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Study of the effect of *trans* fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease[1–3]

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ABSTRACT

Background: The intake of *trans* fatty acids (TFA) from industrially hydrogenated vegetable oils (iTFA) is known to have a deleterious effect on cardiovascular health, the effects of TFA from ruminants (rTFA) are virtually unknown.

Objective: The purpose of the present study was to compare the effects of rTFA and iTFA on plasma LDL concentrations and other cardiovascular disease risk factors in healthy subjects.

Design: In a double-blind, randomized crossover controlled study, 38 healthy men were fed each of 4 experimental isoenergetic diets lasting 4 wk each. The 4 diets were high in rTFA (10.2 g/2500 kcal), moderate in rTFA (4.2 g/2500 kcal), high in iTFA (10.2 g/2500 kcal), and low in TFA from any source (2.2 g/2500 kcal) (control diet).

Results: Plasma LDL-cholesterol concentrations were significantly higher after the high- rTFA diet than after the control ($P = 0.03$) or the moderate- rTFA ($P = 0.002$) diet. Plasma LDL-cholesterol concentrations also were significantly ($P = 0.02$) higher after the iTFA diet than after the moderate-rTFA diet. Plasma HDL-cholesterol concentrations were significantly ($P = 0.02$) lower after the high-rTFA diet than after the moderate-rTFA diet. Finally, all risk factors were comparable between the control and the moderate-rTFA diets.

Conclusions: These results suggest that, whereas a high dietary intake of TFA from ruminants may adversely affect cholesterol homeostasis, moderate intakes of rTFA that are well above the upper limit of current human consumption have neutral effects on plasma lipids and other cardiovascular disease risk factors. *Am J Clin Nutr* 2008;87:593–9.

KEY WORDS *trans* Fatty acids, ruminants, industrial sources, plasma lipids, lipoproteins, cardiovascular disease, healthy men

INTRODUCTION

Dietary *trans* fatty acids (TFA) come mostly from the industrial hydrogenation of unsaturated vegetable oils; they are found in manufacturing products such as cookies, pastries, and salad dressings. TFA also are formed during the natural bacterial hydrogenation of unsaturated fatty acids that occurs in the rumen of polygastric animals such as cattle, sheep, and goats. TFA from ruminants are mainly found in dietary products derived from the animals' milk and meat. A number of epidemiologic studies have provided virtually undisputed evidence that the consumption of TFA from industrial sources (iTFA) increases the risk of cardiovascular disease (CVD) (1). The results of clinical studies have clearly shown that a greater dietary intake of iTFA has a deleterious effect on lipoprotein-lipid concentrations (1). Specifically, the consumption of iTFA has been associated with higher plasma concentrations of total cholesterol, LDL cholesterol, and triacylglycerols and lower HDL-cholesterol concentrations than is consumption of unsaturated vegetable oils (2). We have also previously reported a greater proportion of small, dense LDL after the intake of iTFA than after that of butter and unsaturated vegetable oils (3).

These deleterious effects of iTFA on cardiovascular health are well established (4), but the effects of TFA from ruminants (rTFA) on CVD risk factors have not been as extensively studied. Results from a few epidemiologic studies have suggested that rTFA may be less detrimental to heart health than are iTFA (5–8). However, 2 epidemiologic studies observed that rTFA had the same negative effect on CVD risk factors as iTFA (9, 10). Previous clinical studies, of which there are only a few, have not yet been able to address this issue with consistent results, largely because of major differences in study design and experimental approaches (11, 12). The purpose of the present study was to compare for the first time on a gram-for-gram basis the effect of naturally produced rTFA and iTFA on plasma LDL-cholesterol concentrations and other CVD risk factors in healthy men. Another objective was to verify the extent to which an exposure corresponding to an achievable but still high intake of rTFA may also lead to changes in plasma LDL-cholesterol concentrations and other CVD risk factors.

SUBJECTS AND METHODS

Population

Forty-eight healthy men were recruited in the Québec City area to participate in the study. Of the 38 participants who completed the study, 36 were white and 2 were black. Subjects were

[1] From the Institute of Nutraceuticals and Functional Foods, Laval University, Quebec, Canada (AM-B, AC, GG, PP, YC, SL, and BL), and the Lipid Research Center, Centre Hospitalier Universitaire de Québec Research Center, Québec, Canada (PC).

