Consumption of *Trans* Fatty Acids Is Related to Plasma Biomarkers of Inflammation and Endothelial Dysfunction[1]

Esther Lopez-Garcia,*[2] Matthias B. Schulze,* James B. Meigs,** JoAnn E. Manson,†‡†† Nader Rifai,‡‡ Meir J. Stampfer,*†‡†† Walter C. Willett,*‡ and Frank B. Hu*†‡

*From Departments of *Nutrition and †Epidemiology, Harvard School of Public Health; **General Medicine Division, Department of Medicine, Massachusetts General Hospital and Department of Medicine, Harvard Medical School; ‡the Channing Laboratory and ††Division of Preventive Medicine, Harvard Medical School; ‡‡Department of Laboratory Medicine, Children's Hospital and Department of Pathology, Harvard Medical School*

ABSTRACT *Trans* fatty acid intake has been associated with a higher risk of cardiovascular disease. The relation is explained only partially by the adverse effect of these fatty acids on the lipid profile. We examined whether *trans* fatty acid intake could also affect biomarkers of inflammation and endothelial dysfunction including C-reactive protein (CRP), interleukin-6 (IL-6), soluble tumor necrosis factor receptor 2 (sTNFR-2), E-selectin, and soluble cell adhesion molecules (sICAM-1 and sVCAM-1). We conducted a cross-sectional study of 730 women from the Nurses' Health Study I cohort, aged 43–69 y, free of cardiovascular disease, cancer, and diabetes at time of blood draw (1989–1990). Dietary intake was assessed by a validated FFQ in 1986 and 1990. CRP levels were 73% higher among those in the highest quintile of *trans* fat intake, compared with the lowest quintile. IL-6 levels were 17% higher, sTNFR-2 5%, E-selectin 20%, sICAM-1 10%, and sVCAM-1 levels 10% higher. *Trans* fatty acid intake was positively related to plasma concentration of CRP ($P = 0.009$), sTNFR-2 ($P = 0.002$), E-selectin ($P = 0.003$), sICAM-1 ($P = 0.007$), and sVCAM-1 ($P = 0.001$) in linear regression models after controlling for age, BMI, physical activity, smoking status, alcohol consumption, intake of monounsaturated, polyunsaturated, and saturated fatty acids, and postmenopausal hormone therapy. In conclusion, this study suggests that higher intake of *trans* fatty acids could adversely affect endothelial function, which might partially explain why the positive relation between *trans* fat and cardiovascular risk is greater than one would predict based solely on its adverse effects on lipids. J. Nutr. 135: 562–566, 2005.

KEY WORDS: • *trans fatty acids* • *inflammation* • *endothelial dysfunction* • *Nurses' Health Study*

In prospective studies, *trans* fatty acid intake has been associated with higher risk of cardiovascular disease and type 2 diabetes mellitus (1–5). This relation can be explained by several mechanisms. Metabolic studies showed that *trans* fatty acid intakes above the population range of consumption raise LDL cholesterol, lower HDL cholesterol, and increase lipoprotein (a) and plasma triglyceride levels. *Trans* fatty acids can influence thrombogenesis through the eicosanoid synthesis pathway, and may also promote insulin resistance (6).

Increasing evidence indicates the important role of endothelial dysfunction in the development of cardiovascular disease and that dietary factors might influence cardiovascular risk through modulation of endothelial function (7). Recently, Mozaffarian et al. (8) found that intake of *trans* fatty acids was associated with plasma concentrations of biomarkers of systemic inflammation, but they did not evaluate their effects on endothelial adhesion molecules, which are markers of endothelial activation and dysfunction. In this study, we examined intake of *trans* fatty acids in relation to plasma concentration of biomarkers of inflammation and endothelial dysfunction, including C-reactive protein (CRP),[3] interleukin-6 (IL-6), soluble tumor necrosis factor receptor (sTNFR-2), E-selectin, and soluble intercellular and vascular cell adhesion molecules (sICAM-1 and sVCAM-1, respectively) among apparently healthy women.

SUBJECTS AND METHODS

Subjects. The Nurses' Health Study cohort was established in 1976 with 121,700 female registered nurses residing in the United

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[2] To whom correspondence should be addressed.
E-mail: elopezga@hsph.harvard.edu.

