

Trans Fat Diet Induces Abdominal Obesity and Changes in Insulin Sensitivity in Monkeys

Kylie Kavanagh, Kate L. Jones, Janet Sawyer, Kathryn Kelley, J. Jeffrey Carr, Janice D. Wagner, and Lawrence L. Rudel

Abstract

KAVANAGH, KYLIE, KATE L. JONES, JANET SAWYER, KATHRYN KELLEY, J. JEFFREY CARR, JANICE D. WAGNER, AND LAWRENCE L. RUDEL. Trans fat diet induces abdominal obesity and changes in insulin sensitivity in monkeys. *Obesity*. 2007;15:1675–1684.

Objective: There is conflicting evidence about the propensity of trans fatty acids (TFAs) to cause obesity and insulin resistance. The effect of moderately high intake of dietary monounsaturated TFAs on body composition and indices of glucose metabolism was evaluated to determine any pro-diabetic effect in the absence of weight gain.

Research Methods and Procedures: Male African green monkeys (*Chlorocebus aethiops*; $n = 42$) were assigned to diets containing either *cis*-monounsaturated fatty acids or an equivalent diet containing the *trans*-isomers (~8% of energy) for 6 years. Total calories were supplied to provide maintenance energy requirements and were intended to not promote weight gain. Longitudinal body weight and abdominal fat distribution by computed tomography scan analysis at 6 years of study are reported. Fasting plasma insulin, glucose, and fructosamine concentrations were measured. Postprandial insulin and glucose concentrations, and insulin-stimulated serine/threonine protein kinase (Akt), insulin receptor activation, and tumor necrosis factor- α concentrations in subcutaneous fat and muscle were measured in subsets of animals.

Results: TFA-fed monkeys gained significant weight with increased intra-abdominal fat deposition. Impaired glucose

disposal was implied by significant postprandial hyperinsulinemia, elevated fructosamine, and trends toward higher glucose concentrations. Significant reduction in muscle Akt phosphorylation from the TFA-fed monkeys suggested a mechanism for these changes in carbohydrate metabolism.

Discussion: Under controlled feeding conditions, long-term TFA consumption was an independent factor in weight gain. TFAs enhanced intra-abdominal deposition of fat, even in the absence of caloric excess, and were associated with insulin resistance, with evidence that there is impaired post-insulin receptor binding signal transduction.

Key words: fatty acids, animal models, insulin resistance, diabetes

Introduction

Production of partially hydrogenated vegetable oils containing trans fatty acids (TFAs)¹ was developed because of their low cost, long shelf life, and suitability for commercial frying and transport. Partial hydrogenation, the industrial hardening of edible oils, causes some double bonds to be saturated, whereas others are changed from *cis* to *trans* configuration. The end products typically contain >20 new isomers of oleic and linoleic acids, which can make up 40% or more of the total fat (1). Elaidic acid (t18:1n9) is the major component of industrially produced monounsaturated TFAs (2). Natural sources of TFAs are milk, butter, and beef fat, produced by ruminant bacterial isomerases that can convert the double bonds of dietary fat into a *trans* configuration (3–5). Industrial processes produce much higher amounts of monounsaturated TFAs, which can be found in manufactured products such as margarine, shortenings, and fats used for frying (3).

Received for review July 14, 2006.

Accepted in final form January 5, 2007.

The costs of publication of this article were defrayed, in part, by the payment of page charges. This article must, therefore, be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Address correspondence to Kylie Kavanagh, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157

E-mail: kkavanag@wfubmc.edu

Copyright © 2007 NAASO

¹ Nonstandard abbreviations: TFA, trans fatty acid; T2DM, type 2 diabetes; MS, metabolic syndrome; CIS, diet containing primarily monounsaturated fatty acids in the *cis* conformation; TRANS, diet containing a partial substitution of *cis* fatty acids for *trans* isomers; CV, coefficient of variation; CT, computed tomography; IR, insulin receptor; Akt, serine/threonine protein kinase; TNF- α , tumor necrosis factor- α ; HOMA, homeostasis model assessment; NCEP ATP III, National Cholesterol Education Program's Adult Treatment Panel.

Intake of TFAs is currently estimated at <7% of dietary fat and, on average, 3% of total energy intake (6–9). The Food and Drug Administration has recognized the harmful effects of TFAs, and, as of 2006, a regulation was passed to enforce declaration of TFA content on the nutrition label of conventional foods and dietary supplements. The decision to regulate TFA labeling has been driven by extensive data showing TFA association with cardiovascular disease (10). Before this ruling, the U.S. and Canada did not require manufacturers to reveal the trans content of their products and allowed labeling as “low saturated fat” and “low in cholesterol” regardless of their trans fat content (1).

A diet high in fat content is a well-known risk factor for development of type 2 diabetes (T2DM) (11). The Nurse’s Health Study provides epidemiologic evidence of increasing TFA intake and risk of T2DM (8,12), and smaller studies in obese individuals and animals have shown increased insulin secretion after TFA ingestion (3,13,14). Data suggest that the incidence of T2DM would be reduced by >40% if these oils were consumed in their original, unhydrogenated form (8,12). Conflicting evidence exists, however, with short-term intake of diets containing 5% to 9% TFAs having no adverse effects on insulin sensitivity and glucose metabolism (15,16). Additionally, both the Health Professional’s Follow-up Study and the Iowa Women’s Health Study showed that there was no increase in risk of T2DM with increasing TFA intake when total energy intake, all fats consumed, and body weight were factored into analysis (17,18).

