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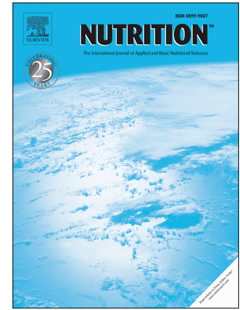

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DISRUPTED FATTY ACID DISTRIBUTION IN HDL AND LDL ACCORDING TO APOLIPOPROTEIN E ALLELE

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Running title: *APOE* isoform-dependent metabolism of omega-3 fatty acids

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ABSTRACT

BACKGROUND: Omega-3 polyunsaturated fatty acid (*n*−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 *n*−3 PUFA distribution in the high and low density lipoproteins is *APOE*-genotype dependant before and after supplementation with *n*−3 PUFA.

SUBJECTS/METHODS: Eighty participants, aged between 20 to 35 years old were recruited and supplemented with 900 mg of eicosapentaenoic acid + 680 mg of docosahexaenoic acid for 4-weeks. Over the 4-wk intervention, blood samples were collected and HDL and LDL particles were obtaind using sucrose gradient ultracentifugation. Fatty acid profiles of the HDL and LDL fractions were performed by gas chromatography.

RESULTS: Baseline anthropometric characteristics of participants was not significantly different between the two *APOE*-groups (*E4*+, N = 10; *E4*−, N = 70). At baseline, in the LDL of *E4*+, *n*−6/*n*−3 PUFA ratio was 17% higher than *E4*−. At week-4, *n*−6/*n*−3 PUFA ratio was significantly higher in LDL of *E4*+ than *E4*−. There was a significant genotype × time interaction for 16:0 in HDL and LDL and for 18:2 *n*−6 in HDL. DHA in the HDL was positively correlated to HDL-C levels pre- and post-supplementation in *E4*− only.

CONCLUSIONS: Contrary to what we anticipated, *n*−3 PUFA in HDL and LDL was not *APOE* isoform-dependant in young participants. However, young *E4*+ had already a tendency towards lower baseline-DHA levels in LDL particles as well as a more atherogenic *n*−6/*n*−3 PUFA ratio in LDL pre and post-supplementation.

KEYWORDS: Omega-3 fatty acid; DHA, EPA, Plasma lipoproteins, Omega-6/Omega-3 fatty acid ratio, DHA metabolism, APOE isoforms.

ABBREVIATIONS:

AD, Alzheimer's disease; ALT, alanine transferase; APOE, apolipoprotein E; AST, aspartate transaminase; CVD, cardiovascular diseases; DHA, docosahexaenoic acid; *E4+*, carriers of APOE ε4 allele; *E4−*, non-carriers of APOE ε4 allele; EPA, eicosapentaenoic acid; LDL-R, low density lipoprotein receptor family; $n-3$ PUFA, omega 3 fatty acid; $n-6$ PUFA, omega-6 fatty acid; TC, total cholesterol; TG, triglyceride; TSH, thyroid stimulating hormone.

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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 factor of 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 through consumption of omega-3 fatty acids (*n*−3 PUFA) from fatty fish [2]. A low balance of *n*−6/*n*−3 PUFA seems to be contributing in decreasing the risk of inflammatory-related diseases and may promote heart and brain health [3]. However, *E4+* do not seem to be protected against CVD [4] and cognitive decline [5, 6] when consuming *n*−3 PUFA. Recent evidences suggest that this lack of protection could be related to dysfunction of *n*−3 PUFA metabolism and kinetics [7, 8].

