A novel self-micro-emulsifying delivery system enhances enrichment of eicosapentaenoic acid and docosahexaenoic acid after single and repeated dosings in healthy adults in a randomized trial\textsuperscript{1-3}

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Supplementary data (2 tables and 3 figures) are submitted

Running title: Self-micro-emulsifying delivery system for omega-3
Supplemental Table 1, Supplemental Table 2, Supplemental Figure 1, Supplemental Figure 2 and Supplemental Figure 3 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.

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Abbreviations used: BHT, butylated hydroxytoluene; BMI, body mass index; C_{max}, maximum concentration change; DHA, docosahexaenoic acid; EE, ethyl ester; EPA, eicosapentaenoic acid; iAUC, incremental area under the curve; MNC, peripheral blood mononuclear cell; RBC, red blood cell; SMEDS, self-micro-emulsifying delivery system; T_{max}, time at which C_{max} occurs.

Clinical trial registration: ISRCTN96459690 at www.isrctn.com
Abstract

Background. A self-micro-emulsifying delivery system (SMEDS) promotes spontaneous emulsification of omega-3 ethyl esters (EEs) into microdroplets in the stomach.

Objective. The objective was to compare the effect of SMEDS preparations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) EEs with standard EEs on EPA and DHA concentrations in the bloodstream following a single dose and repeated daily dosing.

Methods. Eighty healthy subjects aged 18 to 65 y were randomly assigned to SMEDS-EPA, EE-EPA (both providing more EPA than DHA), SMEDS-DHA or EE-DHA (both providing more DHA than EPA). They consumed a single dose (1.23–1.33 g EPA+DHA) without a meal and EPA and DHA were measured in plasma over the following 24 h. Participants continued to take a single dose each morning before breakfast for 12 wk. EPA and DHA were measured in fasting plasma, mononuclear cells (MNCs) and red blood cells (RBCs).

Results. EPA and DHA were higher in plasma in the 24 h after a single dose of SMEDS-EPA or -DHA than after consuming the comparator EE ($P < 0.001$ for both). Compared with the EE form, repeated daily dosing of the SMEDS formulations for 12 wk resulted in higher concentrations of EPA and DHA in plasma ($P = 0.086$ and 0.005), MNCs ($P < 0.001$ and 0.020) and RBCs (both $P < 0.001$). The omega-3 index increased over 12 wk from $5.1 \pm 0.9$ to $7.9 \pm 0.9$ in the SMEDS-EPA group, from $5.3 \pm 1.1$ to $9.0 \pm 1.2$ in the SMEDS-DHA group, from $4.8 \pm 0.8$ to $6.4 \pm 0.9$ in the EE-EPA group and from $5.2 \pm 0.9$ to $7.2 \pm 1.0$ in the EE-DHA group (all $P < 0.001$). Omega-3 index was higher with SMEDS than with comparator EE at 12 wk (both $P < 0.001$).

Conclusion. Compared with standard EEs, a SMEDS results in greater incorporation of EPA and DHA into blood pools after a single dose and with repeated daily dosing in healthy adults. A SMEDS enhances delivery of bioactive omega-3 fatty acids.

Clinical trial registration: ISRCTN96459690 at www.isrctn.com
Key words: Omega-3, Fish oil, Eicosapentaenoic acid, Docosahexaenoic acid, Omega-3 index, Bioavailability, Emulsification, SMEDS
**Introduction**

Long chain omega-3 fatty acids have been linked to many health benefits such as reduced risk of heart disease (1, 2), most likely due to an improved risk factor profile (3, 4), less inflammation (5, 6) and improvements in psychological, psychiatric and cognitive outcomes (7-12). Both eicosapentaenoic acid (EPA)\(^6\) and docosahexaenoic acid (DHA) have beneficial effects (13-15). As a result of these benefits, many governments, regulatory authorities and scientific societies have issued recommendations for western populations to consume oily fish, an important source of EPA and DHA, or to have a minimum intake of EPA + DHA (typically around 250 to 500 mg/d) (see 16). However, in many countries including the United Kingdom and the United States, intake of oily fish is low. Supplements that contain EPA and DHA can provide an alternative source of bioactive omega-3 fatty acids to oily fish. Irrespective of their source, the biological actions of EPA and DHA require their delivery to the bloodstream, to cells and to tissues (16). Limited delivery would result in limited biological impact and might explain why some studies fail to find beneficial effects of EPA and DHA. Thus, there is great interest in strategies to enhance EPA and DHA delivery.

Altering the chemical form in which EPA and DHA are administered (triglyceride, ethyl ester (EE), free fatty acid) has only limited impact on delivery to the bloodstream and blood cells when meals containing fat are being consumed (17, 18). However, from studies performed to date, the EE form of EPA and DHA shows little incorporation into blood lipids and cells if consumed without food or following a low fat meal (19, 20). This is important in the context of meal skipping or where meals contain little fat, both of which would limit digestion and absorption of esterified forms of omega-3 fatty acids from supplements. In contrast, the free fatty acid form is superior to esterified omega-3 fatty acids in the absence of a fatty meal (21), because unlike the esterified forms of EPA and DHA, the free fatty acid form is less reliant upon the machinery of digestion and
absorption that is promoted by having fat in the meal. There is also some discussion around the impact of the phospholipid form of EPA and DHA on their delivery to the bloodstream (22).

Preformed emulsions of oil containing EPA and DHA in triglyceride form resulted in greater EPA and DHA appearance in plasma triglycerides over the postprandial period (over 6-8 h post ingestion) when compared to standard unemulsified oil (23). This is possibly due to increased enzymatic hydrolysis of the preformed emulsion in the duodenum due to the lipid droplet size. A self-micro-emulsifying delivery system (SMEDS) has been developed which promotes spontaneous emulsification of omega-3 EEs into microdroplets in the gastric environment (24). This may aid EPA and DHA digestion and absorption in the absence of a fatty meal. Very recently, a SMEDS preparation of omega-3 EEs was shown to enhance EPA and DHA appearance in total plasma lipid over 48 h compared with standard EEs (25).

