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

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6

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14

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21

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26

27 **Abstract**

28 Objectives: to evaluate the impact of three specific ruminant (R) milk fats resulting from
29 modification of the cow's diet on cardiovascular risk factors in healthy volunteers. R-milk fats were
30 characterized by increased content in **total** *trans* fatty acids (R-TFA) and parallel decrease in
31 saturated fatty acids (SFA).

32

33 Methods: 111 healthy, normolipemic men and women have been recruited for a monocentric,
34 randomised, double-blind, and parallel intervention, 4-week controlled study. Volunteers consumed
35 3 experimental products (butter, dessert cream and cookies) made with one of the 3 specific milk
36 fats (55 g fat/day). During the first week (run-in period), the subjects consumed on a daily basis
37 dairy products containing 72% SFA/**2.85%** R-TFA (called "L0"). For the next 3 weeks of the study
38 (intervention period), the first group continued to consume L0 products. The second group received
39 dairy products containing 63.3% SFA/**4.06%** R-TFA (called "L4"), and the third group received
40 dairy products containing 56.6% SFA/**12.16%** R-TFA (called "L9").

41

42 Results: plasma concentrations of HDL-cholesterol was not significantly altered by either diet ($p =$
43 0.38). Compared to L0 diet, L4 diet contributed to reduce LDL-cholesterol (-0.14 ± 0.38 mmol/L, $p =$
44 0.04), total cholesterol (-0.13 ± 0.50 mmol/L, $p = 0.04$), LDL-cholesterol/HDL-cholesterol ($-$
45 0.14 ± 0.36 , $p = 0.03$) and total cholesterol/HDL-cholesterol (-0.18 ± 0.44 , $p = 0.02$).

46

47 Conclusion: different milk fat profiles can change cardiovascular plasma parameters in human
48 healthy volunteers. A limited increase of the R-TFA/SFA ratio in dairy products is associated with
49 an improvement in some cardiovascular risk factors. However, a further increase in R-TFA/SFA
50 ratio has no additional benefit.

51

52 **Keywords:** Human Nutrition, Lipids, *trans* fatty acids, milk fat, cardiovascular risk factors,
53 cholesterol.

54

55 Introduction

56 Over 2 million people in EU are dying from Cardiovascular disease (CVD) every year (European
57 Heart Network, 2008). The subsequent cost is estimated to €192 billion /y including direct and
58 indirect cost. Thus, the reduction of the number of death from CVD is a huge target which could be
59 reached by a limiting exposure to CVD risk factors. In this respect, dietary fatty acids represent key
60 factors having a significant impact on health, especially on CVD. Specific effects of clusters or
61 isolated fatty acids have been extensively studied, with a particular attention paid to saturated (SFA)
62 and *trans* (TFA) fatty acids (Ascherio et al., 1999; Gebauer et al., 2007; Hu et al., 1997; Katan et
63 al., 1995). Reports from different health authorities and agencies recommend a reduction of SFA
64 and TFA intake (Afssa, 2005; Scientific Panel on Dietetic Products, 2004; Stender and Dyerberg,
65 2003).

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

80 Recently, two concomitant studies were published. In the first one, 38 healthy men were provided 3
81 meals/d based on 4 experimental diets: high R-TFA (3.7% of daily energy, ≈ 13.3 g/d), moderate R-
82 TFA (1.5% of daily energy, ≈ 5.6 g/d), high IP-TFA (3.7% of daily energy, ≈ 13.3 g/d), and
83 “control” low total TFA (0.8% of daily energy) for 4 weeks. The consumption of the high IP-TFA
84 and high R-TFA diets had similar consequences, *i.e.* elevated LDL-cholesterol concentrations and
85 decreased HDL-cholesterol levels compared to the consumption of moderate R-TFA or low total
86 TFA diets (Motard-Belanger et al., 2008). The second one is the TransFact study (Chardigny et al.,
87 2008) where 40 healthy subjects consumed food items containing either R-TFA or IP-TFA (11–12
88 g/d, $\approx 5\%$ of daily energy intake). Different effects on CVD risk factors are reported according to the
89 2 sources of TFA but the HDL cholesterol–lowering property of TFA was concluded to be specific
90 to IP-TFA.

