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Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* Fatty Acids Collaboration (TRANSFACT) study[1–4]

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ABSTRACT

Background: The consumption of monounsaturated *trans* fatty acids (TFAs) increases the risk of cardiovascular disease (CVD). Putative differences between the effects of TFAs from industrially produced and natural sources on CVD risk markers were not previously investigated in healthy subjects.

Objective: We aimed to compare the effects of TFAs from industrially produced and natural sources on HDL and LDL cholesterol, lipoprotein particle size and distribution, apolipoproteins, and other lipids in healthy subjects.

Design: In a randomized, double-blind, controlled, crossover design, 46 healthy subjects (22 men and 24 women) consumed food items containing TFAs (11–12 g/d, representing ≈5% of daily energy) from the 2 sources.

Results: Forty subjects (19 men and 21 women) completed the study. Compared with TFAs from industrially produced sources, TFAs from natural sources significantly ($P = 0.012$) increased HDL cholesterol in women but not in men. Significant ($P = 0.001$) increases in LDL-cholesterol concentrations were observed in women, but not in men, after the consumption of TFAs from natural sources. Apolipoprotein (apo)B and apoA1 concentrations confirmed the changes observed in LDL and HDL cholesterol. Analysis of lipoprotein subclass showed that only large HDL and LDL concentrations were modified by TFAs from natural sources but not by those from industrially produced sources.

Conclusions: This study shows that TFAs from industrially produced and from natural sources have different effects on CVD risk factors in women. The HDL cholesterol–lowering property of TFAs seems to be specific to industrial sources. However, it is difficult in the present study to draw a conclusion about the effect of TFAs from either source on absolute CVD risk in these normolipidemic subjects. The mechanism underlying the observed sex- and isomer-specific effects warrants further investigation. *Am J Clin Nutr* 2008;87:558–66.

KEY WORDS Cardiovascular disease risk factor, cholesterol, lipoprotein, nutrition in public health, *trans* fatty acids

INTRODUCTION

The consumption of monounsaturated *trans* fatty acids (TFAs) has been associated with a greater risk of cardiovascular disease [(CVD) 1, 2]. Two dietary sources exist for the TFAs present in the food supply—industrial production, in which TFAs are mainly derived from partially hydrogenated vegetable oils (PHVOs), and natural sources, in which TFAs are found in smaller amounts in ruminant-derived food products (3). Unfortunately, very little scientific research to date has compared the specific health effects of TFAs from industrial and natural sources (1–3).

Epidemiologic studies that have allowed for a comparison of TFAs from different sources clearly showed a positive association between CVD risk and the intake of TFAs from industrial sources but not between CVD risk and TFAs from natural sources (4–11). Several public health agencies have implemented labeling or ingredient restrictions on *trans* fats (11, 12). In only a few countries, however, do these restrictions discriminate between TFAs from industrially produced and natural sources (13). The decision not to discriminate is based on the assumption that *trans* fats from the 2 sources have the same

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biological and nutritional effects. It is important to note, however, that the TFAs originate from different sources, are present in different food products (1, 14), and would require very different means to reduce or eliminate them from the food supply.

PHVOs can contain ≈1–65% of TFAs, of which isomers of elaidic acid (*trans*-9 and *trans*-10 18:1) are the 2 most common isomers (14). On the other hand, dairy products contain smaller amounts of TFAs (≈1–8% of total fatty acids in milk fat), and the main isomer is vaccenic acid (*trans*-11 18:1) (14). In ruminants (eg, cow, ewe, and goat), TFAs are produced as intermediates during the biohydrogenation of dietary polyunsaturated fatty acids by anaerobic bacteria in the rumen (15). Moreover, humans can utilize vaccenic acid, in the endogenous synthesis of rumenic acid (*cis*-9, *trans*-11 18:2) (16), a fatty acid that may not have a negative effect on biomarkers of CVD risk (17–19). These 2 sources of TFAs differ in their TFA isomer distribution and contribution to dietary intake (20), and, as a consequence, they also may have different biological effects.

Initial studies of TFAs took advantage of the ability to distinguish plasma LDL and HDL cholesterol as a means of providing evidence that *trans* fats increased the risk of heart disease (21, 22). During the 15 y since those studies, lipoprotein biology continued to elaborate the variation in size and composition of plasma LDL and HDL particles (23–29). These investigations have provided evidence that the small dense LDL are responsible for most of the harmful consequences of LDL cholesterol (23, 24) and that increases in large HDL may be responsible for most of the protective effects of HDL cholesterol (25, 26).

Until recently, it was not possible to produce ruminant fat with sufficient quantities of TFAs to allow a direct comparison with TFAs from an industrially produced source in humans. Developments, however, have shown that, by appropriate management of dairy cows, milk fat naturally enriched in TFAs can be produced (30). Because of the lack of human intervention studies and the interesting, although underpowered epidemiologic observations (3), we designed a human intervention study in healthy, free-living men and women to address the following question: do TFAs from industrially produced and natural sources have similar effects on CVD risk factors in healthy subjects? The rationale for the study, the experimental protocol, and a description of the population, treatments, and analytic procedures were reported elsewhere before the clinical study was begun (31).

