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3 Associations between dairy intake and metabolic risk parameters in a healthy French-Canadian 4 population

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24

25 **Abstract**

26 **Background:** Observational studies support that dairy product intake is associated with a reduced risk
27 of developing type 2 diabetes; however, several clinical studies report conflicting results on the
28 association between dairy product consumption and metabolic parameters. The aim of this study was to
29 determine associations between dairy product consumption and metabolic profile. **Methods:** Dietary
30 data, using a validated food frequency questionnaire, and fasting blood samples were collected from
31 233 French-Canadians. Plasma phospholipid (PL) fatty acids (FA) concentrations were determined by
32 gas chromatography. **Results:** Subjects consumed 2.5 ± 1.4 portions of dairy products daily, including
33 1.6 ± 1.3 portions of low-fat (LF) and 0.90 ± 0.70 portions of high-fat (HF) dairy products. *Trans*-
34 palmitoleic acid level in plasma PL was related to HF dairy consumption ($r=0.15$; $p=0.04$). Total
35 ($r=-0.21$; $p=0.001$) and LF dairy ($r=-0.20$; $p=0.003$) intakes were inversely correlated with fasting
36 plasma glucose level. Total dairy intake was inversely associated to systolic blood pressure (SBP)
37 ($r=-0.17$; $p=0.008$) and diastolic blood pressure (DBP) ($r=-0.14$; $p=0.03$). LF dairy intake was also
38 inversely correlated with SBP ($r=-0.17$; $p=0.009$). Total dairy intake was correlated with plasma C-
39 reactive protein (CRP) ($r=0.15$; $p=0.03$). No association was found between HF dairy consumption and
40 the risk factors studied. **Conclusion:** Dairy intake is inversely associated with glycaemia and BP; yet, it
41 may modify CRP levels. Moreover, *trans*-palmitoleic FA levels in plasma PL may be potentially used
42 to assess full-fat dairy consumption.

43

44 **Keywords:** Dairy products, blood pressure, blood glucose, *trans* fatty acids, plasma phospholipids, sex
45 differences

46

47

48

49 **Introduction**

50 Dairy consists in a wide variety of milk-based products including milk, yogurt, cheese, cream
51 and butter. These products are often categorized as either HF dairy products (cheese, butter, cream,
52 whole milk) or LF dairy products (skim milk, low-fat yoghurt). Most of dietary guidelines worldwide
53 suggest that consumption of 2-4 portions of dairy per day is part of a healthy diet as dairy mineral
54 content, especially calcium content, helps to maintain healthy bones. Recent studies demonstrated that
55 dairy consumption may have beneficial effects on metabolic health and thus be linked to a reduced risk
56 of metabolic diseases such as obesity, metabolic syndrome (MetS) or type 2 diabetes (T2D) (Aune et
57 al. 2013; Elwood et al. 2010; Kalergis et al. 2013; Tong et al. 2011; Tremblay and Gilbert 2009).

58 MetS is characterized by a cluster of metabolic disorders such as hyperglycemia, deteriorated
59 lipid profile, elevated BP and abdominal obesity (International Diabetes Federation 2013). Subjects
60 suffering of MetS have an increased risk of developing T2D and cardiovascular diseases (CVD)
61 (National Cholesterol Education Program (NCEP) Expert Panel on Detection Evaluation and Treatment
62 of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002). The prevalence of T2D is
63 increasing worldwide and it is estimated that 522 million people will suffer of T2D by 2030 (Whiting
64 et al. 2011). Although T2D is a multifactorial disease, diet and exercise remain major risk factors and
65 the cornerstone of prevention and treatment (Stumvoll et al. 2010). Specifically, most of healthy dietary
66 patterns associated with lower incidence of T2D include dairy products (Ley et al. 2014).

67 Dairy consumption may have beneficial effects on a deteriorated glycemic profile, by increasing
68 insulin secretion and lowering glycaemia (McGregor and Poppitt 2013), and on lipid profile by
69 lowering plasma triglycerides and cholesterol (Hidaka et al. 2012). Further, dairy products incorporated
70 within an energy-restriction diet may increase weight loss (Chen et al. 2012). Recently, obese and

71 diabetic subjects have been found to be in a low-grade chronic inflammation state with increased
72 plasma C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor (TNF)- α levels. Dairy
73 consumption may improve inflammatory profile by lowering these inflammatory markers
74 (Panagiotakos et al. 2010). Finally, dairy intake, especially LF dairy intake has been found to reduce
75 SBP in overweight and obese subjects (Ralston et al. 2012; van Meijl and Mensink 2011). There is
76 currently no evidence that dairy intake affects men and women differently. Nevertheless, a recent study
77 reported that an increased LF dairy consumption for 6 weeks improved metabolic syndrome markers
78 differently in men and women; after intervention, men had lower blood glucose whereas women had
79 lower body weight, waist circumference and body mass index (BMI) (Dugan et al. 2014). Although
80 beneficial effects of dairy consumption are often attributed to LF dairy consumption, there is currently
81 no clear evidence that HF dairy consumption leads to a deteriorated metabolic profile (Kratz et al.
82 2013).

