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Randomized controlled study of the effect of a butter naturally enriched in *trans* fatty acids on blood lipids in healthy women[1–3]

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ABSTRACT

Background: Whereas the negative effect of consuming *trans* fatty acids found in partially hydrogenated vegetable oils on cardiovascular disease (CVD) risk is well established, the effect of *trans* fatty acids from ruminant sources (rTFAs) on CVD risk factors has not yet been established, particularly among women.

Objective: We investigated the effects of a butter naturally enriched in rTFAs, of which vaccenic acid is the predominant isomer, on plasma lipid concentrations among healthy women.

Design: In a double-blind, randomized, crossover controlled study, 61 healthy women aged 19–70 y were fed 2 isoenergetic diets lasting 4 wk each. The 2 diets were defined as moderately high in rTFAs (3.7 g/d, 1.5% of daily energy) and control (0.9 g/d, 0.3% of daily energy).

Results: No significant effect of the rTFA diet was found on total plasma cholesterol, LDL cholesterol, apolipoprotein B, apolipoprotein A-I, and triglyceride concentrations compared with the control diet. There was a small yet statistically significant reduction in plasma HDL-cholesterol concentrations with the rTFA diet (-2.8%; $P = 0.004$), which was significant (P for the BMI $\times$ treatment interaction = 0.006) among women with a BMI (in kg/m^2) $\geq$25 (-5.2%; $P = 0.004$; $n = 18$) but not among women with a BMI $<$25 (-1.2%; $P = 0.13$; $n = 43$).

Conclusions: These results suggest that an increase in dietary rTFAs equivalent to $\sim$1% of daily energy has no significant effect on LDL but may be associated with a reduction in plasma HDL-cholesterol concentrations, particularly in overweight women. This trial is registered at clinicaltrials.gov as NCT00930137. *Am J Clin Nutr* 2012;95:318–25.

INTRODUCTION

Whereas the negative effect of consuming TFAs[4] found in PHVO on health, particularly on CVD risk, is well established and recognized (1), the association between dietary rTFAs and CVD risk has yet to be more formally established. A recent quantitative review has suggested that TFAs from PHVOs and rTFAs may have similar effects on plasma lipids (2), but this conclusion remains uncertain because it is based on only 5 studies (3–7). Data in particular subgroups of the population are also utterly lacking. Specifically, women are greatly underrepresented in the 5 rTFA clinical studies on blood lipids published to date, representing only 11% of subjects. Interestingly, in the one study that did include both sexes, the increase in the LDL-/HDL-cholesterol ratio in women after consumption of rTFAs (5% of energy) was 2.4 times the change seen in men (4). In contrast, results from epidemiologic studies suggest that women may respond more favorably to dietary rTFA exposure than men (8, 9). These differences emphasize the need for further research documenting the effect of rTFAs in various subgroups of the population, particularly among women.

In a previous controlled feeding study in men, we showed that a moderately elevated intake of rTFAs (1.5% of daily energy) had no effect on CVD risk factors, which suggests that, even at the upper limit of human consumption, rTFAs may have little effect on CVD risk factors (3). This is consistent with previous epidemiologic studies in men, which have generally reported no effect of rTFAs on CVD risk across usual dietary intakes (8, 10, 11). The extent to which a moderate increase in dietary intake of rTFAs has an effect (positive or negative) on plasma lipid concentrations in women has not been studied yet. Hence, this study was designed to investigate the effect of a high but yet achievable intake of rTFAs on plasma lipid concentrations among healthy women of various ages and with a broad range of plasma LDL-cholesterol concentrations.

SUBJECTS AND METHODS

Population

Seventy-two women were recruited in the Québec City metropolitan area through the media and electronic newsletter to participate in the study. Recruitment took place at the Institute of Nutraceuticals and Functional Foods between September 2009 and April 2010. Subjects were initially screened on the basis of

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[4] Abbreviations used: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CLA, conjugated linoleic acid; CVD, cardiovascular disease; EL, endothelial lipase; FSH, follicle-stimulating hormone; HL, hepatic lipase; PHVO, partially hydrogenated vegetable oil; rTFA, ruminant *trans* fatty acid; TFA, *trans* fatty acid.

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a complete physical examination and medical history. The subjects recruited for the study had to be 18–70 y of age, to have a BMI (in kg/m^2) between 18 and 30, and to have a plasma LDL-cholesterol concentration within 2.0–4.2 mmol/L. Exclusion criteria were the presence of a previous history of CVD, type 2 diabetes, monogenic dyslipidemia, or any diagnosed endocrine disorders; the use of medications for hyperlipidemia or hypertension; and a significant weight change within the 6 mo before the experiment. Among premenopausal women, only those with a regular menstrual cycle (25–35 d) for the past 3 mo were included. This combined with the fact that measurements were performed at the end of each 4-wk diet maximized the chance of having women being tested within the same phase of their menstrual cycle. All postmenopausal women were eligible, irrespective of their hormone supplementation status. Women with nutritional habits such as vegetarianism, with food allergies or aversion to foods included in the experimental diets, with alcohol consumption of >2 drinks/d, and elite athletes were excluded. Smokers and women who use contraceptive agents were not excluded from the study. FSH measurements were performed to confirm the premenopausal or postmenopausal status (FSH <20 IU/L or FSH >25 IU/L). 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 the TFA-enriched milk fat

