Replacement of Dietary Saturated Fatty Acids by *Trans* Fatty Acids Lowers Serum HDL Cholesterol and Impairs Endothelial Function in Healthy Men and Women

Nicole M. de Roos, Michiel L. Bots, Martijn B. Katan

Abstract—We tested whether *trans* fatty acids and saturated fatty acids had different effects on flow-mediated vasodilation (FMD), a risk marker of coronary heart disease (CHD). Consumption of *trans* fatty acids is related to increased risk of CHD, probably through effects on lipoproteins. *Trans* fatty acids differ from most saturated fatty acids because they decrease serum high-density lipoprotein (HDL) cholesterol, and this may increase the risk of CHD. We fed 29 volunteers 2 controlled diets in a 2×4-week randomized crossover design. The "Trans-diet" contained 9.2 energy percent of *trans* fatty acids; these were replaced by saturated fatty acids in the "Sat-diet." Mean serum HDL cholesterol after the Trans-diet was 0.39 mmol/L (14.8 mg/dL), or 21% lower than after the Sat-diet (95% CI 0.28 to 0.50 mmol/L). Serum low density lipoprotein and triglyceride concentrations were stable. FMD+SD was 4.4±2.3% after the Trans-diet and 6.2±3.0% after the Sat-diet (difference −1.8%, 95% CI −3.2 to −0.4). Replacement of dietary saturated fatty acids by *trans* fatty acids impaired FMD of the brachial artery, which suggests increased risk of CHD. Further studies are needed to test whether the decrease in serum HDL cholesterol caused the impairment of FMD. **(*Arterioscler Thromb Vasc Biol.* 2001;21:1233-1237.)**

Key Words: lipoproteins ■ HDL ■ *trans* fatty acids ■ endothelium ■ arteriosclerosis

When liquid oils are partially hydrogenated to form solid margarines and shortenings, *trans* isomers of fatty acids are formed. In countries such as the United States[1,2] and the Netherlands,[3] *trans* fatty acids (TFAs) constitute 4% to 7% of dietary fat intake. A high intake of TFAs is associated with an increased risk of coronary heart disease (CHD).[4–6] One probable cause is the effect of TFAs on serum lipoproteins. Like saturated fatty acids, TFAs increase the concentration of serum LDL cholesterol.[7,8] Moreover, and unlike saturated fatty acids, TFAs decrease serum HDL cholesterol (HDL-C).[7–11] This might be harmful, inasmuch as there is increasing evidence that HDL-C is inversely related to CHD.[12,13]

We investigated whether the intake of *trans* fat would indeed increase the risk of CHD more than the intake of saturated fat by comparing the effects of these fats on endothelial function, a surrogate cardiovascular end point.[14–16] We assessed endothelial function as flow-mediated vasodilation (FMD) of the brachial artery, because this is a noninvasive measurement that correlates well with known risk factors[17–22] and other markers of CHD.[23–25] Moreover, 2 longitudinal studies show an association between FMD in the past with future CHD events.[26,27] The diets were given for a minimum of 3 weeks, a time period long enough to establish changes in serum lipids[28] and FMD.[21] We hypothesized that FMD would be lower after the diet rich in *trans* fat than after the diet rich in saturated fat because of the expected difference in serum HDL-C.

Methods

Subjects

The Medical Ethical Committee of Wageningen University approved the study aim and design. Each volunteer signed an informed consent form. We recruited 39 nonsmoking men and women and assessed their health by using a questionnaire; we eliminated 1 person because of use of medication, 2 because of missing information, and 1 because of poor veins for venipuncture. All subjects had normal concentrations of serum cholesterol and triglycerides and normal amounts of protein and glucose in their urine. We excluded 2 subjects because we could not obtain clear ultrasound images of their brachial arteries. One other subject withdrew before the start of the study; in the end, 32 subjects were enrolled. They all completed the study.

Study Design

We provided 2 controlled diets for 4 weeks, each in a randomized crossover design. The diets consisted of conventional food items supplemented with special margarines and were given in a 28-day menu cycle. On Mondays through Fridays, subjects came to our dining room and ate a hot meal under our supervision. All other foods (bread; margarine; meat and/or cheese; honey, jam, or sprin-

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From the Division of Human Nutrition and Epidemiology (N.M.d.R., M.B.K.), Wageningen University, Wageningen, the Netherlands; Julius Center for Patient Oriented Research (M.L.B.), University Medical Center, Utrecht, the Netherlands; and The Wageningen Center for Food Sciences (M.B.K.), Wageningen, the Netherlands.

