Differential postprandial incorporation of 20:5n-3 and 22:6n-3 into individual plasma triacylglycerol and phosphatidylcholine molecular species in humans

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Abbreviations: BFO, blended fish oil; CE, cholesteryl ester; CSO, transgenic *Camelina sativa* oil; FAME, fatty acid methyl ester; LPL, lipoprotein lipase; LCAT, lecithin: cholesterol acyl transferase; LCPUFA, long chain polyunsaturated fatty acids; MTBE, methyl-tert-butyl-ether; PC, phosphatidylcholine; TG, triacylglycerol; VLDL, very low density lipoprotein.

ABSTRACT

The mechanisms by which digested fat is absorbed and transported in the circulation are well documented. However, it is uncertain whether the molecular species composition of dietary fats influences the molecular species composition of meal-derived lipids in blood. This may be important because enzymes that remove meal-derived fatty acids from the circulation exhibit differential activities towards individual lipid molecular species. To determine the effect of consuming oils with different molecular compositions on the incorporation of 20:5n-3 and 22:6n-3 into plasma lipid molecular species. Men and women (18 - 30 years) consumed standardised meals containing 20:5n-5 and 22:6n-3 (total 450mg) provided by an oil from transgenic *Camelina sativa* (CSO) or a blended fish oil (BFO) which differed in the composition of 20:5n-3 and 22:6n-3 – containing molecular species. Blood was collected during the subsequent 8 hours. Samples were analysed by liquid chromatography-mass spectrometry. The molecular species composition of the test oils was distinct from the composition of plasma triacylglycerol (TG) or phosphatidylcholine (PC) molecular species at baseline and at 1.5 or 6 hours after the meal. The rank order by concentration of both plasma PC and TG molecular species at baseline was maintained during the postprandial period. 20:5n-3 and 22:6n-3 were incorporated preferentially into plasma PC compared to plasma TG. Together these findings suggest that the composition of dietary lipids undergoes extensive rearrangement after absorption, such that plasma TG and PC maintain their molecular species composition, which may facilitate lipase activities in blood and/or influence lipoprotein structural stability and function.

Key words: Phosphatidylcholine, triacylglycerol, docosahexaenoic acid, eicosapentaenoic acid, postprandial, molecular species

1. Introduction

The mechanisms by which dietary fatty acids are absorbed, enter the blood stream and are turned over in the circulation are well described [1,2]. Briefly, dietary triacylglycerol (TG) is hydrolysed in the small intestine, primarily by the activity of pancreatic lipase, to yield fatty acids, derived from cleavage at the *sn*-1/3 positions, and *sn*-2 monoacylglycerol (2-MG) [3] which are absorbed from the unstirred water layer interface between the gut lumen and the apical surface of enterocytes. Fatty acids and 2-MG derived from hydrolysed TG are re-esterified in enterocytes to form primarily TG [4] or phosphatidylcholine (PC) before assembly into chylomicrons. In human enterocytes, between 70% and 90% of TG is synthesised via the MG pathway during the postprandial period [5], while the remainder is formed by the glycerol-3-phosphate pathway [4,6]. Enterocytes synthesise PC by the CDP:phosphocholine cytidylyltransferase pathway [7,8] and by re-acylation of biliary lysoPC [7, 8]. PC synthesis is required for absorption of digested lipids, and for the assembly and formation and secretion of chylomicrons [7,9]. However, the relevance of these experiments in rodents to human postprandial lipid metabolism and to understanding the relative contributions of these pathways to chylomicron metabolism under physiological conditions is uncertain. Recent findings show that in addition to contributing to chylomicron structure, PC may be an important carrier of meal-derived long-chain polyunsaturated fatty acids (PUFA), namely 20:5n-3 and 22:6n-3, from enterocytes to the blood stream [11, 12]. One possible implication of these findings is that the partitioning of fatty acids between chylomicron TG and PC may be an important influence on the composition of fatty acids available to peripheral tissues after a meal. This suggestion is supported by the observation that the fatty acid composition of chylomicron TG can influence the uptake of meal-derived fatty acids by peripheral tissues [13]. In this context, the molecular specificity of enterocyte TG and PC synthesis may be an important factor in the postprandial metabolism of blood lipids.

The two principal enzymes that hydrolyse esterified fatty acids in blood; lipoprotein lipase (LPL) and lecithin-cholesterol acyltransferase (LCAT) exhibit differential activity towards TG and PC molecular species, respectively, according to fatty acid chain length, unsaturation and the distribution of fatty acids between *sn* positions on the glycerol moiety of TG [14, 15]. LCAT preferentially hydrolyses *sn*-1 16:0 PC molecular species when paired with *sn*-2 polyunsaturated fatty acids (PUFA) including 20:4n-6 or 22:6n-3 [16]. The fatty acid composition and *sn* position within the glycerol moiety chylomicron TG can influence particle size [17] and LPL activity [15, 18-20] which, in turn, can influence rate of clearance of meal-derived lipids from the circulation [21, 22]. This may have implications for health as the

rate of chylomicron clearance is a risk factor for cardiovascular disease [23]. Thus, it is possible that the molecular species composition of chylomicron PC and TG may influence their turnover by LPL and LCAT within the circulation, and consequently the uptake of fatty acids derived from the meal by peripheral tissues.

There is limited information about the postprandial incorporation of fatty acids into individual lipid molecular species in human blood. The molecular species composition of chylomicron and VLDL TG during the postprandial period from women who consumed palm oil or transesterified palm oil suggested that the plasma lipids reflected the molecular species of the ingested lipids [24], although the reporting of the fatty acid compositions of individual species was incomplete. Moreover, the findings of a study in which men consumed inter-esterified fats containing differing amounts of saturated or *trans* fatty acids, or 18:1n-9 or 18:2n-6 also suggested that the composition of chylomicron TG molecular species reflected that of the dietary oils, although individual molecular species were not reported [25]. In contrast, others have shown rearrangement of fatty acids in chylomicron TG compared to dietary TG [26] and that the molecular structure of ingested lipids containing 20:5n-3 and 22:6n-3 did not influence the concentrations of these fatty acids in blood during the postprandial period [11].

The purpose of the present study was to investigate whether differences in the molecular species composition of TG in dietary oils influenced composition of plasma lipids during the postprandial period. We compared the molecular species composition of two oils, a commercially prepared blended fish oil (BFO) with an oil from transgenic *Camelina sativa* (CSO) [27] that were the sole sources of 20:5n-3 and 22:6n-3 in each of two standardised test meals, with the molecular species composition of plasma TG during the postprandial period in healthy men and women. We also investigated the impact of consuming these test meals on the concentrations of PC molecular species that contained 20:5n-3 and/or 22:6n-3.

2. Materials and Methods

2.1. Preparation of seed oil from transgenic C. sativa

Seed oil from transgenic *C. sativa* plants that produce 20:5n-3 and 22:6n-3 was prepared as described previously [12, 27]. Briefly, seeds were harvested from a homozygous T3 generation of transgenic *C. sativa* plants grown in an environmentally controlled containment glasshouse. After threshing, the oil was extracted by cold-pressing and solvent extraction, and then refined, bleached and deodorised (POS Bio-Sciences, Canada).

2.2. Ethics statement

Ethical approval for the study was granted by the South Central – Hampshire B Research Ethics Committee (REC reference 15/SC/0627). The study is registered at ClinicalTrials.gov (Identifier: NCT03477045). All participants provided written informed consent.

2.3. Human postprandial study

The data reported here were obtained from the analysis of samples collected as part of a postprandial crossover study that compared the incorporation into blood lipids of 20:5n-3 and 22:6n-3 provided as either a commercially prepared BFO (Simply Timeless®, Seven Seas, Feltham, UK) or as CSO [12]. Details of the study design, including participant characteristics and inclusion criteria, are described elsewhere [12]. Briefly, healthy men and women aged between 18 to 30 years consumed one of two test breakfasts after fasting overnight for approximately 12 hours. The test meals had similar nutrient contents and fatty acid compositions (details of the nutrient compositions and ingredients used are described elsewhere [12]). Both test meals contained approximately 450 mg 20:5n-3 plus 22:6n-3 from either BFO (452 mg) or CSO (455mg). The total fat content of the test meals was BFO 49.5g and CSO 50.2g [12]. The amount of 20:5n-3 plus 22:6n-3 was based on current UK recommended daily intakes [28], while the total fat content of the test meals relfected 50% of the daily total fat intakes across the range reproted in European countries [29]. Thus the findings are potential relevance to the dietary habits of western populations. The meals were consumed in random order at least 14 days apart. Venous blood samples were collected at baseline and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 and 8 hours after the meal. No other food was consumed during this period, but water was consumed *ad libitum*. Samples collected at baseline and at 1.5 and 6 hours after consuming the test meal were used for detailed analysis of lipid molecular species compositions. Samples collected at 1.5 hours and 6 hours corresponded to peak 20:5n-3 and 22:6n-3 concentrations in TG and PC determined by gas chromatography [12]. Cells were removed from blood by centrifugation at 4^{o}C [11] and the plasma fraction then stored at -80^{o}C.

