Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent

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Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. Am J Physiol Gastrointest Liver Physiol 295: G987–G995, 2008. First published September 4, 2008; doi:10.1152/ajpgi.90272.2008.—The aims of this study were to determine whether combining features of a western lifestyle in mice with trans fats in a high-fat diet, high-fructose corn syrup in the water, and interventions designed to promote sedentary behavior would cause the hepatic histopathological and metabolic abnormalities that characterize nonalcoholic steatohepatitis (NASH). Male C57BL/6 mice fed ad libitum high-fat chow containing trans fats (partially hydrogenated vegetable oil) and relevant amounts of a high-fructose corn syrup (HFCs) equivalent for 1–16 wk were compared with mice fed standard chow or mice with trans fats or HFCs omitted. Cage racks were removed from western diet mice to promote sedentary behavior. By 16 wk, trans fat-fed mice became obese and developed severe hepatic steatosis with associated necroinflammatory changes. Plasma alanine aminotransferase levels increased, as did liver TNF-α and procollagen mRNA, indicating an inflammatory and profibrogenic response to injury. Glucose intolerance and impaired fasting glucose developed within 2 and 4 wk, respectively. Plasma insulin, resistin, and leptin levels increased in a profile similar to that seen in patients with NASH. The individual components of this diet contributed to the phenotype independently; isocaloric replacement of trans fats with lard established that trans fats played a major role in promoting hepatic steatosis and injury, whereas inclusion of HFCs promoted food consumption, obesity, and impaired insulin sensitivity. Combining risk factors for the metabolic syndrome by feeding mice trans fats and HFCs induced histological features of NASH in the context of a metabolic profile similar to that of patients with NASH. Because dietary trans fats promoted liver steatosis and injury, their role in the epidemic of NASH needs further evaluation.

nonalcoholic steatohepatitis; insulin resistance; obesity

The prevalence of obesity in the United States is rising, and with it the frequency of fatty liver disease, nonalcoholic steatohepatitis (NASH), and type 2 diabetes mellitus. Large-cohort studies indicate that up to 30% of adults and 13% of children have excessive hepatic steatosis (>5% fat), while NASH is present in ~3% of adults. NASH is a necroinflammatory disease that can progress to cirrhosis, with subsequent liver failure and an increased risk of hepatocellular carcinoma. To gain insights into the pathogenesis of NASH and develop effective therapy, animal models have been developed that typically rely on genetic manipulation (e.g., ob/ob mouse), toxic injury [e.g., methionine- and choline-deficient (MCD) diet], or dietary extremes (e.g., abnormally high-fat or high-fructose diets) (3), none of which fully reflects the characteristics of the American fast-food diet. Although some of the histological changes that develop in these models exhibit features of human nonalcoholic fatty liver disease (NAFLD), the underlying pathogenesis of fat accumulation and consequent cellular injury may not reflect the mechanisms of human disease. For example, the frequently used MCD diet model induces many histological abnormalities described as similar to human NASH, but the model is not associated with insulin resistance, and rodents treated with this diet typically lose, rather than gain, weight (23, 32). Although much has been learned from this model, its relevance to human NASH has been questioned (3). The hyperphagic leptin-deficient ob/ob mouse develops fatty liver and has provided important insights into the pathogenesis of NAFLD, but leptin plays an important role in regulating inflammation and fibrosis, and its absence may compromise the ultimate utility of this as an appropriate model for identifying effective therapy. The fact that leptin deficiency is a rare human disorder raises additional questions about the relevance of this model to human disease. Similarly, models that utilize dietary extremes, such as 60% of calories from fat or 60% of calories from fructose, do not reflect commonly encountered dietary habits, and such dietary models typically focus on just one dietary component without including the recognized sedentary behaviors that may be common in patients with NASH (4, 26, 29). None of the models developed to date has included trans fats. Little is known about the role of trans fats in promoting liver injury in the setting of NASH, yet the consumption of trans fats has escalated in parallel with the epidemic of fatty liver disease. Because consumption of trans fats has been associated with an increased risk of developing insulin resistance (17), this relatively recent dietary aberration could be playing a role in the expanding number of NASH patients being identified.

