Trans fat feeding results in higher serum alanine aminotransferase and increased insulin resistance compared with a standard murine high-fat diet

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Koppe SW, Elias M, Moseley RH, Green RM. Trans fat feeding results in higher serum alanine aminotransferase and increased insulin resistance compared with a standard murine high-fat diet. Am J Physiol Gastrointest Liver Physiol 297: G378–G384, 2009. First published June 18, 2009; doi:10.1152/ajpgi.90543.2008.—Diets high in trans fats are associated with an increased risk of cardiovascular disease and components of the metabolic syndrome. The influence of these toxic fatty acids on the development of nonalcoholic fatty liver disease has not been significantly examined. Therefore, we sought to compare the effect of a murine diet high in trans fat to a standard high-fat diet that is devoid of trans fats but high in saturated fats. Male AKR/J mice were fed a calorically identical trans fat diet or standard high-fat diet for 10 days, 4 wk, and 8 wk. Serum alanine aminotransferase (ALT), lipid, insulin, and leptin levels were determined and the quantitative insulin-sensitivity check index (QUICKI) was calculated as a measure of insulin resistance. Additionally, hepatic triglyceride content and gene expression of several proinflammatory genes were assessed. By 8 wk, trans fat-fed mice exhibited higher ALT values than standard high-fat-fed mice (126 ± 16 vs. 71 ± 7 U/l, P < 0.02) despite similar hepatic triglyceride content at each time point. Trans fat-fed mice also had increased insulin resistance compared with high-fat-fed mice at 4 and 8 wk with significantly higher insulin levels and lower QUICKI values. Additionally, hepatic interleukin-1β (IL-1β) gene expression was 3.6-fold higher at 4 wk (P < 0.05) and 5-fold higher at 8 wk (P < 0.05) in trans fat-fed mice compared with standard high-fat-fed mice. Trans fat feeding results in higher ALT values, increased insulin resistance, and elevated IL-1β levels compared with standard high-fat feeding.

Elaidic acid; nonalcoholic fatty liver disease; interleukin-1β; quantitative insulin-sensitivity check index, trans fat

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is the most common cause of liver disease in the United States with an estimated prevalence of 5–14% (10). NAFLD describes a range of disease, from simple steatosis to fibrosing steatohepatitis that can progress to cirrhosis. NASH is associated with insulin resistance, central adiposity, dyslipidemia, and hypertension and is considered by many to be the hepatic manifestation of the metabolic syndrome (10, 31, 39).

Trans fats are a form of unsaturated fats that are relatively rare in nature but are present in abundance in “fast food” and many processed foods that are excessively consumed in developed nations. Trans fats differ from the majority of unsaturated fatty acids because of a double bond in the “trans” configuration instead of the standard “cis” configuration. This structural change results in a fatty acid species that is straighter and more closely resembles the structure of a saturated fatty acid. This structural change is thought to play a particularly important role in the toxicity of trans fats (41). The major source of trans fats in our diet is from the partial hydrogenation of vegetable oils, which is an industrial process used to convert oils into semisolid fats for use in baked goods, deep-fried fast foods, and margarines. Consumption of just one or two servings of these products can often exceed the recommended daily intake of trans fats. Trans fats account for 2–3% of total calories consumed by Americans, but it has been recommended that intake be no greater than 1% of total calorie consumption (2, 35).

Trans fat consumption is associated with an increased risk of coronary heart disease (5, 36, 37). Additionally, trans fats may exacerbate diabetes, as suggested by increased insulin resistance in trans fat-fed rodents, although data linking trans fats to diabetes in humans are conflicting (16, 18, 34, 51). Insulin resistance and vascular disease are commonly found in persons with NAFLD, and these diseases share common risk factors. Several recent studies have demonstrated an increase in cardiovascular events in patients with NAFLD independent of conventional risk factors (4, 13, 19, 49). Although consumption of a high-fat diet by rodents leads to the development of experimental fatty liver disease, the specific importance of trans fats on either experimental or human fatty liver disease has not been previously addressed. Therefore, we sought to compare the effect of a trans fat diet consisting primarily of trans-18:1, n-6, the species found in greatest abundance in trans fats consumed from industrial sources, with a calorically identical standard high-fat (trans fat-free) diet on the development of experimental fatty liver disease.

