Therapeutic role of niacin in the prevention and regression of hepatic steatosis in rat model of nonalcoholic fatty liver disease

Shobha H. Ganji, Gary D. Kukes, Nils Lambrecht, Moti L. Kashyap,* and Vaijinath S. Kamanna*

Department of Veterans Affairs Healthcare System, Long Beach, California and the University of California, Irvine, California

Submitted 13 June 2013; accepted in final form 14 December 2013

Ganji SH, Kukes GD, Lambrecht N, Kashyap ML, Kamanna VS. Therapeutic role of niacin in the prevention and regression of hepatic steatosis in rat model of nonalcoholic fatty liver disease. Am J Physiol Gastrointest Liver Physiol 306: G320–G327, 2014. First published December 19, 2013; doi:10.1152/ajpgi.00181.2013.—Non-alcoholic fatty liver disease (NAFLD), a leading cause of liver damage, comprises a spectrum of liver abnormalities including the early fat deposition in the liver (hepatic steatosis) and advanced nonalcoholic steatohepatitis. Niacin decreases plasma triglycerides, but its effect on hepatic steatosis is elusive. To examine the effect of niacin on steatosis, rats were fed either a rodent normal chow, chow containing high fat (HF), or HF containing 0.5% or 1.0% niacin in the diet for 4 wk. For regression studies, rats were first fed the HF diet for 6 wk to induce hepatic steatosis and were then treated with niacin (0.5% in the diet) while on the HF diet for 6 wk. The findings indicated that inclusion of niacin at 0.5% and 1.0% doses in the HF diet significantly decreased liver fat content, liver weight, hepatic oxidative products, and prevented hepatic steatosis. Niacin treatment to rats with preexisting hepatic steatosis induced by the HF diet significantly regressed steatosis. Niacin had no effect on the mRNA expression of fatty acid synthesis or oxidation genes (including sterol regulatory element-binding protein 1, acetyl-CoA carboxylase 1, fatty acid synthase, and carnitine palmitoyltransferase 1) but significantly inhibited mRNA levels, protein expression, and activity of diacylglycerol acyltransferase 2, a key enzyme in triglyceride synthesis. These novel findings suggest that niacin effectively prevents and causes the regression of experimental hepatic steatosis. Approved niacin formulation(s) for other indications or niacin analogs may offer a very cost-effective opportunity for the clinical development of niacin for treating NAFLD and fatty liver disease.

niacin; nicotinic acid; nonalcoholic fatty liver disease; hepatic steatosis; nonalcoholic steatohepatitis

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is increasingly recognized as a leading cause of liver disease and significantly contributes to premature mortality (4, 11). In addition to its high risk for developing liver disease, NAFLD is a major pathophysiological component of metabolic syndrome, obesity, and type 2 diabetes and is associated with increased risk for cardiovascular disease (27). The prevalence of NAFLD is estimated at 30% of the general population and affects up to 75% of patients with obesity and type 2 diabetes (4, 11).

NAFLD encompasses a spectrum of hepatic pathology ranging from simple fatty liver (intracellular lipids >5%) to a progressive nonalcoholic steatohepatitis (NASH) with lobular inflammation, fibrosis, and cirrhosis and increases the risk for hepatocellular carcinoma (4, 11). Despite its severity and prevalence, little is known about the pathogenesis of NAFLD and treatment modalities. Excessive accumulation of triglycerides in hepatocytes is the hallmark of NAFLD, which is strongly associated with hepatic insulin resistance (32, 1). Increased triglyceride synthesis has been shown in fatty livers that accompany obesity and type 2 diabetes mellitus (31). Hepatic fat accumulation leads to hepatic insulin resistance by stimulating gluconeogenesis and activating PKC-ε and JNK1 signaling pathways (33). A two-hit hypothesis has been proposed to understand the pathogenesis of NAFLD: the first hit includes excess fat accumulation in the liver, and second hit consists of oxidative stress and lipid peroxidation with increased generation of inflammatory cytokines (12, 13). Evidence indicates that hepatic steatosis is a risk factor for NASH and fibrosis and implicates steatosis as a direct contributor to NASH (12, 13, 38, 25).