[2] Supported by an unrestricted grant from the Dairy Farmers of Canada, by Novalait Inc, and by a grant from the Natural Sciences and Engineering Research Council of Canada. BL holds a Canada Research Chair in Nutrition and Cardiovascular Health. The Canadian Dairy Commission donated the low-rTFA control butter; Provigo-Loblaws donated the foods used in the present study.

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initially screened on the basis of a complete physical examination and medical history. The subjects recruited for the study had to be nonsmokers, to be 18–65 y old, and to have a body mass index (BMI; in kg/m^2) between 18 and 30. Exclusion criteria were the presence of a monogenic dyslipoproteinemia or any diagnosed endocrine disorder; the use of any medication including those known to affect lipid metabolism; the presence of a chronic, metabolic, or acute disease; and significant weight change within the 6 mo before the experiment. Men with food allergies, with an aversion to foods included in the experimental diets, or with a usual alcohol consumption of >2 drinks/d were also excluded.

The study protocol was fully explained to all participants, who gave written informed consent at the beginning of the study. The protocol was approved by the Clinical Research Ethics Committee of Laval University.

Production of *trans* fatty acids from ruminants and control fat

TFA-enriched butter was generated from milking cows whose diet had been modified as described previously (13). Briefly, a group of 28 cows were fed a total mixed diet composed of grass silage, energy and protein supplements, and 4% safflower oil. After 4 wk, milk samples from each cow were obtained, and the 18:1*t* content of cow-milk fat was measured as described below. Cows with the greatest concentration of C18:1*t* ($n = 7$) were kept on this diet for the purpose of milk collection and the manufacturing of rTFA-enriched butter. Milk was accumulated in a refrigerated (4 °C) bulk tank at the Laval University Experimental Farm and transported twice a week to our Research Institute Pilot Plant. Raw milk was first separated into skim milk and cream with the use of a cream separator (model #619; De Laval, Peterborough, Canada). Cream was then standardized to 39% fat, immediately pasteurized at 78 °C for 16 s by using a heat exchanger (model CF125; Chalinox Ltd, Sorel, Canada), and then churned at 0–1 °C to obtain butter. The control low-rTFA milk fat was butter obtained from the Canadian Dairy Commission (winter 2006 production). Commercially available shortenings were used as a source of iTFA, and products were selected on the basis of their iTFA content. Characterization of the fatty acid composition of experimental butterfat and shortenings was carried out with a gas chromatograph (HP 5890; Hewlett-Packard Co, Palo Alto, CA) equipped with a 100-m CP Sil 88 capillary column (Chrompack, Middelburg, Netherlands) and a flame ionization detector (Hewlett-Packard Co) (14). Feeding safflower oil to dairy cows increases the total TFA content of milk fat without modifying its isomeric distribution. The absolute amount of vaccenic acid (18:1*t*-11), the predominant TFA in the TFA-enriched butter, was >4 times that in the control butter (**Table 1**). This particular isomer represented 41%, 43%, and 16% of total TFA in the control butter, the rTFA-enriched butter, and the shortening, respectively (**Figure 1**).

TABLE 1
Fatty acid composition of the experimental butter enriched with *trans* fatty acids (TFA) from ruminants (rTFA), the control butter, and the shortening[1]

	Experimental fat		
	rTFA-enriched butter	Control butter	Shortening
	g/100 g		
SFA			
4:0	3.9	3.8	—[2]
6:0	1.9	2.1	—
8:0	1.0	1.2	—
10:0	2.0	2.7	—
12:0	2.2	3.1	0.4
14:0	7.8	9.8	0.4
15:0	0.8	1.2	—
16:0	17.8	26.2	15.6
17:0	1.5	2.4	0.4
18:0	10.6	8.0	9.8
20:0	0.1	0.1	0.4
Total	49.6	60.6	27.0
MUFA *cis*			
14:1*c*	0.8	1.0	—
16:1*c*	1.2	1.6	0.1
18:1*c* (total)	18.8	15.8	27.5
Total	20.8	18.4	27.6
TFA			
18:1*t* (total)	10.0	2.4	28.0
18:2*t* (total)	0.5	0.1	0.7
Total	10.5	2.5	28.7
PUFA			
18:2*c*	2.4	1.7	11.8
18:3*c*	0.3	0.6	0.6
Total	2.7	2.3	12.4
CLA	2.0	0.5	0.2

[1] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated fatty acids.
[2] No content of this fatty acid above detectable concentrations (all such).

