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ORIGINAL ARTICLE

n-3 PUFA Esterified to Glycerol or as Ethyl Esters Reduce Non-Fasting Plasma Triacylglycerol in Subjects with Hypertriglyceridemia: A Randomized Trial

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Abstract To date, treatment of hypertriglyceridemia with long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) has been investigated solely in fasting and postprandial subjects. However, non-fasting triacylglycerols are more strongly associated with risk of cardiovascular disease. The objective of this study was to investigate the effect of long-chain n-3 PUFA on non-fasting triacylglycerol levels and to compare the effects of n-3 PUFA formulated as acylglycerol (AG-PUFA) or ethyl esters (EE-PUFA). The study was a double-blinded randomized placebo-controlled interventional trial, and included 120 subjects with non-fasting plasma triacylglycerol levels of 1.7–5.65 mmol/L (150–500 mg/dL). The participants received approximately 3 g/day of AG-PUFA, EE-PUFA, or placebo for a period of eight weeks. The levels of non-fasting plasma triacylglycerols decreased 28 % in the AG-PUFA group and 22 % in the EE-PUFA group ($P < 0.001$ vs. placebo), with no significant difference between the two groups. The triacylglycerol lowering effect was evident after four weeks, and was inversely correlated with the omega-3 index (EPA + DHA content in erythrocyte membranes). The omega-3 index increased 63.2 % in the AG-PUFA group and 58.5 % in the EE-PUFA group ($P < 0.001$). Overall, the heart rate in the AG-PUFA group decreased by three beats per minute ($P = 0.045$). High-density lipoprotein (HDL) cholesterol increased in the AG-PUFA group ($P < 0.001$). Neither total nor non-HDL cholesterol changed in any group. Lipoprotein-associated phospholipase A2 (LpPLA2) decreased in the EE-PUFA group ($P = 0.001$). No serious adverse events were observed. Supplementation with long-chain n-3 PUFA lowered non-fasting triacylglycerol levels, suggestive of a reduction in cardiovascular risk. Regardless of the different effects on heart rate, HDL, and LpPLA2 that were observed, compared to placebo, AG-PUFA, and EE-PUFA are equally effective in reducing non-fasting triacylglycerol levels.

Keywords Non-fasting · Triacylglycerols · n-3 Polyunsaturated fatty acid · Omega-3 ethyl ester · Omega-3 acylglycerol · Docosahexaenoic acids · Eicosapentaenoic acid · Lipoprotein-associated phospholipase A2 · Heart rate

Abbreviation

AE	Adverse events
AG	Acylglycerol
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
BMI	Body mass index
DHA	Docosahexaenoic acid
EE	Ethyl ester
EPA	Eicosapentaenoic acid
FA	Fatty acids
HDL-C	High-density lipoprotein cholesterol
HgbA1c	Glycated hemoglobin A1
ITT	Intention-to-treat
LDL-C	Low-density lipoprotein cholesterol
LDL-P	LDL particle number
LpPLA2	Lipoprotein-associated phospholipase A2
n-3 PUFA	Omega-3 polyunsaturated fatty acids
PP	Per protocol
PUFA	Polyunsaturated fatty acids
QTc	Corrected QT interval
VLDL-C	Very-low-density lipoprotein cholesterol

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Introduction

For decades, a high level of plasma cholesterol has been considered a major risk factor for cardiovascular disease [1, 2], and treatment has focused on lowering total cholesterol and low-density lipoprotein cholesterol (LDL-C). However, several observational studies and a meta-analysis [3, 4] have indicated that high plasma triacylglycerols may be an independent atherogenic risk factor. All interventional studies to date have been based upon fasting or postprandial triacylglycerols, yet non-fasting triacylglycerols are more strongly associated with cardiovascular disease than fasting triacylglycerols [5, 6], as was substantiated in a Mendelian randomization study [7]. Treatments for hypertriglyceridemia are limited to lifestyle changes, niacin, fibrates, long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), and, to a lesser degree, statins. Niacin, fibrates, and statins have a number of side effects, whereas n-3 PUFA are generally well-tolerated [8, 9]. The beneficial effects of n-3 PUFA were first suggested in the 1970s by Bang and Dyerberg [10–12], who observed that natives of Greenland had low triacylglycerols, low incidence of cardiovascular mortality, and high dietary marine long-chain n-3 PUFA. Several interventional trials have documented that long-chain n-3 PUFA have a significant lowering effect on fasting triacylglycerol levels [13–17]. Consequently, dietary supplements with marine long-chain n-3 PUFA have been used in treating hypertriglyceridemia. Additionally, consumption of n-3 PUFA has been found to have beneficial effects on heart rate and blood pressure, as well as inflammation [15, 17–19], although some of these effects have been challenged [20]. Acylglycerol and ethyl ester formulations are the two most available types of marine n-3 PUFA. Acylglycerol n-3 PUFA (AG- PUFA) is the typical formulation for "over-the-counter" dietary supplementation containing the long-chain n-3 PUFA eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). In the U.S. and Europe, an ethyl ester n-3 PUFA formulation (EE-PUFA) also containing EPA and DHA (Lovaza®/Omacor®) has been approved for prescription for some years for the treatment of severe hypertriglyceridemia. An ethyl ester formulation (Vascepa®) containing only EPA, as well as a formulation (Epanova®) containing DHA and EPA as free fatty acids, has been approved in the U.S.

Nonetheless, the effect of any n-3 PUFA formulation on non-fasting triacylglycerols remains to be seen. The present study investigates the effect of long-chain n-3 PUFA on plasma triacylglycerols in non-fasting subjects with moderate hypertriglyceridemia, comparing the effect of acylglycerol (Lipomar®) and ethyl ester (Lovaza®/Omacor®) formulations.