2.4. Analysis of the fatty acid composition of blood lipids by gas chromatography

The fatty acid composition of plasma PC, TG and CE was measured by gas chromatography as described [11,30]. Briefly, dipentadecanoyl PC (100 μg), tripentadecanoin (100 μg) and CE heptadecanoate (100 μg) internal standards were added to 0.8 ml plasma (800 μl). Plasma total lipids were extracted with chloroform/methanol (2:1 (v/v)) and individual lipid classes isolated

by solid phase extraction [12,30]. Fatty acids within purified lipid classes were converted to fatty acid methyl esters (FAME) by reaction with methanol containing 2% (v/v) sulphuric acid at 50 ^{o}C for 2 hours [30]. FAME were resolved on a BPX-70 fused silica capillary column (30 m x 0.25 mm x 25 µm) using an Agilent 6890 gas chromatograph equipped with flame ionisation detection as described [12]. Fatty acid concentrations (µmol/ml) were calculated as described [30].

2.5. Analysis of the molecular species composition of the test oils and of blood lipids by mass spectrometry

Total lipids were extracted from plasma (200 µl) using methyl-tert-butyl-ether (MTBE) [31] and stored under nitrogen at -20 ^{o}C prior to analysis. Briefly, MTBE (5 ml) was added to plasma (200 µl) and shaken at room temperature for 60 minutes. Water (1.25 ml) was then added, the mixture vortex mixed and then centrifuged at 700 x g for 10 minutes. The organic phase was collected and the aqueous phase then re-extracted with MTBE/methanol/water (10:3:2.5, v/v/v). The organic phases were combined and dried under nitrogen at 35^{o}C, dissolved in chloroform/methanol (9:1, v/v) and stored at -20^{o}C. TG and PC molecular species compositions were analysed by high resolution / accurate mass (HR/AM) lipidomics [32-34] using a Vanquish - Q Exactive Plus UPLC-MS/MS system (Thermo Fisher Scientific).

Plasma total lipids were diluted 1 in 100 in chloroform:methanol (1:1, v/v). Internal standard, tripentadecanion (TG15:0/15:0/15:0; 0.857µM) and 1,2-dinervonoyl-sn-glycero-3-phosphocholine (PC24:1/24:1; 0.857µM) were added and 20µl injected into the UPLC. Lipids were separated using an Accucore C18 (2.1 x 150 mm, 2.6 mm) column (Thermo Fisher Scientific) at 35^{o}C with autosampler tray temperature at 10^{o}C and flow rate 400 µl min^{-1}. Mobile phase: A = 10 mM ammonium formate in 50% acetonitrile + 0.1% formic acid, B = 2 mM ammonium formate in acetonitrile:propan-2-ol:water (10:88:2 v/v/v) + 0.02% formic acid. The elution gradient ran for 28 minutes from 35% B at start to 100% at 24 mins. The Thermo Q Exactive HESI II probe was in use probe position in C. Conditions were adjusted for separate positive and negative runs. Running samples in a single polarity facilitated identification of more molecular species. LC/MS full-scan at 140K resolution and data-independent HCD MS2 experiments at 35,000 resolution were performed in positive and negative ion modes. The top 15 most abundant peaks from full scan with a mass resolution of 140,000 for m/z 150-1200 were used for fragmentation. Higher-energy collision dissociation with a 35,000 mass resolution was performed using an isolation window of 1 m/z, maximum integration time of 75 ms and dynamic exclusion window of 8 s. The stepped collision energy

was 25, 30, 40 eV replacing 25 with 30 eV negative ion mode. Sheath gas set to 60, Aux gas 20, sweep gas 1, spray voltage 3.2 KV in positive ion mode with small adjustments in negative ion mode, capillary temperature 320^{o}C and aux gas heater set to 370^{o}C. The LipidSearch 4.2 experimental workflow (Thermo Fisher Scientific) was used for lipid characterisation and potential lipid species were identified separately from positive or negative ion adducts. The data for each biological replicate were aligned within a chromatographic time window by combining the positive and negative ion annotations and merging these into a single lipid annotation.

Following normalisation to internal standards, peak areas corresponding to individual PC or TG molecular species that contained either 20:5n-3 and/or 22:6n-3 were expressed as a proportion of the total species containing 20:5n-3 and/or 22:6n-3 in either lipid class. The proportion of each molecular species was then used to calculate its concentration from the concentrations of 20:5n-3 or 22:6n-3 measured by gas chromatography in either plasma PC or TG. Molecular species that contained both 20:5n-3 and 22:6n-3 were present at low levels and were included with the 22:6n-3 -containing species. It was not possible to assign fatty acids to specific glycerol *sn* positions of individual TG molecular species which are reported as combinations of fatty acids separated by an underscore [35]. Assignment of fatty acids to the *sn*-1 and *sn*-2 positions of PC was based on human plasma PC molecular species [36]. Raw lipid molecular species composition data can be found at the EMBL-EBI MetaboLights database (http://www.ebi.ac.uk/metabolights)[37] with the identifier MTBLS1348.

2.6. Statistical analysis

Based on our previous findings [12], ten participants per group provided 85% power to detect a 10% difference in peak postprandial 20:5n-3 or 22:6n-3 concentrations in plasma PC or TG with $\alpha = 5\%$ in a two tailed analysis. This level of statistical power was confirmed by retrospective power calculations. Statistical comparisons were by ANOVA with sex and test oil as fixed factors, and time as a repeated measure with Tukey's *post hoc* correction for multiple testing. The distribution of the data was tested by analysis of residuals by the Shapiro–Wilk test. According to Mauchly's Test of Sphericity, the assumption of sphericity was not met for any of the molecular species measured and hence the Greenhouse-Geisser correction was applied to ANOVA analyses. Estimated marginal means were calculated to allow comparison of the concentrations of individual molecular species between time points with adjustment for any interaction effects of participant sex or the type of test oil. *Post hoc*

pairwise testing was by Tukey's test. All data are presented as mean ± SEM and statistical significance was assumed at $P < 0.05$.

3. Results

3.1. Triacylglycerol molecular species compositions of the test oils

Two hundred and seventy-eight TG molecular species were identified in the test oils of which 40 contained 20:5n-3 in CSO and 40 in BFO (Table 1). All the molecular species that contained 20:5n-3 were present in both oils. However, the relative contribution of each species differed between the test oils. The top three most abundant TG species accounted for approximately one quarter of all 20:5n-3 in both oils, but these molecular species differed between CSO (TG18:1_20:5_20:5 > TG18:0_20:5_20:5 > TG20:1_18:1_20:5; 26.4%) and BFO (TG16:0_18:1_20:5 > TG16:0_16:1_20:5 > TG16:0_20:5_20:5; 25.2%). TG18:4_18:4_20:5, TG18:3_17:1_20:5 and TG20:5_20:5_21:1 had the same position in the rank order of the proportions of TG molecular species in both CSO and BFO, but accounted for a greater proportion of 20:5n-3 in BFO compared with CSO (Table 1)

The same sixty-six TG molecular species that contained 22:6n-3 were identified in both CSO and BFO (Table 2). TG18:0_16:0_22:6 > TG18:2_18:2_22:6 > TG16:0_18:1_22:6 were the most abundant 22:6n-3 -containing TG species in CSO, while the 22:6n-3 –containing species present in the greatest proportion in BFO were TG16:0_16:0_22:6 > TG16:0_18:1_22:6 > TG18:0_16:0_22:6. The top three most abundant species accounted for approximately one third of all 22:6n-3 -containing species in each of the test oils (CSO, 30.6%; BFO 33.7%). TG18:0_22:6_20:0, TG18:0_22:6_22:6, TG18:1_22:5_22:6, TG24:1_18:2_22:6, TG20:0_22:5_22:6, TG22:0_22:6_22:6 and TG24:0_20:3_22:6 had the same position in the rank order of 22:6n-3 –containing TG species but were present in greater proportions in BFO compared with CSO (Table 2).

Ten TG molecular species were identified in both test oils that contained 20:5n-3 and 22:6n-3 (Table 3). TG16:0_20:5_22:6 accounted for approximately two thirds of 20:5n-3 and 22:6n-3 species in both test oils. TG20:5_22:6_22:6 and TG20:5_17:1_22:6 occupied the same rank position in both test oils, but accounted for a greater proportion of TG species in BFO compared to CSO.

