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A novel *n*-3 glyceride mixture enhances enrichment of EPA and DHA after single dosing in healthy older adults: results from a double-blind crossover trial

Helena L. Fisk[1,2]*, Grete M. Kindberg[3], Svein O. Hustvedt[3] and Philip C. Calder[1,2]

[1]*School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK*
[2]*NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton SO16 6YD, UK*
[3]*BASF Norge AS, Oslo 0283, Norway*

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Abstract

A glyceride mixture of monoglyceride, diglyceride and TAG increases solubilisation and enhances emulsification of *n*-3 fatty acid (FA)-containing lipids in the stomach. This allows for better access of digestive enzymes, pivotal for the release of bioactive *n*-3 FA. The objective was to compare the effect of a glyceride formulation and an ethyl ester 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 years consumed a single dose (2·8 g EPA + DHA) of each EPA + DHA formulation without a meal in random order separated by a 2-week washout 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-fold greater Δ concentration of EPA, 3-fold greater Δ concentration of DHA and 5-fold greater Δ concentration of EPA + DHA were seen with the glyceride-EPA + DHA. The time at which the maximal concentrations of *n*-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 mixture of monoglyceride, diglyceride and TAG 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 *n*-3 FA.

Key words: ***n*-3: Fish oil: EPA: DHA: Emulsification: Monoglyceride: Diglyceride**

n-3 Long chain PUFA (*n*-3 LCPUFA) have been widely studied due to their associations with benefits to human health including lowering inflammation[1,2], decreasing the risk of CVD and events[3–8] and improving cognition[9,10]. The bioactive *n*-3 LCPUFA are EPA and DHA; for these fatty acids (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 FA α-linoleic acid 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/d (two × approximately 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 250–600 mg/d (see Table 5 of Calder[11]). However, the UK population, in addition to those of the USA and many other countries, consumes 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 TAG, ethyl esters (EE) or NEFA. 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 *n*-3 LCPUFA[18].

Abbreviations: *n*-3 LCPUFA, *n*-3 long chain PUFA; C_{max}, maximal concentration; DPA, docosapentaenoic acid; EE, ethyl ester; FA, fatty acid; iAUC, incremental AUC; SMEDS, self-micro-emulsifying delivery system; T_{max}, time at which maximal concentration occurs.

* **Corresponding author:** Helena L. Fisk, email h.fisk@soton.ac.uk

Considering this, the 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 *n*-3 LCPUFA in TAG form and self-micro-emulsifying delivery systems (SMEDS) which promote spontaneous emulsification of *n*-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 with other TAG 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 mixture of monoglyceride, diglyceride and TAG[22,23]. Investigation in rats showed improved appearance of DHA in the lymph using monoglyceride and diglyceride formulations in comparison with TAG and EE formulations[23], but no such improvement was seen when using a diglyceride and TAG DHA formulation in comparison with diglyceride and TAG[24]. A single study in humans reported significantly enhanced bioavailability of EPA using a mixture of monoglyceride, diglyceride and TAG in comparison with TAG and monoglyceride formulations[25]. These studies suggest the use of monoglycerides in combination with diglyceride and TAG is key in *n*-3 LCPUFA bioavailability, but this requires further evaluation as evidence for both EPA and DHA, and comparison with bioavailability of EE *n*-3 LCPUFA in humans is lacking.

Therefore, the present study aimed to compare the use of a novel alternative glyceride formulation of EPA + DHA, comprising of a mixture of monoglyceride, diglyceride and TAG 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 years were enrolled into the study. Inclusion criteria for participation were as follows: age between 50 and 75 years, BMI (kg/m^2) 20–35, self-reported oily fish intake of <1 portion/week and an omega-3 index (erythrocyte 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 *n*-3 FA or other lipid supplements. The study took place between October 2019 and February 2020.

