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Effect of Sofosbuvir and Ribavirin Treatment on Peripheral and Hepatic Lipid Metabolism in Chronic HCV, Genotype-1 Infected Patients

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Abstract

Hepatitis C virus (HCV) modulates intrahepatic cholesterol biosynthetic pathways to promote viral replication. Chronic HCV infection is associated with altered metabolism, including dyslipidemia and insulin resistance, which contributes to disease progression and influences response to therapy. To further understand the impact of HCV infection on host metabolism, we examined changes in serum lipid profiles and intrahepatic expression of lipid-related genes during interferon (IFN)-free treatment of chronic HCV, genotype-1 infection with sofosbuvir and ribavirin (RBV), and explored associations with treatment outcome. Serum lipids (total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides) and hemoglobin A1C (HbA1C) were measured during treatment, while gene expression of lipid-related genes was assessed using paired pre- and end of treatment (EOT) liver biopsies from 8 patients (n=7 sustained virologic response (SVR), n=1 relapse) and unpaired EOT liver biopsies from 25 patients (n= 17 SVR, n=8 relapse). Serum LDL concentration and particle size increased early in therapy, while triglyceride concentration and very low density lipoprotein (VLDL) particle size decreased concomitantly, irrespective of treatment outcome. While LDL increased in

patients regardless of treatment outcome, average LDL concentration was lower at baseline and post-treatment in patients who relapsed. Analysis of paired liver biopsies revealed altered expression of genes associated with lipid transport, assembly, and signaling. In unpaired EOT liver biopsies, intrahepatic expression of fatty acid metabolism and lipid transport genes was lower in patients who experienced treatment relapse.

Conclusion—Clearance of HCV using an IFN-free antiviral regimen results in rapid changes in peripheral and intrahepatic metabolic pathways, implicating a direct effect of HCV replication on lipid homeostasis.

Keywords

LDL; cholesterol; viral hepatitis; SVR; relapse

Chronic HCV infection is associated with metabolic complications, including insulin resistance, hepatic steatosis, hypobetalipoproteinemia, and hypocholesterolemia (1, 2). Hepatic steatosis is most prevalent in patients infected with genotype (GT)-3 virus; however, it is also observed in HCV GT-1 infection that is prevalent in the United States (1, 3, 4). Compared to HCV GT-1 infection, GT-3 infection is associated with relatively lower serum LDL, while GT-1 infection is associated with higher LDL and insulin resistance (3, 5, 6). Clinical metabolic complications of HCV infection improve after treatment-induced clearance of HCV (sustained virologic response, or SVR), implicating a causative role of ongoing viral replication (1, 7). Addition of an HMG-CoA reductase inhibitor (statin) to IFN-based therapy can improve rates of SVR and reduce hepatic steatosis, supporting the relevance of host metabolism in HCV treatment outcome and pathogenesis (8–10). In addition, baseline LDL concentration is associated with interferon lambda gene nucleotide variants (*IFNL3* and *IFNL4*) in HCV infection, but not in healthy volunteers, and baseline LDL, *IFNL3/4* genotype, and presence of insulin resistance predict odds of achieving SVR with IFN-based therapy (1, 5, 6, 11).

HCV is also known to utilize host lipid metabolic pathways during replication. Viral assembly and envelope acquisition during hepatocyte budding require the VLDL biosynthetic pathway, and circulating HCV is complexed with VLDL in lipoviral particles containing host apolipoproteins, including APOB, APOE, and APOC3 (1, 12–16). Upregulation of the *MTTP* gene, whose protein product modulates VLDL production, is observed in HCV-infected liver, and a high association of HCV virions with LDL and VLDL particles is correlated with increased viral infectivity (1, 7, 12). HCV infection is also associated with intrahepatic transcriptional upregulation of *SREBP* genes, master transcriptional regulators of fatty acid metabolism, leading to enhanced HCV-lipid droplet association and viral assembly (17–22).

