Farnesoid X Receptor Agonism, Acetyl-Coenzyme A Carboxylase Inhibition, and Back Translation of Clinically Observed Endpoints of De Novo Lipogenesis in a Murine NASH Model

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A promising approach for the treatment of nonalcoholic steatohepatitis (NASH) is the inhibition of enhanced hepatic de novo lipogenesis (DNL), which is the synthesis of fatty acids from nonlipid sources. This study assesses three approaches to DNL suppression in a newly developed dietary NASH mouse model: i) dietary intervention (switch from NASH-inducing diet to normal diet); ii) inhibition of acetyl-coenzyme A carboxylase (ACC), the enzyme catalyzing the rate-limiting step in DNL; and iii) activation of farnesoid X receptor (FXR), a major transcriptional regulator of DNL. C57BL/6j mice on a high-fat diet combined with ad libitum consumption of a fructose–sucrose solution developed several of the liver histologic features seen in human disease, including steatosis, inflammation, and fibrosis, accompanied by elevated fibrosis biomarkers and liver injury enzymes. Obesity and metabolic impairments were associated with increased intestinal permeability and progression to adenoma and hepatocellular carcinoma. All three approaches led to resolution of established NASH with fibrosis in mice; however, some differences were noted, e.g., with respect to the degree of hepatic steatosis attenuation. While ACC inhibition resulted in elevated blood triglycerides and peripheral obesity, FXR activation prevented peripheral obesity in NASH mice. Comparative transcriptome analysis underlined the translatability of the mouse model to human NASH and revealed novel mechanistic insights into differential regulation of lipid, inflammatory, and extracellular matrix pathways by FXR agonism and ACC inhibition.

Conclusion: Novel insights are provided on back translation of clinically observed endpoints of DNL inhibition by targeting ACC or FXR, which are promising therapeutic options for the treatment of NASH, in a newly developed diet-induced NASH mouse model. (Hepatology Communications 2020;4:109-125).

Elevated hepatic de novo lipogenesis (DNL), which is the synthesis of new fatty acids from nonlipid sources, is central to the development of nonalcoholic fatty liver disease (NAFLD) and the progression of nonalcoholic steatohepatitis (NASH).1 Whereas in healthy individuals DNL contributes up to 5% to hepatic triglycerides, its contribution in NAFLD is estimated as 3.5-fold higher, with about

Abbreviations: ACC, acetyl-coenzyme A carboxylase; Akt, protein kinase B; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BAT, brown adipose tissue; Col, collagen; DNL, de novo lipogenesis; FXR, farnesoid X receptor; GTT, glucose tolerance test; H&E, hematoxylin and eosin; HCC, hepatocellular carcinoma; HDL-C, high-density lipoprotein cholesterol; HF, high fat; IBA1, ionized calcium-binding adapter molecule 1; ITT, insulin tolerance test; MRI, magnetic resonance imaging; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ND, normal diet; ns, not significant; Otud7b, ovarian tumor deubiquitinase 7B; PIII-NP, N-terminal procollagen III propeptide; Scarb1, scavenger receptor class B member 1; Scd, stearoyl-coenzyme A desaturase; SREBF1, sterol regulatory element-binding transcription factor 1; SREBP, sterol regulatory element-binding protein; TEM, transmission electron microscopy; TGR5, G protein-coupled bile acid receptor 1; TIMP-1, tissue inhibitor of metalloproteinase 1; TGFβ, transforming growth factor beta; TNFα, tumor necrosis factor alpha; WAT, white adipose tissue.

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26% of triglycerides derived from hepatic DNL. (2,3) Because the increase in hepatic lipids in patients with NAFLD is substantially attributed to DNL, pharmacologic inhibition of DNL represents a promising approach for NASH. The conversion of acetyl-coenzyme A (CoA) to malonyl-CoA by acetyl-CoA carboxylase (ACC) is rate limiting, and enzymatic activity of ACC is a key regulatory step in DNL. (4) Hepatic DNL was elevated in mice with constitutively active ACC and was associated with the development of fatty liver and fibrosis, while antisense oligonucleotide ACC inhibitors prevented hepatic steatosis. (5,6)

Pharmacologic inhibition of ACC in patients with NASH using GS-0976, a liver-targeted ACC inhibitor, reduced DNL and liver fat content, and improved noninvasive markers of fibrosis but unexpectedly resulted in a significant rise in plasma triglycerides. (7,8)