The butter enriched with rTFAs was obtained by altering the cows' diets as previously described (3), with slight modification. Briefly, a group of 31 cows were fed a mixed diet composed of concentrates + alfalfa and corn silages with the addition of 3.6% corn oil. Milk samples were taken after 4 wk, and the 13 cows with the highest concentration of *trans*-18:1 continued consuming the diet for the purpose of milk collection and the manufacturing of the rTFA-enriched butter. The control low-rTFA butter was obtained from the Canadian Dairy Commission (March–April 2009 production). The characterization of the fatty acid composition of experimental butterfat was carried out by gas chromatography (HP 5890; Hewlett-Packard Co) with a 100-m CP Sil 88 capillary column (Chrompack; Middelburg) and a flame ionization detector (12). Feeding corn oil to dairy cows increased the total TFA content of milk fat without significantly modifying its isomeric distribution. The absolute amount of vaccenic acid (18:1*t*-11), the predominant TFA in the TFA-enriched butter, was 3.7 times that in the control butter and accounted for 37.1% and 47.5% of total rTFAs in the control butter and the rTFA-enriched butter, respectively (**Table 1**). The 16:1*t* isomers were not measured.

TABLE 1
Fatty acid composition of the experimental butter enriched with rTFAs and of the control butter[1]

	Control butter	rTFA butter
SFAs	68.4	54.2
4:0	4.7	3.7
6:0	2.3	1.5
8:0	1.3	0.8
10:0	2.9	1.6
12:0	3.3	1.9
14:0	11.0	7.9
15:0	1.3	0.8
16:0	28.4	20.8
17:0	2.1	1.5
18:0	10.8	13.5
20:0	0.2	0.2
22:0	0.1	0.1
MUFAs	23.0	27.2
14:1*c*	1.1	0.8
16:1*c*	1.4	1.1
18:1*c* (total)	20.5	25.0
20:1*c*	0.2	0.2
PUFAs	3.1	3.0
18:2*c*	2.1	2.1
18:3*c*	0.5	0.4
Others[2]	0.6	0.5
TFAs	4.6	13.2
18:1*t* (total)	4.1	12.4
18:1*t*-11 (vaccenic acid)	1.7	6.3
18:2*t* (total)[3]	0.5	0.8
18:2*c*-9*t*-11 (CLA)	0.8	2.4

[1] Values are percentage of butter fat. 16:1*t* isomers were not measured in either of the experimental butters. CLA, conjugated linoleic acid; rTFA, *trans* fatty acid from ruminant sources; TFAs, *trans* fatty acids.
[2] 18:4*c*, 20:2*c*, 20:3*c*, 20:4*c*, 22:2*c*, 20:5*c*, 22:4*c*, and 22:5*c*.
[3] 18:2*t*-9*t*-12, 18:2*c*-9*t*-12, 18:2*t*-9*c*-12, and 18:2*t*-11*c*-15.

Diets and study design

The current study was undertaken as a double-blind, randomized, crossover controlled study in which each participant was assigned to 2 sequences of 2 different isocaloric diets lasting 4 wk each. Randomized treatment sequences were computer-generated by one of the investigators (BL) and then passed on to the research team without any knowledge of participant's status and eligibility. Each diet was separated by a 3-d period (weekend). The 2 experimental diets used in the current study were the rTFA diet (3.7 g/d) and the control diet low in rTFAs (0.9 g/d, control diet), with a difference between the 2 diets of 2.8 g rTFA. Sources of rTFAs, such as ruminant meat (lamb, veal, or beef) and nonskim dairy products, were not used in the formulation of the diets so that differences in the rTFA content between diets came exclusively from the experimental butters. Inclusion of any other foods with a high SFA content was also minimized to achieve the prescribed 10% SFA content. Specific vegetable and animal oils and fat were incorporated into each diet to attenuate differences in the amounts of SFAs and unsaturated fatty acids between treatments (*see* Supplemental Table 1 under "Supplemental data" in the online issue). As a result, the 2 experimental fats used to formulate the predetermined diets contained relatively comparable amounts of every major type of SFA and PUFA, whereas rTFAs were mainly substituted by 18:1*c* fatty acids. Both diets were identical in terms of menus, calories, and macronutrient composition, with the exception of rTFAs and 18:1*c* (**Table 2**). On average, the 2 experimental diets were formulated to provide 54% of daily energy from carbohydrate, 15% from protein, and 33% from fat. The rTFA diet provided 1.5% (exact value: 1.474%) of daily energy intake as rTFAs, and the control diet provided 0.3% (exact value: 0.342%) of daily energy intake as rTFAs. Because some of the foods used in the diets did contain traces of TFAs from PHVO, there was