SUBJECTS AND METHODS

Subjects

Forty-six normolipidemic subjects with waist size < 102 cm (men) or < 88 cm (women) were initially recruited, as described previously (31). All of the volunteers replied to a medical questionnaire and then underwent an examination performed by the principal investigator or by a coinvestigator. Six subjects who failed to complete the study were excluded from the per-protocol dataset: 2 subjects dropped out during the run-in period, 1 subject dropped out during period 2, and 3 subjects did not meet compliance standards for both periods. Twenty-one women and 19 men completed the study; their demographic data are shown in **Table 1**. Baseline data for risk factors associated with CVD in the subjects enrolled in the study and values from the French general population as determined in the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (WHO-MONICA) study (32) are shown in **Table 2**.

TABLE 1
Characteristics of the subjects at baseline[1]

Characteristic	Men (n = 19)	Women (n = 21)	All subjects (n = 40)
Age (y)	27.7 ± 6.8	27.5 ± 7.6	27.6 ± 7.1
Weight (kg)[2]	72.2 ± 9.0	57.2 ± 7.0	64.3 ± 11.0
BMI (kg/m)[2]	22.9 ± 2.6	21.1 ± 2.0	22.0 ± 2.4
Waist measurement (cm)[2]	79.4 ± 6.1	69.6 ± 5.0	74.3 ± 7.4
Systolic BP (mm Hg)[2]	121.6 ± 7.2	111.2 ± 7.2	116.2 ± 8.9
Diastolic BP (mm Hg)[2]	68.9 ± 5.9	63.3 ± 4.6	66.0 ± 5.9
Heart rate (beat/min)	65.1 ± 9.7	63.6 ± 5.6	64.3 ± 7.8

[1] All values are $\bar{x}$ ± SD. BP, blood pressure.
[2] Significant sex differences ($P < 0.05$).

Written informed consent was obtained from all participants. The study protocol was approved by the Comité Consultatif pour la Protection des Personnes se pretant la Recherche Biomedicale (CCPPRB) Auvergne (Clermont-Ferrand, France; CCPPRB no. AU 599). The Clinical Trial Registration number is NCT00439582.

Study design and treatment composition

The study used a randomized, double-blind, controlled crossover design. After a 1-wk run-in period, volunteers were randomly allocated to 1 of the 2 treatments for 3 wk, with a 1-wk wash-out period before they received the other treatment (31). Sex was the only stratification factor used in the randomization. During the run-in period, subjects received regular food items. During the experimental periods, subjects consumed the foods with TFAs from the 2 different sources; daily intake of these 3 foods was 20 g butter (80% fat content), 100 g cheese (31% fat content), and 22 g cookies (31% fat content). The lipids from the experimental products represented a mean ± SD 67.3 ± 8.8% of the daily energy intake provided by fat. Details on the fatty acid distribution of the 2 experimental fats are provided in **Table 3**.

The dietitian provided instructions to the volunteers to avoid consumption of additional food items containing TFAs during both 3-wk experimental periods. Subjects recorded their food consumption (study products and other items) 3 times during the study period—at week 0 (run-in period) and at weeks 3 and 7 (the last week of the 2 intervention periods). Dietary records were analyzed by a dietitian using MICRO 6 diet analyzer software (version 6.0; GENI, Villers-Les-Nancy, France), and the daily energy intake and the proportion of energy intake from different nutrient sources were calculated (**Table 4**). The baseline values reported in Table 4 are representative of the nutrient intake and distribution during the run-in period.

The experimental fats did not provide adequate concentrations of essential fatty acids (linoleic and α-linolenic acids). To address this, 2 vegetable oils balanced in linoleic and α-linolenic acids were provided to the subjects (1 L of each oil for each subject each month). These oils were suitable for cooking or for use in salad dressings, and their consumption was monitored by the dietitian.

Assessment of subject compliance

Subject compliance was assessed by a questionnaire and by analysis of the concentration of TFAs in plasma cholesteryl esters (CEs). The mean baseline TFA concentration in CEs was

TABLE 2
Comparison between cardiovascular risk factors in women and men in the TRANSFACT study and values from the WHO-MONICA study conducted on the general French population[1]

	Women		Men	
	WHO-MONICA[2]	TRANSFACT	WHO-MONICA	TRANSFACT
HDL cholesterol (mg/dL)	62.0 ± 15.0	79.6 ± 13.8	50.0 ± 14.0	61.7 ± 12.5
LDL cholesterol (mg/dL)	130.0 ± 33.0	99.6 ± 29.2	150.0 ± 40.0	91.8 ± 25.9
Total cholesterol (mg/dL)	208.0 ± 34.0	195.6 ± 33.1	224.0 ± 40.0	169.1 ± 29.1
Triacylglycerol (mg/dL)	83.0 ± 47.0	82.0 ± 30.1	127.0 ± 108.0	77.7 ± 25.7

[1] All values are $\bar{x}$ ± SD. TRANSFACT, *trans* Fatty Acids Collaboration; WHO-MONICA, World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease. $n = 21$ and 19 for women and men, respectively, in the TRANSFACT study; $n = 511$ and 527 for women and men, respectively, in the WHO-MONICA study.
[2] Data adapted from Savés et al (32).