Treatment of chronic HCV infection is transitioning from IFN-based to IFN-free regimens composed of directly acting antiviral agents (DAAs). DAA therapy results in improved rates of SVR and reduces the predictive ability of previously described baseline characteristics for SVR with IFN-based therapy, although baseline LDL has remained a predictor in some DAA studies (5, 23). The association of baseline metabolic characteristics with treatment outcome and changes in lipid metabolism during IFN-free DAA treatment has not been fully

assessed. To explore these associations, we used samples collected during a phase 2 clinical trial of treatment naïve, HCV GT-1 infected subjects (24). We asked whether peripheral lipoparticle composition and intrahepatic lipid-related gene expression changed during IFN-free therapy, and attempted to identify potential markers of favorable outcomes to DAA therapy.

Experimental Procedures

Study Population

Sixty treatment-naïve, chronic HCV GT-1 patients were treated with sofosbuvir (400 mg daily) and low dose (600 mg daily) or weight-based (400mg QAM with 600mg QPM if < 75kg or 600mg BID if >75kg) RBV for 24 weeks on the NIH/NIAID SPARE protocol (NCT01441180), as previously described (24). Clinical data from 54 patients who completed 24 weeks of therapy, and 1 patient who completed 12 weeks of therapy, were utilized for this study. Biospecimens for analysis were selected based on availability and treatment outcome. Data for serum lipid analysis were excluded if patients newly started or increased the dose of statin therapy on-study. One patient receiving insulin therapy was excluded from analysis of HbA1C. Data from patients receiving stably dosed, long-standing lipid-modulating drugs or metformin therapy for diabetes were included. Fourteen patients who relapsed on SPARE were retreated on the SYNERGY protocol (NCT01805882) with 12 weeks of sofosbuvir and ledipasvir (48), and lipid measurements were collected longitudinally. All patients provided written or oral informed consent approved by the NIAID/NIH Institutional Review Board, and the study protocols conformed to the ethical guidelines of the Declaration of Helsinki.

Serum cholesterol and HbA1C measurements

Measurements for serum total cholesterol, HDL, LDL, triglycerides, and HbA1C were performed in the NIH Clinical Research Center laboratory on the day of collection. ApoB/ApoA1 ratios were determined using serum stored at -80C and later thawed for analysis. Patients were instructed to fast prior to blood draws, but adherence was not confirmed. Homeostatic model assessment-insulin resistance (HOMA-IR) score was calculated as $(\text{glucose} \times \text{insulin})/22.5$ using fresh (glucose) or stored serum (insulin) (25).

The Vantera Clinical Nuclear Magnetic Resonance (NMR) Analyzer was used to assess lipoprotein particle size and distribution and generate a LipoProfile using stored patient serum (26). Briefly, serum is subjected to a short pulse of radio frequency energy within a magnetic field, and the distinctive radio frequency signal generated by each particle is quantified to determine lipoprotein size and number using proprietary Vantera Clinical Analyzer software.

Liver and PBMC gene expression analysis

Liver biopsies—Liver biopsy processing, RNA isolation, and approach to microarray analysis have been previously described (27).

Peripheral blood mononuclear cells (PBMC)—Whole blood samples were collected at baseline, day 6–11 of treatment initiation, and at EOT. PBMC were isolated, stored, and used for RNA extraction and qRT-PCR, as previously described (27).

Bioinformatic analysis

Significant canonical pathways with representation in the microarray datasets were determined by Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, California) using input of the top 1% of differentially expressed genes, as described (27). $-\log(p\text{-values})$ of 1.3 ($p = 0.05$) represent the cut-off for significance.

qRT-PCR

Expression analysis of select genes was performed with pre-designed assays in custom-designed 96-well plates or 384-well microfluidic cards (Life Technologies, Grand Island, NY). Total RNA isolated from liver or PBMC was reverse-transcribed using random primers with the High Capacity cDNA Reverse Transcriptase Kit (Life Technologies, Grand Island, NY), and between 1 and 5 ng of RNA in duplicate was used for each qRT-PCR reaction. *GAPDH* Ct values (endogenous control) for all samples were distributed between 20 and 25. Relative expression of targets normalized by *GAPDH* expression (dCt) was calculated as $dCt = Ct_{(GAPDH)} - Ct_{(target)}$.