The current study aimed to compare the influence of SMEDS preparations of EPA and DHA EEs with standard EE forms on EPA and DHA concentrations in blood plasma following a single dose and in blood plasma, peripheral blood mononuclear cells (MNCs) and red blood cells (RBCs) following repeated dosing. The primary hypothesis was that the SMEDS formulations would result in higher concentrations of EPA and DHA in RBCs after repeated dosing with a higher omega-3 index (EPA + DHA in RBCs). The secondary hypotheses were that the SMEDS formulations would result in higher concentrations of EPA and DHA in blood plasma after a single dose and in blood plasma and MNCs after repeated dosing. As far as we are aware, this is the first report of repeated dosing of a SMEDS formulation of omega-3 fatty acids.
Subjects, materials and methods

Subjects

All procedures involving human subjects were approved by the South Central - Hampshire A National Health Service Research Ethics Committee (REC 15/SC/0775). The trial was conducted according to the principles of the Declaration of Helsinki and all participants signed written informed consent prior to enrolment. The study is registered at www.isrctn.com (study ID ISRCTN96459690).

Eighty healthy participants (evenly stratified for sex and age) were enrolled into the study.

The inclusion criteria for participation were: age between 18 and 65 y, body mass index (BMI) between 20 and 35 kg/m², self-reported dietary oily fish intake < 1 portion per wk, and omega-3 index (EPA+DHA in RBCs) measured in a screening blood sample < 6.5. Exclusion criteria were any chronic medical condition; gastrointestinal problems; allergies to fish or soya products; smoking; pregnancy or lactation; or consumption of any omega-3 fatty acid supplements. The study took place between April 2016 and May 2017.

Study design and supplements

This study was a double blind, parallel design, randomized trial that investigated two encapsulated SMEDS formulations of EEs of EPA and DHA (SMEDS-EPA and SMEDS-DHA, respectively) alongside the standard EE forms acting as controls (EE-EPA and EE-DHA, respectively). All formulations contained both EPA and DHA but SMEDS-EPA and EE-EPA were richer in EPA than DHA while SMEDS-DHA and EE-DHA were richer in DHA than EPA (see below). Participants consumed three capsules per d of either SMEDS or standard EE. All formulations were presented in soft gelatine capsules of similar appearance. The SMEDS and EE forms were matched for EPA and DHA content as closely as possible and the total amount of EPA+DHA provided in all four groups was 1.23 to
1.33 g per d. SMEDS-EPA provided 726 mg of EPA and 576 mg of DHA in three capsules, while EE-
EPA provided 684 mg of EPA and 549 mg of DHA. SMEDS-DHA provided 408 mg of EPA and 918 mg
of DHA in three capsules while EE-DHA provided 381 mg of EPA and 888 mg of DHA.

Blinding, randomization and supplement packaging were completed by the Research
Pharmacy at Southampton General Hospital, Southampton, UK by individuals independent of the
researchers conducting the study. The randomization process was achieved by manually drawing
counters from a pot which randomly allocated the 80 participants into the 4 treatment groups,
whilst stratifying for age and sex to ensure an even distribution of males aged 18 to 40 y, males
aged 41 to 65 y, females aged 18 to 40 y and females aged 41 to 65 y across the groups. The
researchers maintained treatment group blinding until statistical analysis of all data was complete.
Participants attended the National Institute for Health Research Wellcome Trust Clinical
Research Facility, Southampton General Hospital, Southampton, UK on five occasions. The first
was a screening visit during which patients provided written informed consent prior to having
their weight and height measured and providing a blood sample. The latter was used to determine
the RBC omega-3 index (EPA+DHA) with a value < 6.5 being required for study entry. Volunteers
who met all inclusion/exclusion criteria were enrolled into the study and randomized. These
participants made a further four clinic visits all in the fasted state (≥ 10 h without food or drink
other than water). The first of these four visits was at approximately 0730 h when a cannula was
inserted into a forearm vein. Participants provided a zero-time blood sample after which they
ingested a single dose (i.e. three capsules) of the study supplement with water. A member of the
research nursing team observed capsule ingestion to ensure compliance and the time of
consumption was accurately recorded. Further blood samples were collected at 0.5, 1, 1.5, 2, 3, 4,
6, 8, 12 and 24 h post-supplement ingestion. Low fat meals with decaffeinated tea or coffee were
given directly after the 3, 6 and 12 h blood samples were collected. The 3 h meal consisted of 2
slices of toast without spread but with jam accompanied by tea or coffee made with skimmed
(0.1% fat) milk. The 6 h meal was identical to the 3 h meal but with the addition of an apple or
orange. The 12 h meal was a light meal of sandwiches, fruit or cake and a juice drink. The 3 and 6 h
meals contained 22 g fat each while the 12 h meal contained < 40 g fat. Participants were asked to
fast from after the 12 h time point meal until the 24 h sample was collected. They were allowed to
leave the clinical facility between the 12 and 24 h time points. After this 24 h investigation,
participants were requested to continue taking 3 capsules daily for 12 wk, following a ≥ 10 h
overnight fast and at least 30 min prior to breakfast consumption. They returned to the Clinical
Research Facility for fasted blood sample collections following 1 wk, 4 wk and 12 wk
supplementation.

Sample preparation
Blood was collected into tubes containing EDTA and directly stored on ice before being processed
within one h of collection. Plasma was prepared by centrifugation of blood samples collected at all
time points at 3000 x g for 15 min at 4°C and analysed for EPA and DHA contents. An enzyme
inhibitor cocktail containing sodium fluoride, L-ascorbic acid and 5-methylisoxazole-3-carboxylic
acid was added to an additional plasma aliquot prepared from blood collected at the first 0, 0.5, 1,
1.5, 2, 3, 4, 6, 8, 12, and 24 h time points; this was used for free EPA and DHA determination.
Samples were stored at -80°C prior to analysis. Peripheral blood MNCs and RBCs were isolated
from whole blood at baseline (zero-time blood sample) and following 1, 4 and 12 wk of
supplementation. This was achieved by centrifugation of blood layered onto Histopaque (Sigma-
Aldrich, Poole, UK) at 1500 x g for 10 min at room temperature and with no brake used to slow the
centrifuge. Isolated cells were washed twice in phosphate buffered saline before storage at -80°C
prior to analysis.
Fatty acid composition analysis

