Lipid structure does not modify incorporation of eicosapentaenoic and docosahexaenoic acids into blood lipids in healthy adults: a randomised control trial

Annette L. West[1], *Graham C. Burdge*[1]* *and Philip C. Calder*[1,2]

[1]Academic Unit of Human Development and Health, University of Southampton, Southampton, United Kingdom.

[2]National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton National Health Service Foundation Trust and University of Southampton, Southampton, United Kingdom.

*To whom correspondence should be addressed. Professor Graham C. Burdge, Academic Unit of Human Development and Health, IDS Building (MP887), Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK. E-mail g.c.burdge@soton.ac.uk; Telephone +44 (0)2318205259

Supplemental tables and figures are available from the "Online Supporting Material" URL

Running head: EPA and DHA supplement bioavailability

Key words: Eicosapentaenoic acid, docosahexaenoic acid, postprandial, lipid structure, polyunsaturated fatty acids

Abstract

Dietary supplementation is an effective means to improve eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) status. However, it is unclear whether lipid structure affects EPA+DHA bioavailability. We determined the effect of consuming different EPA and DHA lipid structures on their concentrations in blood during the postprandial period and during dietary supplementation compared to unmodified fish oil triglyceride (uTAG). In a postprandial crossover study, healthy men (*n* 9) consumed in random order test meals containing 1.1 g EPA + 0.37 g DHA as either uTAG, re-esterified TAG, free fatty acids (FFAs) or ethyl esters (EEs). In a parallel design supplementation study, healthy men and women (*n* 10/ sex/ supplement) consumed one supplement type for 12 weeks. Fatty acid composition was determined by gas chromatography. EPA incorporation over 6 hours into TAG or phosphatidylcholine (PC) did not differ between lipid structures. EPA enrichment in non-esterified fatty acids (NEFAs) was lower from EEs than from uTAG ($P = 0.01$). Plasma TAG, PC or NEFA DHA incorporation did not differ between lipid structures. Lipid structure did not affect TAG or NEFA EPA incorporation, and PC or NEFA DHA incorporation following dietary supplementation. Plasma TAG peak DHA incorporation was greater ($P = 0.02$) and time to peak shorter ($P = 0.02$) from FFAs than from uTAG in men. In both studies, the order of EPA and DHA incorporation was PC > TAG > NEFA. In conclusion, EPA and DHA lipid structure may not be an important consideration in dietary interventions.

Introduction

Dietary supplementation with eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) confers well-established changes in the biophysical properties of cell membranes and cell signalling processes that are associated with positive effects on health[1]. EPA and DHA are consumed in the diet primarily as components of the muscle of oily fish. Consequently, several organisations have published recommendations for oily fish consumption in order to provide sufficient EPA+DHA for health benefits[2]. However, low levels of oily fish consumption in some populations have limited their effectiveness[3]. Dietary supplementation with encapsulated oils containing EPA+DHA provides an alternative means of increasing their intake[4], which may be facilitated by highly purified EPA+DHA preparations that reduce the volume of oil required to achieve health benefits[4]. It is, therefore, important to identify the preparations that are most effective in increasing EPA+DHA status.

The lipid structures of EPA+DHA used most commonly in dietary supplements are unmodified fish oil triacylglycerol (uTAG) from fish body oil or cod liver oil, re-esterified triacylglycerol (rTAG), krill oil phospholipid, free fatty acids (FFAs) and ethyl esters (EEs). Differences in the lipid structure in which EPA+DHA are ingested may influence their bioavailability and accumulation within lipid pools. The rank order of the increment in EPA+DHA status after consuming 3.3 g EPA+DHA daily for 2 weeks in men and women was rTAG > fish body oil > FFAs > cod liver oil > EEs[4]. Similarly, the increment in omega-3 index[5] was greater when EPA+DHA (1.68 g daily) were consumed for 6 months as rTAG compared to EEs in moderately hypertriglyceridaemic subjects[6; 7]. EPA incorporation into plasma TAG over 24 hours has been shown to greater when consumed as *sn*-2 monoacylglycerol, TAG or FFA compared to EEs [8; 9]. Postprandial incorporation of EPA+DHA into plasma TAG was greater when ingested as FFA than as TAG (approximately 38%) or EE (80%)[10; 11]. In addition, the bioavailability of EPA from rTAG was shown to be greater than from *sn*-2 monoacylglycerol[12], although others have not found this[13; 14]. EPA and DHA bioavailability may also be influenced by the total fat composition of a meal[11; 15]. Furthermore, the magnitude of EPA and DHA accumulation appears to differ between plasma lipid classes: phospholipids > cholesteryl esters > TAG[2].

Differences in study design have produced uncertainty in the literature about the effect of lipid structure on EPA and DHA bioavailability during dietary supplementation. The purpose of this study was to compare directly the effect of different lipid preparations of EPA+DHA in increasing their concentrations in blood lipids. We measured EPA and DHA concentrations in plasma TAG, non-esterified fatty acids (NEFAs) and phosphatidylcholine (PC) after consumption of EPA+DHA as rTAG, FFAs or EEs in a single meal and compared this to uTAG that is used commonly in commercial dietary supplements. We then investigated whether lipid structure altered

EPA and DHA incorporation into blood after daily consumption. We also assessed the effect of enteric protective capsules containing rTAG on postprandial EPA+DHA incorporation into blood lipids and of sex and body mass index (BMI) on EPA+DHA assimilation during 12 weeks dietary supplementation.

Methods

Ethical approval and study registration

The study was conducted in accordance with the declaration of Helsinki. The postprandial study was approved by the Isle of Wight, Portsmouth and South East Hampshire Research Ethics Committee B (approval number 09/H0501/98) and the dietary supplementation study was approved by the Southampton and South West Hampshire Research Ethics Committee B (approval number 11/SC/0049). The postprandial study is registered as ISRCTN11656280 and the dietary supplementation study is registered as ISRCTN46532656 at www.controlled-trials.com.

Postprandial study: participants, design, sample collection and specimen processing

Twelve volunteers showed initial interest in the study; two of these did not complete the screening process. One of the ten participants who completed the study was subsequently found to have elevated fasting blood glucose concentration (> 7 mmol/l) and their data were not included in the final analyses (Supplemental Fig.1). The participants in the postprandial study were nine healthy men with median age (years) 26 (range 22 – 38), BMI (kg/m^2) 24 (20 – 30), and fasting plasma TAG ($mmol*L^{-1}$) 0.8 (0.4 – 2.2), NEFA ($mmol*L^{-1}$) 0.3 (0.2 – 0.9) and glucose ($mmol*L^{-1}$) 5.3 (4.5 – 6.0) concentrations. All included participants self-reported that they were low consumers of oily fish (< one meal per week) and that they did not use fish oil supplements. The double-blinded postprandial study was based upon our previous single-blinded crossover design[16]. Each participant took part in five postprandial study days in random order, with an interval of at least 14 days between each study day. Capsule containers were labelled with anonymised codes and the order in which the participants took the supplements was assigned using a random number generator (Random.org) by an independent member of staff. The nine participants consumed their habitual diet throughout the study. On the day preceding any postprandial study day, participants were asked to consume their evening meal by 21.00 hours and to fast until the postprandial study commenced. Participants arrived at the National Institute for Health Research Wellcome Trust Clinical Research Facility, Southampton General Hospital, Southampton, UK at approximately 07.00 hours. A cannula was inserted into a forearm vein and a baseline blood sample (5 ml) collected into an evacuated tube containing heparin sulphate as anticoagulant. Participants consumed the same test meal on each occasion. This contained 4.3 MJ total energy derived from

carbohydrate (117 g), protein (15 g) and a blend of fats (55 g) from sunflower oil, double cream, flaxseed oil and olive oil which provided a fatty acid pattern that was representative for the typical UK diet as described previously [16]. The fat and protein component of the test meal was administered in an emulsion (total volume made up to 160 ml with water). The carbohydrate component of the meal was given as toast with marmalade. Participants consumed the test meal within 15 minutes. On each postprandial study day, participants consumed one of the following supplements; gelatin encapsulated uTAG, rTAG, EEs or FFAs each of which provided approximately EPA 1.1 g + DHA 0.37 g (Table 1). The rTAG, EE and FFA preparations contained less saturated and monounsaturated fatty acids and 18:2n-6 compared to uTAG (Table 1). In order to determine whether exposure to the gastric environment affected the bioavailability of EPA and DHA, on one occasion participants consumed rTAG (providing EPA 1.1 g + DHA 0.37 g) encapsulated in an enteric-resistant coating. Blood samples (5 ml) were collected at 0·5, 1, 1·5, 2, 2·5, 3, 4, 5 and 6 h after consumption of the test meal finished. Participants remained resting and were allowed free access to water throughout the study. Plasma was isolated form blood samples by centrifugation and stored at -80^{o}C [16].

Supplementation study design, sample collection and specimen processing

The supplementation study had a double blinded, parallel design. Two hundred volunteers enquired about the study, of which 120 agreed to undergo initial screening. Of these, twenty subsequently either declined further participation or were found to be ineligible for reasons not covered in the questionnaire and a further twenty were randomised to an arm of the study that was not included in the analysis design (Supplemental Figure 2). These participants consumed capsules of medium chain TAG from palm oil. Because the palm oil did not contain EPA or DHA, this arm of the study was excluded from the final analysis because it did not provide a direct comparator for the other lipid structures. Eighty participants (*n* 40 men and *n* 40 women) were randomised to receive one of the four supplements that contained EPA and DHA (Supplemental Figure 2). Two participants withdrew during the study because they found taking capsules unpleasant and one more was unable to attend appointments.