0.16 ± 0.06 g/100 g total fatty acids. During the industrial and the natural TFA experimental periods, the average concentrations of TFAs found in CEs were 0.51 ± 0.16 and 0.50 ± 0.17 g/100 g total fatty acids, respectively. On the basis of these data, compliance was estimated to be ≥75% according to the mathematical model of Zock et al (33). A significant ($P = 0.004$) period effect was observed: consumption of TFAs from both sources was lower in the second intervention period (−0.6 ± 0.2% of daily energy) than in the first. However, because of the crossover design, the period effect was not treatment dependent ($P = 0.25$).

Biochemical analysis

Blood samples were drawn in the morning, after a 12-h fast. Water was still allowed, but volunteers were asked to limit water intake before the visit. For the analysis of the lipid profiles (ie, triacylglycerols, total cholesterol, and HDL cholesterol), lipoprotein concentrations and subclasses, and apoA1 and apoB, blood samples were collected in tubes containing EDTA or heparin. Blood samples were centrifuged (3500 RPM, 10 min, 4 °C; GR4.12; JOUAN SA, St Herblain, France), and plasma was

TABLE 3
Composition and daily intake of fatty acids provided by the experimental *trans* fats in men and women[1]

	TFAs from industrially produced sources		TFAs from natural sources	
Fatty acids	Composition	Daily energy	Composition	Daily energy
	g/100g fat	*%*	*g/100g fat*	*%*
4:0	0.00	0.00	3.41	0.84
6:0	0.00	0.00	1.75	0.43
8:0	0.09	0.02	0.90	0.22
10:0	0.06	0.02	1.82	0.45
12:0	1.18	0.29	2.09	0.52
14:0	1.23	0.30	8.02	1.98
16:0	31.52	7.80	22.94	5.68
18:0	14.81	3.67	5.82	1.44
Σ(12:0 + 14:0 + 16:0)	33.93	8.39	33.05	8.18
cis-9 18:1	22.76	5.63	13.37	3.31
Other *cis* 18:1	1.69	0.42	1.99	0.49
trans-4 18:1	0.00	0.00	0.09	0.02
trans-5 18:1	0.00	0.00	0.09	0.02
trans-6/8 18:1	3.37	0.83	0.78	0.19
trans-9 18:1	9.83	2.43	0.64	0.16
trans-10 18:1	4.42	1.09	2.42	0.60
trans-11 18:1	2.32	0.57	13.87	3.43
trans-12 18:1	0.80	0.20	0.78	0.19
trans-13/14 18:1	1.26	0.31	0.98	0.24
trans-15 18:1	0.50	0.12	0.29	0.07
trans-16 18:1	0.07	0.02	0.16	0.04
Σ(*trans*-9 + *trans*-10 + *trans*-11)	16.57	4.09	16.93	4.19
cis-9, *cis*-12 18:2	2.01	0.50	2.35	0.58
trans 18:2	0.87	0.22	1.99	0.49
cis-9, *trans*-11 18:2	0.00	0.00	4.44	1.10
cis-9, *cis*-12, *cis*-15 18:3	0.04	0.01	0.32	0.08

[1] $n = 19$ men, 21 women. TFAs, *trans* fatty acids.

TABLE 4
Nutrient sources for the daily intake of energy in men and women at baseline and after receiving *trans* fatty acids (TFAs) from natural or industrially produced sources for 3 wk[1]

	Baseline		TFAs from industrially produced sources (experimental period)		TFAs from natural sources (experimental period)	
Constituent	Men	Women	Men	Women	Men	Women
Fat (% of energy)	37 ± 5	39 ± 5	36 ± 4	37 ± 6	36 ± 4	40 ± 4
Carbohydrate (% of energy)	45 ± 5	44 ± 6	46 ± 5	47 ± 7	46 ± 5	45 ± 4
Protein (% of energy)	15 ± 2	16 ± 2	16 ± 2	15 ± 1	15 ± 2	15 ± 1
Alcohol (% of energy)	3 ± 2	1 ± 1	2 ± 2	1 ± 1	2 ± 3	1 ± 1
Total energy						
kcal/d	2235 ± 322	1788 ± 188	2096 ± 299	1891 ± 242	2155 ± 390	1895 ± 271
MJ/d	9 ± 1	7 ± 1	9 ± 1	8 ± 1	9 ± 2	8 ± 1