Another therapeutic approach to inhibiting DNL is by its transcriptional down-regulation. Gain/loss-of-function studies identified sterol regulatory element-binding protein (SREBP)-1 as a critical lipogenic transcription factor and a major driver of hepatic DNL. (9,10) The activation of farnesoid X receptor (FXR) suppresses the transcription of SREBP-1c and its lipogenic target genes, thus repressing DNL. (11) Preclinical and clinical studies support a crucial role of FXR in hepatic triglyceride homeostasis, bile acid, and glucose metabolism as well as hepatic inflammation and fibrogenesis. (12) FXR-deficient mice exhibited marked hepatosteatosis, while FXR agonists prevented liver steatosis in rodent studies. (12,13) Obeticholic acid (OCA), a clinically advanced semi-synthetic bile acid, improved hepatic steatosis and other histologic features in human NASH. (14) Tropifexor (LJN452) is a highly selective and potent nonbile acid FXR agonist that is in development for NASH. (15) LJP305 is its close analog with similar in vivo potency and pharmacokinetic properties and a useful tool for preclinical studies. (15)

This study assesses three approaches to DNL suppression in a newly developed dietary NASH mouse model: dietary intervention (switch from NASH-inducing diet to normal diet [ND]) and pharmacologic inhibition of DNL by targeting activity (through ACC inhibition by GS-0976) or transcriptional regulation (through FXR activation by LJP305) of enzymes involved in DNL.

Materials and Methods

Animal Studies

Studies were performed in compliance with Swiss guidelines for animal experimentation. Adult male
C57BL/6J mice were housed with *ad libitum* access to water and food. Mice were fed a high-fat (HF)/NASH diet (40 kcal% fat, 2% cholesterol, 40 kcal% carbohydrate [D09100301; Research Diets] or SSniff Special Diets, supplemented with a fructose–sucrose solution [42 g/L, 55% fructose and 45% sucrose by weight] in drinking water). Age-matched animals were maintained on regular chow (ND) (#3892; Kliba Nafag) and received drinking water.

Total body fat and lean mass were analyzed by nuclear magnetic resonance, and liver fat was measured by magnetic resonance imaging (MRI). Mice on the HF/NASH diet did not gain weight and total fat mass on a uniform basis; mice that gained less than 10 g body weight and 7 g total body fat at week 7 of the HF/NASH diet (HF/NASH slow progressors, ~15%-20% of all animals) were excluded from intervention studies but analyzed for progression to NASH and hepatocellular carcinoma (HCC).

**Dietary Intervention and Drug Treatment Studies**

Mice fed HF/NASH diet for 8 weeks were switched to ND and water for 12 weeks. Mice exposed to HF/NASH diet for 20 weeks were treated with LJP305 (1 mg/kg), GS-0976 (10 mg/kg), or vehicle (0.5% methylcellulose, 0.5% Tween 80 in water) orally once daily for 12 weeks while remaining on the HF/NASH diet. At the end of the studies, blood and tissue samples were collected for analyses.

**TISSUE ANALYSIS**

Liver samples were analyzed for collagen (Quickzyme), lipids, and triglycerides (Cayman). Relative gene expression was calculated by normalizing Ct values of genes of interest against 18s (liver) or isocitrate dehydrogenase 3 oxidized nicotinamide adenine dinucleotide beta (Idh3b) (fat, ileum). Serum tissue inhibitor of metalloproteinase 1 (TIMP-1; R&D Systems Inc.), N-terminal pro-collagen III propeptide (PIII-NP, Cusabio Biotech Co.), α-fetoprotein (R&D Systems Inc.), leptin, and adiponectin (Meso Scale Discovery) were measured.

Paraffin-embedded tissue was stained with hematoxylin and eosin (H&E) and picrosirisus red. Ionized calcium-binding adapter molecule 1 (IBA1)-positive crown-like structures (ab178846; Abcam), lymphocyte antigen 6 complex, locus B (Ly6b) (MCA771; BioRad), uncoupling protein 1 (UCP-1) (ab109839; Abcam) or Zona occludens-1 (ZO-1) (14-9776-80; Invitrogen) were stained (Ventana Discovery XT). This was followed by digital (Aperio ScanScope XT; Leica Biosystems) and automated image analysis (HALO; Indica Labs).

