Nutrition 31 (2015) 807–812

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Disrupted fatty acid distribution in HDL and LDL according to apolipoprotein E allele

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ARTICLE INFO

Article history:
Received 29 August 2014
Accepted 25 November 2014

Keywords:
Omega-3 fatty acid
DHA
EPA
Plasma lipoproteins
Omega-6/omega-3 fatty acid ratio
DHA metabolism
APOE isoforms

ABSTRACT

Objectives: Omega-3 polyunsaturated fatty acid (ω-3 PUFA) metabolism seems to be disrupted in carriers of the epsilon 4 allele of apolipoprotein E (*E4+*). The objective of this study was to investigate whether the ω-3 PUFA distribution in the high and low density lipoproteins is *APOE*-genotype dependant before and after supplementation with ω-3 PUFAs.
Methods: Eighty participants, aged between 20 and 35 y old were recruited and supplemented with 900 mg of eicosapentaenoic acid plus 680 mg of docosahexaenoic acid for 4 wk. Over the 4-wk intervention, blood samples were collected and HDL and LDL particles were obtained using sucrose gradient ultracentifugation. Fatty acid profiles of the HDL and LDL fractions were performed by gas chromatography.
Results: Baseline anthropometric characteristics of participants were not significantly different between the two *APOE*-groups (*E4+*, N = 10; *E4−*, N = 70). At baseline, in the LDL of *E4+* subjects, the ω-6/ω-3 PUFA ratio was 17% higher than *E4−* subjects. At week 4, the ω-6/ω-3 PUFA ratio was significantly higher in the LDL of *E4+* than *E4−* subjects. There was a significant genotype × time interaction for 16:0 in HDL and LDL and for 18:2 ω-6 in HDL. DHA in the HDL was positively correlated to HDL-C levels pre- and postsupplementation in *E4−* only.
Conclusions: Contrary to what we anticipated, ω-3 PUFAs content? in HDL and LDL were not *APOE* isoform-dependant in young participants. However, young *E4+* participants already had a tendency toward lower baseline-DHA levels in LDL particles as well as a more atherogenic ω-6/ω-3 PUFA ratio in LDL pre- and post-supplementation.

Introduction

Cardiovascular diseases (CVD) are one of the leading causes of death worldwide. Carrying the apolipoprotein E epsilon 4 allele (*E4+*) is one of the most important genetic risk factors for developing age-related chronic diseases such as CVD and Alzheimer's disease (AD) [1]. One environmental factor likely capable of decreasing the risk of CVD and AD is the consumption of omega-3 fatty acids (ω-3 PUFA) from fatty fish [2]. A low balance of ω-6/ω-3 PUFAs seems to be contributing in decreasing the risk of inflammatory-related diseases, and may promote heart and brain health [3]. However, *E4+* subjects do not seem to be protected against CVD [4] and cognitive decline [5,6] when consuming ω-3 PUFAs. Recent evidence suggests that this lack of protection could be related to dysfunction of ω-3 PUFA metabolism and kinetics [7,8].

In humans, there are three isoforms of the apoE protein, namely apoE2, apoE3, and apoE4, resulting from six genotypes (i.e. ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4) [9]. The sequence variations found between *APOE* isoforms induce structural modifications of the apoE protein that ultimately modulate low-density lipoprotein receptor family (LDL-R) binding activity [10]. The structural conformation of apoE4 explains its preferential bind to triacylglycerol rich lipoproteins (i.e., VLDL and LDL) as opposed to apoE3 and apoE2, which preferentially bind to high-density lipoproteins (HDL) [10,11]. Therefore, *E4+* subjects usually have higher plasma triacylglycerols (TGs), total cholesterol, and smaller and denser LDL than *E4−* subjects [10,12]. Because apolipoprotein E (apoE) plays important roles in the regulation,