Pharmacokinetic data: determination of total plasma and non-esterified EPA and DHA concentrations following single dosing

Total lipid was extracted from plasma using chloroform/methanol (1:1; vol/vol). EPA and DHA were released from esterified lipids and simultaneously derivatized to methyl esters by incubation with 1% H₂SO₄ in methanol for a minimum of 16 h at 60°C. The samples were then cleaned up with a liquid/liquid extraction using 5% (w/v) KCl/KHCO₃ solution and hexane, followed by solid phase extraction using a SI silica cartridge (Agilent). The samples were then analysed on a liquid chromatography-tandem mass spectrometer, using an Accucore PFP 100 mm x 2.1 mm x 2.6 µm column (Thermo Scientific). Internal standards (eicosapentaenoic acid-d₅ and docosahexaenoic acid-d₅ (Cayman Chemicals)) were used for quantification purposes and butylated hydroxytoluene (BHT) was present to prevent fatty acid oxidation.

Free EPA and DHA were isolated from the plasma lipid extract using solid phase extraction on NH₂ cartridges (VWR); the free acids were eluted using diethyl ether/acetic acid (100:2, v/v). Methyl esters were formed by incubation with 1% H₂SO₄ in methanol. The samples were then analysed on a liquid chromatography-tandem mass spectrometer fitted with a Halo C₁₈ column (50 mm x 2.1 mm x 2.7 µm, manufactured by Hichrom). Internal standards (eicosapentaenoic acid-d₅ and docosahexaenoic acid-d₅ (Cayman Chemicals)) were used for quantification purposes and BHT was present to prevent fatty acid oxidation.

Determination of plasma total lipid EPA and DHA concentrations following repeated dosing
Lipid was extracted from plasma using 5 mL of chloroform/methanol (2:1; v/v) containing 0.2 M BHT. 1 M sodium chloride (1 mL) was added and the sample vortexed and then centrifuged. The lower solvent phase was aspirated and evaporated to dryness under nitrogen at 40°C. The lipid extract was redissolved in 0.5 mL toluene and fatty acids were released from esterified lipids and simultaneously derivatized to methyl esters by incubation with 1 mL 2% H₂SO₄ in methanol for a minimum of 2 h at 50°C to form fatty acid methyl esters. The samples were then neutralized and fatty acid methyl esters transferred into hexane for analysis by gas chromatography. Fatty acid methyl esters 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. Gas chromatography run conditions were as described elsewhere (26, 27). Dipentadecanoyl phosphatidylcholine added into the initial plasma sample was used as an internal standard for quantification purposes and a Supelco® 37 Component FAME Mix was used as a calibration reference standard (Sigma-Aldrich).

**Determination of MNC and RBC EPA and DHA following repeated dosing**

EPA and DHA in MNCs and RBCs were determined using the same methods as described above for total plasma except that the lipid extraction was performed on frozen cell pellets and no internal standard was used.

**Other laboratory analyses**

The plasma concentrations of triglycerides, cholesterol, HDL-cholesterol, non-esterified fatty acids and glucose were measured using enzyme-linked colourimetric assays (Alpha laboratories, UK; and Microgenics GmbH, Germany) on a Konelab 20 auto-analyser in accordance with manufacturer’s instructions. LDL-cholesterol concentration was calculated using the Friedwald equation. Plasma
insulin concentration was measured by ELISA (Access ultrasensitive Insulin kit; Beckman Coulter, UK). Plasma C-reactive protein concentration was measured by using a high-sensitivity ELISA kit (CRP Latex kit; Beckman Coulter, UK).

Statistical analysis

The study sample size was estimated according to the anticipated change in EPA + DHA content of RBCs (Omega-3 index). Based upon previous studies of this sort, a standard supplement providing 1 to 1.5 g EPA plus DHA was expected to increase the omega-3 index by 3 (e.g. from 6.5 to 9.5). It was estimated that the SMEDS formulation would increase the omega-3 index by a further 30% i.e. by 4. Using a SD of 1.5 for both changes, a sample size of 15 per group was estimated to give 90% power of detecting this difference as statistically significant, by a pairwise comparison and setting \( P < 0.05 \). In order to allow for a drop-out rate of 25%, 20 subjects per group were recruited (80 participants in total).

EPA and DHA in plasma are expressed as absolute concentration (\( \mu g/mL \) plasma) while EPA and DHA in MNCs and RBCs are expressed as relative concentration (% of total fatty acids). All fatty acid data were normalized against the baseline concentrations and the distribution of all data sets was checked. Any skewed data were normalized by logarithmic transformation. Incremental area-under-the-curve (iAUC), maximum concentration change (\( C_{\text{max}} \)) and time point at which \( C_{\text{max}} \) was achieved (\( T_{\text{max}} \)) were calculated using GraphPad Prism version 7 (GraphPad, USA). Repeated measures ANOVA was completed on all time course data and the analyses controlled for the possible confounding effects of age and sex. One-way ANOVA was used to compare baseline characteristics between treatment groups and a univariate analysis was used to test circulating blood cell fatty acid concentrations whilst controlling for age and sex. Kruskal Wallis tests were used to compare plasma iAUC, \( C_{\text{max}} \) and \( T_{\text{max}} \) as log transformation was unable to correct the
skewed nature of these data. All statistical analyses were carried out using SPSS version 20 (IBM, USA). In all cases a value for \( P < 0.05 \) was taken to indicate statistical significance while a value for \( P < 0.10 \) but \( > 0.05 \) was taken to indicate a trend.

269 Results

270 Participant characteristics

271 Figure 1 illustrates the flow of participants through the study and numbers of participants in each treatment group. A total of 80 participants were randomized equally across the four treatment groups. Two participants withdrew from the study prior to completion: one withdrew during the “single dose” clinic visit because he did not like being so long without food while the other stopped taking study capsules prior to elective surgery. Compliance to study supplements was checked by a count of returned capsules at the end of the intervention. According to this, the average compliance amongst the 78 participants who completed the study was 99.8\% and this did not differ among the four groups.