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

95 Modifications of cows’ feeding are able to up-regulate the R-TFA content in milk fat with a
96 concomitant reduction in SFA (Chilliard and Ferlay, 2004). These changes in milk fat composition
97 can be considered as a beneficial output (Hu et al., 1997). In that respect during a 5 week
98 intervention study, Tholstrup et al. showed that a butter rich in vaccenic acid (3.6g/d – around 1%
99 daily energy intake) and monounsaturated FAs, significantly decreased total and HDL-cholesterol
100 concentrations in comparison with a conventional butter high in SFA (Tholstrup et al., 2006). From

101 these combined data, the importance of improving R-TFA to SFA ratio in dairy products is
102 suggested. The present study aimed at evaluating in healthy volunteers, the impact on CVD risk
103 factors of milk fats presenting varying ratio between R-TFA and SFA but also MUFA and PUFA.

104 In this respect, a clinical trial where two-thirds of daily fat intake came from experimental dairy fat
105 was designed.

106 **Materials and Methods**

107 **Materials.** Three experimental dairy fats differing in their fatty acid profiles were obtained from
108 cows fed or not linseed extruded grain or oil, the detailed fatty acid profiles are presented in Table
109 1. The first one, called “L0” (no linseed supplementation) is the dairy fat with the lowest R-
110 TFA/SFA ratio, i.e. 2.9 and 72 g/100g of fatty acids respectively. The milk was obtained from dairy
111 cows fed a maize silage diet with cereals based concentrate and soybean meal. The second dairy fat,
112 “L4” obtained from cows supplemented with 4.1% on DM basis of extruded linseed (Tradi-Lin®
113 Valorex SAS Combourtillé, France) contained around 4.1 and 63.3 g/100g of R-TFA and SFA,
114 respectively. Finally, “L9” obtained from cows grazing on autumn grass based on a mixture of
115 white clover and perennial rye grass and supplemented with 1 kg of linseed oil (SA huilerie
116 Vandeputte, Mouscron, Belgium) mixed with 5 kg of fresh maize silage. The milk contained around
117 12.2 and 56.6g /100g of R-TFA and SFA, respectively.

118

119 **Subjects.** Volunteers meeting the following criteria: age 18-50 y, waist circumference < 94 cm for
120 men and < 80 cm for women, HDL-C > 1 mmol/L, LDL-C < 4.1 mmol/L and TG < 1.7 mmol/L
121 were enrolled. The eligibility criteria also included non-smoking, and for women, effective
122 contraception. Characteristics of the volunteers are summarized in Table 2.

123

124 **Sample size recruitment.** The main criterion justifying the number of recruited subjects was the
125 expected L9-induced increase of HDL-cholesterol compared to L0. The difference between L9 and
126 L0 was calculated using the predictive equation of HDL-cholesterol (Yu et al., 1995) and averaged
127 $\delta=2.17$ mg/dL. Sample size (n) was then calculated using the formula $n = (z_{\alpha}+z_{\beta})^2(\sigma/\delta)^2$ for
128 comparison of two averages (significance level α was chosen to be 5 % two-sided, leading to $z_{\alpha} =$
129 1.96, β was 1-power, and power was set to 80%, leading to $z_{\beta} = 0.84$). According to the TransFact
130 trial (Chardigny et al., 2006) the within subject standard deviation (SD) on this parameter is 4.5
131 mg/dL. Therefore, 34 subjects per group were needed to detect significant statistical differences

132 (p<0.05 two- sided test). To take into account putative drop outs, 37 subjects per group were finally
133 recruited i.e. a total of 111 healthy volunteers (57 men and 54 women).