Study design and supplements

The present study was a double-blind randomised crossover trial that investigated an *n*-3 glyceride formulation with 500 mg EPA + 200 mg DHA per capsule (BASF AS), alongside an EE formulation (also providing 500 mg EPA + 200 mg DHA per capsule) (BASF AS) both in soft gelatin capsules. The FA compositions of the EE and glyceride mixtures were identical. They contained (g/100 g FA): 18 : 1*n*-9, 0·8; 18 : 2*n*-6, 1·5; 18 : 3*n*-3, 0·9; 18 : 4*n*-3, 3·7; 20 : 3*n*-3, 0·2; 20 : 4*n*-6, 2·3; 21 : 5*n*-3, 1·8; 22 : 5*n*-6, 0·8; 20 : 5*n*-3 (EPA), 53·2; 22 : 5*n*-3 (docosapentaenoic acid (DPA)), 3·0; 22 : 6*n*-3 (DHA), 23·4; and other minor FA (all < 0·4), 3·1. Both formulations provided about 28 mg of DPA. The distribution of EPA and DHA within the monoglyceride, diglyceride and TAG is not known.

As this was a crossover design, all participants received both treatments but in random order. Blinding, randomisation and supplement packaging were completed by the Research Pharmacy at Southampton General Hospital, Southampton, UK, 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, UK, on three 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 erythrocyte omega-3 index (EPA + DHA as a % of total erythrocyte 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 two 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 approximately 07.30 hours at which a cannula was inserted into a forearm vein. Participants provided a 0 h blood sample after which they ingested a single dose (four 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 approximately 10 h. These meals consisted of two 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 weeks after the second clinic visit (the average time between the second and third clinical visits was 26·1 (SD 2·4) d); 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 ***g*** for 15 min at 4°C and analysed for EPA and DHA contents. Plasma and erythrocyte samples were stored at −80°C before analysis.

Fatty acid composition analysis

Total lipid was extracted from 0·5 ml plasma with 5 ml chloroform–methanol (2:1, v/v) containing 0·2 M BHT[27]. A quantity of 1 ml of 1 M sodium chloride was added and the sample vortex mixed prior to centrifugation at 1500 ***g*** for 5 min at room temperature. The lower solvent phase was aspirated and evaporated under N_2 at 40°C. The lipid extract was re-dissolved in 0·5 ml toluene and FA 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[28]. The samples were then neutralised and fatty acid methyl esters transferred into hexane for analysis by GC; fatty acid methyl esters were separated on a BPX-70 fused silica capillary column (30 m × 0·2 mm × 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 was used as a calibration reference standard (Sigma-Aldrich).

Other laboratory analyses

The plasma concentrations of TAG, 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 twenty was estimated to give 90 % power to detect this difference as statistically significant by pairwise comparison with significance defined as $P \leq 0{\cdot}05$.

EPA, DPA and DHA in plasma are expressed as relative (percentage of total FA) and absolute (μg/ml 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}) and time point at which C_{max} was achieved (T_{max}) 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 ($P \leq 0{\cdot}172$ and $\leq 0{\cdot}659$, 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} 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} 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 $P \leq 0{\cdot}05$.

Results

Participant characteristics

Fig. 1 shows the flow of participants through the study and numbers of participants in each treatment group. Twenty participants (ten male and ten 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 (SD 0·7) years (range 50–70 years), mean BMI of 26·95 (SD 0·73) kg/m^2, consumed on average 2·55 (SD 0·31) portions of oily fish per month and had a mean screening omega-3 index of 5·35 (SD 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 erythrocyte 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, docosapentaenoic acid and DHA incorporation in plasma with single dosing

The glyceride-EPA + DHA supplement resulted in a significantly greater increase in the absolute (μg/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 $P < 0{\cdot}001$, Figs. 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 ($P \leq 0{\cdot}013$, Figs. 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 ($P \leq 0{\cdot}023$, Figs. 2 and 3, Table 2). An average 9·7-fold greater Δ concentration of EPA, 3·0-fold greater Δ concentration of DPA, 2·8-fold greater Δ concentration of DHA and 5·3-fold greater Δ concentration of EPA + DHA were achieved with single dosing of the glyceride supplement in comparison with the EE supplement