The participants who completed the supplementation study were men aged between 19 and 38 years with BMI 20 to 30 kg/m^2 and women aged between 19 and 44 years with BMI 20 to 31 kg/m^2 who were in good general health, and who did not consume fish oil or other oil supplements and did not eat more than one portion of oily fish meal per week (Table 2). There were no significant differences within each sex in age, BMI, or in fasting plasma TAG or NEFA concentrations between supplement groups. Participants consumed their habitual diet throughout the study. At study entry, participants were asked to fast from 21:00 until after a blood sample (20

ml) had been collected between approximately 08:00 and 10:00. Participants (*n* 10 per sex and per supplement) were then assigned at random to consume one of four dietary supplements used in the postprandial study for 12 weeks. The identity of the supplements was anonymised and the allocation of the participants randomised using a random number generator by the manufacturer of the supplements (Vifor Pharma Ltd., Glattbrugg, Switzerland). The supplements were gelatin encapsulated uTAG, EEs, or FFAs, or rTAG encapsulated in an enteric-resistant coating which each provided approximately EPA (1.1 g per day) and DHA (0.35 g per day). The gelatin encapsulated rTAG dietary supplement was omitted from the supplementation study due to lack of differences in EPA and DHA incorporation into blood lipids between coating materials in the postprandial study. Fasting blood samples (20 ml) were collected during the supplementation period after 1, 2, 4, 8 and 12 weeks. Plasma was isolated from blood samples by centrifugation and stored at -80^{o}C [16].

Analysis of plasma lipid fatty acid composition

The fatty acid composition of plasma TAG, NEFAs and PC was determined by gas chromatography[16]. Briefly, internal standards (dipentadecanoyl PC (100 μg), tripentadecanoin (100 μg) and heneicosanoic acid (50 μg)) were added and total lipids were extracted from plasma (0.8 ml) with chloroform and methanol[17] and dried under nitrogen. The total lipid extracts were dissolved in chloroform and individual lipid classes were then isolated by solid phase extraction using BondElut 100 mg aminopropylsilica cartridges (Agilent Technologies, Oxford, UK)[18]. The purified lipid classes were dissolved in toluene and fatty acid methyl esters (FAMEs) were synthesised by heating the purified lipids at 50^{o}C in the presence of methanol containing 2% (v/v) sulphuric acid [18]. FAMEs were recovered by extraction with hexane and resolved on a BPX-70 fused silica capillary column (32 m x 0.25 mm x 25 μm (SGE Analytical Science, Milton Keynes, UK) using an Agilent 6890 gas chromatograph equipped with flame ionisation detection [16]. The concentrations of EPA and DHA in blood lipid classes, expressed as μmol*L^{-1}, were calculated from the ratio of their peak area to the internal standard, multiplied by the amount of standard and corrected for the volume of plasma extracted.

Statistical analysis

The researchers, participants and staff involved in care of the participants (for example research nurses) were blinded to the allocation of supplements until data analysis had been completed. Data were analysed using SPSS v21 (SPSS Inc., Chicago, IL). The data sets were not distributed normally and did not approximate a normal distribution after log transformation. Therefore, non-parametric analyses were used throughout. Comparison of data sets involving repeated measures was by Friedman's test with comparisons between groups at specific time points by the Wilcoxon

Signed-rank test. Comparison of single time point measurements between groups of different subjects was by Kruskal-Wallis test or Mann-Whitney U-test. Comparison of baseline and end of study total lipid concentrations in the supplementation study was by the Wilcoxon Signed-rank test For all comparisons, the gelatin coated uTAG was used as the reference data set. Because we intended to model the effect of lipid structure in a manner relevant to the general population, the amount of EPA and DHA consumed by each participant was not adjusted for BMI. Because the data were not normally distributed, it was not possible to carry out analysis of co-variance. However, we assessed the relationship between the incremental area-under-the curve (iAUC) for EPA and DHA, and BMI using Spearman's rank order correlation test. Age has been shown to be related to incorporation of EPA and DHA[19]. Therefore, the relationship between age and the iAUC for EPA and DHA was also assessed by Spearman's rank order correlation test.

The postprandial study was powered according to the anticipated change in EPA content of plasma TAG. Based upon our previous study[16], a sample size of 9 would give a statistical power of 85% power to detect an increase in plasma TAG EPA concentration of 15 μmol*L^{-1} at 4 hours with a probability of <0.05. The dietary supplementation calculations are based on the anticipated change in plasma PC EPA from 1.7% of total fatty acids to 2.9%[20]. Consequently, a sample size of 10 was expected to provide 90% statistical power of detecting this increment in plasma PC EPA concentration with a probability of $P<0.05$.

Results

Postprandial incorporation of EPA and DHA into blood lipids

There were no significant differences between supplements in the concentrations or EPA or DHA at baseline (Supplemental Table 1). The changes in the concentrations of EPA and DHA in plasma TAG, NEFAs and PC are summarised in Supplemental Fig. 3 and Table 3. There were no significant differences between supplements in the incremental area-under-the-curve (iAUC), maximum concentration (C_{max}) or time to C_{max} (T_{max}) for incorporation of EPA into plasma TAG or PC (Table 3). There were no significant differences between supplements in iAUC or T_{max} for incorporation of EPA into plasma NEFAs (Table 3). However, C_{max} for the incorporation of EPA into plasma NEFAs differed significantly ($\chi 2$ (4, *n* 16) = 12.1, p = 0.02) between groups (Table 3). Pairwise testing showed that the C_{max} for the EE preparation, but not the other molecular structures, was lower than that for the uTAG (Z = -2.549, p = 0.011) with a large effect size (r = -0.9).

There were no significant differences between supplements in iAUC or C_{max} for incorporation of DHA into plasma TAG or PC (Table 3). However, T_{max} for the incorporation of DHA into plasma PC differed significantly ($\chi 2$ (4, *n* 18) = 11.1, p = 0.03) between groups (Table 2), although this was not supported by pairwise testing between groups (Wilcoxon Signed-rank test).

There were no significant differences between supplements in iAUC, T_{max} or C_{max} for incorporation of DHA into plasma NEFAs (Table 3).

Incorporation of EPA and DHA during the postprandial period differed between plasma lipid classes such that the rank order of EPA iAUC was PC > TAG > NEFAs and of EPA C_{max} was TAG > PC > NEFAs irrespective of the structure of the supplement (Table 3, Supplemental Table 2). There was no statistically significant difference in EPA T_{max} among lipid classes (Table 3). The rank order of DHA iAUC and C_{max} was PC > TAG > NEFAs, and of DHA T_{max} was NEFAs > TAG > PC irrespective of the structure of the supplement (Table 3, Supplemental Table 2).

There was a significant positive association between the incorporation of EPA from rTAG(EC) into plasma PC and BMI (Supplemental Table 3). There was a significant negative association between the incorporation of EPA from rTAG into plasma PC and BMI (Supplemental Table 3). There was a significant negative association between the incorporation of EPA EE into plasma PC and age (Supplemental Table 3). The was a significant positive association between incorporation of DHA from rTAG(EC) into plasma PC and age (Supplemental Table 3). There were no other significant associations between BMI or age and the incorporation of EPA and DHA into plasma lipids (Supplemental Table 3).

Incorporation of EPA and DHA into blood lipids during dietary supplementation

There were no significant differences between groups in the concentrations or EPA or DHA at baseline (Supplemental Table 1). There were no significant changes in the concentrations of total plasma TAG, PC or NEFAs between the start and end of the supplementation study (Supplemental Table 4). The changes in EPA and DHA concentrations in plasma lipids during 12 weeks supplementation in men and women are summarised in Supplemental Figures 4 and 5, and in Tables 4 and 5, respectively. There were no significant differences between supplements in iAUC, C_{max} or T_{max} for incorporation of EPA into plasma TAG in men or women (Tables 4, 5). There was a significant difference between molecular structures of the supplements in the incorporation of DHA into plasma TAG in men (iAUC $\chi 2$ (3, *n* 40) = 8.8, p = 0.03; T_{max} $\chi 2$ (3, *n* 40) = 8.3, p = 0.04), but there was no difference in C_{max} in men (Table 4), and no differences in any of these parameters in women (Table 5). Pairwise comparisons showed that the incorporation of DHA into plasma TAG in men was significantly greater (122 $\mu mol*week^{-1}*L^{-1}$, P = 0.02) and the peak concentration occurred 4 weeks earlier (P = 0.02) for the FFA supplement than for the uTAG, while the other supplements did not differ significantly from uTAG.

T_{max} ($\chi 2$ (3, *n* 40) = 10.2, p = 0.02), but not C_{max} or iAUC, for incorporation of EPA into plasma PC differed significantly between supplements in men (Table 4), but not women (Table 5). However, this was not supported by pairwise testing. There were no significant differences

between supplements in iAUC, C_{max} or T_{max} for incorporation of DHA into plasma PC in men or women (Tables 4, 5).

There were no significant differences between supplements in iAUC, C_{max} or T_{max} for incorporation of EPA or DHA into plasma NEFAs in men or women (Tables 4, 5).

There were no significant differences between men and women in EPA or DHA iAUC, C_{max} or T_{max} within a supplement molecular structure and plasma lipid class (Tables 4 and 5, Supplemental Tables 5 and 6). Comparisons between lipid classes showed significant differences in iAUC and C_{max}, but not T_{max}, in postprandial and long-term EPA and DHA incorporation such that PC > TAG > NEFAs in both men and women who consumed rTAG(EC), FFA and uTAG (Tables 4 and 5; Supplemental Tables 5 and 6). However, there was no significant difference in EPA or DHA C_{max} in men who consumed EEs (Table 5; Supplemental Table 5).

There were no significant associations between BMI or age and the incorporation of EPA or DHA into plasma lipids in men (Supplemental Table 7). There was no significant association between age and EPA or DHA incorporation into plasma lipids in women. However, there was a significant positive association between BMI and incorporation of EPA and DHA into plasma TAG, but not PC or NEFA, irrespective of lipid structure in women (Supplemental Table 7).

Discussion

The overall findings of this research are that the lipid structure of ingested EPA and DHA has, at most, a limited effect on the incorporation of these fatty acids into blood lipids during the postprandial period and during 12 weeks dietary supplementation. In addition, the incorporation of EPA and DHA into blood lipids during dietary supplementation did not differ between men and women. However, the findings suggest differential incorporation of EPA+DHA into individual plasma lipid classes.

There were inconsistent associations in terms of lipid structure, plasma lipid class and subject sex between BMI or age in the postprandial and supplementation studies. This suggests that neither of these factors was a major determinant of incorporation of EPA and DHA incorporation into plasma lipids.