[1] All values are $\bar{x}$ ± SD. n = 19 men, 21 women.

removed and stored at −80 °C until it was analyzed. Plasma concentrations of HDL cholesterol, triacylglycerols, total cholesterol, and apoA1 and apoB were measured by enzymatic assays, and LDL cholesterol was calculated according to the Friedewald method as described previously (31). For calibration and quality control, the following standards were used according to the guidelines provided by the manufacturer (Thermo Electron Corporation, Cergy-Pontoise, France): lipotrol as a normal control, sCal for the calibration of the substrates, and eCal for the enzymatic calibration. Plasma lipoprotein(a) [Lp(a)] and LDL and HDL particle sizing and distribution profiles were measured by using nuclear magnetic resonance (NMR) spectrometry (Liposcience, Raleigh, NC) (29). Plasma activity of CE transfer protein (CETP) was measured by fluorimetry (34), and fatty acid profiles of plasma CEs were analyzed by gas-liquid chromatography (33). Fasting body weight and blood pressure were measured at each visit; no changes in these variables were observed during the study.

Statistical analysis

Statistical analyses were performed with SAS software (version 8.2; SAS Institute Inc, Cary, NC). The number of subjects enrolled in the study was based on detection of a difference of 2.11 mg/dL in HDL cholesterol, with a planned within-subject variability of 4.5 mg/dL, a significance level of 5% (2-sided), and a power setting of 80%. Results are presented on the per-protocol data set. Plasma variables from the last week of each intervention period were analyzed by using a mixed model, with treatment as the fixed effect, subject as the random effect, and sex as the covariate. A secondary analysis was performed to examine the sex × treatment interaction. Given that conclusions about effects were the same for both models, only the results of the model with interaction are presented; effects were declared significant at $P <$ 0.05.

RESULTS

The experimental products represented 67.3 ± 8.8% of the mean daily energy provided by dietary lipids. The total of the most prevalent TFA isomers—*trans*-9, *trans*-10, and *trans*-11 18:1—was balanced in both treatments to represent ≈4.2 ± 0.5% of daily energy (Table 3). Treatment effects after 3 wk of consumption of TFAs from the industrially produced source were compared with those after equal consumption of TFAs from the natural source; results for the clinical outcomes are presented in **Table 5**. For all clinical outcomes, no significant treatment effects were observed for men; significant treatment effects were observed for women.

Plasma concentrations of HDL cholesterol, selected as the primary outcome, were significantly (P = 0.012) lower in women with consumption of TFAs from the industrially produced source than with consumption of TFAs from the natural source. Because of the magnitude of the effect and the balanced sex distribution across experimental periods, a significant (P = 0.037) differential effect was found for all subjects. The NMR analysis of the plasma lipoprotein size and distribution confirmed that, in women, consumption of TFAs from industrially produced sources significantly (P = 0.011) reduced the total number of HDL particles, primarily through a significant ($P <$ 0.001) reduction in the quantity of large HDL particles (**Table 6**). Analysis of the HDL particle distribution showed that, because of the magnitude of the effect observed for women, the treatment effect on HDL particle distribution was significant ($P < 0.001$) in all subjects, but this effect was largely a consequence of the magnitude of the effect observed in women. Analysis of plasma apoA1 confirmed the sex difference in the response to treatments. Accordingly, concentrations of apoA1 were significantly ($P < 0.001$) lower in women consuming TFAs from the industrially produced source than in those consuming TFAs from the natural source.

The concentration of LDL cholesterol (Table 5) was significantly (P = 0.001) higher in women consuming TFAs from the natural source than in those consuming TFAs from the industrially produced source, but not in men (P = 0.99). The increase in LDL cholesterol observed in women was a result of a significant (P = 0.009) increase in large LDL particles, and there was a slight, nonsignificant (P = 0.064) increase in the number of LDL particles. Analysis of the concentration of apoB confirmed these results in women ($P < 0.001$). VLDL particle concentrations, measured by NMR, were not significantly affected by the treatments (results not shown). Plasma concentrations of total cholesterol and triacylglycerol were significantly ($P < 0.001$ and P = 0.001, respectively) higher in women consuming TFAs from the natural source than in those consuming TFAs from the industrially produced source (Table 5). The diagnostic ratios of total to HDL cholesterol and of apoA1 to apoB, usually used to assess CVD risk, were not significantly modified during the study (Table 5). The plasma concentration of Lp(a) and the activity of the CETP also were not changed after 3 wk of consumption of TFAs from either source.