3.2. Postprandial incorporation of 20:5n-3 and 22:6n-3 into plasma triacylglycerol molecular species

One hundred and eighty-two TG molecular species were identified in plasma collected at baseline and at 1.5 and 6 hours during the postprandial period. Of these, ten molecular species contained 20:5n-3 (Fig. 1), three contained 20:5n-3 and 22:6n-3 and twenty-five contained 22:6n-3 (Fig. 2). At baseline, TG16:0_18:2_20:5 was the most abundant 20:5n-3 –containing species, TG16:0_20:5n-3_22:6n-3 was the most abundant species that contained both 20:5n-3 and 22:6n-3, and TG16:0_18:1_22:6 was the most abundant species that contained 22:6n-3 (Fig.s 1 - 3). The concentration of TG16:0_20:5_22:6 was approximately 10% of that of TG16:0_18:2_20:5 and 4% of TG16:0_18:1_22:6 (Fig.s 2,3). There was a significant effect of time after the test meal was consumed on the concentrations of all TG molecular species that contained 20:5n-3, 22:6n-3 or 20:5n-3 and 22:6n-3 (Fig.s 1 - 3, Table 4). TG16:0_18:2_20:5, TG18:1_18:2_20:5 and TG20:5_18:2_18:2 showed the greatest overall increase in concentration compared to baseline (approximately 1 to 3 μmol/l), while the increase in concentration of all other 20:5n-3 and 22:6 –containing species was less than 0.2 μmol/1 (Figure 2). There was no significant single factor effect of the type of oil consumed on the concentrations of any 20:5n-3 or 22:6n-3 –containing molecular species during the postprandial period (Table 4). There was a significant sex*time effect on the concentrations of TG16:0_18:2_20:5, TG20:5_18:2_18:2, TG16:1_18:2_20:5, TG18:1_20:5_20:5 and TG16:0_12:0_20:5 (Table 4). There was a significant interactive effect of time*oil type*sex on TG18:1_20:5_20:5 concentration and a tendency towards a three-way interactive effect on TG16:0_18:2_20:5 and TG16:0_20:5_20:5 concentrations (Table 4).

There was a significant effect of time after the test meal was consumed on the concentrations of all TG molecular species that contained 22:6n-3 except TG18:1_22:6_22:6 and TG18:2_22:6_22:6 (Table 4). There was a significant sex*time interaction effect on the concentrations of TG18:2_18:2_22:6, TG16:0_18:3_22:6, TG16:1_18:2_22:6 and TG18:1_12:0_22:6, and a tendency towards an effect on TG18:1_18:2_22:6 (Table 4). There was no significant singe factor effect of the type of oil consumed on the concentrations of any of the 22:6n-3 –containing molecular species (Table 4). There were no 3-way interactive effects on the concentrations of any of the 22:6n-3-containing species that were measured. The increments at 6 hours in the concentrations of 22:6n-3 –containing species tended to be less than for 20:5n-3 -containing species.

There was no overlap in the rank order of TG molecular species containing 20:5n-3 and/or 22:6n-3 in CSO and BFO (Tables 1 - 3) with that of plasma TG molecular species at 1.5 hours after consuming the respective test oils, when the concentrations of recently secreted chylomicrons were highest [12] (Tables 1,2; Fig.s 1 - 3).

3.3 Postprandial incorporation of 20:5n-3 and 22:6n-3 into plasma phosphatidylcholine molecular species

Fifty-four PC molecular species were identified in all plasma samples, of which four species contained 20:5n-3 and eight species contained 22:6n-3 (Fig. 4). No species containing both 20:5n-3 and 22:6n-3 were detected. At baseline, PC16:0/20:5 and PC 18:0/20:5 were the most abundant 20:5n-3 -containing species. The two *sn*-1 alkyl species that contained 20:5n-3, PC16:1Alkyl/20:5 and PC18:1Alkyl/20:5, were present in markedly lower concentrations than the equivalent ester-linked species (Fig. 4). The major 22:6n-3 -containing PC species at baseline were, in order of decreasing concentration, PC16:0/22:6 > PC18:0/22:6 > PC18:1/22:6 (Fig. 4). The two alkyl species that contained 22:6n-3, PC16:1Alkyl/22:6 and PC18:0Alkyl/22:6 were present in lower concentrations that the ester-linked counterparts (Fig. 4).

There was a significant effect of time on the concentrations of all 20:5n-3 and 22:6n-3 –containing PC species that were measured, except PC16:1Alkyl/22:6 (Table 5). However, there were no interactive effects of sex or the type of oil consumed on any of the 20:5n-3 and 22:6n-3 –containing PC species that were measured. The change in PC16:0/20:5 concentration at 6 hours compared to baseline was between 2-fold and 3- fold greater than PC18:0/22:5. The increment in PC16:0/22:6 concentration at 6 hours was approximately 4-fold greater than for PC18:0/22:6.

3.4 Incorporation of 20:5n-3 and 22:6n-3 into plasma cholesteryl esters

There was a significant effect of time ($P < 0.0001$) on the concentration of cholesteryl-16:0, but not on cholesteryl-20:5n-3 or cholesteryl-22:6n-3 concentrations (both $P = 0.2$) (Figure 4).

3.5 The time course of the postprandial incorporation of 20:5n-3 and 22:6n-3 into plasma phosphatidylcholine and triacylglycerol molecular species

Based on estimated marginal means, the concentration of PC16:0/20:5 was significantly greater at 1.5 hours than at baseline, and at 6 hours than 1.5 hours (Fig. 6A). In contrast, PC18:0/20:5, PC16:0/22:6 and PC18:0/22:6 concentrations were greater at 1.5 hours than at

baseline, but did not change significantly between 1.5 hours and 6 hours (Figure 6 A,B). Other minor PC 20:5 and 22:6 -containing molecular species that changed significantly over time also increased significantly at 1.5 hours compared to baseline, without further change. However, PC15:0/22:6 concentration was significantly lower at 1.5 hours and 6 hours compared to baseline.

The concentrations of seven out of ten 20:5n-3 -containing TG species that changed significantly over time were greater at 1.5 hours than at baseline, but did not differ further between 1.5 hours and 6 hours (Fig. 6C). In contrast, TG16:0_20:5_20:5, TG18:3_18:2_20:5 and TG20:5_18:2_20:5 concentrations were significantly lower at 1.5 hours than at baseline and did not change significantly between 1.5 hours and 6 hours. The concentrations of twenty out of twenty-two 22:6n-3 -containing TG molecular species that changed significantly over time were greater at 1.5 hours than at baseline, but did not differ significantly between 1.5 hours and 6 hours, while TG18:1_18:3_22:6, TG18:3_18:2_22:6 concentrations were significantly greater at 6 hours than at baseline or at 1.5 hours (Fig. 6D).

4. Discussion

The findings of this study show that the molecular composition of dietary fats had little, if any, relationship to the molecular composition of blood lipids during the postprandial period. Instead, these observations suggest that processes involved in the post-absorptive assembly of LCPUFA into TG and PC result in the complete rearrangement of the molecular composition of the ingested lipids in a manner that could influence the partitioning of these fatty acids between metabolic fates. Moreover, these findings also showed that 20:5n-3 and 22:6n-3 are preferentially incorporated during the postprandial period into plasma PC compared to plasma TG or CE.

CSO and BFO were composed of complex mixtures of TG molecular species. Despite similarities in the overall proportions of 20:5n-3 and 22:6n-3 in CSO and BFO [12] and their combinations with other fatty acids in individual molecular species, the relative contribution of each molecular species to the overall 20:5n-3 and 22:6n-3 content differed markedly between the test oils. For example, in CSO the most abundant 20:5n-3 and 22:6n-3 -containing TG species tended to also contain other unsaturated fatty acids. In BFO TG, 20:5n-3 and 22:6n-3 had a greater tendency to be combined with saturated fatty acids than in CSO. Previous analysis of the positional isomers of TG from an unspecified FO showed that almost all of the 22:6n-3 and 44% of the 20:5n-3 were esterified at the *sn*-2 position [38]. In contrast, 20:5 and

22:6n-3 have been shown to be esterified predominately at the *sn*-1/3 position in CSO [27]. In rodents, ingested fatty acids that are esterified at the *sn*-1/3 position of TG have greater bioavailability than those at the *sn*-2 position due to the lower accessibility of the *sn*-2 position to pancreatic lipases [39, 40]. In contrast, the present results and our previous findings [12] show no significant differences between the test oils in the incorporation of 20:5n-3 and 22:6n-3 into plasma total TG or individual plasma TG molecular species. Thus, any differences between the test oils in the distribution of 20:5n-3 and 22:6n-3 between TG *sn* positions have little influence their assimilation.

The differences in the TG molecular species compositions of the test oils were not reflected in the molecular composition of plasma TG during the postprandial period. This suggests that the TG molecular species composition of ingested lipids has little, if any, influence on composition of 20:5n-3 and 22:6n-3 –containing TG molecular species that were secreted into the circulation during the postprandial period. This is consistent with studies which showed that the structure of ingested lipids does not influence the postprandial incorporation of 20:5n-3 and 22:6n-3 into blood lipids [41, 42]. One interpretation is that the composition of meal-derived plasma TG containing 20:5n-3 and 22:6n-3 may be determined primarily by the selectivity of TG synthesis and could reflect, at least in part, the substrate specificity of acyl-CoA:diacylglycerol acyltransferases [43] in enterocytes and the incorporation of newly synthesised TG into chylomicrons. However, the present findings do not exclude the possibility that the molecular species composition of TG from a meal could be modified by the activities of lipases in blood.