TABLE 2
Mean nutritional composition of the 2 experimental diets[1]

	Control diet	rTFA diet
Energy (kcal/d)[2]	2279 ± 268	2280 ± 233
Carbohydrates (% of energy)	54.4	54.3
Proteins (% of energy)	15.0	15.0
Lipids (% of energy)	33.0	33.0
SFAs	9.9	10.3
4:0	0.4	0.4
6:0	0.2	0.2
8:0	0.1	0.1
10:0	0.2	0.2
12:0	0.3	0.2
14:0	0.9	1.0
16:0	5.4	5.0
17:0	0.2	0.2
18:0	2.1	2.7
20:0	0.1	0.1
22:0	0.2	0.2
MUFAs	14.2	12.8
14:1*c*	0.1	0.1
16:1*c*	0.4	0.4
18:1*c* (total)	13.6	12.1
20:1*c*	0.2	0.2
PUFAs	5.9	5.8
18:2*c*	5.2	5.1
18:3*c*	0.6	0.6
Others	0.1	0.0
TFAs from butters[3]	0.3	1.5
18:1*t* (total)	0.3	1.4
18:1*t*-11 (VA)	0.1	0.7
18:2*t* (total)	0.0	0.1
TFAs from PHVO[4]	0.3	0.3
16:1*t* (total)	0.0	0.0
18:1*t* (total)	0.2	0.2
18:2*t* (total)	0.0	0.0
Total fibers (g/d)	25.7	25.7
Cholesterol (mg/d)	292	294

[1] Numbers are based on the nutritional composition of the formulated diets estimated by using the food and nutrient database. Only the individual fatty acids presented in Table 1 are reproduced here, with the exception of the TFAs from PHVO, which have been added to the table. This explains why the sum of the individual fatty acids do not add up to the exact total percentage of the main fats in the diets. The rounding of the numbers to the first decimal point may also have the effect that the sum of the individual fatty acids in one category does not add up to the exact total of that category. PHVO, partially hydrogenated vegetable oil; rTFA, *trans* fatty acid from ruminant sources; TFAs, *trans* fatty acids; VA, vaccenic acid.

[2] Values are means ± SDs.

[3] The composition of the experimental butters was incorporated into our food database to derive the percentage of energy from the experimental butters as part of the whole diet.

[4] 0.7 g TFAs from foods containing PHVO are shown separately from rTFAs. The same foods in the same amounts contributed to the intake of TFAs from PHVO (0.3% of energy) in both diets.

a residual 0.7 g TFA from PHVO foods accounted for in each of the 2 diets. These TFAs from PHVO specifically were obtained by calculation and not chemical analysis based on the food database and food analysis software used for the study (Nutrition Data System, version 4.03_31; Nutrition Coordinating Center). Estimates of usual energy intake were ascertained at baseline by using a validated food-frequency questionnaire (13). 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 intakes 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 at least one member of the research team, who was blinded to subject's treatment. At that time, they were also provided with 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. The breakfast meal represented 26% of the daily energy intake, whereas the lunch and dinner meals each provided 37% of 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 within a limit of 2 cups/d (500 mL/d). Supplementation with vitamins and natural health products, as well as alcohol consumption, was 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. The subjects were asked to remain sedentary on the 3 d preceding blood sampling at the various stages of the study.

Compliance

A 7-d cyclic menu was developed to reflect a typical Canadian diet to optimize compliance 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 with the use of 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 at the end (days 25 and 26) of each experimental period in 77% of the participants. The mean of the 2 postdiet values were used for all variables in these subjects. In the remaining 33% of subjects, blood samples were taken only once on the last day (day 26) of each diet. Assessment of the plasma lipid profile was performed according to methods described previously (14–17). The CV for plasma lipid concentrations measured on samples taken on 2 consecutive days averaged 2.7% for total cholesterol, 3.6% for LDL cholesterol, 3.0% for HDL cholesterol, and 9.8% for triglycerides.

CETP mass, apo A-I, and apo-B concentrations

Serum apo A-I and apo B were measured with ELISA Imunoassay kits (Alerchek Inc). The plasma CETP mass concentration

was determined by using a commercial sandwich ELISA immunoassay kit (Wako Chemicals Inc).

Hepatic and endothelial lipases

Serum HL and EL concentrations were measured using commercial ELISA kits according to the manufacturer's protocol (USCN Life Science Inc).

Anthropometric measures 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 (18). Systolic and diastolic blood pressures were averaged from 3 measurements taken after a 10-min rest in the sitting position with an automated blood pressure monitor (BPM 300-BpTRU model; Vital Signs Monitor).