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http://dx.doi.org/10.1016/j.nut.2014.11.019
0899-9007/

transport and clearance of fatty acids, carrying the apoE4 isoform may modulate the efficiency of apoE in accomplishing its essential role in lipoprotein metabolism. Moreover, the lack of protection against CHD and AD when *E4*+ subjects are supplemented with ω-3 PUFA may potentially be explained by disrupted postprandial kinetics of docosahexaenoic acid (DHA; 22:6 ω-3), an ω-3 PUFA [7]. Other studies reported gene-by-diet interaction in the uptake of ω-3 PUFA and cholesterol metabolism in *E4*+ supplemented with 3 g/d of ω-3 PUFAs [4,12,13]. Because *E4*+ subjects supplemented with ω-3 PUFA have greater sensitivity of fasting triacylglycerols [14] and increased total cholesterol [4,13] when compared to *E4*− subjects, we speculate ω-3 PUFA distribution in plasma lipoproteins is *APOE* isoform-dependant. We also hypothesize that ω-3 PUFA are mainly incorporated into LDL particles in *E4*+ subjects as opposed to HDL particles in *E4*− carriers. The aim of this study was to determine the longitudinal FA profile in the HDL and LDL of young *E4*+ and *E4*− participants receiving 680 mg/d of DHA +900 mg/d of eicosapentaenoic acid (EPA; 20:5 ω-3) over a 4-wk intervention.

Material and methods

Subjects and study design

Eighty-two healthy men and women (N = 82) aged 20–35 y old, from the Sherbrooke area, were recruited. Subjects were excluded if they smoked, were medicated, (with the exception of contraception pills), had a history of psychiatric difficulties or depression, were allergic to seafood, were pregnant or breast-feeding, or were already supplemented with ω-3 PUFA capsules. Each participant gave their informed written consent before participating in the study. This study was approved by the ethics committee of the Health and Social Services Center, Sherbrooke University Geriatrics Institute. This study is registered on clinicaltrial.org (NCT-01544855).

Participants were asked to consume two capsules of ethyl ester fish oil (450 mg of EPA + 340 mg of DHA/capsule) daily for 4 wk (Ocean Nutrition, Dartmouth, NS, Canada). This dose corresponds to three times the current ω-3 PUFA consumption in young French Canadian adults [15]. Participants were instructed to record their daily consumption of fish, alcohol, and natural products in a logbook. Compliance was measured by counting the capsules returned to the research staff each week.

Participants came to our research facility once per week, for 4 wk, and a fasted blood sample was collected. Plasma was separated from red and white blood cells by centrifugation (3500 × g for 10 min at 4°C). Whole blood was kept for subsequent DNA extraction and *APOE* genotyping. Separation of HDL and LDL was performed as follows: 800 μL of plasma was added to a sucrose gradient as described in Cooper et al. [16]. Briefly, 105 mg of sucrose was added to plasma to obtain 12.5% sucrose in plasma. The sucrose gradient was created by successive layers, from top to bottom: 500 μL of PBS, 12.5% of sucrose + plasma, and 333 μL of both 25% and 47% sucrose in PBS solutions (w/w). Ultracentrifugation was performed at 201 000 × *g*, 12°C for 26 h using a Beckman Optima TLX ultracentrifuge equipped with a TLS-55 rotor (Beckman Coulter, Brea, CA, USA). The following fractions were pooled together: 700 μL of LDL (fraction 3−9, *P* = 1.04–1.07 g/mL) and 600 μL of HDL fractions (fraction 10−15, *P* = 1.07–1.23 g/mL). Blood biochemistry, including glucose, albumin, total cholesterol (TC), TG, thyroid stimulating hormone (TSH), aspartate transaminase (AST), alanine transferase (ALT), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and creatinine, was assessed at the Centre Hospitalier Universitaire de Sherbrooke.

Fatty acid analysis

Fatty acid (FA) profile of HDL and LDL particles was performed as previously described [17]. Briefly, total lipids were extracted from HDL and LDL using a 2:1 chloroform:methanol solution. The total lipid extract was then saponified using 1 M KOH/methanol and heated at 90°C for 1 h, thereby releasing the FAs from cholesteryl esters and glycerolipids. The transmethylation of FA into FA-methyl-esters was done by adding boron trifluoride/methanol (14%; Sigma-Aldrich, St. Louis, MO, USA) and heating to 90°C for 30 min. Analysis was performed using a gas chromatograph equipped with a 50-m BPX-70 fused capillary column (SGE, Melbourne, Australia; 0.25-mm inner diameter, 0.25-μm film thicknesses). FAs were identified using external standard (NuChek 68 A, NuChek 411, and NuChek 455; NuChek Prep, Inc., Elysian, MN, USA and a custom mixture of saturated FA standards).