Statistical analysis

Changes from baseline serum lipid measurements were assessed at weeks 4, 12, 24, 36, and 48. The Hotelling's t-statistic was used to test the overall on-treatment (Week 4, Week 12, Week 24) and post-treatment means (Week 36, Week 48) in relation to baseline values (Day 0), with means and standard errors presented in the text. NMR Lipoprofile data were analyzed by ANOVA with a multiple test correction. Longitudinal lipid data for patients retreated on the SYNERGY protocol were analyzed using a linear mixed effects model. Gene expression analysis was performed with nonparametric assumptions using the Mann-Whitney or Wilcoxon matched-pairs signed rank test for two-group comparisons, and the Kruskal-Wallis non-parametric ANOVA for multi-group comparisons. Prism 6.0 (GraphPad Software Inc., La Jolla, CA) and Spotfire S+ 8.2 (TIBCO, Boston, MA) software were used for statistical analysis and data presentation.

Results

In this phase-2 clinical trial, 38 of 55 patients (69%) achieved SVR with undetectable HCV 24 weeks after treatment with sofosbuvir and ribavirin, while 17 of 55 patients (31%) relapsed (24). A summary of baseline patient demographics, metabolic parameters, and pertinent medications is shown in Table 1. Early in therapy, serum LDL in the entire cohort increased by week 4 of treatment (91 ± 4 to 104 ± 5 mg/dl, $p=0.0027$), a change sustained during treatment at week 12 (102.6 ± 5 mg/dl, $p=0.0029$) and week 24 (EOT, 106.3 ± 5 mg/dl, $p=0.008$), as well as post-treatment (week 36: 107 ± 7 mg/dl, $p=0.0015$; week 48: 101 ± 5 mg/dl, $p=0.039$) (Figure 1A). When results were analyzed by treatment outcome, mean serum LDL was lower in patients who relapsed vs. achieved SVR at baseline (78.7 vs. 97.7 mg/dl, $p=0.031$) and at post-treatment weeks 36 (81.6 vs. 116.6 mg/dl, $p=0.0033$) and

48 (82.2 vs. 109.4, $p=0.005$), with no differences observed on treatment (Figure 1B). In contrast to serum LDL, serum triglycerides decreased by week 4 of therapy (136.7 ± 9 to 98.2 ± 9 mg/dl, $p<0.0001$), a decrease sustained during treatment (Figure 1C). When results were analyzed by treatment outcome, only post-treatment week 36 triglyceride values differed (78.6 ± 8 mg/dl relapse vs. 123.7 ± 13 mg/dl SVR, $p=0.0055$) (Figure 1D). A similar on-treatment pattern for LDL and triglycerides was observed irrespective of HCV subtype (genotype 1a vs. 1b), and the results did not differ when patients on lipid-modifying agents were excluded (data not shown). ApoB/ApoA1 values, a ratio of serum lipoproteins correlated with the risk of coronary heart disease (28), increased early in therapy by week 4 ($0.56 \pm .03$ to 0.63 ± 0.04 , $p=0.029$) paralleling changes in LDL, a difference not sustained by EOT and that did not differ by treatment outcome (Figure 1E). Patient weight, serum albumin, HDL, and total cholesterol did not change significantly during therapy or differ by treatment outcome (data not shown). Additionally, there was no correlation between baseline triglyceride or LDL levels with viral load, hepatic steatosis, hepatic inflammation grade, or fibrosis stage (data not shown). No difference at baseline in host lipid parameters based on the *IFNL4*-rs368234815 dinucleotide variant was observed, although there were limited patients with favorable TT/TT genotype (Table 1, data not shown).