We sought to develop and characterize a mouse model of NASH based on the nutritional makeup of commonly consumed fast food, using non-genetically altered mice kept in conditions designed to promote sedentary behavior. We hypothesized that combining dietary and lifestyle risk factors for NASH would lead to significant liver pathology, and we designed experiments that would allow us to identify the relative contributions of each component of the diet to the development of obesity and liver disease. Our results indicate that mice kept...
in cages without wire racks and fed ad libitum a high-fat chow that included trans fats and relevant amounts of high-fructose corn syrup (HFCS) in their drinking water became obese and developed reduced glucose tolerance, hyperinsulinemia, and substantial hepatic steatosis associated with a necroinflammatory and profibrogenic response. While fructose contributed to the development of obesity, the consumption of trans fats contributed substantially to the development of hepatic steatosis and injury. Because the diet was similar to commonly consumed fast food and the mice were kept in conditions designed to promote sedentary behavior, this model was termed the American Lifestyle-Induced Obesity Syndrome (ALIOS) model.

MATERIALS AND METHODS

Animal treatment. Male C57BL/6 mice ~5 or 6 wk old (Harlan, Indianapolis, IN) were handled and cared for in accordance with a protocol approved by the Animal Care Committee of Saint Louis University. This strain was chosen because of its predisposition to developing insulin resistance (2, 14, 18) and the availability of genetically manipulated mice on this background for future studies. Mice were kept on a 12:12-h light-dark schedule at 23°C, housed in cages of five, and weighed weekly.

ALIOS mice were fed a diet similar in composition to an American fast-food diet, with 45% calories in the chow from fat and 30% of the fat in the form of partially hydrogenated vegetable oil (28% saturated, 57% monounsaturated fatty acids (MUFA), 13% polyunsaturated fatty acids (PUFA)); trans fat custom diet TD06303, Harlan Teklad, Madison, WI). Control mice were fed standard rodent chow containing 13.6% of calories from fat in the form of soybean oil (15% saturated, 23% MUFA, 61% PUFA; 20185, Harlan Teklad). ALIOS mice were also given HFCS equivalent (55% fructose, 45% glucose by weight) in drinking water at a concentration of 42 g/l. The drinking water was provided as gel-water [93% water, 2.8% gelatin (pork skin gelatin, 57% monounsaturated fatty acids (MUFA), 13% polyunsaturated fatty acids (PUFA); trans fat custom diet TD06303, Harlan Teklad) and 4.2% HFCS] in dishes on the cage floor. This allowed removal of cage racks in order to discourage physical activity in the ALIOS mice. Control mice fed standard chow were kept in cages with wire racks and given gel-water without HFCS. Food and water consumption was measured by weighing new and remaining food and water three times weekly.

A total of 50 mice were treated with ALIOS conditions and compared with 40 mice treated with control conditions; groups of 8–10 mice from each treatment group were killed at time points from 1 wk to 16 wk of treatment, except that control mice were not killed at 2 wk. Mice were killed by carbon dioxide inhalation at 1, 2, 4, and 8 wk. Because of stress-induced metabolic changes observed with ketamine and xylazine anesthesia.

To examine the roles of individual components of the ALIOS diet, groups of 5-wk-old mice (n = 10/group) treated for 16 wk were kept in cages without wire racks and studied by omitting single components of the diet: one group was fed the regular ALIOS diet; a second group had trans fats omitted and was instead fed chow containing 45% of calories as lard (39% saturated, 50% MUFA, 11% PUFA) along with HFCS; a third group had HFCS omitted and was given control gel-water along with the trans fat chow. A group fed standard chow but without cage racks was not included to examine the role of this intervention.

Plasma prepared with EDTA was separated and frozen at ~80°C. Livers were removed, weighed, and divided into sections that were fixed in phosphate-buffered formalin (Sigma Aldrich, St. Louis, MO), frozen in liquid nitrogen, or submersed in RNA stabilization solution (RNAlater, Ambion, Austin, TX).