APOE genotype analysis

APOE genotyping was performed using a derived method of Hixson and Vernier [18]. Genomic DNA was first isolated from whole blood by Qiagen DNA Blood Mini Kit (Qiagen Ltd, Crawley, UK). After, *APOE* polymorphism was determined by polymerase chain reaction-fragment length polymorphism. The 244 pb amplified PCR fragments were then digested with the restriction enzyme *Hha1* (New England Biolab, Ipswich, MA, USA). Fragments were separated through migration on a 20% polyacrylamide gel, poststained with gel red, and visualised under UV-light (Image analyser body mass index [BMI] lab equipment, MBI sigma, Kirland, Canada).

Statistical analysis

Sample size calculation was based on the relative percentage of DHA in plasma TG at baseline in *E4*+ (0.82% ± 0.25%) and *E4*− subjects (0.53% ± 0.31%) as reported in Plourde et al. [8]. This metric was used because no data are currently available in literature, to our knowledge, on baseline-DHA content of HDL or LDL according to *APOE* genotype. An unequal sample size in each group was expected for two reasons: 1) our institution does not allow prescreening for *E4*+ genotype, and 2) 15–25% of Canadians are known to be carriers of at least one epsilon 4 allele of *APOE* [18]. To achieve a statistical power of 80% ($\alpha = 0.05$), we determined 10 (N = 10) *E4*+ subjects were needed. Therefore, based on the lowest frequency of *APOE* epsilon 4 allele in Canadians (15%), the number of participants to be recruited was 67 (N = 67), but with an anticipated dropout of 20% [19], 80 participants (N = 80) were recruited.

Normal distribution and homogeneity of variance were evaluated before further statistical analysis. All data were analysed for statistical differences of the FA profile in HDL and LDL using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY, USA). When assumptions of homogeneity of the covariance matrixes were rejected (i.e., Mauchly's Test of Sphericity), Greenhouse-Geisser corrections were used. The main effect of genotype at baseline and after supplementation was analysed using the Kruskal-Wallis non-parametric analysis of variance. Univariate spearman correlation analysis was used to investigate associations among outcomes. The balance of ω-6/ω-3 PUFA was calculated using the sum of the following FA: The sum of linoleic acid (LA), di-homo-gamma linolenic acid (DGLA), and arachidonic acid over the sum of alpha-linolenic acid (ALA), EPA, docosapentaenoic acid, and DHA. *P* values ≤0.05 were considered statistically significant, and the *P* value for trends was set as ≤ 0.08. FA profiles are presented as mean percentage (%) of total FA ± SEM and as percentage (%) compared to control, meaning *E4*− subjects, using the following equation:

$$\text{Compared to control (\%)} = [(E4+ \text{ value}) \div (E4- \text{value})] \times 100$$

Table 1
Baseline characteristics of participants carrying (*E4*+) or not carrying (*E4*−) the apolipoprotein E epsilon 4 allele

Characteristics*	*E4*+ (N = 10)	*E4*− (N = 70)	*P*
Age	26 ± 4	27 ± 4	0.509
Sex (men/women)	(5/5)	(29/41)	0.801
Weight (kg)	68 ± 13	70 ± 13	0.651
Body mass index (kg/m^2)	24 ± 3	24 ± 4	0.951
Waist circumference (cm)	82 ± 10	85 ± 9	0.195
Natural product (%)†	10	23	0.302
Alcohol consumption (%)			0.752
Never	10	10	
2−3/mo	20	26	
1/wk	20	31	
2−3/wk	50	29	
Almost everyday	0	4	
Physical activity (%)			0.370
Never	0	2	
2−3/mo	20	7	
1/wk	0	19	
2−3/wk	40	46	
Almost everyday	40	26	

* Values are presented as mean ± SD.

† Percentage (%) of subjects taking natural products in each group. Natural product included: Homeopathic products, vitamin B6, C, and D, protein, aloes, probiotics, multivitamins, multiminerals, orange triads, echinacea, collagen, creatinin phosphate, oregano oil, branched chain amino acids, hypericum. Supplementation with ω-3 PUFA was not permitted.