Correspondence to Dr Martijn B. Katan, Division of Human Nutrition and Epidemiology, Wageningen University, Bomenweg 2, 6703 HD Wageningen, Netherlands.

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TABLE 1. Fatty Acid Composition of Margarines Used in Diet Rich in TFAs and Diet Rich in Saturated Fatty Acids

	Margarine, g/100 g Fatty Acid	
Fatty Acid	Trans-Diet	Sat-Diet
Saturated	30.5	63.1
Lauric acid (C12:0)	ND	24.5
Myristic acid (C14:0)	0.1	10.2
Palmitic acid (C16:0)	10.5	17.0
Stearic acid (C18:0)	18.5	7.4
Cis-Monounsaturated	18.6	20.9
Oleic acid (*cis*-C18:1n-9)	8.0	19.9
Trans-Monounsaturated	41.4	0.6
Trans-C18:1	40.9*	0.3
Polyunsaturated	8.7	15.0
Linoleic acid (*cis,cis*-C18:2)	8.2	14.6
Others	1.3	0.6

ND indicates not detected.
*Mainly n-10 (22%), n-9 (20%), and n-11 (17%) isomers.

kles; fruit; milk and/or yogurt) were packed for consumption at home, as was food for the weekends.

Habitual energy intake of the subjects was estimated from a food-frequency questionnaire. We designed menus for 14 levels of energy intake, ranging from 7 to 20 MJ/d, and allocated the subjects to an intake level close to their habitual energy intake. We provided 90% of energy (en%); all this food was weighed out for each subject. We measured body weight twice a week; if body weight changed >1 kg, subjects were switched to a different energy intake level. The remaining 10 en% had to be chosen from a list of low-fat food items and recorded in a diary. Subjects received the diets for 21 to 32 days (mean 27.5 days).

Diets

The experimental diets differed in margarine only (Table 1). The composition of the diets was calculated by using food composition tables[3,29,30] and checked by collecting duplicates of all meals (Table 2). The analyzed values were similar to the calculated composition.

The margarine in the diet rich in TFAs (Trans-diet) was a blend of 70 parts partially hydrogenated soy oil, containing 44% *trans*-C18:1 (Gouda's Glorie, Van Dijk Foods), 14 parts vegetable oil containing 63% linoleic acid and 23% oleic acid (Becel, Unilever), and 16 parts water. The margarine in the diet rich in saturated fat (Sat-diet) was a blend of 60 parts palm kernel fat (Loders Croklaan) and 40 parts commercially available margarine made from a blend of vegetable oils and solid vegetable fats (Blue Band, Van den Bergh BV). Both margarines were produced at NIZO Food Research. The margarines were used as shortenings in bread and cookies, in sauce and gravy, and as a spread. They supplied 62% of fat in the diets; the remaining 38% was mainly derived from meat, cheese and other dairy products, eggs, and salad dressings.

Blood Lipids

We took blood samples after an overnight fast on 2 separate days after day 19 of each diet. All 4 blood samples of each subject were analyzed in duplicate within 1 run. Total cholesterol and triglycerides (Cholesterol Flex and Triglycerides Flex reagent cartridge, Dade Behring) and HDL-C (*Liquid* N-geneous HDL-C assay, Instruchemie BV) were measured, and LDL cholesterol was calculated with the Friedewald formula. The coefficient of variation of 64 duplicate measurements was 0.4% for total cholesterol, 1.5% for triglycerides, and 1.1% for HDL-C.

Brachial Artery Measurements

All subjects had an overnight fast of at least 12 hours before the measurements. We measured FMD of the brachial artery as described by Celermajer et al[22] and Sorensen et al.[31] We used the diameter of the artery at rest and at maximum vasodilation to calculate the percentage increase or FMD. All measurements were performed at end diastole by the use of the R wave of the ECG. The ultrasound images were made by 1 technician with a 7.5-MHz linear array transducer of an Ultramark 9 HDI duplex scanner. All images were stored on super-VHS videotapes for offline analysis.