MATERIALS AND METHODS

Animals and experimental protocol. Male AKR/J mice were purchased from Jackson Laboratory (Bar Harbor, ME), and mice were 9–10 wk of age at the start of each experiment. Animals were housed in a temperature-controlled room (22°C) with 14-h light:10-h dark cycling and fed Harlan Teklad chow (Madison, WI) prior to experimentation, with free access to food and water. Mice were administered either a trans fat, standard high-fat, or control diet (Research Diets, New Brunswick, NJ) for 10 days (n = 6–8 per group), 4 wk (n = 5–6 per group), or 8 wk (n = 4–5 per group). Separate cohorts of mice were used for each experimental time point.

The high-fat and trans fat diets were calorically identical; both consisting of 20 kcal% protein, 35 kcal% carbohydrate, and 45 kcal% total fat (Table 1). The diets differed in their content of trans fatty acids: the high-fat diet contained no trans fats, whereas the trans fat diet contained 22 kcal% trans fat; the most common species of trans fat was trans-18:1, n-6 (elaidic acid). The fatty acid composition of the
Table 1. Diet compositions

<table>
<thead>
<tr>
<th></th>
<th>Control Diet, kcal %</th>
<th>Standard High-Fat Diet, kcal %</th>
<th>Trans-Fat Diet, kcal %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>70</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Overall fat</td>
<td>10</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Saturated</td>
<td>2.5</td>
<td>16.2</td>
<td>10</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>4</td>
<td>8.6</td>
<td>4.5</td>
</tr>
<tr>
<td>PUFA-to-SFA ratio</td>
<td>1.6</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Monounsaturated (Cis-)</td>
<td>3.5</td>
<td>20.3</td>
<td>10.5</td>
</tr>
<tr>
<td>Trans-</td>
<td></td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Diet compositions as a measure of insulin sensitivity; QUICKI lipoproteins were fractionated with an AKTA fast-protein liquid chromatography system using equal volumes of pooled plasma from mouse cohorts on tandem Superose 6 FPLC columns (Amersham Biosciences, Piscataway, NJ). The columns were then eluted with 200 mmol/l sodium phosphate (pH 7.4), 50 mmol/l NaCl, 0.03% (wt/vol) EDTA, and 0.02% (wt/vol) sodium azide at a flow rate of 0.4 ml/min. The content of cholesterol in the eluted fractions was measured with a microplate assay technique. The cholesterol of the serum and of the eluted fractions was determined with an enzymatic assay reagent kit (Sigma, St. Louis, MO). Apo B-100 levels of pooled LDL fractions were determined by using rabbit polyclonal antibodies (1:500) directed against mouse apoB-100 (Biodiesign International, Saco, ME) as described previously (40).

Quantitative real-time PCR. RNA was isolated from livers by using Trizol reagent purchased from Invitrogen (Carlsbad, CA). First strand cDNA synthesis was performed by reverse transcription of 2 μg of total RNA with an iScript cDNA synthesis kit from Bio-Rad (Hercules, CA). Real-time PCR was performed using 2 μl of the total RNA in a 25-μl reaction containing QuantiTect SYBRGreen PCR Master Mix (Qiagen, Valencia, CA) (primer sequences listed in Table 2). Amplification was performed in duplicate for each sample in an Applied Biosystems 7300 Sequence Detector (Foster City, CA) and the amount of mRNA was normalized with GAPDH used as the endogenous control.

Statistical analysis. Data are presented as means ± SE. Comparisons between groups were performed with one-way ANOVA and the Holm-Sidak method for multiple pairwise comparisons. SigmaStat 3.0 was utilized to calculate results, and significance was defined as P < 0.05.

RESULTS

Trans fat-fed mice have higher ALT values compared with standard high-fat-fed mice after 8 wk. There were no differences in serum ALT values between any of the groups at 10 days or 4 wk, but after 8 wk both trans fat-fed and standard high-fat-fed mice had significantly higher ALT values compared with mice on the control diet (Fig. 1). At 8 wk, the trans fat group also had a significantly higher ALT value than the high-fat group (126 ± 16 vs. 71 ± 7 U/l, P < 0.05). No significant histological differences were noted between the high-fat and trans fat groups. After 8 wk on the diet both groups demonstrated significant steatosis compared with the control group, but there was no inflammatory infiltrate or fibrosis identified in either group.