Currently, there are no approved agents available for the treatment of NAFLD. Modifications of risk factors, such as weight reduction and dietary fat consumption, are commonly recognized treatment modalities for NAFLD (2, 3). Pilot studies have indicated that insulin sensitizers, such as thiazolidinediones, and antioxidants, such as vitamin E, improve clinical and histological features of NASH (6, 34, 7, 30, 35, 24, 19). Because of the lack of larger clinical trials, the value of these drugs for the treatment of NASH remains uncertain. Niacin is one of the commonly used therapeutic agents for treating dyslipidemia and cardiovascular disease (26). Using in vitro studies, alterations in hepatocyte micromasomal activity of diacylglycerol acyltransferase 2 (DGAT2) and/or adipocyte triglyceride lipolysis are thought to contribute to the action of niacin on reducing plasma triglycerides (8, 18). Additionally, we showed that niacin, by increasing the redox potential, decreased cellular oxidative stress (17). Because initial fat accumulation in liver and subsequent oxidative stress/inflammatory events are involved in the pathogenesis of NAFLD, here we propose that niacin can prevent and regress high-fat induced experimental hepatic steatosis and NAFLD via inhibiting hepatic triglyceride synthesis and oxidative/inflammatory processes.

MATERIALS AND METHODS

Materials

Nicotinic acid (niacin) and all other chemicals used were from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated.

Animal studies. Male Sprague-Dawley rats (250–300 g) were obtained from Charles River Laboratories (Wilmington, MA). Custom-prepared diets including control (Lab Dyets no. 100000), high-fat (HF) (Lab Dyets #101447), and HF diet containing 0.5% and 1.0% niacin were obtained from Dyets (Bethlehem, PA). Control diet is a low-fat diet consisting of 12% of total calories from fat as corn oil,
Thiobarbituric acid-reactive substances (TBARS) were measured using commercially available Free Fatty Acid Assay Kit (Cayman Chemical, Ann Arbor, MI). Measurements of liver thiobarbituric acid-reactive substances (TBARS) were measured as an index of lipid peroxidation products as described previously (17).

Hepatic Lipogenic and Fatty Acid Oxidation Gene Expression

Liver mRNA expression of sterol-regulatory element-binding protein 1c (SREBP1c), fatty acid synthase (FAS), acetyl-CoA carboxylase 1 (ACC1), DGAT2, and carnitine palmitoyltransferase 1 (CPT1) were performed by real-time PCR (iCycler real-time PCR detection system; Bio-Rad, Hercules, CA) using previously published specific primer sequences (10). mRNA expression levels were calculated by dCT method using β-actin as an internal control.

Liver histology. Liver samples were fixed in 10% buffered formalin and embedded in paraffin wax. The histology of liver tissue was examined in paraffin-embedded 5-μm hematoxylin and eosin (H&E)-stained sections. Coded H&E-stained sections were analyzed by experienced pathologists (N. Lambrecht and G. Kukes) for hepatic steatosis (microvesicular and macrovesicular), inflammation, and necrosis. Semiquantitative total fat accumulation scores (range 0–4) were determined by adding the fat score for microvesicular and macrovesicular fat as reported previously (29). In brief, fat accumulation scores are calculated as follows: score 1, presence of intrahepatic fat droplets in <5% of hepatocytes; score 2, presence of intrahepatic fat droplets in 5–33% of hepatocytes; score 3, presence of intrahepatic fat droplets in 33–66% of hepatocytes; and score 4, presence of intrahepatic fat droplets in >67% of hepatocytes.

Statistical Analysis

The data presented are means ± SE. One-way ANOVA and Turkey’s posttest were used for multiple comparisons for histological data in Fig. 4. Student’s t-test was used for comparisons between two groups for all other figures and tables. Statistical comparisons were made between control and HF or between HF and HF + niacin groups. A P value of <0.05 was considered significant.