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Table 1. Characteristics of the participants at study entry*
(Median values and 25th, 75th percentiles; percentages)

	Males (*n* 10)		Females (*n* 10)		
	Median	P25, P75	Median	P25, P75	*P*
Age (years)	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		100		
Plasma TAG (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 (erythrocyte 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

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

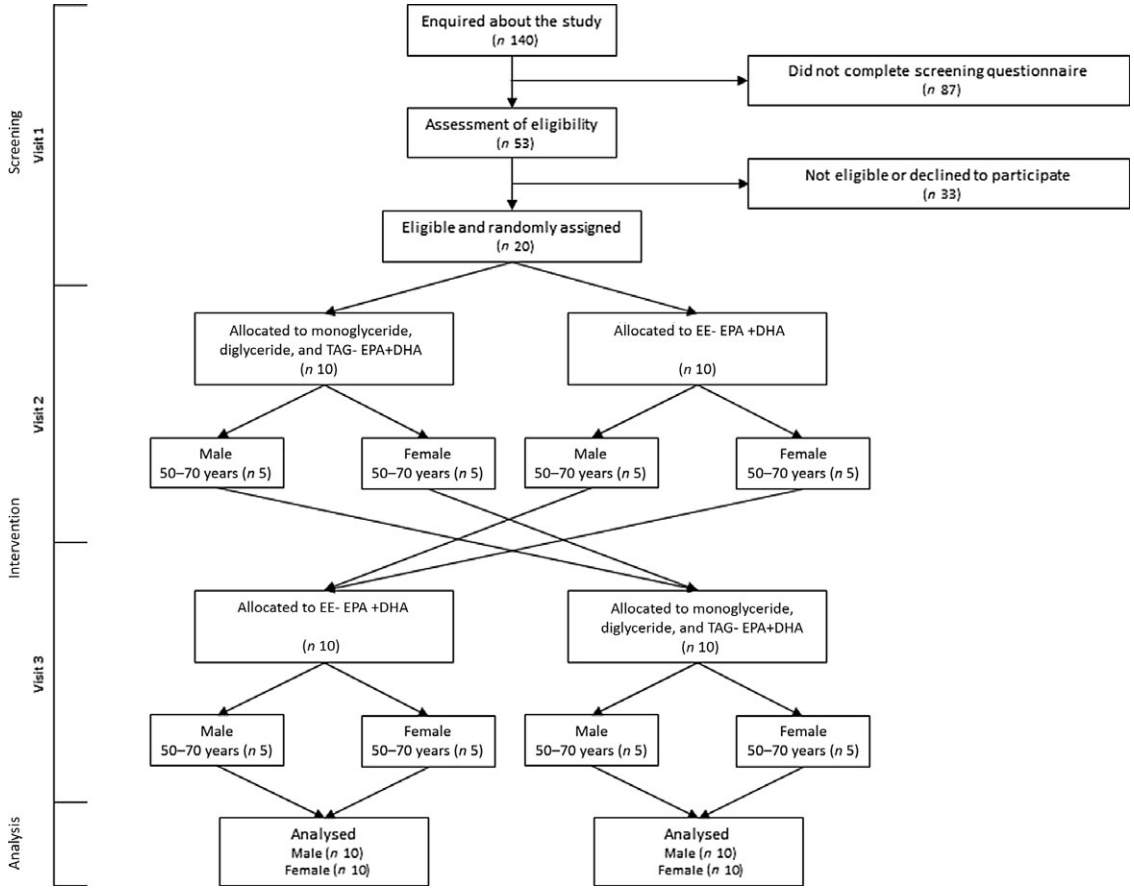

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) diagram of participant inclusion and flow through the study. EE, ethyl ester; glyceride, mixture of monoglyceride, diglyceride and TAG EPA + DHA.