Previous studies have shown that the appearance of EPA and DHA in blood lipids during the postprandial period and during dietary supplementation can be influenced by the lipid structure in which these fatty acids were ingested[10; 11; 12], although not all studies have found this [14]. The present findings are consistent with these observations in that the peak post-prandial concentration of EPA provided as an EE, though not of DHA EE, was approximately half that of the TAG reference supplement (uTAG) in the plasma NEFA pool. This suggests differential metabolism of EE with fatty acids of different chain length and level of unsaturation, presumably within the

gastrointestinal tract, and may reflect differential activity of esterases. In addition, the meal context in which the supplements were consumed may have modified the postprandial metabolism of the n-3 fatty acids. For example, consuming EPA+DHA with a high fat meal increased their bioavailability during the postprandial period irrespective of the lipid structure of these fatty acids [11; 15]. The meal used in the present study contained more than twice as much fat as used in previous studies [5; 14] and so the overall absorption of EPA+DHA would be expected to be greater. It is possible that such an increase in uptake could mask differences in bioavailability between molecular structures of EPA+DHA reported in previous studies.

Incorporation of EPA+DHA from rTAG encapsulated in an enteric resistant coating did not differ significantly from that from gelatin coated capsules. This suggests that the losses of EPA+DHA in the stomach are relatively small and that enteric protective coating does not either increase or decrease the bioavailability of EPA+DHA supplements.

There were relatively small, but statistically significant, differences between molecular structures of EPA and DHA for their incorporation into blood lipids during the 12 week supplementation trial. This suggests that longer term intake of EPA+DHA may have masked any differences between molecular structures seen for acute fatty acid uptake. Previous studies have reported lower omega-3 index[6; 7] and EPA and DHA concentration[21] following consumption of EPA and DHA EEs compared to rTAG in patients with hyperlipidaemia or coronary artery disease, respectively. However, three studies in healthy, nomolipidaemic subjects reported that all lipid structures tested were equally effective in increasing EPA and DHA status [13; 14; 22], although one study reported a pharmaceutical preparation of FFAs to be more effective than EEs [9]. These findings, together with those of the present study, suggest that differences in the lipid structure of EPA+DHA may have, at most, modest effects on the increase in these fatty acids in blood lipids in healthy individuals. It is possible that the differences between lipid structures reported in some studies [6; 7; 21] may reflect impaired lipid metabolism associated with dyslipidaemia. In this context, the lipid structure in which EPA and DHA are consumed may have little effect on the increment in status in the general population, both men and women, although this may be an important consideration in specific groups of patients.

Previous studies measured either the incorporation of EPA and DHA consumed in different lipid structures into total plasma lipids [9; 13; 14; 23; 24; 25] or into single plasma lipid classes [21; 26; 27; 28]. One previous study compared EPA and DHA incorporation into plasma PC and cholesteryl esters during dietary supplementation, but did not report direct comparison between lipid classes [22]. Thus, the present findings report for the first time differential incorporation of EPA and DHA into individual plasma lipid classes during both the postprandial period and long-term supplementation. Incorporation of EPA and DHA into plasma PC was greater in terms of iAUC than into plasma

TAG and, in turn, into NEFAs. The greatest concentration of EPA after a meal was in plasma TAG, compared to plasma PC and in turn to plasma NEFAs. However, this incorporation difference between lipid classes was not found for DHA. Twelve weeks supplementation resulted in greater concentrations of EPA and DHA in plasma PC, when compared to plasma TAG and in turn NEFAs. However, the differential plasma lipid pool enrichment may reflect differences in the lipoprotein composition of fasting compared to postprandial blood. Although there was no difference between lipid classes in the time to reach maximum EPA concentration, the appearance of DHA in plasma NEFAs was slightly slower than in plasma TAG and PC for some lipid structures. Together, these findings suggest that, as may be expected, EPA and DHA are initially incorporated into plasma TAG and PC, probably in the form of chylomicrons. Appearance in plasma NEFAs may reflect incomplete entrapment of fatty acids released by lipoprotein lipase catalysed hydrolysis of chylomicrons[29]. However, because the amount of EPA and DHA in each supplement was not equal, it was not possible to assess whether there was differential incorporation of these fatty acids between lipid classes that has been reported previously[30; 31].

After an initial early postprandial increase in plasma TAG EPA concentration, EPA and DHA were preferentially incorporated into plasma PC compared with TAG or NEFAs in both the postprandial period and when consumed as dietary supplements. One possible explanation is that during the postprandial period EPA and DHA from the diet are primarily incorporated into the chylomicron TAG, then released by lipoprotein lipase activity to form NEFAs and subsequently are rapidly incorporated into PC, probably in the liver [31]. This is supported by the similar time course of incorporation of EPA and DHA into NEFAs to incorporation into TAG and PC which is consistent with incomplete entrapment of these fatty acids following release by lipoprotein lipase activity [29]. These findings are consistent with plasma PC marking long-term EPA and DHA status [32].

The main strengths of this study are that several commercially available EPA and DHA lipid structures were compared directly. In addition, three blood lipid classes were analysed. In the postprandial study, all individuals consumed each of the different types of supplements. One limitation of the postprandial study was that it did not include women. The strength of the dietary supplementation study was that included both men and women, and so increased the generalisability of the findings. However, this study had a parallel rather than a crossover design. Other limitations include the exclusion of pregnant women and children who may have different nutritional requirements for EPA and DHA. Pregnant women may also metabolise EPA+DHA differently compared to non-pregnant women because of well known adaptations to metabolism[33]. Furthermore, we did not study the effect of EPA and DHA provided in the phospholipid form.

There are claims of enhanced bioavailability of omega-3 fatty acids provided as phospholipids [26; 27]. It will be important to explore this using the study designs used in the current research.

Together these findings show that in healthy individuals, neither the lipid structure nor the overall fatty acid composition of supplements that contain EPA and DHA significantly influence their bioavailability during dietary supplementation, despite the apparently lower postprandial bioavailability of EPA+DHA EEs compared to TAG or FFAs. One possible explanation is that any postprandial variation in EPA and DHA bioavailability is lost during longer term supplementation. Furthermore, there were, at most, small effects of sex on the incorporation of EPA into plasma lipids, which is in agreement with the findings of previous studies[19]. One implication of these findings is that choice of the lipid structure of EPA and DHA is not a primarily consideration in the design of interventions to improve EPA and DHA status of healthy adults.

Acknowledgments

This work was supported by a grant from Vifor Pharma, Switzerland. Supplements were donated by Vifor Pharma.

Conflicts of interest

PCC serves as an advisor to Pronova BioPharma, Dutch State Mines (DSM), Cargill, Smartfish and Danone and has previously served as an advisor to Vifor Pharma and Aker Biomarine. GCB serves as an advisor to BASF, and currently or has previously received research funding from Abbott Nutrition and Nestle. ALW has no conflicts of interest to declare. The funder of the studies (Vifor Pharma) agreed the design, but had no input into the conduct of the studies, the analysis or interpretation of the data, or the writing of the manuscript.

Author's contributions

GCB and PCC designed the studies; ALW recruited subjects and conducted both studies including all laboratory work under the supervision of GCB and PCC; ALW and GCB analysed the data; GCB drafted the manuscript; all authors provided intellectual input into the manuscript and agree its content.

References

1. Calder PC (2015) Functional roles of fatty acids and their effects on human health. J Parenter Enteral Nutr **39**, 18S-32S.
2. Kris-Etherton PM, Harris WS *et al.* Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106**, 2747-2757.
3. Givens DI & Gibbs RA (2008) Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. *Proc Nutr Soc* **67**, 273-280.
4. Dyerberg J, Madsen P, Moller JM *et al.* (2010) Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukot Essent Fatty Acids* **83**, 137-141.
5. Harris WS & Von Schacky C (2004) The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med* **39**, 212-220.
6. 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.
7. Neubronner J, Schuchardt JP, Kressel G *et al.* (2011) Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. *Eur J Clin Nutr* **65**, 247-254.
8. 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.
9. Offman E, Marenco T, Ferber S *et al.* (2013) Steady-state bioavailability of prescription omega-3 on a low-fat diet is significantly improved with a free fatty acid formulation compared with an ethyl ester formulation: the ECLIPSE II study. Vasc Health Risk Manag **9**, 563-573.
10. Lawson LD & Hughes BG (1988) Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters. *Biochem Biophys Research Comm* **152**, 328-335.
11. 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((R)) compared to Lovaza((R)) in a pharmacokinetic single-dose evaluation) study. *J Clin Lipidol* **6**, 573-584.
12. 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.

13. Laidlaw M, Cockerline CA & Rowe WJ (2014) A randomized clinical trial to determine the efficacy of manufacturers' recommended doses of omega-3 fatty acids from different sources in facilitating cardiovascular disease risk reduction. *Lipids Health Dis* **13**, 99.
14. Krokan HE, Bjerve KS & Mork E (1993) The enteral bioavailability of eicosapentaenoic acid and docosahexaenoic acid is as good from ethyl esters as from glyceryl esters in spite of lower hydrolytic rates by pancreatic lipase in vitro. *Biochim Biophys Acta* **1168**, 59-67.
15. Lawson LD & Hughes BG (1988) Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. *Biochemical and biophysical research communications* **156**, 960-963.
16. Burdge GC, Powell J & Calder PC (2006) Lack of effect of meal fatty acid composition on postprandial lipid, glucose and insulin responses in men and women aged 50-65 years consuming their habitual diets. *Br J Nutr* **96**, 489-500.
17. Folch J, Lees M & Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497-509.
18. Burdge GC, Wright P, Jones AE *et al.* (2000) A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction. *Br J Nutr* **84**, 781-787.
19. Walker CG, Browning LM, Mander AP *et al.* (2014) Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans. *The Br J Nutr* **111**, 679-689.
20. Caslake MJ, Miles EA, Kofler BM *et al.* (2008) Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. *Am J Clin Nutr* **88**, 618-629.
21. Reis GJ, Silverman DI, Boucher TM *et al.* (1990) Effects of two types of fish oil supplements on serum lipids and plasma phospholipid fatty acids in coronary artery disease. *Am J Cardiol* **66**, 1171-1175.
22. Hansen JB, Olsen JO, Wilsgard L *et al.* (1993) Comparative effects of prolonged intake of highly purified fish oils as ethyl ester or triglyceride on lipids, haemostasis and platelet function in normolipaemic men. *Eur J Clin Nutr* **47**, 497-507.
23. Ramprasath VR, Eyal I, Zchut S *et al.* (2013) Enhanced increase of omega-3 index in healthy individuals with response to 4-week n-3 fatty acid supplementation from krill oil versus fish oil. *Lipids Health Dis* **12**, 178.
24. Yurko-Mauro K, Kralovec J, Bailey-Hall E *et al.* (2015) Similar eicosapentaenoic acid and docosahexaenoic acid plasma levels achieved with fish oil or krill oil in a randomized double-blind four-week bioavailability study. *Lipids Health Dis* **14**, 99.