It is assumed generally that chylomicron TG is the primary means by which PUFA, including 20:5n-3 and 22:6n-3, enter the circulation [1], although incorporation of 20:5n-3 and 22:6n-3 into plasma PC may be quantitatively more important than TG for transport of dietary LCPUFA from enterocytes into the bloodstream [11, 12]. The present findings agree with this view and extend these observations by showing that 20:5n-3 and 22:6n-3 were incorporated into specific PC molecular species. For example, the concentration of PC16:0/20:5, the predominant 20:5n-3 –containing PC species, at 1.5 hours was approximately 6.5 -fold greater than the most abundant 20:5n-3-containing TG species; TG16:0_18:2_20:5. The concentration of PC16:0/22:6, the principal 22:6n-3 –containing PC species, was between 23 and 26 -fold greater than the most abundant 22:6n-3 -containing TG molecular species; TG16:0_18:1_22:6. Similarly, when participants consumed 20:5n-3 and 22:6n-3 in different molecular forms the incorporation during the postprandial period of 20:5n-3 and 22:6n-3 into plasma PC was 30% greater than into plasma TG, irrespective of the structure of the ingested LCPUFA [11]. One

interpretation is that 20:5n-3 and 22:6n-3 primarily enter the circulation on the surface of chylomicrons which could facilitate their availability for hydrolysis by lipoprotein lipase, although this preferentially cleaves PC at the *sn*-1 position [15]. One further implication is that studies that measure postprandial incorporation of 20:5n-3 and 22:6n-3 into plasma TG alone, and exclude the contribution of PC, may underestimate the assimilation of these LCPUFA.

The present findings and those reported previously [36] show that 20:5n-3 and 22:6-3 are incorporated primarily into PC molecular species containing *sn*-1 16:0 compared to species containing *sn*-1 18:0. LCAT preferentially transfers 16:0 from the *sn*-1 position of PC to cholesteryl esters when the *sn*-2 position is occupied by LCPUFA, in particular 22:6n-3 [16]. For example, dietary supplementation for 6 weeks with 20:5n-3 and 22:6n-3 increased the proportion of *sn*-2 22:6n-3 in plasma PC, but also increased the proportion of 16:0, but not 18:0, in plasma CE [44]. Thus, preferential partitioning of 20:5n-3 and 22:6n-3 into *sn*-1 16:0 PC species may facilitate CE synthesis by LCAT during the postprandial period. The present findings showed a rapid increase in cholesteryl- 16:0 during the early postprandial period, while there was no significant enrichment of plasma CE with either 20:5n-3 or 22:6n-3. This suggests incorporation of meal derived LCPUFA into PC may be important for subsequent CE synthesis during the postprandial period. Dietary supplementation with either fish oil for 21 days [45] or an algal oil for 29 days [46] induced differential incorporation of 20:5n-3 and 22:6n-3 into individual plasma PC molecular species, including those which showed the greatest increments during the postprandial period in the present study. Together these findings suggest that plasma PC composition may also be determined by the specificity of PC synthesis, including the relative contributions of the Kennedy and acyl-remodelling mechanisms [47]. This agrees with previous studies that have shown that the molecular species composition of plasma PC is regulated physiologically rather than by diet. For example, pregnancy in humans and rodents is associated with a differential increase in plasma and/ or hepatic PC16:0/22:6 concentration [48, 49], which in rodents involves decreased hepatic acyl remodelling of newly synthesised *sn*-1 16:0 to *sn*-1 18:0 PC species [49].

The greatest postprandial increments in 20:5n-3 and 22:6n-3 were in TG and PC molecular species that were present at the highest concentrations at baseline, namely TG16:0_18:2_20:5, TG16:0_18:1_22:6, PC16:0/20:5 and PC16:0/22:6. Moreover, the rank order of the concentrations of individual 20:5n-3 and 22:6n-3 –containing TG and PC species at baseline was essentially maintained during the postprandial period. Plasma TG and PC in baseline samples represents primarily VLDL derived from the liver. Enterocytes can secrete VLDL-like particles in the fasting state, although these are likely to only be a minor

contributor to the total VLDL pool in humans [2]. However, PC and TG carrying fatty acids derived from a meal enter the circulation as chylomicrons [1]. If the specificity of TG and PC synthesis is a primary determinant of the composition of these lipids in plasma, then in order for the rank order of the molecular species in these lipids to be maintained throughout the fasting and postprandial periods the specificity of TG and PC synthesis would need to be similar in enterocytes and hepatocytes. This view is supported in part by one study in rodents that suggested rapid postprandial equilibration of chylomicron phospholipid molecular species with those of endogenous lipoproteins [50]. Thus, the present findings may suggest that in human chylomicron TG and PC molecular species equilibrate rapidly with PC and TG carried by VLDL. If so, one implication of these findings is that there is a metabolically favourable molecular species composition of lipoprotein TG and PC which is determined, at least in part, by the specificity of TG [51] and PC synthesis [47]. One alternative interpretation of the present findings, that does not exclude former suggestion, is that the persistence of specific molecular species throughout the postprandial period may represent those that are resistant to hydrolysis and so accumulate during the early postprandial period but are not turned over rapidly. The presence in cell membranes of 'pivot' PC molecular species that are resistant to turnover has been proposed as a mechanism to control membrane structure and function [52]. In the present context, it is possible that maintaining particular PC and TG molecular species compositions during the postprandial period may be important for conferring structural stability and function of lipoproteins.

The main limitations of the study were that individual lipoprotein classes were not isolated and so interpretation of the findings in terms of the metabolism of specific lipoproteins is speculative and the *sn* positions of the fatty acids in TG could not be determined.

6. Conclusions

In conclusion, these findings show that the molecular species composition of dietary lipids undergoes extensive rearrangement after absorption. Whether this facilitates alignment with the substrate specificities of key enzymes that mediate postprandial hydrolysis of dietary fatty acids in TG-rich lipoproteins and/or reflects the persistence of specific molecular species that are important for lipoprotein structure or function remains to be determined. These findings may have implications for understanding variation between individuals in the effectiveness of dietary interventions to increase 20:5n-3 and 22:6n-3 status and for identifying mechanisms by which specific fatty acids are supplied to individual tissues.

Author contributions

G.C.B., P.C.C., E.A.M., K.A.L. and J.A.N designed and supervised the study; L.H., O.S., R.P.H, L.V.M., R.G. and A.L.W carried out the experiments; G.C.B, A.L.W. L.VM. analysed the data; G.C.B. wrote the first draft of the manuscript with input from all authors.

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Declaration of Competing interests

GCB has received research funding from Nestle, Abbott Nutrition and Danone. He has served as member of the Scientific Advisory Board of BASF and is member of the BASF Asia Grant Panel. PCC acts as a consultant to BASF AS, Smartfish, DSM, Danone and Fresenius-Kabi. JAN has provided *ad hoc* consultancy services to BASF. The other authors state they have nothing to declare.

