

A novel omega-3 glyceride mixture enhances enrichment of eicosapentaenoic acid and docosahexaenoic acid after single dosing in healthy older adults: results from a double-blind crossover trial

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Running heading: Enrichment of EPA and DHA after single dosing

List of abbreviations:

ω -3, omega-3; ALA, alpha-linoleic acid; BMI, body mass index; C_{max} , maximal concentration; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EE, ethyl-ester(s); EPA, eicosapentaenoic acid; FA, fatty acid(s); FAME, fatty acid methyl esters; FFA, free fatty acid(s); Glyceride formulation, mix of mono, di and triglycerides; HDL, high density lipoprotein; HOMA2-IR, homeostatic model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein; iAUC, incremental area under the curve; ω -3 LCPUFA, omega-3 long chain polyunsaturated fatty acid(s); SMEDS, self-micro-emulsifying delivery system; TG, triglyceride(s); T_{max} , time at which maximal concentration occurs.

Abstract

A glyceride mix of mono, di, and triglycerides increases solubilisation and enhances emulsification of omega-3 (ω -3) fatty acid (FA) containing lipids in the stomach. This allows for better access of digestive enzymes, pivotal for the release of bioactive ω -3 FA.

The objective was to compare the effect of a glyceride formulation and an ethyl ester (EE) formulation of EPA+DHA on concentrations of EPA and DHA in plasma following single dosing.

We conducted a double-blind crossover trial in which twenty healthy adults aged 50-70 y consumed a single dose (2.8 g EPA+DHA) of each EPA+DHA formulation without a meal in random order separated by a two-week wash out period. EPA and DHA were measured in plasma total lipid over the following 12 h.

EPA and DHA in plasma total lipid increased over 12 h with both formulations. A 10 x greater Δ concentration of EPA, 3 x greater Δ concentration of DHA, and 5 x greater Δ concentration of EPA+DHA was seen with the glyceride-EPA+DHA. The time at which the maximal concentrations of ω -3 FA occurred was 4 h earlier for EPA, 1 h earlier for DHA, and 2 h earlier for EPA+DHA when consuming glyceride-EPA+DHA.

A mix of mono, di, and triglycerides results in greater and faster incorporation of EPA and DHA into blood plasma lipid in the absence of a fatty meal. This may provide benefit to individuals on a low fat diet or with digestive impairments and could result in greater efficacy in clinical trials using ω -3 FA.

Keywords: omega-3, fish oil, eicosapentaenoic acid, docosahexaenoic acid, emulsification, monoglyceride, diglyceride, triglyceride

Introduction

Omega-3 long chain polyunsaturated fatty acids (ω -3 LCPUFA) have been widely studied due to their associations with benefits to human health including lowering inflammation [1, 2], decreasing the risk of cardiovascular disease and events [3-8] and improving cognition [9, 10]. The bioactive ω -3 LCPUFA are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); for these FA to exert their actions, they require incorporation into the bloodstream and delivery to cells and tissues [11].

Synthesis of EPA and DHA from the essential fatty acid alpha-linoleic acid (ALA) is low in humans, and therefore consumption of preformed EPA and DHA from the diet is preferred [12]. In the UK it is recommended that adults consume EPA and DHA in the form of fish to provide the equivalent of at least 450 mg of EPA+DHA/day (two x ~140 g portions per week, one of which is to be oily/fatty fish) [13]. Other global or national recommendations for EPA and DHA intake by adults are in the range 250 to 600 mg/day (see Table 5 of [11]). However, the UK population, in addition to those of the US and many other countries, consume well below the recommended amounts of oily fish and of EPA and DHA [14]. It is estimated that only 18.9% of countries achieve the minimum daily recommendation of 250 mg EPA+DHA [14]. Here, supplementation would be an alternative option to diet to increase intake of bioactive EPA and DHA.