134

135 **Human intervention design.** This study was a controlled, double-blind, randomized trial. It has
136 been approved by the French authorities “Comité Protection des Personnes” (CPP Auvergne,
137 Clermont-Ferrand, France, agreement #AU684). For all subjects, written informed consent was
138 obtained. The Clinical Trial Registration number is NCT00685581. The study design is provided in
139 Figure 1. During the 3 week duration of the intervention, the volunteers consumed three different
140 food items prepared with the 3 experimental fats: butter (20 g/d, 80% fat content), dessert cream
141 (100 g/d, 25% fat content), and cookies (59 g/d, 24% fat content) which corresponded to a total
142 intake of 55 g of lipid (i.e. two-thirds of the total daily lipid intake). Within a day, the experimental
143 products could be consumed during any meal or snack. The three food items were prepared with the
144 three different experimental milk fats (see above). The products were manufactured using the same
145 batch of experimental fat. Microbiological tests and measurement of both total fat and fatty acid
146 (FA) profiles were performed before starting the clinical investigation.

147 During the run-in period (first week, W0), all subjects had to consume L0 food items (Table 1).

148 Thereafter, the volunteers were randomly allocated to one of the three experimental groups after
149 gender stratification was performed. For the following 3 week intervention period, the first group
150 was maintained on the L0 dietary supplementation, whereas the second and the third groups
151 received food items produced from the L4 and the L9 experimental fats, respectively (Figure 1).

152 Fatty acid profile of L9 fat (Table 1) was designed so that the total TFA intake contributed to
153 around 3.1% of daily energy intake (Table 3), which is 2.1% higher than the level recommended by
154 the French authorities (i.e. 2% of TFA excluding CLA of daily energy intake (Afssa, 2005)).

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

158

159 **Measurements.** Subjects attended the laboratory for measurements and blood samples the day after
160 W0 (day 1 of W1) and the day after W3 (day 1 of W4) (Figure 1). Weight was measured at each
161 visit after an overnight fast of at least 12 h, using the same calibrated digital scale with participant
162 dressed in light indoor clothing without shoes. Blood were sampled after an 11h to 15h overnight
163 fast. Plasma was obtained by centrifugation, aliquoted and stored at -80°C until further analyses.
164 The subjects recorded their dietary intake (foods and drinks) during 5 consecutive days, including 3
165 week days and 2 week end days, during the run-in period (W0) and during the last week of the
166 intervention (W3). Data were coded and analyzed by a dietician using computerized nutrient
167 databases (GENI Micro6.0, Villers-les- Nancy, France).

168

169 **Biochemical analyses.** HDL-cholesterol, total cholesterol, triglycerides, apolipoprotein A1,
170 apolipoprotein B were measured by enzymatic assays using a Konelab 20 analyser (Thermo
171 Electron SA, Cergy-Pontoise, France). LDL-cholesterol concentration was calculated by the
172 Friedewald equation. In order to assess the compliance, plasma phospholipids FA profiles were
173 characterized after plasma lipid extraction and fatty acid methylation. Fatty acid methyl ester
174 profiles were analysed and identified by gas chromatography (Trace GC 2000 Series,
175 ThermoFinnigan, France). The detailed analytical conditions were already reported (Roy et al.,
176 2006). Cholesteryl ester transfer protein (CETP) activity was measured by fluorimetry using
177 commercial kits. Fibrinogen was assessed using a turbidimetric assay (BioDirect, La Villeneuve,
178 France).

179

180 **Assessment of subjects' compliance.** Subject compliance was assessed by a questionnaire and by
181 analysis of the concentration of total *trans*-18:1 and vaccenic acid in plasma phospholipids
182 (Mansour et al., 2001). The mean baseline vaccenic acid concentration in phospholipids was 0.098
183 \pm 0.027 (mean \pm standard deviation) g/100 g total fatty acids with no significant effect observed

184 between groups. At the end of the experimental periods, the average concentrations of vaccenic acid
185 found in plasma phospholipids were 0.160 ± 0.045 , 0.252 ± 0.077 and 0.616 ± 0.184 g/100 g total
186 fatty acids for L0, L4 and L9 diet respectively. It was statistically different between the 3 groups (2-
187 way ANOVA, diet: $p < 0.0001$, gender $p = 0.489$ interaction $p = 0.473$; post-hoc tests: L0, L4 $p =$
188 0.002 ; L0, L9 $p < 0.0001$ and L4, L9 $p < 0.0001$).