Table 2
Blood biochemistry values before (Baseline) and after 4-wk of supplementation with ω-3 polyunsaturated fatty acids in carriers (*E4+*) and non-carriers (*E4−*) of the apolipoprotein E epsilon 4 allele*

	Baseline		Week 4		*P* values†		
	E4+	*E4−*	*E4+*	*E4−*	Interaction	Genotype	Time
Glucose (mmol/L)	4.29 ± 0.14	4.12 ± 0.05	4.34 ± 0.15	4.18 ± 0.06	0.908	0.660	0.134
Albumine (g/L)	46.17 ± 0.71	45.40 ± 0.33	44.46 ± 0.48	45.34 ± 0.33	**0.021**	0.998	(0.015)
AST (UI/L)	22.00 ± 2.06	20.60 ± 0.61	24.67 ± 2.58	21.89 ± 0.89	0.874	0.196	0.215
ALT (UI/L)	19.70 ± 3.36	20.86 ± 1.20	24.78 ± 3.91	21.21 ± 1.43	0.267	0.603	0.180
TSH (UI/L)	2.20 ± 0.37	2.45 ± 0.14	2.41 ± 0.41	2.60 ± 0.14	0.996	0.639	0.280
Total-C (mmol/L)	4.81 ± 0.27	4.39 ± 0.10	4.84 ± 0.24	4.38 ± 0.09	0.572	0.057	0.497
TG (mmol/L)	0.95 ± 0.18	1.16 ± 0.07	0.85 ± 0.14	1.06 ± 0.05	0.698	0.281	0.110
HDL-C (mmol/L)	1.71 ± 0.10	1.42 ± 0.04	1.78 ± 0.11	1.49 ± 0.04	0.838	**0.022**	**0.021**
LDL-C (mmol/L)	2.67 ± 0.20	2.45 ± 0.08	2.68 ± 0.19	2.40 ± 0.08	0.702	0.182	0.269
Total-C/HDL-C	2.84 ± 0.13	3.26 ± 0.12	2.76 ± 0.12	3.10 ± 0.11	0.984	0.270	**0.029**
Creatinine (μmol/L)	82.90 ± 5.76	73.39 ± 1.24	81.44 ± 4.05	73.11 ± 1.20	**0.047**	(0.006)	(0.025)

AST, aspartate transferase; ALT, alanine transferase; TSH, thyroid stimulating hormone; C, cholesterol; TG, triacylglycerol
* Values are presented as mean relative percentages ± SEM.
† *P* values were obtained using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Bold characters indicate significant differences ($P \leq 0.05$). When interactions were found, significant *P* values for the independent genotype and time effects are indicated in parenthesis.

Results

Participants

The characteristics of *E4+* and *E4−* are presented in Table 1. Ten participants were carrying one allele of *E4+* (N = 6, ε4/ε3 and N = 4, ε4/ε2) whereas the remaining participants were classified as *E4−* (N = 59, ε3/ε3 and N = 11, ε3/ε2). There were an equal number of men and women in the *E4+* group, whereas men represented 41% of the *E4−* group. Two individuals were ε2/ε2 and were excluded from our statistical analysis to avoid any bias because *APOE2* homozygous commonly have dyslipidemia [9]. There was no significant difference in baseline anthropometric values, alcohol consumption or physical activity levels between both groups. Consumption of fish oil was well tolerated by the participants.

Variation of biomarkers between baseline and week 4

As shown in Table 2, there was a genotype × time interaction ($P = 0.021$) for albumin and for creatinine ($P = 0.047$), a biomarker of kidney function. There was an independent

Table 3
Fatty acid profiles in high density lipoproteins (HDL) before (Baseline) and after 4-wk of supplementation with ω-3 polyunsaturated fatty acids in carriers (*E4+*) and non-carriers (*E4−*) of the apolipoprotein E epsilon 4 allele*

