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

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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 acyltrasferase 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 microsomal activity of diacylglycerol acyltrasferase 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,

and most of the fat is linoleic acid. The HF diet contained 60% of total calories as lard and 2% corn oil and is enriched in oleic acid and the saturated fatty acids palmitic and stearic, which has been previously used to induce NAFLD in rats (9). Seven rats in each of the four groups were randomized and fed the following diets for 4 wk: *group 1*: control (C) diet; *group 2*: HF diet, *group 3*: HF + 0.5% niacin diet; *group 4*: HF + 1.0% niacin.

For regression studies, rats were first fed the HF diet for 6 wk to induce hepatic steatosis. Seven rats in each group were randomized and fed either the HF diet or HF + 0.5% niacin diet for an additional 6 wk. The rats were placed on a 12-h:12-h light/dark cycle, pair-fed based on the amount of diet consumed by HF + niacin-fed rats, and provided with ad libitum access to water. The study was approved by the institutional IACUC. After the feeding period, rats were fasted 16–18 h and anesthetized, and blood and liver samples were collected for biochemical and histological analyses.

Triglyceride, cholesterol, and nonesterified fatty acids. Total lipid was extracted from liver samples (0.25 g) with chloroform-methanol mixture (2:1) and washed with 0.73% sodium chloride solution. The organic phase containing total lipid was dried under nitrogen and reconstituted in isopropanol. Triglycerides and total cholesterol were measured using assay kits from Wako Diagnostics (Richmond, VA). Serum nonesterified fatty acid (NEFA) levels were measured by a commercially available Free Fatty Acid Assay Kit (Cayman Chemical, Ann Arbor, MI).

Measurement of liver thiobarbituric acid-reactive substances. 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.

Measurements of DGAT2 Protein and Activity

Liver samples were homogenized in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.1% SDS, 1 mM DTT, 1.0% NP-40, protease inhibitor cocktail). Protein expression levels were measured by Western blot analysis using anti-DGAT2 antibodies (GeneTex, Irvine, CA). Semiquantitative measurements of DGAT2 protein expression was performed by densitometric analysis of Western blots and normalized to β-actin. DGAT2 activity was measured in microsomal preparations as described in our previous publication (18) using [14C]oleoyl-CoA and sn-1,2-dioleoylglycerol as substrates.

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. 1*A*). 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. 1*B*). Addition of niacin at 0.5% or 1.0% levels completely blocked gain in liver weight induced by the HF diet (Fig. 1*B*).

Niacin Prevents Triglyceride Accumulation in Liver and Hepatic Steatosis

The HF diet caused a significant and marked deposition of triglycerides in the liver (about 4-fold increase) compared with rats fed a control diet (Fig. 2, *left*). Inclusion of niacin in the HF diet significantly reduced liver triglyceride deposition by 40% compared with rats fed the HF diet (Fig. 2, *left*). Triglyceride levels in serum were also markedly higher (about 5-fold) in rats fed the HF diet compared with rats on a control diet (Fig. 2, *right*). Niacin at 1.0% significantly decreased serum triglyceride levels (Fig. 2, *right*).
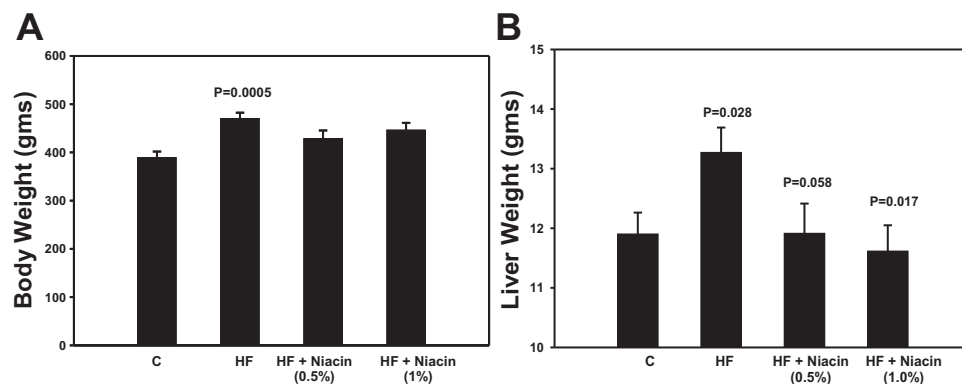