Advanced liver disease is associated with lower serum LDL concentration (29). We previously identified elevated baseline HCV viral load and advanced liver disease as risk factors for relapse in a bivariable model of treatment outcome in this study (24). In a logistic regression analysis of viral load ($<800K=0$, $800K=1$), liver disease (HAI fibrosis 0–2=0, HAI fibrosis 3–4=1), and LDL, elevated viral load ($p=0.038$) and advanced liver disease ($p=0.018$) remained significant predictors of outcome, while baseline LDL did not ($p=0.11$). While these results should be interpreted with caution given the small sample size of the study, they suggest that lower serum LDL may reflect the presence of other risk factors for treatment relapse.

To further investigate changes in cholesterol concentration, we performed an NMR LipoProfile analysis using patient serum to quantify the absolute number of distinct lipoparticles in serum by size. Larger HDL and LDL particle size are associated with decreased cardiovascular risk, and are often inversely correlated with triglyceride concentration, as VLDL-associated triglycerides can be transferred to HDL and LDL by the CETP enzyme where they undergo lipolysis, leading to reduced LDL and HDL particle size (30). Average LDL particle size increased early in therapy at week 4 ($21.08 \pm .09$ to $21.43 \pm .09$ nanometers, $p=0.0001$), accounted for by an increase in large LDL and a decrease in small LDL particles (Figure 2A–B), before returning to baseline levels later in treatment. In contrast, average VLDL particle size decreased early during therapy at week 4 (49.64 ± 1.08 to $45.12 \pm .91$ nanometers, $p<0.0001$), due to an increase in small VLDL and a decrease in medium VLDL particles (Figure 2C–D). During the time points tested (day 0, week 4, week 24 (EOT), week 36), there was no difference in LDL or VLDL particle size based on treatment outcome. Average HDL particle size did not change over the course of therapy (Figure 2E–F).

Because all patients on the SPARE protocol received RBV, the relative effect of HCV clearance versus a medication effect on the changes in lipoproteins could not be determined.

Of the 17 patients who relapsed on SPARE, 14 subsequently enrolled in a retreatment arm of the NIAID SYNERGY study (NCT1805882) and received 12 weeks of sofosbuvir combined with ledipasvir, an HCV NS5A inhibitor, with all 14 patients achieving SVR12 (48). Longitudinal analysis of serum LDL concentration over the course of the SPARE and SYNERGY trials in these 14 SPARE relapsers revealed LDL values that closely paralleled changes in serum HCV viral load, implicating a direct effect of viral replication (Figure 3). Less consistent changes in serum total cholesterol, triglycerides, and HDL in this subset of patients were apparent (Supplemental Figure 1).

In addition to dyslipidemia, chronic GT-1 HCV infection is associated with insulin resistance and an increased risk of diabetes, and glycemic control improves after SVR with interferon-based therapy (1, 7, 31). Because patients in SPARE received RBV, which can cause hemolysis and affect the reliability of HbA1C measurements, we assessed glycemic control pre-treatment and 24 weeks after the EOT (week 48). We found a consistent decrease in HbA1C by week 48 ($5.58 \pm .08$ to $5.45 \pm .91$ %, $p=0.0046$) irrespective of treatment outcome in patients with paired values available (Figure 4A). No difference in HOMA-IR score, a more direct measurement of insulin resistance (25, 32), was detected during therapy (Figure 4B). Notably, the majority of these patients were not diabetic or pre-diabetic, and while fasting prior to blood draws was recommended to patients, adherence was not confirmed.

We next asked what effect HCV clearance with an IFN-free regimen might have on intrahepatic expression of lipid-related genes, as HCV modulates transcription of lipid genes in cell lines, primary human hepatocytes, and the chimpanzee infection model (22, 33–35). We previously reported a microarray analysis of paired pre- and EOT liver biopsies available from 8 patients treated on the SPARE protocol, 7 who achieved SVR and one who relapsed (27). One patient who achieved SVR was on stable statin therapy at the time of study enrollment. In addition to changes in the endogenous interferon pathway, analysis of gene expression changes during treatment revealed significant modulation in the LXR/RXR and FXR/RXR pathways (Figure 5A, previously published in part in reference 27), with genes from these pathways in the top 1% of differentially expressed microarray genes displayed in heatmaps (Supplemental Figure 2). The LXR/RXR pathway is associated with regulation of lipid metabolism and inflammation, including control of the *SREBF1* gene, a master regulator of fatty acid metabolism upregulated in liver in chronic HCV infection (22, 36, 37), while the FXR/RXR pathway regulates bile acid, lipoprotein, lipid, and glucose metabolism (38).