In human, 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 triglyceride 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 triglycerides (TG), total cholesterol, and small and dense LDL than *E4−* [10, 12]. Because apolipoprotein E (apoE) plays important roles in the regulation, transport and clearance of fatty acids, carrying apoE4 isoform may modulate the efficiency of apoE in accomplishing its essential role in lipoprotein metabolism. Moreover, the lack of protection against CHD

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and AD when *E4+* are supplemented with *n*−3 PUFA may potentially be explain by a disrupted postprandial kinetics of dososahexaenoic acid (DHA; 22:6 *n*−3), an *n*−3 PUFA [7]. Other studies reported gene-by-diet interaction in the uptake of *n*−3 PUFA and cholesterol metabolism in *E4+* supplemented with 3 g/d of *n*−3 PUFA [4, 12, 13]. Because *E4+* supplemented with *n*−3 PUFA have greater sensitivity of fasting triglycerides [14] and increased total cholesterol [4, 13] when compared to *E4−* subjects, we speculate that *n*−3 PUFA distribution in plasma lipoproteins is *APOE* isoform-dependant. We also hypothesize that *n*−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/day of DHA + 900 mg/d of eicosapentaenoic acid (EPA; 20:5 *n*−3) over a 4-weeks intervention.

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MATERIAL AND METHODS

Subjects and study design

Eighty-two healthy men and women (N = 82) aged between 20−35 years 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 breastfeeding, or were already supplemented with *n*−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 in 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 weeks (Ocean Nutrition, Dartmouth, NS, Canada). This dose corresponds to three times the current *n*−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 weeks, and a fasted blood sample was collected. Plasma was separated from red and white blood cells by centrifugation (3500 × *g* during 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% of sucrose in plasma. The sucrose gradient was created by successive layers, from top to bottom: 500

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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 during 26 h using a Beckman Optima TLX ultracentrifuge equipped with a TLS-55 rotor (Beckman Coulter, Brea, California, United State). The following fractions were pooled together: 700 μL of LDL (fraction 3−9, ρ=1.04–1.07 g/mL) and 600 μL of HDL fractions (fraction 10−15, ρ=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

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 1M 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, Missouri, USA) and were heated at 90°C during 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 68A, NuChek 411, and NuChek 455; NuChek Prep, Inc., Elysian, MN, USA and a custom mixture of saturated FA standards).

APOE genotype analysis

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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 (PCR-RFLP). 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, post-stained with gel red, and visualised under UV-light (Image analyser 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−* (0.53% ± 0.31%) as reported in Plourde *et al* [8]. This metric was used since no data are currently available in literature 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 pre-screening for *E4+* genotype; 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 that ten (N = 10) *E4+* were needed. Therefore, based on the lowest frequency of *APOE* epsilon 4 allele in Canadians (15%), the number of participants to be recruited was sixty seven (N = 67), but with an anticipated dropout of 20% [19], eighty 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 using a Factorial Repeated Measures (Split-Plot) ANOVA in

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SPSS version 22.0 (IBM Corp., Armonk, NY). 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 Kruskal-Wallis non-parametric analysis of variance. Univariate spearman correlation analysis was used to investigate associations among outcome. The balance of $n-6/n-3$ PUFA was calculated using the sum of the following FA: the sum linoleic acid (LA), di-homo-gamma linolenic acid (DGLA), and arachidonic acid (AA) over the sum of alpha-linolenic acid (ALA), EPA, docosapentaenoic acid (DPA), and DHA. P values ≤ 0.05 were considered statistically significant, and P value for trends was set as ≤ 0.08. FA profiles are presented as means percentage (%) of total FA ± SEM and as percentage (%) compared to control, meaning *E4−* subjects, using the following equation:

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

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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 since *APOE2* homozygous commonly have dyslipidemia [9]. There was no significant difference in baseline anthropometrics 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 genotype ($P = 0.022$) and time effect ($P = 0.021$) on HDL-cholesterol (HDL-C). *E4+* had 20% higher HDL-C level at baseline ($P = 0.015$) when compared to *E4−*. 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+* 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

