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Review

Effects of Age, Sex, Body Mass Index and APOE Genotype on Cardiovascular Biomarker Response to an n-3 Polyunsaturated Fatty Acid Supplementation

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Abstract
Objectives: To test whether age, sex, body mass index (BMI), and the apolipoprotein E (APOE) genotype are associated with the metabolic response to an n-3 polyunsaturated fatty acid (PUFA) supplementation. ***Methods:*** 210 subjects followed a 2-week run-in period based on Canada's Food Guide and underwent a 6-week 5 g/day fish oil supplementation (1.9 g of eicosapentaenoic acid and 1.1 g of docosahexaenoic acid). Cardiovascular disease risk factors were measured. ***Results:*** n-3 PUFA supplementation was associated with a decrease of plasma triglyceride levels ($p = 0.0002$) as well as with an increase of fasting glucose (FG) levels ($p = 0.02$). Age was associated with post-intervention plasma total cholesterol ($p = 0.01$), low-density lipoprotein cholesterol ($p = 0.007$), apolipoprotein B ($p = 0.04$), and insulin ($p = 0.002$) levels. Sex was associated with post-intervention plasma high-density lipoprotein cholesterol levels ($p = 0.02$). BMI was associated with plasma FG ($p = 0.02$) and insulin levels ($p < 0.0001$) after the supplementation. APOE genotype was associated with FG ($p = 0.001$) and C-reactive protein levels ($p = 0.03$) after the supplementation. ***Conclusion:*** Results suggest that age, sex, BMI, and the APOE genotype contribute to the inter-individual variability observed in the metabolic response to an n-3 PUFA supplementation.

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Introduction

Nine out of 10 individuals (90%) in Canada have at least one cardiovascular disease (CVD) risk factor [1]. The prevalence of multiple risk factors increases at 20–29 years along with an exponential increase in 30–39 years concomitant with an increase in body mass index (BMI) [2]. Dietary omega-3 (n-3) polyunsaturated fatty acids (PUFAs) have multiple beneficial effects on CVD risk factors [3]. Over the last 20 years, randomized controlled trials and epidemiological studies have demonstrated the cardioprotective action of fish oil fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [4–8]. High intakes of EPA and DHA (>2 g/day) have shown positive effects on endothelial function, vascular reactivity, blood pressure, inflammation, and plasma lipid levels [9–13]. The effect of fish oil supplementation on blood glucose concentrations has been investigated and two meta-analyses concluded that the effect is marginal [14] or not statistically significant [15].

Moreover, sex differences have been reported for plasma lipid levels, inflammatory markers, and glycemic markers. It is well documented that men and women have significant differences in their blood lipid profiles [16]. Carroll et al. [17] have observed that total cholesterol (total-C) levels increase with age, reaching a peak in the age group of 50–59 years in men and in the age group of 60–69 years in women. Moreover, men aged between 30 and 49 years had higher total-C levels than women in the same age group and lower levels of total-C after 60 years of age. Plasma high-density lipoprotein cholesterol (HDL-C) levels of women were consistently higher than those observed in men, whereas plasma triglyceride (TG) levels were lower in women [17]. These sex differences could be partly explained by ovarian hormones [18], by a greater DHA synthesis in women that results in higher DHA concentrations in plasma lipids [18], and by a considerably higher conversion rate of α-linolenic acid to long-chain n-3 PUFAs in women than in men [19]. After an n-3 supplementation (4.5 g EPA and 1.9 g DHA/day during 3 weeks), Mueller et al. [20] observed differences in plasma lipid response in men and women. TG levels decreased significantly for men after the n-3 PUFA supplementation, while HDL-C levels increased significantly for women [20].

Another contributor to the heterogeneity in plasma lipid levels in the population is the *APOE* genotype. The *APOE* genotype is among the most widely studied with respect to the impact of genetic variations on plasma lipoprotein levels [21–23]. The *APOE* genotype partly influences the blood lipid response after a fish oil supplementation [24]. In APOE4 carriers, the hypotriglyceridemic effects may be counteracted by a potential proatherogenic shift in the cholesterol profile [24]. Therefore, the aim of the present study was to evaluate the effect of age, sex, BMI, and the *APOE* genotype on the metabolic response to an n-3 PUFA supplementation.