Subjects and Methods

Subjects

Individuals aged 18 years and older of both genders were identified and recruited from the department's clinical database and laboratory information system based on a history of fasting triacylglycerol levels of >1.7 mmol/L. All data were registered in electronic case report forms (OpenClinica Community Edition version 3.1.3; OpenClinica, LLC, Waltham, MA, USA). The medical histories and previous laboratory results of 1,460 individuals were evaluated. Of these, 660 were considered eligible to receive an invitational letter to join the study. Some 261 replied, and 198 were invited to an interview, including clinical and laboratory examination. Of these, 120 had non-fasting plasma triacylglycerols of 1.7–5.65 mmol/L (150–500 mg/dL) and were enrolled, provided they were willing to terminate any fish oil treatment at least four weeks prior to randomization. Furthermore, participants were required to limit fish intake to a maximum of two meals a week and to maintain weight and physical activity levels throughout the study. Enrolment was performed by investigators AH and PBS.

Exclusion criteria were as follows: history of pancreatitis; gallstone disease, unless treated with cholecystectomy; cancer treatment within the past two years; uncontrolled diabetes (glycosylated hemoglobin [HgbA1c] ≥75 mmol/mol [≥9 %]); hypertension (≥160 mmHg systolic and/or ≥100 mmHg diastolic); hypothyroidism (thyroid-stimulating hormone [TSH] ≥5 mlU/L); nephrotic syndrome; cardiovascular, pulmonary, liver, gastrointestinal, or immunological diseases; pregnancy; use of systemic steroids, fibrates, or niacin; or simultaneous participation in another clinical study. Informed written consent was obtained from all participants. The study was approved by the Ethical Committee of Copenhagen (H1-2012-071), by the European Union Drug Regulating Authorities Clinical Trials register (EudraCT number 2012-003029-11), and by The Danish Health and Medicines Authority with Protocol Code Number EVT-2012-489. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983, and its later amendments, and was monitored by the Copenhagen University Hospital Good Clinical Practice Unit. The study started in February 2013 and was terminated in November 2013.

The participants were allocated to one of the three treatment arms at inclusion and instructed to consume two capsules of study medication twice daily (at the subjects' discretion) with meals for a period of eight weeks (±2 weeks), considering the kinetic profiles of DHA and

EPA [21]. The daily dose of four capsules (soft gels) consisted of either 5,564 mg AG-PUFA (Lipomar® [Marine Ingredients, Mount Bethel, PA, USA], a re-esterified n-3 PUFA consisting of approximately 60 % triacylglycerols and 40 % diglycerides and monoglycerides, containing 1,930 mg DHA, 767 mg EPA, and 2,885 mg total n-3 PUFA); 4,000 mg EE-PUFA (Omacor/Lovaza® [Pronova BioPharma, Lysaker, Norway], containing 1,382 mg DHA, 1,702 mg EPA, and 3,306 mg total n-3 PUFA); or 4,600 mg olive oil as placebo ([Marine Ingredients] containing no n-3 PUFA). All capsules were prepackaged and sealed in similar containers labeled with randomization numbers by the Capital Region Pharmacy in Denmark. Follow-up visits occurred at weeks 4 and 8 (±1 week). Compliance, medical history, medication status, and adverse events were registered; weight, resting heart rate, and blood pressure were measured; and blood and urine samples were collected. The compliance was evaluated by the number of returned capsules at each follow-up visit and measurement of erythrocyte membrane fatty acid content. Electrocardiogram (ECG) and clinical examination were performed at the screening visit and at week 8.

Study Design

The trial was performed at the Department of Clinical Biochemistry, Copenhagen University Hospital Gentofte, Denmark, as an investigator-initiated, prospective, randomized, double-blind, placebo-controlled, parallel three-arm interventional trial, with block sizes of nine subjects at an allocation ratio of 1:1:1. The consecutive randomization sequence was independently generated by a statistician. The primary aim of the study was to evaluate and compare the effect of AG-PUFA and EE-PUFA on non-fasting plasma triacylglycerol levels. The primary outcomes were changes in non-fasting plasma triacylglycerol level, omega-3 index (content of EPA + DHA in the erythrocyte membrane), and heart rate from beginning to end of the study. Secondary outcomes were changes in total plasma cholesterol, high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol (non-HDL-C: LDL + very-low-density lipoprotein cholesterol [VLDL]), triacylglycerol/HDL-C ratio, non-HDL-C/HDL-C ratio, apolipoprotein A1 (ApoA1) and B (ApoB), apolipoprotein B/A1 ratio (ApoB/A1), lipoprotein-associated phospholipase A2 (LpPLA2), LDL particle number, non-fasting plasma glucose, blood pressure, and safety test results.

Methods

Hematology, coagulation, chemistry including lipids, TSH, and glycosylated hemoglobin were analyzed within four hours of sampling, with the exception of apolipoproteins, which were analyzed weekly in batches. Analysis was performed at the Copenhagen University Hospital Gentofte laboratory, according to ISO-15189 certification. Blood and plasma samples were stored at −80 °C. At the end of the study, samples were shipped to Health Diagnostic Laboratory, Inc., (Richmond, VA) for analysis of plasma LDL particle concentration, LpPLA2, and whole-blood fatty acid composition. Fatty acid methyl esters generated by treatment with boron trifluoride-methanol were analyzed using a capillary gas chromatograph (GC-2010 Plus; Shimadzu, Kyoto, Japan) equipped with a 100-m SP-2560 column (Supelco, Bellefonte, PA, USA), and identified by comparison with a known standard (GLC-727; Nu-Chek Prep, Elysian, MN, USA). Blood fatty acids were expressed as a percentage of total fatty acids. The coefficient of variation for EPA + DHA was 5–6 %. The omega-3 index was derived from the DHA and EPA content of whole blood and converted to red blood cell values by multiplying by 1.0366 and adding 0.57 % ($r = 0.96$) [22]. Quantification of LDL particle number was performed using nuclear magnetic resonance spectroscopy. LpPLA2 in plasma was determined using a colorimetric activity method (CAM assay; diaDexus, San Francisco, CA, USA). QT intervals were obtained from a resting 12-lead ECG (Cardiofax Q ECG-9132K; Nihon Kohden, Tokyo, Japan). The QT interval was corrected (QTc) using the Bazett formula, or Fridericia's formula in the case of a heart rate above 70 beats per minute. Clinically significant QTc prolongation was defined as a QTc interval of 500 ms or greater or an increase of more than 25 % from baseline.

