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ORIGINAL ARTICLE

Differential impact of milk fatty acid profiles on cardiovascular risk biomarkers in healthy men and women

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Background/Objectives: The objective of this study was to evaluate the impact of three specific ruminant (R) milk fats resulting from modification of the cow's diet on cardiovascular risk factors in healthy volunteers. R-milk fats were characterized by increased content in total *trans* fatty acids (R-TFAs) and parallel decrease in saturated fatty acids (SFAs).
Subjects/Methods: A total of 111 healthy, normolipemic men and women have been recruited for a monocentric, randomized, double-blind and parallel intervention, 4-week controlled study. Volunteers consumed three experimental products (butter, dessert cream and cookies) made with one of the three specific milk fats (55 g fat per day). During the first week (run-in period), the subjects consumed on a daily basis dairy products containing 72% SFA/2.85% R-TFA (called 'L0'). For the next 3 weeks of the study (intervention period), the first group continued to consume L0 products. The second group received dairy products containing 63.3% SFA/4.06% R-TFA (called 'L4'), and the third group received dairy products containing 56.6% SFA/12.16% R-TFA (called 'L9').
Results: Plasma concentrations of high-density lipoprotein (HDL)-cholesterol were not significantly altered by either diet ($P = 0.38$). Compared to L0 diet, L4 diet contributed to reduce low-density lipoprotein (LDL)-cholesterol (-0.14 ± 0.38 mmol/l, $P = 0.04$), total cholesterol (-0.13 ± 0.50 mmol/l, $P = 0.04$), LDL-cholesterol/HDL-cholesterol (-0.14 ± 0.36, $P = 0.03$) and total cholesterol/HDL-cholesterol (-0.18 ± 0.44, $P = 0.02$).
Conclusions: Different milk fat profiles can change cardiovascular plasma parameters in human healthy volunteers. A limited increase of the R-TFA/SFA ratio in dairy products is associated with an improvement in some cardiovascular risk factors. However, a further increase in R-TFA/SFA ratio has no additional benefit.
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Introduction

Over 2 million people in European Union are dying from cardiovascular disease (CVD) every year (European Heart Network, 2008). The subsequent cost is estimated to 192 billion per year including direct and indirect cost. Thus, the reduction of the number of death from CVD is a huge target that could be reached by a limiting exposure to CVD risk factors. In this respect, dietary fatty acids represent key factors having a significant impact on health, especially on CVD. Specific effects of clusters or isolated fatty acids have been extensively studied, with a particular attention paid to saturated fatty acids (SFAs) and *trans* fatty acids (TFAs) (Katan *et al*., 1995; Hu *et al*., 1997; Ascherio *et al*., 1999; Gebauer *et al*., 2007). Reports from different health authorities and agencies recommend a reduction of SFA and TFA intake (Stender and Dyerberg, 2003; Scientific Panel on Dietetic Products, 2004; Afssa, 2005).

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Two meta-analyses tabulating different intervention studies clearly stated that TFAs are more deleterious than SFAs, when considering fatty acids' impact on cardiovascular risk factors (Ascherio *et al*., 1999; Mensink *et al*., 2003). Consequently, the relationship between the consumption of dietary TFA and the increased risk of CVD has been clearly highlighted (Gebauer *et al*., 2006; Booker and Mann, 2008; Dalainas and Ioannou, 2008). However, all these studies considered industrially produced TFA (IP-TFA) isomers resulting from partial hydrogenation of oils, but TFAs are also present naturally in ruminant milk fat and meat (R-TFA). R-TFA and IP-TFA have different isomeric profiles. In IP-TFA, *trans*-9 18:1 (elaidic acid) and *trans*-10 18:1 are the most important isomers whereas *trans*-11 18:1 (vaccenic acid) is the major R-TFA isomer (Stender and Dyerberg, 2004). The R-TFA term comprises total TFAs (all the geometrical isomers of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) having nonconjugated, carbon–carbon double bonds in the *trans* configuration, except the conjugated linoleic acids, according to the definition by Afssa (2005). Until now, only few clinical trials have studied the specific isomeric effects of TFAs (IP-TFA vs R-TFA) on CVD.

Recently, two concomitant studies were published. In the first one, 38 healthy men were provided three meals per day based on four experimental diets: high R-TFA (3.7% of daily energy, $\approx$13.3 g/day), moderate R-TFA (1.5% of daily energy, $\approx$5.6 g/day), high IP-TFA (3.7% of daily energy, $\approx$13.3 g/day) and 'control' low total TFA (0.8% of daily energy) for 4 weeks. The consumption of the high IP-TFA and high R-TFA diets had similar consequences; that is, elevated low-density lipoprotein (LDL)-cholesterol concentrations and decreased high-density lipoprotein (HDL)-cholesterol levels compared to the consumption of moderate R-TFA or low total TFA diets (Motard-Belanger *et al*., 2008). The second one is the TRANSFACT study (Chardigny *et al*., 2008), where 40 healthy subjects consumed food items containing either R-TFA or IP-TFA (11–12 g/day, $\approx$5% of daily energy intake). Different effects on CVD risk factors are reported according to the two sources of TFAs but the HDL-cholesterol-lowering property of TFA was concluded to be specific to IP-TFA.

Moreover, the consumption for 6 weeks of dairy products naturally enriched in vaccenic acid (the major R-TFA isomer) (around 1.6% daily energy intake) had no effects on most CVD risk parameters in middle-aged men (Tricon *et al*., 2006). Finally, an 18-year follow-up study found no association between R-TFA intake and CVD risk factors (Jakobsen *et al*., 2008).