25. Ulven SM, Kirkhus B, Lamglait A *et al.* (2011) Metabolic effects of krill oil are essentially similar to those of fish oil but at lower dose of EPA and DHA, in healthy volunteers. *Lipids* **46**, 37-46.
26. Schuchardt JP, Schneider I, Meyer H *et al.* (2011) Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations--a comparative bioavailability study of fish oil vs. krill oil. *Lipids Health Dis* **10**, 145.
27. Kohler A, Sarkkinen E, Tapola N *et al.* (2015) Bioavailability of fatty acids from krill oil, krill meal and fish oil in healthy subjects--a randomized, single-dose, cross-over trial. *Lipids Health Dis* **14**, 19.
28. Nordoy A, Barstad L, Connor WE *et al.* (1991) Absorption of the n-3 eicosapentaenoic and docosahexaenoic acids as ethyl esters and triglycerides by humans. *Am J Clin Nutr* **53**, 1185-1190.
29. Evans K, Burdge GC, Wootton SA *et al.* (2002) Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* **51**, 2684-2690.
30. Burdge GC, Sala-Vila A, West AL *et al.* (2007) The effect of altering the 20 : 5n-3 and 22 : 6n-3 content of a meal on the postprandial incorporation of n-3 polyunsaturated fatty acids into plasma triacylglycerol and non-esterified fatty acids in humans. *Prostaglandins Leukot Essent Fatty Acids* **77**, 59-65.
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. Browning LM, Walker CG, Mander AP *et al.* (2012) Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. *Am J Clin Nutr* **96**, 748-758.
33. Herrera E (2000) Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *EurJ Clin Nutr* **54 Suppl 1**, S47-S51.

Table 1. Fatty acid compositions of the lipid supplements

	Intake of fatty acids from supplements per test meal or per day (mg)				
	FFA	uTAG	rTAG(EC)	EE	rTAG
14:0	0	355	0	0	0
16:0	6	785	6	5	6
16:1n-7	2	425	2	2	2
18:0	2	151	27	1	27
18:1n-9	44	483	63	40	63
18:1n-7	3	143	17	7	19
Unknown	7	164	0	3	0
18:2n-6	289	1030	165	261	165
18:3n-6	188	144	127	170	127
18:3n-3	8	48	12	7	12
20:0	1	4	0	1	1
20:1n-9	64	182	26	59	26
20:2n-6	3	12	5	3	5
20:3n-6	4	9	5	4	5
20:4n-6	68	72	51	60	50
22:0	3	0	0	3	0
20:4n-3	30	59	42	27	41
EPA	1284	1391	1199	1159	1190
24:0	31	47	54	27	53
24:1n-9	10	13	14	9	13
22:5n-3	37	104	70	33	70
DHA	397	379	384	355	394
EPA+DHA	1681	1771	1583	1514	1584
Peroxide value (mmol/kg)	< 0.9	< 0.6	< 2.1	< 0.9	< 2.1

Concentrations of contaminants: heavy metals (Cd, Hg, Pb, As) < 0.1 ppm; polychlorinated biphenyls < 0.09 ppm; dioxins < 1.5 pg*Teq*g^{-1}; pesticides < 1 ppm. EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified triglyceride

Table 2. Characteristics of the participants in the dietary supplementation study

	Descriptive statistics											
	uTAG			FFA			rTAG(EC)			EE		
	25^{th}	50^{th}	75^{th}	25^{th}	50^{th}	75^{th}	25^{th}	50^{th}	75^{th}	25^{th}	50^{th}	75^{th}
Men												
Age, Years	19	26	38	25	28	37	21	26	34	20	28	37
BMI, $kg*m^{-2}$	21	24	30	21	25	29	21	25	32	21	25	29
Fasting plasma total TAG, $mmol*L^{-1}$	0.9	1.6	4.6	0.6	1.2	2.7	1	2.0	3.8	0.7	1.6	4.8
Fasting plasma total NEFAs, $mmol*L^{-1}$	0.2	0.3	0.5	0.2	0.4	0.9	0.2	0.4	1.4	0.2	0.4	0.6
Women												
Age, Years	22	28	44	19	25	39	20	26	44	21	28	42
BMI, $kg*m^{-2}$	20	24	29	21	23	30	21	23	29	21	24	31
Fasting plasma total TAG, $mmol*L^{-1}$	0.8	1.3	4.8	0.8	1.8	2.6	0.8	1.3	4.2	0.7	1.3	4.2
Fasting plasma total NEFAs, $mmol*L^{-1}$	0.2	0.4	1.1	0.3	0.6	0.8	0.2	0.4	0.8	0.2	0.4	0.5

Values are median (50^{th} percentile), 25^{th} and 75% percentiles. EE, ethyl ester; FFA, free fatty acid; uTAG, unmodified triglyceride; rTAG(EC), enteric protected re-esterified triglyceride.

Table 3. Postprandial changes in EPA and DHA concentrations in plasma triacylglycerol, phosphatidylcholine and non-esterified fatty acids in healthy men

Lipid structure of supplement	uTAG			FFA			rTAG (EC)			EE			rTAG		
	25%	50 %	75%	25%	50%	75%	25%	50%	75%	25%	50%	75%	25%	50%	75%
Plasma lipid class, parameter															
TAG															
EPA iAUC, μmol*h^{-1}*L^{-1}	140	230	328	140	226	462	172	216	293	151	202	276	150	183	432
EPA C_{max}, μmol*L^{-1}	22	51	75	20	76	173	32	48	82	30	53	67	25	38	119
EPA T_{max}, h	3	4	5	3	5	6	4	5	5	3	5	6	3	4	6
DHA iAUC, μmol*h^{-1}*L^{-1}	484	499	527	486	513	575	491	514	535	479	503	523	484	515	578
DHA C_{max}, μmol*L^{-1}	11	17	26	9	23	54	10	22	32	10	13	22	9	16	42
DHA T_{max}, h	2	3	4	3	3	5	4	5	5	2	4	6	3	4	5
PC															
EPA iAUC, μmol*h^{-1}*L^{-1}	335	359	501	310	370	401	330	361	431	346	386	440	321	368	417
EPA C_{max}, μmol*L^{-1}	11	18	52	11	20	33	15	21	37	14	31	37	15	21	35
EPA T_{max}, h	3	4	6	3	5	5	3	5	6	5	5	6	4	5	6
DHA iAUC, μmol*h^{-1}*L^{-1}	484	589	686	471	541	580	473	551	608	427	540	616	511	584	714
DHA C_{max}, μmol*L^{-1}	26	31	53	21	26	37	14	32	70	22	27	57	17	41	78
DHA T_{max}, h	3	4	4	1	1	3	2	2	4	1	2	3	3	4	5
NEFAs															
EPA iAUC, μmol*h^{-1}*L^{-1}	31	34	40	33	37	42	33	34	39	30	33	36	32	35	37
EPA C_{max}, μmol*L^{-1}	2	3	5	2	4	6	2	3	4	1	2†	3	2	3	4
EPA T_{max}, h	4	5	6	3	5	6	5	5	6	4	5	6	4	5	6
DHA iAUC, μmol*h^{-1}*L^{-1}	45	54	62	53	58	61	52	58	65	55	55	58	51	56	64
DHA C_{max}, μmol*L^{-1}	1	4	5	1	4	4	2	4	4	1	2	3	2	3	6
DHA T_{max}, h	4	5	6	2	5	5	4	5	5	4	5	6	4	6	6

Values are median (50^{th} percentile), 25^{th} and 75% percentiles, *n* 9/group. EE, ethyl ester; FFA, free fatty acid; uTAG, unmodified triglyceride; rTAG(EC), enteric protected re-esterified triglyceride; rTAG, re-esterified triglyceride. †Median significantly different ($p < 0.05$) from uTAG (Mann-Whitney U-test).

Table 4. Longitudinal changes in EPA and DHA concentrations in plasma triacylglycerol, phosphatidylcholine and non-esterified fatty acids in healthy men

Lipid structure of supplement	uTAG			FFA			rTAG(EC)			EE		
	25%	50 %	75%	25%	50%	75%	25%	50%	75%	25%	50%	75%
Plasma lipid class, parameter												
TAG												
EPA iAUC, μmol*week^{-1}*L^{-1}	614	674	762	611	800	877	621	719	798	532	638	748
EPA C_{max}, μmol*L^{-1}	3	10	29	6	15	45	88	15	25	0	4	19
EPA T_{max}, weeks	1	3	8	1	4	6	1	3	8	0	1	12
DHA iAUC, μmol*week^{-1}*L^{-1}	508	680	706	682	802	888	650	707	797	554	646	709
DHA C_{max}, μmol*L^{-1}	3	8	18	8	24	34	8	12	16	0	7	16
DHA T_{max}, weeks	1	3	7	4	8	12	1	5	9	0	1	8
PC												
EPA iAUC, μmol*week^{-1}*L^{-1}	808	1180	1692	1470	1759	2073	1231	1460	1665	1225	1389	1867
EPA C_{max}, μmol*L^{-1}	73	100	145	120	134	45	99	108	143	99	113	176
EPA T_{max}, weeks	3	4	7	4	8	12	1	2	3	1	2	9
DHA iAUC, μmol*week^{-1}*L^{-1}	595	1103	1458	1000	1073	1280	1059	1260	1395	967	1284	1434
DHA C_{max}, μmol*L^{-1}	18	44	94	31	39	59	47	60	80	32	50	76
DHA T_{max}, weeks	1	4	7	2	4	10	2	4	8	2	5	9
NEFAs												
EPA iAUC, μmol*week^{-1}*L^{-1}	50	62	77	54	65	84	68	70	77	62	68	84
EPA C_{max}, μmol*L^{-1}	1	2	4	2	2	5	3	3	4	2	3	5
EPA T_{max}, weeks	1	4	11	4	8	12	2	2	9	2	6	12
DHA iAUC, μmol*week^{-1}*L^{-1}	47	70	82	63	72	100	58	70	79	68	75	90
DHA C_{max}, μmol*L^{-1}	1	2	4	1	3	5	2	2	3	2	4	5
DHA T_{max}, weeks	2	6	12	2	4	12	1	2	8	2	4	9

Values are median (50th percentile), 25th and 75% percentiles, *n* 9/group. EE, ethyl ester; FFA, free fatty acid; uTAG, unmodified triglyceride; rTAG(EC), enteric protected re-esterified triglyceride.