Methods

Subjects

A total of 254 subjects were recruited between September 2009 and December 2011 from the greater Quebec City metropolitan area through advertisements in local newspapers as well as by electronic messages sent to university students/employees. To be eligible, subjects had to be non-smokers and free of any thyroid or metabolic disorders requiring treatment such as diabetes, hypertension, severe dyslipidemia, and coronary heart disease. Participants had to be aged between 18 and 50 years with a BMI between 25 and 40. Subjects were excluded from the study if they had taken n-3 PUFA supplements for at least 6 months prior to the start of the study. A total of 210 unrelated subjects completed the n-3 PUFA supplementation period. Two subjects had missing pre-supplementation values and were excluded from subsequent analyses. The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This trial was registered at clinicaltrials.gov as NCT01343342.

Study Design and Diets

Subjects followed a run-in period of 2 weeks. Personalized dietary instructions were given by a trained dietitian to achieve the recommendations from Canada's Food Guide. Subjects were asked to follow these dietary recommendations and to maintain their body weight stable throughout the protocol. Some specifications were given regarding the n-3 PUFA dietary intake: no more than two fish or seafood servings per week (maximum of 150 g/week), to prefer white-flesh fishes instead of fatty fishes (examples were given), and to avoid enriched n-3 PUFA dietary products such as some milks, juices, breads, and eggs. Subjects were also asked to limit their alcohol consumption during the protocol; two regular drinks per week were allowed. In addition, subjects were not allowed to take n-3 PUFA supplements (such as flaxseed), vitamins or natural health products during the protocol.

After the 2-week run-in period, each participant received a bottle containing the needed n-3 PUFA capsules for the following 6 weeks. They were invited to take 5 g/day of fish oil (Ocean Nutrition, Mulgrave, N.S., Canada), providing a total of 3 g/day of n-3 PUFAs (1.9–2.2 g of EPA and 1.1 g of DHA). Compliance was assessed from bottle returning. Subjects were asked to report any deviation during the protocol and to write down their alcohol and fish consumption as well as the side effects. Before each phase, subjects received detailed written and oral instructions on their diet.

A dietitian administrated, to each participant, a validated food frequency questionnaire (FFQ) before the run-in period [25]. This FFQ was based on typical food items available in Quebec and contained 91 items; 27 items had between 1 and 3 sub-questions. Moreover, subjects completed a 3-day food diary before and after the n-3 PUFA supplementation period. Dietary intakes were analyzed using the Nutrition Data System for Research (NDS-R) software v.4.02 developed by the Nutrition Coordinating Center (University of Minnesota, Minneapolis, Minn., USA).

Anthropometric Measurements

Body weight, height, and waist girth were measured according to the procedures recommended by the Airlie Conference [26] and were taken by a dietitian at each visit at the clinical investigation unit; during the run-in period and before and after the n-3 PUFA supplementation. BMI was calculated as weight in kilograms per meter squared (kg/m^2).

Biochemical Parameters

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after 12 h overnight fast and 48 h alcohol abstinence. Blood samples were taken before the run-in period to identify and exclude individuals with any metabolic disorders. Afterwards, selected participants had blood samples taken before and after the n-3 PUFA supplementation period. Plasma was separated by centrifugation (2,500 *g* for 10 min at 4°C) and samples were aliquoted and frozen for subsequent analyses. Plasma total-C and TG concentrations were measured using enzymatic assays [27, 28]. The HDL-C fraction was obtained after precipitation of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) particles in the infranatant with heparin manganese chloride [29]. LDL cholesterol (LDL-C) was calculated with the Friedewald formula [30]. Plasma C-reactive protein (CRP) was measured by nephelometry (Prospec equipment, Behring, Siemens Healthcare Diagnosis, Deerfield, Ill., USA) using a sensitive assay, as described previously [31]. Apolipoprotein B-100 (Apo-B) concentrations were measured in the plasma by the rocket immunoelectrophoretic method of Laurell [32], as previously described. Fasting insulinemia was measured by radioimmunoassay with polyethylene glycol separation [33]. Fasting glucose (FG) concentrations were enzymatically measured [34].

Genotype Analysis

Genetic analyses were performed on genomic DNA isolated from human leukocytes. The common three allele (E2/E3/E4) variations in the *APOE* gene were analyzed with the PCR-RFLP method described by Hixson and Vernier [35].

