Replacement of Dietary Saturated Fat With *Trans* Fat Reduces Serum Paraoxonase Activity in Healthy Men and Women

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A high intake of saturated fat and of *trans* isomers of unsaturated fat is associated with increased risk of cardiovascular disease. Recently, we found that replacement of saturated fat by *trans* fat in a dietary controlled study with 32 men and women decreased serum high-density lipoprotein (HDL)-cholesterol and impaired endothelial function, suggesting that *trans* fats have stronger adverse effects than saturated fats. To investigate this further, we measured the activity of serum paraoxonase (PON1) in serum samples of the same volunteers after consumption of both diets. PON1 protects lipoproteins from oxidative damage, and higher PON1 activity appears to be related to lower cardiovascular disease risk. PON1 activity (mean ± SD) was 195.9 ± 108.9 U/L after 4 weeks of consuming a diet with 22.9% of energy (en%) from saturated fat and 184.5 ± 99.3 U/L when 9.3 en% from saturated fat was replaced by *trans* fat ($P = .006$). Thus, replacement of dietary saturated fat by *trans* fat not only decreased serum HDL-cholesterol and impaired endothelial function, but also decreased the activity of serum paraoxonase. Whether the changes in serum paraoxonase activity caused the changes in endothelial function needs to be further investigated.

INTAKE OF *trans* and saturated fatty acids is associated with increased risk of cardiovascular disease,[1] probably because of their effects on serum lipoproteins. *Trans* fatty acids increase serum low-density lipoprotein (LDL)-cholesterol to a similar extent as saturated fatty acids. In addition, they decrease serum high-density lipoprotein (HDL)-cholesterol,[2-9] resulting in a undesirably high LDL to HDL ratio. In a recent study,[10] we compared the effects of saturated and *trans* fatty acids on serum HDL-cholesterol and endothelial function in a controlled dietary intervention. Endothelial function was assessed as flow-mediated vasodilation of the brachial artery, a noninvasive marker for coronary artery disease risk.[11] We found that serum HDL-cholesterol was 20% lower after the diet rich in *trans* fatty acids and that flow-mediated vasodilation was impaired. Serum concentrations of LDL-cholesterol and triglycerides remained constant. It is probable that the decrease in HDL-cholesterol paralleled a decrease in the concentration of HDL in serum, because *trans* fatty acids have also been shown to decrease the concentration of apolipoprotein A-I, the major protein of HDL, in serum.[4,8] HDL can inhibit the formation of oxidized LDL in vitro,[12-15] and LDL isolated from subjects with low HDL concentrations appears to be more susceptible to oxidation than LDL isolated from subjects with high HDL concentrations in serum.[16] In subjects whose LDL is more susceptible to oxidation, endothelial function appears to be impaired.[16] Thus, a decrease in HDL might result in impaired endothelial function through its antioxidant effect on LDL. The antioxidant capacity of HDL is thought to be largely,[17-19] although not entirely,[15] affected by paraoxonase-1 (PON1), an esterase that is closely attached to the HDL-particle. PON1 was shown to catalyze hydrolysis of lipid peroxides in oxidized lipoproteins.[17] The activity of serum PON1 is genetically determined,[19] but can be influenced by changes in the diet[20-23] and smoking.[24] There are several indications that serum PON1 activity is associated with cardiovascular disease. First, PON1-knockout mice are more susceptible to atherosclerosis than their wild-type littermates.[25] This is in accordance with several epidemiologic studies showing that cardiovascular disease patients have lower serum PON1 activity or concentrations than healthy controls.[19] In addition, a transgenic mouse model carrying the human PON1 gene showed higher PON1-concentrations and less atherosclerosis than the wild-type after feeding both types of mice an atherogenic diet for 16 weeks.[26] There is also some evidence that PON1 polymorphisms are associated with cardiovascular disease.[19] For example, a prospective study among participants in the Kuopio ischemic heart disease risk factor study showed that men who were MM homozygous for the Met54Leu polymorphism had a more than 3-fold risk of a first myocardial infarction than men who were LL homozygous.[27]

We hypothesized that a diet rich in *trans* fatty acids that lowers serum HDL-cholesterol would also decrease PON1 activity in serum. We tested this in the same study for which we previously reported serum lipoproteins and flow-mediated vasodilation.[10]

SUBJECTS AND METHODS

The study was approved by the Medical Ethics Committee of Wageningen University. Each volunteer signed an informed consent form after oral and written explanation of the study.