[3] Abbreviations used: CRP, C-reactive protein; HT, hormone therapy; IL-6, interleukin-6; sICAM-1, soluble intercellular adhesion molecule 1; sTNFR-2, soluble tumor necrosis factor receptor 2; sVCAM-1, soluble vascular cell adhesion molecule 1.

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States. Every 2 y, follow-up questionnaires were sent to update information on potential risk factors and to identify newly diagnosed cases of chronic diseases. The present study includes 730 women who were selected as control subjects for an earlier nested case-control study of diabetes. These women had not been diagnosed with cardiovascular disease, cancer, or diabetes mellitus at the time of blood draw in 1989–1990. The mean age of women at the time of blood collection was 56 y (range: 43–69).

Blood collection and assessment of biomarkers. Blood was collected between 1989 and 1990. Women willing to provide blood specimens were sent instructions and a phlebotomy kit. Sodium heparin was used as an anticoagulant. Blood specimens were returned by overnight mail on ice, centrifuged (1200 × *g*, 15 min) on arrival to separate plasma from buffy coat and red cells, and frozen in liquid nitrogen until analysis; 97% of samples arrived within 26 h of phlebotomy. Quality control samples were routinely frozen along with study samples to monitor changes due to long-term storage and assay variability. All biomarkers were measured in the Clinical Chemistry Laboratory at Children's Hospital in Boston. High-sensitivity CRP levels were measured by a high-sensitivity latex-enhanced inmunonephelometric assay on a BNII analyzer (Dade Behring). IL-6 was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit) and sTNFR-2 by an ELISA kit utilizing immobilized monoclonal antibody to human TNFR-2 (Genzyme). Levels of E-selectin, sICAM-1, and sVCAM-1 were measured by Theyare ELISA (R&D Systems). The interassay CVs for each biomarker were: CRP, 3.4–3.8%; IL-6, 5.8–8.2%; sTNFR-2: 3.6–5.1%; E-selectin, 6.4–6.6%; sICAM-1, 6.1–10.1%; sVCAM-1, 8.5–10.2%. Processing times did not substantially affect concentration of the biomarkers (9).

Assessment of dietary intake. In 1986 and 1990, a semiquantitative FFQ was mailed to participants. The FFQ included 116 food items with specified serving sizes that were described by using natural portions or standard weight and volume measures of the servings commonly consumed in this study population. For each food item, participants indicated their average frequency of consumption over the past year in terms of the specified serving size by checking 1 of the 9 frequency categories ranging from "almost never" to "≥6 times/d." Detailed information about types of fat or oil used for frying, baking, and at the table, and the type, brand, and year of consumption of margarine was also collected. The average daily intake of nutrients was calculated by multiplying the frequency of consumption of each item by its nutrient content per serving and totaling the nutrient intake for all food items. Values for the total *trans* isomer fatty acid contents of foods were based on analyses by Enig et al. (10) and Slover et al. (11) and updated using data from the USDA, food manufacturers, and analyses of commonly used margarines, shortenings, and baked products performed at the Harvard School of Public Health (Department of Nutrition). We included the *trans* isomers of 16- and 18-carbon fatty acids. Nutrient intakes were adjusted for total energy intake by the residual approach (12). We calculated the means of nutrient intakes in 1986 and 1990 to represent long-term dietary consumption and to reduce measurement error.

Glycemic load was calculated for each food by multiplying the carbohydrate content of 1 serving by the food's glycemic index value. We then multiplied this glycemic load value by the frequency of consumption and summed these products over all food items to produce the dietary glycemic load (13).

The reproducibility and validity of the FFQ were described in detail elsewhere (14). The questionnaire provided a reasonable measure of intake of total and specific types of fats compared with multiple 1-wk diet records. The correlation coefficients between the calculated dietary fatty acid intake from the FFQ and the proportion of the fatty acids in adipose tissue were 0.40 ($P < 0.001$) for total *trans* fats, 0.40 ($P < 0.001$) for polyunsaturated fats, and 0.16 ($P > 0.05$) for saturated fatty acids (15).

