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The Strange and Critical Intersection of Hepatitis C and Lipoprotein Metabolism: “C-zing” the Oil

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Since its discovery in 1989, a substantial amount of research has emerged regarding the close association of hepatitis C virus (HCV) replication and hepatic lipoprotein metabolism. Even before the identification of the HCV virus, a link to fat metabolism was suspected based on clinical observations regarding steatosis as a common feature of what used to be known as “non-A, non-B hepatitis.”¹ This relationship was later extended in studies that revealed that HCV-infected patients with steatosis often lack common risk factors for fatty liver, such as obesity.² These early observations, along with subsequent studies that identified circulating viral particles in low-density, more-buoyant plasma fractions (partitioned with plasma centrifugation in sucrose or iodixanol gradients), fueled a now extensive and growing body of literature on this key aspect of HCV replication.³ Investigations performed over the past 23 years have defined replicative pathways of HCV involving mechanisms and biochemical pathways right out of the “play book” of fatty liver disease (FLD).

Seemingly unique among the single-stranded RNA *Flaviviridae* viruses (which include yellow fever, dengue, and West Nile viruses), the hepatotropism of HCV rests, in part, at least on its replicative dependence on hepatic very-low-density lipoprotein (VLDL) synthesis. However, much of the evidence in support of this relationship has been established through studies using surrogate cell-culture and animal models of HCV replication, albeit with methodology that is truly elegant and sophisticated. These studies have been summarized in several recent review articles, including works by Negro and Ye.^{4,5} Nonetheless, with some notable exceptions, which are discussed below, there have been limited physiological data in humans infected with HCV.

In the present report from Lambert et al, the investigators have narrowed this gap with a detailed study of fasting and postprandial lipid metabolism in 5 non-diabetic patients with chronic hepatitis C versus 9 uninfected control subjects.⁶ At baseline, although of similar

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age, body mass index (approximately 25–27), and body-fat percentage, HCV-infected patients demonstrated lower total cholesterol and low-density lipoprotein (LDL) cholesterol and borderline lower high-density lipoprotein (HDL) cholesterol, whereas triglyceride (TG) and glucose levels were similar and insulin and homeostasis model assessment values were higher consistent with insulin resistance (IR) in the infected subjects. Using deuterium uptake with isotope ratio spectrometry and gas chromatography and with patients on a defined diet, the investigators measured fasting and postprandial fatty acid (FA) and cholesterol synthesis and FA composition as well as a number of relevant metabolic parameters in chronic HCV.

The key findings in this study were that fasting, but not postprandial, *de novo* FA synthesis was significantly higher in HCV-infected patients, whereas both fasting and postprandial (8-hour) cholesterol synthesis were significantly lower, whether measured as whole-body or hepatic cholesterol synthesis. In addition, FA lipogenesis estimated from plasma TG was lower than that measured from VLDL synthesis in controls, but this difference was not evident among HCV-infected subjects, indicating a significant physiologic change in VLDL metabolism in HCV infection. FA composition of VLDL TG also showed diminished polyunsaturated FAs in HCV patients, whereas palmitic acid, the primary endpoint of *de novo* FA synthesis, was higher in plasma TG among HCV-infected patients. Finally, total plasma phospholipid FA content was lower in the HCV-infected subjects, compared to controls. So, what are the implications of these results?

