A High–*Trans* Fatty Acid Diet and Insulin Sensitivity in Young Healthy Women

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Epidemiological and experimental studies suggest that a diet rich in saturated fat affects insulin sensitivity. Monoenes and dienes that have an usaturated bond with the *trans* configuration (*trans* fatty acids) resemble saturated fatty acids with respect to structure, but no published data are available on the effect of *trans* fatty acids on insulin sensitivity. Therefore, the effects of diets high in *trans* fatty acids (TFA diet) and oleic acid (monounsaturated fat [MUFA] diet) on glucose and lipid metabolism were studied in 14 healthy women. Subjects consumed both experimental diets for 4 weeks according to a randomized crossover study design. Both experimental diet periods were preceded by consumption of a standardized baseline diet for 2 weeks. The diets provided 36.6% to 37.9% of energy (E%) as fat. In the TFA diet, there was 5.1 E% *trans* fatty acids, and in the MUFA diet, 5.2 E% oleic acid, substituted for saturated fatty acids in the baseline diet. A frequently sampled intravenous glucose tolerance test (FSIGT) was performed at the end of the experimental diet periods. Glucose effectiveness (S_G) and the insulin sensitivity index (S_I) did not differ after the two experimental diet periods. There was also no difference in the acute insulin response between the diets. The total cholesterol to high-density lipoprotein (HDL) cholesterol ratio and serum total triglyceride, HDL, and low-density lipoprotein (LDL) triglyceride and apolipoprotein B (apoB) concentrations were higher ($P < .05$) after the TFA diet. In conclusion, in young healthy women, the TFA diet resulted in a higher total/HDL cholesterol ratio and an elevation in triglyceride and apo B concentrations but had no effect on glucose and insulin metabolism compared with the MUFA diet.

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MONOENES AND DIENES that have an unsaturated bond with the *trans* configuration (*trans* fatty acids) are formed concomitantly with saturated fatty acids when vegetable oils or fish oils are hardened by partial hydrogenation. *Trans* fatty acids are also naturally present in the butterfat and meat of ruminants. The main dietary sources of *trans* fatty acids in the Western world are vegetable oil–based margarines, partially hydrogenated shortenings, and cooking oils and foods containing hydrogenated fat. The intake of *trans* fatty acids in the developed countries varies from very low levels to 5% to 6% of total fatty acids.

With respect to the effect on serum lipids and lipoproteins, *trans* fatty acids resemble more saturated fatty acids than the respective monounsaturated or polyunsaturated fatty acids with *cis*-double bonds. Several previous studies have shown that *trans* fatty acids increase the concentration of serum total and low-density lipoprotein (LDL) cholesterol in a dose-dependent manner as compared with unhydrogenated vegetable oils.[1-6] In some studies, a *trans* fatty acid–enriched diet also decreased the concentration of high-density lipoprotein (HDL) cholesterol,[4,6] whereas in other studies, no effect on HDL cholesterol has been found.[2,3,5,7] High dietary intake of saturated fatty acids has been associated with a deterioration of glucose tolerance in healthy subjects and in different patient groups.[8-11] It has been suggested that this effect is mediated by a dietary fat–induced change in the fatty acid composition of structural membrane lipids in skeletal muscle, which in turn would relate to insulin action.[12] In the Swedish population, a high proportion of saturated fatty acids in the muscle membranes was associated with an increased risk to develop non–insulin-dependent diabetes during follow-up evaluation.[13] Since *trans* fatty acids resemble saturated fatty acids with respect to structure, it can be speculated that the effect on glucose metabolism could also be similar. In a recent study, a diet high in *trans* fatty acids was shown to alter postprandial C-peptide and insulin responses compared with a diet high in *cis*-monounsaturated fatty acids.[14] However, there are no previous data on the effect of *trans* fatty acids on insulin sensitivity. The purpose of the present study was to examine the effect of *trans* fatty acids on insulin sensitivity in young healthy women. In addition, serum lipids and lipoproteins and coagulation factors were examined.

SUBJECTS AND METHODS

Subjects

Fifteen healthy women participated in the study. One subject was excluded because of pregnancy during the study. The mean age of 14 subjects who completed the study was 23 ± 3 years (mean ± SD), and the mean body mass index (BMI) was 20.8 ± 2.1 kg/m^2 (range, 18.3 to 26.4). All had normal liver, kidney, and thyroid function tests before the study. None of the subjects were taking medication known to affect lipid metabolism. Eight subjects used low-estrogen oral contraceptives, and two had a hormone-releasing intrauterine device during the study. All subjects had regular eating and exercise habits, and they were asked to maintain their weight and exercise habits unchanged during the study. Subjects provided informed consent to participate in the study. The study plan was approved by the Ethics Committee of the University of Kuopio.