Statistical Analyses

A sample size of 120 subjects (40 per arm) was calculated to provide at least 80 % power to detect a 25 % change in triacylglycerols in the n-3 PUFA groups compared to placebo, assuming a common standard deviation of 50 %, a two-sided alpha = 0.05, an average triacylglycerol value of 2.5–3.5 mmol/L (221–310 mg/dL) before treatment, and a 10 % drop-out rate. This sample size was also calculated to provide 80 % power to detect a 10 % non-HDL-C and HDL-C change in the n-3 PUFA groups compared to placebo, assuming a common standard deviation of 30 %.

Patient demographics, baseline variables, and changes in measurements among the groups were compared using the ANOVA post hoc Tukey test (in cases of equal variance) or Tamhane test (in cases of unequal variance). Changes within groups were evaluated with paired t tests. Linear regression models were used for association testing. Two-tailed $P < 0.05$ for primary endpoints and $P < 0.01$ for secondary endpoints were considered significant (modified Bonferroni correction for multiple testing). All data

analyses were performed using SPSS version 19.0 software (IBM Corp., Armonk, NY, USA).

All analyses were performed on the "intention-to-treat" (ITT) population. For the analysis of efficacy endpoints, the method of last observation carried forward was utilized. Some "per-protocol" (PP) analyses were performed for comparison to the ITT population (data not shown). Subjects were excluded from the PP population for violations of inclusion criteria, exclusion criteria, or other deviations that could confound or influence the results, such as major lifestyle changes.

Results

A total of 120 subjects were enrolled in the study and allocated to one of the three treatment arms: AG-PUFA ($n = 40$), EE-PUFA ($n = 40$), or placebo ($n = 40$). There were no significant differences with regard to age, body mass index, therapy, treatment, or prior diseases among the three groups of subjects. There were significantly fewer women in the AG-PUFA group ($n = 3$) than in the EE-PUFA ($n = 14$) and placebo groups ($n = 8$). There were no differences in any of the characteristics between the ITT and PP populations. For additional demographics, see Table 1.

A total of 111 subjects completed the study; four withdrew due to adverse events, two due to compliance failure, one due to personal reasons, and one did not show up for examinations. Eighteen subjects had insufficient compliance, defined as consumption of less than 3.5 or more than 4.5 capsules per day (of these, seven were among the eight who dropped out). A flow chart is shown in Fig. 1. The mean duration of study participation was 54.5 days in the AG-PUFA group, 54.4 days in the EE-PUFA group, and 56.5 days in the placebo group, corresponding to a median intake of 3.7, 3.7, and 3.8 capsules per day, respectively, indicating good and equal compliance among groups.

Table 1 Patient demographics and baseline characteristics in the intention-to-treat (ITT) population

	Total ($n = 119$)	AG-PUFA ($n = 39$)	EE-PUFA ($n = 40$)	Placebo ($n = 40$)
Characteristics				
Age (years) ± SD	62.4 ± 10.0	63.3 ± 7.5	60.4 ± 11.2	63.6 ± 10.8
Male, *n* (%)	94 (79)	36 (92.3)	26 (65)	32 (80)
BMI (kg/m^2) ± SD	29 ± 3.5	29 ± 3.2	28 ± 3.0	29 ± 4.1
Female weight (kg) (range)	75.9 (53.3–98.3)	82.1 (68–98.3)	71.1 (53.5–86)	81.8 (55–96)
Male weight (kg) (range)	91.3 (71–120)	93.1 (75–120)	89.8 (71–117)	90.4 (72–118)
History (self-declared)				
Cardiovascular disease, *n* (%)	92 (77.3)	30 (76.9)	30 (75)	32 (80)
Dyslipidaemia, *n* (%)	95 (79.8)	30 (76.9)	35 (87.5)	30 (75)
Hypertension, *n* (%)	61 (51.3)	22 (56.4)	19 (47.5)	20 (50)
Diabetes mellitus type 2, *n* (%)	19 (16.0)	5 (12.8)	6 (15)	8 (20)
Psychiatric disorder, *n* (%)	4 (3.4)	1 (2.6)	2 (5)	1 (2.5)
Therapy				
Statin therapy, *n* (%)	88 (74.9)	30 (76.9)	29 (72.5)	29 (72.5)
Ezetimibe, *n* (%)	9 (7.6)	3 (7.7)	3 (7.5)	3 (7.5)
Hypertension therapy, *n* (%)	83 (69.7)	27 (69.2)	26 (65)	30 (75)
Psychopharmaca, *n* (%)	10 (8.4)	3 (7.7)	5 (12.5)	2 (5)
Blood pressure				
Systolic (mmHg) ± SD	144 ± 13.8	145 ± 13.8	141 ± 13.8	145 ± 13.9
Diastolic (mmHg) ± SD	84 ± 8.9	84 ± 8.8	83 ± 9.8	85 ± 8.0
Heart rate (beats per minute) ± SD	65 ± 10.3	67 ± 12.6	63 ± 8.9	65 ± 8.9
Values				
Cholesterol (mmol/L) ± SD (mg/dL)	4.91 ± 1.23 (190)	4.79 ± 1.34 (185)	5.03 ± 1.28 (195)	4.92 ± 1.09 (190)
HDL cholesterol (mmol/L) ± SD (mg/dL)	1.14 ± 0.30 (44)	1.13 ± 0.29 (44)	1.15 ± 0.33 (44)	1.14 ± 0.29 (44)
Non-HDL cholesterol (mmol/L) ± SD (mg/dL)	3.77 ± 1.18 (146)	3.66 ± 1.19 (141)	3.88 ± 1.24 (151)	3.78 ± 1.11 (146)
Triacylglycerol (mmol/L) ± SD (mg/dL)	2.98 ± 1.10 (264)	2.93 ± 1.03 (260)	3.01 ± 1.11 (267)	2.99 ± 1.17 (265)
Omega-3 index (mol%) ± SD	6.5 ± 1.67	6.8 ± 1.93	6.5 ± 1.46	6.1 ± 1.59