Table 5. Longitudinal changes in EPA and DHA concentrations in plasma triacylglycerol, phosphatidylcholine and non-esterified fatty acids in healthy women

Lipid structure of supplement	uTAG			FFA			rTAG(EC)			EE		
	25%	50 %	75%	25%	50%	75%	25%	50%	75%	25%	50%	75%
Plasma lipid class, parameter												
TAG												
EPA iAUC, μmol*week^{-1}*L^{-1}	522	679	726	626	675	739	300	695	763	412	487	641
EPA C_{max}, μmol*L^{-1}	1	8	21	2	12	27	0	12	32	0	5	8
EPA T_{max}, weeks	1	4	8	1	8	12	0	3	12	0	2	4
DHA iAUC, μmol*week^{-1}*L^{-1}	543	665	746	577	634	692	426	687	768	383	611	754
DHA C_{max}, μmol*L^{-1}	3	11	12	0	0	9	3	12	24	2	4	12
DHA T_{max}, weeks	0	4	13	0	4	12	1	8	12	0	1	3
PC												
EPA iAUC, μmol*week^{-1}*L^{-1}	1650	1792	1955	1125	1535	2161	1220	1538	2177	1403	1545	2158
EPA C_{max}, μmol*L^{-1}	140	152	182	76	140	179	93	115	215	114	139	234
EPA T_{max}, weeks	2	4	8	3	4	8	2	8	12	2	4	12
DHA iAUC, μmol*week^{-1}*L^{-1}	955	1185	1424	911	1252	1687	754	1113	1425	1090	1209	1415
DHA C_{max}, μmol*L^{-1}	39	58	87	27	73	94	14	42	83	32	58	79
DHA T_{max}, weeks	4	4	8	2	4	12	2	8	8	2	4	5
NEFAs												
EPA iAUC, μmol*week^{-1}*L^{-1}	50	62	77	54	65	84	68	70	77	62	68	84
EPA C_{max}, μmol*L^{-1}	1	2	4	2	2	5	3	3	4	2	3	5
EPA T_{max}, weeks	1	4	11	4	8	12	2	2	9	2	6	12
DHA iAUC, μmol*week^{-1}*L^{-1}	47	70	82	63	72	100	58	70	79	68	75	90
DHA C_{max}, μmol*L^{-1}	1	2	4	1	3	5	2	2	3	2	4	5
DHA T_{max}, weeks	2	6	12	2	4	12	1	2	8	2	4	9

1

Values are median (50th percentile), 25th and 75% percentiles, n = 9. uTAG, unmodified triglyceride, uTAG; EE, ethyl ester; FFA, free fatty acid; rTAG(EC), enteric protected re-esterified triglyceride.

Online Supplemental Material

Supplemental table 1. EPA and DHA concentrations at baseline in the postprandial and dietary supplementation studies

Lipid structure	Fatty acid concentration (μmol*L^{-1})									
	uTAG		FFA		rTAG(EC)		EE		rTAG	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
	Postprandial study									
TAG										
EPA	9	39	8	15	6	13	6	10	8	9
DHA	12	149	13	24	9	15	9	13	10	11
PC										
EPA	60	149	58	101	47	82	47	174	52	69
DHA	140	149	132	185	143	132	145	212	110	203
NEFAs										
EPA	2	5	2	3	2	10	2	3	2	3
DHA	7	23	7	8	6	20	7	5	6	8
	Supplementation study									
	Men									
TAG										
EPA	5	21	10	27	10	31	10	44		
DHA	16	20	12	21	12	24	12	26		
PC										
EPA	43	67	47	56	56	51	37	56		
DHA	99	123	121	145	107	78	126	107		
NEFAs										
EPA	1	1	1	3	1	2	1	1		
DHA	3	4	4	4	3	8	3	6		
	Women									
TAG										
EPA	16	37	14	21	9	50	20	46		
DHA	13	40	16	32	11	49	19	56		
PC										
EPA	37	86	40	27	27	97	39	125		
DHA	121	147	96	236	125	92	114	157		
NEFAs										

EPA	1	2	1	2	1	3	1	2
DHA	3	6	5	9	4	5	4	4

Values are median and range, *n* 9/group. EE, ethyl ester; FFA, free fatty acid; uTAG, unmodified triglyceride; rTAG(EC), enteric protected re-esterified triglyceride; rTAG, re-esterified triglyceride. There were no significant differences in the concentrations of EPA and DHA between groups at baseline within each study

Supplemental Table 2. Comparisons of postprandial EPA and DHA incorporation into different lipid classes

	Friedman's test				Wilcoxon Signed-rank test								
					TAG vs PC			TAG vs NEFA			PC vs NEFA		
Comparison between lipid classes per supplement type	X^2	DF	n	P	Z	P	r	Z	P	r	Z	P	r
EPA iAUC, μmol*h^{-1}*L^{-1}													
Unmodified TAG	14.9	2	9	<0.01	-2.3	0.02	-0.5	-2.7	0.01	-0.6	-2.7	0.01	-0.6
FFA	14.0	2	9	<0.01	-1.2	0.21	-0.3	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG (EC)	18.0	2	9	<0.01	-2.7	0.01	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
EE	18.0	2	9	<0.01	-2.7	0.01	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG	14.9	2	9	<0.01	-1.5	0.14	-0.4	-2.7	0.01	-0.6	-2.7	0.01	-0.6
EPA $C_{max,}$ μmol*L^{-1}													
Unmodified TAG	14.2	2	9	<0.01	-1.6	0.11	-0.4	-2.7	0.01	-0.6	-2.4	0.02	-0.6
FFA EPA	16.2	2	9	<0.01	-2.3	0.02	-0.5	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG (EC)	14.9	2	9	<0.01	-2.3	0.02	-0.5	-2.7	0.01	-0.6	-2.7	0.01	-0.6
EE	14.9	2	9	<0.01	-1.6	0.11	-0.4	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG	14.3	2	8	<0.01	-2.0	0.04	-0.5	-2.5	0.01	-0.6	-2.5	0.01	-0.6
EPA $T_{max,}$ h													
Unmodified TAG	3.1	2	9	0.22	-0.3	0.75	-0.1	-1.8	0.08	-0.4	-0.52	0.61	-0.1
FFA	1.6	2	9	0.45	-0.1	0.92	-0.02	-1.3	0.18	-0.3	-0.4	0.67	-0.1
rTAG (EC)	1.2	2	9	0.55	-0.5	0.61	-0.1	-0.8	0.48	-0.2	-1.4	0.16	-0.3
EE	1.2	2	9	0.54	-0.6	0.57	-0.1	-0.4	0.71	-0.1	-0.4	0.67	-0.1
rTAG (EC^1)	3.6	2	8	0.17	-0.2	0.83	-0.1	-2.1	0.04	-0.5	0.0	1.00	0.0
DHA iAUC, μmol*h^{-1}*L^{-1}													
Unmodified TAG	13.6	2	9	<0.01	-1.5	0.14	-0.4	-2.7	0.01	-0.6	-2.7	0.01	-0.6
FFA	14.0	2	9	<0.01	-0.4	0.68	-0.1	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG (EC)	13.6	2	9	<0.01	-0.7	0.52	-0.2	-2.7	0.01	-0.6	-2.7	0.01	-0.6
EE	13.6	2	9	<0.01	-0.7	0.52	-0.2	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG	13.6	2	9	<0.01	-1.4	0.17	-0.3	-2.7	0.01	-0.6	-2.7	0.01	-0.6
DHA C_{max}, μmol*L^{-1}													
Unmodified TAG	10.8	2	8	0.01	-1.5	0.14	-0.4	-2.1	0.04	-0.5	-2.5	0.01	-0.6
FFA	13.0	2	8	<0.01	-0.9	0.34	-0.2	-2.7	0.01	-0.7	-2.5	0.01	-0.6
rTAG (EC)	14.0	2	9	<0.01	-1.1	0.29	-0.3	-2.7	0.01	-0.6	-2.7	0.01	-0.6

EE	14.3	2	8	<0.01	-2.2	0.03	-0.6	-2.7	0.01	-0.7	-2.5	0.01	-0.6
rTAG	12.0	2	8	<0.01	-1.5	0.14	-0.4	-2.5	0.12	0.6	-2.5	0.12	-0.6
DHA $T_{max,}$ h													
Unmodified TAG	9.2	2	8	0.01	-0.5	0.60	-0.1	-2.3	0.02	-0.6	-2.4	0.02	-0.6
FFA	5.8	2	8	0.05	-1.6	0.12	-0.4	-1.9	0.06	-0.5	-1.7	0.09	-0.4
rTAG (EC)	5.6	2	9	0.06	-1.5	0.13	-0.4	-1.4	0.16	-0.3	-2.2	0.03	-0.5
EE	11.6	2	8	<0.01	-1.8	0.08	-0.5	-1.8	0.07	-0.5	-2.5	0.01	-0.6
rTAG	7.7	2	8	0.02	-0.3	0.75	-0.1	-2.3	0.02	-0.6	-2.2	0.03	-0.6

EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG, re-esterified TAG; rTAG(EC), enteric protected r TAG.

Supplemental Table 3. Relationship between BMI and the iAUC for incorporation of EPA and DHA into individual plasma lipid classes during the postprandial period.