We performed qRT-PCR using liver RNA of select genes associated with lipid transport, assembly, and signaling, including those previously implicated in the HCV life cycle. Expression of *APOC3* and *SREBF1* increased over the course of therapy, while expression of *APOL3*, *LEPR*, and *MTTP* decreased. Expression of *APOB*, *APOE*, *LDLR*, *HMGCR*, *HMGCS2*, *CH25H*, and *PPARA* did not change significantly (Figure 5B and data not shown). We next measured gene expression of *SREBF1* in total PBMC over the course of treatment in 21 patients with available samples ($n=11$ SVR, $n=10$ relapsers), as lower PBMC expression of *SREBF1* has been observed in non-responders to IFN-based therapy (34), and whole blood is more easily accessible for gene expression analysis. We found a

significant decrease in expression by week 24 (Figure 6A), while there was a trend ($p=0.051$) towards lower pre-treatment PBMC expression of *SREBF1* in relapsers (Figure 6B), similar to the association of lower pre-treatment serum LDL with relapse (Figure 1B).

We next asked whether lipid pathways might be differentially modulated in end of treatment liver of patients with respect to treatment outcome. We previously identified interferon-stimulated genes (ISGs) as having lower expression in EOT liver biopsies of patients who experienced treatment relapse ($n=17$ SVR, $n=8$ relapse) (27). Similarly, the LXR/RXR pathway was amongst the most differentially expressed canonical pathways with lower gene expression in relapsers (Supplemental Figure 3, previously reported in reference (27)). Four of the 16 patients achieving SVR were on stable statin therapy during the study, and one patient who relapsed was on stable gemfibrozil therapy. Utilizing the Diseases and Biological Function feature of IPA, we identified fatty acid metabolism and lipid transport as differentially regulated pathways between relapse and SVR at EOT (Figure 6A–B, Supplemental Figure 3), with lower predicted activity in relapsers. Sufficient RNA was available from 16 patients who achieved SVR and 8 who relapsed for qRT-PCR confirmation of findings. All four genes tested from the LXR/RXR pathway had lower expression in relapsers at EOT (*ACACA*, *CETP*, *PLTP*, *S100A8*). qRT-PCR also verified lower expression of additional fatty acid metabolism and transport genes (*LYN*, *SQLE*, *STAT3*), although there was no difference in expression of *SREBF1* or other genes identified by the microarray analysis within these pathways (*ACADSB*, *ALDH5A1*, *FGF2*, *NAAA*, *SIPR1*, *SRD5A1*, *TEF*) (Figure 6C and data not shown).

Discussion

We demonstrate rapid changes in serum lipoprotein particle concentration during treatment of chronic HCV, genotype-1 infected patients with an IFN-free regimen of sofosbuvir and RBV. The rapid increase in LDL and decrease in VLDL observed early in therapy likely reflect a shift in lipid metabolism in the setting of inhibition of HCV replication. Serial changes in LDL concentration amongst patients treated first with sofosbuvir and RBV (SPARE protocol) and after relapse with sofosbuvir and ledipasvir (SYNERGY protocol) implicate a direct viral effect on serum lipoprotein concentrations. Importantly, lower baseline serum LDL and lower expression of fatty acid metabolism and lipid transport genes at EOT were associated with relapse, suggesting the relevance of host metabolism for treatment outcome with this DAA combination in HCV genotype-1 infection.