279 The mean age of the 78 participants who completed the study was 40.1 ± 13.2 y (range 18 to 65 y) and mean BMI was 26.2 ± 4.2 kg/m\(^2\) (range 20 to 35 kg/m\(^2\)). Seventeen participants had a BMI between 30 and 35 kg/m\(^2\). The participants had a mean omega-3 index of 5.1 ± 0.9. Detailed participant characteristics are presented in Table 1. The baseline concentrations of EPA and DHA in both plasma and circulating cells were not significantly different among the participants in the different treatment groups (Table 1).

285 Pharmacokinetic patterns of EPA and DHA (single dose)

286 Both formulations of SMEDS resulted in a rapid increase in the concentrations of EPA and DHA in the plasma total lipid pool (Figure 2). This resulted in significantly higher maximum concentration
changes ($C_{\text{max}}$) and greater iAUC for both EPA and DHA in the plasma of participants taking the
SMEDS formulation when compared to those taking the corresponding EE ($P \leq 0.002$ for all; Table
2). While EPA reached its $C_{\text{max}}$ at a similar time with both SMEDS and standard EE formulations,
SMEDS-EPA resulted in DHA reaching its $C_{\text{max}}$ in total plasma lipid 4 h earlier than in the EE-EPA
group, while for SMEDS-DHA, this was 8 h earlier when compared to the EE-DHA group (Table 2).
Both SMEDS formulations resulted in greater iAUC and higher $C_{\text{max}}$ for both EPA and DHA
within the plasma free fatty acid pool when compared to the EE controls ($P < 0.031$ for all; Figure
3; Table 2).

**EPA and DHA incorporation patterns in plasma with repeated dosing**

**SMEDS-EPA vs EE-EPA**

Both SMEDS-EPA and EE-EPA supplements resulted in a significant increase in the concentration of
EPA within the plasma total lipid pool over the 12 wk supplementation period (Figure 4A; $P$ for
effect of time < 0.001), but SMEDS-EPA resulted in significantly greater EPA enrichment when
compared to EE-EPA ($P$ for effect of treatment = 0.002; $P$ for time x treatment interaction = 0.003).
Consequently, SMEDS-EPA resulted in a higher maximum concentration change of EPA than EE-
EPA ($P = 0.096$; Table 3). SMEDS-EPA also resulted in a significantly higher maximum concentration
change of DHA than EE-EPA ($P = 0.005$; Figure 4B; Table 3).

**SMEDS-DHA vs EE-DHA**

Both SMEDS-DHA and EE-DHA supplements resulted in a significant increase in the concentration
of DHA within the plasma total lipid pool over the 12 wk supplementation period (Figure 4D; $P$ for
effect of time < 0.001), but SMEDS-DHA resulted in significantly greater DHA enrichment when
compared to EE-DHA ($P$ for effect of treatment = 0.004; $P$ for time x treatment interaction =
Consequently, SMEDS-DHA resulted in a higher maximum concentration change of DHA than EE-DHA (P = 0.005; Figure 4D; Table 3). SMEDS-DHA also resulted in a significantly higher maximum concentration change of EPA than EE-DHA (P = 0.033; Figure 4C; Table 3).

EPA and DHA incorporation patterns in MNCs and RBCs with repeated dosing

The concentrations of EPA and DHA increased in MNCs following 12 wk supplementation with both SMEDS and standard EE supplements, with significantly greater incorporation seen following the SMEDS supplements compared to the EEs (P ≤ 0.020 in all cases; Figure 5; Table 3). The concentrations of EPA and DHA increased in RBCs following 12 wk supplementation with both SMEDS and standard EE supplements, with significantly greater incorporation seen following the SMEDS supplements compared to the EEs (P ≤ 0.003 in all cases; Figure 6; Table 3). Consequently, the SMEDS groups showed greater increases in the omega-3 index compared to the EE groups at 12 wk (P < 0.001; Figure 7; Table 3). When looking at the EPA and DHA assimilation into RBCs separately at the 12 wk time point, there was a 2- or 3-fold increase in RBC EPA concentration when the supplement was consumed in SMEDS form compared to EE (SMEDS-EPA vs. EE-EPA P < 0.001; SMEDS-DHA vs. EE-DHA P = 0.002). The SMEDS supplement resulted in a 1.5-2-fold difference in RBC DHA concentration when compared to the EE (SMEDS-EPA vs. EE-EPA P < 0.001; SMEDS-DHA vs. EE-DHA P < 0.001). The SMEDS-EPA induced a 2.8 point increased the omega-3 index from a mean (+ SD) of 5.1 ± 0.9 to 7.9 ± 0.9, compared to the EE-EPA which caused a more modest increase of 1.6, from 4.8 ± 0.8 to 6.4 ± 0.9 (both P < 0.001). The SMEDS-DHA caused a greater increase in omega-3 index with a rise of 3.7 bringing the omega-3 index up from 5.3 ± 1.1 to 9.0 ± 1.2 (P < 0.001). The EE-DHA induced a 2 point increase bringing the index from 5.2 ± 0.9 to 7.2 ± 1.0 (P < 0.001). At the end of the supplementation period omega-3 index was higher in both SMEDS groups than in the respective EE groups (both P < 0.001). In the SMEDS-EPA
group 50.0% of participants achieved an omega-3 index of ≥ 8, while in the EE-EPA group this was 10.5%. Likewise, in the SMEDS-DHA group 70.0% of participants achieved an omega-3 index of ≥ 8 while in the DHA-EE group this was 25.0%.

