RESEARCH ARTICLE

Comparison of the impact of *trans* fatty acids from ruminant and industrial sources on surrogate markers of cholesterol homeostasis in healthy men

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Scope: Mechanisms by which *trans* fatty acids (TFA) from industrial (iTFA) and ruminant (rTFA) sources alter cholesterol homeostasis are virtually unknown. We compared the impact of dietary iTFA and rTFA on surrogate markers of cholesterol absorption (β-sitosterol and campesterol) and synthesis (lathosterol) in healthy men.
Methods and results: In a randomized, controlled double-blind crossover study, 38 healthy men consumed three experimental isoenergetic diets for 4 wk each. The three diets were (i) high in iTFA (10.2 g/2500 kcal), (ii) high in rTFA (10.2 g/2500 kcal) and (iii) control diet low in TFA from any source (2.2 g/2500 kcal). The sum of plasma β-sitosterol and campesterol concentrations was significantly reduced after the iTFA diet compared with the control diet (−12%, $p = 0.050$). The reduction in combined β-sitosterol and campesterol levels was larger in magnitude after the rTFA diet (−29% versus the control diet and −20% versus the iTFA diet, $p < 0.0001$). The TFA-rich diets had no impact on plasma lathosterol concentrations.
Conclusions: Very high intakes of rTFA and iTFA decrease cholesterol absorption but have no impact on cholesterol synthesis. Consumption of rTFA reduces cholesterol absorption to a greater extent than iTFA, but this difference does not ultimately affect plasma cholesterol concentrations.

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1 Introduction

Dietary *trans* fatty acids (TFA) are derived mostly from partially hydrogenated vegetable oils found in many industrially prepared foods. TFA also occur naturally in meat and dairy products from ruminants. This is attributable to the biohydrogenation of unsaturated fatty acids in the animals' rumen. Consumption of TFA from industrial sources (iTFA) is recognized as an important dietary risk factor for cardiovascular disease (CVD) [1]. On a per-calorie basis, iTFA appear to raise CVD risk more than any other macronutrient [2]. On the other hand, the impact of TFA from ruminant sources (rTFA) on CVD risk is still not clearly established. A few prospective observational studies have suggested that rTFA consumption has no impact on CVD risk [3–5]. On the other hand, our group has recently shown that on a gram-for-gram basis, very high intakes of rTFA and iTFA have comparable LDL-cholesterol (LDL-C) raising effects [6].

Mechanisms underlying this apparently similar effect of both sources of TFA on plasma LDL-C concentrations are unknown. Plasma cholesterol concentrations are finely regulated through feedback mechanisms implicating, among other factors, endogenous synthesis and intestinal

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Abbreviations: C, cholesterol; **CVD,** cardiovascular disease; **iTFA,** industrial TFA; **LDL-C,** LDL-cholesterol; **MetS,** metabolic syndrome; **PPAR,** peroxisome proliferator-activated receptor; **rTFA,** ruminant TFA; **TFA,** *trans* fatty acids

absorption of cholesterol [7]. A disruption in any one of these processes implicated in cholesterol homeostasis can alter the balance and result in disease progression, particularly if cholesterol accumulates in circulation and tissues [8, 9]. The cholesterol precursor lathosterol has proved to be a reliable marker for whole body cholesterol synthesis, while plant sterols β-sitosterol and campesterol are both recognized as valid indicators of cholesterol absorption [10–12]. These cholesterol homeostasis markers have been shown to predict CVD risk, independent of variations in traditional lipid risk factors [8, 13, 14]. Several studies have indeed demonstrated that reduced cholesterol absorption and high cholesterol synthesis are associated with impaired metabolic states predisposing to CVD, such as insulin resistance and metabolic syndrome (MetS) [15, 16]. Interestingly, several of the metabolic effects attributed to iTFA consumption are similar to features of the MetS [1].

The purpose of the present study was to compare for the first time the impact of a very high dietary intake of iTFA and rTFA on surrogate markers of cholesterol absorption and synthesis in healthy men in a double-blind randomized crossover study. We have hypothesized that because iTFA and rTFA have different isomeric profiles [17], their impact on the regulation of cholesterol homeostasis may differ.