83 Even though numerous studies found a beneficial effect of dairy consumption, several authors
84 report no effect of dairy on metabolic health (Benatar et al. 2013; Bendtsen et al. 2014; Bowen et al.
85 2005; Crichton et al. 2012; Palacios et al. 2011; Van Loan et al. 2011), leading to conflicted results
86 about the effect of dairy consumption. In their meta-analysis of cohort studies, Tong et al. (2011)
87 suggested that dairy may have a dose-dependent effect. Reliable biomarkers of dairy consumption are
88 thus needed to study precisely its health effects. Biomarkers can be specific dairy components such as
89 *trans*-palmitoleic acid and *trans*-vaccenic acid, which are *trans* FA occurring naturally in raw milk.

90 The aim of this study was to determine associations between dairy intake, plasma PL FA and
91 specific metabolic risk factors, including anthropometric status, plasma glucose, plasma lipid profile,
92 inflammatory markers and BP, in a healthy French-Canadian population.

93

94 **Subjects and Methods**

95

96 *Study population*

97 A total of 254 participants from the greater Quebec City metropolitan area were recruited. Study
98 inclusion and exclusion criteria's have been described in a previous article (Rudkowska et al. 2014).

99 The following data was collected from each of the study participants during the visit: anthropometric
100 measurements, fasting blood samples, and the food frequency questionnaire (FFQ). The protocol was
101 approved by the ethics committees of Laval University Hospital Research Center and Laval University.

102 This trial was registered at clinicaltrials.gov as NCT01343342.

103

104 *Anthropometric measurements*

105 Body weight, height, waist, and hip circumferences were measured according to the procedures
106 recommended by the Airlie Conference (Callaway et al. 1988). BMI was calculated as weight in
107 kilograms divided by height in meters squared. Resting BP measurements were performed after a 5-
108 min rest in a sitting position, phases I and V of Korotkoff sounds being respectively used for SBP and
109 DBP. Measurements were performed in duplicate and the mean was used for analyses.

110

111 *Dietary intake*

112 Dietary intake of the past month was determined by a 91-item validated FFQ (Goulet et al.
113 2004) based on food habits of Quebecers, administered by a registered dietitian (RD). The RD asked
114 participants how often they consumed each type of food: daily, weekly, monthly or none at all during
115 the last month. To make sure each participant estimated correctly the portion eaten, examples of
116 portion size were provided. Data obtained from FFQ were analysed using the Nutrition Data System for

117 Research software version 2011, developed by the Nutrition Coordination Center (University of
118 Minnesota, Minneapolis, MN). All the information was compiled and similar food items from the FFQ
119 were grouped, as previously described (Paradis et al. 2009). Three criteria were used to form these
120 groups: first, the similarity of nutrient profiles, second, the culinary usage of different types of food
121 (similar to groups used in a previous study (Hu et al. 1999)) and third, the consideration of groups
122 utilized in other studies to maintain consistency (Newby and Tucker 2004). Specifically, the LF dairy
123 product subgroup included < 2 % - fat dairy products whereas HF dairy product subgroup included > 2
124 % - fat dairy products (**Table 1**). The total dairy product intake was defined as the sum of LF and HF
125 dairy intakes.

126

127 *Biochemical parameters*

128 Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA
129 after 12-h overnight fast and 48-h alcohol abstinence. Blood samples were taken to identify and
130 exclude individuals presenting exclusion criteria such as metabolic disorders, as mentioned. Plasma
131 was separated by centrifugation (2500 x g for 10 min at 4°C) and samples were aliquoted and frozen at
132 -80°C for subsequent analyses. Plasma total cholesterol (TC) and triglyceride (TG) concentrations were
133 measured using enzymatic assay (Burstein and Samaille 1960; McNamara and Schaefer 1987). The
134 high-density lipoprotein cholesterol (HDL-C) fraction was obtained after precipitation of very low-
135 density lipoprotein and low-density lipoprotein (LDL) particles in the infranatant with heparin
136 manganese chloride (Albers et al. 1978). LDL cholesterol (LDL-C) was calculated with the Friedewald
137 formula (Friedewald et al. 1972). Fasting insulinemia was measured by radioimmunoassay with
138 polyethylene glycol separation (Desbuquois and Aurbach 1971). Fasting glucose concentration was
139 measured enzymatically (Richterich and Dauwalder 1971). Insulin resistance was calculated using the

140 homeostatic model of the assessment of insulin resistance (HOMA-IR) and was obtained by applying
141 the following formula: HOMA-IR = fasting insulin (IU/mL) x fasting blood glucose (mmol/L) / 22.5
142 (Matthews et al. 1985). Plasma concentrations of IL-6 and TNF- α were measured with high-sensitivity
143 enzyme-linked immunosorbent assay (ELISA) kits including: Human IL-6 Quantikine HS ELISA Kit
144 (R&D Systems, Minneapolis, MN, United States (HS600B)) and Human TNF- α Quantikine HS ELISA
145 Kit (R&D Systems, Minneapolis, MN, United States (HSTA00D)). Plasma CRP was measured by
146 nephelometry (Prospec equipment Behring) using a sensitive assay, as described previously (Pirro et al.
147 2001).