EPA and DHA are commercially available in supplements in multiple chemical forms as triglycerides (TG), ethyl esters (EE) or free fatty acids (FFA). The chemical form in which EPA and DHA are administered has been shown to have some impact on their incorporation into the bloodstream, cells and tissues, most noticeably when consumed in the absence of a fatty meal. This is particularly observed when EPA and DHA are administered as EE, which are used in many clinical trials in which unsupervised daily dosing of capsules occurs over several weeks/months [15-17]. This suggests that the optimal absorption of these FA is dependent on digestive processes, which act in response to intake of dietary fat. Therefore, variation in the fat content of the meal consumed alongside the capsules or if the capsules were taken on an empty stomach, may be one of the reasons why some studies fail to find beneficial effects of ω -3 LCPUFA [18].

Considering this, development of alternative delivery systems to improve the bioavailability of EPA and DHA in the absence of dietary fat has been of great interest. Pre-emulsification of ω -3 LCPUFA in TG form and self-micro-emulsifying delivery systems (SMEDS) which promote spontaneous emulsification of ω -3 FA in EE form in the gastric environment have been shown to result in superior incorporation into blood and cell lipid pools in comparison to other TG and EE formulations, respectively [17, 19-22]. Other approaches to enhancing uptake of EPA and DHA are less well researched but include the use of a mix of mono-, di-, and triglycerides [22, 23].

Investigation in rats showed improved appearance of DHA in the lymph using monoglyceride and diglyceride formulations in comparison to TG and EE formulations [23] but no such improvement was seen when using a di- and triglyceride DHA formulation in comparison to diglyceride and TG [24]. A single study in humans reported significantly enhanced bioavailability of EPA using a mix of mono-, di- and triglycerides in comparison to TG and monoglyceride formulations [25]. These studies suggest the use of monoglycerides in combination with di- and triglycerides is key in ω -3 LCPUFA bioavailability, but this requires further evaluation as evidence for both EPA and DHA, and comparison with bioavailability of EE ω -3 LCPUFA in humans is lacking.

Therefore, the current study aimed to compare the use of a novel alternative glyceride formulation of EPA+DHA, comprising of a mix of mono-, di-, and triglycerides in a ratio of 1:2.5:1 thought to enhance solubility, digestion, and therefore availability of EPA and DHA, with an EE-EPA+DHA formulation, in the absence of dietary fat, on EPA and DHA concentrations in blood plasma over a period of 12 h following single dosing. It was hypothesised that the glyceride formulation would result in higher concentrations of EPA and DHA in blood plasma and that the maximal concentration would be achieved faster than seen with the EE-EPA+DHA formulation.

Methods

Subjects

All procedures involving human subjects were approved by the London - Brighton & Sussex Research Ethics Committee (REC 19/LO/0939) and the study is registered at www.isrctn.com (study ID: ISRCTN18364209). The trial was conducted according to the principles of the Declaration of Helsinki, and all participants gave written informed consent prior to enrolment.

Ten healthy male and ten healthy female participants aged 50-70 y were enrolled into the study. Inclusion criteria for participation were as follows: age between 50-75 y, body mass index (BMI; kg/m²) 20-35, self-reported oily fish intake of <1 portion/wk, and an omega-3 index (red blood cell (RBC) EPA+DHA [26]) determined from a screening blood sample of ≤ 6.5 . Exclusion criteria were any chronic medical condition, cancer within the last 2 years, gastrointestinal problems, allergy to fish, smoking, pregnancy or lactation, or consumption of any ω -3 FA or other lipid supplements. The study took place between October 2019 and February 2020.

Study design and supplements

This study was a double blind randomised crossover trial that investigated an ω -3 glyceride formulation with 500 mg EPA + 200 mg DHA per capsule (BASF AS, Norway), alongside an EE formulation (also providing 500 mg EPA + 200 mg DHA per capsule) (BASF AS, Norway) both in soft gelatin capsules. The fatty acid compositions of the EE and glyceride mixtures were identical. They contained (g/100 g FA): 18:1n-9, 0.8; 18:2n-6, 1.5; 18:3n-3, 0.9; 18:4n-3, 3.7; 20:3n-3, 0.2; 20:4n-6, 2.3; 21:5n-3, 1.8; 22:5n-6, 0.8; 20:5n-3 (EPA), 53.2; 22:5n-3 (docosapentaenoic acid; DPA), 3.0; 22:6n-3 (DHA), 23.4; and other minor FAs (all < 0.4), 3.1. Both formulations provided about 28 mg of DPA. The distribution of EPA and DHA within the mono-, di- and triglycerides is not known.