2 Materials and methods

2.1 Subjects

Forty-eight healthy men aged between 18 and 65 years were initially recruited, as described previously [6]. All subjects underwent a complete physical examination and answered a medical history questionnaire. 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 months before the study. Men with food allergies, with an aversion to foods included in the experimental diets or with a usual alcohol consumption of >2 drinks/day were also excluded.

Written informed consent was obtained from all participants at the beginning of the study. The Clinical Research Ethics Committee of Laval University approved the study protocol (♯2005–233).

2.2 Study design and diets composition

This study used a randomized, controlled, double-blind crossover design. A detailed description of the study design and the diets composition has been provided previously [6]. Briefly, volunteers were randomly assigned to four experimental isocaloric diets lasting 4 wk each. A wash-out period of 3–12 wk separated each diet. For the purpose of the present study, only three of the four diets were examined. These diets were high in iTFA (10.2 g/2500 kcal), high in rTFA (10.2 g/2500 kcal) or low in TFA from any source (2.2 g/2500 kcal) (control diet). The fourth diet containing ''moderate'' amounts of rTFA (4.2 g/2500 kcal) was not included in the present analyses because it had no impact on plasma LDL-C concentrations [6]. Commercially available shortenings were used as the main source of iTFA. A TFA-enriched butter generated from milking cows whose diet had been modified to include safflower oil was used as the source of rTFA. The low-rTFA butter found in the control diet was obtained from the Canadian Dairy Commission

Table 1. Nutritional composition of the three experimental diets for the 38 subjects[a)]

Variable	Diet		
	Control	iTFA	rTFA
Energy (kcal)	3196 ± 519[b)]	3268 ± 525	3213 ± 527
Carbohydrates (%)	50.1	50.2	48.8
Proteins (%)	14.0	14.0	14.0
Lipids (%)	37.0	37.0	38.1
SFA (%)	18.5	18.0	19.4
MUFA (%)	11.8	10.1	10.0
PUFA (%)	4.6	4.0	3.5
TFA (%)	0.8	3.7	3.7
Total fibers (g/2500 kcal)	20.8	20.8	20.7
Cholesterol (mg/2500 kcal)	299	299	303
Phytosterols (mg/2500 kcal)	317	241	200

a) TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. These data (except phytosterol content) have been reported in a previous publication [6].
b) Mean ± SD (all such values).

(winter 2006 production). As shown in Table 1, the three diets provided 50% of daily energy intake from carbohydrate, 14% from protein and 37% from fat. iTFA and rTFA provided 3.6% of daily calories in the TFA diets. The control diet provided 0.8% of daily energy intake from rTFA and 0% from iTFA. Several vegetable and animal oils and fat were mixed in each diet to minimize differences in the amounts of saturated and unsaturated fatty acids between the diets [6]. Phytosterol content of each diet was estimated using data from the United States Department of Agriculture National Nutrient Database for Standard Reference. When phytosterol amounts were not available in the United States Department of Agriculture Database, they were derived from tables presented by Normén et al. [18, 19], Lange [20] or Kritchevsky [21].

Participants were each assigned to a level of energy intake estimated by a validated food frequency questionnaire [22], and the level was revised when required to keep the body weight constant. All meals were provided to participants according to a 7-day cyclic menu. Subjects came on week days to the Clinical Investigation Unit at the Institute of Nutraceuticals and Functional Foods to consume their lunch. At that time, they were also given their dinner and the next day's packaged breakfast to take home. Participants had free access to water and to caffeine-free diet beverages. Consumption of tea and coffee was limited to 2 cups/day (500 mL/day). Alcohol consumption and supplementation with vitamins and natural health products were forbidden during all dietary phases.

2.3 Compliance

Compliance has been evaluated with a food checklist provided weekly to all participants. The checklist was used to identify foods that had or had not been consumed as well as unlisted but permitted food items (e.g. tea, coffee) and the use of any medication. As described previously [6], on the basis of the completed checklists, which represented 84% of all distributed checklists, compliance to the diets was estimated to be $>99\%$.

2.4 Anthropometric and clinical measures

The subjects' body weight was recorded on all week days just before lunch. Waist and hip circumferences and blood pressure were also measured at the beginning and at the end of each experimental diet, according to standardized procedures [23].