148

149 *Fatty acid composition of plasma phospholipids*

150 Plasma PL FA composition was assessed in 210 participants (97 men and 113 women). Plasma
151 lipids were extracted with chloroform:methanol (2:1, v/v) according to a modified Folch method
152 (Shaikh and Downar 1981). Total PL were separated by thin layer chromatography using a
153 combination of isopropyl ether and acetic acid and FA of isolated PL were then methylated. Capillary
154 gas chromatography was then used to obtain FA profiles. The technique used for plasma analyses has
155 been previously validated (Kröger et al. 2009). Values of FA concentrations are expressed as percent of
156 total FA in plasma PL.

157

158 *Statistical analysis*

159 Means (\pm SDs) were calculated for dietary intakes, plasma PL percentages and other subjects'
160 characteristics. Relationships between dairy intake and plasma PL FA or metabolic parameters were
161 assessed either by Pearson's or Spearman's correlation coefficients, depending on the normality of the
162 variables. Correlations were computed for all participants and for men and women separately. Unless

163 otherwise stated, correlations coefficients were adjusted for age and BMI. Statistical analyses were
164 performed with SAS statistical software, version 9.3 (SAS Institute Inc, Cary, NC). *P*-values ≤ 0.05
165 were considered statistically significant.

166

167 **Results**

168

169 *Subjects' characteristics*

170 Analyses were performed on the 233 participants (105 men and 128 women) who met all the
171 eligibility criteria's for the study. Subject's characteristics are presented in **Table 2**. Subjects had a
172 mean BMI over 25 kg.m^{-2} which suggests that the study population was slightly overweight. However,
173 subjects had healthy glycemic and lipid profiles according to the NCEP-ATP III guideline
174 recommendations (National Cholesterol Education Program (NCEP) Expert Panel on Detection
175 Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002)). TC
176 was within the desirable range ($< 5.17 \text{ mmol.L}^{-1}$). LDL-C (mean \pm SD) was $2.76 \pm 0.79 \text{ mmol.L}^{-1}$
177 which is near the optimal range (desirable, $< 2.58 \text{ mmol.L}^{-1}$) and HDL-C was $1.44 \pm 0.36 \text{ mmol.L}^{-1}$
178 (optimal level $> 1.55 \text{ mmol.L}^{-1}$). Subjects also had normal plasma TG levels ($< 1.7 \text{ mmol.L}^{-1}$). Further,
179 subjects had optimal fasting plasma glucose (desirable, $< 5.6 \text{ mmol.L}^{-1}$ (International Diabetes
180 Federation 2013)) and BP (SBP $\leq 130 \text{ mmHg}$ and DBP $\leq 85 \text{ mmHg}$). In this study, women participants
181 had a healthier metabolic profile compared to men, with lower SBP, waist-to-hip ratio, fasting plasma
182 glucose, LDL-C and TC:HDL-C ratio and a higher HDL-C.

183 Men had a significantly greater energy intake than women; however, proportions of energy
184 intake coming from proteins, carbohydrates and fats are similar in men and women (**Table 3**).

185

186 *Dairy consumption among subjects*

187 Subjects consumed 2.5 ± 1.4 portions of dairy products per day, including 1.6 ± 1.3 portions of
188 LF dairy products and 0.90 ± 0.70 portions of HF dairy products (**Table 3**). Dairy consumption was not
189 significantly different between men and women. Among the 233 participants, 104 (44.6 %) including

190 54 men (51.4 %) and 50 women (39.0%) had declared consuming less than 2 portions of dairy products
191 a day.

192 Mean plasma PL FA percentages are shown in **Table 4**. Significant sex differences were
193 observed for plasma PL myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) percentages,
194 which are the three main saturated FA in milk fat. Women had significantly higher plasma PL
195 percentages of 14:0 and 16:0 whereas men had a higher plasma PL 18:0 percentage. Specific dairy
196 *trans* FA (*trans*-palmitoleic 9t-16:1 and *trans*-vaccenic 11t-18:1 acids) were found in the lowest plasma
197 PL percentages in both men and women (less than 0.20 %). Correlations made between plasma PL FA
198 and dairy intake (**Figure 1**) in all participants showed that HF dairy intake was positively correlated
199 with the percentage of *trans*-palmitoleic FA in plasma PL ($r = 0.15$; $p = 0.04$). Similarly, LF dairy
200 intake was found related to plasma PL 16:0 percentage ($r = 0.14$; $p = 0.05$). Although the *trans*-
201 palmitoleic acid and *trans*-vaccenic acid levels in plasma PL were found strongly correlated ($p <$
202 0.0001), dairy intake was not associated with the percentage of *trans*-vaccenic acid in plasma PL (data
203 not shown).