Fig. 1. Effect of niacin on body and liver weight. Rats were fed control diet (C), high-fat diet (HF), HF + 0.5% niacin, or HF + 1.0% niacin for 4 wk. *A*: body weight of rats. *B*: liver weight of rats. Data presented are means ± SE. Statistical analysis for the 2 groups were made using a 2-tailed Student's *t*-test, and *P* < 0.05 was considered statistically significant. *P* values shown on HF group represent comparison to control group and on HF + niacin groups are compared with HF group.

Fig. 2. Niacin prevents increased liver and serum triglycerides in rats fed the HF diet. Rats were fed control diet, HF diet, HF + 0.5% niacin, or HF + 1.0% niacin for 4 wk. Liver and serum triglycerides are expressed as mg/g of liver and mg/dl of serum, respectively. *P* values shown on HF group represent comparison to control group and on HF + niacin groups are compared with HF group.
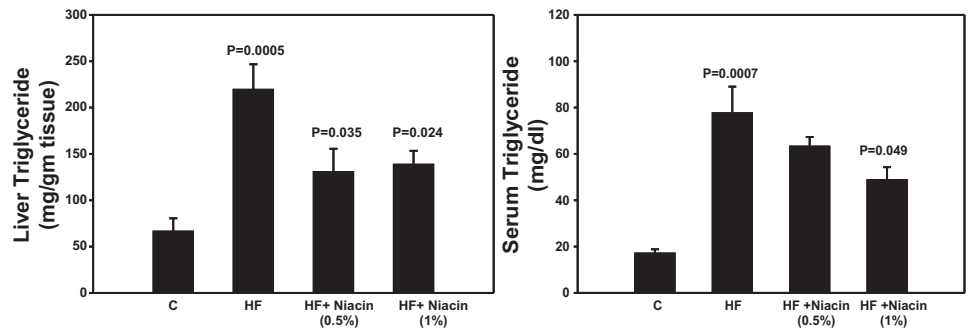
Liver and serum cholesterol levels were also significantly increased in rats fed the HF diet compared with rats on a control diet (Fig. 3, *left* and *right*). Inclusion of niacin (0.5% and 1.0%) in the HF diet significantly decreased serum cholesterol (Fig. 3, *right*), and niacin at 0.5% significantly 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. 4*B*) compared with controls (Fig. 4*A*). 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 lympho-cytes. 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

Fig. 3. Niacin prevents increased liver and serum total cholesterol in rats fed the HF diet. Rats were fed control diet, HF diet, HF + 0.5% niacin, or HF + 1.0% niacin for 4 wk. *P* values shown on HF group represent comparison to control group and on HF + niacin groups are compared with HF group.
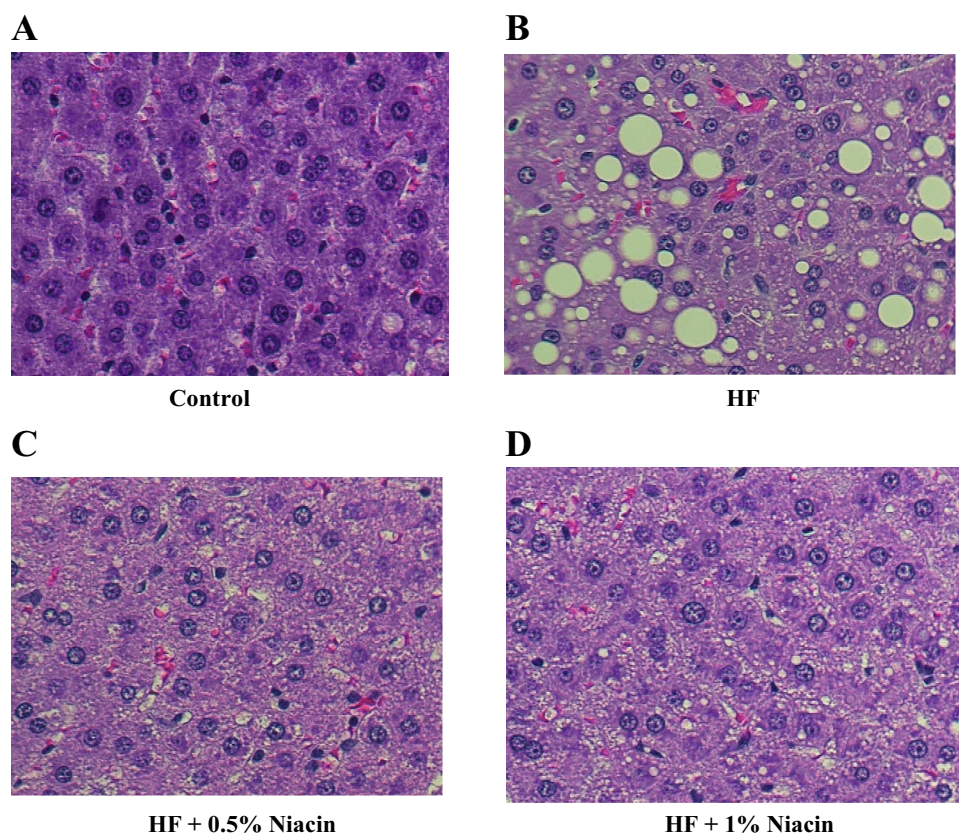
A