	Spearman's Correlation			
Comparison between lipid classes per supplement type	BMI (*n* 9/ supplement)		Age (*n* 9/ supplement)	
	β	P	β	P
Plasma TAG EPA iAUC, μmol*h^{-1}*L^{-1}				
Unmodified TAG	-0.03	0.93	-0.47	0.20
FFA	0.26	0.50	0.00	1.00
rTAG (EC)	0.42	0.26	-0.19	0.63
EE	-0.08	0.83	-0.67	0.05
rTAG	0.42	0.26	0.03	0.93
Plasma TAG DHA iAUC, μmol*h^{-1}*L^{-1}				
Unmodified TAG	-0.03	0.95	-0.45	0.35
FFA	0.18	0.65	-0.02	0.97
rTAG (EC)	0.53	0.15	0.05	0.90
EE	-0.04	0.92	-0.54	0.14
rTAG	0.18	0.65	-0.03	0.93
Plasma PC EPA iAUC, μmol*h^{-1}*L^{-1}				
Unmodified TAG	-0.3	0.43	-0.13	0.74
FFA	0.17	0.67	0.23	0.56
rTAG (EC)	0.68	0.05	0.54	0.14
EE	-0.23	0.54	-0.68	0.04
rTAG	-0.75	0.02	-0.51	0.16
Plasma PC DHA iAUC, μmol*h^{-1}*L^{-1}				
Unmodified TAG	-0.45	0.22	-0.17	0.67
FFA	0.41	0.27	0.48	0.19
rTAG (EC)	0.47	0.20	0.83	<0.01
EE	-0.27	0.49	0.14	0.71
rTAG	0.04	0.92	0.24	0.54
Plasma NEFA EPA iAUC, μmol*h^{-1}*L^{-1}				
Unmodified TAG	-0.23	0.54	-0.29	0.44
FFA	0.28	0.47	0.11	0.78

rTAG (EC)	0.42	0.26	0.06	0.89
EE	-0.32	0.40	-0.25	0.51
rTAG	0.11	0.78	-0.40	0.28
Plasma NEFA DHA iAUC, $\mu mol*h^{-1}*L^{-1}$				
Unmodified TAG	-0.22	0.57	-0.56	0.12
FFA	0.51	0.16	0.43	0.25
rTAG (EC)	0.30	0.43	0.07	0.86
EE	-0.03	0.93	-0.44	0.24
rTAG	-0.05	0.90	0.03	0.95

EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified TAG.

Supplemental table 4. Total plasma lipid concentrations at baseline and end of dietary supplementation study

Lipid structure	Plasma lipid concentration (μmol*L$^{-1)}$															
	uTAG				FFA				rTAG(EC)				EE			
	Start		End		Start		End		Start		End		Start		End	
	Med	Range	Med	Range	Med	Range	Med	Range	Med	Range	Med	Range	Med	Range	Med	Range
								Men								
Total TAG	1648	3732	2555	7414	1192	2043	1499	4845	1974	2833	1724	3960	1639	4092	1852	2249
Total PC	4241	1936	4319	6077	4120	2519	3128	5161	4415	2161	3948	4015	4587	2822	4067	2280
Total NEFA	314	347	369	669	409	673	344	1654	399	1203	350	427	365	408	416	1528
								Women								
Total TAG	1310	4045	1411	1744	1809	1761	1809	3659	1326	3438	934	1438	1264	3435	3830	3191
Total PC	4262	3901	3824	3011	4527	4084	3948	4469	4366	3256	3566	6273	3830	3191	3639	3817
Total NEFA	359	920	360	573	554	589	310	1091	421	588	404	547	390	276	317	1312

Values are median (Med) and range, *n* 9/group. EE, ethyl ester; FFA, free fatty acid; uTAG, unmodified triglyceride; rTAG(EC), enteric protected re-esterified triglyceride; rTAG, re-esterified triglyceride. There were no significant differences between the start and end of the study in any of the lipid classes that were measured.

Supplemental Table 5. Comparisons of EPA and DHA incorporation into different lipid classes following 12 weeks' supplementation in healthy men

	Friedman's test				Wilcoxon Signed-rank test								
					TAG vs PC			TAG vs NEFA			PC vs NEFA		
Comparison between lipid classes per supplement type	X^2	DF	n	P	Z	P	r	Z	P	r	Z	P	r
EPA iAUC, μmol*weeks^{-1}*L^{-1}													
Unmodified TAG	18	2	9	<0.01	-2.7	0.01	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
FFA	18	2	9	<0.01	-2.7	0.01	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG(EC)	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EE	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EPA C_{max}, μmol*L^{-1}													
Unmodified TAG	16	2	9	<0.01	-2.7	0.01	-0.6	-2.4	0.02	-0.6	-2.7	0.01	-0.6
FFA	15	2	9	<0.01	-2.7	0.01	-0.6	-2.3	0.02	-0.5	-2.7	0.01	-0.6
rTAG(EC)	18	2	10	<0.01	-2.8	0.01	-0.6	-2.7	<0.01	-0.6	-2.8	0.01	-0.6
EE	15	2	10	<0.01	-2.8	0.01	-0.6	-1.0	0.33	-0.2	-2.8	0.01	-0.6
EPA T_{max}, weeks													
Unmodified TAG	0.2	2	9	0.90	-0.3	0.74	-0.1	-0.2	0.83	-0.1	-0.3	0.80	-0.1
FFA	4.7	2	9	0.10	-1.7	0.09	-0.4	-1.5	0.13	-0.4	-0.3	0.79	-0.6
rTAG(EC)	0.4	2	10	0.82	-0.6	0.54	-0.1	-0.3	0.74	-0.1	-1.0	0.33	-0.2
EE	1.6	2	10	0.44	-0.4	0.68	-0.1	-1.1	0.29	-0.2	-0.9	0.35	-0.2
DHA iAUC, μmol*weeks^{-1}*L^{-1}													
Unmodified TAG	16	2	9	<0.01	-2.4	0.02	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
FFA	18	2	9	<0.01	-2.7	0.01	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG(EC)	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EE	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
DHA C_{max}, μmol*L^{-1}													
Unmodified TAG	8	2	9	0.02	-2.3	0.02	-0.5	-2.2	0.03	-0.5	-2.5	0.01	-0.6
FFA	16	2	9	<0.01	-2.1	0.04	-0.5	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG EC)	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EE	12	2	10	<0.01	-2.6	0.01	-0.6	-1.3	0.20	-0.3	-2.8	0.01	-0.6
DHA T_{max}, weeks													
Unmodified TAG	<1	2	9	0.80	-0.4	0.73	-0.1	-1.1	0.29	-0.3	-0.6	0.53	-0.1

FFA	2	2	9	0.37	-1.2	0.23	-0.3	-0.8	0.44	-0.2	-0.5	0.62	-0.2
rTAG (EC)	3	2	10	0.25	-0.6	0.53	-0.1	-0.8	0.44	-0.2	-1.1	0.29	-0.2
EE	3	2	10	0.21	-1.4	0.18	-0.3	-1.3	0.21	-0.29	-0.1	0.92	<0.1

EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified TAG.

Supplemental Table 6. Comparisons of EPA and DHA incorporation into different lipid classes following 12 weeks' supplementation in healthy women

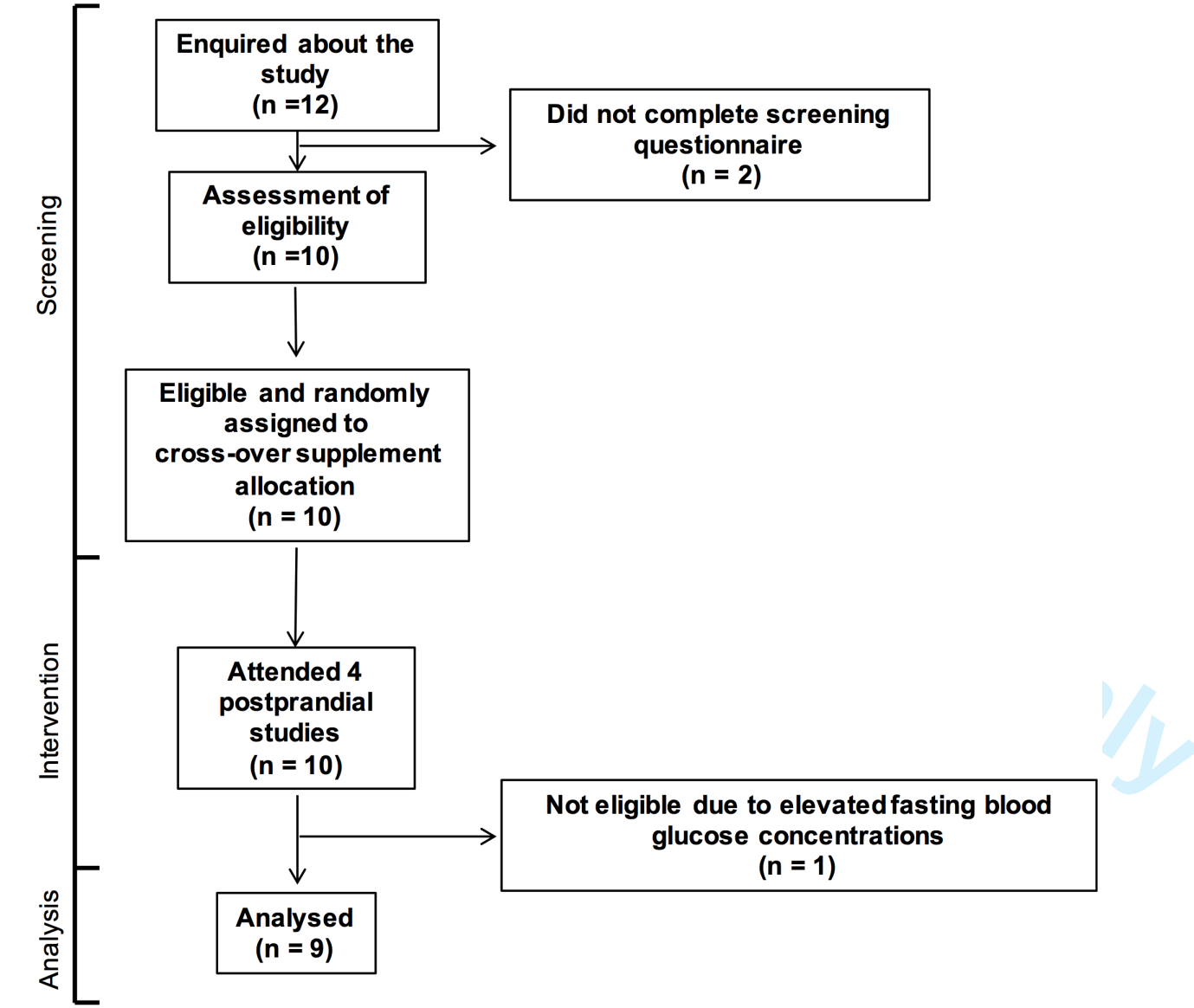
	Friedman's test				Wilcoxon Signed-rank test								
					TAG vs PC			TAG vs NEFA			PC vs NEFA		
Comparison between lipid classes per supplement type	X^2	DF	n	P	Z	P	r	Z	P	r	Z	P	r
EPA iAUC, µmol*weeks^{-1}*L^{-1}													
Unmodified TAG	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
FFA	16	2	9	<0.01	-2.4	0.02	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG (EC)	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EE	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EPA C_{max}, µmol*L^{-1}													
Unmodified TAG	16	2	10	<0.01	-2.8	0.01	-0.6	-1.5	0.14	-0.3	-2.8	0.01	-0.6
FFA	15	2	9	<0.01	-2.7	0.01	-0.6	-1.8	0.07	-0.4	-2.7	0.01	-0.6
rTAG (EC)	16	2	10	<0.01	-2.8	0.01	-0.6	-2.2	0.03	-0.5	-2.8	0.01	-0.6
EE	15	2	10	<0.01	-2.8	0.01	-0.6	-0.4	0.72	-0.1	-2.8	0.01	-0.6
EPA T_{max}, h													
Unmodified TAG	1.3	2	10	0.52									
FFA	1.3	2	9	0.53									
rTAG (EC)	1.5	2	10	0.48									
EE	9.6	2	10	0.1									
DHA iAUC, µmol*weeks^{-1}*L^{-1}													
Unmodified TAG	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
FFA	18	2	9	<0.01	-2.7	0.01	-0.6	-2.7	0.01	-0.6	-2.7	0.01	-0.6
rTAG (EC)	18	2	10	<0.01	-2.7	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
EE	20	2	10	<0.01	-2.8	0.01	-0.6	-2.8	0.01	-0.6	-2.8	0.01	-0.6
DHA C_{max}, µmol*L^{-1}													
Unmodified TAG	13	2	10	<0.01	-2.8	0.01	-0.6	-1.8	0.07	-0.4	-2.7	0.01	-0.6
FFA	11	2	9	<0.01	-2.5	0.01	-0.6	-0.1	0.95	<-0.1	-2.7	0.01	-0.6
rTAG (EC)	6	2	10	0.05	-1.5	0.14	-0.3	-2.2	0.03	-0.5	-2.7	0.01	-0.6
EE	15	2	10	<0.01	-2.8	0.01	-0.6	-0.6	0.58	-0.1	-2.8	0.01	-0.6
DHA T_{max}, h													