RESULTS

Food Intake, Body Weight, and Liver Weight

Rats in each group consumed an average of 20 g of diet. After 4 wk of the feeding period, rats in the HF-fed group exhibited significantly higher body weight than in control diet-fed rats (Fig. 1A). Inclusion of niacin at 0.5% or 1.0% level in the HF diet had no effect on body weight of rats compared with HF-fed rats. Rats fed the HF diet had significantly higher liver weight compared with rats fed control diet (Fig. 1B). Addition of niacin at 0.5% or 1.0% levels completely blocked gain in liver weight induced by the HF diet (Fig. 1B).
Liver and serum cholesterol levels were also significantly increased in rats fed the HF diet compared with rats on a control diet (Fig. 3, left and right). Inclusion of niacin (0.5% and 1.0%) in the HF diet significantly decreased serum cholesterol (Fig. 3, right), and niacin at 0.5% significantly decreased liver cholesterol levels (Fig. 3, left) when compared with rats fed the HF diet. Niacin had no effect on serum levels of NEFA. Serum NEFA levels (mEq/l) were as follows: control (0.35 ± 0.13), HF = 0.36 ± 0.06, HF + 0.5% niacin = 0.4 ± 0.09, HF + 1.0% niacin = 0.44 ± 0.08.

Figure 4 displays representative histological images of H&E-stained liver sections. Prominent and significant hepatic steatosis (micro- and macrosteatosis with intrahepatic lipid droplets of about 4–10 μm diameter) was observed in the HF diet-fed rats (group 2, Fig. 4B) compared with controls (Fig. 4A). Niacin at both doses (0.5% and 1.0%) markedly prevented hepatic steatosis in liver sections of rats fed the HF diet. Semiquantitative calculations from the histological data showed that the relative fat content in HF diet-fed animals was markedly higher compared with normal chow (control)-fed animals. Addition of niacin (0.5 and 1.0%) to the HF diet to a large extent prevented intracellular hepatic fat accumulation. Analysis by ANOVA between control and treatment groups demonstrated that the fat content in the HF diet-fed animals was significantly greater than in control animals fed normal chow diet (P = 0.03). Intrahepatic fat content in rats fed the HF + niacin was significantly less than in rats fed the HF diet (P = 0.038). ANOVA analysis showed no significant differences between control and the HF diet + niacin-treated animals.

Increased numbers of sporadic inflammatory foci were observed in the HF diet-fed rats. These were panacinar collections of up to 10 mononuclear cells, predominantly lymphocytes. No animal, however, exhibited the minimum number of intralobular, chronic inflammatory foci (2 foci/field) to be scored on the NASH activity score inflammation scale. Trichrome-stained sections showed no rat with increased centrilobular (zone 3) or perisinusoidal fibrosis. Some animals exhibited grade I-II periportal fibrosis, out of grades I-IV, but that degree of fibrosis was observed in several control animals and judged nonspecific.

Liver Lipid Peroxidation Products

Niacin addition to the HF diet (at 0.5% and 1.0% level) significantly inhibited liver TBAR levels compared with rats fed the HF diet (Fig. 5).

Hepatic Inflammatory Events

Expression of TNF-α, a prototypic inflammatory cytokine involved in hepatic inflammation, was similar in liver extracts of control, HF, and HF + niacin-fed animals. TNF-α mRNA expression (Ct values, TNF-α/β-actin) were as follows: control = 1.52 ± 0.06, HF = 1.64 ± 0.06, HF + 0.5% niacin = 1.68 ± 0.09, HF + 1.0% niacin = 1.52 ± 0.08. Serum levels of alanine transaminase (ALT, as measured by ALT assay kit; BioVision Research Products, Mountain View, CA) were also unchanged between groups receiving control, HF diet, and HF + niacin. Serum ALT values (U/ml) were as follows: control = 31 ± 3.0, HF = 32 ± 2.0, HF + 0.5% niacin = 34 ± 2.9, HF + 1.0% niacin = 33 ± 1.5.

Niacin Regressed Liver Fat Content and Hepatic Steatosis

In addition to the prevention of hepatic steatosis studies, we further assessed whether niacin regressed preexisting hepatic steatosis. For these studies, rats were first fed the HF diet for 6
wk to induce hepatic steatosis. These rats were then treated with niacin (0.5% in the diet) while they continued on the HF diet for 6 wk. As shown in representative histological images of H&E-stained liver sections, feeding the HF diet for the first 6 wk caused marked hepatic steatosis as assessed by deposition of fat droplets (Fig. 6, HF). Treating HF-fed rats with niacin robustly regressed preexisting steatosis (Fig. 6, niacin) compared with rats on the HF diet (Fig. 6, HF). Semiquantitative lipid accumulation scores in H&E-stained liver sections include the following: control = 0.125 ± 0.04; HF = 2.5 ± 0.28; HF + niacin = 1.12 ± 0.12 (P = 0.04 HF vs. HF + niacin). Biochemical analysis of triglyceride content in liver extracts also showed that treatment with niacin significantly caused regression of liver triglyceride content (Fig. 7). As shown in Table 1, treating HF-fed rats with niacin also decreased liver weight, serum triglycerides, and liver TBAR levels. Niacin had no effect on serum NEFA (Table 1).