As this was a crossover design, all participants received both treatments but in random order. Blinding, randomization, and supplement packaging were completed by the Research Pharmacy at Southampton General Hospital, Southampton, United Kingdom, by individuals independent of the researchers involved in the study. Treatment group blinding was maintained until completion of statistical analysis of all data.

Participants attended the National Institute for Health Research Wellcome Trust Clinical Research Facility, Southampton General Hospital, Southampton, United Kingdom, on 3 occasions. The first was a screening visit during which participants provided written informed consent prior to having their weight and height measured and providing a non-fasting blood sample. This sample was used to determine RBC omega-3 index (EPA+DHA as a % of total RBC FA [26]), with a value of ≤ 6.5 required for study enrolment. Participants who met all inclusion and exclusion criteria were enrolled into the study and randomly assigned. These participants made a further 2 clinic visits, both in the fasted state (≥ 10 h without food or drink other than water). At both of these visits, participants attended the clinic at ~0730 h at which a cannula was inserted into a forearm vein. Participants provided a zero h blood sample after which they ingested a single dose (4 capsules totalling 2.8 g EPA+DHA) of one of the study formulations with water. A member of the research nursing team observed capsule ingestion to ensure compliance and record time of consumption. Further blood samples were collected at 1, 2, 3, 4, 5, 6, 8, and 12 h post supplement ingestion. Low-fat meals with decaffeinated tea or coffee were given directly after the 3 and 6 h samples were collected, and again at ~10 h. These meals consisted of 2 slices of toast without butter or alternative spread but with jam, and either a small apple or orange accompanied by tea or coffee made with skimmed (0.1% fat) milk, totalling 2.3 g fat for the entire meal. A more substantial meal was provided after the 12 h blood sample was collected; participants could choose either sandwiches, fruit or cake and a juice drink, or a pre-made meal such as curry or pasta to heat and consume at home. The third clinic visit took place at least 2 wk after the second clinic visit (the average time between the second and third

clinical visit was 26.1 ± 2.4 days); all procedures were the same at this visit except that the alternative supplement was taken.

Sample preparation

Blood was collected into tubes containing EDTA and directly stored on ice before being processed within 1 h of collection. Plasma was prepared by centrifugation of blood samples collected at all time points at $3000 \times g$ for 15 min at 4°C and analysed for EPA and DHA contents. Plasma and RBC samples were stored at -80°C before analysis.

FA composition analysis

Total lipid was extracted from 0.5 mL plasma with 5 mL chloroform:methanol (2:1: vol:vol) containing 0.2 M BHT [27]. 1 mL of 1 M sodium chloride was added and the sample vortex mixed prior to centrifugation at $1500 \times g$ for 5 min at room temperature. The lower solvent phase was aspirated and evaporated under nitrogen at 40°C . The lipid extract was re-dissolved in 0.5 mL toluene and fatty acids were released from esterified lipids and simultaneously derivatised to methyl esters by incubation with 2% H_2SO_4 in methanol for 2 h at 50°C to form fatty acid methyl esters (FAME) [28]. The samples were then neutralised and FAME transferred into hexane for analysis by gas chromatography; FAME were separated on a BPX-70 fused silica capillary column (30 m x 0.2 mm x 0.25 μm ; manufactured by SGE) in a HP6890 gas chromatograph fitted with a flame ionisation detector. GC run conditions were as described elsewhere [28]. Dipentadecanoyl phosphatidylcholine added into the initial plasma sample was used as an internal standard for quantification purposes and a Supleco 37 Component FAME Mix as used as a calibration reference standard (Sigma-Aldrich).