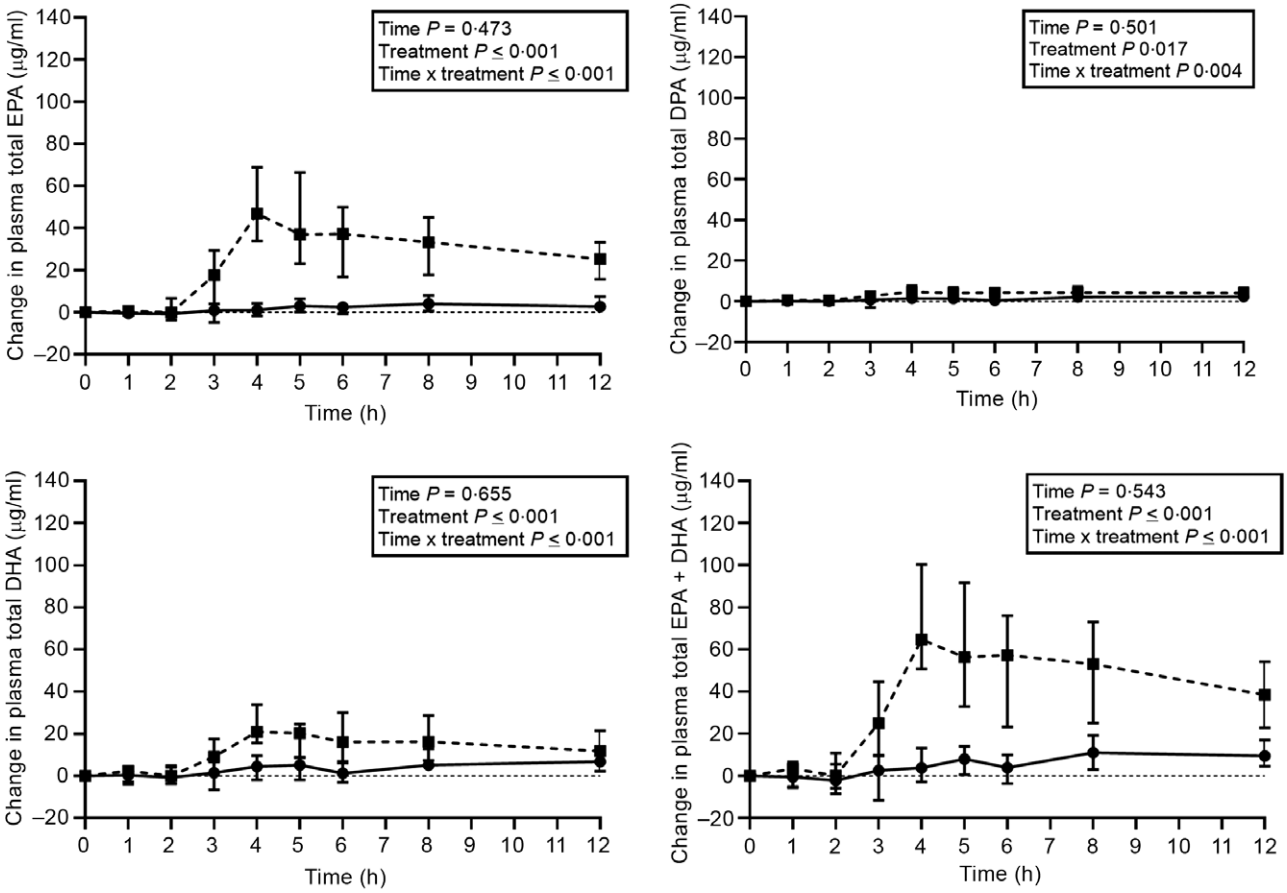

Fig. 2. Changes from baseline in absolute concentration of plasma total EPA, docosapentaenoic acid (DPA), DHA and EPA + DHA after a single dose of glyceride-EPA + DHA or EE-EPA + DHA in healthy older adults. Values are medians and interquartile ranges. Glyceride-EPA + DHA *n* 20, EE-EPA + DHA *n* 20. EE, ethyl-ester; glyceride, mixture of monoglyceride, diglyceride, and TAG. —●—, Treatment 1; - -■- -, treatment 2.