Modifications of cows' feeding are able to upregulate the R-TFA content in milk fat with a concomitant reduction in SFAs (Chilliard and Ferlay, 2004). These changes in milk fat composition can be considered as a beneficial output (Hu *et al*., 1997). In that respect during a 5-week intervention study, Tholstrup *et al*. (2006) showed that a butter rich in vaccenic acid (3.6 g/day—around 1% daily energy intake) and MUFAs, significantly decreased total and HDL-cholesterol concentrations in comparison with a conventional butter high in SFAs. From these combined data, the importance of improving R-TFA/SFA ratio in dairy products is suggested. This study aimed at evaluating in healthy volunteers, the impact on CVD risk factors of milk fats presenting varying ratio between R-TFA and SFA but also between MUFA and PUFA. In this respect, a clinical trial where two-thirds of daily fat intake came from experimental dairy fat was designed.

Materials and methods

Materials

Three experimental dairy fats differing in their fatty acid profiles were obtained from cows fed or not linseed extruded grain or oil; the detailed fatty acid profiles are presented in Table 1. The first one, called 'L0' (no linseed supplementation) is the dairy fat with the lowest R-TFA/SFA ratio, that is, 2.9 and 72/100 g of fatty acids, respectively. The milk was obtained from dairy cows fed a maize silage diet with cereal-based concentrate and soybean meal. The second dairy fat, 'L4' obtained from cows supplemented with 4.1% on dry matter basis of extruded linseed (Tradi-Lin; Valorex SAS, Combourtillé, France) contained around 4.1 and 63.3/100 g of R-TFA and SFA, respectively. Finally, 'L9' obtained from cows grazing on autumn grass based on a mixture of white clover and perennial rye grass and supplemented with 1 kg of linseed oil (SA Huilerie Vandeputte, Mouscron, Belgium) mixed with 5 kg of fresh maize silage. The milk contained around 12.2 and 56.6/100 g of R-TFA and SFA, respectively.

Subjects

Volunteers meeting the following criteria: age 18–50 years, waist circumference $<$94 cm for men and $<$80 cm for women, HDL-cholesterol $>$1 mmol/l, LDL-cholesterol $<$4.1 mmol/l and triglyceride $<$1.7 mmol/l were enrolled. The eligibility criteria also included nonsmoking, and for women, effective contraception. Characteristics of the volunteers are summarized in Table 2.

Sample size recruitment

The main criterion justifying the number of recruited subjects was the expected L9-induced increase of HDL-cholesterol compared to L0. The difference between L9 and L0 was calculated using the predictive equation of HDL-cholesterol (Yu *et al*., 1995) and averaged $\delta = 2.17$ mg/100 ml. Sample size (n) was then calculated using the formula $n = (z_\alpha + z_\beta)^2(\sigma/\delta)^2$ for comparison of two averages (significance level α was chosen to be 5% two-sided, leading to $z_\alpha = 1.96$, β was 1-power, and power was set to 80%, leading to $z_\beta = 0.84$). According to the TRANSFACT trial (Chardigny *et al*., 2006), the within-subject standard deviation (s.d.) on this parameter is 4.5 mg/100 ml. Therefore, 34 subjects

Table 1 Fatty acid composition of the different experimental dairy fats (g fatty acid/100 g fatty acids)

Fatty acids	*Fatty acid composition*		
	L0[a]	*L4*[a]	*L9*[a]
C4:0	2.54	2.83	2.94
C5:0	0.04	0.03	0.03
C6:0	1.80	1.76	1.95
C7:0	0.03	0.02	0.02
C8:0	1.20	1.06	1.24
C9:0	0.03	0.03	0.03
C10:0	3.09	2.33	2.86
C10:1	0.30	0.19	0.27
C11:0	0.07	0.04	0.05
C12:0	3.95	2.88	3.31
C13:0	0.22	0.13	0.17
C14:0	12.84	9.75	11.04
C14:1	1.00	0.64	0.94
C15:0	1.26	1.08	0.94
C16:0	34.60	27.94	21.93
C16:1	1.55	1.65	1.02
C17:0	0.73	0.79	0.53
C17:1	0.21	0.35	0.15
C18:0	9.41	12.42	9.43
C18:1 *trans* Total	2.53	3.49	9.50
trans-4	0.01	0.01	0.04
trans-5	0.01	0.01	0.03
trans-6/8	0.21	0.21	0.58
trans-9	0.21	0.23	0.45
trans-10	0.29	0.33	1.23
trans-11	1.00	1.81	4.26
trans-12	0.29	0.29	0.86
trans-13	0.51	0.59	2.04
trans-9 + *trans*-10 + *trans*-11	1.51	2.37	5.94
C18:1 *n*−9	15.53	21.87	17.12
C18:1 *cis*-14 + *trans*-16	0.28	0.35	0.63
C18:1 *cis*-15 + C19:0	0.16	0.21	0.53
Other *cis*-C18:1 isomers	0.94	0.97	1.49
trans-C18:2	0.32	0.57	2.66
CLA	0.42	0.67	1.86
C18:2 *n*−6	1.34	1.31	2.06
C18:3 *n*−3	0.22	0.59	1.22
C20:0	0.11	0.13	0.07
C20:2 *n*−6	0.01	0.01	0.02
C20:3 *n*−6	0.06	0.04	0.05
C20:4 *n*−6	0.09	0.07	0.08
C20:5 *n*−3	0.03	0.08	0.04
C22:0	0.04	0.05	0.02
C24:0	0.02	0.03	0.02
C22:5	0.05	0.09	0.05
Other fatty acids	3.00	3.53	3.72
Sum (12:0 + 14:0 + 16:0)	51.39	40.57	36.27
Total saturated fatty acids	71.97	63.31	56.59
Total *cis*-MUFA	19.52	25.66	20.99
Total *trans* fatty acids[b]	2.85	4.06	12.16
Total *cis*-PUFA	2.21	2.87	5.37