Diets and study design

The present study was undertaken as a double-blind, randomized, crossover controlled study according to a Latin square design, in which each participant was assigned to 4 different isocaloric diets lasting 4 wk each. Thirteen of 24 possible diet sequences were randomly attributed to participants. Each of the 4 diets started 3 or 4 of the 13 sequences. Each diet was separated by a wash-out period of 3 to 12 wk. The 4 experimental diets used in the present study were diets that were high in rTFA (10.2 g/2500 kcal), moderate in rTFA (4.2 g/2500 kcal), high in iTFA (10.2 g/2500 kcal), or low in TFA from any source (2.2 g/2500 kcal) (control diet). Specific vegetable and animal oils and fat were incorporated in each diet to minimize differences in the amounts of saturated fatty acids (SFA) and unsaturated fatty acids between treatments (**Table 2**). As a result, the 4 experimental fats used to formulate the diets contained relatively comparable amounts of every major type of SFA, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (**Table 3**). All diets were identical in terms of menus, calories, and macronutrient composition, with the exception of TFA sources and concentrations (**Table 4**). On average, the 4 experimental diets were formulated to provide 50% of daily calories from carbohydrate, 14% from protein, and 37% from fat. The rTFA and iTFA provided 3.6% of daily energy intake in the high TFA diets, and the rTFA provided 1.5% of daily energy intake in the moderate rTFA diet. Finally, the control diet provided 0.8% of daily energy intake from rTFA and 0% from iTFA. The intake of SFA was similar between diets, but minor differences were observed in the intakes of MUFA and PUFA (Table 3). Experimental diets were formulated by using NUTRITION DATA

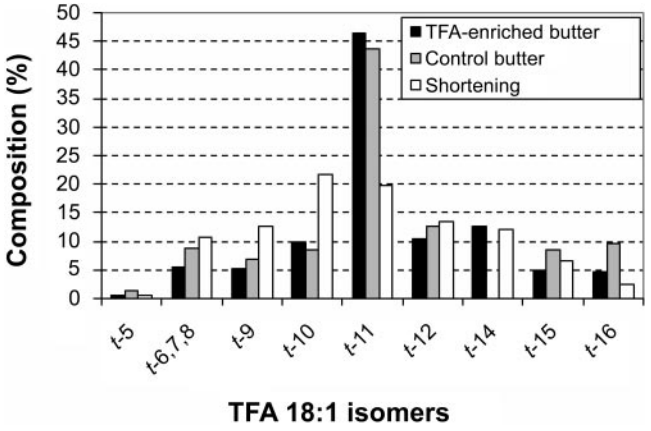

FIGURE 1. Distribution of the 18:1 *trans* fatty acid (TFA) isomers of the butter enriched with TFA from ruminants, the control butter, and the shortening used in the present study.

SYSTEM (NDS) software (version 4.03_31; Nutrition Coordinating Center, Minneapolis, MN).

A validated food-frequency questionnaire was administered to the participants by a registered dietitian at the beginning and at the middle of the study to estimate the energy intake required to keep the body weight constant (15). Each participant was assigned to a value of energy intake that was revised when required to minimize body weight fluctuation during the study. Body weight was recorded on all weekdays just before lunch. All meals were provided to participants so that control for energy and macronutrient intake was optimized. On weekdays, subjects came to the Clinical Investigation Unit at the Institute of Nutraceuticals and Functional Foods at Laval University to consume their lunch under the supervision of ≥1 member of the research team, who was blinded to subject's treatment. At that time, they were also given their dinner and the next day's packaged breakfast to take home. Weekend meals were prepared, packaged, and provided at the Clinical Investigation Unit on the Friday lunchtime visits. All take-home meals were provided in containers that could be heated in a microwave when necessary, which obviated the need to transfer the food from the containers before consumption.