($P < 0{\cdot}001$, Fig. 2, Table 2). The time at which the maximal concentration of EPA, DHA and EPA + DHA was achieved occurred on average 4, 1 and 2 h earlier, respectively, following single dosing with the glyceride supplement in comparison with the EE supplement ($P < 0{\cdot}001$, Fig. 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 ($P \geq 0{\cdot}520$, Fig. 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 *n*-3 LCPUFA ($P < 0{\cdot}001$, Fig. 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 with 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 with the EE supplement ($P < 0{\cdot}001$, Fig. 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 ($P \geq 0{\cdot}172$, all).

Discussion

n-3 LCPUFA are found to be esterified into TAG 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 *n*-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

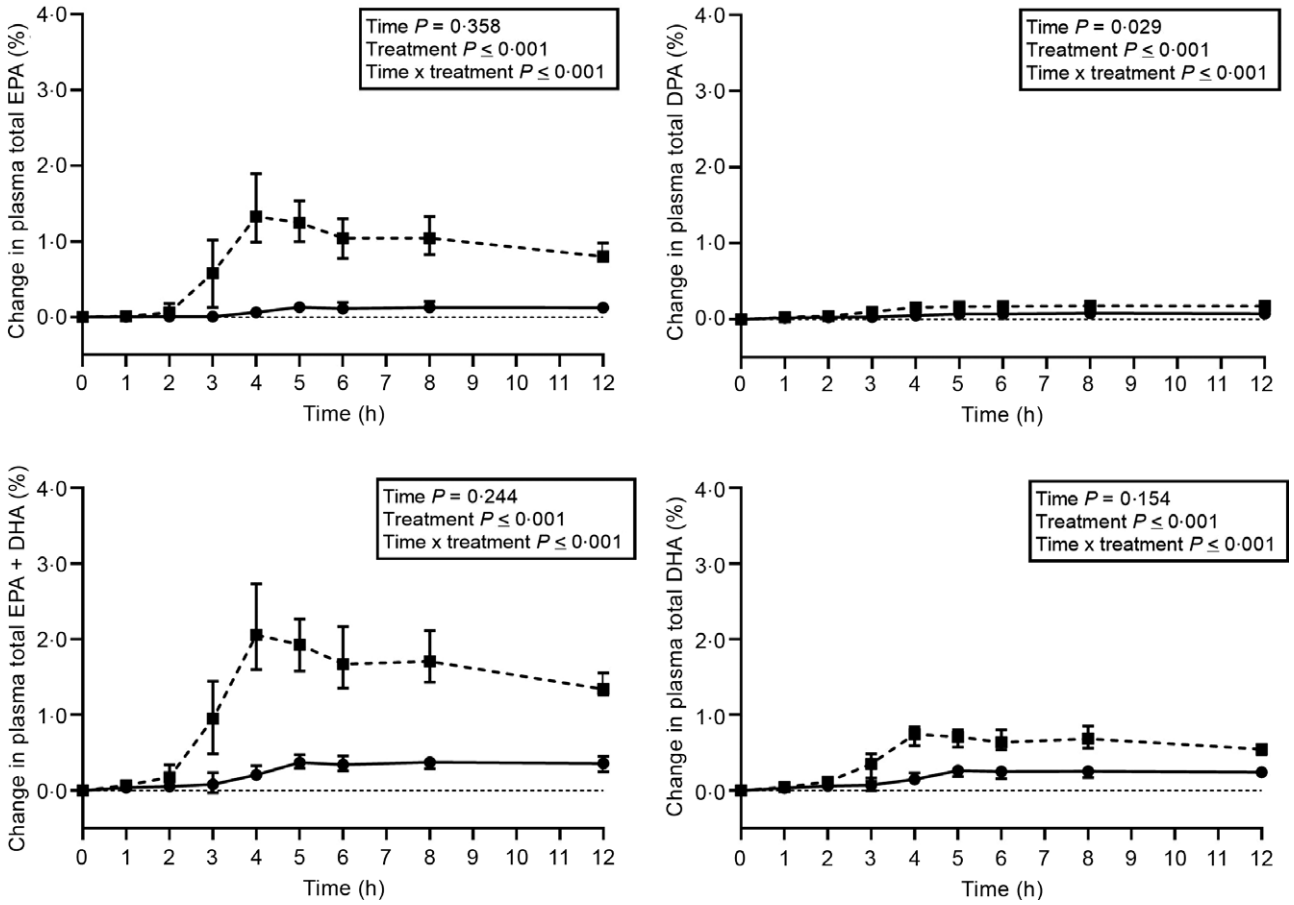

Fig. 3. Changes from baseline in relative concentration of plasma total EPA, docosapentaenoic acid (DPA), DHA and EPA + DHA after a single dose of glyceride-EPA + DHA or EE-EPA + DHA in healthy older adults. Values are medians and interquartile ranges. Glyceride-EPA + DHA *n* 20, EE-EPA + DHA *n* 20. EE, ethyl ester; glyceride, mixture of monoglyceride, diglyceride, and TAG. —●—, Treatment 1; - -■- -, treatment 2.

absorption, and in situ emulsification or 'self-emulsifying' EE *n*-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 with standard EE *n*-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-week period resulted in a greater increase in EPA and DHA in plasma, mononuclear cells and erythrocytes, with a greater increase in omega-3 index, in comparison with standard EE formulation[17].

The present 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 mixture of monoglyceride, diglyceride and TAG 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 *n*-3 LCPUFA may circulate in the bloodstream esterified into TAG as components of chylomicrons, VLDL 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 present 