204

205 *Relations between dairy product intake and metabolic risk factors*

206 Correlation coefficients for dairy product intake and metabolic risk factors are shown in **Figure**
207 **2**. Considering all participants, the strongest association was found between both total and LF dairy
208 intakes and fasting plasma glucose ($r = -0.21$; $p = 0.001$ and $r = -0.20$; $p = 0.003$ respectively). An
209 inverse association was also found between total dairy intake and both SBP and DBP ($r = -0.17$; $p =$
210 0.008 and $r = -0.14$; $p = 0.03$ respectively). LF dairy intake was also inversely correlated with SBP (r
211 $= -0.17$; $p = 0.009$) although the correlation with DBP was not significant ($p = 0.11$). Dairy intake was
212 not found related to IL-6 and TNF- α inflammatory markers; however, total dairy intake was

213 significantly correlated with CRP levels ($r = 0.15; p = 0.03$). No associations were observed between
214 dairy intake and plasma insulin, HOMA-IR or lipid profile and none of the metabolic risk factors
215 studied was associated with HF dairy intake. Correlation coefficients were also computed between
216 dairy intake and BMI or waist circumference and no significant associations were found (data not
217 shown).

218 When subjects was stratified on the basis of sex (**Figure 2**), the inverse association between
219 total dairy or LF dairy intake and fasting plasma glucose remained significant in women ($r = -0.24; p =$
220 0.007 and $r = -0.19; p = 0.03$ respectively) but not in men. LF dairy intake was also related to lower
221 SBP in women ($r = -0.19; p = 0.04$) whereas HF dairy intake was inversely correlated with DBP in
222 men ($r = -0.23; p = 0.02$). Total dairy intake was not related to BP neither in men nor in women. No
223 other significant association was observed between dairy intake and metabolic parameters considering
224 men and women separately, although HF dairy consumption tended to positively correlate with the
225 TC:HDL-C ratio in men ($p = 0.06$). However, a different tendency pattern in men and women was
226 observed for the association between dairy intake and TNF- α level. In men, total dairy intake tended to
227 correlate positively with TNF- α level ($p = 0.06$) whereas LF and total dairy intakes tended to be
228 inversely correlated with TNF- α level in women ($p = 0.09$ and $p = 0.10$ respectively). Total dairy intake
229 was not significantly related to CRP levels neither in men nor in women.

230

231 *Relations between plasma phospholipid fatty acids and metabolic risk factors*

232 Significant correlations between plasma PL dairy *trans* FA and metabolic risk factors are
233 presented in **Figure 3**. *Trans*-palmitoleic acid level in plasma PL was inversely correlated with SBP in
234 all participants ($r = -0.15; p = 0.03$). In men, *trans*-palmitoleic acid ($r = -0.22; p = 0.03$) and *trans*-

235 vaccenic acid ($r = -0.26$; $p = 0.01$) level in plasma PL were inversely correlated to BMI. These
236 correlations were not significant in women (data not shown).

237

238 **Discussion**

239

240 Results from this study suggest that dairy intake is inversely associated with fasting plasma
241 glucose and BP as well as modify CRP levels in healthy subjects. Further, HF dairy consumption may
242 be evaluated by the *trans*-palmitoleic acid level in plasma PL.

243 Dairy consumption assessed by FFQ showed that almost half study participants do not meet the
244 Canada's food guide recommendations concerning consumption of at least 2 portions of dairy products
245 a day. These findings are supported by recent Canadian surveys which highlighted an under
246 consumption of dairy products by the Canadian population (Statistics Canada 2009 and 2006). A recent
247 review suggested that increasing dairy consumption to 3-4 serving per day may have additional
248 beneficial effects on metabolic health (Da Silva and Rudkowska 2014). Additional well-designed
249 intervention studies are needed to ascertain the effects of increased dairy consumption.

250 HF dairy consumption was associated with the percentage of *trans*-palmitoleic acid in plasma
251 PL. However, 54 participants had a null percentage of plasma PL *trans*-palmitoleic even though they
252 had declared consuming HF dairy products (**Figure 1A**). These participants may have a *trans*-
253 palmitoleic acid level below the detection level. Although it is believed that *trans*-palmitoleic acid level
254 in humans comes from dietary sources, Jaudszus et al. recently reported that circulating *trans*-
255 palmitoleic acid can also be synthesized endogenously by chain shortening of dietary *trans*-vaccenic
256 acid (Jaudszus et al. 2014). In the participants of the present study, percentages in plasma PL of these
257 two *trans* FA were strongly correlated. Further, this high presence of zero values suggests that the
258 FFQ-declared dairy consumption may be biased. Indeed these participants might have over-reported
259 their HF dairy consumption. The use of biomarkers have been suggested to lower errors related to self-
260 reporting of diet (Boeing 2013). *Trans*-palmitoleic acid in plasma PL had already been reported to be

261 associated with HF dairy consumption (Mozaffarian et al. 2013; Nestel et al. 2014). Palmitic acid was
262 found correlated with LF dairy intake in the present study. As the major FA in dairy fat, palmitic acid
263 must also be the main FA in LF dairy products.