TABLE 5
Serum lipid, lipoprotein, and apolipoprotein concentrations; cholesterol ester transfer protein (CETP) activity; and diagnostic ratios in men and women at baseline and after receiving *trans* fatty acids (TFAs) from natural or industrially produced sources for 3 wk[1]

		Experimental periods			
Variable and subjects	Baseline values	TFAs from industrially produced sources	TFAs from natural sources	Estimated mean effect[2]	*P*
HDL cholesterol (mg/dL)[3]					
Men	61.7 ± 12.5[4]	58.8 ± 14.8	58.2 ± 14.9	−0.56	0.743
Women	79.6 ± 13.8	73.6 ± 11.9	77.8 ± 13.2	−4.02	0.012
Overall	71.1 ± 15.9	66.6 ± 15.1	68.5 ± 17.0	−2.29	0.037
LDL cholesterol (mg/dL)[3]					
Men	91.8 ± 25.9	87.0 ± 27.4	88.7 ± 31.7	−0.03	0.994
Women	99.6 ± 29.2	89.6 ± 26.5	103.1 ± 30.2	−13.75	0.001
Overall	95.9 ± 27.6	88.3 ± 26.6	96.3 ± 31.4	−6.89	0.015
Total cholesterol (mg/dL)[3]					
Men	169.1 ± 29.1	161.1 ± 31.4	164.0 ± 30.6	−2.02	0.642
Women	195.6 ± 33.1	179.6 ± 30.5	199.5 ± 33.6	−19.98	<0.001
Overall	183.0 ± 33.7	170.8 ± 31.9	182.7 ± 36.5	−11.00	<0.001
Triacylglycerol (log) (mg/dL)					
Men	77.7 ± 25.7	76.3 ± 26.2	85.6 ± 44.2	−0.09[5]	0.994
Women	82.0 ± 30.1	82.1 ± 31.6	93.0 ± 30.3	−0.14[5]	0.001
Overall	80.0 ± 27.8	79.4 ± 28.9	89.5 ± 37.3	−0.05[5]	0.002
ApoA1 (mg/dL)[3]					
Men	1.32 ± 0.17	1.31 ± 0.21	1.30 ± 0.23	0.00	0.943
Women	1.71 ± 0.28	1.60 ± 0.26	1.72 ± 0.32	−0.12	<0.001
Overall	1.53 ± 0.30	1.46 ± 0.28	1.52 ± 0.35	−0.16	0.012
ApoB (mg/dL)[3]					
Men	0.71 ± 0.15	0.70 ± 0.16	0.72 ± 0.16	−0.01	0.778
Women	0.81 ± 0.18	0.77 ± 0.18	0.86 ± 0.20	−0.09	<0.001
Overall	0.76 ± 0.17	0.74 ± 0.17	0.79 ± 0.19	−0.05	−0.005
Lp(a) (nmol/L)					
Men	42.3 ± 53.3	45.6 ± 57.5	42.7 ± 56.1	0.05	0.410
Women	56.2 ± 62.7	60.8 ± 68.9	56.1 ± 62.2	0.06	0.246
Overall	49.6 ± 58.1	53.6 ± 63.4	49.7 ± 59.0	0.05	0.465
CETP activity (pmol $CE \cdot mL^{-1} \cdot h^{-1}$)					
Men	9051 ± 1948	10373 ± 2086	10610 ± 2514	−413.95	0.505
Women	12018 ± 2130	11833 ± 2604	12600 ± 2478	−749.06	0.184
Overall	10609 ± 2516	11140 ± 2456	11655 ± 2661	−581.51	0.165
Total:HDL cholesterol					
Men	2.8 ± 0.6	2.9 ± 0.7	2.8 ± 0.6	−0.05	0.616
Women	2.5 ± 0.5	2.6 ± 0.6	2.5 ± 0.5	−0.14	0.128
Overall	2.7 ± 0.6	2.8 ± 0.7	2.6 ± 0.6	−0.09	0.162
ApoA1:apoB					
Men	0.54 ± 0.11	0.56 ± 0.13	0.54 ± 0.13	−0.014	0.644
Women	0.48 ± 0.11	0.51 ± 0.13	0.49 ± 0.12	−0.021	0.165
Overall	0.51 ± 0.11	0.54 ± 0.13	0.52 ± 0.12	−0.008	0.201

[1] $n = 19$ men, 21 women. Apo, apolipoprotein; Lp(a), lipoprotein(a). Results are presented on the per-protocol data set. All plasma variables were analyzed by using a mixed model in week 3 of treatment, with treatment as the fixed effect, subject as the random effect, and sex as the covariate. $P < 0.05$ was considered to be significant.

[2] Estimate is defined as the difference in clinical outcomes between TFAs from industrially produced and natural sources.

[3] Treatment × sex interaction was significant ($P < 0.05$).

[4] $\bar{x}$ ± SD (all such values).

[5] Evaluated in log-transformed data.