The breakfast meal represented 20% of the daily energy intake; the lunch and dinner meals each provided 40% of the daily energy intake. Subjects were instructed to consume their entire meals. Subjects had free access to water and to caffeine-free diet beverages. Consumption of tea and coffee was allowed with a limit of 2 cups/d (500 mL/d). Supplementation with vitamins and natural health products and alcohol consumption were strictly forbidden during all treatment phases. Throughout the study, participants were asked to maintain their usual level of physical activity, which was evaluated by a weekly questionnaire completed by the subjects.

TABLE 2
Composition of the mixed fat added to the 4 experimental diets[1]

		rTFA		
	Control	Moderate	High	iTFA
		g/2500 kcal		
rTFA-enriched butter	—[2]	24.5	98.4	—
Control butter	75.0	55.3	—	60.0
Shortening	—	—	—	26.6
Canola oil	20.0	20.4	—	—
Fractionated palm oil	—	—	6.0	—
Flax oil	—	—	3.9	3.5
Safflower oil	—	1.0	0.8	—
Coconut oil	—	—	—	1.5
Olive oil	—	—	—	4.9
Peanut oil	4.6	—	—	—
Egg yolk	3.4	2.4	0.8	5.5

[1] TFA, *trans* fatty acids; rTFA, TFA from ruminants; iTFA, TFA from industrial sources.
[2] No content of this fatty acid above detectable concentrations (all such).

TABLE 3
Fatty acid composition of the 4 experimental diets[1]

		rTFA		
Fatty acid	Control	Moderate	High	iTFA
		g/2500 kcal		
SFA				
4:0	2.88	3.10	3.77	2.31
6:0	1.55	1.60	1.78	1.26
8:0	0.92	0.93	0.95	0.85
10:0	2.00	1.95	1.85	1.72
12:0	2.34	2.25	2.06	2.63
14:0	7.5	7.5	7.6	6.4
16:0	23.3	22.1	22.0	23.3
17:0	1.78	1.70	1.45	1.55
18:0	7.5	8.4	11.4	8.7
20:0	0.32	0.27	0.15	0.22
22:0	0.22	0.09	0.02	0.02
Total	50.3	49.9	53.0	49.0
MUFA *cis*				
14:1*c*	0.72	0.73	0.77	0.57
16:1*c*	1.52	1.48	1.37	1.33
18:1*c*	29.9	29.7	25.4	26.2
20:1*c*	0.49	0.44	0.12	0.13
22:1*c*	0.14	0.14	0.02	0.02
Total	32.8	32.5	27.7	28.3
PUFA				
18:2*c*	9.5	9.1	6.5	7.9
18:3*c*	2.74	2.75	2.70	2.76
18:4*c*	0.009	0.009	0.009	0.009
20:4*c*	0.077	0.074	0.069	0.086
20:5*c*	0.046	0.046	0.046	0.046
22:5*c*	0.091	0.079	0.064	0.064
22:6*c*	0.18	0.18	0.16	0.18
Total	12.6	12.2	9.6	11.0
TFA				
16:1*t* (total)	0.02	0.02	0.02	0.02
18:1*t* (total)	2.07	4.03	9.73	9.11
18:2*t* (total)	0.15	0.24	0.50	1.11
Total	2.2	4.3	10.3	10.2

[1] TFA, *trans* fatty acids; rTFA, TFA from ruminants; iTFA, TFA from industrial sources; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values presented in this table are derived from the analysis of the macronutrient content of the experimental fats combined with data from our nutrient database.

Compliance

A 7-d cyclic menu was developed in such a way that it was very similar to a typical Canadian diet, which optimized compliance

TABLE 4
Mean nutritional composition of the four experimental diets for the 38 subjects[1]

Variable	Control	rTFA		iTFA
		Moderate	High	
Energy (kcal)	3196 ± 519[2]	3239 ± 491	3213 ± 527	3268 ± 525
Carbohydrates (%)	50.1	49.7	48.8	50.2
Proteins (%)	14.0	14.0	14.0	14.0
Lipids (%)	37.0	37.4	38.1	37.0
SFA (%)	18.5	18.3	19.4	18.0
MUFA (%)	11.8	11.8	10.0	10.1
PUFA (%)	4.6	4.4	3.5	4.0
TFA (%)	0.8	1.5	3.7	3.7
Total fibers (g/2500 kcal)	20.8	20.9	20.7	20.8
Cholesterol (mg/2500 kcal)	298.6	294.8	303.3	298.7

[1] TFA, *trans* fatty acids; rTFA, TFA from ruminants; iTFA, TFA from industrial sources; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] $\bar{x}$ ± SD (all such values).

with the protocol. A checklist was provided to all participants to identify foods that they had or had not consumed when eating outside the Clinical Investigation Unit. This list also provided space to indicate unlisted but permitted food items that had been consumed in addition to the formulated diets. Concurrent use of medication during the experimental protocol was also tracked by using this list. However, participants had to notify the physician in charge of the clinical aspects of the study before initiating any medication.