Recognition of the most infectious form of circulating HCV (the buoyant, low-density, TG-rich, roughly 100-nm lipoprotein known as an LVP) as more than a simple aggregation of viral particles with circulating native lipoproteins has led to increasing understanding of the remarkable life cycle of HCV.⁷ As presently understood (and in a perhaps oversimplified summary), translation of the positively stranded HCV RNA results in a polyprotein that is subsequently cleaved into 10 proteins, including core protein, envelope glycoproteins (E1 and E2), and a series of non-structural proteins (NS3, 4, and 5) that form a replication complex localized to the endoplasmic reticulum (ER). Through a hydrophobic domain (D2), core protein localizes to the phospholipid monolayer of lipid droplets, altering the distribution of healthy lipid droplet proteins (PAT proteins) and apparently causing microtubule-dependent aggregation of the droplets in the perinuclear region.^{8,9} Core protein is also associated with E1 and E2 in the ER and appears to serve as a carrier of replicated viral RNA. Localization of NS3, 4A, 4B, 5A, and 5B to the nearby ER results in the creation of the replication complex, which usurps the VLDL synthetic pathway through lipidation of apolipoprotein B (apoB; essential for secretion of the viral particle) through the activity of microsomal TG transfer protein (MTP) to create a nascent LVP.^{10,11} Subsequent merging of the nascent LVP with a core-protein-bearing lipid droplet (possibly facilitated by the envelope glycoproteins, E1 and E2) and incorporation of apolipoproteins E and C (apoE and apoC) completes the synthesis of the LVP, which now resembles a slightly larger than usual VLDL particle (Fig. 1). Although variously depicted as a single spherical lipoprotein or a lipoprotein with a smaller viral particle attached, previous electron microscopy studies favor its formation as a single lipid-rich spherical lipoprotein particle of approximately 100 nm in diameter.^{12–13}

However, the circulating form of HCV particles in humans is heterogeneous. In fasting blood samples from infected patients, the LVP-associated fraction of HCV RNA varies to as high as 74%, but constitutes, on average, approximately 24% of the total circulating HCV RNA.¹⁴ Postprandial blood samples reveal decreased total circulating HCV RNA to approximately 80% of baseline, but increased levels of the RNA-positive LVP fraction.¹⁵ Thus, postprandial blood may be the most infectious of all. However, some detectable E1/E2-carrying LVPs lack viral nucleocapsids.¹⁶ Perhaps, this reflects either a defect in its lipoprotein-generating machine or a survival strategy with use of a decoy. The postprandial state is also associated with changes in LVP constituents, possibly as a result of intravascular interactions between circulating lipid-rich particles.¹⁶ To what extent postprandial changes in LVP metabolism represent intestinal secretion of apolipoprotein B48 carrying viral particles remains uncertain.¹⁷ However, the importance of the LV in HCV pathogenesis is underscored not only by its position as the most infectious form of circulating viral particles, but also by its correlation with increased IR, as well as to a ratio of total TG to circulating HDL, to measures of liver stiffness and to response to antiviral therapy. The role of the LVP in higher infectivity is mediated by key surface constituents of the particle (apo-B-100, apoE, apoC, and the viral E1/E2 envelope glycoproteins) and the interaction of these elements with hepatocyte receptors, including glycosaminoglycans, the LDL receptor, and the scavenger receptor B type I receptor.⁴

The seemingly paradoxical role of cholesterol metabolism vis-à-vis FA and TG metabolism in HCV deserves some additional comment. The present study demonstrated enhanced FA and diminished cholesterol synthesis in HCV infection—findings that are consistent with previous studies demonstrating increased expression of sterol regulatory element-binding protein (SREBP)1 (nuclear transcription factor for factors involved with FA synthesis), but not SREBP2 (nuclear transcription factor involved with cholesterol synthesis) in human liver samples.¹⁸ On the other hand, it is fairly clear that higher LDL cholesterol predicts a better response to antiviral therapy, and, paradoxically, statin (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition) therapy, which reduces LDL cholesterol, also favors response to antiviral therapy.¹⁹ Moreover, although HCV lacks gene products that directly mimic or usurp the host pathway of cholesterol metabolism, HCV replication is dependent on geranylgeranyl pyrophosphate, which is an intermediary in the cholesterol synthetic pathway.²⁰ Recently, a relationship between cellular cholesterol metabolism, HCV replication, and autophagy (a key regulator of cellular lipid metabolism) has been proposed based on both human biopsy samples and a cell-culture model of HCV replication, wherein HCV stimulates a “lipid-selective” form of autophagy, and its inhibition leads to accumulation of cholesterol in the infected cell.^{21,22} In addition, changes in inflammatory cytokines may modulate this process. For example, tumor necrosis factor alpha activates the SREBP-signaling pathway in the liver of fasting mice, resulting in significant alterations of lipid homeostasis, such as reduction of HDL cholesterol, an increase in LDL cholesterol, and elevated expression of cholesterogenic genes.²³