Consistent with these findings, higher baseline serum LDL is associated with odds of favorable treatment outcome for IFN-based therapy, including IFN-based regimens that include an HCV protease inhibitor (1, 5, 6). The increase in LDL we observed after suppression of HCV is similar to observations in patients who achieved SVR with IFN-based therapy (39, 40), suggesting a direct effect of viral suppression. While larger LDL particle size is associated with decreased cardiovascular risk, the changes observed in particle size here using the NMR LipoProfile were transient and returned to pre-treatment values by the end of treatment. As such, the observed changes likely reflect a physiologic response to viral clearance, and any impact of viral clearance on atherosclerotic potential requires assessment in larger cohorts followed longitudinally.

We previously observed lower EOT hepatic ISG expression in patients who relapsed in the SPARE trial (27). Here, we report lower expression of fatty acid metabolism genes, including genes central to lipid homeostasis (*CETP* and *PLTP*). These observations suggest a relationship between innate immunity and host lipid metabolic pathways in the presence of suspected low-level HCV replication occurring below assay detection limits at EOT in patients who later relapse. They also suggest that resolution of inflammation, potentially involving lipid mediators, could be important for HCV eradication during treatment with sofosbuvir and RBV (41, 42), or that differential expression of lipid biosynthetic genes may be a marker of residual, ongoing HCV replication.

Favorable *IFNL3/4* genotype and lower intrahepatic ISG expression have been associated with less disturbance of lipid metabolism and lower prevalence of steatosis in HCV genotype-1 infection (43). Differential expression of lipid genes based on *IFNL3/4* genotype in the setting of HCV genotype-1 infection has also been demonstrated (34). In this study, the small number of patients with favorable *IFNL* genotype precluded the ability to assess the relation of *IFNL* genotype to steatosis, hepatic or peripheral gene expression, or treatment outcome (44).

We assessed expression of genes known to have altered expression in HCV infection or whose protein products are incorporated into lipo-viral particles (15, 45). Differential regulation of genes associated with lipid transport (*APOC3*, *APOL3*) and lipid assembly and signaling (*LEPR*, *MTTP*) was observed in patients with paired liver biopsies available for analysis (14, 45). Consistent with these observations, upregulation of *APOL3* and *SQLE* was observed in the chimpanzee model of HCV infection (35). HCV is known to activate transcription of *SREBP* genes, transcriptional regulators of fatty acid and cholesterol metabolism (19, 22, 46). Previous observations in HCV-infected liver revealed increased *SREBF1*, *ApoB100*, *MTTP*, and *LXRα* expression, decreased *LDLR* expression, and no change in *SREBF2* expression (22, 37). As such, the increase in *SREBF1* expression we observed during treatment was unexpected, although prior studies have not evaluated paired biopsies in relation to *SREBF1* gene expression during viral clearance. This increase may reflect a response to complex changes in HMG-CoA reductase activity, LDLR expression, and intracellular lipid composition following removal of virus perturbation of *de novo* lipogenesis. The observed increase in hepatic *APOC3* gene expression (Figure 5B) is noted in the context of an association of low serum APOC3 with chronic HCV infection and the severity of hepatic fibrosis (47). In addition, high APOC3 in HCV lipo-viral particles reduces lipoprotein lipase-mediated HCV inhibition and stimulates VLDL synthesis (45), and as such, HCV-induced down-regulation in chronic infection may explain the observed increase with viral suppression.

Limitations of the study include the small number of paired liver biopsies available for analysis, particularly in relapsers, and the lack of available tissue from healthy volunteers for comparison. Some patients, as indicated, were receiving lipid-modulating agents, which could impact serum and gene expression results observed. In addition, although patients were instructed to fast prior to serum blood draws, adherence could not be confirmed.

In conclusion, serum lipoprotein composition and concentration change dynamically during IFN-free therapy of HCV genotype-1 infection. This likely reflects an altered balance of lipogenesis following removal of host lipid metabolism perturbation induced by HCV. Mechanisms of treatment relapse with DAA therapy are unclear, but are important to understand to optimize treatment allocation and duration. Our data suggest that differential regulation of host lipid metabolic pathways may be associated with treatment relapse, and support further investigation of lipid metabolites as predictors of treatment response to DAA-therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest

John McHutchison and Mani Subramanian are employees of Gilead Sciences, manufacturer of sofosbuvir. Keyur Patel is a consultant for Gilead Sciences. Susanna Naggie reports prior consulting and research funding from Gilead.