Risk factor assessment

Plasma lipid concentrations

Fasting blood samples (12-h fast) were collected from an antecubital vein at the beginning (day 1) and the end (day 26) of each experimental period. Assessments of the basic lipid profile and of lipoprotein-lipid concentrations by ultracentrifugation were performed according to previously described methods (16–19).

Anthropometry and blood pressure

At the beginning and at the end of each experimental diet, body weight and waist and hip circumferences were measured according to standardized procedures (20). Blood pressure was measured after a 5-min rest in the sitting position. It was measured on the right arm with the use of a standard mercury sphygmomanometer and was computed as the mean of 3 readings, separated by a 3-min interval. The Korotkoff sound V was taken as the diastolic blood pressure.

Statistical analyses

The primary analysis compared the values of each outcome measured at the end of the 4 experimental diets according to a Latin square study design. Data were analyzed by using the PROC MIXED procedure for repeated measures in SAS software (version 8.02; SAS, Inc, Cary, NC). The structure of the covariance matrix for each variable (intrasubject autocorrelation across repeated measures) was taken into account in all analyses to ensure the most adequate statistical fit and power. The Tukey adjustment was used to account for the multiple comparisons of the 4 diets. Comparisons of postdiet values for each outcome are presented with and without adjustment for baseline values measured at the beginning of each dietary phase. Carryover effects were tested by introducing terms reflecting the interaction between the sequence of treatments and the treatments per se. Group averages are reported as means ± SDs unless stated otherwise. C-reactive protein (CRP) and triacylglycerol concentrations and the ratios of total to HDL cholesterol (total:HDL cholesterol) and of apolipoprotein (apo)B to apoA1 (apoB:apoA1) were logarithmically transformed before statistical analysis. Four CRP concentrations values >10 mg/L at different time-points in different participants were excluded from analysis because they suggested the presence of bacterial infection or inflammation (21). Differences were considered significant at $P \leq 0.05$.

RESULTS

Characteristics of subjects at baseline and compliance

Of the 48 men enrolled in the study, a total of 10 dropped out: 2 subjects moved during the course of the study, 6 found the study protocol too demanding and decided to stop, 1 was excluded because he was not compliant, and 1 was excluded for health reasons that were not related to the study. Of the 10 dropouts, 7 occurred during or immediately after the first diet, 2 occurred after 2 diets, and 1 occurred after 3 dietary phases. The final study group ($n = 38$) included only the subjects who completed the 4 diets. These 38 men were in good health and had a mean age of 32.8 ± 15.0 y (**Table 5**). Mean blood lipid concentrations were within normal values. None of the subjects had diabetes. Of the food checklists provided weekly to the participants to evaluate compliance, 84% were completed; on the basis of those lists, we calculated that 99.9% of the food provided had been consumed.

Anthropometric measures

There was no significant difference between the subjects' weight at the end of the 4 experimental diets (**Table 6**). The significant difference in waist circumference between diets was no longer significant when baseline values were not included in the analysis. On the basis of that change, because there was no

TABLE 5
Physical characteristics and plasma lipid profile of the 38 subjects at baseline[1]

Variable	Value
Age (y)	32.8 ± 15.0
Weight (kg)	73.8 ± 9.8
BMI (kg/m^2)	23.6 ± 3.3
Waist girth (cm)	81.5 ± 9.9
Systolic BP (mm Hg)	114.1 ± 11.8
Diastolic BP (mm Hg)	72.5 ± 8.1
Fasting glucose (mmol/L)	5.08 ± 0.44
Cholesterol (mmol/L)	4.32 ± 1.03
LDL-C (mmol/L)	2.56 ± 0.86
HDL-C (mmol/L)	1.25 ± 0.23
Triacylglycerol (mmol/L)	1.14 ± 0.81

[1] All values are $\bar{x}$ ± SD. BP, blood pressure; C, cholesterol.