2.5 Plasma lipids and non-cholesterol sterols concentrations

Blood samples were collected in the morning, after a 12-h fast, at the beginning (day 1) and at the end (day 26) of each experimental phase. Analyses of the lipid profile and of lipoprotein-lipid concentrations by ultracentrifugation were performed according to previously described methods [24–27].

Plasma concentrations of lathosterol, β-sitosterol and campesterol were quantified by GC as previously described [13]. Since non-cholesterol sterols are transported in plasma by lipoproteins, their concentrations have been expressed relative to plasma total cholesterol concentrations ($\times 10^2$ μmol/mmol of cholesterol of the same GC run) to correct for the differing number of lipoprotein acceptor particles [10].

2.6 Statistical analyses

The MIXED procedure for repeated measures in SAS (version 9.1; SAS Institute, Cary, NC) was used to compare the plasma concentrations of the various cholesterol homeostasis surrogates at the end of each diet. The structure of the covariance matrix for each outcome variable was taken into account in all analyses to ensure the most adequate statistical fit of the model to the experimental data. The Tukey adjustment was used to account for the multiple comparisons of the three diets. Variables not normally distributed were logarithmically transformed before statistical analysis. Group averages are reported as means $\pm$ SD unless stated otherwise. Differences were considered significant at $p \leq 0.05$.

3 Results

As indicated in a previous publication [6], of the 48 men enrolled in the study, a total of ten subjects dropped out and were excluded from the final analysis: two subjects moved, six found the study protocol too demanding and decided to quit, one was excluded for non compliance and one was excluded for health reasons unrelated to the study. The baseline characteristics of the 38 subjects who completed the three dietary phases of the study are shown in Table 2. The 38 men were in good health; they had a mean BMI within the normal range and mean blood lipid concentrations within normal values.

As reported previously by Motard-Bélanger et al. [6], the iTFA and rTFA diets led to comparable increases in total cholesterol and LDL-C concentrations compared with the control diet (respectively 2.3% versus 3.0% for total-C; 4.3% versus 5.9% for LDL-C; Table 3). However, these changes reached significance only for LDL-C after the rTFA diet ($p = 0.02$).

Table 3 shows that the iTFA and rTFA diets had no impact on plasma lathosterol concentrations compared with the control diet ($p = 0.72$ and $p = 0.56$, respectively). Plasma lathosterol concentrations were also similar after the iTFA and rTFA diets ($p = 0.94$). Plasma concentrations of total

plant sterols (−11.5%, $p = 0.050$), β-sitosterol (−5.4%, $p = 0.59$) and campesterol (−18.5%, $p = 0.006$) were reduced after the iTFA diet compared with the control diet. On the other hand, the reductions in total plant sterols, β-sitosterol and campesterol concentrations after the rTFA diet compared with the control diet were all highly significant (−29.1, −21.5 and −37.6%, respectively, $p < 0.0001$). Plasma concentrations of intestinal surrogates of cholesterol absorption were also significantly lower after the rTFA diet than after the iTFA diet (−17.1 and −23.5% for β-sitosterol and campesterol, respectively, $p < 0.0001$). Similar results were obtained when absorption levels were normalized for synthesis levels using ratios of β-sitosterol to lathosterol and campesterol to lathosterol.

Table 2. Physical characteristics and plasma lipid profiles of the 38 subjects at screening[a)]

Variable	Value
Age (years)	32.8 ± 15
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

a) All values are presented as mean ± SD. BP, blood pressure; C, cholesterol. These data have been reported in a previous publication [6].

4 Discussion

We have previously shown that very high dietary intakes of iTFA and rTFA had comparable effects on blood lipids and lipoproteins [6]. However, mechanisms by which both sources of TFA alter cholesterol homeostasis are unknown. The main purpose of the study was to directly compare for the first time, on a gram-for-gram basis, the effects of iTFA and rTFA on surrogate markers of cholesterol synthesis and absorption in healthy men.