Statistical Methods

Data are shown for participants who completed the study. Variables not normally distributed (TG, CRP, and insulin) were $\log_{10}$ transformed. Nutritional data were analyzed using an analysis of variance adjusted for age and BMI. Since energy intake was significantly different between men and women, nutritional data were subsequently adjusted for it. Comparisons between men and women before and after supplementation were done by Student's paired t test. ANOVA analysis was undertaken on the outcome (end of treatment values) with the baseline (beginning of treatment period) values as covariates to examine the individual and

Table 1. Daily energy and nutrient intakes at baseline[1]

	Total subjects (n = 210)	Men (n = 97)	Women (n = 113)	p value[2]	p value[3]
Energy, kcal	2,499±729	2,792±814	2,248±527	<0.0001	–
Total fat, g	93.9±34.4	105.9±37.2	83.6±28.0	<0.0001	0.50
Saturated fat, g	30.2±11.8	33.9±13.4	26.9±9.1	0.0002	0.11
Monounsaturated fat, g	39.4±15.5	44.6±16.4	34.9±13.2	<0.0001	0.70
Polyunsaturated fat, g	16.9±6.9	18.9±7.6	15.0±5.7	<0.0001	0.75
EPA, g	0.10±0.08	0.11±0.08	0.10±0.07	0.52	0.78
DHA, g	0.20±0.13	0.21±0.14	0.19±0.14	0.47	0.67
Trans fat, g	3.6±1.9	4.11±2.1	3.21±1.6	0.0002	0.82
Cholesterol, mg	357±157	401±186	320±116	0.001	0.52
Carbohydrates, g	303.3±92.0	336.6±106.5	274.6±65.3	<0.0001	0.78
Proteins, g	111.2±35.7	123.1±41.8	100.9±25.6	<0.0001	0.22
Alcohol, g	6.6±8.8	7.9±11.2	5.4±5.9	0.04	0.15
Total fibers, g	26.1±9.0	27.4±9.7	24.9±8.2	0.08	0.08

Values are means ± SD. [1] Dietary intake obtained from a FFQ. [2] Adjusted for age and BMI. [3] Adjusted for age, BMI, and energy.

combined effects of the design variables (i.e. age, sex, BMI, and APOE genotype) on response to treatment. All statistical analyses were performed with SAS statistical software v.9.2 (SAS Institute Inc., Cary, N.C., USA). Statistical significance was defined as $p < 0.05$.

Results

Macronutrients and energy intakes for men and women before the n-3 PUFA supplementation are shown in table 1. Although men had significantly higher energy intakes than women ($p < 0.0001$), no significant differences were observed for total lipids, saturated fat, monounsaturated fat, polyunsaturated fat, EPA, and DHA after further adjustments for energy. Baseline characteristics for men (n = 96) and women (n = 112) are presented in table 2. These results show that men and women were overweight (BMI >25) and had mean plasma TG levels slightly above the cut point value of 1.129 mM recommended by the American Heart Association for optimal plasma TG levels [36]. Moreover, according to the World Health Organization, the average waistline of study participants is considered as a risk factor since it is ≥94 cm for men and ≥80 cm for women [37]. Also, men and women had significantly different body weight as well as plasma LDL-C, HDL-C, FG, and CRP levels. A total of 24 subjects were carriers of the APOE2 allele, 129 subjects were APOE3 homozygotes, and 51 subjects were carriers of the APOE4 allele. Out of the 51 carriers of the APOE4 allele, 4 were homozygotes and 47 were heterozygotes E3/E4. The allele frequencies were of 6.9, 69.1, and 24.0%, respectively, for the APOE2, E3, and E4 allele. These frequencies are similar to those reported in a Caucasian population from Europe [38] and in French-Canadians of northeastern Quebec [39]. We excluded 3 heterozygotes E4/E2 from the analysis because of the small sample size and another subject due to missing values.

Metabolic variations before and after n-3 PUFA supplementation are presented in table 3. A significant intervention effect was observed for plasma TG levels ($p = 0.0002$): 11.89 ± 25.91% of reduction was observed for the total cohort. Moreover, we observed an increase of 2.44 ± 49.55% for FG ($p = 0.002$) with the supplementation (table 3) when men and women