Modulation of Liver Lipogenic and Fatty Acid Oxidation Genes

To understand the in vivo mechanism of action of niacin on hepatic steatosis, we examined mRNA expression levels of key regulatory genes involved in hepatic lipid accumulation. Niacin had no significant effect on the mRNA expression of SREBP1, a transcription factor involved in the regulation of fatty acid and lipid metabolism (Table 2). Other hepatic lipogenic SREBP1 target genes of fatty acid synthesis including ACC and FAS were also not affected by niacin treatment (Table 2). Additionally, treatment of rats with niacin did not alter the mRNA expression of CPT1, a key regulator of fatty acid β-oxidation (Table 2).

Contrary to the effects on fatty acid synthesis/oxidation genes, niacin significantly inhibited mRNA expression of DGAT2, a final and rate-limiting enzyme involved in hepatic triglyceride synthesis (Table 2). We further characterized the effect of niacin on DGAT2 by measuring DGAT2 activity and protein levels. Similar to DGAT2 mRNA expression, niacin significantly inhibited hepatic DGAT2 activity and reduced protein expression in the HF-fed rats (Table 2).
HF diet-fed rats. Triglycerides are expressed as mg/g of liver. With niacin (0.5% in the diet) while they continued on HF diet for 6 wk. Liver sections were stained with H&E and analyzed for hepatic steatosis.

DISCUSSION

Similar to Western diets in humans, rats and mice fed the HF diets (50–75% calories derived from fat) develop hepatic steatosis and signs of early NASH associated with dyslipidemia, insulin resistance, alterations in mitochondria, increased oxidative stress, and early signs of hepatic inflammation (5, 14, 36). Although the HF diet-fed rodent model does not develop severe steatohepatitis, these models closely resemble the pathophysiology observed in human NAFLD associated with moderate hepatic inflammatory events and metabolic abnormalities (36, 5, 14). The HF diet-induced NAFLD model has been extensively used for investigating the pathogenesis of NAFLD and for testing treatment strategies (9, 10, 33, 39). Findings from this study indicate that niacin (at 0.5% and 1.0% in the diet) markedly and significantly prevented the development of hepatic steatosis as assessed by histological deposition of fat vesicles and fat content in liver (triglycerides and cholesterol).

In addition to the steatosis prevention studies, we investigated whether niacin can cause regression of preexisting steatosis, which would be most relevant to the clinical situation in humans. Findings from the regression studies indicated that niacin treatment caused a significant regression of preexisting hepatic steatosis by 42–55%, as assessed by biochemical liver triglyceride content measurements and histological analysis in H&E-stained liver sections. Although niacin did not completely restore hepatic lipid content to the control level, it substantially regressed preexisting hepatic steatosis, and our findings on the extent of regression of hepatic steatosis by niacin in rats are similar to those reported in a pilot uncontrolled clinical trial in hypertriglyceridemic patients with pre-existing NAFLD (21). Based on small clinical trials with a limited number of participants and short duration, commonly recommended treatment strategies such as weight reduction and dietary modification are shown to improve hepatic steatosis by 21–43% (2, 22, 37). If niacin proves to effectively reduce hepatic lipid content in a well-controlled and larger clinical trial, niacin formulations approved for other indications may be an important therapeutic option for treating NAFLD.

Niacin at 0.5% or 1.0% levels completely blocked gain in liver weight induced by the HF diet, and liver weights in HF + niacin-treated rats were comparable to control rats (Fig. 1B). However, niacin inclusion in the HF diet did not completely restore liver triglyceride levels to the control levels (Fig. 2). Niacin also significantly reduced liver cholesterol content in rats fed the HF diet. It is possible that the reduction of both triglycerides as well as cholesterol in liver, to a large extent, may have contributed to the complete reduction of increased liver weight in rats fed the HF + niacin diet.

Based on the diet intake, we estimate that rats consumed 0.1 g and 0.2 g niacin per day with HF diet containing 0.5% and 1.0% niacin. Niacin doses of 0.5% and 1.0% used in the diet were comparable to those reported previously in animal studies.