189

190 **Statistical Methods.** Values are expressed as means \pm Standard Deviation (SD). Statistical analysis
191 was performed using the Statview version 5.0 software (SAS Institute Inc., Cary, NC). The One
192 way ANOVA procedure was used to determine difference in baseline parameters for the groups.
193 Differences between final and baseline measurements among the three groups were tested by a two-
194 way ANOVA, including diet and gender as factors. If the main effects were significant ($p < 0.05$),
195 PLSD Fisher's test was applied for multiple comparisons (post hoc test). We decided to present the
196 results on the per-protocol data set because 3 subjects had already withdrawn during the run-in
197 period before the first measurements (for personal reasons and because of time constraints) and one
198 subject was excluded because he was not compliant. Compliance to the protocol was a primary
199 outcome in the analysis, showing that per-protocol analysis could be performed on the 107 subjects
200 who completed the study (Figure 2).

201

202 **Results**

203

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

207

208 **Plasma lipids, apolipoproteins.** Considering the primary outcome i.e. plasma concentrations of
209 HDL-cholesterol, no significant change was evidenced between the three groups. However

210 compared to L0 diet, L4 diet contributed to reduce total cholesterol (p= 0.037), LDL-cholesterol (p
211 = 0.040), LDL-cholesterol/HDL-cholesterol ratio (p = 0.028), and total cholesterol/HDL-cholesterol
212 ratio (p= 0.016), whereas L9 diet did not alter most of these parameters (Table 4).

213 Plasma ApoB concentration tended to be reduced in the L4 group compared to the L0 group, but
214 without reaching the level of significance (p = 0.065).

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

216

217 **Discussion**

218 The impact of R-TFA on CVD risk markers is a major issue for human nutritional
219 recommendations. Changing the level of R-TFA bio-synthesis in the cows' rumen is associated
220 with a large panel of changes in milk fatty acid content. Our study aimed therefore at examining the
221 metabolic effects of experimental milk fats which represent the widest **range** of putative milk fatty
222 acid profiles resulting from different cows' feeding strategies. Major finding showed that the
223 consumption of dairy fat containing 63.3% SFA and 3.5% *trans*-18:1 (L4 diet) improved some
224 CVD risk factors for healthy volunteers in comparison with a typical dairy fat (72% SFA, 2.5%
225 *trans*-18:1 –L0 diet). It is illustrated by a decrease in total cholesterol, LDL-cholesterol, total
226 cholesterol/HDL-cholesterol ratio and LDL-cholesterol/HDL-cholesterol ratio. We observed a
227 change by 0.18 units in the ratio of total cholesterol/HDL cholesterol between L0 diet and L4 diet.
228 As reported by Stampfer et al. (Stampfer et al., 1991), we calculate that this change can be
229 associated to a 9.5% decrease in the risk of myocardial infarction, which is in the same range as the
230 replacement of 1334 mg *trans* α -linolenic acid by dietary *cis* α -linolenic (Vermunt et al., 2001).
231 Moreover, our results show that the consumption for 3 weeks of the L9-dairy fat, which contains
232 less SFA (56.6%) and more *trans*-18:1 (9.5%) compared to the L0 diet, induces no significant
233 changes in plasma markers of CVD (Table 4). In addition, the ratio between total and HDL-
234 cholesterol was significantly increased after 3 weeks of L9-dairy fat compared to L4 diet (p =
235 0.029). These data suggest that whereas mild increase in R-TFA/SFA ratio in milk fat may be

236 beneficial compared to L0 diet, further increase in R-TFA/SFA ratio does not provide additional
237 benefit regarding the CVD risk factors.

238 In a study where SFA intake was maintained constant (around 18% of energy intake), a 1.5% total
239 energy intake as R-TFA failed to alter any CVD risk factor (Motard-Belanger et al., 2008).