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 reach 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 approximately 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 with standard EE-EPA and -DHA occurred at 4 h suggesting improved gastrointestinal handling of the SMEDS formulation[17]. This has been reproduced in the present study in which we show the largest difference in total plasma EPA and DHA between the glyceride

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Table 2. Summary of changes (Δ) in plasma EPA, docosapentaenoic acid (DPA) and DHA concentrations over 12 h after a single dose of EE-EPA + DHA or glyceride-EPA + DHA in healthy adults*
(Median values and 25th, 75th percentiles (P25, P75))

	Glyceride-EPA + DHA		EE-EPA + DHA			
	Median	P25, P75	Median	P25, P75	Ratio†	*P*
Absolute concentration						
Plasma total EPA						
iAUC (h × μg/ml)	344	189, 405	37	29, 66	9·3	≤0·001
C_{max} (μg/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 × μg/ml)	41	26, 68	25	15, 39	1·6	0·023
C_{max} (μg/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 × μg/ml)	152	96, 245	63	47, 98	2·4	≤0·001
C_{max} (μg/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 × μg/ml)	525	292, 655	98	72, 156	5·4	≤0·001
C_{max} (μg/ml)	79	51, 113	15	7, 24	5·3	0·044
T_{max} (h)	4	4, 5	6	3, 12		≤0·001
Relative concentration						
Plasma total EPA						
iAUC (h × %)	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 × %)	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 × %)	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 × %)	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

EE, ethyl ester EPA + DHA; C_{max}, maximum concentration change; glyceride, mixture of monoglyceride, diglyceride and TAG EPA + DHA; iAUC, incremental AUC; T_{max}, time at which C_{max} occurs.
* *P* values were derived by using the Kruskal–Wallis test.
† The ratio of glyceride-EPA + DHA compared with EE-EPA + DHA for iAUC and C_{max}.

formulation and EE formulation to occur at 4 h also, suggesting there is improved gastrointestinal handling of the glyceride formulation.