**STATISTICAL ANALYSIS**

Statistical analysis was performed by one-way analysis of variance (ANOVA) with post-hoc Dunnett’s test. Repeated measurements (body weight and body composition) were analyzed using two-way ANOVA with post-hoc Tukey’s test. MRI data were analyzed using ANOVA with random effects. Additional details of methods are provided in the Supporting Material.

**Results**

**HF/NASH MICE PROGRESSIVELY DEVELOPED NASH WITH FIBROSIS**

Animals exposed to the HF/NASH diet developed liver injury, as defined by elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Fig. 1A). This was accompanied by an increase in liver weight and total liver fat by MRI (Fig. 1B; Supporting Fig. S1A). Total liver fat progressively increased during the first 8 weeks of HF/NASH feeding and subsequently plateaued at 37%; ND mice at 8-20 weeks of diet reached 5% total liver fat. The data observed by MRI were consistent with biochemical analyses of liver lipids, which were elevated at all measured time points in HF/NASH mice (Fig. 1B). Liver histology demonstrated the presence of microvesicular and macrovesicular steatosis (Fig. 1B). HF/NASH mice reached the maximal degree of steatosis after 8 weeks, and this was maintained until 20 weeks of the HF/NASH diet.

HF/NASH animals had morphologic changes consistent with hepatic inflammation characterized by increased numbers of macrophages (Fig. 1C) and neutrophils (Supporting Fig. S1C). IBA1-positive hepatic crown-like structures progressively increased in HF/NASH animals compared to ND animals (Fig. 1C). The proinflammatory state in liver tissue is often associated with macrophage polarization. Gene expression
analysis confirmed a marked increase of profibrogenic M1 and a decrease of proresolution M2 macrophage markers in HF/NASH livers (Fig. 1C). Liver and serum cytokines were also elevated in HF/NASH mice at selected time points (Supporting Fig. S1B).

HF/NASH mice progressed to fibrosis, with the extent of fibrosis being proportional to the duration of the model (Fig. 1D). HF/NASH mice had greater mean fibrosis severity scores compared to ND animals, and automated image analysis demonstrated a significant increase in collagen deposition in HF/NASH animals compared to ND animals (Fig. 1D). Biochemical collagen assay confirmed increased total collagen content in HF/NASH livers (Fig. 1D). TIMP-1 and PIINP levels were increased from 8 weeks of diet for TIMP-1 (105%) and from 12 weeks of diet for PIINP (27%) in HF/NASH mice (Fig. 1D).

The NASH-like phenotype was further underpinned through transcriptome-wide RNA profiling that showed progressive deregulation of the main processes involved in the etiology of NASH in HF/NASH mice (Fig. 2A-C). The transcriptional profiles highlighted genes previously implicated in the development of NASH (Fig. 2B). In line with the above measurements, lipid metabolism, processing of extracellular matrix, inflammation, and apoptotic processes were deregulated in HF/NASH mice. On a pathway level, gene sets related to immune function and extracellular matrix organization were upregulated whereas metabolism, oxidative phosphorylation, and electron transport chain pathways were down-regulated, mirroring elements of known metabolic dysfunctions observed in human NAFLD and NASH (Fig. 2C; Supporting Fig. S2). Collectively, these results demonstrated progressive development of hepatic steatosis, inflammation, and fibrosis in HF/NASH mice.