Assessment of other variables. Body weight and smoking status were assessed in 1990. BMI was calculated as weight (kg)/height^2 (m). Physical activity was assessed in hours per week spent pursuing common leisure-time physical activities expressed as metabolic equivalent hours per week (MET-h/wk). Alcohol consumption was measured as mean intake (g/d) between 1986 and 1990 (16). Hormone therapy (HT) use was ascertained among postmenopausal women, who were classified as never, past, or current users in 1990.

Statistical analysis. We used PROC GLM in SAS (17) to calculate adjusted geometric means and their 95% CI for the biomarkers according to quintiles of *trans* fat intake. We used the log-transformed biomarkers as the dependent variables. In multivariate models, we adjusted for age (≤45, 46–50, 51–55, 56–60, 61–65, ≥66 y), BMI (<23.0, 23.0–24.9, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m^2), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12.0–20.9, ≥21.0 MET-h/wk), smoking status (never, past, current 1–14 cigarettes/d, current ≥15 cigarettes/d), alcohol consumption (nondrinker, <5.0, 5.0–10.0, >10.0 g/d), and intakes of monounsaturated, polyunsaturated, and saturated fatty acids (in quintiles). We performed sensitivity analyses, adjusting the models also for intake of fiber, cholesterol, total vitamin E, glycemic load (in quintiles), and for use of HT (premenopausal, never, past, current use). We also examined whether the associations were modified by body weight. In addition, we examined the effect of each type of *trans* fatty acid on the biomarkers.

RESULTS

In **Table 1**, we show baseline characteristics of the study population. Mean intakes of *trans* fatty acids according to quintiles of consumption were 1.5, 2.1, 2.4, 2.9, and 3.7 g/d. *Trans* oleic acid was the most abundant *trans* fatty acid consumed. Women in the highest quintile were younger, had a higher BMI, were less likely to be physical active, drink alcohol, or use hormone therapy, and were more likely to smoke than women in the lowest quintile. The intake of *trans* fat was positively associated with the consumption of saturated, polyunsaturated, and monounsaturated fats, and inversely associated with the consumption of total fiber and glycemic load.

We found a trend of increasing plasma concentrations of biomarkers with increasing quintiles of *trans* fat intake (CRP: 1.1–1.9 mg/L, P for trend of medians <0.001; IL-6: 1.8–2.1 ng/L, $P = 0.02$; sTNFR-2: 2339–2466 μg/L, $P = 0.04$; E-selectin: 41.8–50.3 ng/L, $P < 0.001$; sICAM-1: 238–261 μg/L, $P < 0.001$; sVCAM-1: 504–556 μg/L, $P = 0.004$) (**Table 2**). In addition, CRP levels were 73% higher among those in the highest quintile of *trans* fat intake, compared with the lowest quintile; IL-6 levels were 17% higher, sTNFR-2 5%, E-selectin 20%, sICAM-1 10%, and sVCAM-1 levels 10% higher.

Trans fat intake was positively related to plasma concentration of CRP ($P = 0.009$), sTNFR-2 ($P = 0.002$), E-selectin ($P = 0.003$), sICAM-1 ($P = 0.007$), and sVCAM-1 ($P = 0.001$) (**Table 3**). Additional adjustment for other dietary factors such as fiber, cholesterol, total vitamin E intake, and glycemic load did not alter the results.

When we stratified the analyses by BMI with the median as a cutoff point, we found similar associations between *trans* fats and biomarkers for leaner women compared with heavier women (P-values for interactions > 0.05 for all biomarkers).

We also performed analyses considering different types of *trans* fatty acids separately. The intake of oleic acid [*t*18:1(n-9)] appeared to be more strongly associated with the concentrations of biomarkers than *trans* palmitoleic acid [*t*16:1(n-7)] or *trans* linoleic acid [*tt*18:2(n-6)]. *Trans* oleic acid was positively related to levels of sTNFR-2 ($P = 0.002$), E-selectin ($P = 0.001$), sICAM-1 ($P = 0.003$), and sVCAM-1 ($P < 0.001$). *Trans* palmitoleic acid was inversely associated with levels of sTNF-R2 ($P = 0.05$), E-selectin ($P = 0.05$), sICAM-1 ($P < 0.001$), and sVCAM-1 ($P = 0.02$). Intake of *trans-trans* linoleic acid was positively associated with levels of sTNFR-2 ($P = 0.006$), E-selectin ($P = 0.03$), sVCAM-1 ($P = 0.05$), and marginally with sICAM-1 ($P = 0.06$). Finally, intake of *cis-trans* linoleic acid was positively associated with levels of E-selectin ($P = 0.009$) and marginally with sVCAM-1 ($P = 0.08$).