Abbreviations: *cis*-MUFA, *cis*-monounsaturated fatty acids; *cis*-PUFA, *cis*-polyunsaturated fatty acids; CLA, conjugated linoleic acid.
[a]L0, L4 and L9, see Materials and Methods section.
[b]Sum of *trans*-18:1 and *trans*-18:2 acid isomers; CLAs are not taken into account in this calculation.

per group were needed to detect significant statistical differences ($P<0.05$ two-sided test). To take into account putative dropouts, we finally recruited 37 subjects per group; that is, a total of 111 healthy volunteers (57 men and 54 women).

Human intervention design

This study was a controlled, double-blind, randomized trial. It has been approved by the French authorities 'Comité de Protection des Personnes' (CPP Auvergne, Clermont-Ferrand, France, Agreement No. AU684). For all subjects, we obtained written informed consent. The Clinical Trial Registration number is NCT00685581. The study design is provided in Figure 1. During the 3 week duration of the intervention, the volunteers consumed three different food items prepared with the three experimental fats: butter (20 g/day, 80% fat content), dessert cream (100 g/day, 25% fat content) and cookies (59 g/day, 24% fat content), which corresponded to a total intake of 55 g of lipid (that is, two-thirds of the total daily lipid intake). Within a day, the experimental products could be consumed during any meal or snack. The three food items were prepared with the three different experimental milk fats (see above). The products were manufactured using the same batch of experimental fat. Microbiological tests and measurement of both total fat and fatty acid profiles were performed before starting the clinical investigation.

During the run-in period (first week, W0), all subjects had to consume L0 food items (Table 1). Thereafter, the volunteers were randomly allocated to one of the three experimental groups after gender stratification was performed. For the following 3-week intervention period, the first group was maintained on the L0 dietary supplementation, whereas the second and the third groups received food items produced from the L4 and the L9 experimental fats, respectively (Figure 1). Fatty acid profile of L9 fat (Table 1) was designed so that the total TFA intake contributed to around 3.1% of daily energy intake (Table 3), which is 2.1% higher than the level recommended by the French authorities (that is, 2% of TFA excluding conjugated linoleic acid of daily energy intake (Afssa, 2005)).

The dietician gave instructions to subjects in a documented form to avoid foods containing IP-TFA and ruminant fat. The only source of TFA was the experimental products (R-TFA). All the volunteers were asked to avoid canteens or restaurants during the trial.

Measurements

Subjects attended the laboratory for measurements and blood samples the day after W0 (day 1 of W1) and the day after W3 (day 1 of W4) (Figure 1). Weight was measured at each visit after an overnight fast of at least 12 h, using the same calibrated digital scale with participant dressed in light indoor clothing without shoes. Blood were sampled after an 11–15 h overnight fast. Plasma was obtained by centrifugation, aliquoted and stored at −80 °C until further analyses. The subjects recorded their dietary intake (foods and drinks) during five consecutive days, including 3 week days and

Table 2 Baseline characteristics (by study group) of subjects who completed the trial