List of abbreviations

DAA	directly acting antiviral
EOT	end of treatment
HbA1C	hemoglobin A1C
HCV	hepatitis C virus
HDL	high density lipoprotein
HOMA-IR	homeostatic model assessment-insulin resistance
IFN	interferon
GT	genotype
LDL	low density lipoprotein
NMR	nuclear magnetic resonance
RBV	ribavirin
SVR	sustained virologic response
VLDL	very low density lipoprotein

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Figure 1. LDL and triglyceride concentrations change rapidly on therapy

Serum LDL and TG concentrations change rapidly during therapy with sofosbuvir/RBV. Serum LDL and triglycerides are shown from individual patients (A, C; SVR- closed circle, relapse- open square) and grouped by treatment outcome (B, D; SVR- solid line, relapse- dotted line) (SVR: $n=38$, and relapse: $n=17$). Shown are individual values (A, C) and means with standard error (panels A–D). P-values by the Hotelling's t-statistic are indicated for on-treatment (week 4, 12, 24) and post-treatment (week 36, 48) values in relation to baseline values (week 0) (panel A and C). Values significantly different between SVR and relapse for

LDL and triglycerides at the indicated timepoints are indicated by asterisks ($p < 0.05$) as determined by an unpaired t-test with Welch's correction (B, D). ApoB/ApoA1 ratios (E) also change during therapy, with P-values representing values significantly different by one-way ANOVA with a multiple-test correction.

Figure 2. LDL and VLDL particle size change dynamically on therapy

Serum from patients achieving SVR (n=22, closed circles) or who relapsed (n=16, open squares) was analyzed using NMR LipoProfile. Particle size and number for LDL (A–B) and VLDL (C–D) change dynamically on therapy, while HDL (E–F) values are unchanged. Shown are individual measurements with means and standard error for particle size, and means with standard error for particle numbers. P-values represent values significantly different by one-way ANOVA with a multiple-test correction. No differences over time for

any analyte were found based on treatment outcome (2-way ANOVA). Time on the x-axis is weeks since initiation of therapy, with week 24 representing the end of treatment.

Figure 3. Serum LDL correlates with treatment-induced HCV suppression

Viral load and LDL concentration only from the 14 patients who relapsed on the SPARE protocol (sofosbuvir + RBV) and were retreated on the SYNERGY protocol with 12 weeks of sofosbuvir and ledipasvir are shown. Shown are means and standard errors. Longitudinal on-treatment and post-treatment values from the indicated treatment windows, denoted by vertical dotted lines, were compared to baseline values. Statistical analysis was by a linear mixed effects model with p-values indicating differences between the indicated treatment interval and baseline values.

Figure 4. Glycemic control improves in patients treated with sofosbuvir/RBV

HbA1C improves while HOMA-IR does not change with therapy. A) HbA1C was measured at screening and week 48 in patients who achieved SVR (n=26, closed circles) or relapsed (n=13, open squares). P-value is by a paired t-test of values from patients with available data (n=39), with no significant difference detected between SVR and relapse. B) HOMA-IR was calculated using stored serum for 50 patients at the indicated timepoints (n=33 SVR- closed circles, n=17 relapse- open squares). No significant differences were found over time by the Hotelling's t-statistic. EOT= end of treatment (week 24), weeks on the x-axis are time from start of therapy.

Figure 5. Hepatic expression of lipid genes changes with therapy

A) Top IPA canonical pathways whose gene expression differs over the course of treatment, as determined by microarray analysis from 8 patients with paired pre- and post-treatment liver biopsies (previously published in part, reference 27). B) qRT-PCR expression of genes associated with lipid transport (*APOB*, *APOC3*, *APOL3*) and assembly/signaling (*LEPR*, *MTTP*, *SREBF1*). Shown are medians with interquartile range, with statistical analysis by the Wilcoxon paired signed rank-test considering all patients (n=7 SVR, closed circles, n=1 relapse, open square).