TFA ingestion has been associated with the development of abdominal obesity (19), cardiovascular disease (20), and, in some studies, T2DM (8,12). These conditions exist together in the newly defined metabolic syndrome (MS), which suggests that TFA intake may have an etiologic role in the generation of this syndrome. However, from 1980 to 1998, the average intake of TFAs decreased (5,20), whereas the prevalence of obesity and T2DM is still increasing (21). In adults, T2DM is estimated to reach 5.4% of the population, or 300 million worldwide, in 2025 (22), indicating that quality of dietary fat is not likely to be the sole factor in the pathogenesis of T2DM. The aim of this study was to evaluate the effect of moderately high intake of dietary monounsaturated TFAs over a significant lifespan (~15 years human equivalent) in young healthy adult monkeys on body composition and indices of glucose metabolism to determine any pro-diabetic effect in the absence of weight gain.

The use of a non-human primate permits long-term controlled evaluation of a nutritional intervention in a species that closely resembles humans in respect to fatty acid absorption and metabolism (23). Furthermore, the African green monkey has been shown to develop atherosclerosis as a consequence of a high-fat, cholesterol-supplemented diet in a more similar manner than is true for many other primate species (23,24). The ability to model a chronic complex

disease process such as atherosclerosis, which involves gastrointestinal, vascular, hepatic, and inflammatory physiology, lends support to the selection of this species and the ability to show dietary effects on body composition and insulin sensitivity that would be relevant to people.

Research Methods and Procedures

Adult male African green monkeys (*Chlorocebus aethiops*; $n = 42$; average age, 8 years; range, 4 to 13 years) sourced from St. Kitts island were included in this study that was primarily designed to evaluate dietary influences on atherosclerosis. Monkeys were housed inside, in individual cages, under climate-controlled conditions (70°F to 76°F; 40% to 60% humidity) with 12-hour light and dark cycles. They were assigned to groups, stratified by body weight and plasma lipids, to either diets containing primarily monounsaturated fatty acids in the cis conformation (CIS) or an equivalent diet (TRANS) containing a partial substitution of trans isomers for cis fatty acids. Experimental diets were fed for 6 years. Both diets were supplied at 70 kcal/kg per day, divided into morning and afternoon feedings, and the amount fed throughout the study was based on the body weight recorded at study initiation after equilibration to the laboratory environment had occurred. Body weight was monitored over time by weighing the monkeys no less than every 8 weeks. Calories supplied were calculated to provide maintenance energy requirements and were not intended to promote weight gain. Typically rations were completely consumed within 30 minutes of feeding. Study procedures were approved by the Institutional Animal Care and Use Committee of Wake Forest University.

Experimental diets were made in-house and contained fat as 35% kcal (energy), protein as 17% kcal, carbohydrate as 48% kcal, and cholesterol as 0.4 mg/kcal. The main dietary ingredients are listed in Table 1. Dietary fats were supplied from AC Humko Foods (Memphis, TN) as a TFA-enriched blend derived from nickel-catalyzed partially hydrogenated soybean oil or as an equivalent oleic acid-enriched blend of fatty acids. The dietary fatty acid percentage composition, indicating the degree of enrichment in monounsaturated TFAs, is shown in Table 2. TRANS contained ~8% of energy as TFAs, which is within the upper limit of intake estimates for people. Although high, it is not unrealistic and was chosen to permit detection of effects. TFA content of the CIS diet was <1% of supplied energy (Table 3). Dietary content and composition of fatty acids were checked no less than every 8 weeks by gas chromatography (25). Chromatography was achieved by CP-SelectCB column (Varian, Palo Alto, CA) installed in a HP 5890 series II gas chromatograph equipped with an HP 7673 autosampler (Hewlett-Packard Company, Palo Alto, CA). The carrier gas is hydrogen at 20 pounds per square inch (psi), and

Table 1. Ingredient list for the comparable experimental diets fed for 6 years to the CIS and TRANS groups of monkeys

Ingredient	Amount (g/100g diet)
Wheat flour	35
Fat supplied as either CIS or TRANS blend*	16.7
Dextrin	9.6
Sucrose	10
Casein	9
Lactalbumin	5
Alphacel	7
Hegsted mineral salts mix IV†	5
Vitamin mix‡	2.6
Crystalline cholesterol	0.17

CIS, diet containing primarily monounsaturated fatty acids in the cis conformation; TRANS, diet containing a partial substitution of trans isomers for cis fatty acids.

* AC Humko foods (Memphis, TN).

† Harlan Teklad Laboratory Animal Diets (Madison, WI).

‡ Includes vitamin D3 in corn oil.

chromatograms are plotted and peaks identified and integrated using ChromPerfect Spirit software (Justice Laboratory Software, Denville, NJ).

Monkeys were sedated at study initiation and termination (ketamine hydrochloride, 10 to 15 mg/kg, intramuscularly) for collection of blood samples. Plasma was assayed for fasting insulin (Mercodia, Uppsala, Sweden), glucose (Roche, Basel, Switzerland), fructosamine (Roche), and adiponectin (Linco Research, St. Charles, MO) concentrations in all monkeys. Assays for insulin and adiponectin were done using enzyme-linked immunosorbent assay methodol-

ogy. Insulin had intra-assay and inter-assay coefficients of variability (CVs) of <10% and <5%, respectively. Adiponectin had both intra-assay and inter-assay CVs of <5%. Fructosamine and glucose assays were both colorimetric. Glucose measures had both intra-assay and inter-assay CVs of <5%. Fructosamine concentrations had an intra-assay CV of <15% and an inter-assay CV of <10%.

A subset (*n* = 12) were available at termination for the measurement of postprandial insulin concentrations where monkeys were given access to 50% of their daily caloric allotment for 90 minutes; any remaining food was removed, and blood samples were taken 3 hours later.