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16:0 and 18:2 $n-6$ in HDL. There was an independent time effects on EPA and DHA. Levels of EPA and DHA reached a plateau within 2 weeks of supplementation with $n-3$ PUFA. At week-4, the $n-6/n-3$ PUFA ratio tended (P = 0.062) to be higher in *E4+* subjects compared to *E4−* (Figure 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 $n-7$ and DHA were 29% and 19% lower, respectively in the LDL of *E4+* compared to *E4−* (Table 4. At baseline, the $n-6/n-3$ PUFA ratio was 17% higher in the LDL of *E4+* than *E4−* (Figure 1B). There was a genotype effect for 16:1 $n-7$ and ALA. There was a time effect for 18:1 $n-9$, EPA (P < 0.001) and DHA (P < 0.001) (Table 4). At week-4, $n-6/n-3$ PUFA ratio was 31% higher in the LDL of *E4+* compared to *E4−* (Figure 1B). There was no statistically significant correlation between EPA or DHA in LDL and LDL-C, nor at baseline or at week-4.

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DISCUSSION

Contrary to what we anticipated, *n*−3 PUFA in the HDL and the LDL were not *APOE* isoform-dependant in young participants, before and after *n*−3 PUFA supplementation. *N*−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 *n*−6 in the HDL. *N*−6/*n*−3 PUFA ratio in the LDL faction of *E4*+ was significantly higher than *E4*−, both at baseline and at 4-weeks. Thus, the *APOE* isoform-dependant distribution of FA into HDL and LDL particles was more subtle than we anticipated. Absence of genotype × time interactions may be related to the young age of our participants (25–35 years 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*+ aged over 50 years old. Similarly, *n*−3 PUFA metabolism seems to be age-dependant [21] and DHA kinetics is modified by age [22].

In this study, there was higher *n*−6/*n*−3 PUFA ratio (P = 0.048) in the LDL of *E4*+ compared to *E4*− which arises from a tendency towards lower baseline-DHA, without significant changes in *n*−6 PUFA. There are two potential reasons explaining this result: 1) lower dietary intake of *n*−3 PUFA in the *E4*+ group or 2) imbalance in *n*−3 PUFA metabolism as supported by our previous studies [7, 8]. Unfortunately, nor food frequency questionnaires nor 3-d dietary intake recall were administered to participant, thus we can't discard that *E4*+ subjects may have taken a diet lower in *n*−3 PUFA. The importance of the *n*−6/*n*−3 PUFA ratio is currently a source of debate in determining the risk of CVD [23, 24]. Harris *et al* [23] concluded that the *n*−6/*n*−3 PUFA ratio may be a poor biomarker of the risk of CVD compare to the *n*−3 PUFA profile alone. However,

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Simopoulos [25] argue that this ratio is an important factor to consider in primary and secondary prevention of CVD. The higher $n-6/n-3$ ratio in the LDL of *E4+* is therefore potentially contributing to higher CVD risk as reported in literature [4, 11, 26], but this needs to be investigated in another trial.

Previous studies reported that 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 young age of the participants compared to other studies [12, 13, 20]. Contrary to previous studies [27-30], the *E4+* of this study had higher levels of HDL-C at baseline and 4 weeks after receiving the $n-3$ PUFA supplement compared to *E4−* carriers (Table 2). This is potentially related to cultural differences between French Canadians and Europeans populations used in previous published studies (i.e. Lithuanian [27], UK [28] and Finnish [29]), but this thesis is only speculative and need further investigation.

We also reported a positive correlation between DHA in the HDL and HDL-C level, prior and after the supplementation, in *E4−* subjects only. This result is somewhat in line with Liang *et al* [30], showing that *APOE*-allele modifies association between plasma phospholipid DHA and medium size HDL. *N*−3 PUFA supplementation seems to increase hepatic uptake of HDL-C in mice [31] and increase reverse cholesterol transport [32], whereas homozygous mice for *E4+* allele are less 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 to *E4+* carriers are known to reduce lipase activity and thus diminish HDL synthesis [26]. Therefore, this association support that DHA may upregulate HDL production, explaining why *E4−* have higher levels of HDL-

C [27-30]. However, contrary to *E4−* , there is no association between baseline-DHA in the HDL and HDL-C level in *E4+* carriers. This result also supports the thesis 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 with 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 investigation trials.