	Baseline	Supplementation		*P* values†		
	Week 0	Week 2	Week 4	Interaction	Genotype	Time
16:0						
E4+	21.95 ± 0.41	22.38 ± 0.82	19.09 ± 0.72	**0.004**	(0.005)	(0.024)
E4−	22.56 ± 0.28	22.35 ± 0.25	23.85 ± 0.37			
18:0						
E4+	7.38 ± 0.38	9.15 ± 0.89	7.20 ± 0.67	0.176	0.684	0.481
E4−	8.02 ± 0.30	7.80 ± 0.26	8.63 ± 0.37			
16:1 ω-7						
E4+	1.35 ± 0.10	1.33 ± 0.12	0.99 ± 0.16	0.576	0.147	0.453
E4−	1.78 ± 0.12	1.50 ± 0.08	1.33 ± 0.08			
18:1 ω-9						
E4+	16.00 ± 0.54	15.30 ± 0.49	14.72 ± 0.80	0.840	0.995	0.161
E4−	16.48 ± 0.41	15.02 ± 0.27	14.87 ± 0.31			
18:2 ω-6						
E4+	33.61 ± 0.60	29.83 ± 1.37	35.16 ± 1.25	**0.008**	(0.042)	(0.006)
E4−	31.44 ± 0.47	30.49 ± 0.50	29.90 ± 0.53			
20:4 ω-6						
E4+	10.51 ± 0.49	9.96 ± 0.56	10.20 ± 0.75	0.686	0.192	0.604
E4−	9.85 ± 0.26	9.72 ± 0.21	9.12 ± 0.25			
18:3 ω-3						
E4+	0.74 ± 0.05	0.82 ± 0.15	0.64 ± 0.10	0.546	0.127	0.637
E4−	0.84 ± 0.05	0.94 ± 0.05	0.92 ± 0.05			
20:5 ω-3						
E4+	1.06 ± 0.14	3.18 ± 0.31	4.05 ± 0.42	0.199	0.428	**<0.001**
E4−	1.19 ± 0.09	3.45 ± 0.12	3.62 ± 0.14			
22:6 ω-3						
E4+	2.50 ± 0.18	3.58 ± 0.25	3.77 ± 0.24	0.978	0.112	**<0.001**
E4−	2.83 ± 0.10	4.00 ± 0.08	4.04 ± 0.09			

* Values are presented as mean percentages (%) of total FA ± SEM.
† *P* values were obtained using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY, USA). When assumptions of homogeneity of the covariance matrices were rejected (Mauchly's Test of Sphericity), Greenhouse-Geisser corrections were used. Bold characters indicate significant differences ($P \leq 0.05$). When interactions were significant, *P* values for the independent genotype and time effects are indicated in parenthesis.

genotype ($P = 0.022$) and time effect ($P = 0.021$) on HDL-cholesterol (HDL-C). *E4+* subjects had 20% higher HDL-C level at baseline ($P = 0.015$) when compared to *E4−* subjects. Four weeks after starting the supplement, total-C/HDL-C ratio was significantly reduced in both groups ($P = 0.029$) without any genotype effect. Total-C tended to be higher in *E4+* subjects independently of time ($P = 0.057$).

Fatty acid profile in HDL according to APOE-genotype

At baseline, there was no significant difference in the lipid profile of HDL according to genotype (Table 3). There were significant genotype × time interactions for 16:0 and 18:2 ω-6 in HDL. There was an independent time effect on EPA and DHA. Levels of EPA and DHA reached a plateau within 2 wk of supplementation with ω-3 PUFA. At week 4, the ω-6/ω-3 PUFA ratio tended ($P = 0.062$) to be higher in *E4+* subjects compared to *E4−* subjects (Fig. 1A). Baseline DHA in HDL was positively correlated to baseline HDL-C ($r = 0.400$, $P < 0.001$) and week 4 DHA in HDL to week 4 HDL-C ($r = 0.206$, $P = 0.046$) in *E4−* subjects only.

Fatty acid profile in LDL according to APOE-genotype

Baseline 16:1 ω-7 and DHA were 29% and 19% lower, respectively, in the LDL of *E4+* compared to *E4−* subjects (Table 4). At baseline, the ω-6/ω-3 PUFA ratio was 17% higher in the LDL of *E4+* than *E4−* subjects (Fig. 1B). There was a genotype effect for 16:1 ω-7 and ALA. There was a time effect for 18:1 ω-9, EPA ($P < 0.001$) and DHA ($P < 0.001$) (Table 4). At week 4, the ω-6/ω-3 PUFA ratio was 31% higher in the LDL of *E4+* compared to *E4−* subjects (Fig. 1B). There was no statistically significant correlation between EPA or DHA in LDL and LDL-C, nor at baseline or at week 4.