In order to assess the translational relevance of this model, we compared the murine HF/NASH transcriptomes to data derived from human liver biopsies (Fig. 2D). Specifically, these biopsies were obtained from healthy people with obesity, patients with steatosis before and after gastric bypass surgery, and patients with NASH. We hypothesized that HF/NASH mice should progressively become more similar to patients with NASH. At the same time, we also expected opposite trends by comparing HF/NASH mice to either steatosis before surgery or steatosis after surgery; whereas HF/NASH mice should become progressively more similar to steatotic livers, this trend reverses when comparing to postgastric bypass surgery livers. All our hypotheses were confirmed when we investigated the correlations of each mouse to the average human transcriptomic profile in one of the categories mentioned above (Fig. 2D). Furthermore, we quantified the difference of the HF/NASH diet and ND over time by linear models that specifically take into account an interaction between diet and time (Supporting Material). Overall, HF/NASH mice correlated to the two conditions they were supposed to mimic, namely steatosis and NASH. Of particular note was the increase over time in the correlation of HF/NASH mice with human patients with NASH, accompanied with a concomitant decrease for ND mice (interaction effect for diet, \( P = 0.0059 \)). This clearly showed that, over time, the HF/NASH mice developed transcriptomic changes as seen in livers of human patients with NASH. Interestingly, correlations to patients with steatosis before gastric bypass surgery showed a similar trend seen also for NASH in line with a less pronounced phenotype when comparing NASH to steatosis. The trend seen in the comparison to patients with steatosis after gastric bypass surgery further underlines that the biology of the model (development of a NASH phenotype) is the opposite of recovery from NAFLD/NASH by bariatric surgery. In summary, a comparison of the transcriptional profiles of HF/NASH mice and human subjects with steatosis and liver disease confirmed that the overall processes were reproduced in our murine NASH model.
Fig. 2. Transcriptomics analysis of the HF/NASH model and comparison to human NASH. (A) Expression heat maps of genes involved in lipid metabolism, extracellular matrix turnover, inflammation, and apoptosis during the course of 32 weeks dietary feeding. (B) Gene expression profile of the HF/NASH diet versus ND at 20 weeks. (C) Gene set enrichment analysis for the HF/NASH diet versus ND at 20 weeks. (D) Whole transcriptome correlations of each mouse in the HF/NASH model to average patient with different liver phenotypes (healthy obese, steatosis, NASH). Abbreviation: NCBI, National Center for Biotechnology Information.
HF/NASH mice developed obesity, dyslipidemia, and metabolic impairments

The features of metabolic syndrome include abdominal obesity, dyslipidemia, impaired fasting glucose, and insulin resistance. HF/NASH mice progressively gained body weight with significant changes observed from 2 weeks of HF/NASH feeding onwards (Fig. 3A). Body weight gain correlated with an increase in total fat and lean mass and visceral and subcutaneous fat (Fig. 3A; Supporting Fig. S1D).

Leptin and adiponectin are the main metabolic products of adipose tissue implicated in the pathogenesis of NASH. Leptin, reported to positively correlate with peripheral obesity, was elevated in HF/NASH mice compared to ND mice (Fig. 3B). Adiponectin, reported to inversely correlate with obesity and peripheral fat mass, was reduced in HF/NASH mice starting from 12 weeks of HF/NASH feeding (29%, 42%, and 54% decrease in HF/NASH diet vs. ND at 12, 16, and 20 weeks of diet, respectively) (Fig. 3B). Total and high-density lipoprotein cholesterol (HDL-C) were increased while blood triglycerides were reduced.

FIG. 3. Metabolic profile of HF/NASH mice during the course of 20 weeks dietary feeding. (A) Body weight, total body fat, and lean mass. Visceral and subcutaneous fat weight. Data represent means ± SEM. (B) Serum leptin and adiponectin levels. (C) Intraperitoneal GTT and (D) ITT. Data represent means ± SEM. (E) Semiquantitative analysis of Akt pathway activation in liver in mice fasted for 5 hours followed by insulin bolus at week 20 of dietary feeding. (F) Representative TEM photomicrographs and liver glycogen content in nonfasted animals. Data represent means ± SD unless stated otherwise of n = 8–14 mice per group; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; scale bar, 1 μm. ND mice compared to HF/NASH mice. Abbreviations: AU, arbitrary units; AUC, area under the curve; min, minutes; pAkt, phosphorylated protein kinase B; prot, protein; W, weeks.
in HF/NASH mice (Supporting Fig. S1E). Several epidemiologic studies highlighted the role of insulin resistance in the pathogenesis of NAFLD and the progression of simple steatosis to NASH. In the model described here, no changes were observed in fasted glucose and insulin levels except for the 20-week time point when insulin levels were decreased in HF/NASH animals (not shown). To further characterize the kinetics of glucose and insulin metabolism, intraperitoneal glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed (Fig. 3C,D). NASH mice showed an altered response to the GTT, as shown by prolonged hyperglycemia after glucose bolus. There were, however, no differences in the ITT profile between the groups.

To consolidate our observations, we measured activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway after a bolus injection of insulin and measured the glycogen liver content, both known to be regulated by insulin resistance.\(^{19}\) A significant decrease in Akt activation was found in livers from HF/NASH mice at all measured time points concomitant with a significant decrease in phosphorylated Akt levels at 8 weeks and a sustained increase in total Akt levels (Fig. 3E). Muscle and liver glycogen stocks are decreased in patients with diabetes as well as NAFLD animal models as a direct consequence of impaired insulin signaling. Liver glycogen content and glycogen aggregates visualized on transmission electron microscopy (TEM) sections were massively reduced in HF/NASH livers (Fig. 3F).