Statistical analyses

The main hypothesis tested in this study was that an increase in ~1% of energy as rTFA has no significant effect on plasma LDL-cholesterol concentrations with a 2-tailed $\alpha = 0.05$ and $\beta = 0.2$. The pooled SD of the change in plasma LDL-cholesterol concentrations in our previous study of rTFAs in men (similar design and similar diets) was 0.39 mmol/L (3), and we assumed similar numbers in women. On the basis of these numbers and on a baseline LDL-cholesterol concentration estimated a priori at 3.0 mmol/L, 55 participants completing the study were required to be able to detect a ≥5% change in LDL cholesterol with the rTFA compared with the control diet. Secondary outcomes were changes in HDL cholesterol and in the total/HDL cholesterol ratio. On the basis of the pooled SD for the change in HDL cholesterol in our previous study in men (3), 55 participants were sufficient to detect a significant increase or decrease in HDL cholesterol that would be as small as 0.055 mmol/L (ie, 3.9%, 2-tailed $\alpha = 0.05$ and $\beta = 0.2$). A ±5% change in the total/HDL cholesterol ratio was calculated to be significant (2-tailed $P < 0.05$) with a power of 1.0. The study design allowed us to detect a 5-mm Hg difference in systolic and a 3.6-mm Hg difference in diastolic blood pressure between treatments (80% power at $P = 0.05$). Finally, we were able to detect with a power of 80% a correlation of $r > 0.37$ within a sample of $n = 55$ subjects ($P \leq 0.05$). On the basis of an anticipated 15% dropout rate, we had initially planned to recruit 64 women. However, recruitment was very successful, so that 72 women were finally randomly assigned to treatment sequences.

Data are reported as means ± SDs and the percentage of changes from the control diet in the tables, unless stated otherwise. The main analysis compared values of each outcome measured at the end of the 2 isocaloric diets, ie, values after the rTFA diet compared with values after the control diet. Data were analyzed by using the PROC MIXED procedure for repeated measures in SAS (v9.2; SAS Institute Inc). Menopausal status, weight, treatment sequence, and baseline values were included as covariates in all models. Their interaction with the main treatment effect was also tested, but none were significant; therefore, they were removed from final models. Variables with a skewed distribution were log-10 transformed before statistical analysis. Differences at $P \leq 0.05$ (2-sided) were considered significant.

RESULTS

Characteristics of subjects at baseline and compliance

Of the 72 women enrolled in the study, a total of 8 dropped out because they felt the study protocol was too demanding. Of the remaining 64 subjects who completed the intervention, 2 were excluded because their menopausal status changed during the course of the study (assessed on the basis of FSH values) and 1 was excluded because of missing data for several outcomes (**Figure 1**). The clinical data of the 72 women recruited in the study were very similar to the data in the 61 completers included in the final analyses (data not shown). The risk profile of completers at screening is shown in **Table 3**. Women had a mean (±SD) age of 38.3 ± 17.1 y and were generally in good health, with average blood lipid concentrations within normal values. Among premenopausal women, 55.2% (n = 21/38) were using oral contraceptives, whereas 37.7% (n = 23) of the participants were postmenopausal. Few of the women were taking concurrent medication (thyroid, n = 4; antidepressant/antipsychotic, n = 4; nonsteroidal anti-inflammatory, n = 3; proton pump inhibitors, n = 2). Approximately 95% of the weekly food checklists were returned, from which we calculated that 98.9% of the food provided had been consumed.

Anthropometric measures

No significant difference in waist circumference was found after the 2 diets. A small yet significant difference in body weight was found between the 2 diets (Table 3), which was therefore accounted for in all subsequent analyses. Exclusion of the variations in body weight as a covariable in the statistical model had no effect on the results (data not shown). No difference in reported physical activity, expressed as total metabolic equivalent tasks, was found between the diets (P = 0.97; data not shown).

Cardiovascular disease risk factors

No significant changes in total plasma cholesterol, LDL cholesterol, apo B, or triglyceride concentrations were found after the rTFA diet compared with the control diet (Table 3). Plasma HDL-cholesterol concentrations were significantly lower after the rTFA diet than after the control diet (−2.8%; $P = 0.004$), whereas no significant change in plasma apo A-I concentrations was found after the rTFA diet. A significant BMI × diet interaction on HDL-cholesterol concentrations was found (P-interaction = 0.006). Specifically, women with a BMI ≥25 (n = 18) had a significant 5.2% reduction in plasma HDL-cholesterol concentrations after the rTFA diet compared with the control diet (95% CI: −8.5%, −1.7%; change estimate: −0.09 mmol/L; P = 0.004), whereas no change in HDL cholesterol was found among women with a BMI <25 (−1.2%; 95% CI: −3.4, 1.0%; change estimate: −0.03 mmol/L; P = 0.13). No such BMI × diet interaction was seen with LDL cholesterol or apo B. Menopausal status and age showed no significant interaction with diet on HDL-cholesterol or LDL-cholesterol concentrations. Finally, the rTFA diet tended to increase the total/HDL cholesterol and LDL/HDL cholesterol ratios, but not significantly so.