Monkeys (*n* = 34) were anesthetized between 68 and 73 months of study for computed tomography (CT) scanning and volumetric assessment of abdominal adipose tissue distribution. Scanning used a 16-slice General Electric Lightspeed Pro CT scanner (General Electric Healthcare, Waukesha, WI). CT scanning of the entire body using 0.625-mm slice collimation was performed, with the adipose volume calculated from the abdominal region as defined by the area between the level of the thoracolumbar junction and S₁ vertebral body. Volumes of intra-abdominal, intramuscular, and subcutaneous fat volumes were calculated as previously described (26). Briefly, the abdominal section had a threshold of -140 to -40 CT units (i.e., Hounsfield units) applied to isolate the fat-containing voxels. Sections were manually traced for the intra-abdominal cavity every 1.5 cm, and the volume of fat voxels in the entire section was calculated. The procedure was repeated for manually tracing total fat (subcutaneous, intramuscular, and abdominal compartments) and tracing the body wall (abdominal and intramuscular compartments), and the individual volumes were calculated.

A randomly selected subset of animals (*n* = 9) was available for skeletal muscle and subcutaneous fat biopsy and measurement of waist circumference at study termination. Biopsies were collected under basal and insulin-stimulated conditions. Regular insulin was infused into a peripheral vein at 40 U/m² per minute for 10 minutes, predicted

Table 2. Fatty acid composition of diet as measured by gas chromatography, showing enrichment in monounsaturated fats and specific increases in 18:1 trans in the TRANS group compared with CIS, chosen to reflect the trend toward diets enriched in canola oil that are more oleate rich

Diet	Dietary fatty acid percentage compositions (w/w)									
	≤12:0	14:00	16:00	18:00	18:1t	18:1c	18:2t	18:2c	18:30	Other
CIS	0.61	1.25	25.67	5.29	0.82	51.07	0.22	12.06	0.6	2.41
TRANS	0.21	1.42	25.16	3.77	20.4	26.57	2.28	14.23	0.25	5.71

CIS, diet containing primarily monounsaturated fatty acids in the cis conformation; TRANS, diet containing a partial substitution of trans isomers for cis fatty acids; w/w, weight to weight.

Table 3. Fatty acid contribution to dietary energy (as percent total energy) showing specific increases in 18:1 trans in the TRANS group as a substitution for 18:1 cis in the CIS group

Diet	Dietary fatty acid percentage of supplied energy									
	≤12:0	14:00	16:00	18:00	18:1t	18:1c	18:2t	18:2c	18:30	Other
CIS	0.21	0.44	9.98	1.85	0.29	17.87	0.08	4.22	0.21	0.84
TRANS	0.07	0.50	8.81	1.32	7.14	9.30	0.80	4.98	0.09	2.00

CIS, diet containing primarily monounsaturated fatty acids in the cis conformation; TRANS, diet containing a partial substitution of trans isomers for cis fatty acids; t, TRANS; c, CIS.

to increase the average circulating insulin concentration to 100 μ IU/mL (27). Insulin delivery was confirmed by measurement of capillary blood glucose from a toe stick (Precision QID Glucometer; Abbott Laboratories, Alameda, CA) and resulted in average glucose reduction of 20 mg/dL. Biopsy samples were frozen in liquid nitrogen and stored at -80°C until analysis. Protein was extracted from biopsy samples and analyzed for insulin receptor (IR) β subunit, total serine/threonine protein kinase (Akt), phosphorylated IR, and phosphorylated Akt according to manufacturer recommendations (Biosource, Camarillo, CA). Tumor necrosis factor α (TNF- α) was measured in protein isolated from fat biopsy tissue by enzyme-linked immunosorbent assay (Biosource, Camarillo, CA). TNF- α concentrations were not detectable in muscle extracts.

Results are presented as the mean \pm standard error. Homeostasis model assessment ratios (HOMAs) were calculated from the product of insulin and glucose (mM/22.5) and used as an indicator of insulin resistance (28). Statistical comparisons were made by one-way ANOVA or analysis of covariance, if indicated, with significance set as $\alpha \leq 0.05$. Data were transformed if parameters did not satisfy normality assumptions before analysis. Non-parametric statistical comparisons were made for data associated with biopsy endpoints. All statistics were generated using Statistica 6 (StatSoft, Tulsa, OK).

Results

Body Composition

Body weight at study initiation was comparable between the groups ($p = 0.28$). The mean body weight for the CIS group was 6.41 ± 0.12 kg, compared with 6.60 ± 0.11 kg for the TRANS group. Over the 6-year study period, body weight stabilized as expected secondary to being fed a caloric allowance aimed at weight maintenance (Figure 1). Despite the controlled feeding regimen, TRANS-fed monkeys still exhibited significant weight gain ($p < 0.05$; Table 4). The moderate but significant increase in weight at study termination (7% gain as opposed to $<2\%$ seen in the CIS

group) was reflected by increases in both intra-abdominal and subcutaneous fat deposition, where TRANS fed monkeys had 33% greater and 29% greater fat volumes, respectively, measured in their abdominal region (Figures 2A and 3; Table 4) at the end of the 6-year study period. Although both fat volume compartments appeared to increase in the TRANS-fed group to a similar magnitude, the ratio of volumes was highly significantly different, indicating that TRANS-fed monkeys deposit more fat intra-abdominally for every cubic centimeter of fat gained than the CIS-fed monkeys, even when body weight was accounted for (Figure 2B; $p = 0.018$). This difference is shown in Figure 3, where monkeys matched on total body weight at study termination showed greater fat surrounding the viscera in the TRANS compared with the CIS subjects.