AG-PUFA acylglycerol polyunsaturated fatty acids, *BMI* body mass index, *EE-PUFA* ethyl ester polyunsaturated fatty acids, *HDL-C* high-density lipoprotein, *ITT* intention-to-treat

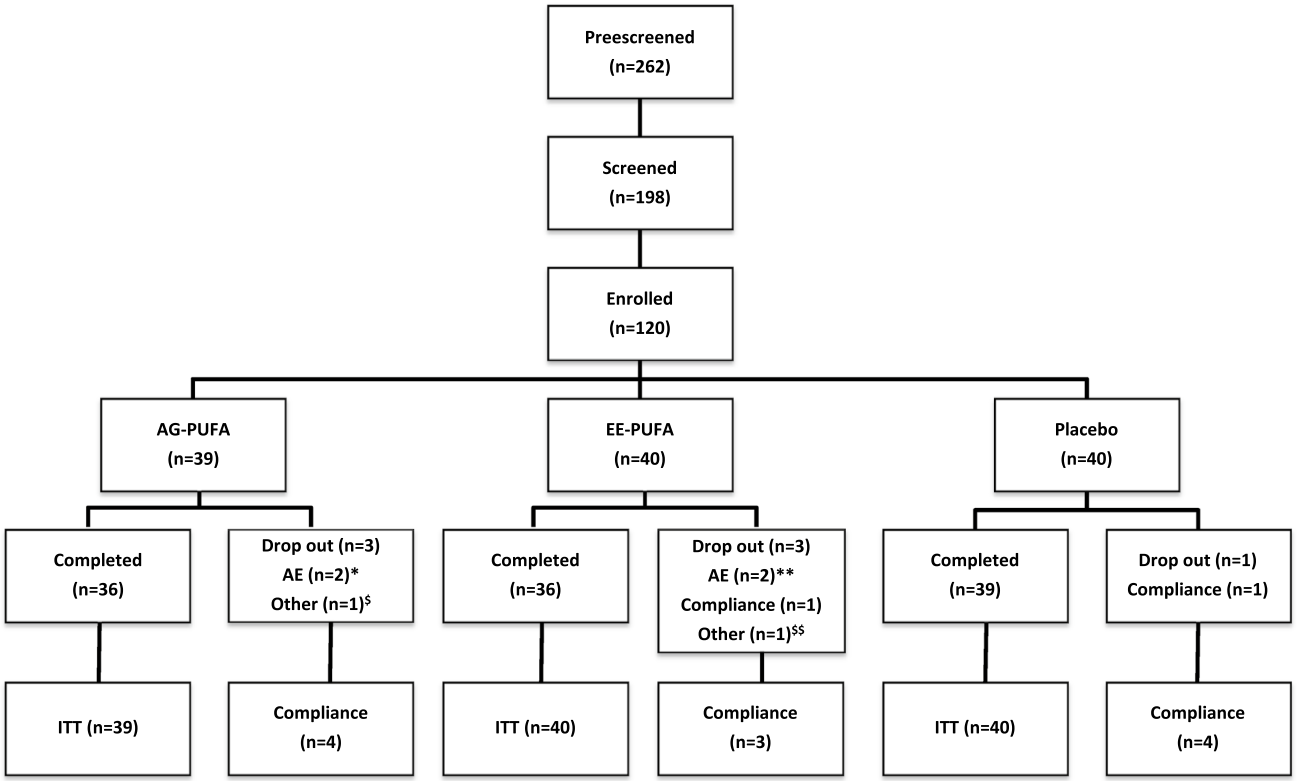

Fig. 1 Flow chart of the process of trial selection. The intention-to-treat population (ITT) consisted of all 119 subjects [AG-PUFA group ($n = 39$); one person lost the study medication the day he received it and was consequently re-randomized), EE-PUFA group ($n = 40$), and placebo group ($n = 40$)]. Participants dropped out due to the following adverse events (AE): *indicates dysphagia due to torticollis ($n = 1$) or bloating ($n = 1$), **peripheral edema and respiratory distress ($n = 1$) or heartburn and acid reflux ($n = 1$), $personal preference ($n = 1$), $$lost to follow-up ($n = 1$)

The primary endpoint non-fasting plasma triacylglycerol decreased significantly in both n-3 PUFA groups compared to placebo ($P < 0.001$), with no significant difference between the n-3 PUFA groups ($P = 0.785$) (Fig. 2). The omega-3 index increased in both PUFA groups compared to placebo ($P < 0.001$), increasing 63.2 % (6.8–11.1 mol%) in the AG-PUFA group and by 58.5 % (6.5–10.3 mol%) in the EE-PUFA group, while remaining unaltered in the placebo group. DHA increased in the AG-PUFA group by 53.8 % (5.2–8.0 mol%) ($P < 0.001$) and in the EE-PUFA group by 29.4 % (5.1–6.6 mol%) ($P < 0.001$). EPA increased in the AG-PUFA group by 93.8 % (1.6–3.1 mol%) ($P < 0.001$) and in the EE-PUFA group by 164.3 % (1.4–3.7 mol%) ($P < 0.001$). The increase in DHA was significantly greater in the AG-PUFA group compared to the EE-PUFA group ($P = 0.043$), while the increase in EPA was significantly higher in the EE-PUFA group compared to the AG-PUFA group ($P < 0.001$). For full fatty acid composition, please consult Table 2. In addition, the heart rate in the AG-PUFA group decreased by three beats per minute compared to placebo ($P = 0.045$), but was unchanged in the EE-PUFA group. There was an inverse dose-dependent relationship between changes in omega-3 index and triacylglycerol level (Fig. 3).