Glucose tolerance test and insulin resistance. Glucose tolerance testing (GTT) was performed in mice fasted for 8–12 h by measuring tail vein glucose levels after intraperitoneal administration of glucose (1 mg/kg) as a sterile 20% solution with a 27-gauge insulin syringe (20). Glucose levels were measured by tail vein sampling with a portable glucometer (Freestyle Flash, Abbott Laboratories, Abbott Park, IL) at 0, 20, 40, 60, and 150 min. The area under the curve (AUC) for the GTT was calculated by the trapezoidal method. After a 6-h fast, insulin tolerance testing was performed by injecting intraperitoneally 0.6 U/kg human regular insulin (Novalin, Abbott Laboratories) at a concentration of 0.2 U/ml with a 27-gauge insulin syringe. Glucose was measured as described above at 0, 20, 40, and 60 min. As an additional estimate of insulin resistance, the quantitative insulin sensitivity check index (QUICKI) of ALIOS and control mice was calculated \[1/\log(gluucose \times insulin)\] with the fasting values (21).

Chemistry. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, and triglycerides were measured with a calibrated clinical analyzer (Cobas Mira Plus Chemistry Analyzer, Roche Diagnostics, Indianapolis, IN). Plasma insulin and adipokines were measured by multiplexed immunoassay (Lino Diagnostics, St. Charles, MO), and adiponectin was measured by radioimmunoassay (Lino Diagnostics). Liver triglyceride content was measured by a glycerol phosphate oxidase method after enzymatic lipolysis (GPO reagent set, Pointe Scientific, Lincoln Park, MI). Tissue was prepared for the assay by first activating 50 mg of previously frozen liver in 1 ml of a buffer containing 0.25 M sucrose, 2 mM EDTA, and 10 mM Tris, pH 7.4 in a glass vial with a 3-mm steel bead at room temperature for 2 h. The samples were taken through two freeze-thaw cycles and aspirated three times through a 25-gauge needle.

RNA analysis. To isolate total RNA, tissue was homogenized within 48 h of death in a phenol-guanidinium-isothiocyanate reagent (TRizol, Life Technologies, Grand Island, NY), extracted with phenol-chloroform-isooamyl alcohol (25:24:1), precipitated with isopropyl alcohol, and washed with ethanol. The concentration of RNA was measured by microspectrophotometry (NanoDrop Technologies, Wilmington, DE). Tumor necrosis factor (TNF)-α and procollagen α1(I) (PCA) mRNA were measured and normalized to 18S RNA by real-time RT-PCR (MyQ, Bio-Rad, Hercules, CA) using SYBR Green. The primer sequences used were PCA forward: gctcctcttaggccacat, RCA reverse: ccccaatggtagcagctgg, TNF-α forward: cecctacactgatacctt, TNF-α reverse: cttgagccgaacgtcagac and 18S forward: tggagccgttagtaagaa, 18S reverse: gtagttacccgttacag.

Pathology. Formalin-fixed portions of livers were paraffin-embedded and sectioned (5 μm). Stains evaluated were hematoxylin and eosin to examine morphological features, Sirius red, and Masson’s trichrome to assess fibrosis.

Statistical analysis. Paired data were analyzed for statistical significance with t-tests, with significance being P < 0.05. Comparisons among groups were performed with one-way ANOVA followed by post hoc pairwise multiple-comparison procedures (Holm-Sidak method) to identify significant differences between groups (P < 0.05) with SigmaPlot 9.0 (Systat, Chicago, IL).

RESULTS

Experimental groups gained weight quickly; by 1 wk the ALIOS mice had already gained 4.3% more weight than the control mice (P < 0.01). By 8 wk the accelerated weight gain of the ALIOS mice began to slow, and subsequent weight gain paralleled that of the control mice (Fig. 1). At 16 wk ALIOS mice weighed 42% more than control mice and 10% more than those without HFCS (P < 0.05); omission of trans fats from the high-fat diet did not significantly alter the weight gain compared with ALIOS mice (Table 1).