DISCUSSION

The baseline values of the subjects enrolled in the present study were compared with values from the general French population (38), and results confirmed that subjects were representative of the general healthy population (Table 2). Women included in the present study were young (28.9 ± 8.3 y old) and did not use postmenopausal hormone therapies. In addition, the consumption of nutritional supplements was one of the exclusion criteria, and medication that can interfere with lipid metabolism was forbidden. The consumption of alcohol was low in both men and women throughout the study, and no significant differences in consumption were recorded between the treatment periods (Table 4).

TABLE 6
Lipoprotein particle subclasses and lipoprotein(a) measured by nuclear magnetic resonance spectroscopy in men and women after receiving *trans* fatty acids (TFAs) from natural or industrially produced sources for 3 wk[1]

Treatment effect	Estimate[2]	Standard error	*P*	Confidence limits	
				Lower	Upper
HDL particles (μmol/L)[3]					
Men	−0.37	0.72	0.607	−1.83	1.09
Women	−1.72	0.65	0.011	−3.04	−0.41
Overall	−1.05	0.48	0.036	−2.02	−0.07
Large HDL particles (μmol/L)[3]					
Men	−0.50	0.32	0.120	−1.14	0.14
Women	−1.22	0.28	<0.001	−1.8	−0.64
Overall	−0.86	0.21	<0.001	−1.29	−0.43
Medium HDL particles (μmol/L)					
Men	0.20	0.67	0.770	−1.16	1.55
Women	0.39	0.60	0.522	−0.83	1.61
Overall	0.29	0.45	0.516	−0.61	1.20
Small HDL particles (μmol/L)					
Men	−0.07	0.84	0.937	−1.77	1.63
Women	−0.89	0.75	0.245	−2.42	0.64
Overall	−0.48	0.56	0.399	−1.61	0.66
LDL particles (nmol/L)					
Men	−43.21	40.04	0.288	−124.35	37.93
Women	−59.02	36.02	0.110	−132.01	13.97
Overall	−51.11	26.77	0.064	−105.36	3.13
Large LDL particles (nmol/L)[3]					
Men	−4.88	29.66	0.870	−64.97	55.22
Women	−73.46	26.68	0.009	−127.52	−19.4
Overall	−39.17	19.83	0.056	−79.34	1.01
Medium-to-small LDL particles (nmol/L)					
Men	−6.89	10.13	0.500	−27.42	13.63
Women	4.25	9.11	0.644	−14.21	22.72
Overall	−1.32	6.77	0.846	−15.04	12.4
Small LDL particles (nmol/L)					
Men	−35.49	43.41	0.419	−123.45	52.47
Women	13.75	39.05	0.727	−65.38	92.88
Overall	−10.87	29.02	0.710	−69.68	47.94
Very small LDL particles (nmol/L)					
Men	−28.60	34.31	0.410	−98.12	40.93
Women	9.50	30.87	0.760	−53.04	72.05
Overall	−9.55	22.94	0.680	−56.03	36.93
Lipoprotein(a) (nmol/L) (log)					
Men	0.05	0.05	0.410	−0.06	0.16
Women	0.06	0.05	0.246	−0.04	0.16
Overall	0.05	0.04	0.165	−0.02	0.13

[1] $n = 19$ men, 21 women. Results are presented on the per-protocol data set. All variables were analyzed by using a mixed model in week 3 of treatment, with treatment as the fixed effect, subject as the random effect, and sex as the covariate. $P < 0.05$ was considered significant.
[2] Defined as the difference in clinical outcomes between TFAs from industrially produced and natural sources.
[3] Treatment × sex interaction, $P < 0.10$.

In the present study, we used PHVOs as a positive control. This fat was selected from among different commercially available products. The criteria used for the selection of PHVOs were the absolute amount and the isomeric profile of the TFA present in the oil. This resulted in the provision by both experimental fats of an identical total combined amount of *trans*-9, *trans*-10, and *trans*-11 18:1 (Table 3). The lack of effects of TFAs from the industrial source was surprising. Numerous studies, including a meta-analysis (2), have reported that the consumption of TFAs from PHVOs increases the plasma concentrations of LDL cholesterol. In some, but not all, randomized clinical trials, a decrease in the HDL-cholesterol concentration was observed in conjunction with the increase in LDL cholesterol (21, 22).