Measures of Carbohydrate Metabolism

There were no significant differences in the fasting measures of insulin and glucose or the index of insulin sensitivity calculated from these fasting measures (HOMA; Table 5) between the groups at the end of study; however, a non-significant tendency toward higher fasting glucose was noted in the TRANS-fed monkeys ($p = 0.15$). The TRANS-fed monkeys had significantly higher concentrations of fructosamine ($p = 0.002$) than CIS-fed monkeys. Only a subset of monkeys was available for measurement of insulin and glucose concentrations under non-fasted conditions; however, results (Figure 4) showed significant group differences in insulin concentrations. Glucose concentrations were similarly elevated between the groups, consistent with a postprandial state, but the insulin response was exaggerated in the TRANS-fed monkeys, where concentrations were >3 -fold that of CIS ($p = 0.015$). Consistent with postprandial hyperinsulinemia representing an insulin-resistant state, postprandial insulin concentrations were highly associated with fructosamine ($r = 0.71$, $p = 0.009$). A relationship of glycemic control with intra-abdominal fat accumulation is suggested by the loss of significance in group fructosamine differences after addition of intra-abdominal fat as a covariate. Additionally, insulin resistance

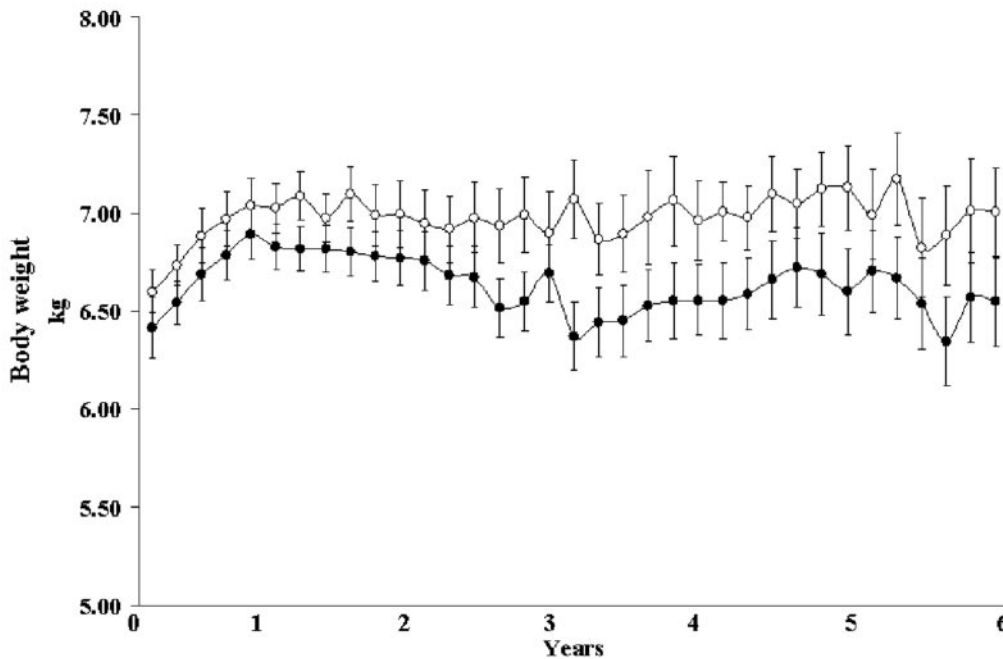


Figure 1: Body weights (mean ± standard error) measured approximately every 2 months in CIS (filled circle) and TRANS (open circle) monounsaturated fat-fed monkeys for a period of 6 years. Diet was supplied in amounts intended to maintain weight (70 kcal/kg/d; 35% of calories supplied as fat). Group body weights at study end showed that TRANS-fed monkeys were, on average, 0.45 kg heavier ($p < 0.05$) and had gained, on average, 7% body weight compared with 1.78% in the CIS group ($p < 0.05$).

index was correlated with intra-abdominal fat volumes (Figure 5; $r = 0.59$, $p < 0.001$).

Insulin Receptor Signaling Efficacy

TRANS-fed monkeys showed significant reduction in muscle Akt activation with insulin stimulation (Table 6), because the phosphorylation amounts were nearly one

quarter that of CIS ($p = 0.02$). No impairment in insulin receptor activation was detected in either fat or muscle, suggesting a post-receptor defect. TNF- α is known to interfere with Akt and insulin-receptor substrate phosphorylation and be elevated in obesity; however, this was not shown to be different between the CIS- and TRANS-fed animals evaluated. Results for fat concentrations are

Table 4. Anthropometric measures in monkeys after 6 years of feeding CIS or TRANS monounsaturated diets supplied in amounts calculated to supply the energy required (70 kcal/kg/d) to maintain body weight

		N		CIS mean (SE)	TRANS mean (SE)	p
		CIS	TRANS			
Body weight at study end	kg	20	21	6.55 (0.20)	7.00 (0.27)	0.049*
Body weight change from study initiation	%	20	21	1.78 (1.95)	7.20 (2.70)	0.049
Waist circumference	cm	3	6	42.50 (2.01)	43.50 (5.20)	0.420
Intra-abdominal fat volume	cm ³	17	17	178.41 (38.94)	237.60 (43.11)	0.110
Subcutaneous fat volume	cm ³	17	17	138.07 (28.59)	177.46 (42.71)	0.330
Intramuscular fat volume	cm ³	17	17	46.40 (12.05)	47.55 (10.73)	0.480
Intra-abdominal:subcutaneous fat volume		17	17	1.36 (0.09)	1.67 (0.14)	0.018*

CIS, diet containing primarily monounsaturated fatty acids in the cis conformation; TRANS, diet containing a partial substitution of trans isomers for cis fatty acids; SE, standard error.

* p values reflect comparisons between groups, with body weight used as a covariate in analysis.