With respect to the secondary endpoints, neither total cholesterol nor non-HDL-C changed significantly compared to placebo in any of the groups. HDL-C increased significantly in the AG-PUFA group ($P < 0.001$) (Fig. 2). The triacylglycerol/HDL-C ratio decreased significantly in both PUFA groups ($P < 0.001$), while the non-HDL-C/HDL-C ratio decreased only in the AG-PUFA group ($P < 0.001$). LpPLA2 decreased in the EE-PUFA group compared to the placebo group. There were no significant changes in LDL particle number, HgbA1c, blood pressure, or any of the other endpoints or safety tests. Data are available upon request.

Treatments were generally well-tolerated, with an even distribution of adverse events among the groups (AG-PUFA, $n = 32$; EE-PUFA, $n = 35$; placebo, $n = 25$). Most of the adverse events were gastrointestinal-related, with no significant difference between the two n-3 PUFA groups regarding gastrointestinal, cardiovascular, dermal, or other adverse events (Table 3). No serious adverse events were observed.

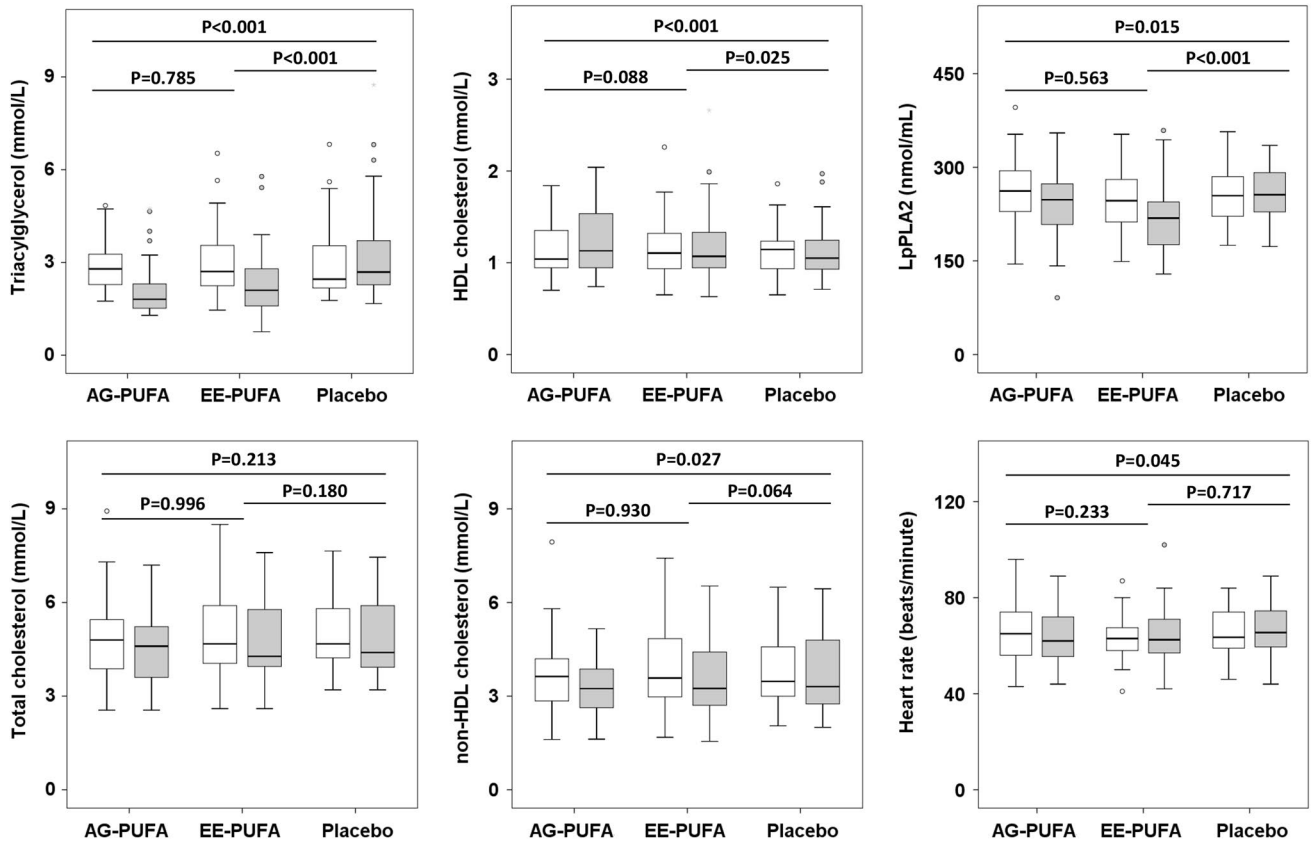

Fig. 2 Effect of treatment with n-3 PUFA on non-fasting triacylglycerol, cholesterols, heart rate, and lipoprotein-associated phospholipase A2. Concentration of non-fasting triacylglycerols, total cholesterol, high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol [low-density lipoprotein (LDL) + very-low-density lipoprotein cholesterol (VLDL)], heart rate, and lipoprotein-associated phospholipase A2 (LpPLA2) before and after eight weeks of treatment with long-chain omega-3 polyunsaturated fatty acids as acylglycerol (Lipomar®, AG-PUFA); as ethyl ester (Lovaza®/Omacor®, EE-PUFA); or as placebo (olive oil). The *white box* represents values before treatment, while the *grey box* represents values after treatment. The *boxes* represent the range of data from the 25th to the 75th percentile, while the *bar* in the middle of each box represents the median value. The "*whiskers*" extending from the box represent the range of values obtained excluding outliers. *Circles* and *asterisks* indicate outliers (1.5 × the interquartile range) and extreme values (3.0 × the interquartile range) outside the central box, respectively. The significance (*P* values, ANOVA) for the change after eight weeks of treatment between each arm is shown above the *box plots*.