These results suggest that the glyceride mixture 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 *n*-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 with 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 mixture, but this could potentially provide greater efficacy of trials using *n*-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 present 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 week. 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 *n*-3 LCPUFA. A third limitation is that we studied adults aged 50–70 years and so we cannot generalise the findings to those of other ages. The glyceride mixture was a ratio of monoglyceride, diglyceride and TAG (1:2·5:1) with an identical FA composition to that of the EE formulation; however, the distribution of EPA, DPA and DHA amongst monoglyceride, diglyceride and TAG within the glyceride mixture 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 mixture of monoglyceride, diglyceride and TAG can provide superior absorption and higher amounts of bioactive EPA and DHA than a commonly available EE formulation.

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References

1. Calder PC (2015) Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochim Biophys Acta* **1851**, 469–484.
2. Calder PC (2017) Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans* **45**, 1105–1115.
3. Mori TA & Woodman RJ (2006) The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Curr Opin Clin Nutr Metab Care* **9**, 95–104.
4. Chowdhury R, Warnakula S, Kunutsor S, *et al.* (2014) Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med* **160**, 398–406.
5. Del-Gobbo LC, Imamura F, Aslibekyan S, *et al.* (2016) ω-3 Polyunsaturated fatty acid biomarkers and coronary heart disease: pooling project of 19 cohort studies. *JAMA Intern Med* **176**, 1155–1166.
6. AbuMweis S, Jew S, Tayyem R, *et al.* (2018) Eicosapentaenoic acid and docosahexaenoic acid containing supplements modulate risk factors for cardiovascular disease: a meta-analysis of randomised placebo-control human clinical trials. *J Hum Nutr Diet* **31**, 67–84.
7. Innes JK & Calder PC (2018) The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review. *Int J Mol Sci* **9**, 532.
8. Innes JK & Calder PC (2020) Marine omega-3 (*n*-3) fatty acids for cardiovascular health: an update for 2020. *Int J Mol Sci* **21**, 1362.
9. Frensham LJ, Bryan J & Parletta N (2012) Influences of micronutrient and omega-3 fatty acid supplementation on cognition, learning, and behavior: methodological considerations and implications for children and adolescents in developed societies. *Nutr Rev* **70**, 594–610.
10. Ciappolino V, Mazzocchi A, Botturi A, *et al.* (2019) The role of docosahexaenoic acid (DHA) on cognitive functions in psychiatric disorders. *Nutrients* **11**, E769.
11. Calder PC (2018) Very long-chain *n*-3 fatty acids and human health: fact, fiction and the future. *Proc Nutr Soc* **77**, 52–72.
12. Arteburn LM, Hall EB & Oken K (2006) Distribution, interconversion, and dose response of *n*-3 fatty acids in human. *Am J Clin Nutr* **83**, 1467S–1476S.
13. SACN (2004) *Advice on Fish Consumption: Benefits & Risks*. London: TSO.
14. Micha R, Khatibzadeh S, Shi P, *et al.* (2014) Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ* **348**, g2272–g2272.
15. El-Boustani S, Colette C, Monnier L, *et al.* (1987) Enteral absorption in man of eicosapentaenoic acid in different chemical forms. *Lipids* **22**, 711–714.
16. Schuchardt JP, Neubronner J, Kressel G, *et al.* (2011) Moderate doses of EPA and DHA from re-esterified triacylglycerols but not from ethyl-esters lower fasting serum triacylglycerols in statin-treated dyslipidemic subjects: results from a six month randomized controlled trial. *Prostaglandins Leukot Essent Fatty Acids* **85**, 381–386.
17. West AL, Kindberg GM, Hustvedt SO, *et al.* (2018) A novel self-micro-emulsifying delivery system enhances enrichment of eicosapentaenoic acid and docosahexaenoic acid after single and repeated dosing in healthy adults in a randomized trial. *J Nutr* **148**, 1704–1715.
18. Rice HB, Bernasconi A, Maki KC, *et al.* (2016) Conducting omega-3 clinical trials with cardiovascular outcomes: proceedings of a workshop held at ISSFAL 2014. *Prostaglandins Leukot Essent Fatty Acids* **107**, 30–42.
19. Raatz SK, Johnson LK & Bukowski MR (2016) Enhanced bioavailability of EPA from emulsified fish oil preparations versus capsular triacylglycerol. *Lipids* **51**, 643–651.
20. Qin Y, Nyheim H, Haram EM, *et al.* (2017) A novel self-micro-emulsifying delivery system (SMEDS) formulation significantly improves the fasting absorption of EPA and DHA from a single dose of an omega-3 ethyl ester concentrate. *Lipids Health Dis* **16**, 204.
21. Maki KC, Palacios OM, Buggia MA, *et al.* (2018) Effects of a self-micro-emulsifying delivery system formulation versus a standard ω-3 acid ethyl ester product on the bioavailability of eicosapentaenoic acid and docosahexaenoic acid: a study in healthy men and women in a fasted state. *Clin Ther* **40**, 2065–2076.
22. Bremmell KE, Briskey D, Meola TR, *et al.* (2019) A self-emulsifying Omega-3 ethyl ester formulation (AquaCelle) significantly improves eicosapentaenoic and docosahexaenoic acid bioavailability in healthy adults. *Eur J Nutr* **59**, 2729–2737.
23. Banno F, Doisaki S, Shimizu N, *et al.* (2002) Lymphatic absorption of docosahexaenoic acid given as monoglyceride, diglyceride, triglyceride, and ethyl ester in rats. *J Nutr Sci Vitaminol* **48**, 30–35.