TABLE 6
Body-composition variables and plasma cholesterol concentrations at the end of each dietary intervention in the 38 subjects[1]

	Diet						
		rTFA				*P*	
Variable	Control	Moderate	High	iTFA	Pooled SD[2]	Unadjusted	Adjusted[3]
Weight (kg)	74.2 ± 8.8[4]	74.3 ± 8.7	74.6 ± 8.6	74.6 ± 9.3	1.91	0.41	0.43
Waist girth (cm)	81.1 ± 9.0	80.7 ± 8.7[5]	81.3 ± 8.6	81.2 ± 8.9[6]	2.47	0.15	0.01
Cholesterol (mmol/L)	4.77 ± 0.93	4.72 ± 0.88	4.92 ± 0.98[6]	4.88 ± 0.95[6]	0.45	0.009	0.004
VLDL-C (mmol/L)[7]	0.21 ± 0.16	0.22 ± 0.15	0.23 ± 0.18	0.24 ± 0.22	0.67	0.37	0.56
LDL-C (mmol/L)	3.27 ± 0.80	3.22 ± 0.83	3.47 ± 0.90[5,6]	3.42 ± 0.89[6]	0.39	0.002	0.001
HDL-C (mmol/L)	1.25 ± 0.24	1.28 ± 0.28	1.22 ± 0.26[6]	1.23 ± 0.24	0.16	0.066	0.046
HDL_2-C (mmol/L)	0.59 ± 0.21	0.59 ± 0.21	0.54 ± 0.19[5]	0.56 ± 0.18	0.12	0.08	0.04
HDL_3-C (mmol/L)	0.66 ± 0.14	0.69 ± 0.13	0.68 ± 0.11	0.67 ± 0.13	0.16	0.34	0.32
TG (mmol/L)[7]	0.98 ± 0.45	0.95 ± 0.41	0.99 ± 0.43	0.97 ± 0.54	0.35	0.84	0.87
ApoB (g/L)	0.94 ± 0.23	0.91 ± 0.21	0.96 ± 0.23[6]	0.94 ± 0.22[6]	0.12	0.03	0.009
ApoA1 (g/L)	1.51 ± 0.19	1.53 ± 0.18	1.49 ± 0.18[6]	1.52 ± 0.18	0.13	0.08	0.049
Total/HDL-C[7]	3.97 ± 1.16	3.86 ± 1.16	4.23 ± 1.32[6]	4.16 ± 1.39[6]	0.14	0.002	0.003
LDL-C/HDL-C	2.75 ± 1.00	2.67 ± 1.01	3.02 ± 1.15[5,6]	2.94 ± 1.17	0.51	0.003	0.002
ApoB/apoA1[7]	0.63 ± 0.21	0.60 ± 0.17	0.65 ± 0.19	0.63 ± 0.17	0.16	0.009	0.040
CRP (mg/L)[7]	0.99 ± 1.49	0.79 ± 1.37	0.72 ± 0.69	0.74 ± 0.96	1.18	0.60	0.61

[1] rTFA, *trans* fatty acids from ruminants; iTFA, TFA from industrial sources; C, cholesterol; TG, triaclyglycerol; apo, apolipoprotein; CRP, C-reactive protein.
[2] Pooled SD represents the SD of the change in each variable when subjects switched from the control diet to any of the other 3 experimental diets. A greater value generally reflects a greater intraindividual variability in the response to the moderate-rTFA, high-rTFA, and iTFA diets.
[3] Adjusted for diet-specific baseline values.
[4] $\bar{x}$ ± SD (all such values).
[5] Significantly different from the control diet, $P < 0.05$.
[6] Significantly different from the moderate-rTFA diet, $P < 0.05$.
[7] Analysis was performed on log-transformed values. For the apoB/apoA1 ratio, there was a significant difference between diets, but the only specific difference that neared significance was that between the moderate- and the high-rTFA diets ($P = 0.057$).

difference in body weight between diets, and because each dietary treatment was only 4 wk, we think that these marginal but significant ($P = 0.01$) changes in waist girth may have had essentially no influence in the present study.