Fig. 6. Niacin causes regression of preexisting hepatic steatosis as shown in representative histological images of liver sections stained with H&E. Rats were first fed the HF diet for 6 wk to induce hepatic steatosis. These rats were then treated with niacin (0.5% in the diet) while they continued on HF diet for 6 wk. Liver sections were stained with H&E and analyzed for hepatic steatosis.

Fig. 7. Niacin causes regression of liver triglyceride content. Rats were first fed the HF diet for 6 wk to induce hepatic steatosis. These rats were then treated with niacin (0.5% in the diet) while they continued on HF diet for 6 wk. Liver triglycerides are expressed as mg/g of liver. *P values shown are compared with HF diet-fed rats.

Table 1. Effect of niacin on liver weight, serum triglycerides, liver TBARS, and serum NEFA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HF</th>
<th>HF + 0.5% Niacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight, g</td>
<td>14.92 ± 0.35</td>
<td>17.53 ± 2.9</td>
<td>13.27 ± 1.0*</td>
</tr>
<tr>
<td>Serum TG, mg/dl</td>
<td>54.98 ± 9.8</td>
<td>144.7 ± 33.9</td>
<td>68.8 ± 27.6*</td>
</tr>
<tr>
<td>Liver TBARS, nMol</td>
<td>329.4 ± 47.0</td>
<td>553.6 ± 37.5</td>
<td>407.4 ± 110.1*</td>
</tr>
<tr>
<td>Serum NEFA, mEq/l</td>
<td>0.55 ± 0.04</td>
<td>0.54 ± 0.02</td>
<td>0.54 ± 0.09</td>
</tr>
</tbody>
</table>

Values are shown as means ± SE. Serum triglycerides, liver thiobarbituric acid-reactive substances (TBARS), and serum nonesterified fatty acid (NEFA) levels were measured as described in MATERIALS AND METHODS. HF, high-fat diet. *P < 0.05 (HF + 0.5% niacin vs. HF).
related to the effect of niacin on plasma lipids (20). Although the effective dose of niacin (0.5% in the diet) used (per kg body wt) in our rat studies are much higher (4.6-fold) than the currently recommended niacin doses in humans (up to 3 g/day) for treating cardiovascular disease, our data provide proof of concept that niacin can be used as a preventive therapy for NAFLD. Because 0.5% niacin in the diet caused maximal and similar effects on preventing hepatic steatosis compared with 1.0% niacin, it is likely that niacin at lower doses may prevent hepatic steatosis. However, additional niacin dose-response studies are needed to determine the minimal effective dose of niacin required to prevent experimental hepatic steatosis.

We explored potential in vivo mechanisms by which niacin decreased liver triglyceride content and hepatic steatosis. Niacin did not affect mRNA expression of SREBP1 transcription factor, a key factor involved in lipidogenesis, and SREBP1 target genes ACC and FAS that regulate fatty acid synthesis. Similarly, niacin had no effect on the mRNA expression of CPT1, a key enzyme involved in fatty acid oxidation. Furthermore, niacin had no effect on serum levels of NEFA in rats fed the HF diet. Our findings on NEFA in rats are consistent with human studies. Although in humans niacin transiently (within minutes) decreases NEFA, subacutely (within 1 h) it causes a profound rebound in NEFA levels such that serum NEFA levels are normal to slightly increased over 24 h (reviewed in Ref. 23).