TABLE 2. Analyzed Composition of the 2 Experimental Diets

Fatty Acid	Trans-Diet	Sat-Diet
Carbohydrate, en%	48.6	45.6
Protein, en%	14.0	13.5
Total fat, en%	37.4	41.0
Saturated	12.9	22.9
Lauric acid (C12:0)	0.3	6.8
Myristic acid (C14:0)	0.8	3.8
Palmitic acid (C16:0)	5.7	7.8
Stearic acid (C18:0)	5.3	3.1
Monounsaturated, total	18.2	8.8
Cis-C18:1	8.4	7.9
Trans-C18:1	9.2	0.3
Total *trans*	9.4	0.4
Polyunsaturated	4.7	6.9
Linoleic acid (*cis,cis*-C18:2)	4.1	5.9
Linolenic acid (*cis,cis,cis*-C18:3)	0.3	0.7
Cholesterol		
mg/MJ	27.0	26.8
mg/d	248.4	253.5
Fiber		
g/MJ	3.2	3.1
g/d	29.4	29.3
Energy		
MJ/d	9.20	9.46
kcal/d	2199	2261

Subjects were made to lie down in a temperature-controlled room (range 20°C to 23°C) with the right arm in 2 arm support cushions. An inflatable cuff was placed around the lower arm. The transducer was held in position at the site of the antecubital crease with a specially developed transducer arm holder (method developed by R. Meijer's group, Vascular Imaging Center, The Julius Center for Patient Oriented Research UMC, Utrecht, the Netherlands).

We first obtained an optimal 2D B-mode ultrasound image of the brachial artery at rest and recorded 3 images to measure the diameter. We then inflated the cuff to 250 mm Hg and kept this pressure constant for 5 minutes to induce ischemia in the forearm and hand. After 5 minutes, the cuff was deflated. The image of the brachial artery was optimized, and changes in the diameter of the artery were recorded during the next 5 minutes. Every 15 seconds, a frozen image was stored on videotape. At the end of the second feeding period, we also measured endothelium-independent vasodilation after a sublingual dose of 400 μg of nitroglycerin.

One reader who was blinded to the treatment read all the images at the Vascular Imaging Center of the University Medical Center in Utrecht. The reader rated the quality of the images from class 1 (perfect) to class 4 (unfit for use). All 32 subjects were measured twice on both diets, so we had 4 measurements per subject. Of these 128 measurements, 24 were rated as perfect, 71 as fair, 26 as marginal, and 2 as unfit. Five measurements were missing. We used only measurements that were rated perfect or fair, which left us with 29 subjects for whom we had observations on both diets. At a mean FMD of 5.3% of the resting diameter, the SD within subjects was 2.6% points, so the corresponding coefficient of variation was 49%. The largest difference in a duplicate FMD measurement was 18.2%

TABLE 3. Concentration of Serum Lipids After 4-wk Consumption of the 2 Diets

	Trans-Diet	Sat-Diet	Difference (95% CI)
Total cholesterol, mmol/L	4.97±0.94	5.34±0.95	−0.37 (−0.24–−0.50)
HDLs, mmol/L	1.48±0.33	1.87±0.45	−0.39 (−0.28–−0.50)
LDLs, mmol/L	3.04±0.80	3.05±0.81	−0.01 (−0.14–0.11)
Triglycerides, mmol/L	0.98±0.41	0.90±0.36	0.08 (−0.04–0.20)

Values are mean±SD. The 29 subjects consumed both diets for 4 weeks in random order. To convert values for total, HDL, and LDL cholesterol to milligrams per deciliter, multiply by 38.67. To convert triglycerides to milligrams per deciliter, multiply by 88.54.

points (FMD 2.6% and 20.8% of the resting diameter); the smallest difference was 0.16% points (measurements were 7.2% and 7.4% of the resting diameter). The coefficient of variation of the resting and maximum diameter was 8%.

Statistical Analysis

We averaged the duplicate measurements in each dietary period and tested for order effects by ANOVA, with diet and order as main effects in the model.[32] Because the order of the 2 diets did not significantly contribute to the model, we then calculated for each subject the difference between treatments. We tested whether these differences were significantly different from zero by the Student *t* test for paired samples. We give 2-sided 95% CIs for the differences.