Normalization according to the amount of EPA and DHA given

The data shown in Figures 2 to 7 and Tables 2 and 3 do not take into account that the SMEDS formulations had slightly more EPA and DHA than the EE comparators (see Subjects, materials and methods). All data were therefore recalculated normalising for this (with the amount of EPA and DHA provided in g/d). Selected normalized data are shown in Supplemental Figures 1, 2 and 3 (Plasma total EPA and DHA after single dosing, RBC EPA and DHA after repeated daily dosing, and omega-3 index after repeated daily dosing, respectively) and a summary of the normalized data after single dosing and after repeated dosing is shown in Supplemental Tables 1 and 2, respectively. There was very little effect of this normalization of the data on the responses to the single oral dose: measures of statistical significance for plasma total EPA, DHA and EPA+DHA and for plasma free EPA were hardly changed and no comparisons lost significance (Supplemental Figure 1; Supplemental Table 1), while for plasma free DHA and EPA+DHA previously significant comparisons between SMEDS-EPA and EE-EPA for iAUC and Cmax became borderline significant ($P = 0.050$ to 0.076; Supplemental Table 1). Normalization of the data following repeated daily dosing resulted in some previously significant differences in summary data for plasma omega-3 fatty acids becoming borderline significant but all comparisons for MNCs and RBCs remained significant (Supplemental Figure 2, Supplemental Figure 3 and Supplemental Table 2). Taking these findings into consideration, it is apparent that normalization of data for the amount of omega-3 fatty acid provided (in g) does not materially alter the findings or conclusions of the study.
The current study used a SMEDS formulation rich in either EPA or DHA EEs to test the hypothesis that enrichment of blood pools with EPA and DHA would be greater than seen with the parent EEs. Both single dosing and repeated dosing approaches were used. It was shown that, compared with the standard EEs, use of SMEDS significantly increases incorporation of both EPA and DHA into blood pools after a single dose and with repeated daily dosing, so improving the omega-3 index over the period of several wk.

In foods and many supplements, omega-3 fatty acids are found esterified into triglycerides and phospholipids. Supplemental forms of omega-3 EEs are also available. Esterified forms require solubilization and hydrolysis in the upper gastrointestinal tract ("digestion") prior to the omega-3 fatty acids being available for absorption. Digestion involves the release into the gastrointestinal lumen of bile providing emulsifying bile salts and of pancreatic secretions including pancreatic lipase that hydrolyses the esterified lipid substrate freeing the omega-3 fatty acids. One of the most important stimuli for the release of bile and pancreatic lipase is fat in the meal. Hence, taking supplements of esterified omega-3 fatty acids without a meal or with a meal that is very low in fat significantly impairs uptake of EPA and DHA into the bloodstream compared to if the meal contains fat (21). The health benefits of EPA and DHA require that EPA and DHA are delivered to the bloodstream and beyond into cells and tissues (16). If individuals chose to obtain EPA and DHA from esterified forms within supplements, then those supplements probably need to be taken with a meal containing fat. Indeed, it has been argued that the failure of some omega-3 fatty acid clinical trials is because participants consumed their supplements in the absence of a fatty meal, for example around the time of a low fat breakfast or late in the evening (28). Interestingly, use of a supplement with free EPA and DHA, which would require less emulsification and no hydrolysis to permit EPA and DHA absorption, resulted in greater appearance of EPA and DHA in the
bloodstream after a single dose with a low fat meal than seen with the EE form (21). The
superiority of the free form of omega-3 fatty acids over the EE form in terms of delivery of EPA
and DHA to the bloodstream was abolished when the supplements were consumed with a fatty
meal (21). The current study supports an alternative approach that enhances availability of EPA
and DHA from EEs in the absence of a fatty meal. Self-emulsification of EEs in situ resulted in
higher concentrations of both EPA and DHA in plasma in the h following a single dose compared
with what was seen with the normal EE formulations. This observation supports the recently
reported findings with the SMEDS preparation of omega-3 EEs (25). Furthermore, a similar
approach to in situ emulsification of omega-3 EE oils has been shown to improve the poor EPA and
DHA appearance in blood lipids over a 24 h period seen when EEs are consumed with a low fat
meal (29, 30). Hence, in situ self-emulsification of omega-3 EEs results in greater appearance of
both EPA and DHA in the bloodstream in the h after their consumption in the absence of a fatty
meal. Given that the appearance of EPA and DHA in the bloodstream in the absence of a fatty
meal is enhanced by both the free fatty acid forms of omega-3 fatty acids (21) and the SMEDS
formulation (25, current study), it will be interesting to directly compare these two approaches.
Following the single dose, we measured omega-3 fatty acids in total plasma and in the
plasma non-esterified fatty acid fraction. After their absorption, fatty acids are esterified into
triglycerides which are released into the lymph and then the bloodstream as components of
chylomicrons. Triglyceride fatty acids are depleted from the chylomicrons as they circulate in the
bloodstream and remnant particles are formed which are taken up by the liver. The liver also
releases triglycerides as components of very low density lipoproteins, which also become fatty
acid depleted as they circulate, resulting in formation of cholesteryl ester rich lipoproteins that are
cleared by the liver. All lipoproteins have a phospholipid monolayer that stabilizes them in the
aqueous environment. Hence, over the period of 24 h, as studied here, EPA and DHA may circulate
in the bloodstream in esterified form as components of triglycerides, cholesteryl esters and phospholipids and it is the combination of these forms that is measured in total plasma. It is likely that gut-derived (i.e. the newly absorbed) EPA and DHA appear in the bloodstream over the first 4 h or so and that after that liver derived recycling of EPA and DHA dominates (31, 32). In the current study, the largest difference in the concentrations of EPA and DHA in total plasma after single dosing between the SMEDS and EE groups was at 4 h, consistent with the notion of much improved gastrointestinal handling of the SMEDS formulation.

In the current study, we also measured non-esterified EPA and DHA, which increased in concentration over the first 4 h after consuming the single dose. Non-esterified fatty acids are released from triglyceride-rich lipoproteins like chylomicrons and very low density lipoproteins as a result of the action of lipoprotein lipase. Most of these fatty acids are taken up by tissues like adipose tissue, but some escape, a process described as “lack of [tissue] entrapment”. The higher concentrations of free EPA and DHA with the SMEDS formulations than with the standard EEs over the period of 2 to 4 h after the single dose is entirely consistent with these free omega-3 fatty acids coming from the esterified gut-derived (i.e. newly absorbed) lipids in the circulation.