TABLE 1

Baseline characteristics by quintiles of trans fatty acid intake in the Nurses' Health Study[1]

	Quintile of *trans* fatty acid intake				
	1 (lower)	2	3	4	5 (higher)
n	147	145	146	146	146
Total *trans* fat, *g/d*	1.5 ± 0.3	2.1 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	3.7 ± 0.6
Total *trans* fat, *% energy*	0.9 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	2.1 ± 0.4
Total *trans* fat, *% fat*	3.1 ± 0.7	3.9 ± 0.5	4.3 ± 0.6	4.9 ± 0.6	5.9 ± 0.9
Palmitoleic acid (*trans* 16:1), *g/d*	0.1 ± 0.04	0.1 ± 0.04	0.2 ± 0.04	0.2 ± 0.04	0.2 ± 0.04
Oleic acid (*trans* 18:1), *g/d*	1.1 ± 0.2	1.6 ± 0.1	1.9 ± 0.1	2.3 ± 0.2	3.0 ± 0.5
Linoleic acid (*trans trans* 18:2), *g/d*	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.02	0.2 ± 0.1
Linoleic acid (*cis trans* 18:2), *g/d*	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Age, *y*	57.6 ± 6.6	57.4 ± 6.7	55.8 ± 6.8	55.4 ± 6.6	55.0 ± 7.1
BMI, *kg/m*^{2}	25.1 ± 4.6	26.1 ± 6.1	26.4 ± 6.3	26.6 ± 5.6	27.3 ± 7.3
Physical activity,[2] *MET-h/wk*	16.3 ± 18.2	12.9 ± 13.1	12.0 ± 15.0	12.0 ± 17.2	11.1 ± 12.4
Alcohol consumption, *g/d*	8.5 ± 14.6	5.4 ± 8.5	6.7 ± 9.4	4.3 ± 6.6	3.4 ± 6.0
Current smoker, %	8.3	9.8	12.4	11.1	20.8
Current postmenopausal HT use, %	41.4	40.7	27.7	28.8	26.4
Energy intake,[3] *kcal/d*	1726 ± 428	1723 ± 413	1806 ± 461	1792 ± 477	1861 ± 510
Saturated fat, *g/d*	16.7 ± 3.9	19.0 ± 3.2	20.2 ± 3.4	20.8 ± 3.4	22.3 ± 3.5
Polyunsaturated fat, *g/d*	10.0 ± 2.9	10.4 ± 2.3	10.5 ± 2.0	11.1 ± 2.4	11.7 ± 2.2
Monounsaturated fat, *g/d*	17.7 ± 3.5	20.3 ± 2.9	21.5 ± 3.0	22.4 ± 2.6	25.0 ± 3.0
Total fiber, *g/d*	21.5 ± 7.6	18.5 ± 4.3	17.9 ± 4.6	17.0 ± 3.6	15.7 ± 3.4
Cholesterol, *mg/d*	218.0 ± 73.8	234.9 ± 56.0	243.2 ± 66.6	230.6 ± 45.1	240.1 ± 64.6
Total vitamin E, *mg/d*	7.6 ± 4.7	6.7 ± 3.3	6.7 ± 2.8	6.3 ± 2.7	6.7 ± 2.9
Glycemic load[4]	106 ± 23	102 ± 18	101 ± 18	102 ± 15	99 ± 14

[1] Values are means ± SD or %.
[2] MET, metabolic equivalent (energy need/kilogram body weight per hour of activity divided by the energy need/kilogram body weight per hour at rest).
[3] 1 kcal = 4.184 kJ.
[4] Glycemic load was defined as an indicator of blood glucose induced by an individual's total carbohydrate intake. Each unit of glycemic load represents the equivalent of 1 g carbohydrate from white bread.