Study Design

All subjects consumed both a high–*trans* fatty acid diet (TFA diet) and a high–oleic acid diet (MUFA diet) for 4 weeks according to a randomized crossover design. Eight subjects started with the TFA diet and six with the MUFA diet. Both experimental diet periods were preceded by consumption of a baseline diet for 2 weeks. The standardized baseline diet approximated the average Finnish diet. Body weight and blood pressure were measured and samples for laboratory analyses (serum lipid, lipoprotein, and apolipoprotein concentrations and coagulation factors) were collected after a 12-hour fast at the beginning and end of each diet period (four times altogether). A frequently sampled intravenous glucose tolerance test (FSIGT) was performed at the end of the experimental diet periods (two times altogether) after a 12-hour fast.

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Diets

Both experimental diets and the baseline diet supplied 36% of energy (E%) as fat, 50 E% as carbohydrate, and 15 E% as protein. The cholesterol content of all three diets was about 25 mg/MJ and fiber content about 3 g/MJ. The fatty acid composition of the baseline diet was 16 E% from saturated, 12 E% from monounsaturated, and 6 E% from polyunsaturated fatty acids. During both the TFA diet and MUFA diet, 5% of energy intake from saturated fatty acids in the baseline diet was replaced by added *trans* fatty acids or oleic acid, respectively.

A *trans* fatty acid–enriched margarine (total *trans* fatty acid content 28.1% of fatty acids, including 22.3% of the *trans* isomer of oleic acid [C18:1t] and 2.4% of the mono-*trans* isomers [C18:2c,t] and 3.4% of the *trans-trans* isomers of linoleic acid [C18:2t,t]) was used as a source of *trans* fatty acids and olive oil as a source of oleic acid. Both diets contained a small amount of sunflower oil; in addition, the MUFA diet also contained a small amount of cocoa butter. During the standardized baseline diet, butter was used as a main source of fat together with olive oil and a small amount of sunflower oil. These vegetable oils were used in addition to the main fat sources to obtain a constant content of linoleic and α-linolenic acids in the diets. Medium-fat dairy products were consumed during all diet periods, except for low-fat milk and cheese in the MUFA diet. Because the baseline diet included milk fat and, as a result, more dietary cholesterol than other fat sources in this study, egg yolk (3 to 6 g/d) was added to the TFA and MUFA diets to keep the intake of dietary cholesterol constant during the whole study. Except for the *trans* fatty acid–enriched margarine and cocoa butter, the diets were composed of common Finnish foodstuffs. The subjects received fat products, vegetable oils, and dairy products free of charge. This together with frequent dietary counseling promoted good compliance.

The energy requirement of the subjects was estimated by 3-day food records kept before the study. The subjects received detailed written instructions about the diets, specifying the number of servings of individual foodstuffs by main food groups (dairy products, cereals, vegetables and roots, fruit and berries, meat and meat products, as well as sugar and sweets) or number of grams (fats). It was stressed that the subjects should retain a stable body weight and unchanged exercise habits during the study.

To control compliance to the diets, the subjects kept 7-day food records (5 weekdays and 2 weekend days) during both experimental diet periods. During the baseline and washout periods, 4-day food records (3 weekdays and 1 weekend day) were kept. The subjects were asked to weigh everything they ate on a digital scale when possible. For situations where weighing would not be possible, the subjects were instructed to estimate portion sizes in household measures. In addition, the subjects collected double portions of everything they ate except for coffee, tea, and tap water on 1 day during both experimental diet periods for analysis of the fatty acid composition of the diets.

The diets were planned and the nutrients in food records were calculated using the Micro-Nutrica dietary analysis program (The Social Insurance Institution, Turku, Finland). The food composition tables were based on values obtained from Finnish food analyses and values from international food composition tables.[15]

In addition to food records and double portions, determination of the fatty acid composition of serum triglycerides was used as an indicator of compliance to the experimental diets during the study.

Serum Lipids and Lipoproteins

To remove the very–low-density lipoprotein (VLDL) fraction, lipoproteins were separated by ultracentrifugation for 18 hours at a density of 1.006 kg/L. High-density lipoprotein (HDL) in the infranatant was separated from LDL by precipitation of LDL with dextran sulfate and magnesium chloride.[16] LDL cholesterol was calculated as the difference between the mass of cholesterol in the infranatant and HDL. Enzymatic colorimetric methods were used for the determination of cholesterol and triglycerides from the whole serum and lipoprotein lipids with commercial kits (Monotest Cholesterol and Triglyceride GPO-PAP; Boehringer, Mannheim, Germany) using an automated instrument (Kone Specific Clinical Analyzer; Kone, Espoo, Finland). The coefficient of variation (CV) between measurements for serum total cholesterol using two different standards was 0.8% to 1.2%, and for total triglycerides 0.9% to 1.2%. The CV for HDL cholesterol was 0.9% to 2.0% (two standards) and for HDL triglycerides 1.3% (one standard).

Serum samples for apolipoprotein A-I (apoA-I) and apoB were stored at −78°C until analysis at the end of the study. Analyses were based on the measurement of immunoprecipitation enhanced by polyethylene glycol at 340 nm. An automated Kone Specific Clinical Analyzer and apoA-I and apoB reagents from Kone Instruments were used in the analyses. The CV for apoA-I within the measurement was 0.6% to 1.0% (two standards), and for apoB 2.2% to 3.0% (two standards).