Other laboratory analyses

The plasma concentrations of TG, total cholesterol, HDL-cholesterol, and glucose at study entry were measured using enzyme linked colorimetric assays (Alpha laboratories and Microgenics GmbH) on a Konelab 20 autoanalyzer in accordance with manufacturer's instructions. Non-HDL-cholesterol at study entry was calculated by subtracting HDL-cholesterol from total cholesterol measurements. Plasma insulin concentration at study entry was measured by ELISA (Access Ultrasensitive Insulin kit; Beckman Coulter). Plasma C-reactive protein concentration at study entry was measured by using a high sensitivity ELISA kit (CRP Latex kit; Beckman Coulter).

Statistical analysis

The study sample size was estimated according to the anticipated change in EPA+DHA content of plasma over the 12 h period following single dosing. Assuming a similar difference between the two formulations as was seen in the previous study comparing SMEDS and a standard EE formulation [17], a sample size of 20 was estimated to give 90% power to detect this difference as statistically significant by pairwise comparison with significance defined as $P \leq 0.05$.

EPA, DPA, and DHA in plasma are expressed as relative (percentage of total FA) and absolute (micrograms per millilitre of plasma) concentrations. All FA data were normalised against baseline concentrations and the distribution of all data checked. Any skewed data were normalised by logarithmic transformation. Incremental AUC (iAUC), maximum concentration change ($C_{max,r}$), and time point at which C_{max} was achieved ($T_{max,r}$) were calculated using GraphPad Prism version 7 (GraphPad). Repeated measures ANOVA was performed on all time-course data controlling for possible confounding effects of sex. There were no significant confounding effects of age or BMI on either relative or absolute EPA, DPA, DHA or EPA+DHA time-course data ($P \leq 0.172$ and ≤ 0.659 respectively). Therefore, these variables were not controlled for in the analyses. The Kruskal-Wallis test was performed to compare baseline characteristics between sexes and to compare plasma iAUC, $C_{max,r}$, and T_{max} between treatments as log transformation was unable to correct the skewed distribution of these data. Correlation of metabolic measurements at study entry with iAUC, $C_{max,r}$, and T_{max} data was investigated using Spearman's correlation. All statistical analyses were conducted using SPSS version 26 (IBM Corporation), and significance was defined as $P \leq 0.05$.

Results

Participant characteristics

Figure 1 shows the flow of participants through the study and numbers of participants in each treatment group. Twenty participants (10 male and 10 female) consumed both single dose treatments at least 2 weeks apart with the order of these randomly assigned. Capsule ingestion was observed by research nurses resulting in 100% compliance. Table 1 details participant characteristics at study entry. Participants included in the study had a mean age of 59.1 ± 0.7 y (range 50-70 y), mean BMI of 26.95 ± 0.73 kg/m², consumed on average 2.55 ± 0.31 portions of oily fish per month, and had a mean screening omega-3 index of 5.35 ± 0.19 . Female participants had significantly higher BMI and total cholesterol, and lower blood glucose than male participants (Table 1). There were no

significant differences in RBC omega-3 index or in baseline relative or absolute concentrations of EPA, DPA, DHA, or EPA+DHA between male and female participants (Table 1).

EPA, DPA, and DHA incorporation in plasma with single dosing

The glyceride-EPA+DHA supplement resulted in a significantly greater increase in the absolute ($\mu\text{g/ml}$) and relative (%) concentrations of EPA, DPA, DHA and EPA+DHA in the plasma total lipid pool within 12 h following single dosing than seen with the EE-EPA+DHA supplement (all $P < 0.001$, Figure 2 and 3). Significantly higher C_{max} , lower T_{max} , and greater iAUC for concentrations of EPA and DHA in the plasma following single dosing with the glyceride supplement were observed when compared to single dosing with the EE supplement ($P \leq 0.013$, Figure 2 and 3, Table 2). Significantly higher C_{max} and greater iAUC for concentrations of DPA in the plasma following single dosing with the glyceride supplement were also observed when compared to single dosing with the EE supplement ($P \leq 0.023$, Figure 2 and 3, Table 2). An average 9.7 fold greater Δ concentration of EPA, 3.0 fold greater fold Δ concentration of DPA, 2.8 greater Δ concentration of DHA, and 5.3 fold greater Δ concentration of EPA+DHA was achieved with single dosing of the glyceride supplement in comparison to the EE supplement ($P < 0.001$, Figure 2, Table 2). The time at which the maximal concentration of EPA, DHA and EPA+DHA was achieved occurred on average 4 h, 1 h, and 2 h earlier, respectively, following single dosing with the glyceride supplement in comparison to the EE supplement ($P < 0.001$, Figure 2, Table 2). The time at which the maximal concentration of DPA was achieved did not differ following single dosing with the glyceride supplement or the EE supplement ($P \geq 0.520$, Figure 2, Table 2).