Food and water consumption were measured from week 10 to week 16 in all groups. ALIOS mice consumed the same amount of food by weight as control mice, but because of the
higher caloric density of the high-fat chow, the daily caloric consumption per mouse was 30% greater than that of control mice (Table 2). Water consumption was equal in all groups, indicating that there was neither a preference for nor an aversion to the HFCS water. The amount of fructose consumed by the ALIOS mice was the equivalent of a 70-kg person consuming about eight cans of HFCS-sweetened soda daily, a relevant amount for some individuals. The HFCS gel-water contained 0.28 kcal/ml from both carbohydrate and protein, whereas control gel-water contained 0.112 kcal/ml from protein; the average consumption was 3.9 ml/day. The presence of HFCS in the diet of ALIOS mice contributed 5.7% of their

caloric intake, thus accounting for only 24% of their excess caloric consumption compared with control mice. The role of HFCS in causing increased solid chow consumption was confirmed when HFCS was omitted from the ALIOS conditions (Fig. 2). ALIOS mice ate 8.2% more calories from solid chow and 13.7% more total calories than mice with HFCS omitted from the ALIOS diet ($P < 0.05$, Table 2). The mice fed lard as an isocaloric replacement for trans fats had the same caloric consumption as the ALIOS mice, indicating that, unlike inclusion of HFCS in the diet, the presence of trans fats did not alter food intake (Table 2). The ALIOS mice consumed 10 g/kg of trans fat per day, which is an ~20-fold higher amount than a person eating five servings of common snack foods (39).

Histological examination of livers from ALIOS mice demonstrated the progressive development of substantial steatosis with necroinflammatory changes. Small fat droplets were present in zone 1 hepatocytes at 1 wk (Fig. 3), but these did not

Table 1. Contribution of trans fats and high-fructose corn syrup to morphological, biochemical, and histological abnormalities of the ALIOS model at 16 wk

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ALIOS</th>
<th>ALIOS Minus Trans Fats</th>
<th>ALIOS Minus HFCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>27.7±2.9a</td>
<td>39.2±2.5b</td>
<td>37.3±2.6abc</td>
<td>35.6±3.4a</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>0.91±0.08a</td>
<td>1.67±0.23b</td>
<td>1.19±0.099</td>
<td>1.41±0.294a</td>
</tr>
<tr>
<td>Liver triglyceride, mg/g</td>
<td>43±9a</td>
<td>123±72b</td>
<td>69±25abc</td>
<td>100±53b</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>31±6a</td>
<td>83±34b</td>
<td>41±15a</td>
<td>83±32b</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dl</td>
<td>80±6a</td>
<td>143±12b</td>
<td>136±19b</td>
<td>138±21b</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>59±8a</td>
<td>70±12a</td>
<td>106±14b</td>
<td>66±17a</td>
</tr>
<tr>
<td>GTT AUC</td>
<td>2.098±632a</td>
<td>1.876±234b</td>
<td>1.709±461a</td>
<td></td>
</tr>
<tr>
<td>GTT glucose at 80 min, mM</td>
<td>15.2±4.1a</td>
<td>14.9±3.6a</td>
<td>11.2±1.8b</td>
<td></td>
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</tbody>
</table>

Variance statistic is SD. Superscript letters denote significant differences among the treatment groups (i.e., there is no difference among values annotated with the same letter), $P < 0.05$ by ANOVA.