Our results showed that consumption of TFA, from any dietary source, had no effect on cholesterol endogenous synthesis estimated by plasma lathosterol concentrations compared with a control diet low in TFA. Very few studies have investigated the impact of dietary TFA from any source on cholesterol synthesis. Cuchel et al. [28] have shown that feeding moderately hypercholesterolemic men and women with corn oil margarine in stick form compared to a corn oil diet increased plasma lipid and lipoprotein levels but had no significant effect on de novo cholesterol rate of synthesis. Matthan et al. [29] have shown in 14 moderately hypercholesterolemic postmenopausal women that increasing the degree of fat hydrogenation in the diet reduced the fractional and absolute synthetic rate of free cholesterol. The highest intake of iTFA in that study was 20 g/day, which is approximately twice the amount used in the present study. Inversely, consumption of iTFA (13 g/day with 7.2 g/day from elaidic acid) in comparison to a mixture of palmitic and oleic acids increased plasma cholesterol concentrations apparently by increasing endogenous synthesis of cholesterol in normolipidemic women [30]. Inconsistent results between studies regarding the impact of TFA on cholesterol synthesis can be attributed to several factors. First, studies

Table 3. Plasma sterol concentrations at the end of each dietary intervention in the 38 subjects[a)]

Variable	Diet					p[c)]
	Control	iTFA	% versus control[b)]	rTFA	% versus control[b)]	
Plasma lipids[d)]						
Cholesterol (mmol/L)	4.77 ± 0.93[e)]	4.88 ± 0.95	2.3%	4.92 ± 0.98	3.0%	0.17
LDL-C (mmol/L)	3.27 ± 0.80	3.42 ± 0.89	4.3%	3.47 ± 0.90[f)]	5.9%	0.02
Plasma non-cholesterol sterols						
Lathosterol[g)]	142.0 ± 48.2	137.7 ± 45.4	−3.0%	135.9 ± 43.2	−4.3%	0.57
β-Sitosterol[g)]	153.8 ± 85.5	145.5 ± 72.8	−5.4%	120.7 ± 74.9[f),h)]	−21.5%	<0.0001
Campesterol[g)]	135.3 ± 84.2	110.2 ± 64.8[f)]	−18.5%	84.3 ± 60.5[f),h)]	−37.6%	<0.0001
Total plant sterols[g)]	289.0 ± 166.0	255.7 ± 135.3[f)]	−11.5%	205.0 ± 132.4[f),h)]	−29.1%	<0.0001
β-Sitosterol/lathosterol	1.39 ± 1.05	1.34 ± 0.89	−3.3%	1.11 ± 0.85[f),h)]	−19.8%	0.0008
Campesterol/lathosterol	1.16 ± 0.97	0.99 ± 0.72	−14.6%	0.74 ± 0.60[f),h)]	−36.1%	<0.0001

a) C, cholesterol; Total plant sterols, sum of β-sitosterol and campesterol.
b) Percent change between each TFA diet and the control diet.
c) *p*-Values representing the main diet effect, determined by the MIXED procedure.
d) These data have been reported in a previous publication [6].
e) Mean ± SD (all such values).
f) Significantly different from the control diet ($p \leq 0.05$), as determined by MIXED models.
g) Expressed as × 10^2 μmol/mmol of cholesterol.
h) Significantly different from the iTFA diet ($p \leq 0.05$), as determined by MIXED models.

varied in their levels of control of the experimental diets with regards to nutritional factors other than TFA, such as saturated and polyunsaturated fatty acids. In our study, the key and most important difference between diets was the source and content of TFA. In previous studies, TFA were substituted by other fat sources such as polyunsaturated fat. Second, inconsistencies of the results can be attributed to the method (direct versus indirect) used to assess cholesterol synthesis. Most importantly, while studies in men including our own study showed no effect of iTFA as well as of rTFA on cholesterol endogenous synthesis, studies in women have been more inconsistent. Chardigny et al. [31] and Jakobsen et al. [32] have suggested that TFA effects on CVD risk factors and coronary heart disease risk may differ between men and women but this possibility requires further investigation. Nevertheless, the nutritionally controlled crossover design of our study and the tight adjustment of the diets for most fatty acids other than TFA demonstrated that TFA, from any source, exert no effect on estimated cholesterol synthesis in healthy men.