Increase in the absolute concentrations of EPA, DPA and DHA over the 12 h period was reflected in an increase in the relative concentration of these ω -3 LCPUFA ($P < 0.001$, Figure 3, Table 2). The C_{max} and iAUC for the relative concentration of EPA, DPA and DHA in the total plasma lipid pool were greater in participants when consuming the glyceride supplement in comparison to the EE supplement. The time at which the maximal concentrations of EPA and DHA were achieved occurred on average 1 h earlier with the glyceride supplement in comparison to the EE supplement ($P < 0.001$, Figure 3, Table 2). There was no difference in the time at which the maximal concentration of DPA was achieved between the glyceride and the EE supplement.

Metabolic measures were not significantly correlated with iAUC, C_{max} , or T_{max} data for either relative or absolute EPA, DHA or EPA+DHA ($P \geq 0.172$, all).

Discussion

Omega-3 LCPUFA are found to be esterified into TG and phospholipids in many commercially available supplements and in food. In this form, the FA require solubilisation and hydrolysis in the upper gastrointestinal tract, known as digestion, before they are available for absorption. The digestive process involves the secretion of bile and pancreatic enzymes which is stimulated by the presence of fat within a meal. Therefore, consuming supplements on an empty stomach or alongside a meal which is low in fat, impairs the digestion and absorption of EPA and DHA into the bloodstream and so makes less EPA and DHA available to cells and tissues [29].

The incorporation of EPA and DHA into cells and tissues, in addition to the bloodstream, is pivotal for their action and associated benefits to human health [11]. Using esterified ω -3 LCPUFA in trials in which participants undergo chronic intervention may result in varying or null findings, which could be due to consumption of the supplements on an empty stomach or in the absence of a fatty meal, such as alongside a low-fat breakfast. Therefore, an alternative formulation that requires less input from digestive processes to deliver bioactive EPA and DHA could be of great benefit. Free EPA and DHA, which would require less emulsification and no hydrolysis for effective absorption, and in situ emulsification or 'self-emulsifying' EE ω -3 LCPUFA formulations, have been investigated and found to increase the appearance of EPA and DHA in the blood stream after single dosing alongside a low fat meal in comparison to standard EE ω -3 LCPUFA formulations [20-22, 29, 30]. In accordance with this, we have previously shown that SMEDS EE-EPA and -DHA enhanced appearance of EPA and DHA into total plasma and FFA pools in comparison with standard EE-EPA and -DHA over 24 h following single dosing with a low-fat meal [17]. Furthermore, repeated daily dosing of SMEDS EE-EPA and -DHA over a 12 wk period resulted in a greater increase in EPA and DHA in plasma, mononuclear cells and red blood cells, with a greater increase in omega-3 index, in comparison to standard EE formulation [17].

The current study supports the use of an alternative formulation to SMEDS-EE that enhances the availability of EPA and DHA in the absence of a fatty meal. A novel glyceride formulation of EPA+DHA was used comprising of a mix of mono-, di- and triglycerides in a specific ratio (1:2.5:1) that improves solubilisation of the lipids and enhances emulsification of the oil in the stomach. This enhanced emulsification will allow for better access of digestive enzymes, pivotal for the release of free FA and monoglycerides. These FA may then be more readily absorbed during passage through the small intestine resulting in a more effective absorption thereby improving the bioavailability of EPA and DHA in the absence of a fatty meal. Once absorbed, the ω -3 LCPUFA may circulate in the bloodstream esterified into TG as components of chylomicrons, very low-density lipoproteins released from the liver, cholesteryl-rich lipoproteins, and as phospholipids as a component of the phospholipid monolayer that stabilises such lipoproteins in the aqueous environment [31, 32].

