Metabolic Implications of Dietary *Trans*-fatty Acids

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Dietary *trans*-fatty acids are associated with increased risk of cardiovascular disease and have been implicated in the incidence of obesity and type 2 diabetes mellitus (T2DM). It is established that high-fat saturated diets, relative to low-fat diets, induce adiposity and whole-body insulin resistance. Here, we test the hypothesis that markers of an obese, prediabetic state (fatty liver, visceral fat accumulation, insulin resistance) are also worsened with provision of a low-fat diet containing elaidic acid (18:1*t*), the predominant *trans*-fatty acid isomer found in the human food supply. Male 8-week-old Sprague–Dawley rats were fed a 10% *trans*-fatty acid enriched (LF-*trans*) diet for 8 weeks. At baseline, 3 and 6 weeks, *in vivo* magnetic resonance spectroscopy (^{1}H-MR) assessed intramyocellular lipid (IMCL) and intrahepatic lipid (IHL) content. Euglycemic–hyperinsulinemic clamps (week 8) determined whole-body and tissue-specific insulin sensitivity followed by high-resolution *ex vivo* ^{1}H-NMR to assess tissue biochemistry. Rats fed the LF-*trans* diet were in positive energy balance, largely explained by increased energy intake, and showed significantly increased visceral fat and liver lipid accumulation relative to the low-fat control diet. Net glycogen synthesis was also increased in the LF-*trans* group. A reduction in glucose disposal, independent of IMCL accumulation was observed in rats fed the LF-*trans* diet, whereas in rats fed a 45% saturated fat (HF-sat) diet, impaired glucose disposal corresponded to increased $IMCL_{TA}$. Neither diet induced an increase in $IMCL_{soleus}$. These findings imply that *trans*-fatty acids may alter nutrient handling in liver, adipose tissue, and skeletal muscle and that the mechanism by which *trans*-fatty acids induce insulin resistance differs from diets enriched with saturated fats.

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INTRODUCTION

The association between obesity and type 2 diabetes mellitus (T2DM) is widely recognized. These diseases fall under the same umbrella as a number of other pathophysiologic manifestations including dyslipidemia, atherosclerosis, nonalcoholic fatty liver disease, and hypertension, (i.e., the metabolic syndrome). Dietary macronutrients profoundly affect adiposity and insulin action and therefore contribute to metabolic disease (1–4). Caloric excess leading to positive energy balance may directly induce insulin resistance at a cellular level through specific nutrient effects on signal transduction, gene expression, and substrate flux through metabolic pathways. In rodents, a chronic oversupply of calories by way of a high-fat diet induces body weight gain, fat mass accumulation, and insulin resistance (1,5–7). However, the extent to which these phenomena are observed differs with respect to dietary fat type. For instance, diets high in ω-3 fatty acids (fish oil) prevent the induction of insulin resistance (8,9).

Recent data regarding *trans*-fatty acids implicate this lipid subclass as being particularly deleterious to human health. *Trans*-fatty acid consumption in the United States constitutes 2–3% of total caloric intake (10). *Trans*-fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. Historically, *trans*-fatty acids were thought to be metabolized similarly to saturated fatty acids due to their conformational similarity. More recently, *trans*-fatty acid intake has been linked to increased risk of cardiovascular disease through effects on lipoprotein metabolism (11,12). In humans, it has been well established that substituting *trans*-fatty acids for either saturated or polyunsaturated fatty acids results in more deleterious lipid profiles than those observed with saturated fatty acids (12). In addition, *trans*-fatty acids negatively affect endothelial function and cytokine production, further increasing cardiovascular disease risk (13,14). Associative and experimental evidence linking *trans*-fatty acid intake and T2DM, however, is limited. Whereas some prospective studies suggest that consumption of a diet containing *trans*-fatty acids is associated with an increased risk for the development of T2DM (15,16), others have disagreed (17). In human metabolic studies involving obese and/or T2DM patient, *trans*-fatty

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acid intake resulted in an increase in markers of insulin resistance beyond those observed with diets enriched with saturated or monounsaturated fatty acids (18–20). Thus, metabolic maladies arising with greater *trans*-fatty acid intake may only be apparent in certain metabolic settings.

Due to unresolved issues relating to the magnitude of the physiologic effects of dietary *trans*-fatty acids on energy balance and insulin sensitivity, we sought to determine whether dietary *trans*-fatty acids alter adiposity and insulin sensitivity and if so, understand the associated changes in intracellular lipid pools and metabolic profiles. The data here show that a diet low in fat but enriched in *trans*-fatty acids increases visceral adiposity, stimulates intrahepatic lipid (IHL) accumulation, and reduces peripheral insulin sensitivity to an extent similar to that brought about by a diet higher in saturated fats. Although the degree of insulin resistance between the two diets is similar, the mechanism by which insulin resistance occurs appears to differ.