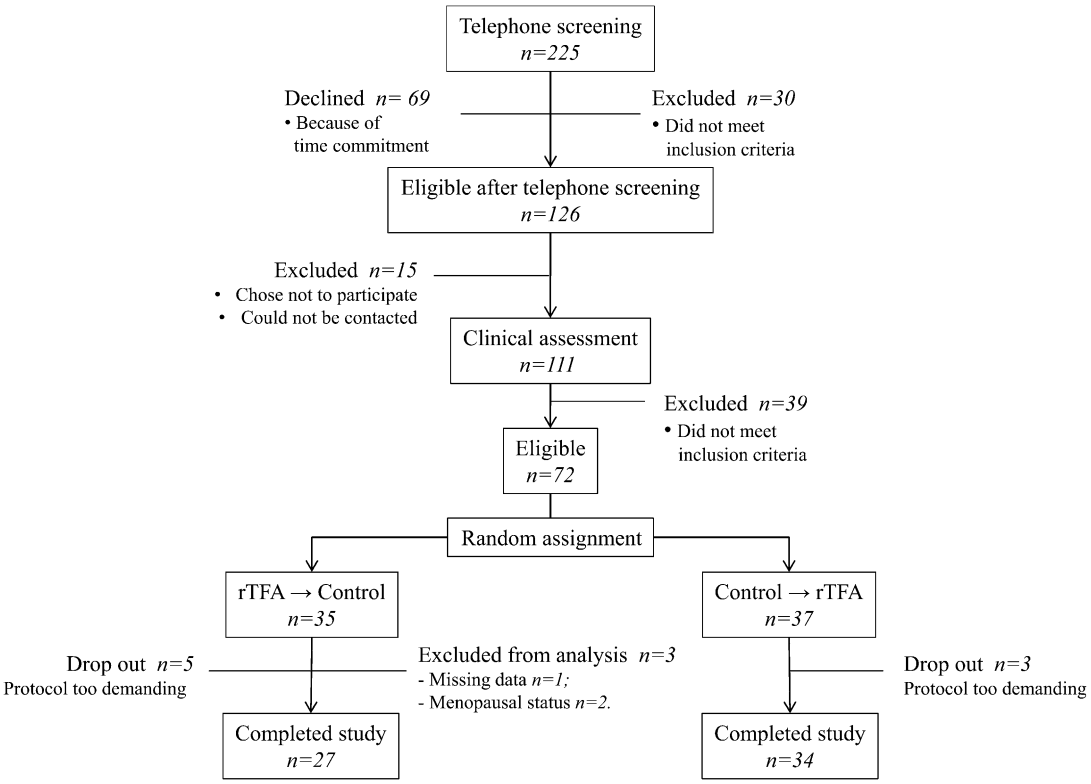

FIGURE 1. Flow of patients throughout the study. rTFA, *trans* fatty acid from ruminant sources.

Plasma concentrations of CETP, HL, and EL

Comparisons between the rTFA and control diets showed no significant differences in mean (±SEM) plasma concentrations of CETP (3.4 ± 0.1 and 3.5 ± 0.1 μg/mL, respectively; $P = 0.3$), HL (112 ± 13 and 110 ± 12 U/L, respectively; $P = 0.6$), and EL (333 ± 107 and 310 ± 96 ng/mL, respectively; $P = 0.8$). There was also no significant correlation between diet-induced changes in plasma HDL cholesterol or apo A-I concentrations and concurrent variations in plasma EL, HL, and CETP concentrations (data not shown).

DISCUSSION

The study investigated how an increase of 1.2% of energy as rTFAs (difference between the rTFA diet and the control diet: 2.8 g rTFA/d) affects plasma lipid concentrations in healthy women. In Canada, the average total TFA intake from any source has declined from 8.4 g/d (3.7% of energy) in the mid-1990s to 4.8 g/d (2.0% of energy) in 2004 and to 3.4 g/d (1.4% of energy) in 2008 (19). A report from 2006 has estimated the specific intake of TFA from ruminant sources in Canada to be ~1 g/d (20), and this value is very unlikely to have changed since then. Thus, our study investigated the effect of a 3-fold increase in dietary rTFAs compared with current estimates of intake in Canada. It can therefore be considered to be relatively high, but theoretically achievable and of nutritional relevance.

A large epidemiologic study among women has shown that, unlike TFAs from PHVO, an increased intake of rTFAs tended to be associated with a lower risk of CVD in women (9). Other data from epidemiologic studies have reported no significant effect of rTFA intake on CVD risk in various populations (8, 10, 21). Data from clinical trials do not entirely support these observations. On the basis of a comprehensive analysis of published clinical trials, Brouwer et al (2) have suggested that rTFAs and TFAs from PHVO may have similar elevating effects on the LDL/HDL cholesterol ratio. We stress that this compounded analyses of the effects of TFAs from PHVO on plasma lipids is based on 23 studies to date, whereas rTFAs have been investigated in only 5 studies so far (2), excluding the current study. We also stress that only 21 women have been included so far in previous clinical studies of rTFAs, representing only 11% of all participants. This is a key point because it has been suggested that men and women may respond differently to nutritional changes. In particular, Chardigny et al (4) have shown that the increase in the LDL/HDL cholesterol ratio with an increased rTFA intake (5% of energy) was in fact 2.4-fold greater in women than was the change seen in men. This emphasizes the importance of having additional adequately powered and optimally controlled studies to draw firm conclusion on the effects of rTFAs on blood lipids, particularly among women.