In the experimental fats, the sum (but not the relative proportion) of the main saturated fatty acids—lauric (12:0), myristic (14:0), and palmitic (16:0) acids—that are known to have effects on HDL and LDL cholesterol, were balanced (Table 3). It was not possible to match the ratio of lauric to myristic acid found in dairy fat with a mixture of vegetable fats and oils. Although vegetable sources rich in lauric acid (eg, palm kernel fat or coconut oil) are widely available, no natural vegetable fat sources contain a substantial concentration of myristic acid. In addition, we observed some differences in the concentrations of oleic (18:1), linoleic (18:2), and conjugated linoleic acids between the experimental fats. Therefore, some of the observed treatment-related variations in plasma lipoproteins may be due to fatty acids other than

TFAs. The effect of saturated and other fatty acids on CVD risk factors can be predicted for the present study by using equations derived from a meta-analysis by Mensink et al (2). The effects of saturated fatty acids with a chain length < C12 on CVD risk factors were not reported in that meta-analysis. The natural TFA experimental fat contained substantial amounts of caprylic (8:0) and capric (10:0) acids (Table 3), so we used the equation reported for lauric acid (12:0) to predict the effect of both caprylic and capric acids in the TFAs from natural sources. (See Table S1 under "Supplemental data" in the current online issue at www.ajcn.org.) Results of these simulations based on robust data derived from the meta-analysis (2) support the idea that observed differences could not be predicted from minor differences in fatty acid composition unrelated to the TFA profile.

The aim of the present study was to compare the effect of TFAs from industrial and natural sources on CVD risk factors. The crossover design and the balance between men and women were sufficient to allow observation of important biological differences. This design could be improved, however, with the addition of a third treatment (a reference group), such as oleic or stearic acid, that is known to have no effect on the clinical outcomes of interest. The comparison of the effects of both dietary TFA sources with the effect of a reference fatty acid could help provide controls for the study. In addition, such a comparison could aid in the understanding of the potential effect on CVD risk factors of the substitution of oleic or stearic acids for TFAs. The statistical analysis showed that almost all of the sex × treatment interactions were significant (Table 5). Overall, in order to provide the analysis planned in the protocol, results for interactions focused on the primary outcome (31). The hypothesis of outliers was carefully assessed during the analysis by looking at the variation observed for cholesterol measurements (Table 5). Analysis indicated that the range and variation were characteristic of the overall population. Therefore, the use of nonparametric statistics was not justified, and mixed models were used as planned a priori (31). The analysis of differences from baseline gave exactly the same results (See Table S2 under "Supplemental data" in the current online issue at www.ajcn.org.) as did the analysis using baseline values as a covariate (Table 5).

Results from the present study show that TFAs from industrially produced sources in the diet of healthy humans results in lower plasma HDL-cholesterol concentrations than do TFAs from natural sources. Further statistical evaluation showed, however, that this effect occurred only in women. Sex differences have rarely been considered in investigations of the effect of dietary TFAs on CVD risk factors. Nevertheless, in a previous randomized controlled clinical trial conducted in an older population (63 ± 6 y old), different responses by sex to diet were observed (35). In the present study, the magnitude and sex specificity of these effects were confirmed by measures of HDL cholesterol, HDL apoA1, and the NMR evaluation of size and distribution of HDL (Table 6). In addition to HDL cholesterol, LDL cholesterol is a known risk factor for heart disease that is affected by the consumption of TFAs from industrial sources (1, 2). Only one previous study examined the size distribution of LDL when TFAs from industrial sources are consumed, and it showed that consumption of the TFAs from an industrial source increased small dense LDL more than did consumption of butter that contains a low amount of TFAs (36). Mechanisms responsible for the more pronounced atherogenicity of small and dense particles than of large LDL have been hypothesized (37). It was suggested that small LDL particles are more prone to oxidative degradation than are large LDL particles (38), but the effective atherogenic effect of LDL particles is not firmly established (39). In the present study, we were able to directly compare the effects of TFAs from industrial and natural sources on LDL size and distribution when consumed at a similar dietary intake; we found that, relative to the TFAs from an industrial source, those from a natural source had no significant effect on the total number of particles but did increase the mean size of LDL.

In the first dietary studies evaluating effects of TFAs on lipid and lipoproteins, men and women were equally responsive to the interventions with respect to plasma concentrations of both LDL and HDL (21, 22). Needless to say, many factors such as dose, prior diets, and population genetics may confound comparisons between diets, especially over a period of almost 20 y. Nonetheless, the lack of response in men in the present study to either the TFAs from industrially produced or natural sources is a significant finding. Because many of the mechanisms that could account for altering LDL and HDL cholesterol have emerged in the past 15 y, it is recommended that future studies examine the various other outcome variables to determine the mechanisms causing the sex-related variation in LDL and HDL responsiveness to diet.