264 In the present study, dairy intake was inversely correlated with fasting plasma glucose and no
265 association was observed between dairy intake and fasting insulin or HOMA-IR parameter in all
266 participants. Observational studies have found that eating patterns incorporating higher amounts of
267 dairy products would decrease the risk of developing T2D on average by 14% (Tong et al. 2011). Two
268 other studies found an inverse association between dairy intake, especially fermented dairy products,
269 and fasting plasma glucose (Nestel et al. 2012; Struijk et al. 2013). In overweight or obese subjects,
270 few authors have also shown an improved insulin profile after dairy intake, with no effect on fasting
271 plasma glucose (Rideout et al. 2013; Stancliffe et al. 2011; Zemel et al. 2005), while numerous clinical
272 trials have reported that dairy intake had no effect on glucose homeostasis (Crichton et al. 2012;
273 Thompson et al. 2005; Van Loan et al. 2011; van Meijl and Mensink 2011). In T2D subjects, yogurt
274 consumption for 12 weeks resulted in an improved glycemic status (Nikooyeh et al. 2011). Concerning
275 gender differences, LF dairy intake was significantly associated to lower blood glucose in women in
276 the present study whereas Dugan et al. (2014) reported a lower blood glucose in men after LF dairy
277 consumption for six weeks. Mechanisms underlying the beneficial effect of dairy intake on T2D
278 incidence remain uncertain. Yet, it is known that milk proteins and peptides can increase postprandial
279 insulin, leading to a decrease in glucose response and a protection against hyperglycemia (Claessens et
280 al. 2008; Goudarzi and Madadlou 2013; Nilsson et al. 2007; Petersen et al. 2009). Milk proteins are a
281 rich source of amino acid leucine which may contribute to metabolic health outcomes through
282 promoted fat oxidation and mitochondrial changes in tissues (Hirahatake et al. 2014; Sun and Zemel
283 2007). Dairy fat components may also play a role. In a recent cross-sectional study, dairy fat intake

284 assessed by *trans*-palmitoleic FA and 17:0 FA levels in plasma PL was found inversely associated with
285 fasting plasma glucose (Kratz et al. 2014) and a multiethnic cohort study showed that *trans*-
286 palmitoleate level in plasma PL was associated with a better metabolic profile with lower fasting
287 plasma glucose (Mozaffarian et al. 2013). Others suggested mechanisms for the positive effects of
288 dairy on glucose homeostasis may implied vitamin D and milk minerals such as calcium (Lacroix and
289 Li-Chan 2014).

290 Dairy intake was also inversely correlated to SBP and DBP in all participants. A meta-analysis
291 of prospective cohort studies suggests that consumption of dairy products (3.4 – 3.7 servings/day) is
292 associated with a 13% reduction in the risk of high BP (Ralston et al. 2012). Several authors also found
293 a positive effect of dairy, especially LF dairy intake, on BP in normotensive subjects (Stancliffe et al.,
294 2011; van Meijl and Mensink 2011; Zemel et al. 2005). Bioactive milk peptides have angiotensin-
295 converting enzyme inhibitory effects which may improve endothelial function and lower BP
296 (McGregor and Poppitt 2013; Rice et al. 2011). Mozaffarian et al. (2013) also reported that *trans*-
297 palmitoleate level in plasma PL was associated with lower SBP. Similarly, our results showed a
298 negative correlation between *trans*-palmitoleic acid level in plasma PL and SBP. It have also been
299 suggested that dairy calcium may modify the calcitonin gene-related peptide hormone action, resulting
300 in a decreased BP (Hirahatake et al. 2014). The positive effect of dairy on BP may also imply vitamin
301 D and others milk minerals (Lacroix and Li-Chan 2014; Rice et al. 2011).

302 Dairy intake was found correlated with CRP plasma levels, though no associations were found
303 between dairy intake and plasma IL-6 or TNF- α . The increased CRP levels are inconsistent with the
304 current literature. A recent cross-sectional survey reported that men and women consuming more than
305 14 serving of dairy products per week had 29 %, 9 % and 20 % lower levels of CRP, IL-6 and TNF- α ,
306 respectively (Panagiotakos et al. 2010). Two clinical trials also reported decreased IL-6 and TNF- α

307 markers after consumption of 3 or 3.5 portions of dairy per day (Stancliffe et al. 2011; Zemel et al.
308 2010). However, consumption of 3 to 5 serving of dairy per day had no effects on IL-6 and TNF- α
309 inflammatory markers in overweight or obese subjects in two clinical trials (Van Loan et al. 2011;
310 Wennersberg et al. 2009). A meta-analysis of randomized studies concluded that dairy foods have not a
311 significant effect on CRP levels (Benatar et al. 2013) and a systematic review of randomized controlled
312 studies reported that dairy intake does not exert adverse effect on inflammatory markers in obese and
313 overweight adults (Labonté et al. 2013). Further, an opposite tendency was observed in the relation
314 between dairy intake and plasma TNF- α among gender, suggesting that men and women may have
315 different inflammatory responses to dairy consumption. Clinical trials as well as mechanistic in-vivo
316 studies are needed to ascertain the effects of dairy intake on inflammation-related outcomes.

317 Dairy intake did not correlate significantly with neither BMI nor waist circumference.
318 Prospective cohort studies have provided evidence of a suggestive but not consistent protective effect
319 of dairy consumption on risk of overweight and obesity (Louie et al. 2013). Oppositely, a meta-analysis
320 of randomized studies reported that both LF and HF dairy intakes caused an increase in body weight
321 (Benatar et al. 2013). In a controlled-diet study, Van Loan et al. reported that dairy intake combined
322 with a moderate energy restricted diet did not increase weight loss (Van Loan et al. 2011). Overall total
323 dairy intake was not associated with long-term changes in body weight or waist circumference in both
324 men and women (Wang et al. 2014). In the present study, *trans*-palmitoleic and *trans*-vaccenic acids
325 which are the major *trans* FA in milk fat were inversely correlated, in men, with BMI. In contrast,
326 Dugan et al. (2014) reported a lower BMI in women after LF dairy consumption for six weeks. Studies
327 assessing gender differences in the relation between dairy intake and adiposity parameters are needed.