We performed a dietary controlled study with a 2 × 4 weeks cross-over design. Subjects were 11 men and 21 women, nonsmoking, aged 18 to 69 years who were free of cardiovascular or kidney disease and who were not taking any drugs known to alter lipid metabolism. At baseline, their mean fasting serum cholesterol was 5.1 ± 1.1 mmol/L and triacylglycerols 1.3 ± 0.6 mmol/L. Serum lipids, flow-mediated vasodilation, and serum paraoxonase activity were measured at the end of the 4-week diet periods. Other details of the study may be found elsewhere.[10,28]

We provided 2 controlled diets that consisted of regular foods

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Table 1. Serum Paraoxonase Activity and Lipoproteins and Lipids of 32 Men and Women After Consuming Two Diets in a Cross-Over Design of 2 × 4 Weeks

	Diet Rich in Saturated Fat	Diet Rich in *Trans* Fat	Difference	*P* Value for Difference
PON-1 activity (U/L)	195.9 ± 108.9	184.5 ± 99.3	11.5 (3.6-19.3)	.006
Total cholesterol (mmol/L)	5.32 ± 0.92	5.01 ± 0.92	0.31 (0.14-0.48)	.0007
HDL-cholesterol (mmol/L)	1.85 ± 0.46	1.49 ± 0.33	0.36 (0.26-0.46)	<.0001
LDL-cholesterol (mmol/L)	3.04 ± 0.78	3.07 ± 0.79	−0.03 (−0.18-0.12)	.66
Triacylglycerols (mmol/L)	0.94 ± 0.51	0.97 ± 0.41	−0.03 (−0.18-0.11)	.64

NOTE. One diet was rich in saturated fatty acids (22.9% of energy); the other rich in *trans* fatty acids (9.3% of energy). Table shows means ± SD for each diet and the mean difference between the 2 diets with 95% CI. To convert mmol/L to mg/dL, multiply by 38.67 for total HDL and LDL cholesterol and multiply by 88.57 for triacylglycerols.

supplemented with special margarine. The foods provided 90% of subjects' energy intake; the remaining 10% of energy was chosen from a list of low-fat food items and recorded in a diary. We used special margarine to obtain a difference in *trans* and saturated fatty acids of about 10% of energy. We collected duplicate diets for analysis of macronutrients. The diet rich in trans fat contained 48.6 en% of carbohydrates, 14.0 en% of protein, and 37.4 en% of fat, with 9.3 en% from *trans* fatty acids and 12.9 en% from saturated fatty acids. The diet rich in saturated fat contained 45.6 en% of carbohydrates, 13.5 en% of protein, and 41.0 en% of fat, with 0.3 en% from *trans* fatty acids and 22.9 en% from saturated fatty acids. Before the study, we estimated the subjects' energy intake by means of a food frequency questionnaire.[29] Body weight was kept stable throughout the study.

Blood was collected after an overnight fast in evacuated collection tubes (Venoject II, Terumo, Leuven, Belgium) from an antecubital vein and allowed to clot for 30 minutes at room temperature. Serum was obtained by centrifugation at 1,187 × g for 10 minutes at 4°C. To eliminate interassay variation, serum was stored at -75°C, and all analyses were performed at the end of the study. PON1 activity was determined using paraoxon as a substrate at pH 8.0.[20] PON1 activity was expressed in U/L, which is equal to 1 μmol of hydrolyzed paraoxon/L serum per minute. The 192 polymorphism of paraoxonase, glutamine (isoform A), or arginine (isoform B) at position 192, was identified by dividing the sodium-chloride-stimulated paraoxon hydrolysis by the hydrolysis of phenylacetate (arylesterase).[20]

We used the average of 2 repeated measurements of serum HDL-cholesterol and 1 measurement of PON1 activity in serum for statistical analysis. Differences between the diets were tested for normality using the Kolmogorov-Smirnov test. [30] We give means, standard deviations, and 95% confidence intervals (CI) of the changes between the 2 diets. In addition, we used the 1-sample *t* test to test whether changes were significantly ($P < .05$) different from zero, and we calculated the Pearson correlation coefficient between changes in HDL-cholesterol and changes in serum paraoxonase activity. We used GraphPad Prism (version 3.0 for Windows) for statistical analyses and graphs (GraphPad Software, San Diego, CA).

RESULTS

PON1 activity was 184.5 ± 99.3 U/L when subjects consumed the diet rich in *trans* fatty acids and 195.9 ± 108.9 U/L when subjects consumed the diet rich in saturated fatty acids ($P = .006$). The difference between the 2 diets was 11.5 U/L (95% CI, 3.6 to 19.3) or 6%. The effect was consistent; in 26 of the 32 subjects, PON1 activity was lower when they consumed the diet rich in *trans* fat than when they consumed the diet rich in saturated fat.