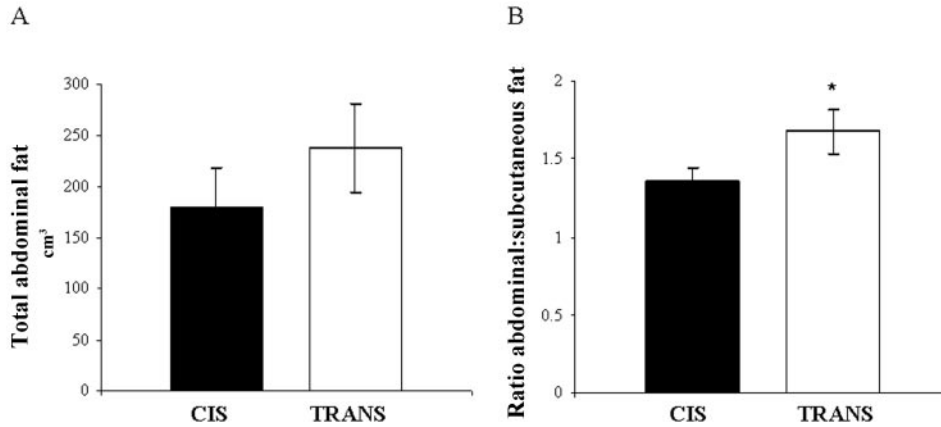


Figure 2: Total abdominal fat volumes (A) and ratio of abdominal:subcutaneous fat volumes (B) measured from CT scans in CIS (black bars) and TRANS (white bars) monounsaturated fat-fed animals. After 6 years of diet consumption of a high-fat diet fed at maintenance requirements (70 kcal/kg/d; 35% of calories supplied as fat), TRANS-fed monkeys had a tendency for a greater volume of abdominal adipose tissue ($p = 0.11$), but as they gained more total fat, they deposited more of it intra-abdominally. The ratio of abdominal:subcutaneous fat was significantly higher (analysis of covariance, $p = 0.02$, adjusted for body weight) in TRANS-fed monkeys.

reported (Table 6) because muscle concentrations of TNF- α were too low to quantify.

Discussion

The major findings of this study showed that, in the absence of caloric excess, TFA induces greater weight gain over time, with enhanced intra-abdominal deposition of fat between the two groups as measured at study termination. There was evidence of impaired insulin sensitivity in the TFA group associated with abdominal obesity and reductions in insulin signal transduction efficiency at the post-receptor binding level compared with monkeys fed the unmodified fat diet at study end. The TFA diet models the trends seen in fats available in grocery stores, which have

become more oleate rich and less TFA rich as canola oil has been increasingly substituted for partially hydrogenated soybean oil. Therefore, a comparison of cis- and trans-monounsaturates better represents the shift in the food fat composition that is already occurring in the U.S. The trans fat used in this study was partially hydrogenated soybean oil, which constitutes the major source (80% to 90%) of TFAs in the American diet (9).

The National Cholesterol Education Program's Adult Treatment Panel (NCEP ATP III) has identified the MS as a clustering of the metabolic complications of obesity (29). MS has significant public health implications because it is associated with a 6-fold increase in risk of developing T2DM (30). Diabetes is the fifth leading cause of death by

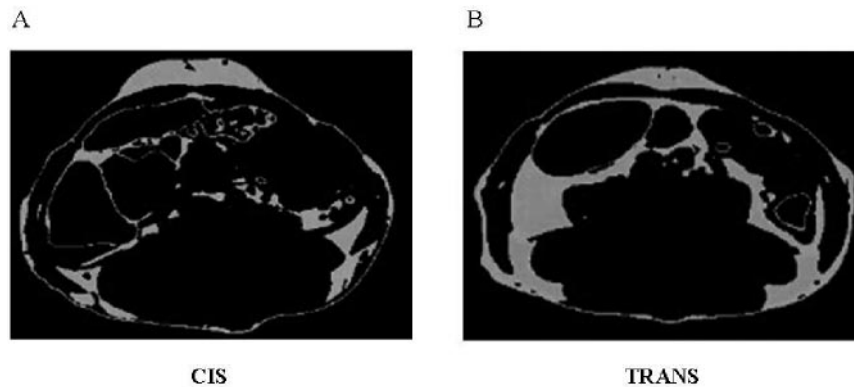


Figure 3: A representative example of abdominal fat distribution from CT scanning of CIS (A) and TRANS (B) monounsaturated fat-fed monkeys that were matched on total body weight. CT scanning was performed at the end of the study to calculate fat volumes from the entire abdomen after application of thresholds to determine adipose tissue.

Table 5. Measures of carbohydrate metabolism in monkeys fed CIS and TRANS monounsaturated fat diets supplied in amounts calculated to maintain body weight

		N	CIS mean (SE)	TRANS mean (SE)	p*
		CIS/TRANS			
Fasting insulin	μIU/mL	21/20	28.15 (4.35)	33.14 (4.77)	0.22
Fasting glucose	mg/dL	21/20	69.51 (2.61)	75.77 (5.37)	0.15
Fructosamine	mM	21/20	168.35 (3.71)	213.83 (14.72)	0.002
HOMA		21/20	5.26 (0.93)	6.50 (1.26)	0.15

CIS, diet containing primarily monounsaturated fatty acids in the cis conformation; TRANS, diet containing a partial substitution of trans isomers for cis fatty acids; SE, standard error; HOMA, homeostasis model assessment. Samples were collected at study termination, after 6 years of experimental diet consumption.

* p values reflect comparisons between groups, with body weight and baseline values used as a covariate in analysis.

disease and the leading cause of permanent disability in the U.S., accounting for more than one half of the national health care expenditures (31). The age-adjusted rate for NCEP-defined MS in the U.S. population is currently 23.7% (30). With the current increasing trend in obesity and diabetes prevalence, these costs are predicted to increase, making study into contributing factors to MS imperative (21).

Our data signify that TFAs are an independent factor in weight gain and abdominal fat distribution, both of which are linked to MS. Our results are consistent with the NCEP ATP III analysis in that they both have shown a significant correlation of insulin resistance and central adiposity (30).