Unmodified TAG	1	2	10	0.62
FFA	1	2	9	0.67
rTAG (EC)	<1	2	10	0.97
EE	6	2	10	0.06

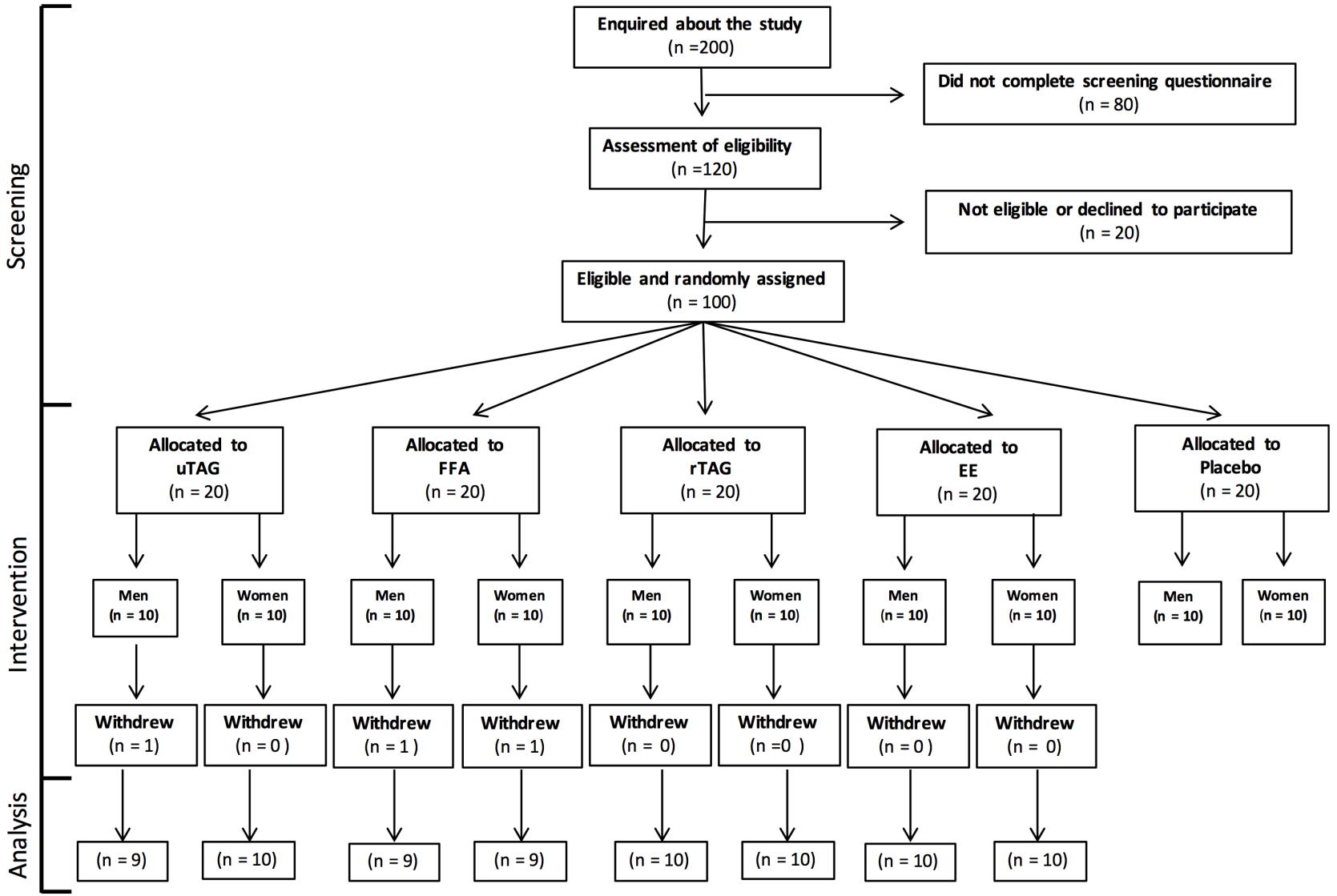
EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified TAG.

Supplemental Table 7. Relationship between BMI and the iAUC for incorporation of EPA and DHA into individual plasma lipid classes during the postprandial period.

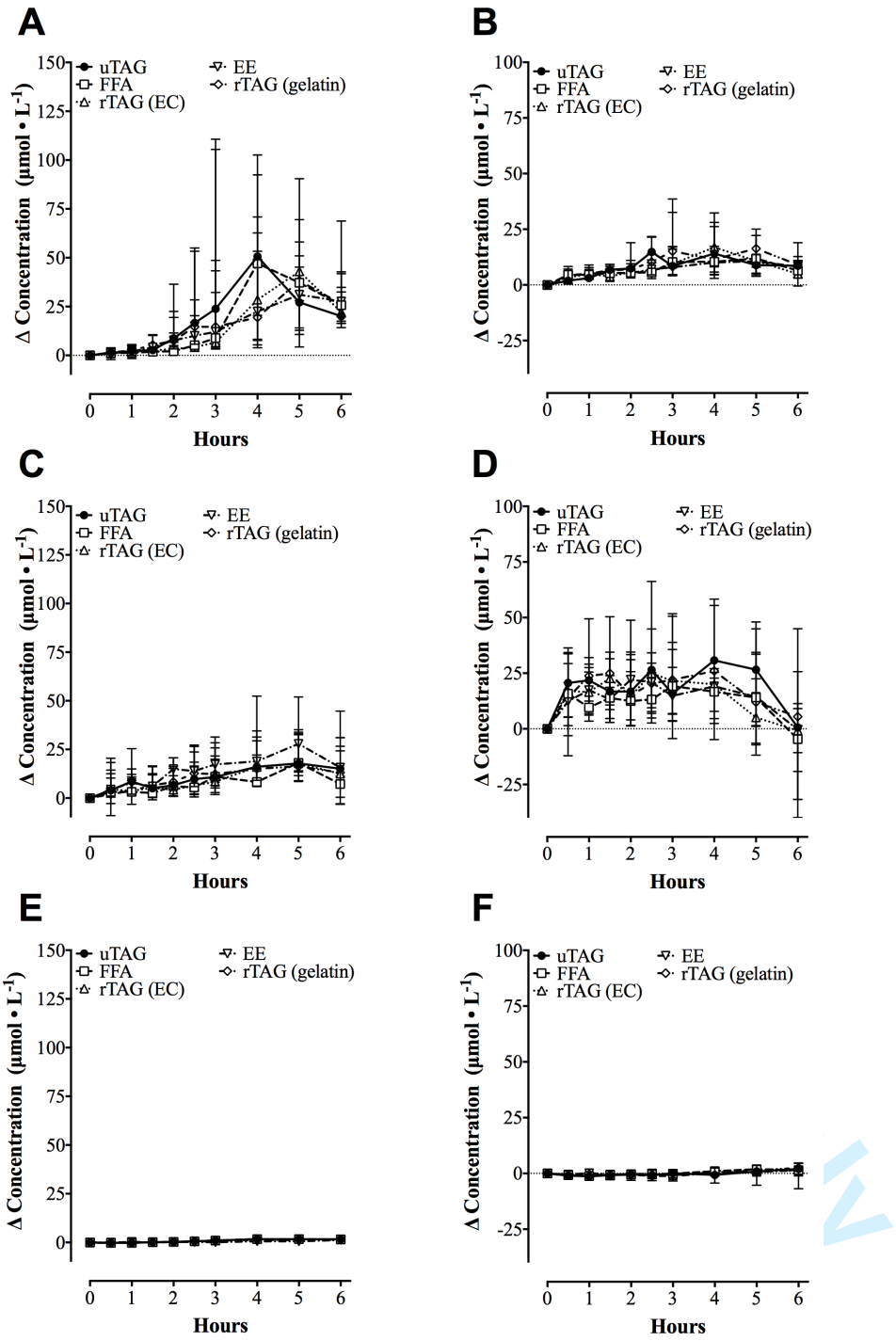
	Spearman's Correlation							
	Men (*n* 38)				Women (*n* 39)			
	BMI		Age		BMI		Age	
	β	P	β	P	β	P	β	P
iAUC (μmol*week^{1}*L^{-1})								
EPA								
TAG	-0.42	0.803	0.151	0.365	0.52	0.001	-0.165	0.315
PC	-0.126	0.451	0.261	0.114	0.262	0.107	0.615	0.315
NEFA	-0.267	0.106	0.081	0.629	0.129	0.434	0.043	0.796
DHA								
TAG	-0.134	0.422	0.05	0.764	0.461	0.003	0.039	0.815
PC	-0.031	0.854	0.026	0.875	0.287	0.077	0.133	0.419
NEFA	-0.141	0.398	-0.051	0.73	0.054	0.746	0.217	0.184