METHODS AND PROCEDURES

Animals and study design

Studies were reviewed and approved by Novartis Animal Care and Use Committee and were in compliance with the Animal Welfare Act Regulations Parts 1, 2, and 3, and other guidelines (Guide for the Care and Use of Laboratory Animals, 1995). Male Sprague–Dawley rats (Charles River Laboratory, Wilmington, MA), 8 weeks of age, were allowed free access to standard, semipurified chow diet and water upon arrival and were individually housed under controlled conditions (12:12 h light–dark cycle; 50–60% relative humidity, 22–23 °C). To examine the effects of *trans*-fatty acid enriched diets on parameters of metabolic syndrome, rats were fed experimental diets for 8 weeks. The experimental diets differed in quantity and quality of fat (**Table 1**), with mineral and vitamin mixes prepared according to guidelines from the American Institute of Nutrition (21). A 10% saturated fat (LF-sat) diet with fat sources being soybean oil (24 g/kg) and lard (19 g/kg) and a 45% saturated fat (HF-sat) diet with the fat source primarily being lard and to lesser extent soybean oil were used as control and insulin resistant diets, respectively. The diet under investigation was a LF-*trans* diet that was identical to the LF-sat diet, but with the lard entirely replaced by elaidic acid (Nu-Chek Prep, Elysian, MN; LF-*trans*). All diets were purchased from Research Diets (New Brunswick, NJ). Food intake and body weight were measured weekly. Rats (n = 8–11/group) were temporally assessed for changes in body fat composition (subcutaneous vs. visceral fat), IHL, and intramyocellular lipid (IMCL) content using *in vivo* magnetic resonance spectroscopy (^{1}H-MR) followed by measurement of tissue metabolites using *ex vivo* ^{1}H-NMR. Whole-body and tissue specific insulin resistance were determined by the euglycemic–hyperinsulinemic clamp.

In vivo magnetic resonance

Three days prior to initiating the diet intervention, and at 3 and 6 weeks of diet feeding, *in vivo* ^{1}H-MR under 2% isoflurane anesthesia was performed to quantify total body fat, visceral and subcutaneous fat, IHL, and IMCL content. All *in vivo* ^{1}H-MR measurements were performed on a Bruker Avance 7.0 T/30 cm wide-bore instrument (Bruker Medical, Billerica, MA) equipped with a 20-cm internal diameter actively shielded gradient insert. Measurement of whole-body adiposity, IHL, and IMCL were done as previously reported (22,23) with adjustments for a 7T magnet.

Euglycemic–hyperinsulinemic clamp

At 7 weeks on diet, rats were cannulated at the carotid artery and jugular vein and were allowed to recover for 7 days. All rats achieved >100% presurgery body weight by this time. At 8 weeks on diet, rats were fasted overnight prior to assessing insulin action by the euglycemic–hyperinsulinemic clamp. Five minutes before initiating infusions, a basal blood sample was taken from the arterial line to determine basal hormone and substrate concentrations. At t = 0 min, a bolus of high-performance liquid chromatography–purified [3-^{3}H]-glucose was given (7.5 µCi) over 1 min into the venous line followed by a constant venous infusion at 0.1 µCi/min. A pancreatic clamp was also initiated by infusion of somatostatin (1.2 µg/kg·min) to suppress endogenous insulin and glucagon production. Insulin (0.24 mU/kg·min) and glucagon (0.8 ng/kg·min) were infused at rates to maintain these hormones at basal concentrations. Arterial blood samples were taken at t = 70, 80, 90 min to assess attainment of tracer steady state and determine basal rates of endogenous glucose appearance (R_a) and disappearance (R_d). At t = 90 min, a second bolus of [3-^{3}H]-glucose was given (3.7 µCi) over 1 min into the venous line and constant infusion rate of [3-^{3}H]-glucose was increased to 0.2 µCi/min for the remainder of the study to minimize changes in blood glucose specific activity with the initiation of the hyperinsulinemic period. A venous infusion of insulin at 3.0 mU/kg·min was also initiated along with a variable infusion of glucose (20% glucose into the jugular vein) while maintaining the pancreatic clamp. Small arterial blood samples

Table 1 Composition of experimental diets

	LF-sat (D12450B)	LF-*trans* (D05040802)	HF-sat (D12451)
Macronutrient (% energy)			
Protein	20	20	20
Carbohydrate	70	70	35
Total fat	10	10	45
Elaidic acid (% energy)	0	4.6	0
Macronutrient sources (g/100 g)			
Casein	19.0	19.0	23.3
L-Cystine	0.3	0.3	0.4
Corn starch	30.0	30.0	8.5
Maltodextrin 10	3.3	3.3	11.7
Sucrose	33.2	33.2	20.2
Soybean oil	2.4	2.4	2.4
Lard	1.9	—	20.7
Elaidic acid	—	1.9	—
Fatty acid profile (g/100 g)			
C14:0, myristic	0.02	—	0.19
C14:1, myristoleic	0.01	—	0.11
C16:0, palmitic	0.68	0.25	5.04
C16:1, palmitoleic	0.08	—	0.78
C18:0, stearic	0.34	0.09	2.87
C18:1*c*, oleic	1.36	0.58	9.21
C18:1*t*, elaidic	—	1.90	—
C18:2, linoleic	1.43	1.27	3.33
C18:3, linolenic	0.21	0.19	0.43
C20:4, arachidonic	0.03	—	0.35
kcal/g	3.85	3.85	4.73

Diets were formulated by Research Diets; diet numbers are provided in the parentheses below each diet's name. All diets were matched by kcal (g/100 kcal) for vitamin mix (0.25), mineral mix (0.25), dye (0.001), choline bitartrate (0.05), potassium citrate (0.41), calcium carbonate (0.14), dicalcium phosphate (0.32), and cellulose (1.23).
HF-sat, 45% saturated fat; LF-sat, 10% saturated fat; LF-*trans*, 10% *trans*-fatty acid enriched.