Results

We analyzed the data of 29 subjects (10 men and 19 women). Their mean (±SD) age was 30±16 years, their mean weight was 69±9 kg, and their mean body mass index was 22.5±2.4 kg/m^2. Prestudy serum cholesterol concentrations were 5.1±1.1 mmol/L, and serum triglycerides were 1.2±0.7 mmol/L.

Body Weight

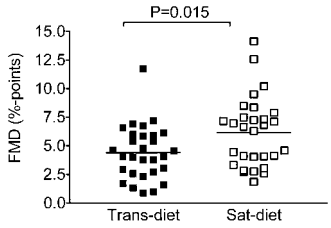
During the 4-week feeding periods, body weight remained basically stable, with mean decreases of 0.4 kg during the Trans-diet and 0.6 kg during the Sat-diet (P=0.43 for difference in change between diets).

Blood Lipids

Serum HDL-C decreased from 1.87±0.46 mmol/L (73.1±17.8 mg/dL) on the diet rich in saturated fats to 1.49±0.33 mmol/L (56.5±12.8 mg/dL) on the diet rich in *trans* fats (Table 3). The decrease was 0.39 mmol/L (95% CI −0.50 to −0.28), or 21%. Serum LDL cholesterol and triglycerides remained stable. The order of the 2 diets hardly affected the change in HDL-C: the mean change was 0.35±0.25 mmol/L in subjects who went from the Trans-diet to the Sat-diet and 0.43±0.32 mmol/L in the subjects who received the diets in the reverse order.

Brachial Artery Measurements

The diameter of the brachial artery at rest was 4.02±0.70 mm on the Sat-diet and 4.08±0.73 mm on the Trans-diet. The maximum diameter was 4.33±0.80 mm on the Sat-diet and 4.19±0.73 mm on the Trans-diet. FMD was 6.2±3.0% on the Sat-diet and 4.4±2.3% on the Trans-diet (P=0.015). Thus, FMD was 1.8% (95% CI −3.2 to −0.4), or 29% lower on the Trans-diet than on the Sat-diet (Figure). The order of the 2 diets hardly affected the results: 15 subjects went from an FMD of 4.8% after the Trans-diet to 6.4% after the Sat-diet,



FMD of the 29 subjects after the diet rich in TFAs (solid squares) and after the diet rich in saturated fatty acids (open squares). The subjects consumed both diets for 21 to 32 days in randomized order.

whereas 14 other subjects went from 5.9% after the Sat-diet to 4.2% after the Trans-diet.

All subjects showed vasodilation after nitroglycerin (range 4.4% to 20.8%). Diet had no effect on nitroglycerin-mediated vasodilation, which was 14.3±3.4% on the Trans-diet and 13.4±5.3% on the Sat-diet (unpaired *t* test, P=0.64).

A decrease in HDL-C went together with a decrease in FMD in 18 of 29 subjects. The correlation between changes in HDL-C and FMD was positive (r=0.12, 95% CI −0.26 to 0.46) but not significant (P=0.55).

Discussion

Consumption of TFAs resulted in lower HDL-C and a smaller FMD than consumption of saturated fatty acids. This might explain the increased risk of cardiovascular disease at high intakes of TFAs. However, whether the impaired vasodilation was attributable to the decrease in HDL-C remains to be determined.

HDL-C, Other Dietary Factors, and Endothelial Function

There is some evidence that changes in HDL-C concentration could change endothelial function. First, higher serum HDL-C is associated with better endothelial function.[24,33,34] This might be due to the proposed antioxidant capacity of HDL,[35] which might prevent oxidation of LDL and therefore prevent adverse effects of oxidatively modified LDL on endothelial function. We know of no other interventions aimed at HDL, but other antioxidants, such as vitamin C,[36,37] were shown to improve FMD. Second, there is ample evidence that reductions in other known risk factors, such as LDL cholesterol[21,25,38] or homocysteine,[39] improve FMD, suggesting that changes in HDL-C could have similar effects. The fact that we did not find a significant correlation between changes in HDL-C and FMD does not rule out a causal relation, because the data were too scarce to correct for possible confounding variables, such as sex and age. On the other hand, a significant correlation would be no proof of a causal relation.