328 No association was found between dairy intake and plasma lipid levels. Others authors have
329 also reported that dairy intake had no effects on plasma lipid levels (Benatar et al. 2013; Van Loan et

330 al. 2011). The participants had a healthy lipid profile; thus, dairy intake should be examined in a
331 population with a more deteriorated lipid profile. To illustrate this, an animal study reported that *trans*-
332 vaccenic acid supplementation for 3 weeks had a lipid lowering effect in obese and insulin-resistant
333 rats but no effect on lipid profile in their lean littermates (Wang et al. 2008).

334 In conclusion, results indicate that near 45 % of the French Canadian population in this study do
335 not meet the dairy intake recommendations. Data from FFQ and plasma PL FA profile suggest that
336 *trans*-palmitoleic acid may be potentially used to evaluate high-fat dairy consumption. Further, dairy
337 intake is associated with lower blood glucose and BP, and higher CRP levels, though no causal
338 relationships can be made due to the cross-sectional design. Further, well-designed clinical and
339 mechanistic studies are needed to ascertain these effects.

340

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349

350 **Authors' contributions to manuscript**

351 MSDS performed statistical analysis, interpreted data and wrote the paper; PJ performed fatty
352 acids analyses; PC was responsible for the medical follow-up; IR, SL, and MCV designed research;

353 MSDS and IR have primary responsibility for final content. All authors read and approved the final
354 manuscript. The authors do not declare any conflicts of interest.

355

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571 **Table captions**

572

573 **Table 1.** Composition of the low-fat and high-fat dairy product subgroups and portions

574 **Table 2.** Subjects' characteristics

575 **Table 3.** Daily dietary intakes

576 **Table 4.** Participants' plasma phospholipid fatty acids

577

578 **Figure captions**

579

580 **Figure 1.** Significant correlations between plasma phospholipid (PL) fatty acids (FA) and dairy intake
581 assessed by a food frequency questionnaire in 210 men and women (model was adjusted for age, sex
582 and BMI). (A) Correlation between plasma trans-palmitoleic acid (9t-16:1) and high-fat dairy intake (r
583 = 0.15; p = 0.04). (B) Correlation between plasma palmitic acid (16:0) and low-fat dairy intake (r =
584 0.14; p = 0.05).

585

586 **Figure 2.** Correlations between low-fat (LF), high-fat (HF) and total dairy intakes and metabolic risk
587 factors in all participants, and in men and women separately. Dash lines correspond to r coefficients in
588 which correlation is significant ($p \leq 0.05$).

589 Abbreviations used: CRP, C-reactive protein; DBP, Diastolic blood pressure; FPG, Fasting plasma
590 glucose; HDL, High-density lipoprotein; IL-6: Interleukin-6; LDL, Low-density lipoprotein; SBP,
591 Systolic blood pressure; TG, Triglycerides; TNF, Tumor necrosis factor; Total-C, Total cholesterol.

592

593 **Figure 3.** Significant correlations between plasma phospholipid (PL) dairy *trans* fatty acids and
594 metabolic risk factors. (A) Correlation between plasma *trans*-palmitoleic acid (9t-16:1) and systolic
595 blood pressure (r = -0.15; p = 0.03). (B) Correlation between plasma *trans*-palmitoleic acid (9t-16:1)
596 and BMI in men participants (r = -0.22; p = 0.03) (model was adjusted for age). (C) Correlation
597 between plasma *trans*-vaccenic acid (11t-18:1) and BMI in men participants (r = -0.26; p = 0.01)
598 (model was adjusted for age).

599

600

Table 1. Composition of the low-fat and high-fat dairy product subgroups and portions

Dairy product subgroup	Products	Fat content	Equivalent to one portion
Low-fat (LF)	Milk	Skim, 1 % or 2 %	250 ml
	Yogurt	Skim, 1 % or 2 %	175 g
	Frozen yogurt	< 2 %	175 g
	Cottage cheese	0 – 2 %	250 ml
High-fat (HF)	Milk	Whole	250 ml
	Cheese	All kinds	50 g
	Yogurt	>2 %	175 g
	Cottage cheese	2 – 4 %	250 ml