Table 2. Pre-supplementation descriptive characteristics

	Total subjects (n = 210)	Women (n = 113)	Men (n = 97)	p value
Age, years	30.8±8.7	30.5±9.1	31.2±8.1	0.55
Weight, kg[1]	81.3±13.9	76.3±12.3	87.1±13.4	<0.0001
BMI[1, 4]	27.8±3.7	28.1±3.8	27.4±3.6	0.13
Waist circumference, cm[1]	93.3±10.5	91.9±10.1	94.8±10.8	0.06
Total-C, mM[2]	4.75±0.90	4.75±0.85	4.75±0.96	0.91
LDL-C, mM[2]	2.76±0.81	2.64±0.73	2.90±0.89	0.02
HDL-C, mM[2]	1.44±0.36	1.57±0.35	1.28±0.30	<0.0001
TG, mM[2, 4]	1.21±0.63	1.17±0.60	1.26±0.66	0.10
Apo-B, g/l[2]	0.84±0.24	0.81±0.22	0.87±0.26	0.08
Glucose, mM[2]	4.95±0.46	4.87±0.45	5.04±0.46	0.02
Insulin, pM[2]	87.1±75.7	84.73±42.10	89.97±102.0	0.17
CRP, mg/l[2, 4, 5]	1.82±2.17	2.41±2.55	1.21±1.48	<0.0001
Genotype[3]				
E2	24	10	14	
E3	129	72	57	
E4	51	27	24	

Values are means ± SD or numbers. [1] ANOVA adjusted for age. [2] ANOVA adjusted for age and BMI. [3] n = 204 for APOE genotype. Three E4/E2 heterozygotes were excluded from statistical analyses. [4] p value derived from $\log_{10}$-transformed values. [5] n = 194; 96 men, 98 women; excluded subject had CRP levels >10 mg/l.

Table 3. Metabolic variations before and after n-3 PUFA supplementation

	Total (n = 208)			Women (n = 112)			Men (n = 96)		
	before suppl.	after suppl.	p value[1]	before suppl.	after suppl.	p value[2]	before suppl.	after suppl.	p value[2]
Weight, kg	81.3±13.9	81.7±14.3	0.001	76.3±12.3	76.0±12.5	NS	87.1±13.4	87.3±13.7	NS
BMI[4]	27.8±3.7	27.9±3.9	0.002	28.1±3.8	28.2±4.0	NS	27.4 ±3.6	27.5±3.7	NS
Waist circumference, cm	93.3±10.5	93.4±10.8	NS	91.9±10.1	91.8±10.6	NS	94.8±10.8	95.1±10.7	NS
Total-C, mM	4.75±0.90	4.72±0.94	NS	4.75±0.85	4.73±0.92	NS	4.75±0.96	4.70±0.98	NS
LDL-C, mM	2.76±0.81	2.78±0.85	NS	2.64±0.73	2.65±0.78	NS	2.90±0.89	2.92±0.91	NS
HDL-C, mM	1.44±0.36	1.47±0.40	NS	1.58±0.36	1.64±0.40	0.001	1.28±0.30	1.29±0.32	NS
TG, mM[4]	1.21±0.63	1.02±0.52	0.0002	1.17±0.60	0.97±0.50	0.003	1.26±0.66	1.07±0.54	0.01
Apo-B, g/l	0.84±0.24	0.87±0.24	NS	0.81±0.22	0.84±0.22	NS	0.87±0.26	0.90±0.25	NS
FG, mM	4.95±0.46	5.06±0.49	0.02	4.87±0.45	4.99±0.53	0.06	5.04±0.45	5.13±0.43	NS
Insulin, pM	87.1±75.7	83.6±40.8	NS	84.7±42.1	86.6±42.8	NS	90.0±102.0	80.1±37.8	NS
CRP, mg/l[3, 4]	1.82±2.17	1.85±2.12	NS	2.41±2.55	2.45±2.48	NS	1.21±1.48	1.24±1.46	NS

Values are means ± SD. [1] ANOVA repeated measures adjusted for age, sex, and BMI. [2] ANOVA repeated measures adjusted for age and BMI. [3] Total n = 194; 96 men, 98 women; excluded subject had CRP levels >10 mg/l. [4] Data not normally distributed were $\log_{10}$-transformed values. suppl. = Supplementation; NS = not significant.

were analyzed together. There was no detectable intervention effect on plasma total-C, LDL-C, HDL-C, Apo-B, insulin, and CRP levels in the total group. When men and women were analyzed separately, the difference in plasma glucose levels was no longer significant and plasma HDL-C levels were increased after supplementation in women and not in men.