(5 µl) were taken every 5–10 min to assess circulating blood glucose concentrations and determine appropriate changes in infusion rate of exogenous glucose necessary to maintain glucose at 100 mg/dl. To assess peripheral glucose uptake into adipose tissue and skeletal muscle, a venous bolus of [U-^{14}C]-2-deoxyglucose (45 µCi in 100 µl of 0.1% BSA/saline, American Radiolabeled Chemicals, St Louis, MO) was administered at t = 135 min over 60 s. Arterial blood samples were taken at t = 137.5, 140, 145, 150, 155, 160, 165, 170, 175, and 180 min for plasma ^{14}C-glucose specific activity. At t = 160, 170, 180 min larger blood samples were collected to assess plasma ^{3}H-glucose specific activity and insulin suppression of glucose R_a. At t = 180 min, rats were anesthetized with sodium pentobarbital (100 mg/kg, i.v.) and tissues were collected and placed in liquid nitrogen. For some animals in each diet group (n = 6–8/group), tissues were collected following the 90-min basal period.

Plasma hormones and substrates

During clamp studies, glucose was estimated with a One-Touch Ultra glucometer (LifeScan, Milpitas, CA). Plasma glucose for the determination of glucose specific activity was assessed using a 2700 YSI Select (Yellow Springs International, Yellow Springs, OH). Insulin, leptin, and adiponectin were measured by enzyme-linked immunosorbent assay (Linco Research, St. Charles, MO). Free fatty acids were measured spectrophotometrically (Wako-C NEFA kit; Wako Chemicals, Richmond, VA). Total plasma triglycerides were estimated fluorometrically using validated in-house enzymatic methods.

Plasma and tissue radioactivity

Plasma samples were deproteinized with $Ba(OH)_2$ and $ZnSO_4$ (0.3 N) and centrifuged (24). One portion was dried to remove all $^{3}H_2O$, reconstituted in distilled deionized water, and counted for ^{3}H and ^{14}C using dual mode liquid scintillation counting (Beckman LS6000IC; Beckman Instruments, Fullerton, CA). Phosphorylated [^{14}C]-2-deoxyglucose concentrations were determined in tibialis anterior (TA), soleus and gastrocnemius muscles, and epididymal fat as described previously (25). In brief, tissues (50–100 mg for muscle; 350 mg for epididymal fat) were homogenized in 0.5% perchloric acid at 4 °C (13,400g, 20 min) and neutralized with 5 N KOH. A portion of the supernatant, representing total [^{14}C]-labeled 2-deoxyglucose, 2-deoxyglucose phosphate and cellular glycogen, was counted. $Ba(OH)_2$ and $ZnSO_4$ (0.3 N) were added to a second portion of the supernatant to precipitate glycogen and 2-deoxyglucose phosphate. Samples were vortexed, centrifuged, and counted, with the difference in ^{14}C-counts representing 2-deoxyglucose phosphate and glycogen. Tissue-specific glucose uptake (R'_g for TA, soleus, gastrocnemius muscles, and epididymal fat) was calculated using accumulation of phosphorylated 2-deoxyglucose and glycogen, which is metabolically trapped in tissues lacking glucose-6-phosphatase activity. The area under the curve for the decay of ^{14}C-glucose specific activity in plasma over the last 45 min of the clamp was also determined. This approach makes the assumption that differences in metabolic handling of 2-deoxyglucose are similar across diet treatments and that the tracer is transported similar to glucose.

Liver glycogen and net liver glycogen synthesis

Liver glycogen content and net liver glycogen synthesis were determined by methods previously described (26).

Calculations

Glucose R_a and R_d were determined using nonsteady state equations as described previously (27) and were expressed as a percent change under hyperinsulinemic conditions relative to basal rates (% hepatic suppression and % disposal). Tissue R'_g was determined by correcting the concentration of tissue [^{14}C]-labeled 2-deoxyglucose-6-phosphate concentration by the area under the curve for plasma ^{14}C-glucose specific activity over the last 45 min of the clamp studies. Glucose infusion rates were determined as a time-weighted average over the last 30 min of clamp studies. Net liver glycogenesis was calculated using a precursor–product relationship, where [^{3}H]-glucose associated with tissue glycogen was corrected for plasma [^{3}H]-glucose specific activity maintained over 180 min (clamp studies).