Biological targets for TFAs have been recently proposed by Mozafarian et al (1); one possible mechanism was TFA regulation of CETP. In the present study, we did not observe any modification of CETP activity by TFAs from either source as assessed ex vivo (Table 5). It has been proposed (1), however, that the effect on CETP activity may be indirect (eg, upstream regulation of CETP synthesis). The *trans* Fatty Acids Collaboration (TRANSFACT) study was not designed to identify biological mechanisms that may account for the detrimental effects of TFAs, because a priori the effects of TFAs from natural sources were unknown. Nevertheless, the robust sex differences that we observed suggest that regulation of lipid metabolism by TFAs may be mediated, at least in part, by sex hormones (40). Almost all epidemiologic studies have reported a positive association between the intake of TFAs from industrially produced sources and CVD risk (1). Some studies have attempted both to isolate intakes of TFAs from different sources as discrete, independent, dietary variables and, in turn, to correlate quantitative intakes with the absolute risk of coronary heart disease (4–11). Two studies included both men and women in a parallel and unbiased fashion (5–6), and one study separated the results in men and women (6). That study, conducted in Scotland, considered sex and TFAs from different sources by using a sex-balanced group of ≈11 000 subjects. The main conclusion of the study was that the data for this population did not support a significant effect of *trans* fats in men, but the results were less clear for women (6). In women, the effects of TFAs on CVD tended to be significant in the middle quintile of total TFA consumption but not in the lower quintiles (6).

The present observations do make it easy to draw conclusions in terms of CVD risk. CVD risk is evaluated by using different biomarkers, such as LDL cholesterol, total cholesterol, HDL cholesterol, apoA1, apoB, or diagnostic ratios (ie, total:HDL cholesterol and apoA1:apoB). The results of the TRANSFACT study suggest that men and women should be considered differently, because significant sex × treatment interactions were found for almost all of the CVD risk factors measured (Table 5). For instance, a significant ($P < 0.001$) effect of TFAs from a

natural source on the total cholesterol was found on the overall population, but, when we evaluated the data, the effect found in men was not significant ($P = 0.642$). The same types of results were observed for LDL cholesterol. In women, a significant elevation was observed in both total and LDL cholesterol when subjects received TFAs from a natural source. In the guidelines of the National Cholesterol Education Program's Adult Treatment Panel III (41), concentrations of total and LDL cholesterol are taken into account to evaluate CVD risk and to follow patients who have critical concentrations of these risk factors. The HDL-cholesterol concentration also plays an important part in this evaluation (41), and that is why total:HDL cholesterol or apoA1:apoB associated with HDL or LDL particles, respectively, is used to evaluate CVD risk (2). In the present study, these 2 important diagnostic ratios were not modified by the experimental treatments. Thus, it is difficult in the present study to draw a conclusion regarding the effect of TFAs from either source on absolute CVD risk in these normolipidemic subjects.

trans Fats do not occur as a single chemical isomer in any natural or industrial food product. Industrial TFAs in foods originate from the use of PHVOs, which contain a range of TFAs. In contrast, TFAs from natural sources, which contain mainly vaccenic acid, occur in low quantities in food products derived from ruminant animals, most principally dairy products and ruminant meats. Vaccenic acid is the predominant TFA in ruminant fat, originating as an intermediate during the biohydrogenation of linoleic and α-linolenic acid. Vaccenic acid is also present in PHVOs, because the hydrogenation process leads to the formation of numerous positional and geometrical isomers of octadecenoic acid. One of the unique characteristics of vaccenic acid is that it is a substrate of the stearoyl Co-A desaturase (SCD). This enzyme can convert vaccenic acid (*trans*-11 18:1) to rumenic acid (*cis*-9,*trans*-11 18:2), a conjugated isomer of linoleic acid. The conversion rate varies, but it has been measured in several animal models and is estimated to be $\approx$20% in humans (11). A relatively high dose of TFAs from natural sources was used in the TRANSFACT study to detect a significant effect on HDL cholesterol. At this dose, no effect was observed on HDL cholesterol, but, compared with consumption of TFAs from an industrially produced source, that of TFAs from a natural source leads to an increase in other plasma lipids, such as LDL cholesterol. This adverse effect may be due to the high dose of natural TFAs consumed in the context of this clinical investigation. This amount does not reflect the consumption in any population. The current estimates for intake in the United States of TFAs from natural sources are $<$5 g/d, whereas the dose used in the present study was $\approx$11–12 g/d.

Research on *trans* fats and their potential effects on human health is of widespread interest to scientists and public health officials. Worldwide, some countries, such as Denmark, have banned outright the use of TFAs from industrial sources, whereas other countries, eg, the United States and Canada, have set limits or established rules and labeling policies for all products and ingredients containing *trans* fats. The decision to take this action was based on convincing scientific evidence that TFAs from industrially produced sources were deleterious to human health—ie, their effect of decreasing plasma HDL cholesterol and increasing LDL cholesterol markedly enhanced the risk of CVD (1, 2). The awareness of the general public of the occurrence of TFAs in natural products and the lack of scientific evidence regarding the effect of TFAs from these sources formed the basis for the current study. The TRANSFACT study is the first to directly compare the effects of food products containing TFAs from industrially produced and those containing TFAs from natural sources on CVD risk markers. The TRANSFACT study shows that TFAs from industrially produced and natural sources have different effects on CVD risk factors. The HDL cholesterol–lowering property of TFAs seems to be specific to that from industrial sources. The observed responses, however, were greater in women than in men, and the mechanism underlying these biological effects warrants further investigation.

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