TFA consumption may exacerbate insulin resistance and for that reason is considered a modifiable risk factor for the MetS, type 2 diabetes and CVD [1]. Several of the metabolic effects attributed to dietary TFA are also similar to features of insulin resistance and the MetS [1, 33]. Not surprisingly, the reduction in cholesterol absorption induced by very high intakes of TFA is consistent with studies that have shown that metabolic states characterizing the MetS and diabetes are associated with decreased intestinal cholesterol absorption [16, 34]. In our study, intake of rTFA reduced cholesterol absorption to a greater extent than iTFA. This suggests that positional isomers of TFA may play a role in defining the effects of the different sources of TFA on cholesterol absorption. We hypothesize that the greater reduction in estimated cholesterol absorption following the consumption of rTFA can be attributable, at least in part, to the presence of and conversion into *c*9, *t*11 conjugated linoleic acid isomer. The *c*9, *t*11 conjugated linoleic acid isomer found primarily in dairy fat is known to be a strong peroxisome proliferator-activated receptor (PPAR) agonist [35, 36] and up regulation of PPAR-α has been shown to inhibit Niemann-Pick C1 like 1 receptor [37], the main cholesterol and phytosterols transporter involved in dietary sterol absorption [38]. Mozaffarian has suggested that TFA per se may act as ligands for nuclear receptors including PPARs, liver X receptors and sterol regulatory element-binding protein 1 [39]. This hypothesis has not yet been formally demonstrated [1], but considering the established role of many fatty acids on gene regulation, TFA on its own or through precursor-product pathways may regulate transcriptional factors, which in turn determine cholesterol absorption. Our data suggest that these effects also appear to be greater for rTFA than iTFA.

The reduced cholesterol absorption with TFA consumption combined with no apparent change in synthesis rate is counterintuitive considering that TFA from both sources raised plasma LDL-C concentrations. The underlying mechanism by which LDL-C increased despite unchanged synthesis and lowered absorption with TFA is likely to be related to a compensatory downregulation of the catabolic pathway of cholesterol-rich lipoproteins [29]. An in vitro study by Niu et al. [40] suggests that the incorporation of TFA in cell membrane phospholipids reduces membrane LDL receptor activity, which would translate into reduced LDL clearance from serum and higher plasma LDL-C concentrations. Accordingly, Matthan et al. [41] have shown using stable isotopes that the LDL-C raising effect of hydrogenated fat was primarily determined by a reduced catabolism of LDL particles. Our data suggest that LDL receptor activity and LDL clearance may have been reduced to a greater extent after rTFA than after iTFA consumption, thereby compensating for the difference in cholesterol absorption rates between the two sources of TFA and resulting in comparable rises in plasma LDL-C concentrations.

The present study has a number of limitations that need to be pointed out. First, the estimated amounts of dietary phytosterols differed between the three diets. We cannot exclude the possibility that the observed reduction in plasma phytosterols concentrations following the intake of iTFA and rTFA may be due to the lower phytosterol content of these diets compared with the control diet. However, we believe that variations in plasma phytosterols can only be partly explained by this difference in phytosterol content between the diets. Miettinen et al. [10] have suggested that a several 100-fold increase in the dietary phytosterol load is required to double plasma β-sitosterol levels in humans. Thus, a 117 mg/2500 kcal difference between diets (largest difference observed) is considered trivial. The difference in dietary phytosterols between the two TFA-rich diets also was only of 41 mg/2500 kcal. The magnitude of the observed reduction in plasma phytosterols between the iTFA and the rTFA diets was larger than what would have been predicted based solely on such a small difference in dietary phytosterols. It is also stressed that cholesterol absorption and synthesis were not directly measured, but were assessed using surrogate markers. However, these markers have been shown to be reliable and valid in previous studies [42, 43]. It must also be emphasized that the intake of rTFA tested in the present study (10.2 g/2500 kcal) is very high and impossible to reach under normal dietary circumstances. Attempts to measure surrogate markers of cholesterol homeostasis after the moderate-rTFA diet (4.2 g/2500 kcal), one of the four diets originally tested [6], led to unreliable data most likely because of significant deterioration in sample quality due to the fact they were analyzed 2 years after. Our data suggest that a moderate intake of rTFA is unlikely to have had an effect on estimated endogenous cholesterol synthesis, while its impact on intestinal cholesterol absorption is unclear.

In summary, we have shown that very high intakes of iTFA and rTFA both decrease intestinal cholesterol

absorption with no effect on cholesterol endogenous synthesis. The effect of rTFA on cholesterol absorption also appears to be greater than the effect of iTFA, but this difference does not translate into differences in plasma LDL-C concentrations between the two sources of TFA. The extent to which differences in the effects of rTFA and iTFA on cholesterol homeostasis affect disease progression is also unknown.

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