High-resolution *ex vivo* ^{1}H-NMR

Tissues were extracted with 7% perchloric acid or chloroform as previously described (28). High-resolution ^{1}H-NMR spectra were acquired using standard pulse sequences at 300 ± 1 K using a Bruker-600 Avance spectrometer (^{1}H frequency of 600.26 MHz). ^{1}H-NMR spectra were acquired with 128–256 free induction decays, 65,536 complex data points, a spectral width of 7.2–8.4 kHz, and a relaxation delay of 3 s. Metabolite assignments were made on the basis of previously reported data (29,30) and, in certain cases, were confirmed by spiking. Metabolite and fatty acid resonances were integrated using the ACD 7.0 package (Advanced Chemistry Development, Toronto, Canada). Resonances for individual fatty acid species were integrated relative to the combined intensities of the FA ω-methyl resonances (tCH_3), including the ω-methyl resonances from ω-3 fatty acid ($^{1}H\delta$ 0.98 ppm), as well as the main fatty acid ω-methyl resonance centered at $^{1}H\delta$ 0.89 ppm. The main fatty acid ω-methyl resonance is composed of resonances from ω-6, ω-9, and all other classes (except ω-3) of fatty acids. It was assumed that each fatty acid molecule contained a single ω-methyl moiety and that the tCH_3 intensity would reflect the total number of fatty acid molecules.

Data analysis

Data are reported as means ± s.d. Two-way repeated measures ANOVA assessed any time dependent effects of diet treatment. One-way ANOVA assessed whether differences existed among diet groups for variables that were not dependent on time or condition. Student–Newman–Kuels multiple comparison test determined between group differences. Significance was set at $P \leq 0.05$.

RESULTS

LF-*trans* diet induces positive energy balance

Body weight differences were observed beginning at 6 weeks of diet where the LF-*trans* and HF-sat groups weighed significantly more than the LF-sat group (510 ± 13, 534 ± 15, 479 ± 16 g; respectively, $P \leq 0.05$). Weekly cumulative weight gain was not different among diet groups until 6 weeks owing to increases in weekly cumulative food intake in the LF-*trans* and HF-sat groups beginning at 2 weeks and continuing through the end of 6 weeks (**Figure 1**).

In vivo ^{1}H-MR assessment of fat distribution, IHL, and IMCL content

The increase in whole-body adiposity was significantly greater ($P \leq 0.05$) in the LF-*trans* ($\Delta_{6\text{week-baseline}} = 0.07 \pm 0.01$ g) and HF-sat groups ($\Delta_{6\text{week-baseline}} = 0.08 \pm 0.02$ g) compared to the LF-sat group ($\Delta_{6\text{week-baseline}} = 0.04 \pm 0.01$ g). The LF-*trans* group accumulated fat primarily in visceral depots, whereas in the HF-sat group, fat accumulation was dispersed between both subcutaneous and visceral fat regions (**Figure 2**). Both HF-sat and LF-*trans* groups had significantly higher IHL at 3 and 6 weeks (diet–time interaction, $P \leq 0.01$) relative to the LF-sat group, representing a sixfold increase from baseline (**Figure 3a**). There was no effect of LF-*trans* diet on IMCL accumulation, whereas the HF-sat group demonstrated an increase in IMCL in the TA ($P \leq 0.01$), a predominantly glycolytic muscle (**Figure 3b**). In contrast, the soleus, a highly oxidative muscle, had no significant diet–time interaction throughout the 6 weeks, although

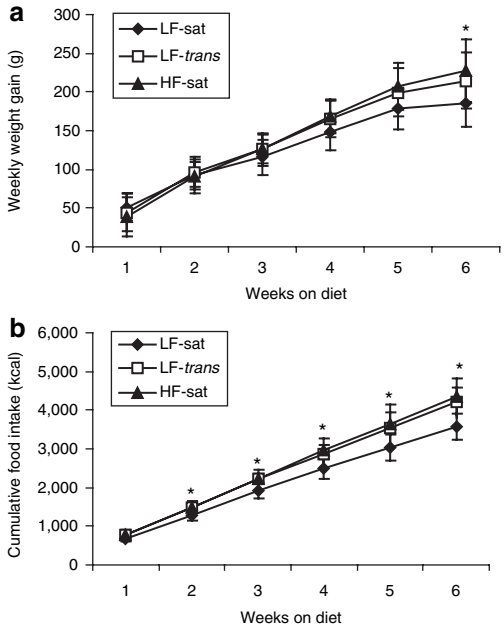

Figure 1 Values are mean ± s.d. (n = 8–9/group). Body weight (BW) and food intake were recorded weekly at 0800 hours. (**a**) Cumulative weekly BW gain and (**b**) cumulative caloric intake (kcal) were calculated based on these measurements. LF-sat, 10% saturated fat; LF-*trans*, 10% *trans*-fatty acid enriched; HF-sat, 45% saturated fat. *LF-*trans* and HF-sat are significantly ($P \leq 0.05$) different from the LF-sat group.