In an ANOVA, we tested whether age, sex, BMI, and the APOE genotype were associated with post-intervention plasma lipid levels. Age was associated with post-intervention levels

Table 4. Effects of age, sex, BMI, and APOE genotype on CVD risk factors after n-3 PUFA supplementation[1] (n = 208)

	Age p value	Sex p value	BMI p value	*ApoE* genotype p value
Total-C	0.01	NS	NS	NS
LDL-C	0.007	NS	NS	NS
HDL-C	NS	0.02	NS	NS
TG[2]	NS	NS	NS	NS
Apo-B	0.04	NS	NS	NS
FG	NS	NS	0.02	0.001
Insulin	0.002	NS	<0.0001	0.08
CRP[2]	NS	NS	NS	0.03

[1] ANOVA analyses undertaken on the outcome data (end-of-treatment values) with the baseline values as covariates. [2] ANOVA derived from $\log_{10}$-transformed values. NS = Not significant.

of total-C, LDL-C, Apo-B, and insulin levels (p = 0.01, 0.007, 0.04, and 0.0002, respectively; table 4). Sex was associated with plasma HDL-C levels (p = 0.02; table 4). Post-intervention plasma HDL-C levels were increased when compared to pre-intervention values, but only in women (table 3). BMI was associated with FG (p = 0.02) and insulin levels (p < 0.0001; table 4). Finally, the *APOE* genotype was associated with FG (p = 0.001) and CRP levels (p = 0.03; table 4). There was no significant effect of the APOE genotype on post-intervention plasma TG levels (table 4). Moreover, total-C, LDL-C, and Apo-B levels were lower in subjects carrying the APOE4 allele, intermediate in homozygotes for the APOE3 allele, and higher in carriers of the APOE2 allele (data not shown).

Discussion

This work presents an interventional study design investigating whether age, sex, BMI, and the APOE genotype are associated with plasma lipid levels as well as with inflammatory and glycemic markers after a supplementation with high doses of n-3 PUFA. The intake of 3 g/day of n-3 PUFAs (1.9–2.2 g of EPA and 1.1 g of DHA) resulted in an 11.89 ± 25.91% decrease in plasma TG levels. n-3 PUFAs are considered to be the most effective way to reduce plasma TG levels via nutrition [40]. The hypotriglyceridemic effect of fish oil is well documented in the literature. A meta-analysis has concluded that 3–4 g/day of EPA and DHA resulted in a 25% decrease of fasting TG levels in normolipidemic subjects and a 34% decrease in hyperlipidemic subjects [11]. According to Skulas-Ray et al. [40], subjects with higher plasma TG levels before n-3 PUFA supplementation reported a greater decrease after supplementation than individuals with lower baseline TG levels. The fact that our study subjects, in spite of being overweight or obese, had only moderate metabolic disturbances could explain why we observed a smaller plasma TG decrease in the present study.

In addition to suppressing VLDL production, n-3 PUFAs are also known to influence VLDL composition with a shift in the distribution of VLDL subclasses towards smaller VLDL particles [24]. Because smaller VLDL particles represent the primary precursor of LDL, this qualitative change in VLDL particles may result in a greater rate of conversion to LDL [24]. Concerns remain regarding the impact of long-term consumption of n-3 PUFA on plasma LDL-C levels [41]. However, in the present study, there was no detrimental effect of n-3 PUFA on plasma LDL-C levels.

A significant increase of FG was observed after the n-3 PUFA supplementation. However, FG remained in the normal range [36]. Woodman et al. [42] reported significantly higher FG concentrations and no effect of high amounts of purified EPA or DHA (4 g/day EPA or DHA) on insulin sensitivity in a 6-week trial. In another trial, Mori et al. [43] also reported a trend toward increased FG levels in overweight, mildly hyperlipidemic men with 4 g/day of purified EPA and significantly increased fasting insulin levels when using a combination of EPA and DHA. In contrast, other randomized trials showed no effect of EPA and DHA on insulin sensitivity [44, 45]. In a recent study, Fink et al. [46] demonstrated that there was no significant association between fish and n-3 PUFA intakes and the risk of type 2 diabetes. Moreover, a study has demonstrated that the risks related to higher FG levels with an n-3 PUFA-enriched diet were probably counteracted by the positive effects on plasma lipids [47]. The difference in the results regarding glucose and insulin sensitivity requires further studies to adequately test the potential impact of n-3 PUFAs on diabetes risk and related risk factors.