The lower levels of 16:0 and 16:1 *n−7* in HDL and LDL of *E4+* compared to *E4−* 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 FA oxidation rate is known to vary according to FA chain-length as well as saturation level. In human [35], FA rate of *β*-oxidation can generally be predicted as follows: lauric acid (12:0) > myristic acid (14:0) > ALA (18:3 *n*−3) > LA (18:2 *n*−6) > OA (18:1 *n*−9) > PA (16:0) > SA (18:0). Previously, *β*-oxidation of ^{13}C-DHA was found to be higher in *E4+* over a 28 days follow-up, supporting a shift in FA substrate selection in *E4+*. Indeed,DHA is usually highly preserved as carnitine palmitoyl-transferase 1 (CPT1), the limiting enzyme of mitochondrial *β*-oxidation [36], possess a greater affinity for EPA, ALA and palmitate [37]. Using *APOE*-targeted replacement mice, Conway *et al* [33] recently reported higher concentration of hepatic CPT1 in *E4+* animals compared to control. Therefore, investigating FA rate of *β*-oxidation according to *APOE*-alleles should be undertaken in humans.

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This trial has strengths and limitations. The participants of this study were young age, therefore excluding bias induce by prescribed medication such as statins that are commonly taken in older *E4+* individual for modifying lipoprotein metabolism [7, 12]. Moreover, another strength is the low body mass index of participants (mean BMI <25 kg/m²), therefore limiting potential confounding effect between BMI and DHA kinetics [19]. There was an important intra-individual 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 *E4+* group (N = 10), it was not statistically possible to stratify our data according to these two *APOE4* genotypes.

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CONCLUSIONS

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

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The authors' responsibilities were as follows – M.P. have 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 data accuracy. All authors report non conflict of interest.

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FIGURE LEGEND

Figure 1. Omega-6 to omega-3 fatty acid ratio (*n*−6/*n*−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 weeks of supplementation with 680 mg/day of docosahexaenoic acid + 900 mg/day 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 $P < 0.08$.

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TABLES

TABLE 1

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

Characteristics [a]	***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 (%) [b]	10	23	0.302
Alcohol Consumption (%)			0.752
Never	10	10	
2−3/month	20	26	
1/wk	20	31	
2−3/wk	50	29	
Almost everyday	0	4	
Physical activity (%)			0.370
Never	0	2	
2−3/ month	20	7	
1/wk	0	19	
2−3/wk	40	46	
Almost everyday	40	26	

[a] Values are presented as mean ± SD.

[b] Percentage (%) of subjects taking natural products in each group. Natural product included: Homeopathic Products, Vitamin B6, C and D, Protein, Aloes, Probiotics, Multi-vitamins, Multi-minerals, Orange Triads, Echinacea, Collagen, Creatinin Phosphate, Oregano Oil, Branched Chain Amino Acids, Hypericum. Supplementation with *n*−3 PUFA was not permitted.

TABLE 2

Blood biochemistry values before (Baseline) and after 4-weeks of supplementation with *n*−3 polyunsaturated fatty acids in carriers (*E4+*) and non-carriers (*E4−*) of the apolipoprotein E epsilon 4 allele [a]

	Baseline		**Week 4**		***P* values** [b]		
	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, triglyceride.

[a] Values are presented as mean relative percentages ± SEM.

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[b] P values were obtained using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY). 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.

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TABLE 3

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

		Baseline	Supplementation		*P* values [b]		
		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 *n*−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 *n*−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 *n*−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 *n*−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 *n*−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 *n*−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 *n*−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			

[a] Values are presented as mean percentages (%) of total FA ± SEM.