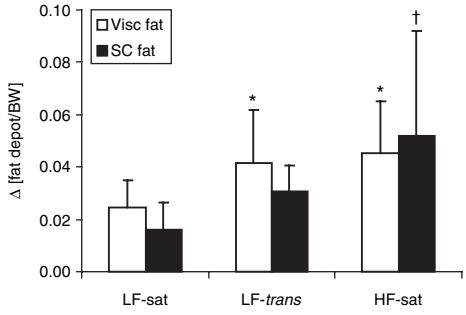

Figure 2 Values are mean change (i.e., delta, Δ) ± s.d. (n = 8–9 rats/group). Change from baseline to 6 week in visceral fat, (Δ[Visc/BW]) and subcutaneous fat (Δ[SC/BW]) is shown. LF-sat, 10% saturated fat; LF-*trans*, 10% *trans*-fatty acid enriched; HF-sat, 45% saturated fat. *$P \leq 0.05$ for Visc fat relative to LF-sat group, †$P \leq 0.05$ for SC fat relative to LF-sat and LF-*trans*.

IMCL content tended to be higher in the HF-sat group (data not shown).

Euglycemic–hyperinsulinemic clamps

Following 8 weeks of diet, whole-body insulin action using the euglycemic–hyperinsulinemic clamp technique was assessed. Baseline plasma parameters were determined immediately prior to the basal period of tracer infusion following an overnight fast (Table 2). Preclamp glucose concentrations were significantly higher in the HF-sat diet ($P \leq 0.01$). No significant diet effects were observed in preclamp insulin, triglyceride, and free fatty acid concentrations. Similarly, there were no differences in preclamp leptin and adiponectin concentrations.

Despite marked increases in whole-body and visceral adiposity with LF-*trans* feeding, there was no significant diet effect on whole-body insulin action as estimated by glucose infusion rate during euglycemic–hyperinsulinemic clamps (Table 2). However, relative to the LF-sat group, % glucose disposal was reduced in both the LF-*trans* and HF-sat groups

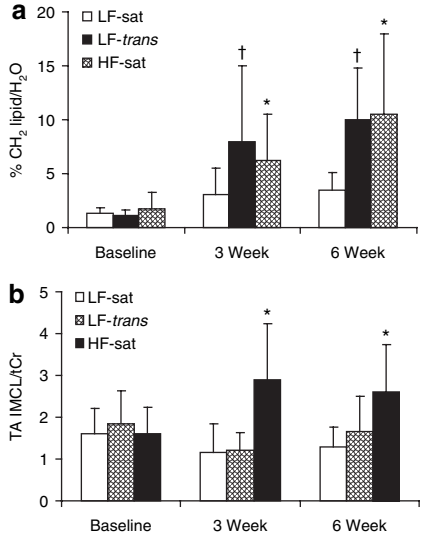

Figure 3 Values are means ± s.d. (n = 8–9/group). LF-sat, 10% saturated fat; LF-*trans*, 10% *trans*-fatty acid enriched; HF-sat, 45% saturated fat. *Significant diet–time interaction ($P \leq 0.01$) at 3 and 6 week in HF-sat group relative to LF-*trans* and LF-sat. †Significant diet–time interaction ($P \leq 0.01$) at 3 and 6 week in LF-*trans* relative to LF-sat group.

Table 2 Euglycemic–hyperinsulinemic clamps

	LF-sat	LF-*trans*	HF-sat
Glucose (mg/dl)	94.4 ± 11.8	102.3 ± 11.6	117.1 ± 11.9*
Insulin (ng/ml)	4.5 ± 0.8	4.5 ± 1.7	3.1 ± 1.4
Triglycerides (mg/dl)	42.9 ± 26.2	30.2 ± 20.4	29.2 ± 14.5
Free fatty acids (μmol/l)	432.9 ± 111.7	444.6 ± 187.1	350.6 ± 68.1
Leptin (pg/ml)	29.5 ± 23.9	39.6 ± 23.9	44.4 ± 36.7
Adiponectin (ng/ml)	13.9 ± 3.4	15.4 ± 3.7	13.4 ± 2.8
Clamp GIR, mg/kg/min	17.0 ± 7.8	13.4 ± 8.9	6.1 ± 15.2
% Hepatic suppression	50.5 ± 47.4	49.7 ± 47.0	30.3 ± 34.8
% Disposal	62.3 ± 18.6	48.1 ± 20.6*	35.4 ± 16.1*

Values are means ± s.d. (n = 8–9/group). Glucose, insulin, triglycerides, free fatty acids, leptin and adiponectin were measured following 15-h fast.
GIR: rate of endogenous glucose infusion necessary to maintain euglycemia, % hepatic suppression and % glucose disposal were determined following a 90-min hyperinsulinemic clamp period.
*$P \leq 0.05$ vs. LF-sat. LF-sat, 10% saturated fat; LF-*trans*, 10% *trans*-fatty acid enriched; HF-sat, 45% saturated fat.