Niacin treatment significantly inhibited hepatic mRNA expression of DGAT2, a rate-limiting enzyme involved in liver triglyceride synthesis. Further characterization of DGAT2 indicated that niacin significantly reduced the hepatic protein expression of DGAT2 and robustly inhibited the activity of DGAT2 in rats fed the HF diet. In human hepatocytes (HepG2 cells), we have previously shown that niacin selectively and noncompetitively inhibited microsomal DGAT2 activity (18). It is likely that the robust inhibition of DGAT2 activity by niacin in livers of rats fed the HF diet could be due to its effect on both mRNA levels as well as noncompetitive inhibition of enzyme activity. Consistent with our current report that niacin reduces hepatic DGAT2 mRNA expression in livers of rats fed the HF diet, in preliminary studies niacin was also found to inhibit DGAT2 mRNA levels in lipid-loaded HepG2 cells (personal observation, S. H. Ganji, M. L. Kashyap, and V. S. Kamanna). Additional studies are needed to understand mechanism(s) by which niacin decreases DGAT2 gene expression in livers of rats fed the HF diet. In a small clinical trial, Hu et al. (21) recently showed that niacin therapy significantly reduced liver fat content compared with their pre-niacin administration baseline in patients with hypertriglyceridemia (21). Liver fat reduction with niacin in these patients was associated with DGAT2 polymorphisms, suggesting that inhibition of DGAT2 by niacin may participate in hepatic fat reduction in dyslipidemic patients (21). These findings suggest that the property of niacin to inhibit DGAT2 may be one of the mechanisms by which niacin prevents hepatic triglyceride accumulation and steatosis. In support of this proposed mechanism, previous studies indicated that overexpression of liver-specific DGAT2 in mice resulted in increased hepatic steatosis (28). Furthermore, knockdown of DGAT2 with antisense oligonucleotides in rats and mice fed HF diet markedly decreased hepatic steatosis (10, 39). It is interesting to note that DGAT2 knockdown paradoxically lowered liver diacylglycerol content (10, 39). This reduction in hepatic diacylglycerol in DGAT2 knockdown rats was, at least in part, attributed to the suppression of lipogenic pathways in response to increased hepatocellular fatty acid content (10). However, further mechanistic studies are warranted to understand the fate of diacylglycerol in niacin-mediated inhibition of DGAT2 in rats fed the HF diet and its impact on hepatocellular signaling events.

Although niacin modestly inhibited lipid peroxidation products in liver, hepatic inflammatory markers including TNF-α and serum levels of ALT were similar in liver of animals fed control, HF, and HF + niacin. Similarly, liver histological analysis also showed no noticeable changes in inflammation and fibrosis between these control, HF diet-fed, and HF + niacin-treated groups. Although longer feeding of HF diet to rats can produce modest hepatic inflammation, 4 wk of the HF diet-feeding protocol used in our study to produce early steatosis was unable to cause noticeable changes in hepatic inflammatory events.

Our current studies in the HF diet-fed animal model of NAFLD indicate the usefulness of this model for studying the efficacy of niacin on this disease process and provide the potential therapeutic usefulness of niacin in prevention and regression of hepatic steatosis. However, very small recent human clinical studies have provided controversial results. A small study with nine patients with NAFLD and obesity using niacin (2 g/day) treatment for 16 wk demonstrated no effect on intrahepatic triglyceride content (16). In another uncontrolled small trial, Hu et al. (21) reported that niacin therapy (2 g/day for 23 wk in 39 patients) significantly reduced liver fat content in patients with hypertriglyceridemia. Liver fat reduction with niacin in these patients was influenced by DGAT2 polymorphisms, suggesting that inhibition of DGAT2 by niacin may participate in hepatic fat reduction in dyslipidemic patients (21). This preliminary report is supportive of our findings and requires confirmation in a controlled trial. It was pointed out that the discrepancy between the two clinical studies may be due to the study design, patient population, and treatment duration (21). Thus additional basic science mechanistic studies are required to address the mechanisms of action of niacin on pathophysiological processes involved in NAFLD. Addi-
nationally, adequately powered larger clinical trials in patients at varying stages of fatty liver disease are required to address the potential therapeutic effects of niacin on the prevention and regression of NAFLD, NASH, cirrhosis, and end-stage liver disease. In view of the increasing cost of developing new pharmacological agents (about $800 million, Ref. 15), immediate-release niacin, which is readily available, or approved niacin formulation(s) designed to reduce flushing (major nuisance adverse effect) may offer a very cost-effective opportunity for the clinical development of niacin for treating NAFLD and fatty liver disease.

In conclusion, the novel findings suggest that niacin effectively prevented and regressed experimental hepatic steatosis. Clinical development of niacin formulations and niacin-related compounds for the treatment of NAFLD will be important in addressing the unmet need for the development of new agents for NAFLD/NASH and other forms of fatty liver disease.

ACKNOWLEDGMENTS

We thank Ximing Xiong, Xiaoming Deng, and Arundhati Biswas for technical assistance.

GRANTS

This study was supported in part by a grant from the Southern California Institute for Research and Education.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