No other study has reported that an increase in TFA composition in diet, without increasing total caloric intake,

can cause increased weight and abdominal fat deposition. Even though the increase in intra-abdominal fat did not reach statistical significance, the 33% gain in intra-abdominal fat volume is biologically significant. Semi-quantitative food-frequency questionnaires have suggested that increasing energy intake by 2% from TFAs, in substitution for carbohydrates and other fats, would be associated with a 0.77-cm waist gain over a 9-year period (19). Our results are consistent with this estimation and eliminate the inaccuracies that may result from survey methods. The lipogenic effect of TFAs is unknown, but interference with essential fatty acid desaturation and elongation and increases in fat cell size have been seen in rodent studies (32). It has been

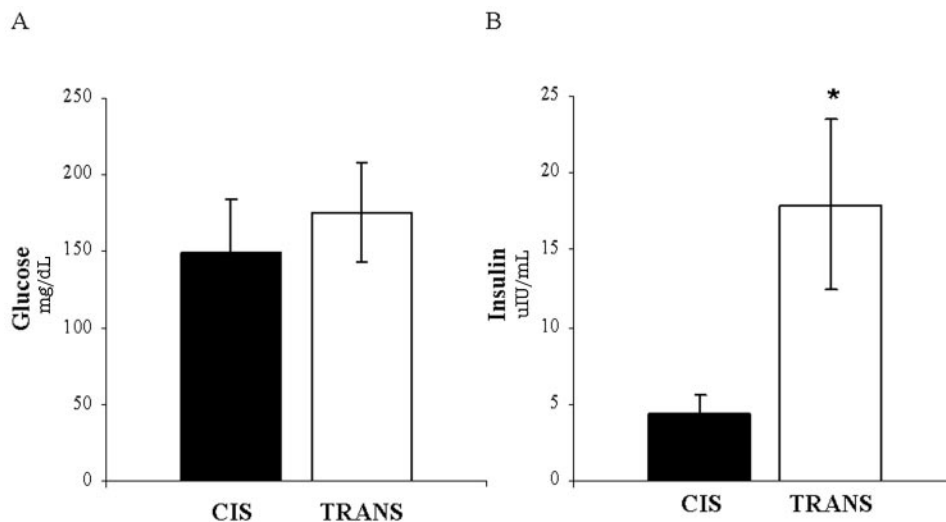


Figure 4: Postprandial glucose (A) and insulin (B) concentrations in a subset of monkeys available for sampling. CIS (black bars) and TRANS (white bars) groups have similar postprandial glucose increases; however, insulin responses are significantly greater in the TRANS monounsaturated fat-fed group, suggestive of insulin resistance.

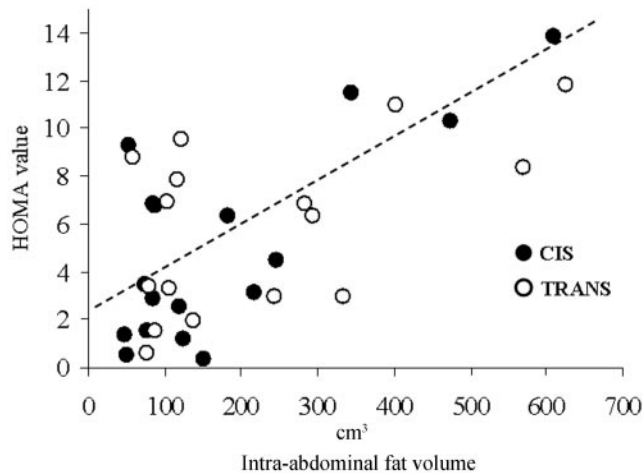


Figure 5: Scatterplot of intra-abdominal fat volumes and their association with HOMA values, a calculated index of insulin resistance from fasting insulin and glucose measures. The association is significant ($R = 0.59$, $p = 0.009$) across the study population, with CIS (filled circle) and TRANS (open circle) individuals indicated.

hypothesized that this interference alters the skeletal-muscle phospholipid composition that leads to insulin resistance and obesity (33).

Impaired glucose tolerance can be defined by post-glucose challenge 2-hour concentrations of 140 mg/dL. This leads to higher postprandial plasma glucose concentrations and is a known risk factor for the development of diabetes (34). Fructosamine is predominantly a measure of glycated plasma albumin, along with other circulating plasma pro-

teins, and is indicative of the average glucose concentration to which these proteins are exposed. Fructosamine concentrations were significantly elevated with TFA feeding, which may suggest impairment of postprandial glucose disposal, because fasting glucose concentrations were comparable. The pattern for postprandial glucose concentrations in the TFA monkeys to be higher on average, although not reaching statistical significance, seems consistent with our observation of significantly elevated fructosamine concentrations. This abnormal handling of glucose is a result of insulin resistance as evidenced by significant postprandial hyperinsulinemia. The postprandial hyperinsulinemia seen is consistent with acute TFA effects observed in short-term studies (3,13,14). Furthermore insulin resistance contributes to hyperinsulinemia, secondary to the increased intra-abdominal adipose tissue, which is anatomically positioned close to the liver, implying greater flux of hepatic non-esterified fatty acids. This, in turn, could interfere with glucose oxidation and hepatic extraction of insulin (13,14,35).

It has been shown that higher fasting plasma glucose levels within the normal glycemic range constitutes an independent risk factor for T2DM among young men (36). Acknowledging this as a risk factor can promote early diagnosis of pre-diabetes and initiate efforts to reverse or delay its onset (34). Trends toward increased fasting glucose concentrations in TFA-fed animals and visceral adiposity were seen in this study. According to Tirosh et al. (36), these factors together enhance the risk of developing T2DM in a healthy male population.