Trans fat-fed mice gained less weight after 8 wk compared with standard high-fat-fed mice but had a similar increase in hepatic steatosis. After 10 days, 4 wk, and 8 wk on their respective diets, the standard high-fat and trans fat-fed mice both had increased weight gain compared with the mice fed the control diet. However, by 8 wk the high-fat-fed mice had gained more weight than the trans fat-fed mice (Fig. 2A). After 8 wk, the high-fat and trans fat groups had a similar increase in hepatic triglyceride content and both groups had significantly higher hepatic triglyceride content than the control group (Fig. 2B).

Table 2. Primers for quantitative real-time PCR

<table>
<thead>
<tr>
<th></th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>5’-CCA GCA TGG GGA CAT GAG AAC-3’</td>
<td>5’-TTT TCT GGA GAC TCA AAT CCC AC-3’</td>
</tr>
<tr>
<td>IL-6</td>
<td>5’-TAG TCC TCC CTA CAA TTC-3’</td>
<td>5’-GGA CCA TCT CTA-3’</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5’-CTG GCA CAG TCT GGG ACC CT-3’</td>
<td>5’-AGA GCA GAC CTG CAT-3’</td>
</tr>
<tr>
<td>IL-10</td>
<td>5’-CAG AAA GCC GGC TCT CAG AA-3’</td>
<td>5’-GCT CAG GAG GGG ACC-3’</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>5’-TCC GAT TAC GCG CCC ATC AGG-3’</td>
<td>5’-CTG CAG CAG TCT GAA AAG CAA-3’</td>
</tr>
<tr>
<td>SOCS-3</td>
<td>5’-CAG AGC AAG TTC CCC GGC GGC GC-3’</td>
<td>5’-GCT CAG CAG CTT GAC ACA-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-ACC ACC ATG GAG GAG GCC GC-3’</td>
<td>5’-CTG CAG GCT CCC AAG CAA CAT GC-3’</td>
</tr>
</tbody>
</table>
Serum cholesterol and triglyceride levels in trans fat-fed mice and standard high-fat-fed mice. Serum cholesterol levels of the high-fat-fed mice were higher than the trans fat-fed mice after 10 days but were not statistically different from the control group (Table 3). After 4 wk and 8 wk of feeding, cholesterol levels were higher in the standard high-fat-fed mice compared with control, but there was no significant difference in the trans fat-fed group compared with the high-fat or control group. Lipoprotein analysis was performed with FPLC and the majority of cholesterol in all groups was high-density lipoprotein (HDL). Levels of low-density lipoprotein (LDL) cholesterol were very low and similar in all groups and there did not appear to be any difference in the amount of apoB-100 in the LDL fraction, suggesting no substantial alteration in the density of LDL particles (Supplemental Fig. S1). Serum triglyceride levels were similar between the trans fat and high-fat group at each time point (Table 3).

Trans fat-fed mice demonstrate more insulin resistance than high-fat-fed mice. Fasting insulin levels of the trans fat-fed mice were higher than those of the control diet-fed mice at each time point and after 8 wk of feeding the standard high-fat-fed mice also had significantly higher insulin levels than the control group. At 4 wk and 8 wk, trans fat feeding resulted in significantly higher fasting insulin levels compared with standard high-fat feeding (4 wk: 5.3 ± 1.2 vs. 1.4 ± 0.2 ng/ml, P < 0.05; 8 wk: 6.2 ± 2.0 vs. 2.0 ± 0.1 ng/ml, P < 0.05) (Fig. 3A). Although there was a trend toward higher glucose levels in the standard high-fat and trans fat groups compared with control, this did not reach statistical significance and there were no significant differences between the trans fat and standard high-fat groups. At 8 wk, the serum glucose levels were 188 ± 13, 215 ± 6, and 215 ± 6 mg/dl in the control, standard high-fat, and trans fat groups, respectively. QUICKI determinations were made based on the fasting glucose and insulin levels at each time point. Although QUICKI determinations were lower in the high-fat group than in the mice fed the control diet, consistent with greater insulin resistance, this only reached statistical significance at 8 wk. The trans fat-fed group, however, demonstrated significantly more insulin resistance at each time point compared with the mice on the control diet. At 4 wk and 8 wk the trans fat-fed mice also demonstrated significantly more insulin resistance compared with the mice on the standard high-fat diet (4 wk: 0.32 ± 0.01 vs. 0.41 ± 0.01, P < 0.05; 8 wk: 0.33 ± 0.02 vs. 0.38 ± 0.00, P < 0.05) (Fig. 3B).