The current study advanced the earlier findings from single dose studies (25) by investigating the effect of repeated daily dosing out to 12 wk. Participants were asked to take their supplements on an empty stomach prior to breakfast. Importantly the repeated dosing study showed higher concentrations of EPA and DHA in plasma, MNCs and RBCs in the SMEDS groups than in the EE groups, suggesting that the greater appearance of EPA and DHA in plasma seen after a single dose ultimately results in higher concentrations of EPA and DHA in cell and tissue pools over time. This enhancement was evident in plasma at one wk and in MNCs and RBCs by 4 wk. Omega-3 index is the sum of EPA plus DHA in RBCs. It is a marker of long term intake of EPA and DHA (33, 34) and also indicates the EPA and DHA content of tissues such as the heart (35).
Omega-3 index is inversely associated with a number of cardiovascular risk factors and with cardiovascular morbidity (36, 37) and mortality (38, 39). Harris and von Schacky (34) suggest that an omega-3 index of 8 or more is associated with optimal cardioprotection. In the current study 65% participants in the SMEDS groups achieved an omega-3 index of 8 or more compared with 17.5% participants in the standard EE groups. Thus, the finding of the current study of significantly higher omega-3 index after daily dosing with the SMEDS formulation of EEs than after daily dosing of the parent EEs themselves is important. It suggests that the SMEDS formulation might have a greater effect on physiology, on risk factors and on cardiovascular morbidity and mortality than the EEs themselves, although this needs to be tested. Another implication of the current findings is that lower amounts of EPA and DHA could be delivered in the SMEDS formulation to achieve the same benefits of a higher amount of EEs. Again this needs to be tested in future research.

The current study has many strengths. First, it combined single dosing, as used by others (21, 25, 29, 30), with repeated daily dosing, the latter being more representative of the real life situation. Second, allocation to study groups was stratified for age and sex, both of which might affect omega-3 fatty acid handling. Third, participants were recruited using omega-3 index as a criterion; a value of ≤ 6.5 was required for inclusion. Fourth, in the single dose study, consumption of the three capsules was observed by a member of the research nursing team to ensure compliance. Fifth, participant retention was high with 78 out of 80 participants completing the repeated dosing study and providing all samples. Sixth, compliance determined by counting of returned capsules was high (over 99%) and similar in all groups. Finally, in the repeated dose study we measured EPA and DHA not only in plasma but also in cells, including in RBCs, a marker considered to reflect tissue levels. These strengths provide significant confidence in our findings.

In conclusion, a SMEDS formulation of EPA and DHA EEs results in higher plasma concentrations of EPA and DHA after a single dose than seen with the parent EEs, and, after
repeated dosing for several wk, results in higher EPA and DHA concentrations in plasma, MNCs and RBCs. SMEDS is an approach to deliver higher amounts of bioactive omega-3 fatty acids than possible with most current formulations.

Conflicts of interest

PCC is an advisor to Pronova BioPharma Norge AS, the funder of this research. GMK and SOH are employees of Pronova BioPharma Norge AS. ALW has no conflicts to declare.

Author’s contributions

PCC, GMK and SOH designed the study. ALW recruited participants, carried out the intervention, processed samples, conducted laboratory and statistical analyses, and drafted the manuscript. PCC supervised all research and finalized the writing of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

We wish to thank Mr Christiaan Gelauf who assisted with the fatty acid analysis.

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Figure captions

Figure 1: Consort diagram of volunteer inclusion and participant flow through the study.
Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Figure 2. Changes from baseline in plasma total EPA (A, C) or DHA (B, D) following a single dose of SMEDS-EPA or EE-EPA (A, B) or SMEDS-DHA or EE-DHA (C, D) in healthy adults. Data are expressed as median with interquartile range as error bars; n 19 for SMEDS-EPA and EE-EPA and n 20 for SMEDS-DHA and EE-DHA. Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Figure 3: Changes from baseline of plasma free EPA (A, C) or DHA (B, D) following a single dose of SMEDS-EPA or EE-EPA (A, B) or SMEDS-DHA or EE-DHA (C, D) in healthy adults. Data are expressed as median with interquartile range error bars; n 19 for SMEDS-EPA and EE-EPA and n 20 for SMEDS-DHA and EE-DHA. Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Figure 4. Changes from baseline in plasma total EPA (A, C) or DHA (B, D) following repeated daily dosing of SMEDS-EPA or EE-EPA (A, B) or SMEDS-DHA or EE-DHA (C, D) in healthy adults. Data are expressed as median with interquartile range error bars; n 19 for SMEDS-EPA and EE-EPA and n 20 for SMEDS-DHA and EE-DHA. Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Figure 5. Changes from baseline in mononuclear cell EPA (A, C) or DHA (B, D) following repeated daily dosing of SMEDS-EPA or EE-EPA (A, B) or SMEDS-DHA or EE-DHA (C, D) in healthy adults.
Data are expressed as median with interquartile range error bars; \( n = 19 \) for SMEDS-EPA and EE-EPA and \( n = 20 \) for SMEDS-DHA and EE-DHA. Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MNC, mononuclear cell.

Figure 6. Changes from baseline in red blood cell EPA (A, C) or DHA (B, D) following repeated daily dosing of SMEDS-EPA or EE-EPA (A, B) or SMEDS-DHA or EE-DHA (C, D) in healthy adults.

Data are expressed as median with interquartile range error bars; \( n = 19 \) for SMEDS-EPA and EE-EPA and \( n = 20 \) for SMEDS-DHA and EE-DHA. Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RBC, red blood cell.

Figure 7. Changes from baseline in omega-3 index (red blood cell EPA + DHA) following repeated daily dosing of SMEDS-EPA or EE-EPA (A) or SMEDS-DHA or EE-DHA (B) in healthy adults. Data are expressed as median with interquartile range error bars; \( n = 19 \) for SMEDS-EPA and EE-EPA and \( n = 20 \) for SMEDS-DHA and EE-DHA. Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RBC, red blood cell.
Table 1. Characteristics of the participants included in the analysis at study entry1.