24. Tamai T, Murota I, Maruyama K, *et al.* (2007) Effects of supplemented diacylglycerol rich in docosahexaenoic acid on serum triacylglycerol in a diet-induced hyperlipidemic model of rats are essentially equivalent to those of triacylglycerol rich in docosahexaenoic acid. *Biol Pharm Bull* **30**, 2381–2388.
25. Wakil A, Mir M, Mellor DD, *et al.* (2010) The bioavailability of eicosapentaenoic acid from reconstituted triglyceride fish oil is higher than that obtained from the triglyceride and monoglyceride forms. *Asia Pac J Clin Nutr* **19**, 499–505.
26. Harris WS (2010) The omega-3 index: clinical utility for therapeutic intervention. *Curr Cardiol Rep* **12**, 503–508.
27. Bligh EG & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
28. Fisk HL, West AL, Childs CE, *et al.* (2014) The use of gas chromatography to analyze compositional changes of fatty acids in rat liver tissue during pregnancy. *J Vis Exp* **13**, 51445.
29. Davidson MH, Johnson J, Rooney MW, *et al.* (2012) A novel omega-3 free fatty acid formulation has dramatically improved bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: the ECLIPSE (Epanova® compared to Lovaza® in a pharmacokinetic single-dose evaluation) study. *J Clin Lipidol* **6**, 573–584.
30. Lopez-Toledano MA, Thorsteinsson T, Daak A, *et al.* (2017) A novel ω-3 acid ethyl ester formulation incorporating Advanced Lipid TechnologiesTM (ALT®) improves docosahexaenoic acid and eicosapentaenoic acid bioavailability compared with Lovaza®. *Clin Ther* **39**, 581–591.
31. Heath RB, Karpe F, Milne RW, *et al.* (2003) Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *J Lipid Res* **44**, 2065–2072.
32. Heath RB, Karpe F, Milne RW, *et al.* (2007) Dietary fatty acids make a rapid and substantial contribution to VLDL-triacylglycerol in the fed state. *Am J Physiol Endocrinol Metab* **292**, E732–E739.
33. Rémond D, Shahar DR, Gille D, *et al.* (2015) Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. *Oncotarget* **6**, 13858–13898.

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