Discussion

This study evaluated the effects of re-esterified n-3 PUFA products containing mixed acylglycerol moieties and n-3 PUFA ethyl esters on non-fasting triacylglycerols. In over 40 years of omega-3 research, most studies regarding the effects of n-3 PUFA on plasma triacylglycerol levels have been performed with subjects in a fasting state, and a few studies have been performed during the postprandial period (with a defined test meal and fixed sampling points), but none with subjects in a non-fasting state. This variable, in contrast to postprandial triacylglycerol values, has been documented as having a strong association with cardiovascular risk [5–7]. A pooled meta-analysis and several interventional studies have found that long-chain n-3 PUFA from both food and dietary supplements has a lowering effect on fasting and postprandial triacylglycerols in healthy volunteers and patients with hyperlipidemia [23–25]. However, most individuals eating three daily meals are in the fasting state (as it relates to triacylglycerol metabolism) for only 2–3 hours per day; hence, the vast majority of time is spent in a non-fasting state. The postprandial setting after test meals is an artificial non-fasting state, and therefore does not reflect everyday living. A number of studies have found that elevated plasma non-fasting triacylglycerols are more strongly associated with cardiovascular disease risk than elevated fasting triacylglycerols. Most recently, results of a Mendelian randomization study investigating 13,957 subjects with genetic variants in the triacylglycerol-degrading enzyme lipoprotein lipase have strongly suggested a causal relationship between non-fasting triacylglycerols, as a surrogate for remnant cholesterol, and cardiovascular mortality [7]. Interestingly, the association was evident even with a reduction in non-fasting triacylglycerol

Table 2 Red blood cell fatty acid composition (mol%)

	AG-PUFA		EE-PUFA		Placebo	
	Pre	Post	Pre	Post	Pre	Post
n-3 fatty acids						
22:6n-3 ± SD	5.2 % ± 1.42	8.0 % ± 1.87	5.1 % ± 1.17	6.6 % ± 1.46	4.8 % ± 1.19	4.7 % ± 1.30
20:5n-3 ± SD	1.6 % ± 0.67	3.1 % ± 1.04	1.4 % ± 0.48	3.7 % ± 1.59	1.3 % ± 0.51	1.2 % ± 0.49
18:3n-3 ± SD	0.3 % ± 0.17	0.3 % ± 0.20	0.3 % ± 0.15	0.3 % ± 0.14	0.3 % ± 0.15	0.4 % ± 0.19
22:5n-3 ± SD	2.0 % ± 0.46	2.0 % ± 0.36	2.0 % ± 0.42	2.5 % ± 0.45	2.0 % ± 0.31	1.9 % ± 0.36
n-6 fatty acids						
20:4n-6 ± SD	11.7 % ± 2.14	10.2 % ± 1.70	12.3 % ± 2.21	11.1 % ± 1.58	13.0 % ± 2.35	12.5 % ± 2.62
18:2n-6 ± SD	13.0 % ± 2.33	12.4 % ± 2.24	14.0 % ± 2.61	12.9 % ± 2.67	14.1 % ± 2.58	14.7 % ± 2.81
18:3n-6 ± SD	0.2 % ± 0.07	0.1 % ± 0.05	0.2 % ± 0.06	0.1 % ± 0.05	0.2 % ± 0.06	0.2 % ± 0.07
20:2n-6 ± SD	0.2 % ± 0.05	0.2 % ± 0.04	0.3 % ± 0.05	0.2 % ± 0.05	0.2 % ± 0.05	0.2 % ± 0.03
20:3n-6 ± SD	1.9 % ± 0.39	1.5 % ± 0.32	1.9 % ± 0.39	1.5 % ± 0.32	1.9 % ± 0.37	1.8 % ± 0.32
22:4n-6 ± SD	1.5 % ± 0.46	1.2 % ± 0.35	1.6 % ± 0.53	1.3 % ± 0.38	1.8 % ± 0.77	1.7 % ± 0.70
22:5n-6 ± SD	0.3 % ± 0.07	0.2 % ± 0.05	0.3 % ± 0.09	0.2 % ± 0.06	0.3 % ± 0.21	0.3 % ± 0.19
Saturated fatty acids						
14:0 ± SD	0.6 % ± 0.31	0.6 % ± 0.24	0.6 % ± 0.28	0.6 % ± 0.26	0.5 % ± 0.22	0.6 % ± 0.30
16:0 ± SD	23.7 % ± 2.85	23.2 % ± 2.54	23.0 % ± 1.78	23.0 % ± 1.81	22.8 % ± 1.74	22.6 % ± 2.18
18:0 ± SD	14.5 % ± 2.12	14.3 % ± 1.51	13.9 % ± 1.59	13.6 % ± 1.14	14.4 % ± 1.59	13.8 % ± 1.32
20:0 ± SD	0.2 % ± 0.05	0.2 % ± 0.04	0.2 % ± 0.05	0.2 % ± 0.04	0.2 % ± 0.04	0.2 % ± 0.04
22:0 ± SD	0.3 % ± 0.09	0.3 % ± 0.07	0.3 % ± 0.10	0.3 % ± 0.07	0.3 % ± 0.08	0.3 % ± 0.07
24:0 ± SD	0.5 % ± 0.16	0.5 % ± 0.13	0.4 % ± 0.11	0.5 % ± 0.14	0.5 % ± 0.12	0.5 % ± 0.15
Cis-monounsaturated fatty acids						
16:1n-7 ± SD	1.1 % ± 0.61	1.0 % ± 0.55	1.1 % ± 0.66	1.0 % ± 0.57	1.0 % ± 0.50	1.0 % ± 0.59
18:1n-9 ± SD	19.8 % ± 1.96	18.9 % ± 2.15	19.6 % ± 2.82	18.9 % ± 2.52	18.9 % ± 2.13	19.9 % ± 2.45
20:1n-9,-11 ± SD	0.3 % ± 0.07	0.4 % ± 0.07	0.3 % ± 0.06	0.3 % ± 0.04	0.3 % ± 0.07	0.3 % ± 0.05
24:1n-9 ± SD	0.7 % ± 0.17	0.7 % ± 0.16	0.6 % ± 0.13	0.6 % ± 0.15	0.6 % ± 0.14	0.7 % ± 0.19
Trans fatty acids						
18:2 ± SD	0.1 % ± 0.04	0.1 % ± 0.04	0.1 % ± 0.04	0.1 % ± 0.03	0.1 % ± 0.04	0.1 % ± 0.04
18:1 ± SD	0.3 % ± 0.09	0.3 % ± 0.10	0.3 % ± 0.09	0.3 % ± 0.08	0.3 % ± 0.12	0.3 % ± 0.10
16:1 ± SD	0.1 % ± 0.04	0.1 % ± 0.05	0.1 % ± 0.04	0.1 % ± 0.04	0.1 % ± 0.03	0.1 % ± 0.04