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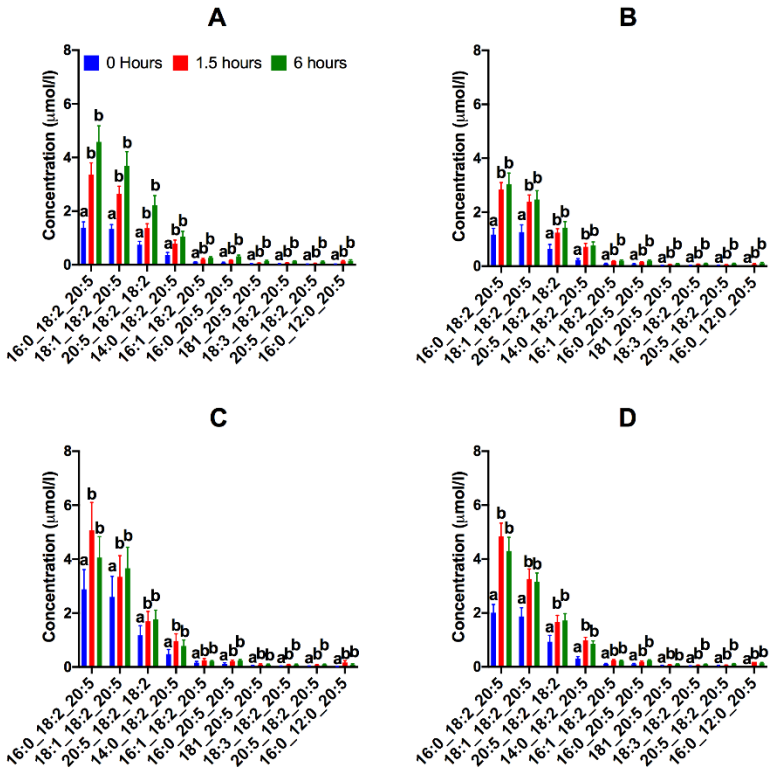
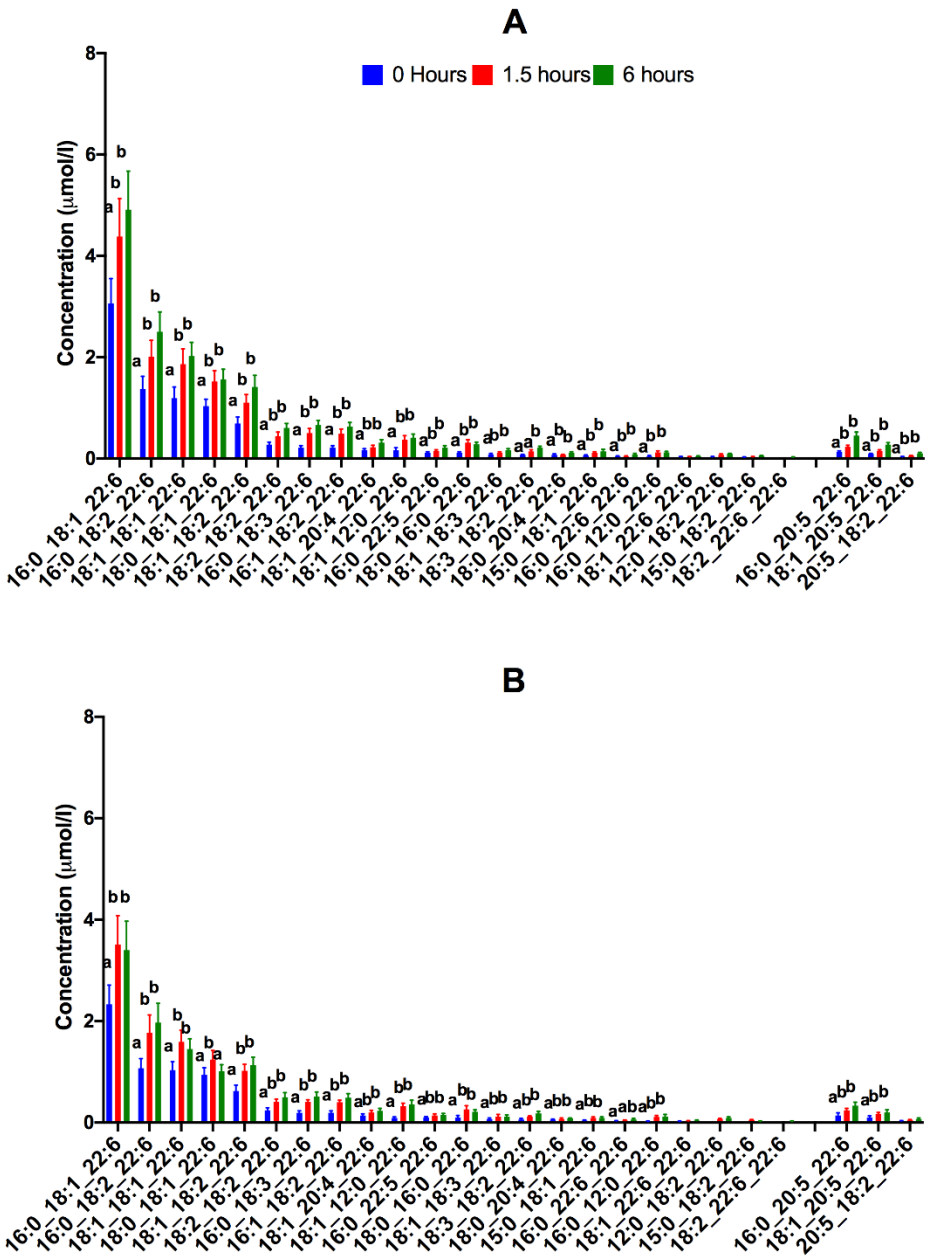
Fig. 1. Concentrations of plasma triacylglycerol molecular species that contained 20:5n-3.



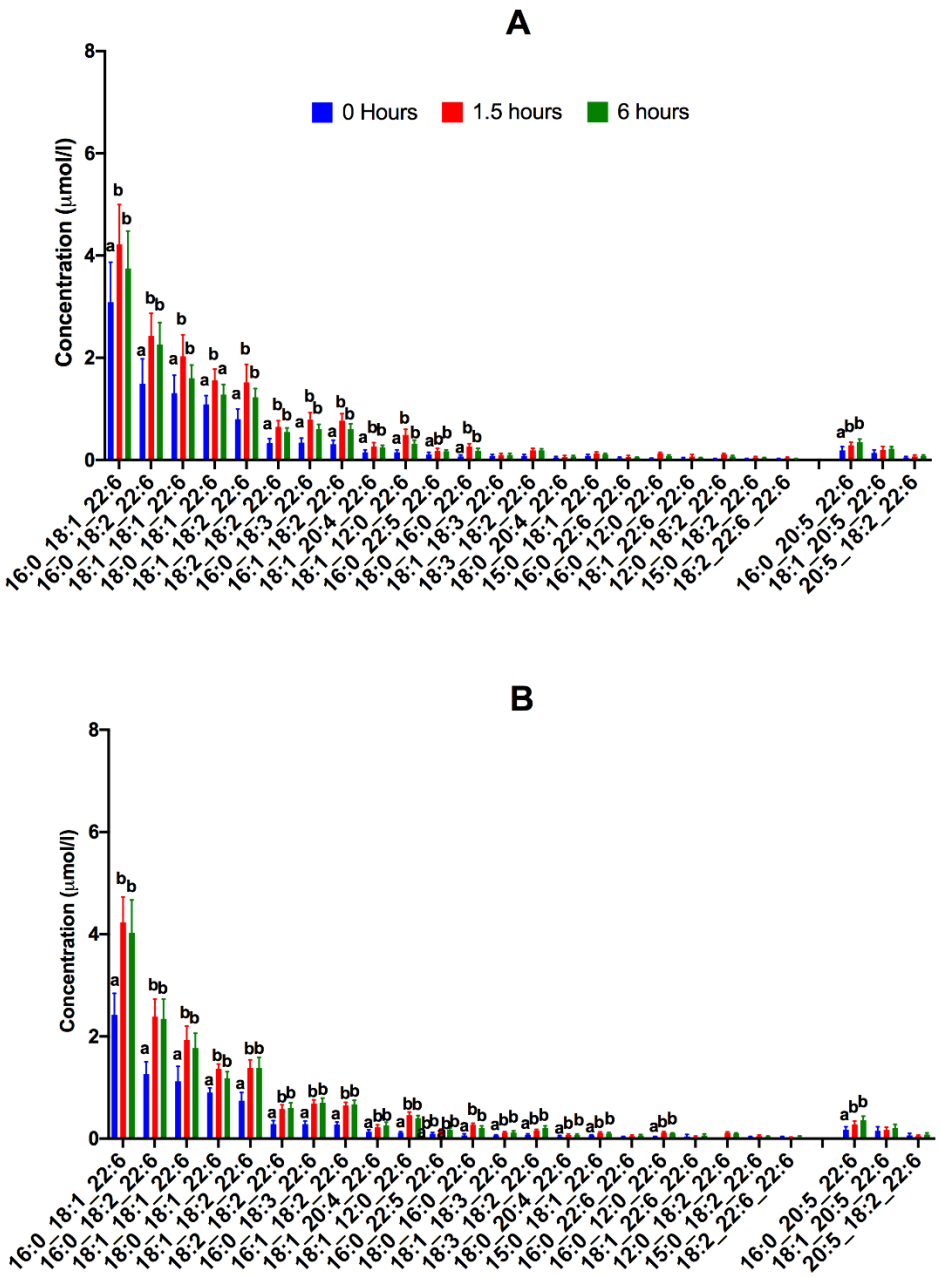
Values are mean ± SEM concentrations of individual plasma TG molecular species that contained 20:5n-3 (n = 10/sex/oil type) in descending rank order of concentration at baseline. (A) and (B) Females, (C) and (D) males; (A) and (C) CSO, (B) and (D), BFO. Data were analysed by ANOVA with type of oil and sex as fixed factors and time as a repeated measure. Tukey's correction for multiple comparisons were applied *post hoc*. Different letters indicate means which differed significantly between time points. The results of the statistical analyses are shown in Table 4. The order of the fatty acids in each molecular species does not indicate the *sn* positions of the fatty acids.

Fig. 2. Concentrations of plasma triacylglycerol molecular species that contained 22:6n-3 or 22:6n-3 and 20:5n-3 in women.