Parameter	*L0 group*	*L4 group*	*L9 group*	P-*value*
Clinical				
Gender (M/F)*	18/18	18/17	18/18	0.990
Age (years)	26 ± 7 (12; 40)	25 ± 6 (13; 37)	28 ± 9 (10; 45)	0.394
Waist (cm)	74.1 ± 9.0 (56.6; 91.7)	74.4 ± 8.1 (58.6; 90.2)	71.3 ± 8.1 (55.3; 87.2)	0.997
Body mass index (kg/m^2)	21.7 ± 2.7 (16.5; 26.9)	22.0 ± 2.3 (17.5; 26.5)	21.9 ± 2.5 (16.9; 26.8)	0.891
Systolic blood pressure (mm Hg)	116 ± 9 (97; 134)	116 ± 8 (100; 132)	116 ± 13 (91; 141)	0.997
Diastolic blood pressure (mm Hg)	73 ± 8 (58; 88)	71 ± 9 (53; 89)	72 ± 9 (54; 90)	0.654
Resting heart rate (beat per min)	67 ± 8 (51; 83)	64 ± 7 (50; 78)	68 ± 10 (48; 88)	0.122
Glucose (mmol/l)	4.6 ± 03 (4.0; 5.3)	4.6 ± 0.4 (3.8; 5.5)	4.7 ± 0.5 (3.7; 5.6)	0.919
Bilirubin (μmol/l)	14 ± 9 (−3; 32)	13 ± 8 (−2; 29)	13 ± 6 (1; 25)	0.709
ASAT (UI/l)	23 ± 5 (13; 32)	23 ± 4 (14; 31)	23 ± 5 (12; 33)	0.927
ALAT (UI/l)	17 ± 8 (1; 33)	18 ± 9 (1; 35)	17 ± 7 (3; 30)	0.685
Phosphatase alkaline (UI/l)	58 ± 14 (31; 85)	59 ± 21 (18; 100)	56 ± 13 (30; 81)	0.653
γ-Glutamyl transpeptidase (UI/l)	14 ± 7 (0; 28)	18 ± 12 (−6; 42)	15 ± 7 (0; 29)	0.135
Na (mmol/l)	142 ± 2 (138; 145)	141 ± 2 (138; 145)	141 ± 2 (138; 145)	0.572
K (mmol/l)	4.3 ± 0.3 (3.6; 4.9)	4.2 ± 0.3 (3.7; 4.8)	4.2 ± 0.3 (3.6; 4.8)	0.687
Cl (mmol/l)	103 ± 2 (100; 106)	103 ± 2 (100; 106)	103 ± 1 (100; 106)	0.919
Urea (mmol/l)	5.1 ± 1.2 (2.8; 7.3)	5.3 ± 1.5 (2.4; 8.3)	4.9 ± 1.3 (2.4; 7.5)	0.429
Creatinin (μmol/l)	75 ± 10 (56; 94)	78 ± 11 (56; 100)	75 ± 12 (52; 97)	0.354
Erythrocytes (T/l)	4.87 ± 0.38 (4.13; 5.61)	4.82 ± 0.36 (4.11; 5.53)	4.79 ± 0.41 (3.99; 5.58)	0.621
Hemoglobin (g/100 ml)	14.3 ± 1.2 (12.0; 16.6)	14.0 ± 1.1 (12.0; 16.1)	14.0 ± 1.2 (11.8; 16.3)	0.525
Hematocrit (%)	42.3 ± 3.1 (36.1; 48.4)	41.6 ± 2.5 (36.8; 46.4)	41.6 ± 3.0 (35.8; 47.4)	0.530
Mean globular volume (fl)	86.8 ± 2.5 (81.9; 91.7)	86.4 ± 3.0 (80.6; 92.2)	87.1 ± 3.8 (79.7; 94.5)	0.624
Platelets (g/l)	224 ± 38^a (148; 299)	255 ± 42^b (172; 337)	247 ± 52^b (146; 349)	0.01
Leukocytes (g/l)	5.96 ± 1.36 (3.29; 8.63)	6.25 ± 1.55 (3.22; 9.28)	5.68 ± 1.28 (3.16; 8.19)	0.234
Neutrophils (g/l)	3.12 ± 1.17 (0.83; 5.40)	3.30 ± 1.14 (1.07; 5.54)	2.98 ± 0.90 (1.22; 4.74)	0.446
Eosinophils (g/l)	0.16 ± 0.10 (0.03; 0.35)	0.16 ± 0.10 (0.03; 0.35)	0.17 ± 0.16 (0.15; 0.49)	0.839
Basophils (g/l)	0.02 ± 0.02 (0.01; 0.06)	0.02 ± 0.01 (0.00; 0.05)	0.03 ± 0.01 (0.00; 0.05)	0.587
Lymphocytes (g/l)	2.14 ± 0.58 (1.00; 3.28)	2.23 ± 0.73 (0.79; 3.66)	2.03 ± 0.65 (0.76; 3.30)	0.454
Monocytes (g/l)	0.52 ± 0.13 (0.27; 0.77)	0.53 ± 0.18 (0.19; 0.87)	0.48 ± 0.13 (0.22; 0.74)	0.337
Fasting chemical lipids				
HDL-C (mmol/l)	1.69 ± 0.33 (1.03; 2.34)	1.76 ± 0.50 (0.79; 2.74)	1.62 ± 0.40 (0.84; 2.39)	0.348
LDL-C (mmol/l)	2.34 ± 0.67 (1.02; 3.66)	2.46 ± 0.75 (0.99; 3.93)	2.35 ± 0.79 (0.80; 3.91)	0.760
Triacylglycerol (mmol/l)	0.81 ± 0.25 (0.31; 1.30)	0.85 ± 0.32 (0.23;1.47)	0.69 ± 0.28 (0.13; 1.25)	0.052
Cholesterol (mmol/l)	4.39 ± 0.69 (3.05; 5.74)	4.61 ± 0.82 (2.99; 6.22)	4.29 ± 0.86 (2.59; 5.98)	0.226

Values are expressed as mean ± s.d. and 95% confidence intervals (95% CIs). Data were analyzed by a one-way ANOVA. Means in a row without common superscript letters besides them differ.
*Number of males and females, respectively.

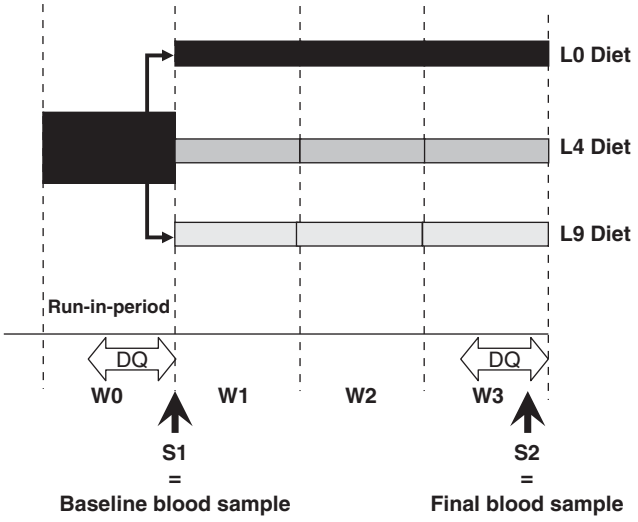

Figure 1 Study design (W, week; S, sample; DQ, dietary questionnaire).