Control

B

HF
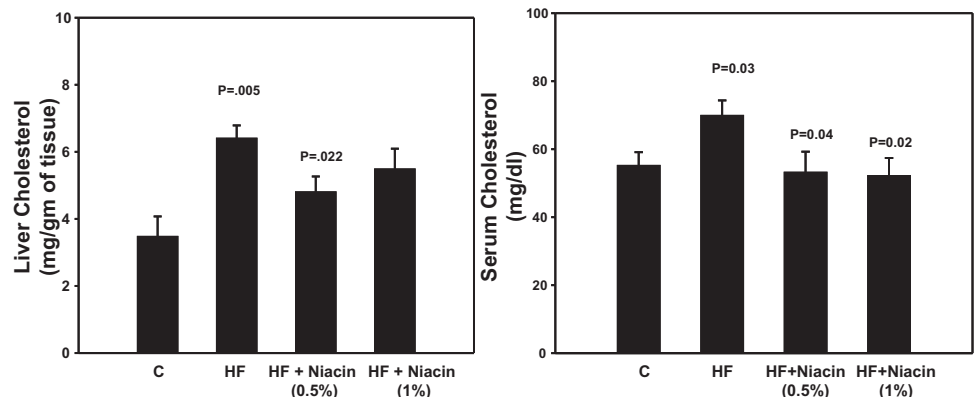
Fig. 4. Niacin prevents hepatic steatosis as shown in representative histological images (×200) of liver sections stained with hematoxylin and eosin (H&E). *A*: rats fed control chow diet. *B*: rats fed HF diet. *C*: rats fed HF + 0.5% niacin. *D*: rats fed HF + 1.0% niacin.

C

HF + 0.5% Niacin

D

HF + 1% Niacin

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).
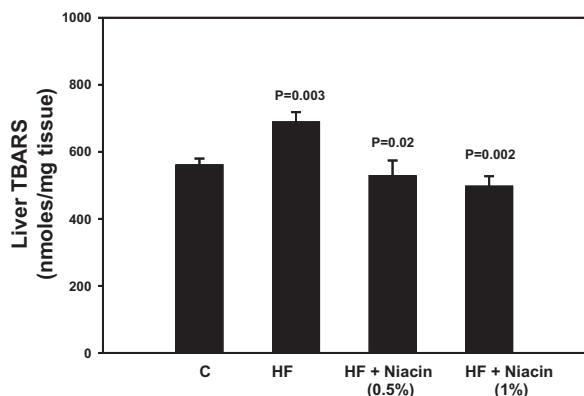

Fig. 5. Niacin inhibits liver lipid peroxidation products in rats fed the HF diet. Rats were fed control diet, HF, HF + 0.5% niacin, or HF + 1.0% niacin for 4 wk. Thiobarbituric acid reactive substances (TBARS) were measured to assess the levels of lipid peroxidation products in liver extracts. *P* values shown on HF group represent comparison to control group and on HF + niacin groups are compared with HF group.