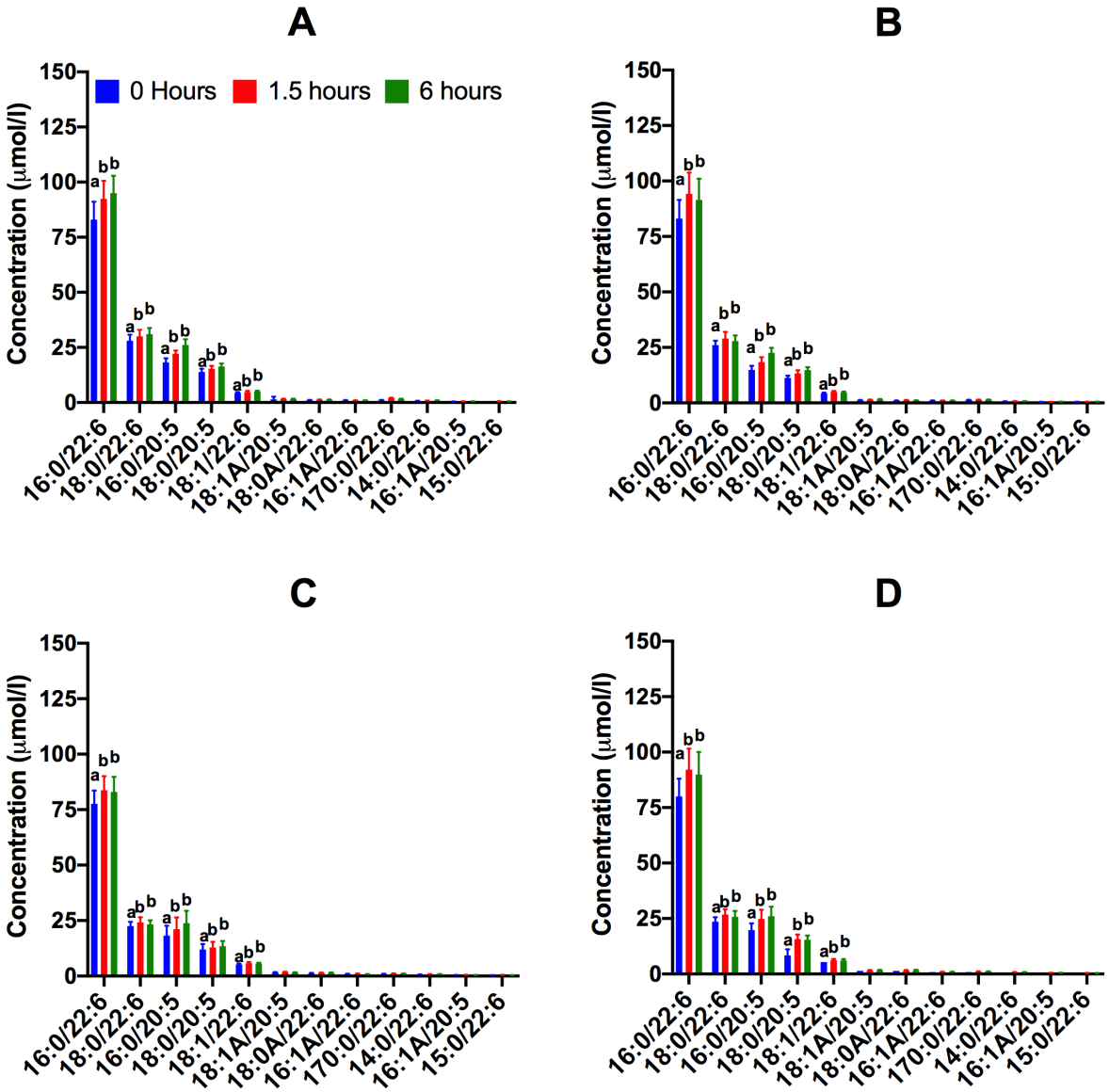
Values are mean ± SEM concentrations of individual plasma TG molecular species that contained 22:6n-3 or 22:6n-3 and 20:5n-3 (n = 10/oil type) in descending rank order of concentration at baseline. (A) CSO and (B) BFO. Data were analysed by ANOVA with type of oil and sex as fixed factors and time as a repeated measure. Tukey's correction for multiple comparisons were applied *post hoc*. Different letters indicate means which differed significantly between time points. The results of the statistical analyses are shown in Table 4. The order of the fatty acids in each molecular species does not indicate their *sn* positions.

Fig. 3. Concentrations of plasma triacylglycerol molecular species that contained 22:6n-3 or 22:6n-3 and 20:5n-3 in men.



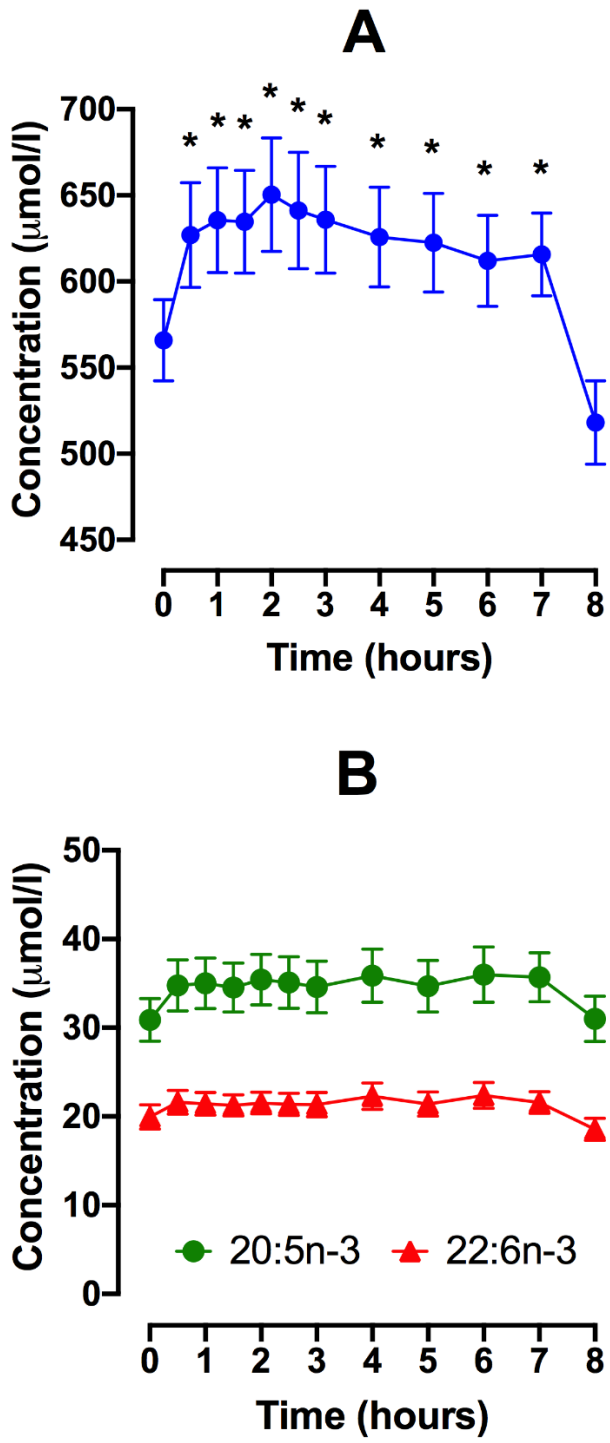
Values are mean ± SEM concentrations of individual plasma TG molecular species that contained 22:6n-3 or 22:6n-3 and 20:5n-3 (n = 10/oil type) in descending rank order of concentration at baseline. (A) CSO and (B) BFO. Data were analysed by ANOVA with type of oil and sex as fixed factors and time as a repeated measure. Tukey's correction for multiple comparisons were applied *post hoc*. Different letters indicate means which differed significantly between time points. The results of the statistical analyses are shown in Table 4. The order of the fatty acids in each molecular species does not indicate their *sn* positions.

Fig. 4. Concentrations of plasma phosphatidylcholine molecular species that contained 20:5n-3 or 22:6n-3.



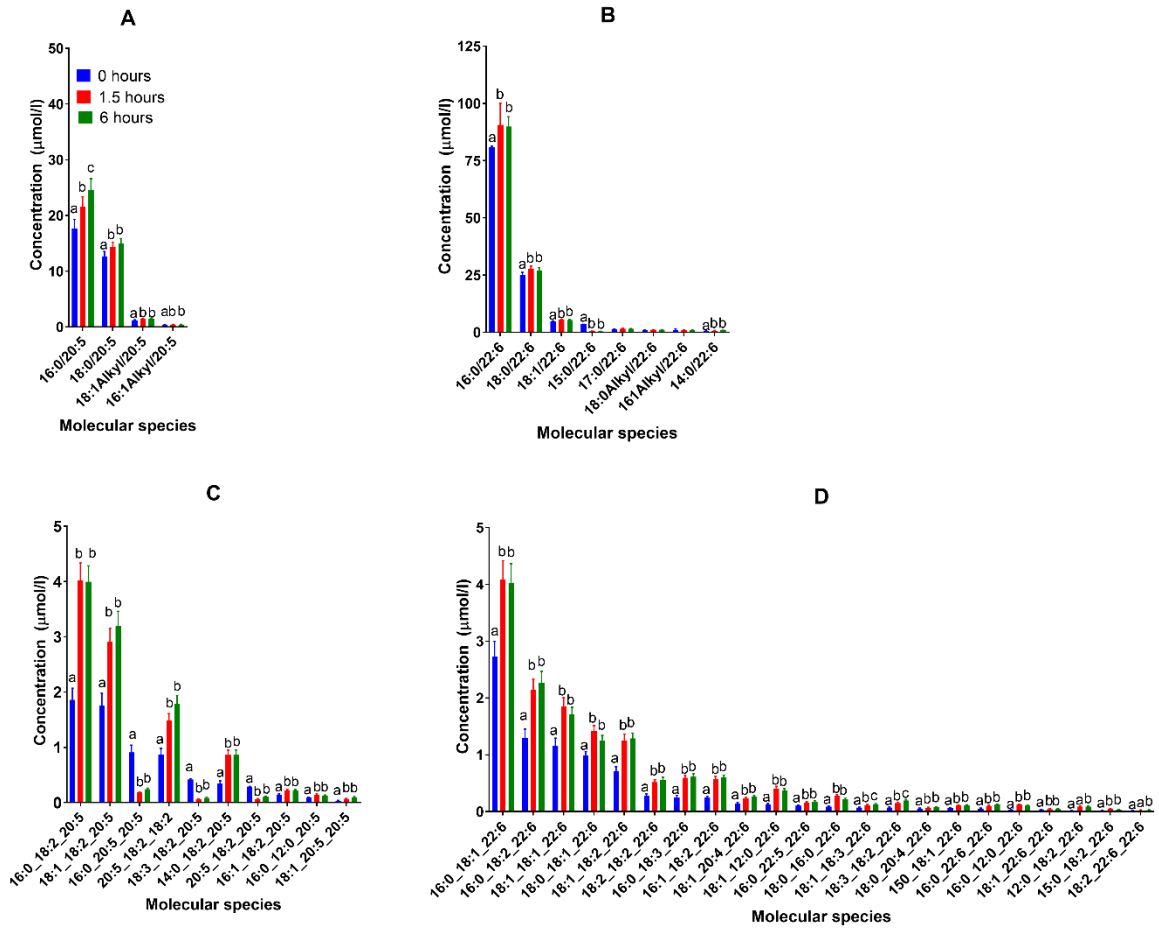
Values are mean ± SEM concentrations of individual plasma PC molecular species that contained 20:5n-3 or 22:6n-3 (n = 10/sex/oil type) in descending rank order of concentration at baseline. Females (A) and (B), males (C) and (D); CSO (A) and (C) CSO, BFO (B) and (D). Data were analysed by ANOVA with type of oil and sex as fixed factors and time as a repeated measure. Tukey's correction for multiple comparisons were applied *post hoc*. Different letters indicate means which differed significantly between time points. The results of the statistical analyses are shown in Table 5. The order of the fatty acids in each molecular species indicates their putative the *sn* positions of the fatty acids.