2 weekend days, during the run-in period (W0) and during the last week of the intervention (W3). Data were coded and analyzed by a dietician using computerized nutrient databases (GENI Micro6.0; Villers-les-Nancy, France).

Biochemical analyses

HDL-cholesterol, total cholesterol, triglycerides, apolipoprotein A1 and apolipoprotein B were measured by enzymatic assays using a Konelab 20 analyzer (Thermo Electron SA, Cergy-Pontoise, France). LDL-cholesterol concentration was calculated by the Friedewald equation. To assess the compliance, we characterized plasma phospholipids fatty acid profiles after plasma lipid extraction and fatty acid methylation. Fatty acid methyl ester profiles were analyzed and identified by gas chromatography (Trace GC 2000 Series; Thermo Finnigan, Illkirch, France). The detailed analytical conditions were already reported (Roy *et al.*, 2006). Cholesteryl ester transfer protein activity was measured by

Table 3 Mean daily intake and 95% CIs of energy and macronutrients in L0, L4 and L9 groups, at baseline and after the 3-week intervention period (follow-up)

	L0 group (n = 36)		*L4 group* (n = 35)		*L9 group* (n = 36)		*ANOVA*		
	Baseline	*Follow-up*	*Baseline*	*Follow-up*	*Baseline*	*Follow-up*	*Gender*	*Diet*	*Gender × diet*
			Mean ± s.d. (95% CIs)				P	P	P
Total energy (kJ/day)	8610 ± 1404 (−5858; 11361)	8782 ± 1602 (−5642; 11923)	8556 ± 1884 (−4864; 12249)	8583 ± 1486 (−5671; 11495)	8104 ± 1539 (−5086; 1121)	8375 ± 1519 (−5398; 11352)	0.225	0.670	0.655
Protein (%en)	14.8 ± 3.0 (−9.0; 20.6)	14.9 ± 2.9 (−9.3 20.5)	15.0 ± 2.7 (−9.7; 20.3)	15.4 ± 2.7 (−10.2; 20.7)	14.1 ± 2.8 (−8.6; 19.6)	14.5 ± 2.4 (−9.8; 19.2)	0.284	0.666	0.968
Carbohydrate (%en)	47.5 ± 5.9 (−36.0; 59.0)	47.4 ± 5.9 (−35.9; 59.0)	45.9 ± 5.3 (−35.5; 56.2)	44.9 ± 5.0 (−36.0; 55.7)	47.2 ± 5.7 (−35.9; 58.4)	46.7 ± 4.9 (−37.2; 56.2)	0.453	0.912	0.485
Total fat (%en)	37.7 ± 5.4 (−27.1; 48.2)	37.7 ± 5.0 (−27.8; 47.5)	39.2 ± 5.0 (−29.4; 49.0)	38.7 ± 5.1 (−28.8; 48.6)	38.8 ± 5.4 (−28.3; 49.3)	38.8 ± 4.8 (−29.4; 48.2)	0.805	0.876	0.612
SFA (%en)	21.5 ± 2.6 (−16.4;26.6)	21.3 ± 2.8[a] (−15.8; 26.8)	22.1 ± 3.0 (−16.2; 28.0)	19.9 ± 2.9[b] (−14.2; 25.5)	22.6 ± 3.3 (−16.1; 29.0)	18.1 ± 2.4[c] (−13.4; 22.9)	0.308	<0.0001	0.965
MUFA (%en)	11.4 ± 2.7 (−6.0; 16.7)	11.8 ± 2.6[a] (−6.7; 16.8)	11.7 ± 2.5 (−6.9; 16.5)	14.0 ± 2.6[b] (−9.0; 19.1)	11.9 ± 2.6 (−6.7; 17.0)	14.3 ± 2.2[b] (−10.1; 18.6)	0.607	0.0003	0.904
PUFA (%en)	3.6 ± 1.3 (−1.1; 6.1)	3.6 ± 1.1[a] (−1.4; 5.7)	3.5 ± 1.2 (−1.1; 5.9)	3.9 ± 1.3[b] (−1.3; 6.4)	3.6 ± 1.2 (−1.3; 5.9)	5.2 ± 1.0[c] (−3.2; 7.1)	0.380	<0.0001	0.531
Total TFA (%en)*	0.70 ± 0.11 (−0.49; 0.91)	0.69 ± 0.11[a] (−0.47; 0.91)	0.72 ± 0.14 (−0.45; 0.98)	1.01 ± 0.18[b] (−0.65;1.36)	0.75 ± 0.13 (−0.49; 1.01)	3.10 ± 0.55[c] (−2.02; 4.18)	0.169	<0.0001	0.148
Total *trans*-18:1 (%en)*	0.62 ± 0.10 (−0.43; 0.81)	0.61 ± 0.10[a] (−0.43; 0.81)	0.64 ± 0.12 (−0.40; 0.87)	0.87 ± 0.16[b] (−0.56; 1.17)	0.67 ± 0.12 (−0.44; 0.90)	2.42 ± 0.43[c] (−1.57; 3.27)	0.236	<0.0001	0.173

Abbreviations: %en, % of total energy; *cis*-MUFA, *cis*-monounsaturated fatty acids; *cis*-PUFA, *cis*-polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, *trans* fatty acids.
All values are means ± s.d. Data (the difference between end of the intervention and baseline) were analyzed using a two-way ANOVA with gender and diet as factors. Means in a row without common superscript letters besides them differ.
*This represents only the percentage of TFA and total *trans*-18:1 in the three different food items (butter, dessert cream and cookies).

fluorimetry using commercial kits. Fibrinogen was assessed using a turbidimetric assay (BioDirect, La Villeneuve, France).