240 Interestingly in healthy moderately overweight men and women, Rivellese et al. showed that
241 decreasing SFA intake by 8% (from 17.6 to 9.6% total energy intake) and increasing in
242 compensation MUFA intake (from 13.1 to 21.2% total energy intake) induced a reduction in plasma
243 LDL-cholesterol concentration (-0.38 mmol/L) (Rivellese et al., 2003). In our present study, milk
244 fats were characterized by different levels in both R-TFA and SFAs, a higher R-TFA level being
245 associated with a lower SFA content. Notably, high R-TFA/SFA ratio was also associated with
246 enhanced MUFA and PUFA intake. These combined changes in milk fat composition could
247 therefore partially explain the LDL-cholesterol reduction observed after the consumption of the L4
248 diet in comparison with L0 (see Table 4). Our present results are in agreement with the results of
249 Poppitt et al. (Poppitt et al., 2002) and Seidel et al. (Seidel et al., 2005). Briefly, Poppitt et al.
250 (Poppitt et al., 2002) reported a significant decrease in both total and LDL-cholesterol in plasma
251 from healthy men after consuming a modified butter-fat (-5 units of percent total energy intake of
252 SFA and +2 units of total energy intake of MUFA) for 3 weeks. Seidel et al. (Seidel et al., 2005)
253 demonstrated beneficial effects regarding the CVD risk, i.e. decreased LDL-cholesterol/HDL-
254 cholesterol ratio, with the consumption of modified milk fat obtained by feeding cows high-fat
255 rapeseed cake (16% oil).

256 By contrast, our study shows that the consumption of R-TFA up to 2.42% (L9 diet) of the daily
257 energy intake has no significant effect on the evolution of the HDL concentration which is different
258 from an IP-TFA intake (Katan et al., 1995). However, the differential effect between IP- and R-
259 TFA sources on the HDL parameter seems to disappear for higher TFA intake (3.5% total energy
260 intake) (Motard-Belanger et al., 2008). Even so, our data suggest that whereas mild increase in R-
261 TFA/SFA ratio in milk fat may be beneficial compared to L0 diet, further increase in R-TFA/SFA

262 ratio does not provide additional benefit regarding the CVD risk factors. Moreover the lack of
263 beneficial effect of the L9 diet could also be due to the huge increase in the *trans* 18:2-isomers. These
264 isomers have been reported to be more deleterious than the *trans* 18:1-isomers (Baylin et al., 2003)
265 , for a review see (Mozaffarian and Clarke, 2009)).

266

267 During our clinical intervention, we found no significant effect of the consumption of these 3
268 different diets on the HDL parameter. This result is in accordance with already published trials.
269 Tricon et al. (Tricon et al., 2006) reported that the consumption for 6 weeks of a dairy product
270 naturally enriched in *cis*-9,*trans*-11 CLA (0.2 g/d to 1.5 g/d) and *trans*-11 18:1 (0.8 g/d to 6.3 g/d)
271 failed to alter plasma triacylglycerol, total cholesterol, LDL-cholesterol, and HDL-cholesterol
272 concentrations and total to HDL cholesterol ratio, in healthy middle aged-men. The lack of
273 differences on the HDL parameter could be related to our calculation of the sample size. Indeed, to
274 calculate the sample size, we use the predictive equation of HDL-cholesterol (Yu et al., 1995) and
275 on the other hand we decided that the predicted difference should be $\delta=2,17$ mg/dL: it was perhaps
276 a too small extent in the change in HDL concentrations.

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

281 To conclude, we confirm that the consumption of R-TFA at nutritional level (1.01 % L4 diet i.e.
282 <2.0% of energy, the level recommended by the French authorities) have no adverse effect related
283 to some cardiovascular risk factors whatever the gender, which is in accordance with most
284 intervention studies (Motard-Belanger et al., 2008; Seidel et al., 2005) and also with the recent
285 epidemiological study (Jakobsen et al., 2008). Moreover, this clinical study underlines the fact that,
286 cows' feeding strategy consisting in decreasing the SFA/TFA ratio (less SFA (56.6%) and more

287 total trans (12.16 %) in fat does not bring any additional benefits regarding the CVD risk in
288 healthy volunteers.