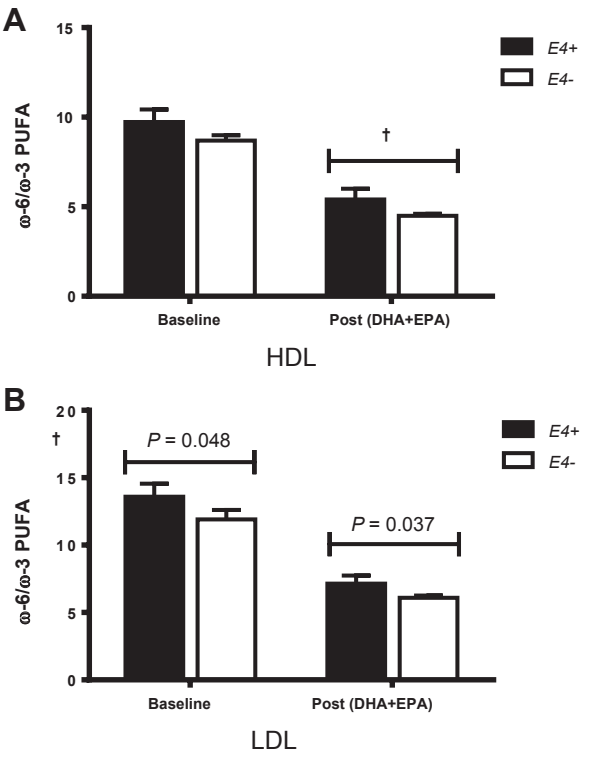

Fig. 1. Omega-6 to omega-3 fatty acid ratio (ω-6/ω-3 PUFA) in (A) HDL and (B) LDL of participants carrying the apolipoprotein E epsilon 4 allele (*E4+*, ■, N = 10) or non-carriers (*E4−*; □, N = 70) before (baseline) and after 4 wk of supplementation with 680 mg/d of docosahexaenoic acid + 900 mg/d of eicosapentaenoic acid. Data are expressed as means ± SEM. *P* values for the independent genotype effect were obtained using a non-parametric Kruskal-Wallis analysis of variance. *P* values ≤0.05 were considered significant. † Trend effect for genotype was set at $P < 0.08$.

Discussion

Contrary to what we anticipated, ω-3 PUFA in the HDL and the LDL were not *APOE* isoform-dependant in young participants, before and after ω-3 PUFA supplementation. ω-3 PUFAs were similarly incorporated into plasma LDL and HDL of *E4+* and *E4−* subjects. However, there was a genotype × time interaction for 16:0 and 18:2 ω-6 in the HDL. The ω-6/ω-3 PUFA ratio in the LDL faction of E4+ subjects was significantly higher than E4− subjects both at baseline and at 4 wk. Thus, the *APOE* isoform-dependant distribution of FA into HDL and LDL particles was more subtle than we anticipated. The absence of genotype × time interactions may be related to the young age of our participants (25–35 y old) compared to prior studies [12,13,20]. Calvalho-Wells et al. [12] showed that disturbance in cholesterol and TG metabolism was only in E4+ subjects aged over 50 y old. Similarly, ω-3 PUFA metabolism seems to be age-dependant [21] and DHA kinetics is modified by age [22].

In this study, there was a higher ω-6/ω-3 PUFA ratio ($P = 0.048$) in the LDL of *E4+* compared to E4− subjects, which arises from a tendency toward lower baseline-DHA, without significant changes in ω-6 PUFA. There are two potential reasons explaining this result: 1) lower dietary intake of ω-3 PUFA in the *E4+* group, or 2) imbalance in ω-3 PUFA metabolism as supported by our previous studies [7,8]. Unfortunately, neither food frequency questionnaires or 3-D dietary intake recall were administered to participants, and thus we cannot discard the possibility that *E4+* subjects may have consumed a diet lower in ω-3 PUFA. The importance of the ω-6/ω-3 PUFA ratio is currently a source of debate in determining the risk of CVD [23,24]. Harris et al. [23] concluded that the ω-6/ω-3 PUFA ratio may be a poor biomarker of the risk of CVD when compared to the ω-3 PUFA profile alone. However, Simopoulos [25] argues that this ratio is an important factor to consider in primary and secondary prevention of CVD. The higher ω-6/ω-3 ratio in the LDL of *E4+* subjects is therefore a potential contributor to higher CVD risk as reported in the Literature [4,11,26], but this needs to be investigated in another trial.