Altogether, these data demonstrate the presence of metabolic impairments in HF/NASH mice, including obesity, dyslipidemia, and disturbances in glucose metabolism and insulin signaling.

DIFFERENCES BETWEEN HF/NASH AND HF/NASH SLOW PROGRESSOR MICE

All animals exposed to an HF/NASH diet developed NASH with fibrosis but differed in the speed and severity of disease progression, similar to what is reported for human NASH.\(^{20}\) Two distinct subsets were identified and classified as HF/NASH and HF/NASH slow progressors. Animals that did not gain at least 7 g of total body fat and 10 g of body weight after 7 weeks of diet were classified as slow progressors (approximately 15%-20% of mice from each study). Slow progressors developed milder liver pathology and obesity compared to HF/NASH mice and were protected from adipose tissue inflammation (Supporting Material and Supporting Fig. S3). Moreover, while intestinal integrity and function were impaired in HF/NASH mice, slow progressors were protected from leaky gut development (Supporting Material and Supporting Fig. S4). When exposed to the HF/NASH diet for up to 43 weeks, HF/NASH mice developed histopathologic changes consistent with liver adenoma and showed a molecular signature of HCC. In contrast, slower progressor mice were protected from adenoma and HCC development (Supporting Material and Supporting Fig. S5).

DIETARY INTERVENTION COMPLETELY RESOLVED NASH WITH FIBROSIS AND OBESITY IN HF/NASH MICE

A sustained weight loss of 10% or more is associated with a reduction in NASH fibrosis.\(^{21}\) As no therapies are approved for NASH, sustained weight reduction can be achieved by lifestyle modifications, including physical activity and caloric restriction, or surgical intervention.

In order to determine the susceptibility of HF/NASH mice to dietary intervention, a group of animals were switched back to an ND (normal chow food and water) after 8 weeks of HF/NASH feeding, for a period of 12 weeks (Fig. 4A), which is similar to the time frame of the pharmacologic intervention described in Fig. 5. The switch to an ND completely restored all investigated serologic parameters (Fig. 4B). Liver weight and liver lipids were also normalized (Fig. 4B). Liver triglycerides were strongly reduced in the switched group but did not come back to the levels observed in ND mice (Fig. 4B). The switch to an ND completely reversed liver fat, as assessed by histology and MRI (Fig. 4C). Interestingly, changes in liver fat achieved by the dietary intervention were rapid, with a maximum reduction achieved in 4 weeks. Remarkably, the switch to an ND completely resolved liver inflammation, as assessed by IBA1-positive macrophages and multiple messenger RNA (mRNA) markers of inflammation (Fig. 4D). Dietary intervention resolved fibrosis and prevented its further progression, as demonstrated by reductions in picrosirius red staining, liver collagen, and serum TIMP-1 (Fig. 4E). Dietary
intervention led to a rapid and sustained decrease in body weight by 14% in the switched group compared to HF/NASH mice (Fig. 4F). Body weight loss was associated with a reduction in visceral and subcutaneous fat masses and the normalization of serum leptin levels (Fig. 4F). However, adiponectin levels were not restored and food intake was increased in switched to ND animals, possibly suggesting a behavioral compensation to caloric deprivation (Fig. 4F).

The proportion of body weight loss achieved by dietary intervention in the HF/NASH model (14%) was similar to the recommendations for body weight loss in patients with NASH. Our study also demonstrated that a sustained body weight loss achieved through caloric restriction resolves liver steatosis, fibrosis, and inflammation, thus underlining an important aspect of clinical translatability of our HF/NASH animal model.