289

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300

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Figure legends

Figure 1 Study design. (Dietary questionnaire)

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

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

Fatty acids	Fatty acid composition		
	L0 ^b	L4 ^b	L9 ^b
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
<i>trans</i> -4	0.01	0.01	0.04
<i>trans</i> -5	0.01	0.01	0.03
<i>trans</i> -6/8	0.21	0.21	0.58
<i>trans</i> -9	0.21	0.23	0.45
<i>trans</i> -10	0.29	0.33	1.23
<i>trans</i> -11	1.00	1.81	4.26
<i>trans</i> -12	0.29	0.29	0.86
<i>trans</i> -13	0.51	0.59	2.04
<i>trans</i> -9+ <i>trans</i> -10+ <i>trans</i> -11	1.51	2.37	5.94
C18:1 n-9	15.53	21.87	17.12
C18:1 <i>cis</i> -14+ <i>trans</i> -16	0.28	0.35	0.63
C18:1 <i>cis</i> -15+C19:0	0.16	0.21	0.53
Other <i>cis</i> -C18:1 isomers	0.94	0.97	1.49
<i>trans</i> -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^a	2.85	4.06	12.16
Total cis-PUFA	2.21	2.87	5.37

^aSum of *trans*-18:1 and *trans*-18:2 acid isomers; Conjugated Linoleic Acid (CLA) are not taken into

account in this calculation; ^bL0, L4, L9 see Method section, **cis-MUFA: cis-monounsaturated fatty**

acids; cis-PUFA: cis-polyunsaturated fatty acids

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 (y)	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±0.3 [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
Haemoglobin (g/dL)	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
Haematocrit (%)	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 ± SD and 95% confidence intervals [95% CIs]* Number of male and females, respectively. Data were analyzed by a one way ANOVA.

Table 3 Mean daily intake and 95% confidence intervals [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 x Diet
	Mean \pm SD [95% CIs]						P	P	P
Total Energy, kJ/d	8610 \pm 1404 [-5858; 11361]	8782 \pm 1602 [-5642; 11923]	8556 \pm 1884 [-4864; 12249]	8583 \pm 1486 [-5671; 11495]	8104 \pm 1539 [-5086; 1121]	8375 \pm 1519 [-5398; 11352]	0.225	0.670	0.655
Protein, %en	14.8 \pm 3.0 [-9.0; 20.6]	14.9 \pm 2.9 [-9.3; 20.5]	15.0 \pm 2.7 [-9.7; 20.3]	15.4 \pm 2.7 [-10.2; 20.7]	14.1 \pm 2.8 [-8.6; 19.6]	14.5 \pm 2.4 [-9.8; 19.2]	0.284	0.666	0.968
Carbohydrate, %en	47.5 \pm 5.9 [-36.0; 59.0]	47.4 \pm 5.9 [-35.9; 59.0]	45.9 \pm 5.3 [-35.5; 56.2]	44.9 \pm 5.0 [-36.0; 55.7]	47.2 \pm 5.7 [-35.9; 58.4]	46.7 \pm 4.9 [-37.2; 56.2]	0.453	0.912	0.485
Total Fat, %en	37.7 \pm 5.4 [-27.1; 48.2]	37.7 \pm 5.0 [-27.8; 47.5]	39.2 \pm 5.0 [-29.4; 49.0]	38.7 \pm 5.1 [-28.8; 48.6]	38.8 \pm 5.4 [-28.3; 49.3]	38.8 \pm 4.8 [-29.4; 48.2]	0.805	0.876	0.612
SFA, %en	21.5 \pm 2.6 [-16.4; 26.6]	21.3 \pm 2.8 ^a [-15.8; 26.8]	22.1 \pm 3.0 [-16.2; 28.0]	19.9 \pm 2.9 ^b [-14.2; 25.5]	22.6 \pm 3.3 [-16.1; 29.0]	18.1 \pm 2.4 ^c [-13.4; 22.9]	0.308	<0.0001	0.965
MUFA, %en	11.4 \pm 2.7 [-6.0; 16.7]	11.8 \pm 2.6 ^a [-6.7; 16.8]	11.7 \pm 2.5 [-6.9; 16.5]	14.0 \pm 2.6 ^b [-9.0; 19.1]	11.9 \pm 2.6 [-6.7; 17.0]	14.3 \pm 2.2 ^b [-10.1; 18.6]	0.607	0.0003	0.904
PUFA, %en	3.6 \pm 1.3 [-1.1; 6.1]	3.6 \pm 1.1 ^a [-1.4; 5.7]	3.5 \pm 1.2 [-1.1; 5.9]	3.9 \pm 1.3 ^b [-1.3; 6.4]	3.6 \pm 1.2 [-1.3; 5.9]	5.2 \pm 1.0 ^c [-3.2; 7.1]	0.380	<0.0001	0.531
Total TFA*, %en	0.70 \pm 0.11 [-0.49; 0.91]	0.69 \pm 0.11 ^a [-0.47; 0.91]	0.72 \pm 0.14 [-0.45; 0.98]	1.01 \pm 0.18 ^b [-0.65; 1.36]	0.75 \pm 0.13 [-0.49; 1.01]	3.10 \pm 0.55 ^c [-2.02; 4.18]	0.169	<0.0001	0.148
Total trans-18:1*, %en	0.62 \pm 0.10 [-0.43; 0.81]	0.61 \pm 0.10 ^a [-0.43; 0.81]	0.64 \pm 0.12 [-0.40; 0.87]	0.87 \pm 0.16 ^b [-0.56; 1.17]	0.67 \pm 0.12 [-0.44; 0.90]	2.42 \pm 0.43 ^c [-1.57; 3.27]	0.236	<0.0001	0.173