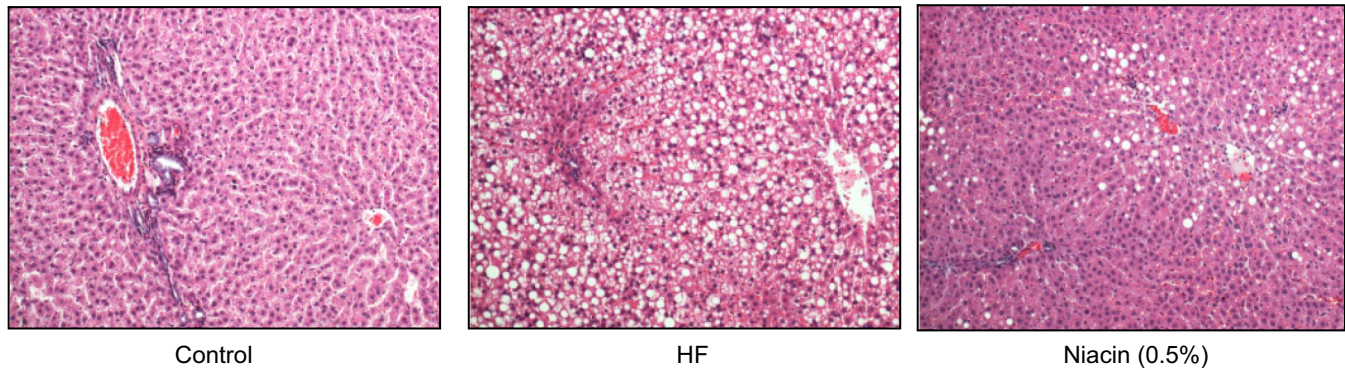Control   HF   Niacin (0.5%)

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.

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 preexisting 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
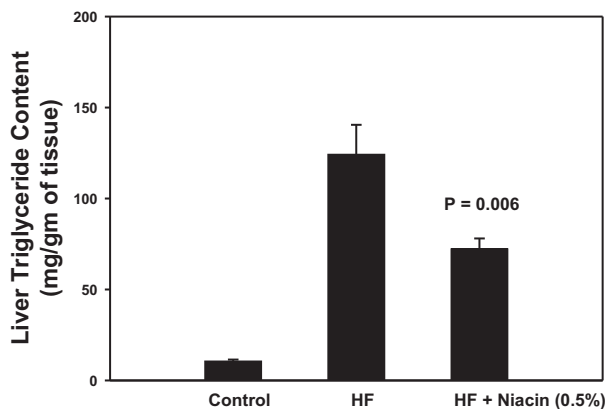

Fig. 7. Niacin causes regression of liver triglyceride content. Rats were first fed the HF diet for 6 wk to induce hepatic steatosis. These rats were then treated with niacin (0.5% in the diet) while they continued on HF diet for 6 wk. Liver triglycerides are expressed as mg/g of liver. P values shown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

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

Parameters	Control	HF	HF + 0.5% Niacin
Liver weight, g	14.92 ± 0.35	17.53 ± 2.9	13.27 ± 1.0*
Serum Triglycerides, mg/dl	54.98 ± 9.8	144.7 ± 33.9	68.8 ± 27.6*
Liver TBARS, nmol	329.4 ± 47.0	553.6 ± 37.5	407.4 ± 110.1*
Serum NEFA, mEq/l	0.55 ± 0.04	0.54 ± 0.02	0.54 ± 0.09

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).