**THERAPEUTIC TREATMENT WITH ACC INHIBITOR GS-0976 OR FXR AGONIST LJP305 RESOLVED ESTABLISHED NASH WITH FIBROSIS BUT HAD DIFFERENTIAL EFFECTS ON LIVER TRANSCRIPTOME AND PERIPHERAL OBESITY IN A CHRONIC HF/NASH MODEL**

In order to establish the relevance of pharmacologic inhibition of DNL to NASH, the therapeutic efficacy of GS-0976, ACC inhibitor, and LJP305, a close analogue of FXR agonist tropifexor, was studied. Mice received an HF/NASH diet for 20 weeks, followed by 12 weeks of treatment with GS-0976 or LJP305 along with the HF/NASH diet (Fig. 5A). Target engagement for ACC inhibitor was demonstrated by a decrease in liver malonyl-CoA in GS-0976-treated mice (44% decrease vs. vehicle) associated with a significant increase in blood triglycerides (70% increase vs. vehicle) (Fig. 5B). Treatment with LJP305 resulted in a robust regulation of FXR target genes in the liver, with a strong up-regulation of small heterodimer partner (SHP) and suppression of cytochrome P450 family 8 subfamily b polypeptide 1 (Cyp8b1) expression (Fig. 5B). Therapeutic treatment with GS-0976 significantly improved liver function, as reflected by a decrease in blood ALT and AST, with no significant changes in LJP305-treated animals (Fig. 5B). A significant decrease in liver weight was observed with both drugs (4.9 g in vehicle vs. 3.8 g and 3.1 g in LJP305 and GS-0976 groups, respectively), which correlated with a reduction in total liver triglycerides (36% and 62% decrease in GS-0976 and LJP305-treated animals, respectively) (Fig. 5B).

Histologic characterization of liver sections showed pronounced steatosis, inflammation, and fibrosis in mice fed an HF/NASH diet for 32 weeks and treated with vehicle (Fig. 5C-E). LJP305 and GS-0976 treatments reversed fatty liver by markedly decreasing liver fat content, as measured by MRI (Fig. 5C). Interestingly, a rebound in liver fat was observed during the last 4 weeks of treatment with GS-0976. Both drugs significantly resolved microvesicular steatosis, but only LJP305 improved macrovesicular steatosis (Fig. 5C).

Both compounds significantly decreased liver inflammation, as measured by IBA1+ hepatic crown-like structures and gene expression of proinflammatory cytokines (Fig. 5D). Moreover, reduction in the inflammation from baseline (week 20 HF/NASH) demonstrated regression of inflammation in HF/NASH liver following GS-0976 and LJP305 treatments.

Therapeutic treatment with GS-0976 or LJP305 prevented fibrosis progression as measured by picrosirius red staining and liver collagen content analysis (Fig. 5E). Interestingly, histologic fibrosis was not significantly reduced below the levels observed in HF/NASH mice at the start of the treatment (week 20 HF/NASH), demonstrating an effect of both drugs...
on inhibition of fibrogenesis. A longer treatment period might be required to demonstrate regression of histologic fibrosis in our model. Systemic TIMP-1 levels were decreased in both GS-0976 and LJP305-treated HF/NASH mice (57% and 37% decrease, respectively, vs. vehicle) (Fig. 5E). Similarly, mRNA liver expression analysis showed a strong down-regulation of fibrogenesis markers in GS-0976- and LJP305-treated animals (Fig. 5E). A decrease in circulating TIMP-1 and molecular markers of fibrosis below the levels observed at treatment start demonstrate an effect of GS-0976 and LJP305 on regression of fibrosis together with inhibition of fibrogenesis in the HF/NASH model.

Surprisingly, chronic treatment with ACC inhibitor GS-0976 resulted in a significant increase in body weight compared to vehicle-treated animals despite similar food intake (Fig. 5F). At the end of the 12-week treatment, the mean body weight of GS-0976-treated animals was 7% above vehicle-treated mice. Consistently, GS-0976 treatment significantly increased total fat mass (27% vs. vehicle, data not shown), visceral and subcutaneous fat (30% vs. vehicle), and substantially elevated circulating leptin levels (2-fold vs. vehicle) (Fig. 5F). In contrast, treatment with the FXR agonist LJP305 improved all measured metabolic readouts, as shown by a sustained decrease in body weight gain (7% vs. vehicle at week 32 HF/NASH) and a reduction in visceral and subcutaneous fat (23% and 26% vs. vehicle, respectively) (Fig. 5F). The decrease in peripheral fat was present despite increased food intake after 6 weeks of LJP305 treatment and was associated with a strong trend of reduction in circulating leptin levels (35% vs. vehicle) (Fig. 5F). Furthermore, while LJP305 treatment did not affect nonfasted blood insulin and glucose levels, both parameters were significantly increased by GS-0976 (117% and 42% increase for insulin and glucose, respectively, vs. vehicle) (Fig. 5F).