Therefore, EPA and DHA in total plasma were measured, as this contains a combination of these forms.

The current study shows that compared with the EE-EPA+DHA formulation, the use of a glyceride-EPA+DHA formulation significantly increases EPA, DPA, and DHA to a greater extent and EPA and DHA reaches a maximal concentration more rapidly over a 12 h period following single dosing. It is thought that EPA and DHA derived from the gut appear in the bloodstream ~4 h following consumption [31, 32]. We previously showed that the largest difference in total plasma EPA and DHA between self-emulsifying EE-EPA and -DHA (SMEDS) in comparison to standard EE-EPA and -DHA occurred at 4 hr suggesting improved gastrointestinal handling of the SMEDS formulation [17]. This has been reproduced in the current study in which we show the largest difference in total plasma EPA and DHA between the glyceride formulation and EE formulation to occur at 4 hr also, suggesting there is improved gastrointestinal handling of the glyceride formulation.

These results suggest that the glyceride mix may be suitable for use in individuals with chronic gastrointestinal problems in which 'normal' absorption is impaired and therefore, EE formulations which are absorbed best with fat may not be appropriate or tolerated. However, this remains to be tested and further investigation into use of this formulation in such individuals would be of great interest. In addition, these results further suggest suitable use for supplementation in older adults in which changes in pancreatic enzyme secretion occur with increasing age and may result in less effective absorption [33].

The use of an ω -3 glyceride formulation in trials in which participants are responsible for their own diet alongside supplementation may also be of benefit as absorption would not be dependent on the fat content of the meal consumed alongside the supplements and these could be taken on an empty stomach. Davidson *et al.* [29] found that the superior absorption of a FFA formulation of EPA and DHA in comparison to EE-EPA and -DHA was negated when consumed with a fatty meal suggesting it would not matter if participants consumed the capsules alongside meals of varying fat content. Further tests would be required to conclude whether similar findings would be observed with the glyceride mix, but this could potentially provide greater efficacy of trials using ω -3 supplements. A final implication of these results is that much lower doses of EPA and DHA would be required if administered via glyceride formulation to achieve similar concentrations achieved by EE supplementation.

The current study has several strengths. First, participants were recruited with the use of the omega-3 index as a criterion; a value of ≤ 6.5 was required for inclusion. Second, consumption of the capsules was observed by a member of the research nursing team to ensure compliance. Third,

participant retention was high; all participants completed both treatments providing all samples. These strengths provide confidence in our findings. One limitation is that we studied only single dosing; however a previous study [17] demonstrated that effects of a SMEDS formulation of EPA and DHA EE shown with single dosing were also seen with repeated daily dosing out to 12 wk. Nevertheless, it will be important to study the new glyceride formulation with repeated daily dosing. Another limitation is that we studied only a single dose of ω -3 LCPUFA. A third limitation is that we studied adults aged 50 to 70 y and so we cannot generalise the findings to those of other ages. The glyceride mix was a ratio of mono-, di-, and triglycerides (1:2.5:1) with an identical FA composition to that of the EE formulation; however, the distribution of EPA, DPA and DHA amongst mono-, di-, and triglycerides within the glyceride mix is not known and is therefore a final limitation of the study.

In conclusion a glyceride formulation of EPA+DHA resulted in higher total plasma EPA and DHA over a 12 h period following single dosing and achieved a maximal concentration faster than observed with an EE formulation of EPA+DHA. Therefore, a mix of mono-, di-, and TG can provide superior absorption and higher amounts of bioactive EPA and DHA than a commonly available EE formulation.