AG-PUFA acylglycerol polyunsaturated fatty acids, *EE-PUFA* ethyl ester polyunsaturated fatty acids, or *placebo* (olive oil). *Pre*: before treatment, *Post*: after treatment

levels below 1 mmol/L. In the present study, long-chain n-3 PUFA reduced non-fasting triacylglycerol levels by 22–28 %, relative to an 8 % increase in the placebo group. A similar reduction has previously been observed in fasting triacylglycerols [26, 27].

A resting heart rate above 65 beats per minute is associated with a higher risk of cardiovascular death [28, 29]. We observed a reduction in heart rate of three beats per minute in the AG-PUFA group vs. placebo, which is consistent with results previously reported in other fish oil studies [30]. The QTc interval, representing depolarization and repolarization of the ventricles, did not change in any of the groups.

The effect of n-3 PUFA on cholesterol is less consistent than its effect on triacylglycerols. The effect of n-3 PUFA on total cholesterol, LDL-C, and HDL-C is equivocal, while VLDL-C (responsible for transporting the vast majority of plasma triacylglycerol) is typically reduced with n-3 PUFA treatment [13, 14, 16, 17, 23, 24]. In the present study, neither total cholesterol nor non-HDL-C were changed. An increase in HDL-C was noted in the AG-PUFA group. There were mixed effects on inflammatory markers, with plasma concentrations of C-reactive protein unaffected by treatment, while LpPLA2 was reduced, but only in the EE-PUFA group. An effect of omega-3 PUFA on LpPLA2, a known risk factor for coronary heart disease [31], has previously been reported in some studies [32], while other studies have reported no effect [33, 34]. Another risk predictor, the ApoB/ApoA1 ratio [35, 36], has been observed to decrease or remain unchanged with n-3

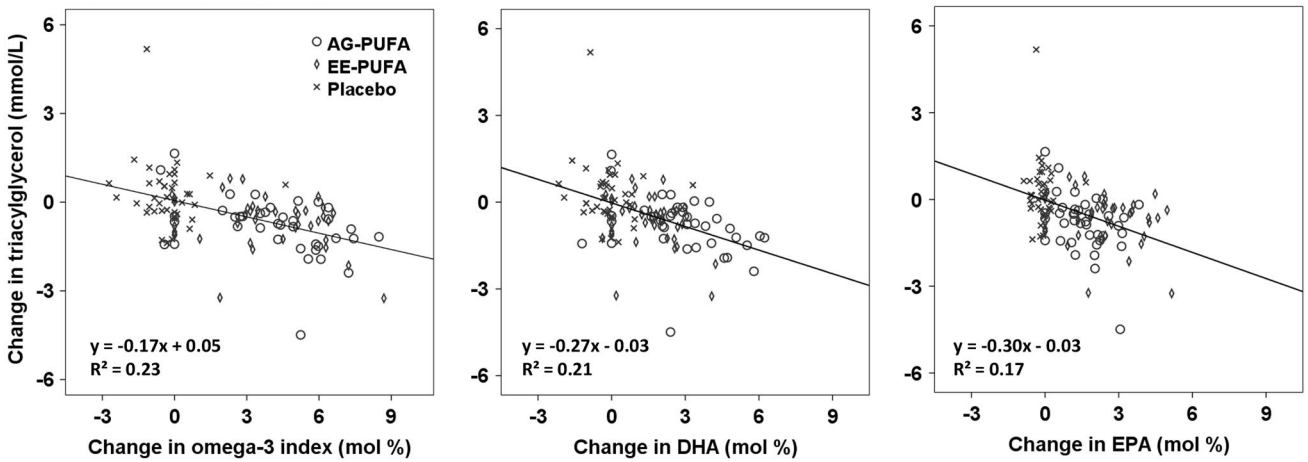

Fig. 3 Change in non-fasting triacylglycerol vs. omega-3 index, DHA, or EPA. The relationship between the change in non-fasting triacylglycerols and omega-3 index, DHA, or EPA after eight weeks of treatment with long-chain omega-3 polyunsaturated fatty acids as acylglycerol (Lipomar®, AG-PUFA); as ethyl ester (Lovaza®/Omacor®, EE-PUFA); or placebo (olive oil). *Regression line* is shown