Fig. 5. Concentrations of cholesteryl-16:0, cholesteryl-20:5n-3 and cholesteryl-22:6n-3 in plasma during the postprandial period.



Values are mean ± SEM estimated marginal mean concentrations at baseline, 1.5 hours and 6 hours corrected for time x sex and time x type of oil interactions. (A) cholesteryl-16:0 (B) cholesteryl-20:5 or cholesteryl-22:6n-3. *Values significantly different ($P<0.05$) from baseline.

Fig. 6. Concentrations of plasma phosphatidylcholine and triacylglycerol molecular species containing either 20:5n-3 or 22:6n-3 during the postprandial period.



Values are mean ± SEM estimated marginal means for concentrations at baseline, 1.5 hours and 6 hours corrected for time x sex and time x oil interactions. Concentrations of plasma (A,B) phosphatidylcholine and (C,D) triacylglycerol molecular species containing either (A,C) 20:5n-3 or (B,D) 22:6n-3 (B,D). Means that differed significantly (all $P<0.001$) by Tukey's *post hoc* test are indicated by different letters. Molecular species are shown in rank order of concentration at baseline.

Table 1

Triacylglycerol composition of the test oils: molecular species containing 20:5n-3.

TG (% total 20:5n-3 –containing TG species)			
CSO		BFO	
Molecular Species	%	Molecular Species	%
18:1_20:5_20:5	10.58±0.07	16:0_18:1_20:5	10.32±0.14
18:0_20:5_20:5	8.42±0.09	16:0_16:1_20:5	8.56±0.03
20:1_18:1_20:5	7.36±0.06	16:0_20:5_20:5	6.34±0.04
20:0_18:2_20:5	6.92±0.05	18:1_20:5_20:5	6.30±0.05
18:0_16:0_20:5	6.55±0.10	16:0_16:0_20:5	6.11±0.02
16:0_18:1_20:5	6.53±0.10	18:3_18:2_20:5	6.01±0.03
16:0_20:4_20:5	6.11±0.04	20:1_18:1_20:5	5.27±0.04
20:5_18:2_22:5	5.56±0.03	20:0_18:2_20:5	5.11±0.04
18:3_20:5_22:5	5.47±0.05	18:3_18:3_20:5	4.73±0.03
20:5_18:2_20:5	5.47±0.06	16:1_16:1_20:5	4.25±0.03
16:0_20:5_20:5	4.52±0.04	18:0_16:0_20:5	4.21±0.02
18:3_18:2_20:5	4.03±0.03	18:4_16:0_20:5	4.18±0.01
18:3_20:5_20:5	3.34±0.28	20:5_18:2_22:5	3.57±0.03
16:0_18:3_20:5	3.16±0.05	18:0_20:5_20:5	2.96±0.01
18:3_18:3_20:5	3.11±0.03	20:0_20:3_20:5	2.85±0.02
16:0_16:0_20:5	2.35±0.02	16:0_18:3_20:5	2.77±0.03
20:0_20:3_20:5	2.30±0.03	16:0_20:4_20:5	2.72±0.01
16:0_16:1_20:5	2.06±0.02	20:5_18:2_20:5	2.52±0.01
20:5_20:5_22:5	1.79±0.04	16:0_14:0_20:5	1.95±0.09
18:4_16:0_20:5	1.67±0.02	20:5_20:5_20:5	1.23±0.05
16:0_20:5_24:0	1.32±0.04	18:3_20:5_22:5	1.07±0.01
18:4_18:3_20:5	1.03±0.05	18:4_20:5_20:5	0.98±0.05
20:5_20:5_20:5	0.98±0.06	18:3_20:5_20:5	0.90±0.05
16:1_16:1_20:5	0.70±0.01	18:4_18:3_20:5	0.86±0.02
18:4_20:5_20:5	0.52±0.03	17:0_18:2_20:5	0.85±0.04
18:4_18:4_20:5	0.15±0.01	18:4_18:4_20:5	0.61±0.01
18:3_20:5_23:0	0.05±0.00	20:5_17:1_20:5	0.47±0.01
17:0_18:2_20:5	0.05±0.00	20:5_20:5_22:5	0.47±0.02
18:3_20:5_23:1	0.03±0.00	18:3_17:1_20:5	0.37±0.01
15:0_18:3_20:5	0.03±0.00	16:0_20:5_24:0	0.34±0.01
18:3_17:1_20:5	0.02±0.01	15:0_18:3_20:5	0.31±0.00
20:5_17:1_20:5	0.02±0.00	18:3_20:5_23:0	0.20±0.00
15:0_20:5_20:5	0.02±0.00	17:0_18:1_20:5	0.12±0.01
24:1_20:5_22:1	0.01±0.00	20:5_17:1_22:5	0.10±0.02
18:3_17:1_20:5	0.01±0.01	15:0_20:5_20:5	0.05±0.00
16:0_14:0_20:5	0.01±0.00	18:3_17:1_20:5	0.04±0.00
18:3_17:1_20:5	0.01±0.00	18:3_17:1_20:5	0.03±0.00
16:0_20:5_23:0	0.01±0.00	24:1_20:5_22:1	0.02±0.00
18:0_20:5_23:0	0.01±0.00	18:3_20:5_23:1	0.02±0.00
20:5_20:5_21:1	0.00±0.00	20:5_20:5_21:1	0.01±0.00

Values are mean ± SEM proportions of individual TG molecular species that contained 20:5n-3 (n = 11 technical replicates) in descending rank order of the proportion in the oils. The order of fatty acids within each molecular species does not represent their *sn* positions.

Table 2

Triacylglycerol composition of the test oils: molecular species containing 22:6n-3.