All values are means \pm SD. Data (the difference between end of the intervention and baseline) were analyzed using a 2-way ANOVA with gender and diet as factors.

Means in a row without common superscript letters differ.

%en: % of total energy, SFA, saturated fatty acids; cis-MUFA: cis-monounsaturated fatty acids; cis-PUFA: cis-polyunsaturated fatty acids; TFA, trans fatty acids. *:

this represents only the percentage of TFA and total trans-18:1 in the three different food items (butter, dessert cream, and cookies).

Table 4 Serum lipids, lipoprotein, apolipoprotein concentrations, cholesterol ester transfer protein (CETP) activity and fibrinogen concentration in the three different groups (L0, L4 and L9 group) **mean and 95% confidence intervals [95% CIs]** at baseline and estimate mean effects after 3 weeks.

Variable and subjects	Baseline values ¹			Estimate mean effects ²			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

¹means ± SD, ² Estimate mean effects is defined as the difference between end of the intervention and baseline. Data are analyzed by using a two-ways anova. Means

in a row without common **superscript** letters **differ**.

Abbreviations

ALT: Alanine aminotransférase

AST: Aspartate aminotransférase

β HCG: Human chorionic gonadotropin

CETP: Cholesteryl ester transfer protein

CPP: Comité de protection des personnes

CRNH: Centre de recherche en nutrition humaine

CRP: C reactive protein

CVD: Cardiovascular disease

DQ: Dietary Questionnaire

EU: European Union

γ GT: Gamma glutamyltransférase

HCV: Hepatitis C virus

IP-TFA: Industrially produced *trans* fatty acids

PHVO: Partially hydrogenated vegetable oils

R-TFA: Ruminant *trans* fatty acids

TFA: *Trans* fatty acids

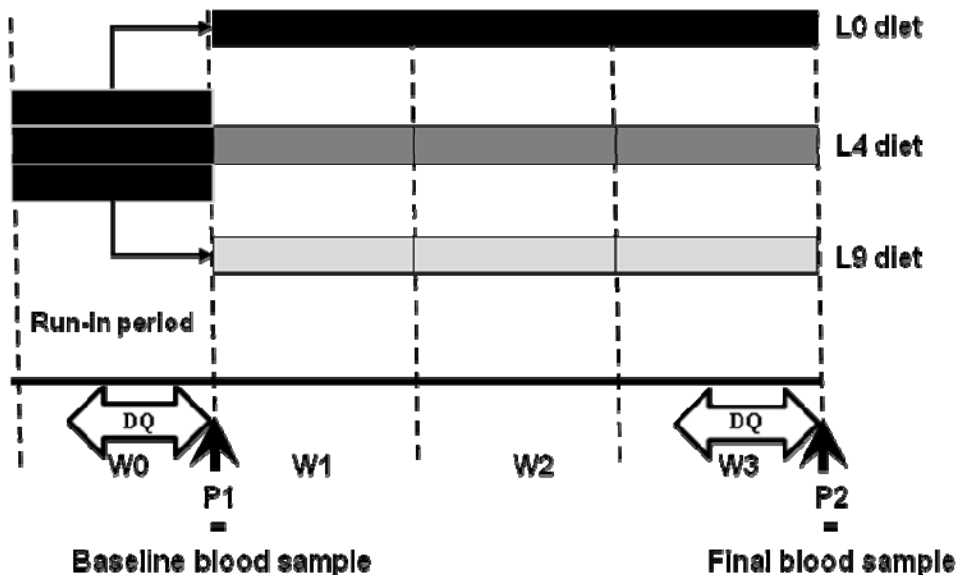


Figure 1

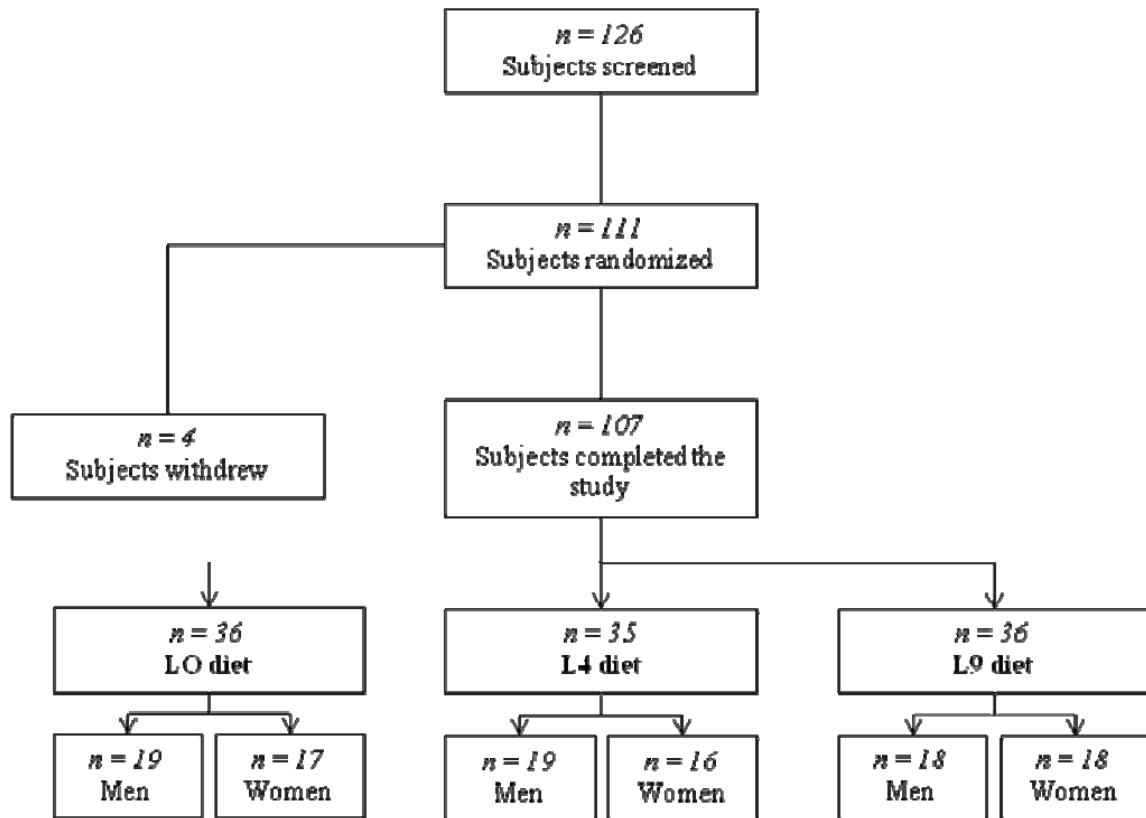


Figure 2