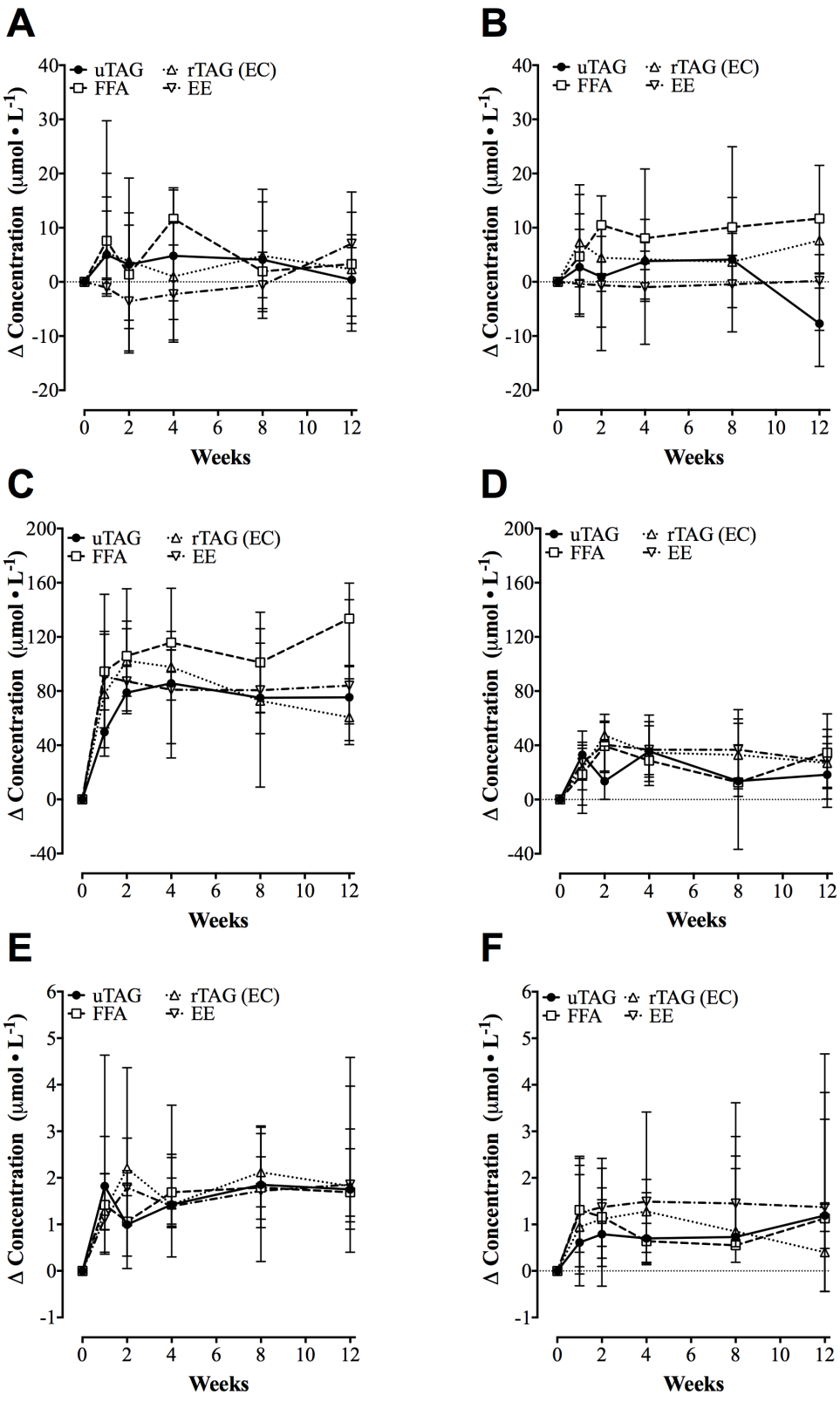
Supplemental Fig. 1. CONSORT flow diagram of the postprandial study.



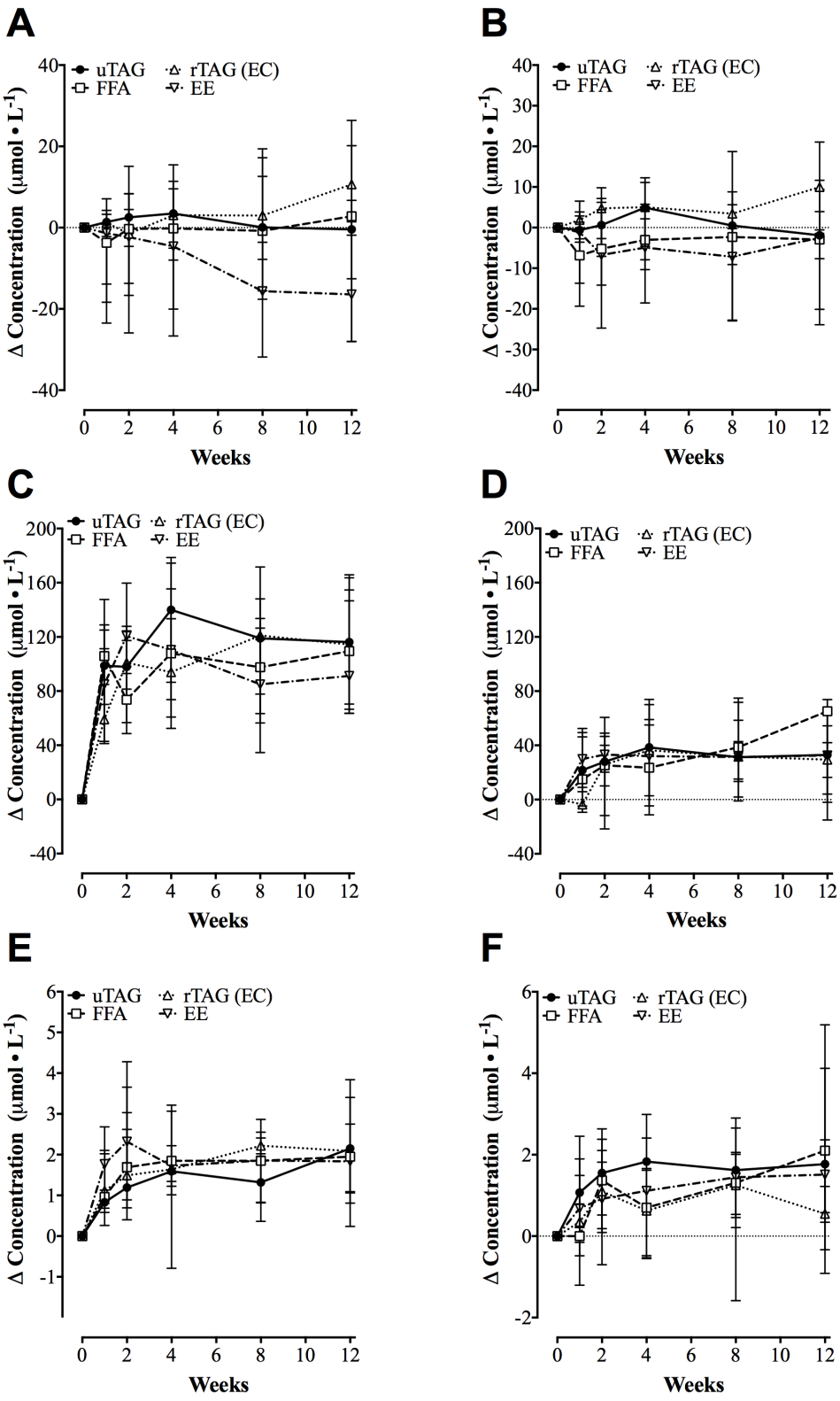
Supplemental Fig. 2. CONSORT flow diagram of the dietary supplementation study. EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG, re-esterified TAG.



Supplemental Fig. 3. Postprandial incremental change in EPA (A,C,E) and DHA (B,D,F) concentration in plasma TAG (A,B), PC (C,D) and NEFA (E,F). Values are median (50th percentile), and 25% and 75% percentiles (n=9 subjects). EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified TAG; rTAG(gelatin), genatin encapsulated tTAG.



Supplemental Fig. 4. Incremental change in EPA (A,C,E) and DHA (B,D,F) concentration during dietary supplementation in plasma TAG (A,B), PC (C,D) and NEFA (E,F) in men. Values are median (50th percentile), and 25% and 75% percentiles (*n* 9 or 10 subjects, Supplemental Figure 2). EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified TAG; rTAG(gelatin), genatin encapsulated tTAG.



Supplemental Fig. 5. Incremental change in EPA (A,C,E) and DHA (B,D,F) concentration during dietary supplementation in plasma TAG (A,B), PC (C,D) and NEFA (E,F) in women. Values are median (50th percentile), and 25% and 75% percentiles (*n* 9 or 10 subjects, Supplemental Figure 2). EE, ethyl ester; FFA, free fatty acid; uTAG , unmodified triglyceride; rTAG(EC), enteric protected re-esterified TAG; rTAG(gelatin), genatin encapsulated tTAG.