Fig. 2. Food and gel-water consumption expressed in terms of daily energy provided. The presence of HFCS in the gel-water was associated with an 8.2% increase in chow consumption compared with ALIOS mice not given HFCS ($P < 0.05$). Caloric intake was similar when trans fats were replaced by lard, indicating that fat substitution did not alter eating behavior. The number of calories provided by the gel-water is shown in the gray stacked bar and demonstrates the relative proportion of calories consumed compared with chow. The calories provided by gel-water not containing HFCS were derived from the collagen used to make the gel-water. Error bars denote SD for calories derived from chow. Significance of differences among groups is shown in Table 2.
fill the cytoplasm. The amount of fat and size of the droplets increased in zone 1 hepatocytes over time such that by 8 wk predominant macrovesicular steatosis filled the periportal zone 1 hepatocytes (Fig. 4). Also by 8 wk, but not at earlier time points examined, small-droplet fat accrued in perivenous zone 3 hepatocytes, and this was more pronounced at 16 wk. At 16 wk the entire cytoplasm of most hepatocytes was distorted by steatosis, and a distinct demarcation of zone 1 and 2 macrovesicular steatosis from zone 3 true microvesicular steatosis was present. Replacing trans fats with lard resulted in a markedly different distribution of steatosis, with macrovesicular steatosis in zone 2 but near-complete sparing of zones 1 and 3 (Fig. 4, C and D, ×10 and ×20, respectively). At 4 wk, larger fat droplets (macrovesicular steatosis) could be appreciated in zone 1 but zone 3 remained uninvolved (E and F, ×10 and ×20, respectively). Hematoxylin and eosin-stained sections. P, portal tract; CV, central vein.

Confirming the histological impressions, the weights of the livers of ALIOS mice progressively increased from 4 wk to 16 wk (Fig. 5) and liver triglyceride content determined biochemically also increased progressively (Fig. 5). The weights of the ALIOS livers were significantly higher compared with mice without trans fats or HFCS (Table 1). Similarly, the liver triglyceride content was elevated threefold in ALIOS mice and elevated twofold in mice fed lard instead of trans fats (Table 1).

Plasma ALT and AST levels progressively increased in ALIOS mice compared with control mice (Fig. 6). AST in the mouse has a high basal level, possibly originating from non-hepatic sources, and the proportional increase over time was less than the increase seen in ALT. Mice fed lard instead of trans fats had nearly normal ALT levels, indicating that the inclusion of trans fats in the diet could play a role in hepatocellular injury in NASH (Table 1). Omission of HFCS had no effect on the ALT elevation in ALIOS mice (Table 1).

Although hypercholesterolemia is unusual in rodents not fed a cholesterol-enriched diet, in ALIOS mice plasma cholesterol levels were significantly increased by 42% and 65% at 8 and 16 wk, respectively, compared with control mice (Fig. 7). Omission of trans fats or HFCS had no effect on the elevated cholesterol levels (Table 1). Plasma triglyceride levels were unchanged in ALIOS mice compared with control mice.
7). However, in the lard-fed mice, plasma triglyceride levels were significantly elevated (52%) compared with ALIOS and control mice (Table 1), indicating that while consuming dietary trans fats contributes to fat accumulation in the liver, it is not associated with hypertriglyceridemia in this model.

To determine whether the obesity and hepatic steatosis that developed in ALIOS mice were associated with reduced glucose tolerance, multiple GTTs were performed over 15 wk. Glucose tolerance was reduced within 2 wk, and by 4 wk the fasting glucose levels in ALIOS mice were also significantly higher than in control mice. Shown in Fig. 8A are the results of a typical GTT demonstrating elevated fasting glucose levels and substantially higher levels than normal following a glucose challenge. Plotting the GTT AUC as a function of duration on diet demonstrates that glucose tolerance was reduced early in the course of treatment and remained at this level throughout the treatment period (Fig. 8B) despite progressively worsening hepatic steatosis. A GTT performed at 14 wk on the mice with dietary components omitted showed better glucose tolerance in the mice lacking HFCS, suggesting that HFCS contributes to the impaired glucose tolerance in ALIOS mice (Table 1). Although the areas under the glucose curves were not significantly different (Table 1), there was significant divergence midway through the 150-min measurement period, when the mean plasma glucose was significantly higher in ALIOS mice compared with ALIOS minus HFCS ($P < 0.05$ at 80 min; Table 1). Insulin tolerance testing at 13 wk confirmed the effect of HFCS on insulin sensitivity. Omission of HFCS from the ALIOS conditions was associated with improved insulin sensitivity (Fig. 9), whereas isocalorically replacing trans fats with lard had an intermediate statistically nonsignificant effect (not shown).