Table 2. Subjects' characteristics

Mean ± SD	All (N=233)	Men (N=105)	Women (N=128)	p [§]
Age (y)	30.5 ± 8.7	30.9 ± 8.2	30.2 ± 9.1	0.56
Body mass index (kg.m ⁻²)	27.7 ± 3.7	27.3 ± 3.5	28.0 ± 3.8	0.12
Waist/hip ratio*	0.86 ± 0.06	0.89 ± 0.06	0.84 ± 0.06	<0.0001
Waist circumference (cm)	93.2 ± 10.5	93.8 ± 11.2	92.7 ± 9.9	0.47
Systolic blood pressure (mm Hg)	112.2 ± 11.7	118.3 ± 11.4	107.2 ± 9.3	<0.0001
Diastolic blood pressure (mm Hg)	68.1 ± 8.7	68.6 ± 8.2	67.6 ± 9.0	0.45
Fasting plasma glucose (mmol.L ⁻¹)*	4.94 ± 0.46	5.03 ± 0.45	4.87 ± 0.46	0.01
Insulin (pmol.L ⁻¹)†	83.3 ± 49.1	82.3 ± 57.7	84.1 ± 41.1	0.26
HOMA-IR †	2.66 ± 1.79	2.68 ± 2.21	2.65 ± 1.37	0.89
Total cholesterol (mmol.L ⁻¹)*	4.76 ± 0.88	4.74 ± 0.93	4.77 ± 0.84	0.68
HDL cholesterol (mmol.L ⁻¹)†	1.44 ± 0.36	1.28 ± 0.29	1.57 ± 0.35	<0.0001
LDL cholesterol (mmol.L ⁻¹)†	2.76 ± 0.79	2.88 ± 0.87	2.64 ± 0.71	0.03
Total cholesterol/HDL-C ratio†	3.49 ± 1.03	3.88 ± 1.10	3.16 ± 0.83	<0.0001
Triglyceride (mmol.L ⁻¹)†	1.22 ± 0.65	1.26 ± 0.67	1.19 ± 0.64	0.37
CRP (mg.L ⁻¹)†	2.29 ± 2.93	1.20 ± 1.46	3.22 ± 3.50	<0.0001
IL-6 (pg.mL ⁻¹)‡	1.38 ± 1.13	1.31 ± 1.38	1.45 ± 0.85	0.0006
TNF-α (pg.mL ⁻¹)‡	1.68 ± 1.41	1.62 ± 1.08	1.74 ± 1.65	0.30

*Women N = 127.

†Men N = 104; Women N = 127.

‡Men N = 97; Women N = 113.

§p value between men and women

Table 3. Daily dietary intakes

Mean ± SD	All (N=233)	Men (N=105)	Women (N=128)	<i>p</i> [‡]
Energy (kcal) [*]	2254 ± 595	2288 ± 615	1981 ± 413	<0.0001
Carbohydrates (% of energy) [*]	50.6 ± 7.2	49.9 ± 7.3	51.2 ± 7.1	0.19
Proteins (% of energy) [†]	17.3 ± 3.4	17.4 ± 3.5	17.3 ± 3.3	0.71
Fat (% of energy) [*]	32.5 ± 6.0	33.0 ± 5.7	32.1 ± 6.3	0.32
Saturated fat (% of energy) [*]	11.1 ± 3.5	11.2 ± 2.8	11.1 ± 4.0	0.71
MUFA (% of energy) [*]	11.8 ± 2.8	11.8 ± 2.7	11.8 ± 3.0	0.99
PUFA (% of energy) [*]	5.9 ± 2.0	6.0 ± 2.1	5.9 ± 1.9	0.77
LF dairy (portions)	1.56 ± 1.31	1.42 ± 1.26	1.68 ± 1.34	0.15
HF dairy (portions)	0.88 ± 0.68	0.86 ± 0.66	0.90 ± 0.70	0.61
Total dairy (portions)	2.44 ± 1.39	2.28 ± 1.35	2.58 ± 1.42	0.11

*Men N = 101; Women N = 123.

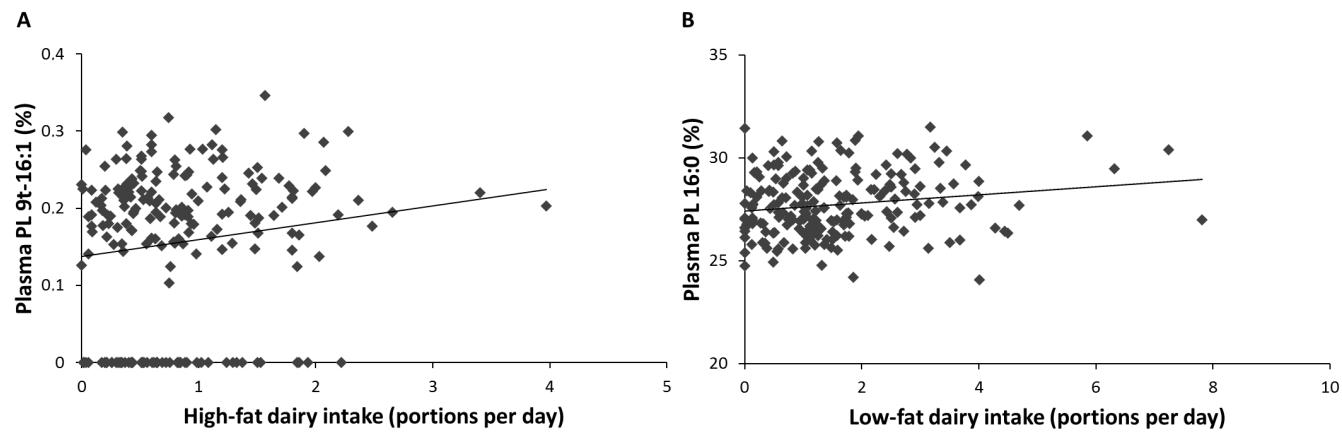
†Men N = 100; Women N = 123.