Lipoprotein(a) and Serum Free Fatty Acids

Apo(a) content in lipoprotein(a) [Lp(a)] was measured using the solid-phase two-site immunoradiometric assay from Mercodia (Uppsala, Sweden). This assay is based on a direct-sandwich principle in which two monoclonal antibodies are directed against separate antigenic determinants on the apo(a) molecule. The assay is calibrated against a highly purified Lp(a) preparation. To transform the concentration of apo(a) to Lp(a), apo(a) concentrations were multiplied by 0.7 and the results are expressed in units per liter. The analytical parameters were validated to meet the criteria as defined by the manufacturer. The method sensitivity is 8.4 U/L (routine standardization at 16.8 to 840 U/L), and levels greater than 840 U/L were reanalyzed after further dilution (measuring range, up to 3,360 U/L). Reasonably low assay imprecision was found: between-assay CVs were 2.5%, 7.1%, 3.5%, and 6.0% at Lp(a) levels of 93.5, 123, 311, and 372 U/L, respectively. The assay is specific to apo(a), and apoB has no measurable cross-reaction. Serum plasminogen up to 5 g/L gives no measurable cross-reaction in the assay. Serum free fatty acids were determined with a turbidometric method and analyzed with the Kone Specific Clinical Analyzer.

Fatty Acid Composition of Serum Triglycerides

For analysis of the fatty acid composition of serum triglycerides, the serum samples were stored at −78°C. Lipids were extracted from 100 μL serum with chloroform-methanol (2:1), and the lipid fractions were separated by solid-phase extraction with an aminopropyl column.[17] The fatty acids of triglycerides were transmethylated with 14% borontrifluoride in methanol. Fatty acid methyl esters were analyzed with a gas chromatograph (HP 5890 Series II; Hewlett-Packard, Waldbronn, Germany) equipped with a fused silica capillary column (SP-2560: 100 m, 0.25-mm ID, 0.25-μm film thickness; Supelco, Bellefonte, PA) for analysis of *trans* fatty acids and with a HP-FFAP capillary column (25 m, 0.20-mm ID, 0.3-μm film thickness; Hewlett Packard) for analysis of other fatty acids. Helium was used as the carrier gas. A more extensive report on the incorporation of *trans* fatty acids in different serum lipid fractions is presented in a separate publication.[18]

Fatty Acid Composition of Double Portions

Double portions were homogenized, and two 100-g samples from each portion were frozen after addition of butyl-hydroxy-toluene extracted with ethanol (1 g/1,000 g food). Samples were stored (−18°C) until all portions were collected. After thawing at +10°C, all samples from the same diet period were pooled. The pooled mass was carefully homogenized, and six aliquots were frozen and stored at −70°C until freeze-dried: From the freeze-dried sample, 50 mg was taken for determination of the fatty acid composition of double portions. The lipids were extracted with dichloromethane-methanol (1:1). The extract

was evaporated in a nitrogen stream, and the lipids remaining in the tube were solved in toluene. The fatty acids were transmethylated and analyzed as the fatty acid composition from the serum sample.

Hemostatic Factors

Blood samples for measurement of hemostatic factors were taken without compression. If the first puncture was not successful, samples were taken from the contralateral arm. All exceptions to the normal procedure such as difficulties in blood collection or preparation of the sample, nausea or fainting, and the use of analgesics by the subject were recorded.

The fibrinogen level was measured with an ACL 300 R coagulometer (Instrumentation Laboratory, Milan, Italy) from the light scattered by the clot during the prothrombin time assay (PT-fibrinogen; Instrumentation Laboratory). A single lot of IL Calibration plasma was used as a standard throughout the study. The intraassay precision of the method is 3.6% and interassay precision 2.3%. The samples were measured in duplicate; the difference between duplicates was required to be within 10% of the mean, or the analysis was repeated using a split sample.[19]

Factor VII coagulant activity (FVII:C) was measured with the one-stage method using rabbit brain thromboplastin (Thromboplastin IS; Baxter Dade, Miami, FL) and human immunodepleted FVII-deficient plasma (Behring, Marburg, Germany). The assays were performed with the ACL 300 R coagulometer. A frozen plasma pool was used as a standard. The intraassay precision of this method is 2.4% and interassay precision 3.9%.[19]

FSIGT

A FSIGT was performed as previously described.[20] First, two intravenous catheters were inserted in the antecubital veins on both arms and the fasting samples were drawn. A glucose dose of 300 mg/kg body weight was administered intravenously as a 50% solution in 1.5 minutes followed by 10 mL 0.9% NaCl solution. Thereafter, a 0.9% NaCl solution was slowly infused until a bolus of 0.03 U insulin/kg was rapidly injected 20 minutes after the glucose dose. NaCl infusion was continued at full speed for 1.5 minutes after the insulin dose. To determine plasma glucose and insulin levels, venous blood samples were collected before the glucose dose (−5 and 0 minutes) and 23 times after the glucose dose (at 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes) via a catheter in the contralateral arm. To arterialize the venous blood, the arm was kept in a 50°C electric pad during the test. The plasma glucose concentration was analyzed by a glucose oxidase method (Glucose Auto & Stat, model GA-110; Daiichi, Kyoto, Japan) and plasma insulin by a radioimmunoassay method (Phadeseph Insulin RIA 100; Pharmacia Diagnostica, Uppsala, Sweden). The data were analyzed by calculating the glucose effectiveness (S_G) and insulin sensitivity index (S_I) with the Minmod program.[21] In addition, the acute insulin response was determined by calculating the area under the insulin curve above the baseline level from 0 to 10 minutes.