The plasma lipid levels and the plasma lipid responses to a nutritional intervention are under the control of several genetic and environmental factors. In the present study, we tested the effect of age, sex, BMI, and the *APOE* genotype on post-intervention values. Age was significantly associated with post-intervention plasma total-C, LDL-C, Apo-B, and insulin levels. In different studies, investigators have shown that advanced age is associated with impaired glucose processing [46, 48]. Estrogen may have protective effects against CVD, as evidenced by the decreased incidence of CVD in premenopausal as compared with postmenopausal women. It seems that aging has a greater impact on sympathetic traffic (muscle sympathetic nerve activity, blood pressure, and heart rate have been measured) in women than in men [49]. Studies in mammals have led to the suggestion that hyperglycemia and hyperinsulinemia are important factors in aging [49].

Gender differences in cardiovascular risk factors are well documented in the literature. In the present study, we observed that the plasma lipid and apolipoprotein responses to a fish oil intervention were influenced by sex [50]. In fact, plasma HDL-C levels increased after supplementation, but only in women. Among the potential mechanisms, there are gender-related differences in peroxisome proliferator-activated receptor α (PPARα) activation. In addition, studies with fenofibrate, a hypolipidemic drug known to have similar effects as n-3 PUFA, showed gender-related differences in the plasma lipid response [51, 52]. A study on rats has demonstrated the involvement of ovarian hormones in the positive regulation of peripheral DHA composition [18]. Also, it has been reported that ovarian steroids can affect lipid metabolism and that these effects are probably mediated by estrogen [47]. The different ability to activate PPARα together with the well-documented effect of sexual hormones on the plasma lipid metabolism may, among other factors, explain why sex was a significant contributor to plasma HDL-C levels after intervention.

BMI was associated with post-intervention FG and insulin levels. *APOE* genotype was associated with FG and CRP levels after the n-3 PUFA supplementation. For post-intervention FG levels, when age, sex, BMI, the APOE genotype, and FG levels before the supplementation were included in the model, BMI, the APOE genotype, and FG levels before the supplementation explained 1.49, 3, and 32.9% of the variance of the trait, respectively. For CRP levels after intervention, 1.21% of variability is explained by the APOE genotype and 31.4% by pre-supplementation plasma CRP levels. The effects of the *APOE* genotype were similar to those previously reported in nutritional studies [53]. A meta-analysis of 48 studies has shown that carriers of the E4 allele (E3/4, E4/4) had an increased risk of coronary heart disease compared with E3/3 individuals [54]. Minihane et al. [24] observed a stronger adverse effect of fish oils on LDL-C levels in carriers of the E4 allele. APOE4 is known to selectively associate with VLDL, which may enhance its catabolism to LDL. In combination with the downregulated state of the LDL receptor in this subgroup, this may explain the greater increase in LDL-C levels among

carriers of the E4 allele. HDL-C was lower in subjects who were carriers of the E4 allele. This finding is in agreement with the lower circulating HDL-C concentrations reported in individuals with the E4/E3 genotype in 17 out of 28 studies examined in a meta-analysis by Dallongeville et al. [55]. In contrast with the results of Minihane et al. [24], we observed no significant effect of the APOE genotype on plasma TG levels before and after supplementation. However, the *APOE* genotype was associated with plasma CRP levels. Kofler et al. [50] observed that the *APOE* genotype modulates plasma CRP levels in the following order: E2 > E3 > E4. In the present study, the same association between *APOE* genotype and plasma CRP levels was observed.

The present study has some limitations. The method used for nutritional assessment may create bias. To limit those errors, we have used an FFQ which had been validated in the Quebec City metropolitan region [25]. Also, the absence of correction for multiple testing in association studies may be seen as a limitation. Since plasma lipid variables under study are strongly correlated, the conventional approaches, such as Bonferroni correction, to correct for multiple testing is probably too stringent and may result in false negatives. Since this study is an explanatory one to identify the potential factors contributing to the inter-individual variability observed in the plasma lipid response to an n-3 PUFA supplementation, we decided not to apply this correction. Finally, the absence of a control group represents a limitation for the results about FG.

In conclusion, results of the present study suggest that age, sex, BMI, and the *APOE* genotype are significant contributors to the metabolic response to an n-3 PUFA supplementation. More studies are needed to better understand the effect of sex on the metabolic response to such interventions.

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Disclosure Statement

The authors declare no conflict of interest.

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