Table 2. *Effect of niacin on lipogenic and fatty acid oxidation gene expression, DGAT2 activity, and protein expression in liver*

Parameters	HF	HF + 0.5% Niacin
mRNA Expression		
SREBP1	100 ± 5.40	96.28 ± 3.40
FAS	100 ± 17.74	100.26 ± 7.65
ACC1	100 ± 6.08	96.27 ± 13.90
CPT-1	100 ± 5.60	105.00 ± 6.50
DGAT2	100 ± 11.90	69.33 ± 7.07 (P = 0.01)
DGAT2 Protein Expression	100 ± 4.03	78.25 ± 3.40 (P = 0.003)
DGAT2 Activity	100 ± 21.7	8.0 ± 0.1 (P = 0.001)

Values are shown as means ± SE. Rats were first fed 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. mRNA expression levels were performed by real-time PCR as noted in MATERIALS AND METHODS. Diacylglycerol acyltrasferase 2 (DGAT2) protein expression and activity were performed as described in MATERIALS AND METHODS. SREBP1c, sterol-regulatory element-binding protein 1c; FAS, fatty acid synthase; ACC1, acetyl-CoA carboxylase 1; CPT1, carnitine palmitoyltransferase 1.

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 lipogenesis, 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-

tionally, 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 therapeutic agents for NAFLD/NASH and other forms of fatty liver disease.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: S.H.G., G.D.K., and N.L. performed experiments; S.H.G., G.D.K., N.L., and V.S.K. analyzed data; S.H.G., G.D.K., and N.L. prepared figures; S.H.G. and V.S.K. drafted manuscript; S.H.G., G.D.K., N.L., M.L.K., and V.S.K. edited and revised manuscript; G.D.K., M.L.K., and V.S.K. interpreted results of experiments; M.L.K. and V.S.K. conception and design of research; M.L.K. and V.S.K. approved final version of manuscript.