Table 3 Tissue-specific glucose disposal (R'_g), liver glycogen, and net glycogen synthesis under clamp conditions

	LF-sat	LF-*trans*	HF-sat
Tibialis anterior (ng/g·min)	246.4 ± 121.9	294.8 ± 137.9	192.8 ± 82.7
Soleus (ng/g·min)	955.9 ± 511.5	1,158.9 ± 587.1	1,022.5 ± 357.9
Gastrocnemius (ng/g·min)	277.2 ± 107.2	238.0 ± 151.7	203.1 ± 65.3
Epididymal fat (ng/g·min)	14.7 ± 13.6	8.7 ± 5.9	15.3 ± 27.8
Liver glycogen content (mg/g tissue)	6.4 ± 2.7	3.8 ± 1.0	5.7 ± 1.7
Liver net glycogenesis (μmol/g·min)	10.5 ± 4.5	19.5 ± 8.9*,**	7.5 ± 2.5

Values are means ± s.d. (n = 8–11/group). Tissues were collected following a 90-min euglycemic–hyperinsulinemic clamp study.
LF-sat, 10% saturated fat diet; LF-*trans*, 10% *trans*-fatty acid diet; HF-sat, 45% saturated fat diet.
*$P \leq 0.05$ vs. LF-sat, **$P \leq 0.05$ vs. HF-sat.

($P \leq 0.01$; Table 2) Despite significantly lower % glucose disposal in the LF-*trans* and HF-sat groups, R'_g was not different in any of the individual skeletal muscles tested (TA, soleus, gastrocnemius); nor was it different in epididymal fat (Table 3). Net hepatic glycogenesis was increased in the livers of rats fed the LF-*trans* diet (Table 3). Unlike % glucose disposal, there was no indication of a diet effect on hepatic insulin action (% hepatic suppression) in that insulin suppression of hepatic glucose release was not different among groups (Table 2). Whole-body rates of glycolysis were also not different among groups under both basal and hyperinsulinemic conditions (data not shown).

Tissue metabolic profiles

Fatty acid composition of tissues was measured using *ex vivo* ^{1}H-NMR. Elaidic acid (18:1*t* n-9) concentrations were significantly higher in the soleus, liver, and epididymal fat in response to the LF-*trans* diet (Table 4), while linoleic acid (18:2n-6) and longer chain polyunsaturated fatty acids, for example docosahexaenoic (22:6n-3, DHA) acid, were significantly reduced in all tissues.

Due to the decrease in % glucose disposal, we examined whether there were differences in tissue diacylglycerol (DAG) pools, a lipid pool mechanistically linked to impaired insulin signaling (31,32), under basal conditions in both the TA and soleus muscles. There was no effect on DAG concentrations in the TA muscle among the three diet groups, but in the soleus muscle, DAG concentrations were increased nearly twofold over the other two diets (Table 4). Water soluble metabolites in the TA and soleus muscles also differed among diets and muscle types. β-hydroxybutyrate (β-hydroxybutyrate/total creatine) was significantly lower ($P \leq 0.05$) with the LF-*trans* diet, but only in the soleus (Table 4). In addition, despite distinct oxidative capacities, TA and soleus glutamine and glutamate concentrations (Glx/total creatine) were significantly ($P \leq 0.05$)

Table 4 Lipid and aqueous phase metabolites in tissues under basal conditions

	LF-sat	LF-*trans*	HF-sat
Organic analytes		% Fatty acid	
Tibialis anterior			
Oleic acid (18:1*c* n-9)	38.4 ± 5.3	44.2 ± 6.1	41.9 ± 5.6
Elaidic acid (18:1*t* n-9)	4.8 ± 0.4	11.9 ± 1.9	7.8 ± 3.9
Linoleic acid (18:2 n-6)	5.9 ± 2.1	3.4 ± 2.3*	3.2 ± 1.9*
Docosahexaenoic acid (22:6 n-3)	12.2 ± 2.8	10.1 ± 4.8	15.0 ± 5.9
Diacylglycerol (μmol/mg tissue)	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.5
Soleus			
Oleic acid (18:1*c* n-9)	59.1 ± 7.3	54.1 ± 14.3	58.8 ± 8.4
Elaidic acid (18:1*t* n-9)	9.4 ± 7.0	17.0 ± 3.3*,**	9.5 ± 4.2
Linoleic acid (18:2 n-6)	7.9 ± 2.4	3.2 ± 3.1*,**	6.4 ± 1.5
Docosahexaenoic acid (22:6 n-3)	7.5 ± 3.4	6.4 ± 2.2	6.9 ± 5.2
Diacylglycerol (μmol/mg tissue)	1.2 ± 0.3	2.3 ± 1.0*	1.3 ± 0.4
Liver			
Oleic acid (18:1*c* n-9)	56.9 ± 6.8	56.6 ± 7.2**	48.2 ± 7.0*
Elaidic acid (18:1*t* n-9)	2.9 ± 1.0	8.5 ± 2.6*,**	4.1 ± 0.5
Linoleic acid (18:2 n-6)	5.5 ± 1.9	4.5 ± 1.6**	13.1 ± 1.9*
Docosahexaenoic acid (22:6 n-3)	8.8 ± 2.6	5.6 ± 2.5*,**	8.5 ± 2.3
Cholesterol	6.0 ± 1.7	6.0 ± 1.5**	8.0 ± 1.1*
Epididymal fat			
Oleic acid (18:1*c* n-9)	66.2 ± 3.3	63.0 ± 1.9*	64.7 ± 1.7
Elaidic acid (18:1*t* n-9)	3.4 ± 2.5	12.1 ± 1.8*,**	4.5 ± 1.5
Linoleic acid (18:2 n-6)	10.8 ± 1.9	9.5 ± 1.3**	15.6 ± 0.7*
Docosahexaenoic acid (22:6 n-3)	2.1 ± 0.5	1.7 ± 0.2	2.0 ± 0.6
Aqueous analytes		Analyte/total creatine	
Tibialis anterior			
Glutamate + glutamine (Glx)	0.8 ± 0.1	0.6 ± 0.1*	0.5 ± 0.2
β-Hydroxybutyrate (β-HB)	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Soleus			
Glutamate + glutamine (Glx)	2.7 ± 0.3	2.0 ± 0.1*	1.8 ± 0.1*
β-Hydroxybutyrate (β-HB)	0.9 ± 0.1	0.6 ± 0.1*	0.5 ± 0.2