Abdominal obesity is associated with insulin resistance and abnormal post-insulin receptor binding signal transduc-

Table 6. Insulin receptor activation, post-receptor Akt activation, and adipose TNF- α concentrations in insulin-stimulated muscle and subcutaneous fat samples collected from a subset of CIS ($n = 6$) and TRANS ($n = 3$) monounsaturated fat-fed monkeys at study end

		CIS mean (SE)	TRANS mean (SE)	<i>p</i>
Muscle insulin stimulated Akt phosphorylation	pg/mL/mg protein	22.4 (9.85)	5.86 (1.95)	0.020
Muscle insulin stimulated IR phosphorylation	U/mL/mg protein	0.22 (0.027)	0.19 (0.013)	0.30
Subcutaneous fat insulin stimulated Akt phosphorylation	pg/mL/mg protein	5.46 (1.66)	3.94 (1.85)	0.220
Subcutaneous fat insulin stimulated IR phosphorylation	U/mL/mg protein	0.16 (0.02)	0.18 (0.02)	0.300
Subcutaneous fat TNF- α	pg/mL/mg protein	10.38 (2.35)	6.36 (2.21)	0.300

Akt, serine/threonine protein kinase; TNF- α , tumor necrosis factor- α ; CIS, diet containing primarily monounsaturated fatty acids in the cis conformation; TRANS, diet containing a partial substitution of trans isomers for cis fatty acids; SE, standard error; IR, insulin receptor. Results indicate that insulin receptor activation is normal; however, post-receptor activation of Akt, and presumably GLUT4 translocation to allow circulating glucose disposal, are impaired in muscle.

tion. Akt is central in connecting signals from insulin receptor phosphorylation to the transport of glucose into the cell through glucose transporter-4 translocation to the cell membrane (37). Our results suggest that post-insulin receptor activation of Akt is impaired in muscle of monkeys fed a high-TFA diet. These data are consistent with the theory that defective Akt signaling is pivotal in the development of insulin resistance (37,38). The significant impairment of Akt in muscle from TFA-fed monkeys contributes to this theory and is worth noting even in our small sample size. Decreased Akt activation, presumably causing decreased glucose import into muscle, may lead to an increase in circulating glucose concentrations and, over time, fructosamine levels, because skeletal muscle glucose uptake is the main determinant of whole body glucose disposal and insulin sensitivity.

TNF- α , an inflammatory adipokine, interferes with Akt phosphorylation through the production of ceramides (39). Limited data from this study suggest that the TFA-associated reduction in Akt activation was not through TNF- α , because TNF- α did not differ between diet groups. However, in this study, we did not expect to detect group differences in TNF- α because monkeys were not fed to induce obesity. Expanding adipocytes secrete more TNF- α , which leads to release of interleukin-6 and monocyte chemoattractant protein 1 from preadipocytes, endothelial cells, and resident macrophages. This results in significant recruitment of more macrophages and synergy in the release of inflammatory mediators and the induction of a pro-inflammatory state (40). TNF- α concentrations usually increase with obesity because there are more macrophages in adipose tissue as it accumulates (41). TNF- α has been proposed as a link between adiposity and the development of insulin resistance, because the majority of T2DM patients are obese and show increased TNF- α expression in fat cells (39,41,42). Furthermore, obese mice lacking TNF- α function have shown protection for developing insulin resistance (39).

This study was unique in that it directly compared the effects of controlled long-term TFA consumption with an equivalent cis-monounsaturated fatty acid diet in a relevant non-human primate model. The TFA consumption caused a 4-fold greater body weight gain despite being fed an individualized weight maintenance diet. Another strength of this study was the accurate body compositional analysis by CT, which allowed precision in determining fat depots. Most nutritional studies use surrogates for adiposity such as BMI and waist circumference. The TFA diet seemed to result in 30% more intra-abdominal fat deposited for every cubic centimeter of fat gained. Furthermore, the increase in intra-abdominal fat was significantly associated with insulin resistance (HOMA and fructosamine).

Several limitations arose throughout the study because the primary goal of the study design was to evaluate atherosclerosis (data to be presented elsewhere). Therefore, not all samples were collected appropriately for the measurement of insulin, and the entire study population was not available for every measurement. Our conclusions could have been strengthened by more sensitive measurements of carbohydrate metabolism such as glucose tolerance testing, hyperinsulinemic-euglycemic clamp methodology, or measuring glycated hemoglobin percentages, which indicate longer-term glycemic control (3 months compared with the 2 weeks that is estimated by fructosamine concentrations).

In conclusion, even in the absence of caloric excess and only very moderate gains in weight, the inclusion of TFA in the diet enhances abdominal obesity and induces abnormalities in glucose metabolism. Although much attention has been drawn to the adverse effects of TFAs on cardiovascular risk factors, little has been emphasized about the effects of consumption on the current “epidemic” of diabetes. The public health significance of TFA-rich diets and its potential contributions to T2DM and MS support the need for labeling requirements of restaurant foods, particularly fast food, where large amounts of TFAs are used in food preparation.

Acknowledgment

This work was supported by NIH Grant HL24736.

