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 States1–2 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 factors17–22 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 lipids28 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-
Table 1. Fatty Acid Composition of Margarines Used in Diet Rich in TFAs and Diet Rich in Saturated Fatty Acids

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Trans-Diet</th>
<th>Sat-Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauric acid (C12:0)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>0.1</td>
<td>10.2</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>10.5</td>
<td>17.0</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>18.5</td>
<td>7.4</td>
</tr>
<tr>
<td>cis-Monounsaturated</td>
<td>18.6</td>
<td>20.9</td>
</tr>
<tr>
<td>Oleic acid (cis-cis-C18:1)</td>
<td>8.0</td>
<td>19.9</td>
</tr>
<tr>
<td>Trans-Monounsaturated</td>
<td>41.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Trans-C18:1</td>
<td>40.9*</td>
<td>0.3</td>
</tr>
<tr>
<td>Polynsaturated</td>
<td>8.7</td>
<td>15.0</td>
</tr>
<tr>
<td>Linoleic acid (cis-cis-C18:2)</td>
<td>8.2</td>
<td>14.6</td>
</tr>
<tr>
<td>Others</td>
<td>1.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

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

Table 2. Analyzed Composition of the 2 Experimental Diets

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Trans-Diet</th>
<th>Sat-Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate, en%</td>
<td>48.6</td>
<td>45.6</td>
</tr>
<tr>
<td>Protein, en%</td>
<td>14.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Total fat, en%</td>
<td>37.4</td>
<td>41.0</td>
</tr>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauric acid (C12:0)</td>
<td>0.3</td>
<td>6.8</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>0.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>5.7</td>
<td>7.8</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>5.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Monounsaturated, total</td>
<td>18.2</td>
<td>8.8</td>
</tr>
<tr>
<td>cis-C18:1</td>
<td>8.4</td>
<td>7.9</td>
</tr>
<tr>
<td>Trans-C18:1</td>
<td>9.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td>9.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Polynsaturated</td>
<td>4.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Linoleic acid (cis-cis-C18:2)</td>
<td>4.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Linolenic acid (cis-cis-cis-C18:3)</td>
<td>0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Cholesterol
- mg/MJ: 27.0 26.8
- mg/d: 248.4 253.5
- g/MJ: 3.2 3.1
- g/d: 29.4 29.3
- Energy
  - MJ/d: 9.20 9.46
  - kcal/d: 2199 2261

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

Blood Lipids
Blood samples were analyzed for cholesterol and triglycerides (Cholesterol Flex and Triglycerides Flex reagent cartridge, Dade Behring) and HDL-C (Liquid N- Junior HDL-C assay, Instrumec 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 and Sorensen et al. We used the FMD of 5.3% of the resting diameter, the SD within subjects was 29 subjects for whom we had observations on both diets. At a mean period, we also measured endothelium-independent vasodilation recorded during the next 5 minutes. Every 15 seconds, a frozen image of the brachial artery was optimized, and changes in the diameter of the artery were recorded 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

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

Values are means±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.

**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.

**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/m². 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).

**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.

**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-C,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-C, 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 used much higher doses, and even at these high doses, most studies did not show an effect. Last, FMD is impaired in diabetes, 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.

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. However, some of these studies are flawed because the low-fat meals had a higher vitamin C content than the fat-enriched meals, which might have improved FMD. 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. 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. 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. 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, but differences in methodology (eg, the position of the inflatable cuff) 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 but higher than values reported by others. 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.

Acknowledgments

This study was financially supported by the Dutch Dairy Foundation on Nutrition and Health. We are indebted to the volunteers who took part in our study. We thank Emer Prof. Dr. C. van der Hoeven from the Erasmus MC for the donation of palm kernel fat. We thank Peter Zock for his advice, Saskia Meyboom for calculating the diets, Els Siebelink for supervising the preparation of the diets, Jan Harryvan for the brachial artery measurements, Rudy Meijer (Radiology Department, University Medical Center Utrecht) for ultrasound training and support, Karin Duijser (Julius Center, University Medical Center Utrecht) for reading the images, Truus Kosmeyer for analysis of the duplicate diets, and all research students for their assistance during the study.

References


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doi: 10.1161/hq0701.092161
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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