Hyperinsulinemia is a compensatory mechanism for insulin resistance. At 8 wk, ALIOS mice had marked hyperinsulinemia compared with control mice (Table 3). Additionally, leptin and resistin were also elevated at 8 wk, as is common in patients, with NASH but adiponectin was unchanged. Tissue plasminogen activator inhibitor-1 (tPAI-1) showed a trend toward elevation, yet the change was not statistically significant. The elevated fasting insulin and impaired insulin-mediated glucose disposal suggest the presence of hepatic and peripheral insulin resistance, respectively. Single-time point parameters are also used to estimate insulin resistance in the clinical setting. Although these have not been validated in mice, the QUICKI was $0.308 \pm 0.010$ in ALIOS mice versus $0.330 \pm 0.021$ in control mice ($P < 0.05$) at 16 wk. This magnitude of difference is similar to that associated with NASH in humans, with lower values reflecting more severe insulin resistance.

Evidence for an inflammatory and fibrogenic response in the liver was sought at the molecular level. Hepatic TNF-$\alpha$ mRNA in ALIOS mice was increased 4.4-fold compared with control mice ($P < 0.05$), and the abundance of liver PCA mRNA was 3.5-fold higher than that of the control mice at 8 wk ($P <$
The increased PCA mRNA abundance suggests early stages of fibrogenesis at the molecular level.

**DISCUSSION**

Maintaining a sedentary lifestyle and consuming excessive portions of food dense in calories and trans fats while drinking beverages that contain substantial amounts of fructose have been identified as major contributing factors in the burgeoning epidemic of obesity and its comorbidities. NAFLD is one such outcome. The pathogenesis of this common disorder, however, remains poorly understood. Moreover, the role of increasing trans fat consumption in promoting the development of NASH has not been explored. In this study, we combined the major dietary and behavioral factors proposed to be contributors to the metabolic syndrome and its comorbidities with the aim of developing a rodent model of NAFLD that closely mimics the American diet and lifestyle.

How NAFLD and NASH should be defined in rodents has not been established, but the development of substantial steatosis with associated necroinflammatory changes in the setting of obesity, insulin resistance, and plasma ALT elevations as seen in this model suggests that the ALIOS model induces important phenotypic features of NASH. The combination of risk factors used in this model led to rapid accumulation of substantial amounts of hepatic steatosis in a pattern initially suggestive of type 2, or pediatric, NAFLD. In contrast to adult NAFLD, in which fat accumulation and injury is often initially localized to zone 3, pediatric NAFLD has been described as accumulation of macrovesicular steatosis in zone 1 with a lack of Mallory bodies or ballooned hepatocytes (37). At the early time points in the ALIOS model, a striking histological feature was the sharp demarcation that developed between the fat droplets in zone 1 and the sparing of zone 3 of hepatic acini. At later time points, macrovesicular fat droplets filled zone 1.

![Fig. 5](image5.png)

*Fig. 5. A: liver weight progressively increased in ALIOS mice from 11% above control at 4 wk to double the weight of control livers at 16 wk. B: liver triglyceride (TG) content progressively increased in ALIOS mice. Week 2 ALIOS liver TG content was greater than week 1 control liver TG content. TG content of ALIOS livers was >4-fold higher than control mice by week 16. Error bars denote SD, n = 8–10 mice/group.*

![Fig. 6](image6.png)

*Fig. 6. Plasma alanine aminotransferase (ALT) was substantially elevated at 8 wk and further increased at 16 wk. Aspartate aminotransferase (AST) was also elevated above a relatively high baseline. Error bars denote SD; n = 8–10 mice/group. *P < 0.01.*

![Fig. 7](image7.png)

*Fig. 7. Plasma cholesterol levels were significantly elevated at 8 and 16 wk, whereas triglyceride levels remained unchanged. The differences between the groups killed at 16 wk compared with earlier time points may be related to the different techniques used to kill animals at these time points (see MATERIALS AND METHODS). Error bars denote SD; n = 8–10 mice/group; *P < 0.01. ND, not done.*
hepatocytes to distension, while there was progressive accumulation of small-droplet fat, microvesicular steatosis, or both in zone 3 hepatocytes. A distinct “border” of macrosteatosis remained between the two zones. Interestingly, these findings were most accentuated in the peripheral areas of the hepatic parenchyma. Inclusion of trans fats in the diet in commonly consumed partially hydrogenated vegetable oil played a major role in the development of steatosis.