Statistical Analysis

The data were analyzed using the SPSS (Chicago, IL) 6.0.1 statistical program.[22] Results from the run-in and washout periods were pooled and are presented as baseline values. The values after both experimental diets were used as the main outcome measures. Before further analysis, normal distribution of the variables was checked with the Shapiro-Wilks test. An ANOVA for repeated measures was performed to assess whether there was any carryover effect. Paired *t* tests were used for two-tailed comparisons, except for analysis of the FSIGT and the triglyceride fatty acid composition, where the Wilcoxon matched-pairs signed-rank test was used. To control the overall α level, Bonferroni adjustment was used. All data are expressed as the mean ± SEM. A *P* value less than .05 was considered statistically significant.

Table 1. Energy Intake, Proportion of Energy Nutrients, and Intake of Dietary Cholesterol and Fiber During the Diet Periods (mean ± SEM, N = 14)

Parameter	Baseline	TFA Diet	MUFA Diet
Energy (kJ)	7,795 ± 375	7,637 ± 390	7,677 ± 382
Fat (E%)	37.2 ± 0.4	36.6 ± 0.6	37.9 ± 0.5
Fatty acids (E%)			
Saturated	15.3 ± 0.2	11.0 ± 0.3	10.9 ± 0.2
Monounsaturated	13.7 ± 0.2	12.5 ± 0.2	18.6 ± 0.2
Polyunsaturated	5.6 ± 0.1	5.2 ± 0.1	5.8 ± 0.2
Trans fatty acids	†	5.1 ± 0.1*	†
Cholesterol (mg/MJ)	25.0 ± 0.6	25.1 ± 1.5	25.4 ± 1.5
Fiber (g/MJ)	3.2 ± 0.2	3.1 ± 0.2	2.9 ± 0.2

NOTE. Data are based on two 4-day food records during the baseline diet and a 7-day food record during TFA diet and MUFA diet.
*Intake from *trans* fatty acid–enriched margarine.
†Not available in the database.

RESULTS

There were no significant changes in body weight during the study (BMI: after baseline diet, 20.8 ± 0.5 kg/m^2; after TFA diet, 20.6 ± 0.5 kg/m^2; after MUFA diet, 20.6 ± 0.5 kg/m^2). Systolic and diastolic blood pressure did not change during the study (data not shown). The goals of the fatty acid composition of the study diets were well achieved. There were also no significant differences in cholesterol or fiber intake during the study. The results of the food records are presented in Table 1. The analyzed fatty acid composition of the diets was comparable with the food record data (Table 2).

Fatty Acid Composition of Serum Triglycerides

The fatty acid composition of serum triglycerides is shown in Table 3. As an indicator of good compliance, the proportions of elaidic acid (C18:1t) and *trans*-octadecadienoate (C18:2t) were significantly higher ($P < .01$) after consumption of the TFA diet compared with the MUFA diet. A similar difference is found when the comparison is made with the baseline diet. As expected, the proportion of oleic acid was highest after the MUFA diet in comparison to the TFA diet ($P < .05$) or baseline diet ($P < .01$).

Table 2. Calculated and Analyzed Fatty Acid Composition of the Diets (% of total fatty acids; mean ± SEM, N = 14)

Fatty Acid	TFA Diet		MUFA Diet	
	Calculated*	Analyzed†	Calculated*	Analyzed†
Saturated‡	32.4	29.8‡	31.0	33.8‡
Monounsaturated	52.3	52.5	52.8	50.9
Oleic§	NA	35.7	NA	44.9
Elaidic (C18:1t)	12.1	15.1	NA	3.8
Polyunsaturated	15.3	17.8	16.3	15.4
Linoleic	13.1	14.9	13.6	12.5
Trans-octadecadienoate (C18:2t)¶	3.1	1.2	NA	0.3

Abbreviation: NA, not available.
*From food records.
†Mean of 7 samples.
‡C14:0-C24:0.
§n-9 + n-7.
¶C18:2c,t + C18:2t,t.