We showed that substituting 1.2% of energy as rTFAs in place of *cis*-MUFAs had no effect on LDL cholesterol (estimated change: 0.01 mmol/L; NS) but reduced plasma HDL-cholesterol concentrations in healthy women (estimated change: 0.05 mmol/L; significant). These effects are not entirely consistent with data from the quantitative review by Brouwer et al (2), which would have predicted an increase of 0.045 mmol/L for LDL cholesterol and a reduction of only 0.009 mmol/L for HDL cholesterol. These discrepancies can be explained by several factors. First, sex may be a factor and women are greatly underrepresented in

the literature on rTFA, because most studies have been conducted in men exclusively, as indicated above. The status of participants at baseline may have also influenced the outcome. In the current study, participants were healthy and nonobese as a group. Although age, risk factor profile at baseline, and menopausal status did not modulate the effects of rTFAs on blood lipids, a significant BMI × diet interaction was found for HDL cholesterol, with the reduction in plasma HDL cholesterol being significantly more pronounced among overweight women than among normal-weight women. This finding is consistent with the concept that adiposity may significantly modulate the effect of nutritional changes on plasma lipid and nonlipid risk factors (22). Consumption of rTFAs led to a small yet significant reduction in body weight. Because there was no concurrent change in waist circumference, we believe that this small change in body weight was most likely coincidental and of little importance in this study.

Previous studies have suggested that the reduction in plasma HDL-cholesterol concentrations with TFA intake (mainly from PHVO) compared with *cis*-MUFAs may be imputed to increased CETP activities. Exploratory analyses suggested that the HDL-lowering effect seen with rTFAs in the current study was apparently not mediated by changes in plasma CETP, HL, and EL concentrations, which is consistent with data from Aro et al (23), who reported no significant effect of TFA on CETP activity compared with diets high in SFAs, despite a significant change in HDL-cholesterol concentrations between diets (23). Whether information on enzyme activities rather than plasma concentrations would have yielded similar results is uncertain. Kinetic studies have shown that the reduction in plasma HDL-cholesterol concentrations with dietary TFAs from PHVO was largely attributable to an enhanced clearance of apo A-I, rather than to a reduced production rate (24). We hypothesize that similar mechanisms may be at play with regard to rTFA. The large variation in the response of apo A-I to treatments may have blunted a significant effect in our study.

The total/HDL cholesterol ratio was nontrivially increased by ~10% after both dietary regimens (control and rTFA diets) compared with values at screening. We analyzed the usual food intake of the participants before undertaking the study in an attempt to identify factors underlying these changes. Their estimated usual intake in saturated fat was 10.1%, whereas their intake in fibers averaged 27.4 g/d. These values are not different from the values achieved during both the control and the rTFA diets (10% of energy for SAT and 25.7 g/d for fibers). Alcohol intake on the other hand was different (screening: 5.1 g/d; study: 0.1 g/d), which may explain at least the slight reduction in plasma HDL cholesterol between screening and the diets and hence in the total/HDL cholesterol ratio as well.

In a recently published double-blind, randomized trial conducted in healthy men and women, a limited increase in the rTFA/SFA ratio (4%/63% compared with 2.8%/72%) in dairy products was associated with improvements in LDL cholesterol and the LDL/HDL cholesterol ratio but not in HDL-cholesterol concentrations (25). A further increase in the rTFA/SFA ratio showed no additional benefit and had similar effects in both sexes. This study was obviously very different from ours, because several changes in many fatty acids were being compared, other than just those between TFAs and *cis*-MUFAs. Tricon et al (7) have shown in men that consumption of a CLA-rich diet in which the rTFA content was close to that attained in our study (4.7 g/d, predominantly vaccenic acid) had no effect on plasma triglycerides, total cholesterol, and LDL cholesterol, but led to a significant increase in the LDL/HDL cholesterol ratio compared with the control diet. A trend toward an increase in the total/HDL cholesterol ratio was also observed with the CLA-rich diet. These effects could not be ascribed to rTFAs or CLAs because both were given concurrently in that study.

TABLE 3
Body-composition variables and plasma lipid concentrations at screening and at the end of the dietary intervention in the 61 subjects[1]