Previous studies reported that the modulation of cholesterol metabolism is *APOE* isoform-dependant [12,13,20]. In this study, TG and LDL-C concentrations at baseline and at week 4 (Table 2) were both independent of genotype, probably because of the younger age of the participants compared to other studies [12,13,20]. Contrary to previous studies [27–30], the *E4+* subjects of this study had higher levels of HDL-C at baseline and 4 wk after receiving the ω-3 PUFA supplement compared to *E4−* carriers (Table 2). This is potentially related to cultural differences between French Canadians and the European populations used in previous published studies (i.e., Lithuanian [27], UK [28], and Finnish [29]), but this idea is only speculative and needs further investigation.

We also reported a positive correlation between DHA in the HDL and HDL-C level, before and after the supplementation, in *E4−* subjects only. This result is somewhat in line with Liang et al. [30], showing that the *APOE*-allele modifies the association between plasma phospholipid DHA and medium size HDL. ω-3 PUFA supplementation seems to increase hepatic uptake of HDL-C in mice [31] and increase reverse cholesterol transport [32], whereas homozygous mice with the *E4+* allele are less

Table 4
Fatty acid profiles in low density lipoproteins (LDL) before (Baseline) and after 4 wk of supplementation with ω-3 polyunsaturated fatty acids in carriers (*E4*+) and non-carriers (*E4*−) of the apolipoprotein E epsilon 4 allele*

	Baseline	Supplementation		*P* values†		
	Week 0	Week 2	Week 4	Interaction	Genotype	Time
16:0						
E4+	18.95 ± 0.56	19.26 ± 0.86	17.15 ± 1.16	0.351	0.238	0.449
E4−	19.92 ± 0.33	19.20 ± 0.28	19.70 ± 0.31			
18:0						
E4+	5.75 ± 0.68	5.82 ± 0.78	6.24 ± 1.16	0.686	0.264	0.831
E4−	5.68 ± 0.30	5.00 ± 0.19	5.16 ± 0.22			
16:1 ω-7						
E4+	1.67 ± 0.23	1.66 ± 0.14	1.25 ± 0.12	0.523	**0.025**	0.137
E4−	2.35 ± 0.11	2.18 ± 0.10	2.13 ± 0.10			
18:1 ω-9						
E4+	21.98 ± 0.98	20.10 ± 1.11	19.73 ± 0.92	0.762	0.706	**0.005**
E4−	21.54 ± 0.40	19.80 ± 0.33	20.31 ± 0.33			
18:2 ω-6						
E4+	37.27 ± 1.24	36.22 ± 1.12	38.21 ± 1.48	0.888	0.385	0.763
E4−	35.45 ± 0.65	35.58 ± 0.57	35.14 ± 0.62			
20:4 ω-6						
E4+	7.08 ± 0.45	7.59 ± 0.52	7.32 ± 0.53	0.864	0.289	0.104
E4−	6.81 ± 0.21	7.08 ± 0.17	6.57 ± 0.16			
18:3 ω-3						
E4+	0.92 ± 0.08	0.88 ± 0.11	0.95 ± 0.07	0.967	**0.008**	0.649
E4−	1.12 ± 0.04	1.20 ± 0.05	1.11 ± 0.05			
20:5 ω-3						
E4+	0.84 ± 0.13	2.45 ± 0.29	2.95 ± 0.40	0.529	0.349	**<0.001**
E4−	0.93 ± 0.07	2.85 ± 0.11	2.83 ± 0.10			
22:6 ω-3						
E4+	1.38 ± 0.12	2.29 ± 0.14	2.45 ± 0.18	0.435	**0.034**	**<0.001**
E4−	1.70 ± 0.07	2.89 ± 0.10	2.75 ± 0.08			

* Values are presented as mean relative percentages ± SEM.
† *P* values were obtained using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY, USA). When assumptions of homogeneity of the covariance matrixes were rejected (Mauchly's Test of Sphericity), Greenhouse-Geisser corrections were used. Bold characters are used to indicate significant effects ($P \leq 0.05$). When interaction terms were found, significant *P* values for the independent terms (i.e. genotype and time) are indicated in parenthesis.