Cardiovascular disease risk factors

There were significant differences between the 4 diets in the concentration of total ($P = 0.004$), LDL ($P = 0.001$), and HDL ($P = 0.046$) cholesterol after adjustment for baseline values. Plasma total cholesterol and apoB concentrations were higher after the consumption of the high- rTFA and -iTFA diets than after consumption of the moderate-rTFA diet. Concentrations of LDL cholesterol were also higher after the high-rTFA diet than after the control ($P = 0.03$) and the moderate-rTFA ($P = 0.002$) diets, whereas the LDL-cholesterol concentrations were higher after the high-iTFA diet than after the moderate-rTFA diet ($P = 0.02$). The moderate-rTFA diet was associated with the most favorable lipid values, although, in comparison with the control diet, none of the differences reached significance. Plasma HDL-cholesterol concentrations were significantly ($P = 0.02$) lower after the high-rTFA diet than after the moderate-rTFA diet. This reduction was attributed to the HDL_2-C subfraction more than to the HDL_3-C subfraction. It must be noted that differences in plasma HDL cholesterol ($P = 0.066$), HDL_2-C ($P = 0.08$), and apoA1 ($P = 0.08$) between diets were not significant when diet-specific baseline values were not included as covariate in the analysis, but general trends were maintained. Total:HDL cholesterol was significantly higher after the consumption of the high-rTFA and -iTFA diets than after consumption of the moderate-rTFA diet ($P = 0.002$ and $P = 0.05$, respectively). Generally similar results were obtained when computing apoB: apoAI or the ratio of LDL to HDL cholesterol. Finally, no significant difference in plasma triacylglycerols and CRP concentrations was observed between the 4 diets.

DISCUSSION

Whereas iTFA are well known to have negative effects on several CVD risk factors, little is known about the effects of rTFA (22, 23). The purpose of the present study was to investigate the effect of rTFA on plasma cholesterol concentrations and other risk factors for CVD. The high-rTFA diet was tested to compare, for the first time in a clinical and controlled nutritional study, the effect of naturally occurring TFA with that of iTFA on a gram-to-gram basis. However, because an intake of 10.2 g rTFA/d (based on 2500 kcal) cannot be reached in a normal diet without the use of a butter purposefully enriched with TFA, the moderate-rTFA diet (1.5% of energy intake) was incorporated into the study design to test the effect of rTFA at a concentration that could possibly be achieved through a very high intake of dairy products and meat from ruminants. The Food and Drug Administration (Internet: http://www.cfsan.fda.gov/~/rd/fr03711a.html) estimated that the adult population's average daily consumption of rTFA was 1.5 g for men and 0.9 g for women—or 1.2 g on average for both sexes—representing 0.5% of calories. Thus, an intake of 1.5% energy as rTFA remains very high in practical terms. Such an intake would correspond to the cumulative 1-d consumption of 4 portions of cheese (4 × 50 g,

33% fat), 2 portions of milk (2 × 250 mL; 3.25% fat), one portion of yogurt (175 g; 3.25% fat), and 8 teaspoons of butter (8 × 5 mL).

Our results showed that a high intake of rTFA was associated with deleterious changes in cholesterol homeostasis, whereas a moderate intake of rTFA had no effects. We also observed that the consumption of iTFA was associated with unfavorable changes in CVD risk factors that were significantly greater than the changes observed with the moderate intake of rTFA, whereas differences observed with the control diet were not significant. Very few studies have investigated the effect of rTFA on the lipid profile and other CVD risk factors in humans. In the Nurses' Health Study, the positive association between total TFA intake and coronary heart disease was entirely due to the intake of partially hydrogenated vegetable oil, and the association between the intake of rTFA and coronary heart disease with nonsignificant and inverse (6). Other epidemiologic studies have shown that the intake of iTFA was positively associated with the risk of coronary heart disease, but no positive association was observed for the intake of rTFA (7, 24). The mean intake of rTFA in epidemiologic studies was estimated to be much less than that in the moderate-rTFA diet tested in the present study.