Figure 6. Altered peripheral gene expression of *SREBF1* over the course of treatment

A) Gene expression of *SREBF1* in PBMC decreases over the course of treatment in a subset of 21 patients tested (n=11 SVR- closed circles, n=10 relapse- open squares). B) Trend towards lower baseline *SREBF1* gene expression of PBMCs in relapsers. Shown are individual measurements with medians and interquartile ranges. P-value is by nonparametric ANOVA with a multiple-test correction (A) or by the Mann-Whitney test (B).

Figure 7. Fatty acid metabolism gene expression differs in relapsers in EOT liver

A) Disease and Biologic Function analysis by IPA of EOT microarray results between patients who achieved SVR (n=17) or relapsed (n=8). Values less than -2 are considered significant, and a negative value reflects lower predicted activity in patients who relapse. B) Heatmap of genes within the Fatty Acid Metabolism Pathway that differ in expression between SVR and relapse. Treatment outcome is noted. Genes with lower relative expression are indicated in blue, genes with higher expression in red. C) qRT-PCR expression of genes associated with the LXR/RXR pathway and/or fatty acid metabolism. Data are from patients achieving SVR (n=16, in black) or who relapsed (n=8, in red). Statistical analysis is by the Mann-Whitney test.

Table 1

Baseline demographics and clinical characteristics of subjects

Characteristics	SVR n = 38	Relapsers n = 17	P-Value
Median age (IQR)	54 (49–57)	55 (52–62)	0.127
Male gender – no. (%)	19 (50)	15 (88)	0.008
Race or ethnicity– no. (%)			
Black	31 (82)	15 (88)	0.705
Non-Black	7 (18)	2 (12)	
Median body mass index –(IQR)	31 (26–34)	28 (26–31)	0.279
Advanced liver disease– no. (%)	5 (13)	8 (47)	0.014
Steatosis Trace– no. (%)	27 (71)	11 (15)	0.717
HCV genotype subtype – no. (%)			
1a	28 (74)	11 (65)	0.533
1b	10 (26)	6 (35)	
<i>IFNL4</i> genotype– no. (%)			
TT/TT	7 (18)	1 (6)	0.411
TT/ G or G/ G	31 (82)	16 (94)	
Median baseline HCV RNA (log₁₀IU/ml)	5.9 (5.3–6.5)	6.3 (6.1–6.6)	0.159
Mean baseline albumin (SD) g/dl	3.8 (0.37)	3.7 (0.29)	0.298
Mean baseline metabolic parameters (SD)			
- Total cholesterol	174.5 (37)	158.7 (32)	0.116
- LDL	97.7 (33)	78.7 (27)	0.031
- Triglycerides	126.8 (67)	157.4 (79)	0.178
- HDL	51.4 (20)	48.7 (18)	0.633
- Glucose	92.9 (18)	103.3 (20)	0.079
- HbA1C	5.69 (0.6)	5.52 (0.5)	0.248
Taking metabolic drugs- no. (%)			
- statins or fibrates	4 (11)	2 (12)	1.000
- metformin or insulin	3 (8)	0 (0)	0.544
Ribavirin dose			
Low-Dose n = 22	12 (55)	10 (45)	0.077
Weight-Based n = 33	26 (79)	7 (21)	

Continuous variables are shown as median (with interquartile range, IQR) with analysis by the Mann-Whitney test or mean (with standard deviation, SD) with analysis by unpaired t-test. Categorical variables are expressed as number of patients (no.) with frequencies (%), with analysis by the Fisher's exact t-test. Race was self-reported. Body mass index is the weight in kilograms divided by the height in meters. Advanced liver disease is HAI-fibrosis of 3–4 with determination by pre-treatment biopsy. Steatosis was graded on liver biopsy. *IFNL4* genotype was determined using the rs368234815 dinucleotide variant as previously described (43).