Assessment of subjects' compliance

Subject compliance was assessed by a questionnaire and by analysis of the concentration of total *trans*-18:1 and vaccenic acid in plasma phospholipids (Mansour *et al*., 2001). The mean baseline vaccenic acid concentration in phospholipids was 0.098 ± 0.027 (mean ± s.d.) g/100 g total fatty acids with no significant effect observed between groups. At the end of the experimental periods, the average concentrations of vaccenic acid found in plasma phospholipids were 0.160 ± 0.045, 0.252 ± 0.077 and 0.616 ± 0.184 g/100 g total fatty acids for L0, L4 and L9 diet, respectively. It was statistically different between the three groups (two-way analysis of variance; diet $P<0.0001$; gender $P=0.489$; interaction $P=0.473$; *post hoc* tests: L0, L4 $P=0.002$; L0, L9 $P<0.0001$ and L4, L9 $P<0.0001$).

Statistical methods

Values are expressed as mean ± s.d.. Statistical analysis was performed using the StatView version 5.0 software (SAS Institute Inc., Cary, NC, USA). The one-way analysis of variance procedure was used to determine difference in baseline parameters for the groups. Differences between final and baseline measurements among the three groups were tested by a two-way analysis of variance, including diet and gender as factors. If the main effects were significant ($P<0.05$), protected least significant difference Fisher's test was applied for multiple comparisons (*post hoc* test). We decided to present the results on the per-protocol data set because three subjects had already withdrawn during the run-in period before the first measurements (for personal reasons and because of time constraints) and one subject was excluded because of non-compliance. Compliance to the protocol was a primary outcome in the analysis, showing that per-protocol analysis could be performed on the 107 subjects who completed the study (Figure 2).

Results

Dietary intake

During the intervention period, the dietary intake was similar in each experimental group with no gender effect (Table 3). As expected, SFA, PUFA and TFA intake were significantly different between L0, L4 and L9 diets with no gender effects (Table 3).

Plasma lipids, apolipoproteins

Considering the primary outcome, that is, plasma concentrations of HDL-cholesterol, we evidenced no significant change between the three groups. However compared to

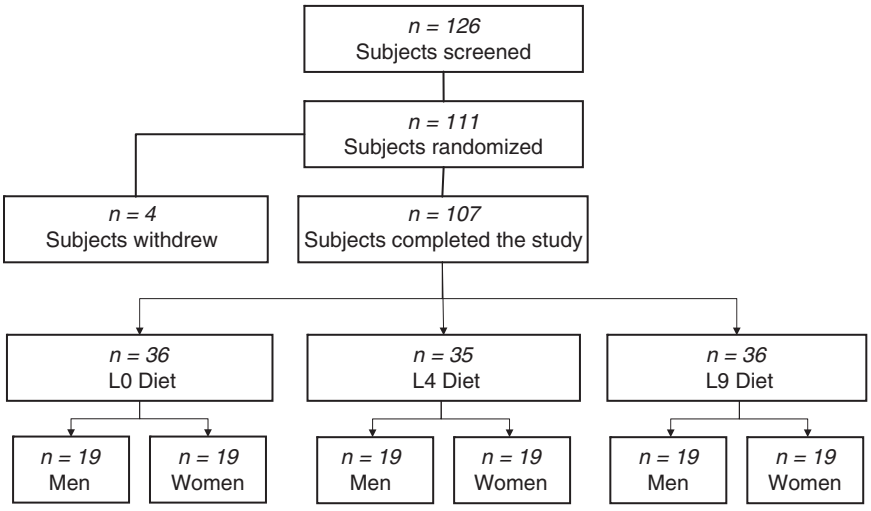

Figure 2 Disposition of subjects ($n=126$) during the study.

L0 diet, L4 diet contributed to reduce total cholesterol ($P=0.037$), LDL-cholesterol ($P=0.040$), LDL-cholesterol/HDL-cholesterol ratio ($P=0.028$) and total cholesterol/HDL-cholesterol ratio ($P=0.016$), whereas L9 diet did not alter most of these parameters (Table 4).

Plasma apolipoprotein B concentration tended to be reduced in the L4 group compared to the L0 group, but without reaching the level of significance ($P=0.065$).

No statistical differences appeared for all the others parameters presented in Table 4.