The basis for the striking demarcations between macrovesicular and microvesicular fat in this model is unknown. Possibilities include differences of lipid metabolism or glucose metabolism or differential responsiveness of hepatocytes to insulin signaling. Zonal hepatocellular differences in Krebs cycle and cytochrome P-450s are well known (30). Additionally, one study has raised the possibility that small-droplet fat could represent abnormal vesicles of smooth endoplasmic reticulum, whereas large-droplet fat is the intended sequestration form of excess hepatocyte triglyceride (7). Common to other C57BL/6 mouse models that use short-term obesity-inducing diets, our model did not result in histologically detectable fibrosis by 16 wk; however, by mRNA analysis evidence of profibrogenic processes was found with increased expression of procollagen mRNA. Whether the length of exposure to the diet was insufficient to observe increased collagen accumulation or robust antifibrotic mechanisms inherent to the mouse are at play is not known.

The consequences of exposure in humans to the individual components of the ALIOS model are well documented. From 1970 to 2003, the United States saw an average increase of 216 calories from fat per person per day (ERS Food Consumption Data System). The caloric distribution of the food consumed by mice in this model was similar to a fast-food meal with 40% of calories derived from fat. In this model, 30% of the fat was trans fats, which is an amount not unlike that in some snack foods (1). Although the use of trans fats is now being reduced by some segments of the food industry because of an unfavorable cardiovascular risk profile (28), they continue to be used to prepare fast foods and baked goods (38), and their consumption has been shown to correlate with insulin resistance and obesity (33). Whether the current regulations allowing foods to contain up to 0.5 g of trans fats per serving yet be labeled as trans fat free is sufficient to prevent these pathological changes in the liver is unknown. The ALIOS model uses far more trans fats in the diet than in the ALIOS model.

Table 3. Insulin and adipokines in mice treated for 8 wk

<table>
<thead>
<tr>
<th></th>
<th>Insulin, pg/ml</th>
<th>Leptin, pg/ml</th>
<th>Resistin, pg/ml</th>
<th>Adiponectin, pg/ml</th>
<th>tPAI-1, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALIOS</td>
<td>1.157±337*</td>
<td>3.335±637*</td>
<td>3.431±961*</td>
<td>11.7±1.9</td>
<td>2.494±1.490</td>
</tr>
<tr>
<td>Control</td>
<td>388±104</td>
<td>805±547</td>
<td>1.575±386</td>
<td>11.8±3.0</td>
<td>1.469±489</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to control.
fat on a per-kilogram basis than would be seen with typical human consumption, so further work is needed to establish where in this spectrum of consumption liver disease might develop. It is worrisome that a recent study of a group of young people eating a fast-food diet for just 4 wk while maintaining a sedentary lifestyle demonstrated marked ALT elevations (22). The authors of that study could only speculate as to the cause, especially since the increase in liver triglyceride was relatively minor, but Marchesini et al. (25) raised the possibility that trans fats could be playing a role.

The mechanisms of cellular dysfunction caused by trans fats continue to be debated. One study demonstrated that trans fats inhibit Δ9-desaturase, an enzyme necessary for converting fatty acids to prostaglandins, thus altering the regulation of cell growth, muscle function, and inflammation (24). Although another study found that trans fats induce zone 3 hepatic steatosis in rats (6), the role of trans fats in promoting hepatocellular injury in NASH has not been examined. In ALIOS mice, the amount of steatosis seen was substantially greater than in the mice fed lard rather than trans fats, as was the plasma ALT level, indicating greater hepatocellular injury related to trans fat consumption compared with an isocaloric diet containing lard (a combination of saturated and natural cis-unsaturated fats). These findings raise the possibility that injury in human NAFLD could be worsened by the consumption of synthetic trans fats.