Table 3. Fatty Acid Composition of Serum Triglycerides (mol % of total) After Consumption of the Baseline Diet and Experimental Diets (mean ± SEM, N = 14)

Fatty Acid	Baseline Diet	TFA Diet	MUFA Diet	P§
Myristic (C14:0)	2.89 ± 0.23	2.45 ± 0.22	2.03 ± 0.19†	NS
Palmitic (C16:0)	26.39 ± 0.48	24.24 ± 0.80†	24.66 ± 0.64†	NS
Palmitoleic (C16:1)	5.55 ± 0.25	6.16 ± 0.29*	5.24 ± 0.23	.030
Stearic (C18:0)	3.05 ± 0.15	2.57 ± 0.11*	2.62 ± 0.10*	NS
Oleic (C18:1, n-9)‖	39.14 ± 0.49	37.41 ± 0.66*	43.56 ± 0.75†	.001
Elaidic (C18:1t)	1.95 ± 0.10	5.26 ± 0.37†	1.59 ± 0.10*	.001
Linoleic (C18:2, n-6)	15.44 ± 0.38	16.11 ± 0.57	15.71 ± 0.50	NS
Trans-octadecadienoate (C18:2t)¶	1.08 ± 0.04	2.12 ± 0.11†	0.96 ± 0.05*	.001
γ-Linolenic (C18:3, n-6)	0.29 ± 0.03	0.27 ± 0.04	0.30 ± 0.04	NS
α-Linolenic (C18:3, n-3)	1.15 ± 0.07	1.05 ± 0.08	1.06 ± 0.08	NS
Dihomo-γ-linolenic (C20:3, n-6)	0.17 ± 0.01	0.15 ± 0.02*	0.19 ± 0.02	.016
Arachidonic (C20:4, n-6)	0.80 ± 0.08	0.62 ± 0.04†	0.68 ± 0.06	NS
Eicosapentaenoic (C20:5, n-3)	0.31 ± 0.04	0.20 ± 0.03	0.19 ± 0.02*	NS
Docosapentaenoic (C22:5, n-3)	0.26 ± 0.02	0.23 ± 0.03	0.25 ± 0.03	NS
Docosahexaenoic (C22:6, n-3)	1.53 ± 0.26	1.16 ± 0.22	0.96 ± 0.13*	NS

*$P < .05$, †$P < .01$: *v* baseline diet (*t* test).
§Difference between the experimental diet periods (*t* test).
‖Includes C18:1, n-7.
¶C18:2c,t + C18;2t,t.

Plasma Glucose, Insulin, and FSIGT

Fasting plasma glucose and insulin levels were similar after the TFA diet and MUFA diet. There were also no differences in S_I or S_G after the two diets. In addition, the acute insulin response was very similar after the experimental diet periods (Table 4).

Serum Lipids, Lipoproteins, and Apolipoproteins

There were no significant differences in serum total, HDL, or LDL cholesterol concentrations after consumption of the experimental diets. VLDL cholesterol tended to be higher after the TFA diet compared with the MUFA diet. However, although the absolute concentrations of total and HDL cholesterol did not differ significantly after consumption of the TFA and MUFA diets, the ratio of total/HDL cholesterol was significantly higher after the TFA diet compared with the MUFA diet ($P < .05$). Furthermore, serum total, HDL, and LDL triglyceride concentrations were higher after the TFA diet. The concentration of apoB was also higher after the TFA diet. There were no differences in apoA-I or Lp(a) concentrations between the experimental diet periods (Table 5).

Table 4. Fasting Plasma Glucose and Insulin, Acute Insulin Response at 0 to 10 Minutes, S_I, and S_G After Consumption of the Experimental Diets (mean ± SEM, N = 14)

Parameter	TFA Diet	MUFA Diet	P
Insulin (mU/L)	8.1 ± 0.6	7.4 ± 0.5	.089
Glucose (mmol/L)	5.0 ± 0.1	5.0 ± 0.1	.488
AIR (mU/L · min)	336 ± 40	339 ± 50	.864
S_I ($\times 10^{-4} \cdot min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$)	5.06 ± 0.41	4.76 ± 0.44	.423
S_G (min^{-1})	0.03 ± 0.001	0.03 ± 0.001	.912

Abbreviation: AIR, acute insulin response.

Table 5. Concentrations of Serum Total and Lipoprotein Cholesterol, Total and Lipoprotein Triglycerides, ApoA-I, ApoB, and Lp(a) After Consumption of the Baseline Diet and Experimental Diets (mean ± SEM, N = 14)

Parameter	Baseline	TFA Diet	MUFA Diet	P*
Cholesterol (mmol/L)				
Total	4.64 ± 0.14	4.23 ± 0.17†	4.12 ± 0.16§	.458
HDL	1.49 ± 0.09	1.31 ± 0.07§	1.37 ± 0.09‡	.158
LDL	2.79 ± 0.14	2.64 ± 0.17	2.53 ± 0.13‡	.371
VLDL	0.36 ± 0.04	0.28 ± 0.02	0.22 ± 0.03§	.058
Total/HDL	3.21 ± 0.16	3.33 ± 0.20	3.11 ± 0.17	.018
Triglycerides (mmol/L)				
Total	0.92 ± 0.08	0.90 ± 0.08	0.77 ± 0.07†	.045
HDL	0.20 ± 0.02	0.19 ± 0.02	0.15 ± 0.01§	.014
LDL	0.26 ± 0.02	0.26 ± 0.03	0.22 ± 0.02†	.035
VLDL	0.45 ± 0.06	0.45 ± 0.05	0.40 ± 0.06	.154
Apolipoproteins				
ApoA-I (g/L)	1.36 ± 0.06	1.22 ± 0.05‡	1.25 ± 0.06‡	.449
ApoB (g/L)	0.74 ± 0.03	0.70 ± 0.04	0.65 ± 0.03‡	.028
Lp(a) (U/L)	226 ± 73	220 ± 73	225 ± 72	.679

*Difference after the TFA diet and MUFA diet.
†$P < .05$, ‡$P < .01$, §$P < .001$: *v* baseline diet.