Discussion

The impact of R-TFA on CVD risk markers is a major issue for human nutritional recommendations. Changing the level of R-TFA biosynthesis in the cows' rumen is associated with a large panel of changes in milk fatty acid content. Therefore, our study aimed at examining the metabolic effects of experimental milk fats that represent the widest range of putative milk fatty acid profiles resulting from different cows' feeding strategies. Major finding showed that the consumption of dairy fat containing 63.3% SFA and 3.5% *trans*-18:1 (L4 diet) improved some CVD risk factors for healthy volunteers in comparison with a typical dairy fat (72% SFA, 2.5% *trans*-18:1—L0 diet). It is shown by a decrease in total cholesterol, LDL-cholesterol, total cholesterol/HDL-cholesterol ratio and LDL-cholesterol/HDL-cholesterol ratio. We observed a change by 0.18 units in the ratio of total cholesterol/HDL cholesterol between L0 and L4 diet. As reported by Stampfer *et al*. (1991), we calculate that this change can be associated to a 9.5% decrease in the risk of myocardial infarction, which is in the same range as the replacement of 1334 mg *trans* α-linolenic acid by dietary *cis* α-linolenic acid (Vermunt *et al*., 2001). Moreover, our results show that the consumption for 3 weeks of the L9-dairy fat, which contains less SFA (56.6%) and more *trans*-18:1 (9.5%) compared to the L0 diet, induces no significant changes in plasma markers of CVD (Table 4). In addition, the ratio between total and HDL-cholesterol was significantly increased after 3 weeks of L9-dairy fat compared to L4 diet ($P=0.029$). These data suggest that whereas mild increase in R-TFA/SFA ratio in milk fat may be beneficial compared to L0 diet, further increase in R-TFA/SFA ratio does not provide additional benefit regarding the CVD risk factors.

In a study where SFA intake was maintained constant (around 18% of energy intake), a 1.5% total energy intake as R-TFA failed to alter any CVD risk factor (Motard-Belanger *et al*., 2008). Interestingly, in healthy moderately overweight men and women, Rivellese *et al*. (2003) showed that decreasing SFA intake by 8% (from 17.6 to 9.6% total energy intake) and increasing compensation MUFA intake (from 13.1 to 21.2% total energy intake) induced a reduction in plasma LDL-cholesterol concentration (−0.38 mmol/l). In our present study, milk fats were characterized by different levels in both R-TFAs and SFAs, a higher R-TFA level being associated with a lower SFA content. Notably, high R-TFA/SFA ratio was also associated with enhanced MUFA and PUFA intake. These combined changes in milk fat composition could, therefore, partially explain the LDL-cholesterol reduction observed after the consumption of the L4 diet in comparison with L0 (see Table 4). Our present results are in agreement with the results of Poppitt *et al*. (2002) and Seidel *et al*. (2005). Briefly, Poppitt *et al*. (2002) reported a significant decrease in both total and LDL-cholesterol in plasma from healthy men after consuming a modified butter fat (−5 units of percent total energy intake of SFA and +2 units of total energy intake of MUFA) for 3 weeks. Seidel *et al*. (2005) showed beneficial effects regarding the CVD risk, that is, decreased LDL-cholesterol/HDL-cholesterol ratio, with the consumption of modified milk fat obtained by feeding cows high-fat rapeseed cake (16% oil).

Table 4 Serum lipids, lipoprotein, apolipoprotein concentrations, CETP activity and fibrinogen concentration in three different groups (L0, L4 and L9 group) mean and 95% CIs at baseline and estimate mean effects after 3 weeks

Variable and subjects	*Baseline values**			*Estimate mean effects***			*P-value*		
	L0 group (n = 36)	*L4 group* (n = 35)	*L9 group* (n = 36)	*L0*	*L4*	*L9*	*Diet*	*Gender*	*Interaction*
HDL-C (mmol/l)	1.70 ± 0.44 (0.85; 2.56)	1.74 ± 0.51 (0.74; 2.74)	1.59 ± 0.32 (0.97; 2.21)	0.01 ± 0.16 (−0.31; 0.33)	0.05 ± 0.17 (−0.29; 0.39)	0.00 ± 0.15 (−0.30; 0.29)	0.378	0.457	0.965
LDL-C (mmol/l)	2.33 ± 0.77 (0.82; 3.83)	2.65 ± 0.83 (1.02; 4.28)	2.55 ± 0.90 (0.78; 4.32)	0.11 ± 0.33^{a} (−0.53; 0.75)	−0.14 ± 0.38^{b} (−0.72; 0.77)	−0.07 ± 0.42^{a,b} (−0.89; 0.76)	0.040	0.759	0.386
Total cholesterol (mmol/l)	4.42 ± 0.78 (2.88; 5.95)	4.88 ± 0.86 (3.19; 6.57)	4.52 ± 0.93 (2.70; 6.34)	0.1 ± 0.42^{a} (−0.68; 0.95)	−0.13 ± 0.50^{b} (−1.11; 0.85)	−0.05 ± 0.42^{a,b} (−0.87; 0.77)	0.037	0.448	0.332
TG (mmol/l)	0.85 ± 0.31 (0.24; 1.47)	1.08 ± 0.53 (0.04; 2.12)	0.82 ± 0.29 (0.25; 1.40)	0.05 ± 0.27 (−0.48; 0.57)	−0.10 ± 0.46 (−0.99; 0.80)	0.04 ± 0.35 (−0.64; 0.72)	0.198	0.629	0.094
ApoA1 (g/l)	1.52 ± 0.25 (1.04; 2.01)	1.63 ± 0.33 (0.98; 2.29)	1.48 ± 0.20 (1.09; 1.88)	0.04 ± 0.13 (−0.21; 0.29)	0.01 ± 0.11 (−0.20; 0.22)	0.00 ± 0.08 (−0.16; 0.16)	0.387	0.980	0.168
ApoB (g/l)	0.79 ± 0.19 (0.42; 1.16)	0.88 ± 0.21 (0.47; 1.28)	0.81 ± 0.22 (0.37; 1.24)	0.02 ± 0.09 (−0.15; 0.20)	−0.03 ± 0.10 (−0.22; 0.16)	0.01 ± 0.12 (−0.22; 0.24)	0.065	0.840	0.221
LDL-C/HDL-C	1.47 ± 0.65 (0.21; 2.74)	1.69 ± 0.70 (0.31; 3.06)	1.68 ± 0.73 (0.25; 3.12)	0.06 ± 0.22^{a} (−0.37; 0.50)	−0.14 ± 0.36^{b} (−0.84; 0.57)	0.00 ± 0.33^{a,b} (−0.66; 0.65)	0.028	0.837	0.587
Total cholesterol/HDL-C	2.73 ± 0.74 (1.27; 4.18)	3.00 ± 0.85 (1.33; 4.68)	2.93 ± 0.79 (1.39; 4.47)	0.07 ± 0.28^{a} (−0.47; 0.61)	−0.18 ± 0.44^{b} (−1.05; 0.68)	0.01 ± 0.39^{a} (−0.74; 0.77)	0.016	0.761	0.293
ApoB/ApoA1	0.53 ± 0.15 (0.23; 0.84)	0.56 ± 0.16 (0.25; 0.87)	0.55 ± 0.17 (0.23; 0.87)	0.00 ± 0.06 (−0.12; 0.12)	−0.03 ± 0.07 (−0.16; 0.11)	0.01 ± 0.08 (−0.14; 0.16)	0.133	0.782	0.577
CETP activity (nmol/h/ml)	16.87 ± 3.97 (9.10; 24.66)	17.04 ± 4.66 (7.91; 26.18)	18.12 ± 4.30 (9.69; 26.56)	0.23 ± 6.83 (−13.15; 13.61)	0.61 ± 8.39 (−15.84; 17.07)	0.03 ± 7.31 (−14.29; 14.36)	0.944	0.630	0.701
Fibrinogen (g/l)	2.68 ± 0.53 (1.64; 3.72)	2.75 ± 0.57 (1.64; 3.86)	2.70 ± 0.53 (1.65; 3.74)	−0.56 ± 0.52 (−1.58; 0.46)	−0.49 ± 0.43 (−1.34; 0.36)	−0.50 ± 0.55 (−1.59; 0.58)	0.843	0.458	0.744