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Conflicts of interest

GMK and SOH are employees of BASF AS. PCC has acted as a consultant to BASF AS. HLF has no conflicts to declare

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Table 1. Characteristics of the participants at study entry

| | Males n= 10 | Females n =10 | P |
|------------------------------------|-----------------------|-------------------------|----------|
| Age, y | 59 (57, 63) | 58 (56, 61) | 0.278 |
| BMI, kg/m ² | 24.90 (24.30, 27.30) | 28.60 (25.10, 31.09) | 0.009 |
| Capsule compliance, % | 100 ± 0 | 100 ± 0 | |
| Plasma TG, mmol/L | 1.05 (0.80, 1.60) | 1.00 (0.80, 1.20) | 0.411 |
| Plasma total cholesterol, mmol/L | 5.45 (5.10, 5.90) | 5.90 (5.60, 7.50) | 0.019 |
| Plasma HDL cholesterol, mmol/L | 1.45 (1.26, 1.78) | 1.72 (1.33, 2.10) | 0.068 |
| Plasma non-HDL cholesterol, mmol/L | 3.92 (3.53, 4.54) | 4.42 (3.88, 5.37) | 0.058 |
| Plasma glucose, mmol/L | 5.40 (5.10, 5.90) | 5.05 (4.90, 5.50) | 0.050 |
| Plasma insulin, mU/L | 5.95 (3.40, 6.90) | 4.40 (3.30, 7.90) | 0.828 |
| HOMA2-IR | 0.70 (0.64, 0.83) | 0.67 (0.61, 0.83) | 0.299 |
| Plasma hsCRP, mg/L | 1.36 (0.44, 2.57) | 2.27 (1.13, 3.32) | 0.129 |
| Omega-3 Index (RBC EPA +DHA), % | 5.78 (4.94, 6.06) | 5.45 (4.44, 5.74) | 0.307 |
| Plasma relative EPA, % | 0.67 (0.60, 0.86) | 0.78 (0.66, 0.95) | 0.267 |
| Plasma relative DPA, % | 0.51 (0.45, 0.58) | 0.50 (0.45, 0.53) | 0.516 |
| Plasma relative DHA, % | 1.66 (1.47, 1.99) | 1.50 (1.41, 1.80) | 0.213 |
| Plasma relative EPA + DHA, % | 2.44 (2.02, 2.85) | 2.48 (2.14, 2.61) | 0.589 |
| Plasma total EPA, µg/mL | 21.89 (19.34, 34.23) | 28.78 (20.02, 33.65) | 0.417 |
| Plasma total DPA, µg/mL | 17.19 (14.85, 20.73) | 17.67 (13.24, 22.43) | 0.935 |
| Plasma total DHA, µg/mL | 57.66 (48.52, 66.09) | 53.66 (47.55, 65.31) | 0.465 |
| Plasma total EPA + DHA, µg/mL | 79.52 (69.15, 105.71) | 80.65 (69.26, 101.91) | 0.935 |

Values are median (25, 27th percentile). All participants consumed both treatments in a crossover design separated by at least a 2-week period. Capsule consumption was supervised resulting in 100% compliance. *P* for comparison of male and female participants; significance defined as ≤ 0.050 .

HOMA2-IR: homeostatic model assessment of insulin resistance, hsCRP: C-reactive protein measured with a high-sensitivity assay

Table 2. Summary of changes (Δ) in plasma EPA, DPA and DHA concentrations over 12 h after a single dose of EE-EPA + DHA or glyceride mix - EPA + DHA in healthy adults¹