Trans fat-fed mice demonstrate altered leptin levels at 8 wk. Serum leptin levels were determined at each time point (Fig. 4). Both standard high-fat feeding and trans fat feeding resulted in higher leptin levels at each time point compared with control diet feeding; however, this only reached significance at 8 wk. Additionally, at 8 wk serum leptin was significantly lower in the trans fat-fed mice compared with the high-fat-fed mice (24.8 ± 3.7 vs. 41.0 ± 4.5 ng/ml, P < 0.05).
Trans fat-fed mice demonstrate significantly increased hepatic IL-1β gene expression as well as increased hepatic IL-1β levels. Hepatic gene expression of several pro- and anti-inflammatory cytokines was determined by quantitative real-time PCR. IL-1β gene expression increased with time in each experimental group. At 4 wk and 8 wk there was a significant elevation in hepatic IL-1β gene expression in the trans fat-fed mice relative to both the high-fat-fed mice and the mice fed the control diet (Fig. 5A). Hepatic IL-1β gene expression of the trans fat-fed mice was 3.6-fold higher at 4 wk ($P < 0.05$) and 5-fold higher at 8 wk ($P < 0.05$) compared with standard high-fat-fed mice. The increase in hepatic gene expression of IL-1β was accompanied by increased hepatic IL-1β protein levels at 8 wk (Fig. 5B). Gene expression of additional cytokines and factors implicated in NAFLD and insulin resistance were also determined [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), suppressor of cytokine signaling-1 (SOCS-1), and suppressor of cytokine signaling-3 (SOCS-3)]. No substantial trends were identified in the other genes analyzed related to time course or between the groups (Table 4).

DISCUSSION

Although trans fats have been linked with coronary heart disease, dyslipidemia, and insulin resistance, the specific impact of trans fats on the liver has not been well studied (5, 16, 18, 34, 36, 37, 51). These experiments demonstrate that mice...
fed a trans fat diet have increased insulin resistance and higher ALT levels compared with mice fed a standard experimental high-fat diet. This occurs despite developing a similar degree of obesity and hepatic steatosis. These observations are accompanied by a substantial increase in IL-1β gene expression and IL-1β cytokine levels in the liver. The increased insulin resistance and more dramatic ALT elevation observed in the trans fat-fed mice suggests this model may translate better to human fatty liver disease than the standard high-fat diet.

Insulin resistance is critical to the development of NAFLD, and the severity of insulin resistance correlates with more advanced liver disease (12, 15, 47). A diet high in saturated fats and low in polyunsaturated fats has been associated with increased insulin resistance. However, there is conflicting evidence regarding the influence of dietary trans fats on the development of insulin resistance and whether they lead to more insulin resistance compared with saturated fats (16, 17). Ibrahim et al. (18) demonstrated that replacing 2% of energy from saturated fatty acids and 1% from cis-monounsaturated fatty acids with 3% trans fats resulted in ~17% higher fasting insulin levels and reduced adipocyte insulin sensitivity in rats. They also showed that trans fats reduced the adipocyte membrane fluidity and suggested this as a possible mechanism of increased insulin resistance in their model. Alstrup et al. (3) also showed that the trans-conformations of 18:1delta-9 and 18:1delta-11 fatty acids led to significantly higher insulin output from isolated mouse islet cells compared with their cis-configurations.

In our experiments, trans fat-fed mice became obese and demonstrated higher insulin levels and more insulin resistance than mice fed a calorically identical diet high in saturated fats. Large human observational studies have examined the association between trans fat intake and the risk of diabetes. The largest study, the Nurses’ Health Study, determined that intake of trans fats was an independent risk factor for developing diabetes (43). Two other studies, however, found no direct association between trans fat intake and the risk of diabetes (34, 32). In addition to having the largest sample size, strengths of the Nurses’ Health Study were its adjustment for the types of fat ingested and a reported intake of trans fats more consistent with previous dietary studies (2, 14). There have been only a few randomized studies in a small number of patients comparing consumption of meals high in trans fats, monounsaturated fats, or saturated fats on the development of insulin resistance. Two studies found no difference in postprandial insulin levels between subjects that consumed a high-trans fat meal vs. a meal high in cis-monounsaturated fats; however, these studies involved healthy, lean subjects (28, 29). In the two studies that have been performed with obese subjects, one of which included diabetic subjects, greater elevations in postprandial insulin levels were demonstrated in meals containing trans fats compared with meals containing cis-monounsaturated fats (8, 25).