		After the diet					
	Screening	Control	rTFAs	Estimated change	95% CI	Change from control	P^2
						%	
Weight (kg)	63.5 ± 8.8[3]	62.8 ± 8.4	62.5 ± 8.3	−0.3	−0.5, −0.1	−0.4	0.013
BMI (kg/m^2)	23.6 ± 2.9	23.3 ± 2.8	23.2 ± 2.8	−0.1	−0.2, −0.0	−0.4	0.013
Waist girth (cm)	81.1 ± 8.8	78.7 ± 8.1	78.6 ± 8.3	−0.1	−1.1, 0.9	−0.1	0.868
Systolic BP (mm Hg)	104.1 ± 9.4	102.2 ± 10.5	103.3 ± 9.5	1.1	−1.1, 3.3	1.1	0.306
Diastolic BP (mm Hg)	68.7 ± 6.3	66.9 ± 6.6	67.6 ± 7.4	0.7	−1.0, 2.4	1.0	0.436
Cholesterol (mmol/L)	5.11 ± 0.97	5.24 ± 0.98	5.21 ± 0.98	−0.04	−0.12, 0.04	−0.8	0.324
LDL-C (mmol/L)	2.84 ± 0.77	3.12 ± 0.82	3.12 ± 0.84	0.01	−0.06, 0.08	0.3	0.771
apo B (g/L)[4]	0.96 ± 0.33	1.00 ± 0.41	1.01 ± 0.42	0.01	−0.12, 0.14	1.2	0.831
HDL-C (mmol/L)	1.78 ± 0.42	1.69 ± 0.37	1.64 ± 0.37	−0.05	−0.08, −0.01	−2.8	0.004
apo A-I (g/L)[4]	1.52 ± 0.73	1.63 ± 0.99	1.46 ± 0.56	−0.18	−0.41, 0.05	−11.0	0.089
Triglycerides (mmol/L)[4]	1.04 ± 0.44	0.96 ± 0.46	0.96 ± 0.49	0.00	−0.06, 0.06	−0.5	0.997
Total/HDL-C[4]	2.95 ± 0.66	3.21 ± 0.81	3.28 ± 0.83	0.07	−0.01, 0.14	2.1	0.053
LDL/HDL-C[4]	1.67 ± 0.56	1.92 ± 0.68	1.99 ± 0.70	0.06	−0.00, 0.13	3.2	0.060
apo B/apo A-I[4]	0.72 ± 0.33	0.71 ± 0.36	0.78 ± 0.39	0.07	−0.05, 0.18	9.5	0.144

[1] apo, apolipoprotein; BP, blood pressure; C, cholesterol; rTFAs, *trans* fatty acids from ruminant sources.
[2] Diets were compared by using mixed models for repeated measures, with adjustment for baseline values (screening), menopausal status, sequence, and weight (on diet). Postdiet values for body weight were not included in the analysis of weight and waist circumference.
[3] Mean ± SD (all such values).
[4] Analysis was performed on log-transformed values.

The current study had strengths as well as limitations. Unlike in previous studies of rTFAs specifically, the large sample size, the controlled feeding nature of the study design, and the repeated measurements at the end of each diet made it powerful to detect very small changes in risk factors. This was also one of the first studies to have investigated the effect of a butter enriched in rTFAs in women specifically. Although the sample size allowed us to detect a $\geq$5% change in LDL cholesterol, it is possible that smaller increases may still affect long-term CVD risk. Unlike in many previous studies of rTFAs, the dietary intake of rTFAs in this study, although still high, was theoretically achievable and therefore of clinical relevance. Although vaccenic acid is the predominant *trans* isomer of rTFAs, it represented $<$50% of all rTFAs in the rTFA-enriched butter. Because other rTFA isomers were increased in the rTFA-enriched butter (eg, 18:2*t* increased by 56% compared with the control butter), it was therefore not possible to ascribe the change (or lack thereof) in plasma lipids in our study to vaccenic acid alone. Plasma concentrations of *trans*-palmitoleate (16:1*t*-7), also found in dairy fat, was found to be associated with lower insulin resistance, the presence of atherogenic dyslipidemia, and incident diabetes in the large Cardiovascular Health Study cohort (26). The specific 16:1*t*-7 and other 16:1*t* isomer contents of the experimental butters in the current study were not measured and their effect on the study outcomes cannot be commented on. These usually represent $<$1% of all fatty acids in dairy fat (27, 28). Finally, the study was conducted over a short-term period, and longer-term effects —particularly on other nonlipid CVD risk factors—cannot be ruled out and need further investigation.

In summary, we showed in healthy women that an increase in the dietary intake of rTFAs corresponding to 1.2% of daily energy had no significant effect on plasma LDL-cholesterol concentrations but had a small HDL-lowering effect, particularly in overweight women. The long-term effect of these changes on CVD risk is unknown. Whether these observations extend to patients with cardiometabolic abnormalities, such as the metabolic syndrome and diabetes, will also need to be addressed in the future.

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The authors' responsibilities were as follows—BL and PC: principal investigators; PP and YL: coordinated the formulation of the TFA-enriched butter; YC: coordinated the production of the enriched milk with the supplements; PC: responsible for the screening and medical supervision of the study participants; A Charest: coordinated the study with the collaboration of EL, LB-G, and A Cyr; and EL: performed the statistical analyses, analyzed the data, and wrote the first draft of the manuscript. BL, PC, PP, and PYC were investigators on the grant from Dairy Farmers of Canada, Dairy Australia, Agriculture and Agri-Food Canada, and the Canadian Dairy Commission, which made the current study possible; PP serves as a consultant on an industrial research project partly supported by Dairy Farmers of Canada. BL is a member of the Scientific Advisory Board of Dairy Farmers of Canada. EL, A Charest, A Cyr, LB-G, and YL had no potential conflict of interest to declare in relation with the content of this article. None of the sponsors had any role in defining the study design or its implementation or in the analysis and interpretation of the data.