References

1. **Ascherio A, Willett WC.** Health effects of trans fatty acids. *Am J Clin Nutr.* 1997;66:1006S–10S.
2. **Mensink RP.** Metabolic and health effects of isomeric fatty acids. *Curr Opin Lipidol.* 2005;16:27–30.
3. **Christiansen E, Schnider S, Palmvig B, Tauber-Lassen E, Pedersen O.** Intake of a diet high in trans monounsaturated fatty acids or saturated fatty acids. Effects on postprandial insulinemia and glycemia in obese patients with NIDDM. *Diabetes Care.* 1997;20:881–7.
4. **Hunter JE, Applewhite TH.** Reassessment of trans fatty acid availability in the US diet. *Am J Clin Nutr.* 1991;54:363–9.
5. **Ascherio A, Willett WC.** Metabolic and atherogenic effects of trans fatty acids. *J Intern Med.* 1995;238:93–6.
6. **Mozaffarian D, Pischon T, Hankinson SE, et al.** Dietary intake of trans fatty acids and systemic inflammation in women. *Am J Clin Nutr.* 2004;79:606–12.
7. **Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC.** Trans-fatty acids intake and risk of myocardial infarction. *Circulation.* 1994;89:94–101.
8. **Salmerón J, Hu FB, Manson JE, et al.** Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr.* 2001;73:1019–26.
9. **Emken EA.** Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. *Annu Rev Nutr.* 1984;4:339–76.
10. **Stender S, Dyerberg J.** Influence of trans fatty acids on health. *Ann Nutr Metab.* 2004;48:61–6.

11. **Sanders TA.** High- versus low-fat diets in human diseases. *Curr Opin Clin Nutr Metab Care.* 2003;6:151–5.
12. **Hu FB, van Dam RM, Liu S.** Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. *Diabetologia.* 2001;44:805–17.
13. **Lefevre M, Lovejoy JC, Smith SR, et al.** Comparison of the acute response to meals enriched with cis- or trans-fatty acids on glucose and lipids in overweight individuals with differing FABP2 genotypes. *Metabolism.* 2005;54:1652–8.
14. **Alstrup KK, Gregersen S, Jensen HM, Thomsen JL, Hermansen K.** Differential effects of cis and trans fatty acids on insulin release from isolated mouse islets. *Metabolism.* 1999;48:22–9.
15. **Louheranta AM, Turpeinen AK, Vidgren HM, Schwab US, Uusitupa MI.** A high-trans fatty acid diet and insulin sensitivity in young healthy women. *Metabolism.* 1999;48:870–5.
16. **Lovejoy JC, Smith SR, Champagne CM, et al.** Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in health adults. *Diabetes Care.* 2002;25:1283–8.
17. **van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB.** Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care.* 2002;25:417–24.
18. **Meyer KA, Kushi LH, Jacobs DR Jr, Folsom AR.** Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care.* 2001;24:1528–35.
19. **Koh-Banerjee P, Chu NF, Spiegelman D, et al.** Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. *Am J Clin Nutr.* 2003;78:719–27.
20. **Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC.** Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. *Am J Epidemiol.* 2005;161:672–9.
21. **Mokdad AH, Ford ES, Bowman BA, et al.** Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA.* 2003;289:76–9.
22. **Hu FB, Manson JE, Stampfer MJ, et al.** Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med.* 2001;345:790–7.
23. **Rudel LL, Sawyer JK, Parks JS.** Dietary fat, lipoprotein structure, and atherosclerosis in primates. *Athero Rev.* 1991;23:41–50.
24. **Rudel LL, Parks JS, Hedrick L, Thomas M, Williford K.** Lipoprotein and cholesterol metabolism in diet-induced coronary artery atherosclerosis in primates. Role of cholesterol and fatty acids. *Prog Lipid Res.* 1998;37:353–70.
25. **Metcalfe LD, Schmitz AA, Pelka JR.** Rapid preparation of fatty acid esters from lipid gas chromatography analysis. *Anal Chem.* 1966;38:514–5.
26. **Wheeler GL, Shi R, Beck SR, et al.** Pericardial and visceral adipose tissues measured volumetrically with computed tomography are highly associated in type 2 diabetic families. *Invest Radiol.* 2005;40:97–101.
27. **DeFronzo RA, Tobin JD, Andres R.** Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214–23.
28. **Bonora E, Targher G, Alberiche M, et al.** Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care.* 2000;23:57–63.
29. **Grundey SM, Brewer B, Cleeman JI, Smith SC, Lenfant C.** Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004;109:433–8.
30. **Carr DB, Utzschneider KM, Hull RL, et al.** Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes.* 2004;53:2087–94.
31. **Hogan P, Dall T, Nikolov P, American Diabetes Association.** Economic costs of diabetes in the U.S. in 2002. *Diabetes Care.* 2003;26:917–32.
32. **Ostlund-Lindqvist AM, Albanus L, Croon B.** Effect of dietary trans fatty acids on microsomal enzymes and membranes. *Lipids.* 1985;20:620–4.
33. **Simopoulos AP.** Is insulin resistance influenced by dietary linoleic acid and trans fatty acids? *Free Radic Biol Med.* 1994;17:367–72.
34. **American Diabetes Association.** Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2006;29(Suppl 1):S43–8.
35. **Parillo M, Riccardi G.** Diet composition and the risk of type 2 diabetes: epidemiological and clinical evidence. *Br J Nutr.* 2004;92:7–19.
36. **Tirosh A, Shai I, Tekes-Manova D, et al.** Normal fasting plasma glucose levels and type 2 diabetes in young men. *N Engl J Med.* 2005;353:1454–62.
37. **Farese RV, Sajan MP, Standaert ML.** Insulin-sensitive protein kinases (atypical protein kinase C and protein kinase B/Akt): actions and defects in obesity and type II diabetes. *Exp Biol Med.* 2005;230:593–605.
38. **Zdychova J, Komers R.** Emerging role of Akt kinase/protein kinase B signaling in pathophysiology of diabetes and its complications. *Physiol Res.* 2005;54:1–16.
39. **Teruel T, Hernandez R, Lorenzo M.** Ceramide mediates insulin resistance by tumor necrosis factor-alpha in brown adipocytes by maintaining Akt in an inactive dephosphorylated state. *Diabetes.* 2001;50:2563–71.
40. **Wellen KE, Hotamisligil GK.** Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest.* 2003;112:1785–8.
41. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW.** Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003;112:1796–808.
42. **Xu H, Barnes GT, Yang O, et al.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003;112:1821–30.