<table>
<thead>
<tr>
<th></th>
<th>SMEDS-EPA (n 19)</th>
<th>EE-EPA (n 19)</th>
<th>SMEDS-DHA (n 20)</th>
<th>EE-DHA (n 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male : Female)</td>
<td>10 : 9</td>
<td>9 : 10</td>
<td>10 : 10</td>
<td>10 : 10</td>
</tr>
<tr>
<td>Age (y)</td>
<td>40.4 ± 13.4</td>
<td>41.0 ± 13.9</td>
<td>38.9 ± 12.9</td>
<td>39.1 ± 13.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 4.5</td>
<td>26.6 ± 4.1</td>
<td>26.0 ± 4.1</td>
<td>26.0 ± 4.1</td>
</tr>
<tr>
<td>Capsule compliance</td>
<td>99.6 ± 4.9</td>
<td>97.0 ± 5.3</td>
<td>99.6 ± 2.9</td>
<td>98.5 ± 8.8</td>
</tr>
<tr>
<td>Plasma NEFA (µmol/L)</td>
<td>617 ± 372</td>
<td>551 ± 291</td>
<td>579 ± 303</td>
<td>686 ± 390</td>
</tr>
<tr>
<td>Plasma TAG (mmol/L)</td>
<td>1.5 ± 1.9</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>Plasma total cholesterol (mmol/L)</td>
<td>5.1 ± 0.9</td>
<td>5.3 ± 1.2</td>
<td>5.2 ± 1.1</td>
<td>5.2 ± 1.3</td>
</tr>
<tr>
<td>Plasma HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Plasma LDL cholesterol (mmol/L)</td>
<td>3.1 ± 0.5</td>
<td>3.3 ± 1.1</td>
<td>3.3 ± 1.0</td>
<td>3.2 ± 1.2</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.9 ± 0.7</td>
<td>6.0 ± 0.7</td>
<td>5.7 ± 0.7</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>Plasma insulin (mU/L)</td>
<td>6.8 ± 5.9</td>
<td>7.6 ± 8.2</td>
<td>6.7 ± 3.5</td>
<td>5.7 ± 2.5</td>
</tr>
<tr>
<td>Plasma hsCRP (mg/L)</td>
<td>3.0 ± 6.1</td>
<td>1.8 ± 2.8</td>
<td>1.5 ± 1.2</td>
<td>1.7 ± 2.1</td>
</tr>
<tr>
<td>Plasma total EPA (µg/mL)</td>
<td>14.4 ± 5.9</td>
<td>14.7 ± 8.9</td>
<td>13.4 ± 8.3</td>
<td>13.5 ± 6.1</td>
</tr>
<tr>
<td>Plasma total DHA (µg/mL)</td>
<td>31.5 ± 8.9</td>
<td>31.8 ± 12.4</td>
<td>27.1 ± 9.6</td>
<td>26.3 ± 9.4</td>
</tr>
<tr>
<td>RBC EPA (%)</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>RBC DHA (%)</td>
<td>4.3 ± 0.9</td>
<td>4.0 ± 0.7</td>
<td>4.4 ± 0.9</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>Omega-3 index</td>
<td>5.1 ± 0.9</td>
<td>4.8 ± 0.8</td>
<td>5.3 ± 1.1</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>MNC EPA (%)</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>MNC DHA (%)</td>
<td>2.0 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>1.8 ± 0.3</td>
</tr>
</tbody>
</table>

1Except for sex, data are mean ± SD

There were no statistically significant differences among the groups.

Abbreviations used: BMI, body mass index; hsCRP, C-reactive protein measured with a high sensitivity assay; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; MNC, mononuclear cell; NEFA, non-esterified fatty acids; RBC, red blood cell; TAG, triglycerides.
Table 2. Summary of change in EPA and DHA concentrations over 24 hours following a single dose of SMEDS-EPA, EE-EPA, SMEDS-DHA or EE-DHA in healthy adults\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>SMEDS-EPA (n 19)</th>
<th>EE-EPA (n 19)</th>
<th>Ratio(^2)</th>
<th>(P)(^3)</th>
<th>SMEDS-DHA (n 20)</th>
<th>EE-DHA (n 20)</th>
<th>Ratio(^2)</th>
<th>(P)(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma total EPA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC (h x (μg/mL))</td>
<td>319 (234, 387)</td>
<td>19.7 (0, 115)</td>
<td>13</td>
<td>&lt; 0.001</td>
<td>253 (175, 345)</td>
<td>44 (14, 71)</td>
<td>6</td>
<td>0.002</td>
</tr>
<tr>
<td>(C_{max}) (μg/mL)</td>
<td>20 (15, 27)</td>
<td>2.2 (0, 8)</td>
<td>9</td>
<td>&lt; 0.001</td>
<td>14 (8, 19)</td>
<td>4 (2, 6)</td>
<td>4</td>
<td>0.001</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>5 (4, 12)</td>
<td>4 (0, 11)</td>
<td>0.234</td>
<td>10 (4, 12)</td>
<td>8 (2, 12)</td>
<td>8 (2, 12)</td>
<td>0.499</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma total DHA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC (h x (μg/mL))</td>
<td>248 (184, 324)</td>
<td>88 (34, 167)</td>
<td>3</td>
<td>&lt; 0.001</td>
<td>421 (329, 613)</td>
<td>136 (78, 207)</td>
<td>3</td>
<td>0.001</td>
</tr>
<tr>
<td>(C_{max}) (μg/mL)</td>
<td>21 (16, 29)</td>
<td>8 (5, 12)</td>
<td>3</td>
<td>&lt; 0.001</td>
<td>38 (29, 48)</td>
<td>11 (7, 17)</td>
<td>3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>8 (4, 11)</td>
<td>12 (8, 24)</td>
<td>0.017</td>
<td>4 (4, 8)</td>
<td>12 (8, 12)</td>
<td></td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma total EPA+DHA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC (h x (μg/mL))</td>
<td>547 (439, 688)</td>
<td>137 (63, 265)</td>
<td>4</td>
<td>&lt; 0.001</td>
<td>681 (480, 999)</td>
<td>178 (82, 330)</td>
<td>4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(C_{max}) (μg/mL)</td>
<td>39 (33, 58)</td>
<td>12 (8, 20)</td>
<td>3</td>
<td>&lt; 0.001</td>
<td>47 (37, 73)</td>
<td>18 (11, 25)</td>
<td>3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>7 (4, 11)</td>
<td>12 (8, 24)</td>
<td>0.041</td>
<td>4 (4, 8)</td>
<td>10 (8, 12)</td>
<td></td>
<td>0.077</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma free EPA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC (h x (μg/mL))</td>
<td>4 (3, 6)</td>
<td>1 (0.3, 2.2)</td>
<td>4</td>
<td>0.009</td>
<td>2 (2, 3)</td>
<td>0.6 (0.4, 1.4)</td>
<td>3</td>
<td>0.003</td>
</tr>
<tr>
<td>(C_{max}) (μg/mL)</td>
<td>0.6 (0.5, 1.1)</td>
<td>0.1 (0.1, 0.2)</td>
<td>6</td>
<td>&lt; 0.001</td>
<td>0.4 (1.2, 0.5)</td>
<td>0.1 (0.1, 0.2)</td>
<td>4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>4 (3, 4)</td>
<td>3 (2, 4)</td>
<td>0.123</td>
<td>3 (2, 4)</td>
<td>12 (3, 12)</td>
<td></td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma free DHA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC (h x (μg/mL))</td>
<td>12 (5, 18)</td>
<td>4 (2, 10)</td>
<td>3</td>
<td>0.031</td>
<td>12 (10, 16)</td>
<td>3 (2, 4)</td>
<td>4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(C_{max}) (μg/mL)</td>
<td>2 (2, 4)</td>
<td>1 (0.4, 2)</td>
<td>2</td>
<td>0.002</td>
<td>3 (2, 4)</td>
<td>1 (0.3, 0.8)</td>
<td>3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>3 (3, 4)</td>
<td>3 (2, 4)</td>
<td>0.335</td>
<td>3 (3, 4)</td>
<td>3 (2, 12)</td>
<td></td>
<td>0.717</td>
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<tr>
<td><strong>Plasma free EPA+DHA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>iAUC (h x (μg/mL))</td>
<td>15 (8, 23)</td>
<td>5 (2, 10)</td>
<td>3</td>
<td>0.012</td>
<td>15 (12, 19)</td>
<td>3 (3, 6)</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td>(C_{max}) (μg/mL)</td>
<td>3 (2, 5)</td>
<td>1 (1, 2)</td>
<td>3</td>
<td>0.001</td>
<td>3 (2, 4)</td>
<td>1 (0.3, 1)</td>
<td>3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>3 (3, 4)</td>
<td>3 (2, 4)</td>
<td>0.617</td>
<td>3 (3, 4)</td>
<td>3 (2, 12)</td>
<td></td>
<td>0.233</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Except for ratio, data are median (25\(^{th}\), 75\(^{th}\) percentile);

