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 and the future risk of 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>Margarine, g/100 g Fatty Acid</th>
<th>Trans-Diet</th>
<th>Sat-Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauric acid (C12:0)</td>
<td>ND</td>
<td>30.5</td>
<td>63.1</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>0.1</td>
<td>24.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>10.5</td>
<td>17.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>18.5</td>
<td>7.4</td>
<td>20.9</td>
</tr>
<tr>
<td>cis-Monounsaturated</td>
<td>8.0</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Oleic acid (cis,cis-C18:1-9)</td>
<td>41.4</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Trans-Monounsaturated</td>
<td>40.9*</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Polynsaturated</td>
<td>8.7</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (cis,cis-C18:2)</td>
<td>8.2</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1.3</td>
<td>0.6</td>
<td></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>12.9</td>
<td>22.9</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 trans</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>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/MJ</td>
<td>27.0</td>
<td>26.8</td>
</tr>
<tr>
<td>mg/d</td>
<td>248.4</td>
<td>253.5</td>
</tr>
<tr>
<td>Fiber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/MJ</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>g/d</td>
<td>29.4</td>
<td>29.3</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MJ/d</td>
<td>9.20</td>
<td>9.46</td>
</tr>
<tr>
<td>kcal/d</td>
<td>2199</td>
<td>2261</td>
</tr>
</tbody>
</table>

Diets

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.

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 (Gouda’s Glorie, Van Dijk Foods), 14 parts vegetable oil containing 70 parts partially hydrogenated soy oil, containing 44% total trans fats (Trans-Diet) was a blend of vegetable oils and solid vegetable fats (Blue Band, Van den Bergh BV). Both margarines were produced at NIZO Food Research. The margarine in the diet rich in saturated fat (Sat-diet) was made from a blend of vegetable oils and solids containing 59% oleic acid and 43% palmitic acid (Loders Croklaan, P Tianil). 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 vegetable oil containing 63% linoleic acid and 23% oleic acid (Becel, Unilever), and 16 parts vegetable oil containing 70 parts partially hydrogenated soy oil, containing 44% total trans fats.

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.

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 128 measurements, 24 were rated as perfect, 71 as fair, 26 as marginal, and 2 as unfit.
The diameter of the brachial artery at rest was 4.02 ± 0.46 mm (95% CI 3.89–4.16) and 4.08 ± 0.25 mm at the Trans-diet (95% CI 3.89–4.28). 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, 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, 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, were shown to improve FMD. Second, there is ample evidence that reductions in other known risk factors, such as LDL cholesterol or homocysteine, 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. 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.\textsuperscript{42–44} Last, FMD is impaired in diabetes,\textsuperscript{46} 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.\textsuperscript{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.\textsuperscript{36,47,48} However, some of these studies\textsuperscript{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.\textsuperscript{49} We know of no short-term effects of TFAs on FMD.

\section*{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 differences between subjects. The order of the 2 diets was randomized to eliminate variation due to differences between subjects. 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 comparable with the variability found in some studies\textsuperscript{52,53} but higher than values reported by others.\textsuperscript{23,31,30,54,55} 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.

\section*{Acknowledgments}

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\section*{References}


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