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[b] P values were obtained using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY). When assumptions of homogeneity of the covariance matrixes 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.

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TABLE 4

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

		Baseline	**Supplementation**		***P* values** [b]		
		Week-0	Week-2	Week-4	*Interaction*	*Genotype*	*Time*
16:0	*E4+*	18.95 ± 0.56	19.26 ± 0.86	17.15 ± 1.16			
	E4−	19.92 ± 0.33	19.20 ± 0.28	19.70 ± 0.31	0.351	0.238	0.449
18:0	*E4+*	5.75 ± 0.68	5.82 ± 0.78	6.24 ± 1.16			
	E4−	5.68 ± 0.30	5.00 ± 0.19	5.16 ± 0.22	0.686	0.264	0.831
16:1 n−7	*E4+*	1.67 ± 0.23	1.66 ± 0.14	1.25 ± 0.12			
	E4−	2.35 ± 0.11	2.18 ± 0.10	2.13 ± 0.10	0.523	**0.025**	0.137
18:1 n−9	*E4+*	21.98 ± 0.98	20.10 ± 1.11	19.73 ± 0.92			
	E4−	21.54 ± 0.40	19.80 ± 0.33	20.31 ± 0.33	0.762	0.706	**0.005**
18:2 n−6	*E4+*	37.27 ± 1.24	36.22 ± 1.12	38.21 ± 1.48			
	E4−	35.45 ± 0.65	35.58 ± 0.57	35.14 ± 0.62	0.888	0.385	0.763
20:4 n−6	*E4+*	7.08 ± 0.45	7.59 ± 0.52	7.32 ± 0.53			
	E4−	6.81 ± 0.21	7.08 ± 0.17	6.57 ± 0.16	0.864	0.289	0.104
18:3 n−3	*E4+*	0.92 ± 0.08	0.83 ± 0.11	0.95 ± 0.07			
	E4−	1.12 ± 0.04	1.20 ± 0.05	1.11 ± 0.05	0.967	**0.008**	0.649
20:5 n−3	*E4+*	0.84 ± 0.13	2.45 ± 0.29	2.95 ± 0.40			
	E4−	0.93 ± 0.07	2.85 ± 0.11	2.83 ± 0.10	0.529	0.349	**<0.001**
22:6 n−3	*E4+*	1.38 ± 0.12	2.29 ± 0.14	2.45 ± 0.18			
	E4−	1.70 ± 0.07	2.89 ± 0.10	2.75 ± 0.08	0.435	**0.034**	**<0.001**

[a] Values are presented as mean relative percentages ± SEM.

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[b] P values were obtained using a Factorial Repeated Measures (Split-Plot) ANOVA in SPSS version 22.0 (IBM Corp., Armonk, NY). 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.