A secondary analysis was made comparing serum lipid, lipoprotein, and apolipoprotein concentrations after the experimental diet periods with concentrations after the baseline diet (Table 5). Compared with the baseline diet, serum total and HDL cholesterol concentrations were reduced by 8.9% and 12.1% after consumption of the TFA diet and by 11.4% and 8.3% after consumption of the MUFA diet, respectively. A significant decrease in LDL cholesterol and VLDL cholesterol was observed only during the MUFA diet (9.2% and 37.5%, respectively). Serum total and lipoprotein triglyceride concentrations were similar to the baseline values after the TFA diet. After the MUFA diet, the concentration of serum total triglycerides was 15.1% lower, HDL triglycerides 26.4% lower, and LDL triglycerides 13.7% lower versus the baseline diet.

A decline in the apoB concentration (12.2%, $P = .001$) was found only during the MUFA diet period compared with the baseline diet. There was a significant reduction in apoA-I concentrations during both the TFA and MUFA diet periods (10.3% and 8.1%, respectively) compared with the baseline concentration.

Coagulation Factors

FVII:C activity and the fibrinogen concentration remained stable during the study period (Table 6).

Table 6. FVII:C Activity and Fibrinogen Concentration After Consumption of the Baseline Diet and Experimental Diets (mean ± SEM, N = 14)

Parameter	Baseline	TFA Diet	MUFA Diet	P*
FVII:C (%)	93.3 ± 4.5	94.5 ± 4.4	94.1 ± 4.9	.877
Fibrinogen (g/L)	3.35 ± 0.09	3.25 ± 0.13	3.22 ± 0.12	.839

*Difference after TFA diet and MUFA diet.

DISCUSSION

In the present study, the effect of *trans* fatty acids on glucose and insulin metabolism, serum lipids and lipoproteins, and clotting factors was evaluated in young healthy females. The experimental diets differed only with respect to fatty acid composition; otherwise, the diets were identical. The dietary goals were well achieved. Between the TFA diet and MUFA diet, the difference in *trans* fatty acid content was 5.1 E%, and for the monounsaturated fatty acids, the difference was 6.0 E%. The changes in the fatty acid composition of serum triglycerides and the fatty acid analysis of the double portions support the data from the food records that the compliance rate in this study was good. The TFA diet in the present study contained 5 E% *trans* fatty acids, which is about five times higher than the habitual intake of *trans* fatty acids in Finland.[23] In addition, the type of *trans* fatty acids in the margarine used corresponds to the reported habitual Finnish intake[24]: approximately 80% *trans* fatty acids as the 18:1t isomer and 20% as the 18:2t isomer.

There are no previous data on the effect of *trans* fatty acids on insulin sensitivity. As to the postprandial glycemic response, *trans* fatty acids increased the insulin and C-peptide response compared with *cis*-monounsaturated fatty acids in non–insulin-dependent diabetics.[14] In the present study, a FSIGT was used to evaluate the effect of *trans* fatty acids on glucose and insulin metabolism. The FSIGT is considered a practical and reproducible method with acceptable intraindividual variation in young healthy subjects.[25] There was no difference between the TFA diet and MUFA diet in any variables derived from the FSIGT in the current study, and there was no difference in fasting plasma glucose or insulin concentrations, although fasting insulin tended to be higher after consumption of the TFA diet ($P = .089$). It can be concluded that the short-term effect of moderate consumption of *trans* fatty acids on glucose and insulin metabolism did not differ from that of oleic acid in healthy young females. However, this does not exclude the possibility that in glucose-intolerant subjects the result might have been different. Furthermore, there are data indicating that both saturated and monounsaturated fat[8,10] or, in some studies, all types of fat[26,27] (with the exception of n-3 fatty acids in rats[26]) could have an adverse impact on glucose and insulin metabolism. However, the data on monounsaturated fat are conflicting, since positive effects on glucose tolerance have also been reported.[28] Moreover, the observed negative associations of monounsaturated fat with glucose tolerance[8,10] could be explained by the strong correlation between the intake of monounsaturated fat and intake of saturated fat in the Western diet. Our study was focused to compare the effects of *trans* fatty acids with those of oleic acid as part of a high-fat diet, and the design does not enable an evaluation of whether the intake of *trans* fatty acids or oleic acid would affect insulin sensitivity when substituted for nonfat constituents such as carbohydrate or protein. Further studies are needed to investigate this aspect.