The mechanism and role of IR in HCV infection also deserves additional consideration. Although obscured by the increasing prevalence of obesity in more-recent studies, HCV appears to have an independent effect on IR.²⁴ Whether or not IR in HCV occurs initially at the level of skeletal muscle (diminished glucose utilization), the hepatocyte (poorly

suppressible glucose production), or adipose tissue (poorly suppressible FA release) remains incompletely resolved.^{25,26} Core protein expression and FLD are associated with altered metabolism of free FAs and mitochondrial function, hepatic lipid accumulation, and IR.²⁷ Because HCV is linked to peripheral IR, it is interesting to speculate that HCV-induced hyperinsulinemia drives hepatic lipogenesis by insulin-induced SREBP1c expression, eventually contributing to fatty liver in some patients and hepatic IR. However, a previous study by Milner et al. demonstrated that hepatic fat accrual is HCV genotype specific, despite similar degrees of hyperinsulinemia and glycemia across HCV genotypes in nonobese patients.²⁸ Specifically 15 HCV patients with genotype 1 demonstrated no hepatic fatty change, compared to the control group (~5% liver fat), whereas 14 patients with genotype 3 had, on average, 15% liver fat by proton nuclear magnetic resonance spectroscopy. Unfortunately, no data were provided for VLDL-associated TG or rates of lipogenesis. Because all patients in both the Milner et al. study and the Lambert et al. study were hyperinsulinemic without hyperglycemia, it still remains unclear whether HCV regulates hepatic lipogenesis entirely by intrinsic or peripheral effects and whether increased lipogenesis is required for LVP formation.

Although the findings of the current study support many of the previous experimental observations, there are several limitations that necessarily temper the conclusions. The small number of study subjects is an obvious limitation, but characteristic of this sort of labor-intensive *in vivo* investigation. In addition, genotype and viral load were not reported on, but would have enhanced the results. Finally, although the patients appear to have been clinically stable (mean native Model for End-Stage Liver Disease: 9.6), it is possible that cirrhosis and associated portosystemic shunting or the presence of hepatocellular cancer (albeit presumably small, given the patients' transplant candidacy) may have influenced the results.

Remarkably, current evidence indicates that HCV replication is imposed on the host liver lipoprotein machine influenced as it is by variable dietary factors, host genetic factors, and the inflammatory milieu. The process can be viewed as akin to molecular mimicry, wherein viral proteins take over or redirect the healthy cellular functions involved with fat-droplet metabolism and VLDL synthesis. Discerning these relations remains a clinical challenge, especially in the setting of confounders that alter lipid metabolism (e.g., obesity, dietary changes, and ethanol). Therapeutically, these studies offer a better understanding of the effects of primary treatment (or potential adjunctive agents) and, possibly, of treatment side effects, because disruption of the HCV lipoprotein complex may have unintended effects on lipid metabolism. Importantly, future studies should investigate genotype-specific changes in lipogenesis and the requirement of lipogenesis for LVP production.

Abbreviations

apoB	apolipoprotein B
apoC	apolipoprotein C
apoE	apolipoprotein E

ER	endoplasmic reticulum
FA	fatty acid
FLD	fatty liver disease
HCV	hepatitis C virus
HDL	high-density lipoprotein
IR	insulin resistance
LDL	low-density lipoprotein
LVP	lipoviroparticle
MTP	microsomal TG transfer protein
NS	nonstructural protein
PUFAs	polyunsaturated FAs
SREBP	sterol regulatory element-binding protein
TG	triglyceride
VLDL	very-low-density lipoprotein

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Fig. 1.

Hypothetical illustration of HCV replication and the formation of the LVP involving cellular components of VLDL synthesis (adopted from Negro,⁴ Ye,⁵ and Icard et al.¹¹). Much remains to be learned about the process, but the principle mechanisms involve localization of the viral core protein to small fat droplets and localization of the viral NS3–5 replication complex (NS3, 4A, 4B, 5A, and 5B) to the ER in proximity to synthetic sites of apoB and MTP, resulting in lipidation of the nascent LVP. The completed particle incorporates surface

structures, including apoB, apoC, apoE, and viral envelope proteins (E1/E2), which facilitates entry into other cells.

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