DISCUSSION

In this study, we examined the relation between intake of *trans* unsaturated fatty acids and plasma concentrations of biomarkers of inflammation and endothelial dysfunction among apparently healthy women. In the range of *trans* fat intake in this population, we found a positive relation with plasma concentrations of CRP, sTNFR-2, E-selectin, sICAM-1, and sVCAM-1. CRP levels were 73% higher among those in the highest quintile of *trans* fat intake, compared with the lowest quintile; IL-6 levels were 17% higher, sTNFR-2 5%, E-selectin 20%, sICAM-1 10%, and sVCAM-1 levels 10% higher. These associations were independent of lifestyle and dietary covariates.

In a 3-y follow-up study, Ridker et al. (18) found that women who later developed cardiovascular events had baseline plasma levels of CRP 50% higher than those who were free of the disease at the end point. In addition, these women also had higher baseline levels of IL-6 and sICAM-1 (27 and 9%, respectively). In our study, we found that differences in concentrations of the biomarkers between extreme quintiles of *trans* fat intake are comparable to the differences in women with and without risk of developing cardiovascular events. Thus, the association between *trans* fat and biomarkers of inflammation and endothelial dysfunction could explain why the epidemiologic studies

TABLE 2

Age-adjusted geometric means (95% CI) of plasma concentrations of biomarkers of inflammation and endothelial dysfunction by quintiles of trans fatty acid intake in the Nurses' Health Study[1]

Quintile	*n*	CRP *mg/L*	IL-6 *ng/L*	sTNFR-2 *μg/L*	E-selectin *ng/L*	sICAM-1 *μg/L*	sVCAM-1 *μg/L*
Trans fatty acids, (*range: g/d*)							
Q1 (0.61–1.87)	147	1.1 (0.9, 1.3)	1.8 (1.6, 2.0)	2339 (2176, 2515)	41.8 (39.0, 44.9)	238 (229, 247)	504 (484, 525)
Q2 (1.88–2.26)	145	1.3 (1.1, 1.6)	1.7 (1.5, 2.0)	2136 (1986, 2298)	41.9 (39.0, 45.0)	246 (236, 256)	520 (499, 542)
Q3 (2.27–2.64)	146	1.5 (1.3, 1.8)	1.8 (1.6, 2.0)	2259 (2102, 2429)	41.9 (39.0, 45.0)	242 (232, 252)	537 (515, 559)
Q4 (2.65–3.13)	146	1.7 (1.4, 2.0)	1.9 (1.7, 2.2)	2338 (2175, 2514)	45.1 (42.0, 48.4)	253 (243, 263)	523 (502, 545)
Q5 (3.14–7.58)	146	1.9 (1.6, 2.3)	2.1 (1.8, 2.3)	2466 (2294, 2651)	50.3 (46.8, 54.0)	261 (251, 272)	556 (533, 579)
P for trend[2]		<0.001	0.02	0.04	<0.001	<0.001	0.004

[1] Mean nutrient intake between 1986 and 1990.
[2] *P* for trend of medians in each quintile.

TABLE 3

Multiple linear regression models for the relation between trans fatty acid intake (g/d) and log-transformed biomarkers of inflammation and endothelial dysfunction in the Nurses' Health Study[1,2]

	Age-adjusted	Multivariate-adjusted[3]
Log CRP	0.19	0.16
(*mg/L*)	(<0.001)	(0.009)
Log IL-6	0.07	0.07
(*ng/L*)	(0.04)	(0.10)
Log sTNFR-2	0.03	0.08
($\mu g/L$)	(0.11)	(0.002)
Log E-selectin	0.09	0.07
(*ng/L*)	(<0.001)	(0.003)
Log sICAM-1	0.04	0.04
($\mu g/L$)	(<0.001)	(0.007)
Log sVCAM-1	0.03	0.05
($\mu g/L$)	(0.01)	(0.001)

[1] Values are β coefficients (*P*-values).
[2] Mean intake between 1986 and 1990.
[3] Adjusted for age (≤45, 46–50, 51–55, 56–60, 61–65, ≥66 y), BMI (<23.0, 23.0–24.9, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m^2), physical activity (<1.5, 1.5–5,9, 6.0–11.9, 12.0–20.9, ≥21 MET-h/wk), smoking status (never smoker, past smoker, current 1–14 cigarettes/d, current ≥cigarettes/d), alcohol consumption (non-drinker, 0–4.9, 5.0–10.0, >10.0 g/d), quintiles of total monounsaturated, polyunsaturated and saturated fatty acids, and postmenopausal hormone therapy.

showed greater risk of developing cardiovascular disease than one would predict based solely on the effects of *trans* fatty acids on blood lipids (4).