Partly divergent from many previous studies,[1-6] the effect of the TFA diet did not differ from the MUFA diet with respect to absolute concentrations of serum total and lipoprotein cholesterol, except for VLDL cholesterol, which tended to be higher after consumption of the TFA diet. Furthermore, the apoB concentration was higher after the TFA diet versus the MUFA diet, and also comparable to previous data,[3,29] the total/HDL cholesterol ratio was significantly higher after the TFA diet compared with the MUFA diet. Interestingly, with this group of young healthy females, total, HDL, and LDL triglyceride concentrations were significantly higher after the TFA diet compared with the MUFA diet. This finding is consistent with previous reports from Judd et al[29] and Mensink and Katan,[4] where oleic acid was compared with elaidic acid (C18:1 *trans* isomer). However, in a similar comparison by Sundram et al,[30] no effect on triglyceride concentrations was observed. For total and LDL cholesterol concentrations, *trans* fatty acids have been reported to result in intermediate concentrations between saturated fatty acids and corresponding *cis* fatty acids.[31] Almost identical triglyceride concentrations after the baseline diet (high in saturated fat) and the TFA diet imply that the *trans* fatty acids may totally lack the potential of the corresponding *cis* fatty acids to reduce triglyceride concentrations when substituted for saturated fat.

An increase in the apoB concentration is in line with previous observations[4,7,30] and indicates that the TFA diet resulted in an elevation of apoB-containing particles in the bloodstream. The elevated apoB concentration in the presence of similar LDL cholesterol and only marginally different VLDL cholesterol concentrations could also indicate a reduced lipoprotein particle size. A tendency to a smaller LDL particle size after a hydrogenated margarine–enriched diet versus a corn oil–enriched diet has been reported by Cuchel et al.[32] Partly different results in the current study and in studies that reported an increase in total cholesterol and LDL cholesterol concentrations may be attributable to the moderate amount of *trans* fatty acids used. They could also be related to the distribution or amount of *trans* fatty acids used in the present study. In contrast to prior studies where mainly different C18:1 isomers have been used, there was a combination of 20% C18:2 and 80% C18:1 *trans* isomers in our study. To our knowledge, there are no data on the specific effects of C18:2 *trans* isomers.

The Lp(a) concentration did not change in the present study. This is in line with recent data from Clevidence et al,[33] where neither 3.8 E% nor 6.6 E% enrichment of *trans* fatty acids in the diet affected the Lp(a) concentration. However, there are several studies where a significant increase in Lp(a) concentration has been reported.[5,34,35] The inconsistency in the findings of different studies could be due to factors relating to study design: characteristics of study subjects, length of intervention, etc. The role of genetic factors in determining the Lp(a) concentration of an individual is thought to be strong, and this could also provide one explanation for the divergent results.

In the present study, there was no change in FVII:C activity or the fibrinogen concentration, which is supported by recent data from Norway on partially hydrogenated soybean oil.[36] However, there may still be a harmful effect of *trans* fatty acids on hemostatic variables, since Almedingen et al[36] reported higher levels of antifibrinolytic plasminogen activator inhibitor type 1 antigen and plasminogen activator inhibitor type 1 activity after consumption of a partially hydrogenated soybean oil diet versus a butterfat diet. However, this was not supported by Mutanen and Aro,[37] who reported no effect on plasminogen activator inhibitor type 1 activity or other coagulation factors of a high *trans* fatty acid diet versus a saturated-fat diet mainly containing butterfat.

In conclusion, the TFA diet did not alter insulin sensitivity compared with the MUFA diet. However, the TFA diet resulted in a higher total/HDL cholesterol ratio and elevated apoB and total, HDL, and LDL triglyceride concentrations, suggesting that the lipid profile of the young healthy women changed to a more atherogenic direction even with a moderate increase of *trans* fatty acid content in the diet.