Table 3 Adverse events

	AG-PUFA	EE-PUFA	Placebo	Total
Gastrointestinal				
Constipation	1	–	1	2
Diarrhoea	7	8	3	18
Dysphagia	–	2	–	2
Indigestion	11	5	14	30
Nausea	1	1	1	3
Cardiovascular				
Palpitation	1	2	1	4
Dermal				
Eczema	4	2	1	7
Other				
Conjunctivitis	–	1	–	1
Cough	1	–	–	1
Dizziness	2	2	1	5
Fatigue	–	2	–	2
Fish odour	–	1	–	1
Leg pain	1	–	–	1
Loss of breath	–	2	–	2
Nosebleed	–	1	–	1
Oedema	1	1	–	2
Perspiration	–	1	–	1
Plugged ears	–	–	1	1
Skeletal pain	1	4	2	7
Vaginal discharge	1	–	–	1

Data are by count according to each type of event

Adverse events in each of the three treatment arms, *AG-PUFA* acylglycerol polyunsaturated fatty acids, *EE-PUFA* ethyl ester polyunsaturated fatty acids, or placebo (olive oil)

PUFA treatment [37, 38]. We observed no change in any of the treatment groups.

With regard to treatment with prescription EE-PUFA, caution is recommended in cases of warfarin use, prior to surgery or with injury, in uncontrolled diabetes, and in cases of decreased liver or kidney function. Early observation of increased bleeding time in natives of Greenland raised the concern that n-3 PUFA supplementation would increase the risk of hemorrhage [39]. This concern, however, has been proven groundless [40], as noted in our observations. There was no effect on glycemic control, liver function, or kidney function in any of our study groups.

The few differences observed between the AG-PUFA and EE-PUFA groups may be explained by several factors. First, a difference in bioavailability in favor of AG-PUFA may account for some of the effect. In our study, consumption of AG-PUFA resulted in a slightly but not significantly higher mean omega-3 index than consumption of EE-PUFA, despite a 15 % lower total n-3 PUFA dose in the AG-PUFA group. Some studies have found better bioavailability with AG-PUFA than EE-PUFA [41–43], while others have reported no difference [26, 44]. There are several possible reasons for the difference in bioavailability. The first is the preferential recognition and cleavage of AG-PUFA versus EE-PUFA by the intestinal lipase. Hydrolysis of the ethanol in EE-PUFA is up to 50 times more resistant to the pancreatic lipase as compared to AG-PUFA [45]. This is supported by studies that have found higher bioavailability of EPA and DHA when ingested as free fatty acids than when ingested as EE-PUFA [46]. (2) Once the dietary n-3 PUFA are absorbed, they must be assembled

as an acylglycerol. Both n-3 PUFA formulations are processed in the small intestine through emulsification by bile salts and hydrolysis by intestinal lipase. The hydrolysis of AG-PUFA produces both free fatty acids and (mono) glycerol molecules, whereas hydrolysis of EE-PUFA produces only ethanol and free fatty acids. AG-PUFA provides its own glycerol substrate, whereas EE-PUFA needs to obtain a glycerol substrate from another source, thereby delaying synthesis [47, 48]. Second, the amount of DHA and EPA differed between the two n-3 PUFA groups, with AG-PUFA containing more DHA and EE-PUFA containing more EPA. DHA and EPA have slightly different effects on certain variables. DHA is suggested to be superior in reducing triacylglycerols and increasing LDL-C [16, 48]. In the present study, DHA and EPA appeared to have the same effect on non-fasting triacylglycerols, judging by the similar slopes in comparisons of change in DHA or EPA (mol%) and change in triacylglycerol (mmol/L) (Fig. 3). We observed an average decrease in triacylglycerol of approximately 0.12 mmol/L per mol% increase in omega-3 index. This corresponds to previous findings of an approximate 0.1 mmol/L reduction in triacylglycerol [49]. It would be interesting to compare the current AG-PUFA and EE-PUFA with the new EPA version of EE-PUFA (Vascepa®).

This study was the first to examine the effects of long-chain n-3 PUFA on non-fasting triacylglycerols in a randomized double-blind placebo-controlled design. One strength of the study is that it was performed at a single site over a short period of time, resulting in low variance in investigator-dependent observations. In addition, the exclusion criteria resulted in a homogenous group reflective of the target population. Among study weaknesses was the fact that most of the samples were analyzed continuously over a period of nine months, resulting in higher assay variation than if they had been analyzed in a batch. In addition, the information on diet, exercise, alcohol consumption, and smoking habits—which could influence several of the endpoints—were self-reported, and the groups were not stratified by gender. Further, EPA and DHA have been shown to have different effects on several variables, and thus some of the effects observed in our study may have been due to differences in the amounts of EPA and DHA administered between the two n-3 PUFA groups. Finally, the eight-week trial period cannot reveal any long-term effects of n-3 PUFA [50–52].

In conclusion, supplementation with long-chain n-3 PUFA has a significant lowering effect on non-fasting triacylglycerol levels, comparable to the effect on fasting triacylglycerols, suggestive of a reduction in cardiovascular risk. Regardless of the different effects on heart rate, HDL, and LpPLA2, we found that AG-PUFA and EE-PUFA were equally effective in reducing non-fasting triacylglycerol. Whether there are any long-term differences in effects between the two formulations remains to be explored. In general, the use of non-fasting measurements in the monitoring of patients receiving fish oil supplements has great logistic benefits and may more accurately reflect a change in cardiovascular risk.

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Conflict of interest JD is scientific advisor for Marine Ingredients. WSH is an employee of Health Diagnostic Laboratory, Inc., and is President of OmegaQuant Analytics, LLC, two laboratories that offer RBC fatty acid testing. AH, PBS, and SS each declare no conflict of interest.

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