| Absolute concentration | Glyceride-EPA+DHA | EE-EPA+DHA | Ratio² | P |
|-------------------------------|--------------------------|-------------------|--------------------------|--------------|
| Plasma total EPA | | | | |
| iAUC, h \times μ g/mL | 344 (189, 405) | 37 (29, 66) | 9.3 | ≤ 0.001 |
| C _{max} , μ g/mL | 58 (35, 76) | 6 (4, 10) | 9.7 | ≤ 0.001 |
| T _{max} , h | 4 (4, 5) | 8 (5, 12) | | ≤ 0.001 |
| Plasma total DPA | | | | |
| iAUC, h \times μ g/mL | 41 (26, 68) | 25 (15, 39) | 1.6 | 0.023 |
| C _{max} , μ g/mL | 6 (4, 10) | 2 (2, 5) | 3.0 | 0.018 |
| T _{max} , h | 4 (4, 5) | 5 (3, 8) | | 0.520 |
| Plasma total DHA | | | | |
| iAUC, h \times μ g/mL | 152 (96, 245) | 63 (47, 98) | 2.4 | ≤ 0.001 |
| C _{max} , μ g/mL | 22 (9, 38) | 8 (5, 14) | 2.8 | 0.013 |
| T _{max} , h | 4 (3, 5) | 5 (3, 8) | | 0.121 |
| Plasma total EPA+DHA | | | | |
| iAUC, h \times μ g/mL | 525 (292, 655) | 98 (72, 156) | 5.4 | ≤ 0.001 |
| C _{max} , μ g/mL | 79 (51, 113) | 15 (7, 24) | 5.3 | 0.044 |
| T _{max} , h | 4 (4, 5) | 6 (3, 12) | | ≤ 0.001 |
| Relative concentration | Glyceride-EPA+DHA | EE-EPA+DHA | Ratio² | P |
| Plasma total EPA | | | | |
| iAUC, h \times % | 18 (14, 23) | 15 (13, 21) | 1.2 | ≤ 0.001 |
| C _{max} , % | 1 (0.2, 2) | 0.6 (0.2, 1) | 1.7 | 0.002 |
| T _{max} , h | 5 (4, 6) | 6 (4, 8) | | ≤ 0.001 |
| Plasma total DPA | | | | |
| iAUC, h \times % | 2 (1, 2) | 0.7 (0.5, 1) | 2.8 | < 0.001 |
| C _{max} , % | 0.2 (0.2, 0.2) | 0.1 (0.1, 0.1) | 2.0 | < 0.001 |
| T _{max} , h | 6 (4, 8) | 5 (4, 8) | | 0.880 |
| Plasma total DHA | | | | |
| iAUC, h \times % | 17 (14, 18) | 15 (14, 18) | 1.1 | ≤ 0.001 |
| C _{max} , % | 0.6 (0.3, 0.8) | 0.4 (0.3, 0.8) | 1.5 | 0.015 |
| T _{max} , h | 5 (4, 8) | 6 (4, 8) | | ≤ 0.001 |
| Plasma total EPA+DHA | | | | |
| iAUC, h \times % | 22 (18, 29) | 18 (15, 27) | 1.2 | ≤ 0.001 |
| C _{max} , % | 2 (0.4, 3) | 1 (0.4, 2) | 2.0 | 0.001 |
| T _{max} , h | 5 (5, 7) | 6 (5, 8) | | ≤ 0.001 |

¹Values are median (25th, 75th percentile). *P* values were derived by using the Kruskal-Wallis test.

²The ratio of glyceride-EPA+DHA compared with EE-EPA+DHA for iAUC and C_{max}. EE, ethyl-ester EPA+DHA; C_{max}, maximum concentration change; glyceride mix, mix of mono, di and triglyceride EPA+DHA; iAUC, incremental AUC; T_{max}, time at which C_{max} occurs.

Figure 1. CONSORT diagram of participant inclusion and flow through the study. CONSORT, Consolidated Standards of Reporting Trials; EE, ethyl ester; glyceride, mix of mono-, di- and triglyceride EPA+DHA.

Figure 2. Changes from baseline in absolute concentration of plasma total EPA, DPA, DHA, and EPA+DHA after a single dose of glyceride-EPA+DHA or EE-EPA+DHA in healthy older adults. Values are median \pm IQR. Glyceride-EPA+DHA n = 20, EE-EPA+DHA n = 20. EE, ethyl-ester; glyceride, mix of mono-, di-, and triglycerides.

Figure 3. Changes from baseline in relative concentration of plasma total EPA, DPA, DHA, and EPA+DHA after a single dose of glyceride-EPA+DHA or EE-EPA+DHA in healthy older adults. Values are median \pm IQR. Glyceride-EPA+DHA n = 20, EE-EPA+DHA n = 20. EE, ethyl-ester; glyceride, mix of mono, di, and triglycerides.