\(^2\)The ratio of SMEDS formulation vs EE for iAUC and \(C_{max}\);

\(^3\)Kruskal Wallis.
Abbreviations used: \( C_{\text{max}} \), maximum concentration change; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; iAUC, incremental area under the curve; \( T_{\text{max}} \), time at which \( C_{\text{max}} \) occurs.
Table 3. Change in EPA and DHA concentration in blood plasma, mononuclear cells and red blood cells following 12 weeks of daily dosing with SMEDS-EPA, EE-EPA, SMEDs-DHA or EE-DHA in healthy adults.1

<table>
<thead>
<tr>
<th></th>
<th>SMEDS-EPA (n 19)</th>
<th>EE-EPA (n 19)</th>
<th>P²</th>
<th>SMEDS-DHA (n 20)</th>
<th>EE-DHA (n 20)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (µg/ml)</td>
<td>28 (17, 37)</td>
<td>16 (12, 33)</td>
<td>0.096</td>
<td>21 (17, 28)</td>
<td>11 (9, 21)</td>
<td>0.033</td>
</tr>
<tr>
<td>DHA (µg/ml)</td>
<td>23 (15, 32)</td>
<td>10 (6, 19)</td>
<td>0.005</td>
<td>39 (27, 47)</td>
<td>23 (14, 30)</td>
<td>0.005</td>
</tr>
<tr>
<td>EPA+DHA (µg/ml)</td>
<td>52 (35, 72)</td>
<td>26 (16, 40)</td>
<td>0.004</td>
<td>59 (42, 71)</td>
<td>30 (23, 51)</td>
<td>0.006</td>
</tr>
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<td><strong>MNCs:</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (%)</td>
<td>1.0 (0.7, 1.3)</td>
<td>0.5 (0.3, 0.7)</td>
<td>&lt; 0.001</td>
<td>0.8 (0.6, 0.9)</td>
<td>0.3 (0.1, 0.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DHA (%)</td>
<td>1.0 (0.7, 1.2)</td>
<td>0.6 (0.3, 0.8)</td>
<td>&lt; 0.001</td>
<td>1.3 (1.0, 1.7)</td>
<td>0.9 (0.7, 1.1)</td>
<td>0.020</td>
</tr>
<tr>
<td>EPA+DHA (%)</td>
<td>2.0 (1.4, 2.3)</td>
<td>0.8 (0.7, 1.6)</td>
<td>&lt; 0.001</td>
<td>2.0 (1.7, 2.5)</td>
<td>1.4 (0.8, 1.7)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>RBCs:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (%)</td>
<td>1.4 (1.1, 1.5)</td>
<td>0.8 (0.5, 1)</td>
<td>&lt; 0.001</td>
<td>1.0 (0.8, 1.2)</td>
<td>0.4 (0.3, 0.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DHA (%)</td>
<td>1.6 (1.1, 1.9)</td>
<td>1.2 (0.7, 1.4)</td>
<td>0.003</td>
<td>2.6 (2.1, 3.1)</td>
<td>1.5 (1.1, 2.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Omega-3 Index (EPA+DHA)</td>
<td>2.8 (2.4, 3.3)</td>
<td>1.6 (1.4, 2.4)</td>
<td>&lt; 0.001</td>
<td>3.7 (3.1, 4.2)</td>
<td>2.0 (1.4, 2.9)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

1 Data are median (25th, 75th percentile);

2 P values determined using multivariate analysis controlling for age and sex.

Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MNC, mononuclear cell; RBC, red blood cell.