Other factors in the diets might account for the effect on FMD. As shown in Table 2, there was a small difference in linoleic acid between the 2 diets, and studies with rats show that TFAs have stronger effects at low intakes of linoleic acid.[40] Although this might apply to humans, those rat studies were performed at very high intakes of TFAs (20 en%), and the adverse effects could be counteracted with a linoleic acid intake as low as 2 en%. Thus, the 4.1 en% provided by linoleic acid in our 9.2 en% Trans-diet was not low compared

with percentages in the rat studies. Also, we think that the difference in linoleic acid between the Sat-diet and Trans-diet was too small to fully explain the effects seen on FMD. Another factor is vitamin E; the different fat mixtures likely differed by 10 to 20 mg/100 g. However, studies that showed an effect of vitamin E on FMD[41] used much higher doses, and even at these high doses, most studies did not show an effect.[42–44] Last, FMD is impaired in diabetes,[45] and if TFAs and saturated fatty acids have different effects on insulin metabolism, this could have biased the results. However, it is unlikely that fasting serum insulin was different between the 2 diets.[46]

We do not know of studies that compared long-term effects of different fats on FMD. Postprandial effects of saturated and *cis*-monounsaturated fats seem to be similar; they all appear to impair FMD compared with preprandial values or compared with low-fat control meals.[36,47,48] However, some of these studies[36,47] are flawed because the low-fat meals had a higher vitamin C content than the fat-enriched meals, which might have improved FMD.[49] We know of no short-term effects of TFAs on FMD.

Study Limitations

We used a crossover design to eliminate variation due to differences between subjects. The order of the 2 diets was balanced and randomized per subject to eliminate bias due to a systematic drift of the outcome variables over time.[32] Although we did not include a washout period, we did not find a significant order effect on any of the blood lipoproteins or for FMD.

We were interested only in differences between the 2 test diets but not in changes from the habitual diet; therefore, no baseline data were collected. We can only speculate on changes in blood lipoproteins and FMD from baseline. Both experimental diets differed in fat content from habitual diets: the amount of TFAs in the Trans-diet was ≈23 g/d, which is 5-fold higher than the estimated 4.8 g/d for men and 3.8 g/d for women in the Netherlands.[3] The amount of saturated fat in the Sat-diet was 58 g/d, which is also higher than the habitual intake of 42 g/d for men and 32 g/d for women in the Netherlands. Because of the low habitual intake of TFAs, replacing them all by saturated fatty acids would probably hardly improve endothelial function. Conversely, our findings imply that replacing all saturated fatty acids by TFAs could impair FMD and should therefore be discouraged.

The inclusion of women in the present study may have increased the variation in FMD response, because changes in serum estradiol concentrations affect FMD.[50] However, we minimized this variation with 4-week study periods, the length of a menstrual cycle. Compared with the men, the women appeared to respond stronger to the diets, with a 2.3 percentage-unit (95% CI 0.4 to 4.2) smaller FMD on the Trans-diet than on the Sat-diet; in the men, the difference was 0.8 percentage units (95% CI −1.3 to 3.0). However, the number of men was small (n=10); therefore, the present study was not powered to test for sex differences. Further studies with larger numbers of men and women are needed to test for differences in response.

Repeatability of the FMD Measurement

We found a mean FMD of 5.3%. This is somewhat lower than values for healthy volunteers reported by others,[16,36,50] but differences in methodology (eg, the position of the inflatable cuff)[51] could account for this. The variability in FMD was high; we found a coefficient of variation of 49%. This is comparable with the variability found in some studies[52,53] but higher than values reported by others.[23,31,50,54,55] However, in most studies it is unclear how the values for variability have been calculated.

In conclusion, we showed that replacement of saturated fatty acids by TFAs in the diet lowered serum HDL-C and impaired FMD. This suggests that TFAs increase the risk of CHD more than the intake of saturated fats, with similar effects on LDL cholesterol. Further studies are needed to verify whether decreases in HDL-C indeed impair endothelial function and thereby explain the increased risk of CHD at high intakes of *trans* fats.

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