Values are means ± s.d. (n = 7–8/group). Note that diacylglycerol is reported in μmol/mg tissue, whereas all other analytes in the organic fraction are reported as a percentage of the total lipid pool.
Glx/total creatine, sum of glutamate and glutamine concentrations expressed relative to total creatine tissue concentrations; β-HF/total creatine, β-hydroxybutyrate/total creatine; LF-sat, 10% saturated fat; LF-*trans*, 10% *trans*-fatty acid enriched; HF-sat, 45% saturated fat.
*$P \leq 0.05$ vs. LF-sat, **$P \leq 0.05$ vs. HF-sat.

reduced in animals given the LF-*trans* and the HF-sat diets compared to those on the LF-sat diet (**Table 4**).

DISCUSSION

In the past 2–3 decades, a fuller description of the metabolic effects of certain types of dietary fats has become available, providing mounting evidence that those fatty acids associated with cardiovascular disease, both in terms of quantity and quality, are also causative to impairments in insulin action and the progression of T2DM in humans. Though there exist abundant evidence that dietary *trans*-fatty acids have a negative impact on the blood lipid profile and the progression of atherosclerotic lesions (11–14), such evidence linking *trans*-fatty acid intake to insulin resistance and obesity are scant. This study compared markers of the metabolic syndrome across three diets, one higher in fat (45% fat) from typical dietary fat sources, and two low in fat (10% fat). The two low-fat diets were matched in all ways except dietary fatty acid composition. The LF-*trans* diet contained elaidic acid that contributed ~4.6% to total calories but comprised ~40% of fat calories, whereas the LF-sat diet consisted primarily of saturated fats. Thus, the untoward metabolic responses observed with the LF-*trans* diet, which included hyperphagia, increased weight gain, expanded visceral and hepatic fat depots, alterations in hepatic deposition of nutrients, and impaired whole-body glucose disposal, were likely explained by the different dietary fat sources, namely saturated vs. *trans*-fatty acids.

Hepatic and visceral fat increased in rats consuming a LF-*trans* diet to levels comparable to that achieved with an HF-sat diet. This was related to an equally profound effect on food intake in these two groups despite the LF-*trans* diet being much lower in percentage of fat calories. The processes determining hyperphagia in rats provided diets high in fat are diverse and vary with dietary fat quantity and quality (33,34). However, the mechanisms by which a diet low in fat but proportionately high in *trans*-fatty acids might increase food intake are unknown, but are likely unrelated to malabsorption of calories (35), particularly given that elaidic acid comprised only 4.6% of the total calories consumed. Likewise, the preferential increase in visceral fat with the LF-*trans* diet, an observation also reported by others with diets enriched with *trans*-fatty acids (35,36), is driven by unknown processes that were not explored in this work. The increase in visceral fat in the LF-*trans* group amounted to ~35% as estimated with *in vivo* NMR over 6 weeks, with elaidic acid contributing ~12% to the total lipid pool by 8 weeks on diet. Thus in visceral fat of the LF-*trans* group, it appears that retention of dietary *trans*-fatty acids could explain a significant portion of this depot's increase. Although it is tempting to speculate that the nearly sixfold increase in hepatic lipids might be due to retention of dietary fatty acids, the percentage of elaidic acid in the hepatic lipid pool suggests that other processes contribute to the hepatosteatosis. The decrease in docosahexaenoic acid (22:6n-3) in the LF-*trans* group could be indicative of lower elongase and desaturase activities, as previously described with *trans*- fat diets (37,38), but whether or how this contributed to the marked increase in hepatic lipids remains to be determined.

Increased hepatic lipid content with *trans*-fatty acid feeding is seldom associated with an increased cholesterol content, but more often with increased liver triglycerides (35,39–41). In our study, the percent that cholesterol contributed to total hepatic fat was not increased in the LF-*trans* group. On an absolute basis, however, liver cholesterol was likely increased compared to the LF-sat group given the nearly sixfold increase in hepatic lipids with the LF-*trans* diet. The majority of the increase in hepatic lipids with the LF-*trans* diet, however, is more likely explained by an increase in triglycerides. Triglyceride accumulation with *trans*-fatty acid feeding has been explained, at least in part, by decreased lipid oxidation (42). Chronic intake of dietary *trans*-fatty acids downregulates CPT1 activity, though there is indication in hepatocytes that *trans*-fats upregulate fat oxidation acutely (43,44), likely through increased peroxisomal oxidation (43). Others have reported increased expression of fatty acid synthase and sterol responsive element binding protein, consistent with increased rates of lipogenesis (45). In our study, the increased rates of net glycogen synthesis in the liver of rats consuming the LF-*trans* diet may imply a role for elaidic acid in hepatic *de novo* lipogenic pathways. More work is required to make definitive statements about the extent to which either of these pathways contribute to the increase in hepatic lipids with the LF-*trans* diet used in the present studies. Beyond net hepatic glycogen synthesis, markers of other metabolic pathways to explain the increase in hepatic lipid content were not evaluated.