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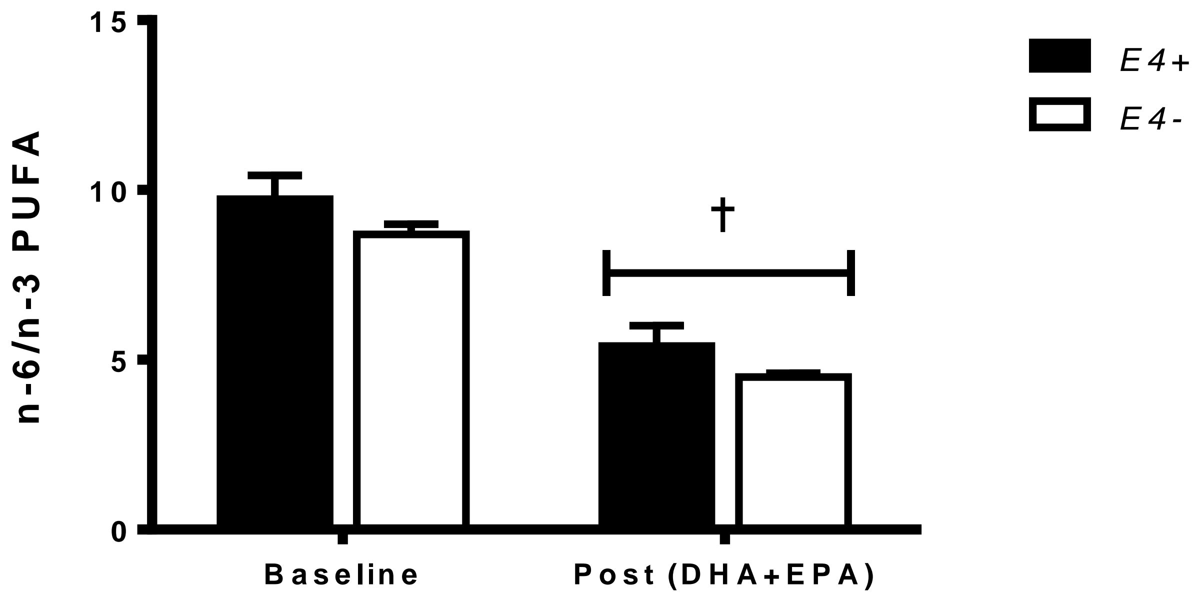

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5
0
n-6/n-3 PUFA
E4+
E4-
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Baseline
Post (DHA+EPA)
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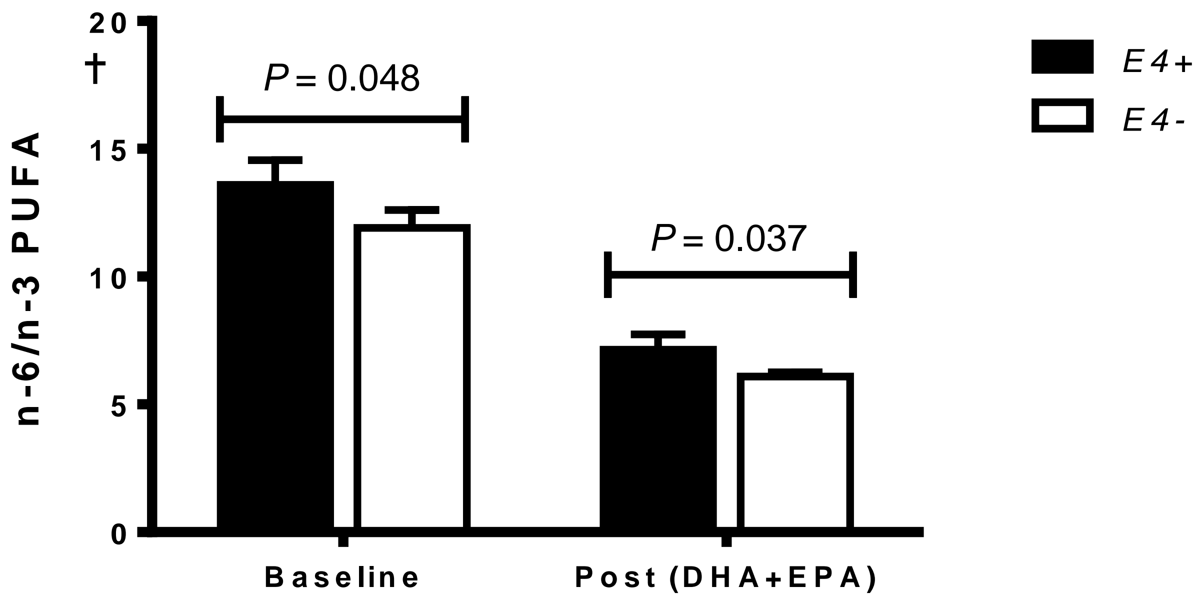
HDL



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P = 0.037
E4+
E4-
Baseline
Post (DHA+EPA)
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