Plasma hypertriglyceridemia did not develop in the ALIOS mice even though there was substantial fat accumulation in the liver. Because plasma triglycerides in the fasting state largely originate from the liver, this observation may reflect impaired hepatic secretion of very low-density lipoprotein. Interestingly, elevations of plasma triglycerides were found when trans fats were eliminated, further suggesting that trans fats may have a role in causing a secretory block of triglyceride secretion from the liver. The cause of the hypercholesterolemia without excessive cholestero feeding also warrants further exploration, as it may reflect impaired hepatic uptake of low-density lipoproteins by the liver or altered gene expression with increased cholesteryl synthesis.

The ALIOS model also included consumption of a HFCS in amounts relevant to that consumed by some Americans. The most commonly used HFCS in soft drinks and other carbohydrate-sweetened beverages is a blend composed of 55% fructose, 41% glucose, and 4% complex polysaccharides, and thus this proportion of fructose was included in the HFCS equivalent in the ALIOS model. Consumption of fructose is associated with adverse alterations of plasma lipid profiles and metabolic changes (10, 12). Fructose was originally touted as a beneficial dietary component because it does not stimulate insulin secretion. But since insulin signaling plays an important role in central mechanisms of satiety, this property of fructose may be undesirable (11, 40). One study has also suggested that, unlike other sugars, fructose may prevent suppression of ghrelin secretion, resulting in impaired satiety mechanisms (40). The observation that the ALIOS mice indeed consumed a greater quantity of food beyond the additional calories consumed from the HFCS when fed HFCS compared with control water supports this observation and reinforces the concerns about the role of fructose in the obesity epidemic (19). In large quantities, fructose can also stress the liver by depleting hepatic energy supplies (41). In one study, normal subjects and patients with NASH exhibited similar depletion of hepatic ATP levels after an injection of fructose, but recovery of ATP levels after depletion was slower in NASH patients compared with healthy patients (8). Why a mixture of the monosaccharides fructose and glucose might induce metabolic abnormalities that differ from sucrose, a disaccharide cleaved to fructose and glucose in the small intestine, is a source of ongoing debate.

The progressively increasing sedentary behavior of children and adults worldwide over the past several decades is well documented. This increasingly inactive lifestyle has been associated with the increasing prevalence of obesity and also a rise in the prevalence of NASH, type 2 diabetes mellitus, insulin resistance, and other components of the metabolic syndrome (15, 16, 34). In one study, rats were shown to improve hepatic steatosis with physical activity (31); exercise has also been shown to prevent hepatic steatosis in rats on a diet that induced steatosis (13). Although formal measurements of exercise were not made in this study, ALIOS mice were likely restricted in their ability to exercise by the removal of the wire racks from their cages and were subjectively found to be sedentary. Additional studies will now be needed to quantify the activity of ALIOS mice and to examine the effects of promoting increased physical activity in this model.

Reduced glucose tolerance in the ALIOS mice was evident by the second week of treatment. Although some investigators have suggested that hepatic steatosis causes insulin resistance (9, 36), our observations are in line with others (5, 27) and suggest that metabolic abnormalities precede the development of steatosis, and may thus be responsible for subsequent hepatic steatosis. Fasting elevations of blood glucose are a marker of hepatic insulin resistance with resulting inappropriate gluconeogenesis (35). In this model, elevated fasting glucose levels were evident early in the course of treatment when liver fat accumulation was relatively minor, suggesting that hepatic insulin resistance develops before the accumulation of much fat.

In summary, mice treated with a combination of factors that have been associated with the development of obesity and NASH develop a severe phenotype of fatty liver disease with necroinflammatory changes and a profibrogenic response at the molecular level in the setting of obesity, hepatic insulin resistance, impaired insulin responsiveness, and hyperinsulinemia. Inclusion of HFCS promoted increased food intake and contributed to impaired insulin sensitivity. Importantly, the presence of trans fats promoted fat retention in the liver and contributed substantially to hepatocellular injury. This clinically relevant model sets the stage for the study of interventions to treat or prevent NAFLD and further the understanding of the role of trans fats in the pathogenesis of NASH.

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REFERENCES