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REFERENCES

1. Anderson JT, Grande F, Keys A: Hydrogenated fats in the diet and lipids in the serum of man. J Nutr 75:388-394, 1961
2. Laine DC, Snodgrass CM, Dawson EA, et al: Lightly hydrogenated soya oil versus other vegetable oils as a lipid lowering dietary constituent. Am J Clin Nutr 35:683-690, 1982
3. Lichtenstein AH, Ausman LM, Carrasco W, et al: Hydrogenation impairs the hypolipidemic effect of corn oil in humans. Hydrogenation, *trans* fatty acids, and plasma lipids. Arterioscler Thromb 13:154-161, 1993
4. Mensink RP, Katan MB: Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol level in healthy subjects. N Engl J Med 323:439-445, 1990
5. Nestel P, Noakes M, Belling B, et al: Plasma lipoprotein lipid and Lp(a) changes with substitution of elaidic acid for oleic acid in the diet. J Lipid Res 33:1029-1036, 1992
6. Zock PL, Katan MB: Hydrogenation alternatives: Effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J Lipid Res 33:399-410, 1992
7. Wood R, Kubena K, O'Brien B, et al: Effect of butter, mono- and polyunsaturated fatty acid–enriched butter, *trans* fatty acid margarine, and zero *trans* fatty acid margarine on serum lipids and lipoproteins in healthy men. J Lipid Res 34:1-11, 1993
8. Feskens EJM, Stengård J, Virtanen SM, et al: Dietary factors determining diabetes and impaired glucose tolerance: A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. Diabetes Care 18:1104-1112, 1995
9. Marshall JA, Shetterly S, Hoag S, et al: Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. The San Luis Valley Diabetes Study. Diabetes Care 17:50-56, 1994
10. Maron DJ, Fair JM, Haskell WL, et al: Saturated fat intake and insulin resistance in men with coronary artery disease. Circulation 84:2020-2027, 1991
11. Uusitupa M, Schwab U, Mäkimattila S, et al: Effects of two high-fat diets with different fatty acid composition on glucose and lipid metabolism in healthy young women. Am J Clin Nutr 59:1310-1316, 1994
12. Storlien LH, Baur LA, Kriketos AD, et al: Dietary fats and insulin action. Diabetologia 39:621-631, 1996
13. Vessby B, Tengblad S, Lithell H: Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 37:1044-1050, 1994
14. Christiansen E, Schnider S, Palmvig B, et al: Effects of postprandial insulinemia and glycemia in obese patients with NIDDM. Diabetes Care 20:881-887, 1997
15. Rastas M, Seppänen R, Knuts L-R, et al: Nutrient Composition of Foods. Helsinki, Finland, Social Insurance Institution, 1992
16. Penttilä IM, Voutilainen E, Laitinen O, et al: Comparison of different analytical and precipitation methods for the direct estimation of high-density lipoprotein cholesterol. Scand J Clin Lab Invest 41:353-360, 1981
17. Ågren JJ, Julkunen A, Penttilä I: Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J Lipid Res 33:1871-1876, 1992
18. Vidgren HM, Louheranta AM, Ågren JJ, et al: Divergent incorporation of dietary *trans* fatty acids in different serum lipid fractions. Lipids 33:955-962, 1998
19. Salomaa V, Rasi V, Pekkanen J, et al: Haemostatic factors and prevalent coronary heart disease; the FINRISK Haemostasis Study. Eur Heart J 15:1293-1299, 1994
20. Bergman R: Toward physiological understanding of glucose tolerance. Minimal model approach. Diabetes 38:1512-1527, 1989
21. Pacini G, Bergman RN: A computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23:112-122, 1986
22. Norusis MJ: SPSS for Windows Base System User's Guide. Release 6.0. Chicago, IL, SPSS, 1993
23. Becker W: Intake of *trans* fatty acids in the Nordic countries. Scand J Nutr 40:16-18, 1996
24. Heinonen M, Lampi A-M, Hyvönen L, et al: The fatty acid and cholesterol content of the average Finnish diet. J Food Composition Analysis 5:198-208, 1992
25. Ferrari P, Alleman Y, Shaw S, et al: Reproducibility of insulin sensitivity measured by the minimal model method. Diabetologia 34:327-330, 1991
26. Storlien LH, Jenkins AB, Chisholm DJ, et al: Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and ω-3 fatty acids in muscle phospholipid. Diabetes 40:280-289, 1991
27. Mayer-Davies EJ, Monaco JH, Hoen HM, et al: Dietary fat and insulin sensitivity in a triethnic population: The role of obesity. The Insulin Resistance in Atherosclerosis Study (IRAS). Am J Clin Nutr 65:79-87, 1997
28. Sarkkinen E, Schwab U, Niskanen L, et al: The effects of monounsaturated-fat enriched diet and polyunsaturated-fat enriched diet on lipid and glucose metabolism in subjects with impaired glucose tolerance. Eur J Clin Nutr 50:592-598, 1996
29. Judd JT, Clevidence BA, Muesing RA, et al: Dietary *trans* fatty acids: Effects on plasma lipids and lipoproteins of healthy men and women. Am J Clin Nutr 59:861-868, 1994
30. Sundram K, Ishmail A, Hayes KC, et al: *Trans* (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. J Nutr 127:514S-520S, 1997 (suppl)
31. Zock PL, Katan MB: Butter, margarine and serum lipoproteins. Atherosclerosis 131:7-16, 1997
32. Cuchel M, Schwab US, Jones PJ, et al: Impact of hydrogenated fat consumption on endogenous cholesterol synthesis and susceptibility of low-density lipoprotein to oxidation in moderately hypercholesterolemic individuals. Metabolism 45:241-247, 1996
33. Clevidence BA, Judd JT, Schaefer EJ, et al: Plasma lipoprotein (a) levels in men and women consuming diets enriched in saturated, *cis*-, or *trans*-monounsaturated fatty acids. Arterioscler Thromb Vasc Biol 17:1657-1661, 1997
34. Almedingen K, Jordal O, Kierulf P, et al: Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp(a) in men. J Lipid Res 36:1370-1384, 1995
35. Mensink RP, Zock PL, Katan MB, et al: Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein(a) levels in humans. J Lipid Res 33:1493-1501, 1992
36. Almedingen K, Seljeflot I, Sandstad B, et al: Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on hemostatic variables in men. Arterioscler Thromb Vasc Biol 16:375-380, 1996
37. Mutanen M, Aro A: Coagulation and fibrinolysis factors in healthy subjects consuming high stearic or *trans* fatty acid diets. Thromb Haemost 77:99-104, 1997