[‡]*p* value between men and women

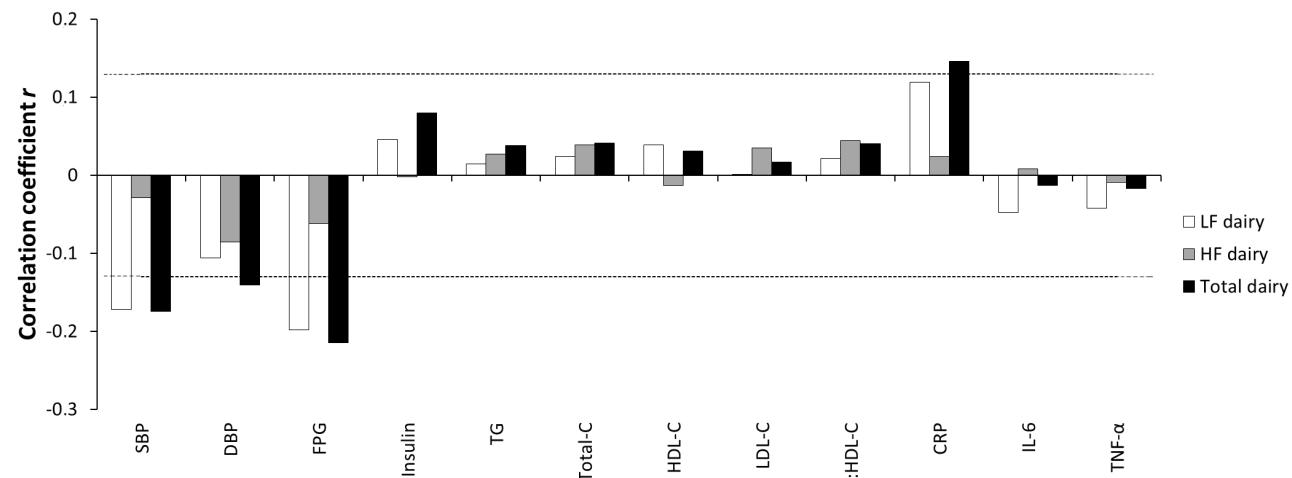
Table 4. Participants' plasma phospholipid fatty acids

Mean ± SD	All (N=210)	Men (N=97)	Women (N=113)	<i>p</i> *
14:0, %	0.38 ± 0.10	0.35 ± 0.08	0.40 ± 0.11	0.0002
16:0, %	27.5 ± 1.5	27.1 ± 1.2	28.3 ± 1.5	<0.0001
18:0, %	13.5 ± 1.3	14.1 ± 1.0	13.1 ± 1.4	<0.0001
9t-16:1, %	0.16 ± 0.10	0.15 ± 0.10	0.16 ± 0.10	0.22
11t-18:1, %	0.12 ± 0.11	0.13 ± 0.11	0.12 ± 0.11	0.63

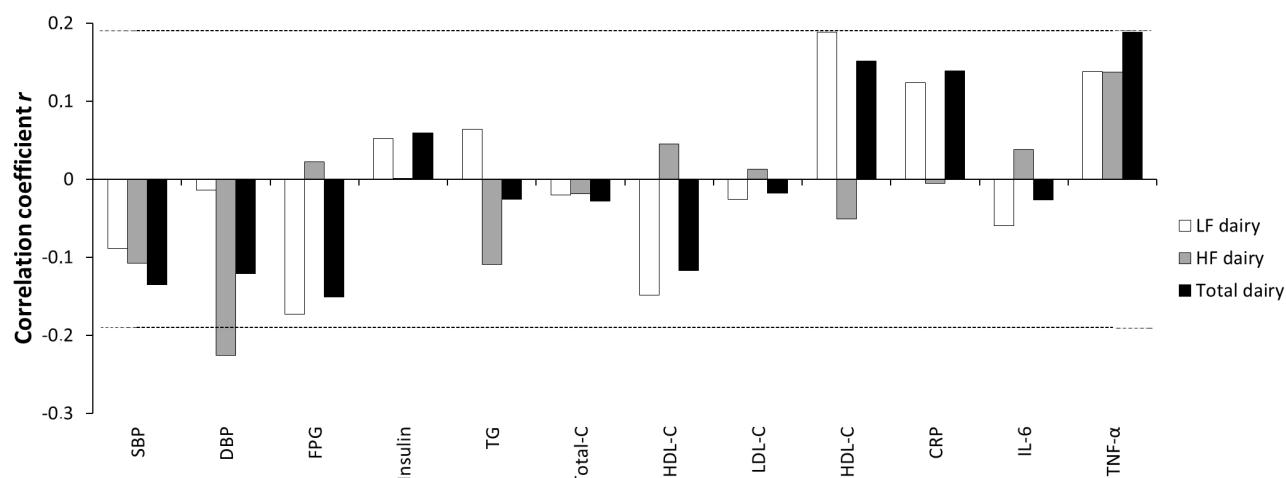
**p* value between men and women



All (N=233)



Men (N=105)



Women (N=128)