In an intervention study conducted with parallel arms in 42 healthy males, Tholstrup et al (11) observed that the consumption of an rTFA-enriched butter (3.6 g/d) lowered total and HDL-cholesterol concentrations by 6% and 9%, respectively, as compared with the concentrations found with use of a control butter low in rTFA and high in SFA; however, plasma LDL-cholesterol concentrations did not differ significantly between the 2 diets. Those authors concluded that the lower concentrations of total and HDL cholesterol after the diet with rTFA-enriched butter may have been due to a higher content of MUFA and a lower content of SFA in the rTFA-enriched butter rather than to the rTFA content per se. In a previous controlled experiment conducted by our group, the consumption of an rTFA-enriched butter (4.7 g TFA/2500 kcal) had no effect on the change from baseline (preexperimental) plasma total cholesterol values and total:HDL cholesterol (−0.02 and −0.00 mmol/L, respectively). There were, however, important differences between that earlier study and the current experiment in the sources of fat that were used to adjust the fatty acid profile of experimental diets (25). In the present study, the major difference in macronutrient content between the control diet and the moderate rTFA diet was the TFA content per se. All of these results, combined with the fact that we did not observe any difference in total or HDL-cholesterol concentrations between the 2 diets, provide further support for the theory that dietary rTFA at a high but achievable intake has no effect on blood lipids.

Many clinical studies have evaluated the effect of iTFA on the CVD risk lipid profile. Lichtenstein et al (2) showed in a randomized controlled study that an increased intake of iTFA was associated with unfavorable changes in plasma cholesterol concentrations. However, it must be stressed that the individual effect of iTFA on CVD risk factors could not be isolated in that landmark study. Indeed, the study compared different margarines with various amounts of TFA, but also of MUFA and PUFA. In present study, several sources of fat were mixed in each diet to minimize the differences attributed to the individual fatty acids and to maximize the difference attributed to the amount and the origin of the TFA. Power calculation based on numbers generated in the present study indicated that differences in plasma LDL-cholesterol concentrations between the iTFA diet and the control diet would have reached significance if we had investigated a total of 52 participants. Finally, although differences between the iTFA and control diets were not significant, it must be recognized that the magnitudes of the observed effects were nevertheless very similar to those seen with the high-rTFA diet.

CRP is a marker of systemic inflammation, and high concentrations of CRP are independent predictors of CVD (26). Our results have shown no significant difference in CRP concentrations between the 4 diets. Power calculations based on values from the present study indicated that >200 subjects would have been required to obtain a significant difference in plasma CRP between the high-rTFA diet (the lowest CRP achieved) and the control diet (the highest CRP achieved). These numbers reflect the large interindividual variability in the CRP response to the diets and the fact that the present study was not a priori designed and powered specifically to investigate the effect of TFA from different sources on plasma CRP concentrations. Other clinical studies have shown that a consumption of iTFA and rTFA had no effect on plasma CRP concentrations (11, 27), whereas a positive association was found between TFA intake and CRP concentrations in the Nurses' Health Study (28) and a controlled clinical study by Baer et al (29).

One of the limitations of the present study was the dietary intakes of total fat and SFA, which were higher than the current recommendations for heart disease prevention (30). However, the total fat and SFA intakes were similar across the 4 experimental diets. The high-rTFA and -iTFA diets also were slightly lower in PUFA and MUFA than was the control diet. Nevertheless, because the dietary TFA represented the most important difference between the 4 diets, we believe that the small differences between the diets in MUFA and PUFA content may have influenced the results in only a minimal way. Another factor of interest in the present study is the differences between the 4 diets in the amount of conjugated linoleic acid (CLA). As is rTFA, CLA is synthesized in the rumen of polygastric animals through the bacterial hydrogenation of unsaturated vegetable oils. The high-rTFA diet thus had a higher CLA content. Because studies that have observed the effects of CLA on health have generated conflicting results (25, 31), it is difficult to identify the potential effect of CLA on our results.

In summary, we showed that a high intake of rTFA may lead to deleterious changes in lipid CVD risk factors, similar to those that have been attributed to TFA from industrial sources. However, data also indicated that an intake of rTFA that may be practically attained by the consumption of large quantities of dairy products had no effect on CVD risk factors. On the basis of these observations and of data from previous studies, we propose that the current intake of rTFA by the population, which corresponds to approximately one-third of that achieved in the moderate-rTFA diet in the present study, is unlikely to lead to deleterious changes in CVD risk. Because this controlled clinical study is among the first to compare the effect of rTFA and iTFA on CVD risk factors, these results will have to be confirmed by additional studies.

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