REFERENCES

1. Katan MB. Trans fatty acids and plasma lipoproteins. Nutr Rev 2000; 58:188–91.
2. Brouwer IA, Wanders AJ, Katan MB. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans–a quantitative review. PLoS ONE 2010;5:e9434.
3. Motard-Bélanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P, Lamarche B. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am J Clin Nutr 2008;87:593–9.
4. Chardigny JM, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE, Lock AL, Barbano DM, Mensink RP, Bezelgues JB, Chaumont P, et al. 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. Am J Clin Nutr 2008;87:558–66.
5. Desroches S, Chouinard PY, Galibois I, Corneau L, Delisle J, Lamarche B, Couture P, Bergeron N. Lack of effect of dietary conjugated linoleic acids naturally incorporated into butter on the lipid profile and body composition of overweight and obese men. Am J Clin Nutr 2005;82:309–19.
6. Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. Am J Clin Nutr 2006;83:237–43.
7. Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, Hall WL, Gerry AB, Leake DS, Grimble RF, et al. Effects of dairy products naturally enriched with cis-9,trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. Am J Clin Nutr 2006;83:744–53.
8. Jakobsen MU, Overvad K, Dyerberg J, Heitmann BL. Intake of ruminant trans fatty acids and risk of coronary heart disease. Int J Epidemiol 2008;37:173–82.
9. Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 1993;341:581–5.
10. Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. Trans-fatty acids intake and risk of myocardial infarction. Circulation 1994;89:94–101.
11. Oomen CM, Ocke MC, Feskens EJ, van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet 2001;357:746–51.
12. Gervais R, Chouinard PY. Effects of intravenous infusion of conjugated diene 18:3 isomers on milk fat synthesis in lactating dairy cows. J Dairy Sci 2008;91:3568–78.
13. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S. Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. Nutr J 2004;3:13.
14. Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L, Breckenridge C, Chow A, Kuba K, Weidman S, et al. Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem 1978;24:853–6.
15. Burstein M, Samaille J. [On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins.] Clin Chim Acta 1960;5:609.
16. Moorjani S, Dupont A, Labrie F, Lupien PJ, Brun D, Gagné C, Giguère M, Bélanger A. Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. Metabolism 1987;36:244–50.
17. Couillard C, Despres JP, Lamarche B, Bergeron J, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. Arterioscler Thromb Vasc Biol 2001;21:1226–32.
18. Lohman T, Roche A, Martorel R. The Airlie (VA) consensus conference. In: Lohman TG, Roche AF, Martorell R, eds. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics, 1988.
19. Ratnayake WM, L'Abbe MR, Farnworth S, Dumais L, Gagnon C, Lampi B, Casey V, Mohottalage D, Rondeau I, Underhill L, et al. Trans fatty acids: current contents in Canadian foods and estimated intake levels for the Canadian population. J AOAC Int 2009;92:1258–76.

20. Nutrition Evaluation Division, Health Canada. TRANSforming the food supply: report of the Trans Fat Task Force—submitted to the minister of health. Ottawa, Canada: Trans Fat Task Force; 2006.
21. Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Am J Epidemiol 1997;145:876–87.
22. Flock MR, Kris-Etherton PM. Dietary Guidelines for Americans 2010: implications for cardiovascular disease. Curr Atheroscler Rep 2011;13 (6):499–507.
23. Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M. Stearic acid, trans fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. Am J Clin Nutr 1997;65:1419–26.
24. Matthan NR, Welty FK, Barrett PH, Harausz C, Dolnikowski GG, Parks JS, Eckel RH, Schaefer EJ, Lichtenstein AH. Dietary hydrogenated fat increases high-density lipoprotein apoA-I catabolism and decreases low-density lipoprotein apoB-100 catabolism in hypercholesterolemic women. Arterioscler Thromb Vasc Biol 2004;24:1092–7.
25. Malpuech-Brugère C, Mouriot J, Boue-Vaysse C, Combe N, Peyraud JL, LeRuyet P, Chesneau G, Morio B, Chardigny JM. Differential impact of milk fatty acid profiles on cardiovascular risk biomarkers in healthy men and women. Eur J Clin Nutr 2010;64:752–9.
26. Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, Hotamisligil GS. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. Ann Intern Med 2010;153:790–9.
27. Destaillats F, Wolff RL, Precht D, Molkentin J. Study of individual trans- and cis-16:1 isomers in cow, goat, and ewe cheese fats by gas-liquid chromatography with emphasis on the trans-delta3 isomer. Lipids 2000;35:1027–32.
28. Jakobsen MU, Bysted A, Andersen NL, Heitmann BL, Hartkopp HB, Leth T, Overvad K, Dyerberg J. Intake of ruminant trans fatty acids in the Danish population aged 1-80 years. Eur J Clin Nutr 2006;60:312–8.