile, changes also significantly differed by race and were related to the amount of PEG-IFN taken. Lastly, the third project found that baseline LDLc, HDLc, and TG levels were significant predictors of treatment efficacy, as were changes in LDLc and TG levels during the first 24 weeks of therapy. However, when lipid profile measures were considered along with other available data collected as part of the Virahep-C study, the racial disparity in treatment efficacy between AAs and CAs remained.

8.1   STRENGTHS AND LIMITATIONS

The primary aim of the Virahep-C study was to examine factors involved in the racial difference in response to antiviral therapy between AAs and CAs. Accordingly, the study enrolled approximately equal numbers from each race group. One limitation, however, was that the cohort was two-thirds male, which limited the ability to assess gender or race/gender relationships with the lipid profile or treatment response. This limitation was further complicated since AA females had significantly larger BMIs and weights, both inversely associated with treatment response. Despite the sample size limitations, the three projects found that gender was associated with TG, HDLc, and TC, and males were less responsive to therapy than females. In the third project of treatment efficacy, there was a significant interaction between gender and HDLc with respect to the relationship with SVR.

Another limitation of the Virahep-C cohort is the highly selected population, which is likely not comparable to the general US population of chronic HCV infected people and limits the generalizability of the findings to other groups with more advanced liver disease. In addition, the study cohort was only infected with genotype 1, which precluded the ability to examine the lipid profile across HCV genotypes. The use of other sampling strategies would enhance the representativeness of the cohort to reflect the US population of people chronically infected with HCV.

Stepwise variable selection was used in multivariable modeling for all three projects, which has limitations. Based on statistical significance, predictors are selected out of subsets of variables for inclusion or exclusion into the model. Given two or more predictors significantly associated with an outcome, automated statistical software programs select those variables that remain significantly associated with the outcome in the presence of other significant variables,

making it possible that more biologically or clinically plausible variables may be excluded. In light of these limitations, two committee members assessed the biological (RWE) and clinical (HSC) relevance and plausibility of multivariable models.

The cross-sectional project 1 has several inherent limitations. First, since the study examined associations between the lipid profile and liver disease measures, it is not possible to assess the direction of these relationships. For example, it is possible that either severe fibrosis or liver fat may have resulted in the observed patterns of the lipid profile, or visa versa. Another limitation of the first project is a result of the lack of a non-HCV exposed control group. Host factors resulting in certain types of chronic liver disease could not be disentangled from the contribution of chronic infection.

This research is the first to characterize the serum lipid profile in a cohort of chronically infected people with HCV genotype 1 that includes a large proportion AAs, which afforded an opportunity to assess the role the role of race. In addition, this was the first study to characterize changes in the lipid profile during therapy in association with many other measures such as the amounts of drug taken and liver disease.

8.2   FUTURE RESEARCH

8.2.1.1 Additional lipid profile studies

Per the original Virahep-C study design, fasting serum samples were collected and stored prior to treatment, 6 months after therapy began, and 6 months after stopping therapy. In addition, among 6 month virological responders, fasting serum samples were collected at 48 weeks of therapy. With additional funding, further research may be able to assess if changes in the lipid profile

measures between 24 and 48 weeks of therapy predict virological breakthrough (n=17 in Virahep-C), defined as undetectable HCV RNA at 6 months of therapy and detectable levels at the end of treatment, which would further clarify the biological relevance of lipid profile measures in treatment induced viral eradication. However, the small sample size limits the power to evaluate relationships between breakthrough and lipid profile in the Virahep-C cohort.

The relationship between HCV viral kinetics and changes in the serum lipid profile during treatment may also warrant further investigation. This evaluation would require more frequent assessments of the lipid profile during the first 28 days of therapy than were available in the Virahep-C study. Findings from this type of analysis would clarify if viral eradication occurs before, after, or in concert with lipid profile measure changes.

8.2.2   Lipid profile sub-fraction and genetic studies

The lipid profile measures examined in this investigation may be related to other factors involved in the racial difference in treatment response. Work to further characterize lipoprotein number and size using nuclear magnetic resonance imaging spectroscopy (NMR LipoProfile®) would yield more specific measures in the evaluation of treatment efficacy. Likewise, the genetic aspects of lipoprotein and cholesterol metabolism may be involved in virological response. These factors may warrant further study using stored samples from Virahep-C or other studies.

8.2.3   Assessments of the role of diet and lipid altering medications

PEG-IFN therapy is associated with numerous side-effects including gastrointestinal disturbances, such as nausea, weight loss, and anorexia[9], and the impact of therapy on changes in

dietary intake as it relates to treatment response has not been well studied. *In vitro* work suggests that certain nutrients (i.e., beta-carotene, vitamin D_2, and linoleic acid) may have inhibitory effects on HCV replication, both with and without combination antiviral therapy.[78] Other work has focused on modifying cholesterol metabolism to inhibit HCV replication using lipid lowering agents, such as statins and fibrates, and have yielded mixed results with respect to viral kinetics and virological response.[13, 76, 77] Prospective studies may be designed to further characterize these relationships and assess if intervention studies are warranted.

8.2.4   Repeated measures of liver disease studies

Project 3 identified significant post-treatment increases in LDLc, TG, and TC above pretreatment levels among sustained virological responders. These findings suggest that eradication of HCV may correspond with recovery from liver disease and subsequent increases in lipoprotein production. In addition, it is unclear if the changes observed over the course of therapy (described in Project 2) are associated with changes in liver disease. To assess these relationships, future studies may incorporate repeated liver disease measures from ultrasound imaging techniques as an alternative or in addition to invasive liver biopsies.

8.2.5   Assessment of cardiovascular disease in chronic HCV

Based on the relevance of the serum lipid profile to biological mechanisms of chronic HCV, this dissertation project focused primarily on each aspect of lipid profile as mutually exclusive measures. Although this was the main aim, the lipid profile measures were combined to create a composite outcome of dyslipidemia, a cardiovascular disease (CVD) risk factor as a secondary

focus. Several other measures of CVD, not available in the Virahep-C study, including pulse wave velocity assessments, inflammatory markers (such as C-reactive protein), carotid intima-media thickness, or coronary calcification would afford an opportunity to examine CVD risk and associations with chronic HCV. Comparisons to an age-, gender-, and race- matched non-HCV exposed control group would suggest if CVD risk differs by infection status. In addition, in chronic HCV infected groups, repeated measures of these additional CVD indicators would allow assessments of changes in CVD risk in relation to virological response and treatment duration.

8.3   PUBLIC HEALTH SIGNIFICANCE OF THIS RESEARCH

This dissertation contributes to the understanding of the relationship between the lipid profile to potential biological mechanisms of HCV and is reflective of findings from *in vitro* work. In addition, the investigation highlights the relevance of the lipid profile in chronic HCV and suggests that lipid metabolism may play a role in predicting treatment efficacy. These contributions may influence future studies in the examination of new therapeutic targets and interventions to improve treatment response, decrease the health care costs associated, reduce the transmission of disease, prevent or slow the progression of chronic liver disease, and improve the quality of life of those with chronic HCV infection.

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