Analysis of white and brown adipose tissue (WAT/BAT) from LJP305-treated mice revealed no differences in mRNA and protein expression of genes related to adipocyte browning, thermogenesis, or activation of G protein-coupled bile acid receptor 1 (TGR5) signaling but showed a decrease in BAT fat mass and lipid droplets in both WAT and BAT (Supporting Fig. S6A-D).

LJP305 and GS-0976 induced a number of transcriptional changes when compared to the vehicle, with the former leading to 2,690 and the latter to 1,220 differentially expressed genes (absolute log2 fold change, >2; adjusted \( p < 0.01 \)) and 926 of these were shared between the two compound treatments. At a pathway level, this also resulted in comparable enrichments seen for gene sets reflecting general and lipid metabolism and biological oxidations, among others (Supporting Fig. S7A,B). Despite pathways seemingly showing the same enrichments, many of the individual genes involved in these processes, however, showed opposite behavior in a direct comparison of LJP306 and GS-0976 (Fig. 6A-C). GS-0976 induced expression of the target it was inhibiting, acetyl-CoA carboxylase alpha (ACACA), along with other components of the early steps of triglyceride synthesis, such as steroyl-CoA desaturase (Scd)1-4 and fatty acyl elongase family member 6 (Elovl6). Whereas these genes were down-regulated with LJP305, other lipid-associated genes, such as scavenger receptor class B member 1 (Scarb1) were up-regulated (Fig. 6A). Scarb1 was shown to be involved in the uptake of HDL-C into hepatocytes. Scarb1 knockout mice as well as humans with a loss-of-function mutation in Scarb1 had elevated HDL-C levels and increased risk for coronary heart disease. Furthermore, Scarb1 up-regulation was proposed as a possible approach to reduction of coronary heart disease risk. Scarb1 is only one of the 1,254 genes that respond differently (absolute log2 fold change, >2; adjusted \( p < 0.01 \)) to LJP305.
Fig. 6. Transcriptomics analysis of ACC inhibitor GS-0976 and FXR agonist LJP305 in HF/NASH mice. (A-C) Expression heat maps of genes involved in lipid metabolism, extracellular matrix turnover, and inflammation. (D) Comparison of SREBF1 target gene expression following treatment with GS-0976 or LJP305. (E) Network of genes highly correlated to SREBF1 target genes. Red, SREBF1 targets; blue, correlated to SREBF1 targets (Spearman coefficient, r > 0.7). (F) Selection of HF/NASH diet-induced genes restored by LJP305 but not GS-0976. Abbreviations: Lpl, lipoprotein lipase; Lpin1, lipin 1; Mogat1, monoacylglycerol o-acyltransferase 1; Scd1, stearoyl- coenzyme A desaturase; tmp, Transcripts Per Kilobase Million.
and GS-0976. Another example is the ovarian tumor deubiquitinase 7B (Otud7b), a negative regulator of nuclear factor kappa B (NF-κB) signaling; decreased expression of Otud7b has been linked to poor prognosis in HCC.\(^\text{24}\) HF/NASH mice showed a 1.7-fold increased expression of this gene with respect to ND mice. Whereas LJP305 did not significantly alter the expression of Otud7b (log2 fold change, 0.14; adjusted \(P = 0.06\), compared to vehicle treatment), it was significantly down-regulated by GS-0976 (log2 fold change, -0.52; adjusted \(P = 3.72 \times 10^{-7}\), compared to vehicle treatment) (Fig. 6C).

Many genes associated with either lipid metabolism, extracellular matrix, or inflammation moved in opposite directions with LJP305 or GS-0976, as evident from Fig. 6A-C. By focusing on the two examples above, Scarb1 and Otud7b, we could shed some light on the underlying biology.

In a subsequent analysis, we specifically focused on sterol regulatory element binding transcription factor 1 (SREBF1) target genes, previously shown to be activated by a liver-specific inhibitor of ACC1/2, MK-4074.\(^\text{25}\) Genes reported to be induced on a transcriptional level in this study (Fig. 6A-C). By focusing on the two examples above, Scarb1 and Otud7b, we could shed some light on the underlying biology.

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with regards to improvement of macrovesicular steatosis and reducing peripheral obesity.