Abbreviations: ApoA, apolipoprotein A; ApoB, apolipoprotein B; CETP, cholesterol ester transfer protein; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides.
*Values are expressed as mean ± s.d.
**Estimate mean effect is defined as the difference between end of the intervention and baseline. Data are analyzed by using a two-way ANOVA. Means in a row without common superscript letters besides them differ.

By contrast, our study shows that the consumption of R-TFA up to 2.42% (L9 diet) of the daily energy intake has no significant effect on the evolution of the HDL concentration that is different from an IP-TFA intake (Katan *et al*., 1995). However, the differential effect between IP-TFA and R-TFA sources on the HDL parameter seems to disappear for higher TFA intake (3.5% total energy intake) (Motard-Belanger *et al*., 2008). Even so, our data suggest that whereas mild increase in R-TFA/SFA ratio in milk fat may be beneficial compared to L0 diet, further increase in R-TFA/SFA ratio does not provide additional benefit regarding the CVD risk factors. Moreover, the lack of beneficial effect of the L9 diet could also due to the huge increase in the *trans*-18:2 isomers. These isomers have been reported to be more deleterious than the *trans*-18:1 isomers (Baylin *et al*., 2003), for a review see Mozaffarian and Clarke (2009).

During our clinical intervention, we found no significant effect of the consumption of these three different diets on the HDL parameter. This result is in accordance with already published trials. Tricon *et al*. (2006) reported that the consumption for 6 weeks of a dairy product naturally enriched in *cis*-9,*trans*-11 conjugated linoleic acid (0.2–1.5 g/day) and *trans*-11 18:1 (0.8–6.3 g/day) failed to alter plasma triacylglycerol, total cholesterol, LDL-cholesterol and HDL-cholesterol concentrations and total cholesterol/HDL-cholesterol ratio, in healthy middle-aged men. The lack of differences on the HDL parameter could be related to our calculation of the sample size. Indeed, to calculate the sample size, we use the predictive equation of HDL-cholesterol (Yu *et al*., 1995) and, conversely, we decided that the predicted difference should be $\delta = 2.17$ mg/100 ml: it was perhaps a too small extend in the change in HDL concentrations.

Moreover, our study was carried out in men and women. To our knowledge, there are few studies that assessed the effect of the consumption of modified dairy fat on female CVD risk factors. In our conditions, we found no gender effect, for the relation between the CVD risk factors and fatty acids profiles of dairy fat.

To conclude, we confirm that the consumption of R-TFA at nutritional level (1.01% L4 diet, that is, $<2.0\%$ of energy, the level recommended by the French authorities) has no adverse effect related to some cardiovascular risk factors whatever the gender, which is in accordance with most intervention studies (Seidel *et al*., 2005; Motard-Belanger *et al*., 2008) and also with the recent epidemiological study (Jakobsen *et al*., 2008). Moreover, this clinical study underlines the fact that cows' feeding strategy consisting in decreasing the SFA/TFA ratio (less SFA (56.6%) and more total *trans* (12.16%)) in fat does not bring any additional benefits regarding the CVD risk in healthy volunteers.

Conflict of interest

The authors declare no conflict of interest.

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