efficient at transferring apoA-I from VLDL to HDL, resulting in less HDL particles than *APOE3* mice [20]. Moreover, the enriched-apoE VLDL particles associated with *E4*+ carriers are known to reduce lipase activity and thus diminish HDL synthesis [26]. Therefore, this association suggests that DHA may upregulate HDL production, explaining why *E4*– subjects have higher levels of HDL-C [27–30]. However, contrary to *E4*– subjects, there is no association between baseline-DHA in the HDL and HDL-C level in *E4*+ carriers. This result also supports the idea of a disrupted lipid metabolism in *E4*+ carriers, but the exact mechanism needs to be clarified in future investigations. As emphasized by Liang et al. [30], the association between EPA or DHA and total cholesterol, LDL-C, and HDL-C is erratic and highly variable between clinical trials and thus, carefulness is needed while interpreting results for such investigational trials.

The lower levels of 16:0 and 16:1 ω-7 in HDL and LDL of *E4*+ compared to *E4*− subjects is an example of *APOE* isoform-dependant modification of FA distribution in plasma lipoproteins. As suggested in previous studies [7,33], this modification may result from a modulation of substrate preference (i.e., FA) undergoing β-oxidation in *E4*+ carriers. Long-chain FAs are preferential substrates for β-oxidation [34] and the FA oxidation rate is known to vary according to FA chain-length as well as saturation level. In humans [35], the FA rate of β-oxidation can generally be predicted as follows: lauric acid (12:0) > myristic acid (14:0) > ALA (18:3 ω-3) > LA (18:2 ω-6) > OA (18:1 ω-9) > PA (16:0) > SA (18:0). Previously, β-oxidation of ^{13}C-DHA was found to be higher in *E4*+ subjects over a 28 d follow-up, supporting a shift in FA substrate selection in E4+ subjects. Indeed, DHA is usually highly preserved as carnitine palmitoyltransferase 1 (CPT1), the limiting enzyme of mitochondrial β-oxidation [36], possesses a greater affinity for EPA, ALA, and palmitate [37]. Using an *APOE*-targeted replacement mice, Conway et al. [33] recently reported a higher concentration of hepatic CPT1 in *E4*+ animals compared to control. Therefore, studies investigating the FA rate of β-oxidation according to *APOE*-alleles should be undertaken in humans.

Among the strengths and weaknesses of the study are the following, therefore excluding bias caused by prescribed medication, such as statins, that are commonly taken by older *E4*+ individuals for modifying lipoprotein metabolism [7,12]. Moreover, another strength is the low body mass index of participants (mean BMI <25 kg/m^2), therefore limiting potential confounding effects between BMI and DHA kinetics [19]. There was an important intraindividual variation in FA distribution into plasma lipoproteins in *E4*+ participants, and this is potentially because the *E4*+ group included two *APOE4* genotypes, namely ε4/ε3 (N = 6) and ε4/ε2 (N = 4). Indeed, a previous study reported that FA metabolism may be different between *APOE4* genotypes (ε2/ε4, ε3/ε4 and ε4/ε4) [12]. Because of the small sample size of the E4+ group (N = 10), it was not statistically possible to stratify our data according to these two *APOE4* genotypes.

Conclusions

In conclusion, baseline level of ω-3 PUFA, as well as ω-3 PUFA level at 4-wk after supplementation, were similar in HDL and LDL fractions of *E4*+ and *E4*− participants. This result suggests that disrupted DHA metabolism in *E4*+ subjects is age-dependant. Therefore, there is room to identify prevention strategies to prevent dysregulation of DHA homeostasis likely occurring in older E4+ individuals.

Acknowledgments

We are grateful to the nurses of the Research Center on Aging for their technical assistance and the expert care provided to the participants. This study was supported by the Centrum Foundation and CIHR (MOP119454). VC is supported by a FRQ-S postdoctoral fellowship and the department of medicine Fellowship from Université de Sherbrooke. M.P. is supported by a Junior 1 FRQ-S salary award.

The authors' responsibilities were as follows: M.P. designed and obtained funding for this study; T.M.D. recruited participants, was in charge of the clinical study, and performed the fatty acid profile of the HDL and LDL; V.C. was responsible for data analysis and interpretation of data. T.M.D and V.C. wrote the manuscript. T.M.D. and V.C. are responsible for data accuracy. All authors report no conflicts of interest.

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