Discussion

Elevated hepatic DNL is a central abnormality in NAFLD and can lead to the development of NASH.\(^1\) We showed that dietary intervention and pharmacologic inhibition of DNL through two different mechanisms, FXR activation and ACC inhibition, reversed established NASH with fibrosis in a newly developed dietary NASH model. This report provides transcriptional, biochemical, and histologic evidence that activation of FXR and inhibition of ACC have overlapping but also differing effects on hepatic steatosis, inflammation, and fibrosis as well as obesity in NASH mice.

Results obtained in this study with the ACC inhibitor GS-0976 mirror related clinical phase 2 NASH studies, where administration of GS-0976 for 12 weeks led to reductions in liver fat, serum TIMP-1, and liver injury.\(^7,8\) We observed elevated serum triglycerides following chronic GS-0976 treatment, a result consistent with hypertriglyceridemia reported in patients with NASH treated with GS-0976\(^8\) or healthy volunteers treated with MK-4704.\(^25\) Studies with MK-4704 in mice revealed hypertriglyceridemia to be attributed to the ACC-mediated suppression of polyunsaturated fatty acid synthesis and \(SREBP-1c\) activation.\(^25\) Also in this study, therapeutic treatment with GS-0976 suppressed DNL while inducing \(SREBP-1c\)-regulated genes and raising serum triglycerides. Interestingly, ACC inhibition and FXR agonism had differing effects on steatosis; while LJP305 decreased both microvesicular and macrovesicular steatosis, the latter persisted in GS-0976-treated animals and was associated with a rebound of liver fat. Steatosis in NAFLD is usually seen as macrovesicular steatosis, while microvesicular steatosis is associated with more advanced fibrosis and impaired mitochondrial \(\beta\)-oxidation.\(^28,29\) Because ACC2 controls mitochondrial \(\beta\)-oxidation, an improvement in microvesicular steatosis by ACC inhibition is conceivable. The persistence of macrovesicular steatosis in ACC inhibitor-treated mice is, however, intriguing. We report that GS-0976 induced multiple genes known to play roles in lipid metabolism, among them genes catalyzing the synthesis of monounsaturated fatty acids, diacylglycerols, and triglycerides. This transcriptional shift in lipid metabolism could explain the histopathologic findings and provide additional biological context as to why ACC inhibition is associated with fewer lipid microdroplets but stable levels of lipid macrodroplets.

Unexpectedly, long-term ACC inhibition increased diet-induced obesity in our study. No significant changes in body weight were observed in patients with NASH treated with GS-0976,\(^8\) and no data were reported for healthy volunteers administered MK-4074.\(^25\) GS-0976 favorably impacted the disease progression in rodents; however, the compound’s effect on body weight was not reported.\(^30\) Because obesity results from an imbalance between energy input and output and most of the excess calories are stored as triglycerides, it is conceivable that ACC inhibition-induced hypertriglyceridemia ultimately results in obesity.

In contrast to ACC inhibition, FXR activation by LJP305 alleviated obesity in our NASH model. Weight loss is reportedly associated with OCA therapy.\(^14,31\) However, understanding of FXR biology in adipose tissue is limited. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity.\(^32\) A similar effect was reported for TGR5, which, when stimulated by bile acids or nonbile acid ligands, increases energy expenditure through browning of WAT and BAT thermogenesis.\(^33\) Interestingly, in our study, a beneficial effect of FXR agonism on diet-induced obesity and lipid reduction in WAT and BAT was not associated with browning, thermogenesis, or activation of TGR5 signaling in adipocyte tissue. Intestinal FXR and TGR5 signaling crosstalk, which may regulate glucagon-like peptide 1 (GLP-1) secretion,\(^34\) was not assessed in our study. Despite the amelioration of liver architecture seen in histopathology, transaminases remained elevated in LJP305-treated mice. For reasons that remain unclear, this contradicts studies reporting beneficial effects of FXR agonism on transaminases.\(^11\)

In summary, we demonstrated that inhibition of DNL is a promising therapeutic option for treatment of NASH. Moreover, given that ACC inhibition and FXR activation exert both synergistic and complementary effects on NASH livers and obesity, this study provides a rationale for ACC/FXR combination...
therapy in NASH. The ongoing NASH trials are critical to further translate the preclinical findings with FXR agonism and ACC inhibition to human NASH and support the utility of the NASH model described here.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1443/suppinfo.