The relevance of inflammatory and endothelial dysfunction biomarkers in the atherogenic process was suggested by several studies. CRP and IL-6 are markers of systemic inflammation and are independent predictors of cardiovascular disease in healthy women (18). Recent data suggest that CRP plays a direct role in atherogenesis (19). In addition, the soluble TNF receptor, which is induced by TNF and other cytokines, is an indicator of inflammatory processes (20) and has been associated with obesity and coronary heart disease (21,22). On the other hand, E-selectin, sICAM-1, and sVCAM-1 are surface and soluble cell adhesion molecules that are overexpressed when the endothelium encounters inflammatory stimuli. The relation between these adhesion molecules and cardiovascular disease has been established, i.e., patients with coronary heart disease had higher levels of E-selectin and sICAM-1 (23), and baseline plasma levels of sICAM-1 were predictors of myocardial infarction among apparently healthy men (24). Finally, sVCAM-1 was a predictor of a more advanced stage in the atherosclerotic process (25).

The biological mechanisms underlying the adverse effects of *trans* fatty acids on endothelial function are not clear. *Trans* fats are incorporated into endothelial cell membranes and thus could alter cellular and macromolecular components acting at the interface of the blood vessel wall. This could result in changes in the antihemostatic properties, altered vascular tone, hyperadhesiveness to blood leukocytes, and increased cytokine and growth factor production, all of which are characteristics of endothelial dysfunction (26).

In a recent study, *trans* fatty acids were found to impair endothelial function assessed by flow-mediated vasodilation (27). Because both the altered vasodilation and the increased production of inflammatory and adhesion molecules indicate endothelial dysfunction, these results are consistent with our findings. However, a high consumption of *trans* fatty acids might also impair endothelial function indirectly by reducing HDL cholesterol concentration (28), which in turn may trigger LDL oxidation (29). We did not adjust for plasma levels of HDL cholesterol in our analyses because we did not measure blood lipids. Nevertheless, adjustment for serum lipid concentrations only partly attenuated the association between intake of *trans* fats and sTNFR-1 and sTNFR-2 in a recent study of younger women (8), suggesting that the effect of *trans* fat on the endothelium is not entirely mediated by HDL cholesterol.

Another recent clinical trial found that the intake of 8% of energy in the diet from *trans* fatty acids at the expense of oleic acid or carbohydrate for 5 wk led to higher CRP and E-selectin levels (30). However, the effects were not observed at a lower level of *trans* fat (4% of energy). Our study suggests that long-term consumption of *trans* fat, even at lower levels, could be detrimental to endothelial function.

Adipose tissue is an important endocrine organ; adipocytes secrete a variety of bioactive proteins, including IL-6 and TNF-α (31). In a previous study of younger women, a positive association between *trans* fat and CRP was observed only among those with a higher BMI (8). However, we found that the effect of *trans* fatty acids on endothelial dysfunction was independent of BMI.

Although some evidence suggests that *trans* isomers of linoleic acid are more strongly related to risk of sudden cardiac death compared with *trans* isomers of oleic acid (32), intake of *trans* oleic acid was more strongly associated with plasma concentrations of biomarkers in our study. *Trans* oleic acid was the most abundant *trans* fatty acid in our population; thus, the different effects might be due to the higher proportion of *trans* oleic acid in the pool of *trans* fats rather than to their different biochemical characteristics.

Our study has several limitations. First, it is cross-sectional; therefore, we cannot infer causality from our results. Second, there is some degree of error in the measurement of food consumption as well as in biochemical measures, although the dietary questionnaire was shown to reflect long-term intake and the biomarker measures are reasonably stable over time. In addition, the use of the repeated measurement of food consumption enabled us to reduce within-person random error. Finally, changes in the *trans* fatty acid content of different foods over time might have resulted in errors in the estimation of *trans* fat intake (33). We minimized these errors by considering the brand and specific type of food when calculating *trans* fatty acid content.

In conclusion, our findings provide strong evidence that *trans* fatty acids adversely affect endothelial function, which might explain in part the association of these fatty acids with the risk of cardiovascular disease. These data lend further support for the recommendation to minimize the content of *trans* fat in the diet.

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