REFERENCES

1. **Abdelmalek MF, Diehl AM.** Nonalocoholic fatty liver disease as a complication of insulin resistance. *Med Clin North Am* 91: 1125–1149, 2007.
2. **Adams LA, Angulo P.** Treatment of non-alcoholic fatty liver disease. *Postgrad Med J* 82: 315–322, 2006.
3. **Ahmed MH, Byrne CD.** Current treatment of non-alcoholic fatty liver disease. *Diabetes Obes Metab* 11: 188–195, 2009.
4. **Angulo P.** Nonalcoholic fatty liver disease. *N Engl J Med* 346: 1221–1231, 2002.
5. **Anstee QM, Goldin RD.** Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 87: 1–16, 2006.
6. **Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K.** A placebo-controlled trial of pioglitazone in subjects with non-alcoholic steatohepatitis. *N Engl J Med* 355: 2297–2307, 2006.
7. **Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL.** A pilot study of thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. *Am J Gastroenterol* 96: 519–525, 2001.
8. **Carlson LA.** Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro. *Acta Med Scand* 173: 719–722, 1963.
9. **Carmiel-Haggai M, Cederbaum AI, Nieto N.** A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J* 19: 136–138, 2005.
10. **Choi CS, Savage DB, Kulkarni A, Yu XX, Liu ZX, Morino K, Kim S, Distefano A, Samuel VT, Neschen S, Zhang D, Wang A, Zhang XM, Kahn M, Cline GW, Pandey SK, Geisler JG, Bhanot S, Monia BP,** **Shulman GI.** Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverse diet-induced hepatic steatosis and insulin resistance. *J Biol Chem* 282: 22678–22688, 2007.
11. **Clark JM, Brancati FL, Diehl AM.** Nonalcoholic fatty liver disease. *Gastroenterology* 122: 1649–1657, 2002.
12. **Day CP, James OF.** Hepatic steatosis: Innocent bystander or guilty party? *Hepatology* 27: 1463–1466, 1998.
13. **Day CP, James OF.** Steatohepatitis: a tale of two "hit"? *Gastroenterology* 114: 842–845, 1998.
14. **Deng QG, She H, Cheng JH, French SW, Koop DR, Xiong S, Tsukamoto H.** Steatohepatitis induced by intragastric overfeeding in mice. *Hepatology* 42: 905–914, 2005.
15. **DiMasi JA, Hansen RW, Grabowski HG.** The price of innovation: new estimates of drug development costs. *J Health Econ* 22: 151–185, 2003.
16. **Fabbrini E, Mohammed BS, Korenblat KM, Magkos F, McCrea J, Patterson BW, Klein S.** Effect of fenofibrate and niacin on intrahepatic triglyceride content, very-low density lipoprotein kinetics, and insulin action in obese subjects with nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 95: 2727–2735, 2010.
17. **Ganji SH, Qin S, Zhang L, Kamanna VS, Kashyap ML.** Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells. *Atherosclerosis* 202: 68–75, 2009.
18. **Ganji SH, Tavintharan S, Zhu D, Xing Y, Kamanna VS, Kashyap ML.** Niacin non-competitively inhibits DGAT2 but not DGAT1 activity in HepG2 cells. *J Lipid Res* 45: 1835–1845, 2004.
19. **Harrison SA, Torgerson S, HayashiP, Ward J, Schenker S.** Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 98: 2485–2490, 2003.
20. **Hernandez M, Wright SD, Cai TQ.** Critical role of cholesterol ester transfer protein in nicotinic acid-mediated HDL elevation in mice. *Biochem Biophys Res Commun* 355: 1075–1080, 2007.
21. **Hu M, Chu WC, Yamshita S, Yeung DK, Shi L, Wang D, Masuda D, Yang Y, Tomlinson B.** Liver fat reduction with niacin is influenced by DGAT-2 polymorphisms in hypertriglyceridemic patients. *J Lipid Res* 53: 802–809, 2012.
22. **Jin YJ, Kim KM, Hwang S, Lee SG, Ha TY, Song GW, Jung DH, Kim KH, Yu E, Shim JH, Lim YS, Lee HC, Chung YH, Lee Y, Suh DJ.** Exercise and diet modification in non-obese non-alcoholic fatty liver disease: Analysis of biopsies of living liver donors. *J Gastroenterol Hepatol* 27: 1341–1347, 2012.
23. **Kamanna VS, Kashyap ML.** Mechanism of action of niacin. *Am J Cardiol* 101: 20B–26B, 2008.
24. **Lavine JE.** Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. *J Pediatr* 136: 734–738, 2000.
25. **Ludwig J, Viggiano TR, McGill DB, Oh BJ.** Nonalcoholic steatohepatitis: Mayo clinic experience with a hitherto unnamed disease. *Mayo Clin Proc* 5: 434–438, 1980.
26. **Meyers CD, Kamanna VS, Kashyap ML.** Niacin therapy in atherosclerosis. *Curr Opin Lipidol* 15: 659–665, 2004.
27. **Misra VL, Khashab M, Chalasani N.** Non-alcoholic fatty liver disease and cardiovascular risk. *Curr Opin Gastroenterol Rep* 11: 50–55, 2009.
28. **Monetti M, Levin MC, Watt MJ, Sajan MP, Marmor S, Hubbard BK, Stevens RD, Bain JR, Newgard CB, Farese RV Sr, Hevener AL, Farese RV Jr.** Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metab* 6: 69–78, 2007.
29. **Morgan K, Uyuni A, Nandgiri G, Mao L, Castaneda L, Kathirvel E, French SW, Morgan TR.** Altered expression of transcription factors and genes regulating lipogenesis in liver and adipose tissue of mice with high fat diet-induced obesity and nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 20: 843–854, 2008.
30. **Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR.** Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 38: 1008–1017, 2003.
31. **Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M.** Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 35: 367–372, 2002.
32. **Postic C, Girard J.** Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 118: 829–838, 2008.

33. **Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, Shulman GI.** Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 279: 32345–32353, 2004.

34. **Sanyal A, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN.** Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 362: 1675–1685, 2010.

35. **Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, Shiffman ML, Clore J, Mills AS.** A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenerol Hepatol* 2: 1107–1115, 2004.

36. **Schattenberg JM, Galle PR.** Animal models of non-alcoholic steatohepatitis: of mice and man. *Dig Dis* 28: 247–254, 2010.

37. **Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K.** Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 27: 103–107, 1997.

38. **Wanless IR, Lentz JS.** Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 12: 1106–1110, 1990.

39. **Yu XX, Murray SF, Pandey SK, Booten SL, Bao D, Song XZ, Kelly S, Chen S, McKay R, Monia BP, Bhanot S.** Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. *Hepatology* 42: 362–371, 2005.