Relationships between the inflammatory cytokines IL-1β, IL-6, TNF-α, and IL-10 and the SOCS-1 and SOCS-3 with insulin resistance are well described (9, 21, 22, 24, 44, 45, 50). Systemically administered IL-1β has been shown to increase hepatic gluconeogenesis; however, the link between IL-1β and insulin resistance has been primarily characterized in adipocytes and pancreatic islet beta cells (20, 23, 26, 30). In our experiments, the marked increase in hepatic IL-1β gene expression at 4 wk and 8 wk, in addition to the significantly higher hepatic IL-1β levels at 8 wk in the trans fat-fed group, suggests a role for IL-1β in the higher ALT and increased insulin resistance observed. In humans, genetic polymorphisms in IL-1β have been reported to be associated with an increased risk of type 2 diabetes in North Indians and also associated with an increased risk of the metabolic syndrome in those with low polyunsaturated fat intake (1, 46). TNF-α, IL-6, IL-10, SOCS-1, and SOCS-3 have also been implicated as links between inflammation and insulin resistance, but there were no significant differences observed in mice fed the trans fat diet.

Leptin levels were greater in the standard high-fat and trans fat groups, but only after 8 wk were they significantly greater than the control group. Leptin levels in the trans fat group, however, remained lower than the standard high-fat group. Leptin levels mirrored percent weight gain and may parallel overall body fat mass. Whereas hepatic fat content was found to be identical between the trans fat and high-fat groups, we cannot exclude the possibility that the fat distribution was different between the groups. The interplay between leptin and insulin is quite complex. Insulin has been shown to directly increase leptin secretion; however, leptin has also been demonstrated to decrease insulin production and the lower leptin levels at 8 wk may have contributed to the higher insulin levels observed in the mice fed the trans fat diet (7, 42). Two studies have directly examined the influence of trans fat consumption on leptin levels. Bray et al. (6) showed unchanged leptin levels in overweight men who ingested a single meal high in trans fats compared with calorically identical meals that were devoid of trans fats. This study, however, involved only a single feeding. Another study demonstrated that rats fed a diet high in trans fats while pregnant and during lactation resulted in significantly lower leptin levels of their offspring despite similar body weights and increased adipose content compared with a control group (38).

Differences in serum cholesterol appear to primarily be related to an increased HDL fraction in the high-fat-fed group.
relative to the trans fat and control diet groups. Although it is possible that trans fat feeding results in lower HDL levels compared with standard high-fat feeding, the small difference in cholesterol content of the diets limits the conclusions one can draw from this finding. The cholesterol content was low in all of the diets and there was no additional cholesterol added to the diets, but there was a slightly greater amount of cholesterol in the high-fat diet compared with the trans fat and control diets. This difference was due to the source of fat used in the diets: lard (i.e., animal) for the high-fat compared with vegetable for the trans fat.

One of the known limitations of the high-fat diet model of NAFLD is its lack of histological evidence of inflammation or fibrosis in mice despite the development of steatosis and insulin resistance. We have also performed an experiment feeding mice trans fats and a standard high-fat diet for up to 6 mo, which did not result in any liver fibrosis. The purpose of our experiment was to compare the effect of trans fats to that of saturated fats on the liver. Although feeding mice a trans fat diet for 8 wk does not invoke progressive steatohepatitis, it does result in a more pronounced elevation of ALT and insulin resistance, sine qua non of human fatty liver disease.

NAFLD is an independent predictor of cardiovascular disease, even after controlling for other features of the metabolic syndrome (13, 48, 49). Several studies correlate trans fat consumption with cardiovascular events, and trans fats have also been implicated as an independent risk factor for endothelial dysfunction, systemic inflammation, and dyslipidemia, frequent comorbid conditions in patients with NAFLD (11, 27, 33, 35). No dietary studies concerning trans fat intake and NAFLD have been performed, and only a few studies involving a small number of patients exist examining dietary fat intake as it relates to NAFLD (32). These data indicate that mice fed a trans fat diet exhibit higher serum ALT values, increased insulin resistance, and increased IL-1β compared with mice fed a standard high-fat diet, despite similar amounts of hepatic steatosis. Additional work is needed to understand the molecular mechanisms by which trans fats may be more hepatotoxic.

**GRANTS**

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