Serum HDL-cholesterol was 1.49 ± 0.33 mmol/L (57.6 mg/dL) after the diet rich in *trans* and 1.85 ± 0.46 mmol/L (71.5 mg/dL) after the diet rich in saturated fatty acids, while other serum lipids remained constant (Table 1). As shown in Fig 1, changes in HDL-cholesterol correlated with changes in PON1 activity ($r = .47$; 95% CI, 0.15 to 0.71; $P = .006$). Subjects with paraoxonase-1 isoform B tended to respond stronger to the change in diet (Table 2). Changes in PON1 activity and changes in the percentage flow-mediated vasodilation were not correlated ($r = -.18$; 95% CI, -0.5 to 0.2, $P > .05$).

DISCUSSION

A diet rich in *trans* fatty acids resulted in a significantly lower PON1 activity towards paraoxon than consumption of a diet rich in saturated fatty acids. This lower enzyme activity might explain the smaller endothelial function we observed previously in the same population after consumption of the diet rich in *trans* fatty acids than after a diet rich in saturated fats.

The size of the effect (6%) was similar to that of alcohol consumption compared with water consumption[20] and about half the difference reported between smokers and nonsmokers.[24] In these studies, the 'high-risk' categories (*trans* fat, water, smoking) had lower serum paraoxonase activities.

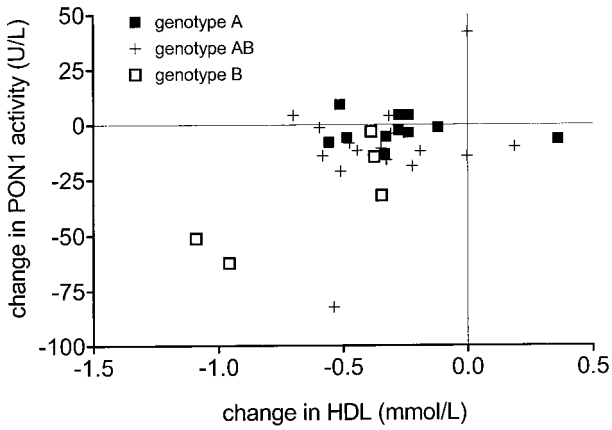

Fig 1. Correlation between changes in HDL-cholesterol and changes in paraoxonase activity in serum of 32 men and women when ~10% of energy from saturated fatty acids is replaced by *trans* fatty acids. Thus, negative values indicate lower values after the diet rich in trans fatty acids. Both diets were consumed by all men and women in a 2 × 4 weeks cross-over design.

Table 2. Serum Paraoxonase Activity (U/L) at the End of the 4-Week Diet Periods for Subjects With PON-1 Polymorphism Isoforms A (glutamine at position 192) and B (arginine at position 192)

Isoform	Diet Rich in Saturated Fat	Diet Rich in *Trans* Fat	No. of Subjects	*P* Value for Difference
A	76.7	74.0	11	.21
AB	234.4	223.5	16	.09
B	335.3	302.6	5	.04

It is still unclear whether the activity of PON1 towards paraoxon is a good marker of the physiologic function of the enzyme.[31] Evidence in favor of this was given by a case-control study that showed that PON1 activity towards paraoxon was significantly lower in 106 male cardiovascular disease patients than in 106 age-matched controls.[32] Also, a study of patients with familial hypercholesterolemia showed that increases in PON1 activity after simvastatin therapy resulted in lower lipid peroxide concentrations in serum.[33] Thus, an increase in the activity of serum PON1 towards paraoxon appears to reflect a physiologic reduction in lipid peroxides and could therefore act as a marker of lipid peroxidation.

We did not find a significant relationship between changes in paraoxonase activity and changes in flow-mediated vasodilation in our subjects. However, our study was not powered to find such a relationship, and the confidence interval for the correlation coefficient was therefore wide. Others have found that serum paraoxonase activity was correlated with endothelial function in 27 patients with angiographically-confirmed atherosclerosis: the percentage constriction after a serotonin provocation test was smaller in patients with higher paraoxonase activity towards paraoxon.[34]

In conclusion, we showed that consumption of *trans* fatty acids, which is related to increased risk of coronary heart disease, decreased serum PON1 activity when compared with consumption of saturated fat. A decreased activity of this enzyme might result in increased concentration of lipid peroxides in serum, which could impair vascular functioning. This effect could be part of the mechanism by which *trans* fatty acids exert adverse effects on coronary heart disease risk.

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