Unlike in other studies using *trans*-fat diets (45,46), fasting glucose, insulin, free fatty acids, and triglycerides were unchanged in the rats fed the LF-*trans* diet. Insulin-stimulated whole-body glucose disposal was reduced, however, though none of the tissues assessed for R'_g offered an obvious site of the defect. The apparent discrepancy in the LF-*trans* group having no reduction in glucose infusion rates but a significant one in insulin-stimulated glucose disposal may relate to their greater hepatic glycogenesis rates. Conceivably, hepatic glucose disposal was increased in order to sustain the higher rate of net hepatic glycogenesis, effectively masking the LF-*trans* diet effect on whole-body glucose infusion rate. Thus, although the lack of effect on glucose infusion rate in the LF-*trans* group indicates insulin action is normal in these animals relative to the LF-sat group, their reduction in glucose disposal suggests insulin responsive tissues are relatively more insulin resistant. The approach used in this study (R'_g) was unable to ascertain which tissue contributed to the reduced glucose disposal, however, although there were trends for a decrease in glucose disposal into epididymal fat for the LF-*trans* group and the TA for the HF-sat diet.

Because skeletal muscle is the primary site of glucose disposal under the hyperinsulinemic conditions used here, we explored R'_g, IMCL, and DAG content in muscles that differed in oxidative capacity. Consistent with previous work in dexamethasone-treated and Zucker rats (22,23), $IMCL_{TA}$ was increased in the HF-sat group, but was not in the soleus. Though not significant, R'_g was also lower in the TA in the HF-sat group. On the other hand, there was no effect on IMCL in either muscle type in the rats fed the LF-*trans* diet, and R'_g was virtually unchanged from that in the LF-sat group. These findings suggest first, that the

insulin resistance brought about with the HF-sat diet occurs through mechanisms similar to those in more severe models of insulin resistance, i.e., dexamethasone-treated and Zucker rats (22,23), possibly via effects of the high fat diet on mitochondrial function (7). Secondarily, despite the increase in soleus DAG content in the LF-*trans* group, there was no indication of insulin resistance in this tissue, in contrast to previous observations using a dramatic lipid load to the muscle to induce insulin resistance (31). Taken together, these data suggest that some tissue besides skeletal muscle is contributing to the decrease in insulin-stimulated glucose disposal. There is some indication that *trans*-fatty acid feeding reduces insulin action at adipose tissue with respect to both glucose uptake and lipolysis (46,47), likely through effects on membrane fluidity (48,49). Indeed, though highly variable, R'_g in epididymal fat in the LF-*trans* group was ~40% reduced from that in the LF-sat group.

In the soleus, β-hydroxybutyrate concentrations were decreased in the LF-*trans* group, and were nearly so in the HF-sat group. Because the soleus is comprised primarily of slow-twitch, highly oxidative fibers, β-hydroxybutyrate may conceivably be a marker of changes in fuel selection in this muscle. Likewise, the lower glutamate and glutamine levels in both the TA and soleus of LF-*trans* rats could be indicative of a switch in fuel preference as well (50). In particular with the LF-*trans* diet, the higher tissue concentrations of elaidic acid, because it is a poor substrate for carnitine palmitoyltransferase I (43), may force a switch in fuel preference in tissues of high oxidative capacity such as the soleus. More detailed work, most likely under a setting of increased energy expenditure (i.e., exercise), are required to better understand the dynamics between these metabolites and tissue fuel selection in the presence of dietary *trans*-fatty acids.

In this study, we observed profound metabolic responses to a low-fat diet enriched with *trans*-fatty acids that were associated with hyperphagia, increased hepatic and visceral fatness, and diminished whole-body glucose disposal, all hallmarks of the metabolic syndrome. Although the percentage of dietary fat in the LF-*trans* diet was low with respect to calories (~4–5%), elaidic acid made up nearly half of the fat consumed, making this LF-*trans* diet somewhat unphysiologic. Thus, any extrapolation to disease should be tentatively made. Despite this, many of the metabolic maladies observed with the HF-sat diet were mimicked by the LF-*trans* diet. Indeed, the caloric value provided by the LF-*trans* diet from *trans*-fatty acids was somewhat comparable to the daily *trans*-fats consumed by US adults (10). Overall, these findings suggest *trans*-fatty acids are uniquely handled by liver, adipose, and muscle in such a way as to induce mild insulin resistance by, as of yet, undiscovered mechanisms.

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