TG (% total 22:6n-3 –containing TG species)			
CSO		BFO	
Molecular Species	%	Molecular Species	%
18:0_16:0_22:6	10.78±0.08	16:0_16:0_22:6	13.61±0.09
18:2_18:2_22:6	10.02±0.12	16:0_18:1_22:6	11.02±0.14
16:0_18:1_22:6	9.83±0.10	18:0_16:0_22:6	9.09±0.05
18:3_18:3_22:6	7.80±0.22	16:0_20:1_22:6	7.83±0.04
16:0_20:1_22:6	7.58±0.04	18:0_18:0_22:6	5.40±0.03
18:1_18:1_22:6	7.22±0.04	16:0_22:6_22:6	5.07±0.04
18:0_18:0_22:6	6.21±0.06	18:1_18:1_22:6	4.91±0.04
16:0_22:6_22:6	5.16±0.07	18:3_18:3_22:6	3.96±0.03
16:0_16:0_22:6	5.02±0.04	18:2_18:2_22:6	3.91±0.05
16:0_22:5_22:6	4.35±0.02	22:0_18:2_22:6	2.86±0.04
20:1_18:3_22:6	3.93±0.03	16:0_22:5_22:6	2.83±0.03
18:3_22:5_22:6	2.54±0.03	16:0_14:0_22:6	2.61±0.11
18:3_22:6_22:6	1.91±0.05	18:0_22:1_22:6	2.40±0.04
18:0_20:0_22:6	1.82±0.05	18:0_20:0_22:6	2.34±0.03
18:1_22:6_22:6	1.82±0.02	16:0_22:0_22:6	2.27±0.04
16:0_22:0_22:6	1.74±0.05	20:1_22:1_22:6	2.04±0.06
18:0_22:6_22:6	1.37±0.01	18:0_22:6_22:6	1.75±0.01
18:1_22:5_22:6	1.31±0.01	18:1_22:5_22:6	1.72±0.01
20:4_22:6_22:6	0.61±0.01	18:1_22:6_24:1	1.64±0.05
22:1_18:2_22:6	0.54±0.00	20:1_18:3_22:6	1.46±0.01
18:3_22:1_22:6	0.53±0.00	18:1_22:6_22:6	1.31±0.01
18:0_22:1_22:6	0.51±0.01	22:1_22:1_22:6	0.83±0.02
20:3_22:6_22:6	0.49±0.01	18:3_22:6_22:6	0.79±0.03
20:4_22:6_22:6	0.46±0.02	18:0_22:0_22:6	0.74±0.03
22:0_18:3_22:6	0.44±0.01	18:1_17:1_22:6	0.65±0.03
18:0_22:0_22:6	0.35±0.02	20:1_22:5_22:6	0.54±0.01
22:0_18:2_22:6	0.32±0.01	17:0_18:2_22:6	0.51±0.01
22:5_22:6_22:6	0.26±0.00	22:1_18:2_22:6	0.48±0.00
20:2_22:6_22:6	0.25±0.00	20:0_22:6_22:6	0.47±0.01
20:1_22:6_22:6	0.23±0.00	18:3_22:5_22:6	0.45±0.01
20:2_22:5_22:6	0.21±0.00	22:0_18:3_22:6	0.42±0.01
22:6_22:6_22:6	0.19±0.01	20:1_22:6_22:6	0.41±0.00
20:1_22:5_22:6	0.16±0.00	20:2_22:5_22:6	0.38±0.00
20:0_22:6_22:6	0.12±0.00	18:3_22:1_22:6	0.37±0.00
20:1_22:1_22:6	0.11±0.01	20:4_22:6_22:6	0.35±0.01
24:1_18:3_22:6	0.11±0.00	20:4_22:6_22:6	0.25±0.01
24:1_18:2_22:6	0.10±0.00	24:1_18:2_22:6	0.21±0.00
18:0_19:0_22:6	0.07±0.00	22:1_22:5_22:6	0.21±0.02
20:0_22:5_22:6	0.07±0.00	20:0_22:5_22:6	0.20±0.00
22:4_22:6_22:6	0.06±0.00	18:0_22:6_24:1	0.19±0.01
18:1_22:6_24:1	0.06±0.00	19:0_18:3_22:6	0.16±0.01
18:0_22:6_24:1	0.05±0.00	20:3_22:6_22:6	0.15±0.00
18:1_17:1_22:6	0.05±0.01	22:1_22:6_22:6	0.14±0.00
18:0_19:0_22:6	0.04±0.00	18:0_19:0_22:6	0.11±0.00

19:0_18:3_22:6	0.03±0.01	24:1_18:3_22:6	0.11±0.00
22:0_22:6_22:6	0.03±0.00	22:0_22:6_22:6	0.10±0.00
18:0_22:6_24:0	0.02±0.00	15:0_18:2_22:6	0.10±0.01
17:0_18:2_22:6	0.02±0.01	22:6_22:6_22:6	0.09±0.00
22:1_22:5_22:6	0.02±0.00	20:2_22:6_22:6	0.08±0.00
18:2_22:6_23:1	0.02±0.00	17:0_22:5_22:6	0.07±0.00
15:0_18:2_22:6	0.02±0.00	24:1_20:3_22:6	0.05±0.00
18:0_22:6_23:1	0.02±0.00	22:5_22:6_22:6	0.05±0.01
24:1_20:4_22:6	0.02±0.00	18:0_22:6_23:1	0.05±0.00
24:1_22:6_22:6	0.01±0.00	18:0_19:0_22:6	0.04±0.00
19:1_22:6_22:6	0.01±0.00	17:0_22:6_22:6	0.04±0.00
24:1_20:3_22:6	0.01±0.00	22:2_22:6_22:6	0.03±0.00
22:1_22:6_22:6	0.01±0.00	22:4_22:6_22:6	0.03±0.00
22:1_22:1_22:6	0.01±0.00	24:1_22:6_22:6	0.03±0.00
22:2_22:6_22:6	0.01±0.00	24:1_20:4_22:6	0.02±0.00
22:4_22:6_22:6	0.01±0.00	18:2_22:6_23:1	0.02±0.00
17:0_22:5_22:6	0.01±0.00	18:0_22:6_23:0	0.02±0.00
16:0_14:0_22:6	0.01±0.00	18:0_22:6_24:0	0.01±0.00
18:0_22:6_23:0	0.01±0.00	19:1_22:6_22:6	0.00±0.00
24:0_20:3_22:6	0.004±0.000	24:0_20:3_22:6	0.004±0.000
17:0_22:6_22:6	0.004±0.000	20:0_22:6_24:0	0.002±0.000
20:0_22:6_24:0	0.003±0.000	24:0_22:6_22:6	0.001±0.000

Values are mean ± SEM proportions of individual TG molecular species that contained 22:6n-3 (n = 11 technical replicates) in descending rank order of their proportions in the test oils. The order of fatty acids within each molecular species does not represent their *sn* positions.

Table 3

Triacylglycerol composition of the test oils: molecular species containing 20:5n-3 and 22:6n-3.

TG (% total 22:6n-3 -containing TG species)			
CSO		BFO	
Molecular Species	%	Molecular Species	%
16:0_20:5_22:6	56.38±0.36	16:0_20:5_22:6	60.98±0.86
18:3_20:5_22:6	19.53±0.09	20:1_20:5_22:6	11.51±0.09
20:5_20:4_22:6	12.11±0.07	20:5_20:5_22:6	9.22±0.41
20:1_20:5_22:6	7.03±0.07	18:3_20:5_22:6	7.88±0.20
20:5_20:5_22:6	4.73±0.29	20:5_20:4_22:6	6.37±0.13
20:5_22:6_22:6	0.61±0.03	20:5_22:6_22:6	3.14±0.18
19:1_20:5_22:6	0.16±0.00	15:0_20:5_22:6	0.53±0.03
24:0_20:5_22:6	0.04±0.00	19:1_20:5_22:6	0.30±0.01
15:0_20:5_22:6	0.03±0.00	24:0_20:5_22:6	0.05±0.00
20:5_17:1_22:6	0.01±0.00	20:5_17:1_22:6	0.02±0.00

Values are mean ± SEM proportions of individual DAG molecular species that contained 20:5n-3 and 22:6n-3 (n = 11 technical replicates) in descending rank order of their proportions in the test oils. The order of fatty acids in each molecular species does not represent their *sn* positions.

Table 4

Results of statistical analysis of triacylglycerol molecular species concentrations during the postprandial period.

	ANOVA			
	Time	Sex*Time	Oil*Time	Oil*Time*Sex
Molecular species	F, P	F, P	F, P	F, P
	Molecular species containing 20:5n-5			
16:0_18:2_20:5	48.1, <0.0001	4.3, 0.02	0.15, 0.9	2.9, 0.06
18:1_18:2_20:5	33.9, <0.0001	1.4, 0.2	1.7, 0.2	1.7, 0.2
20:5_18:2_18:2	49.1, <0.0001	3.8, 0.04	1.7, 0.2	2.9, 0.07
14:0_18:2_20:5	64.6, <0.0001	4.7, 0.12	0.7, 0.5	1.8, 0.2
16:1_18:2_20:5	50.1, <0.0001	5.8, 0.005	0.1, 0.9	3.2, 0.46
16:0_20:5_20:5	68.7, <0.0001	3.5, 0.2	1.7, 0.2	3.2, 0.06
18:1_20:5_20:5	39.7, <0.0001	5.0, 0.01	0.9, 0.4	4.4, 0.02
18:3_18:2_20:5	20.6, <0.0001	3.1, 0.06	0.3, 0.7	1.7, 0.2
20:5_18:2_20:5	38.6, <0.0001	1.8, 0.2	0.2, 0.7	1.6, 0.2
16:0_12:0_20:5	26.7, <0.0001	3.9, 0.03	0.1, 0.9	0.6, 0.5
	Molecular species containing 22:6n-3			
16:0_18:1_22:6	25.2, <0.0001	1.0, 0.4	0.3, 0.8	2.6, 0.09
16:0_18:2_22:6	43.8, <0.0001	2.2, 0.1	0.2, 0.8	0.8, 0.5
18:1_18:1_22:6	32.1, <0.0001	1.4, 0.3	0.1, 0.9	2.4, 0.1
18:0_18:1_22:6	23.7, <0.0001	0.6, 0.5	1.1, 0.4	2.3, 0.1
18:1_18:2_22:6	38.3, <0.0001	3.2, 0.06	0.1, 0.9	1.6, 0.2
18:2_18:2_22:6	47.8, <0.0001	3.7, 0.04	0.1, 0.9	1.5, 0.2
16:0_18:3_22:6	57.7, <0.0001	4.6, 0.02	0.4, 0.6	2.0, 0.1
16:1_18:2_22:6	58.2, <0.0001	4.0, 0.03	0.5, 0.6	1.6, 0.2
18:1_20:4_22:6	32.0, <0.0001	1.3, 0.3	0.2, 0.7	2.2, 0.1
18:1_12:0_22:6	50.5, <0.0001	3.9, 0.03	0.7, 0.5	0.5, 0.6
16:0_22:5_22:6	23.6, <0.0001	1.0, 0.4	0.1, 0.8	2.0, 0.2
18:0_16:0_22:6	37.8 <0.0001	0.4, 0.6	0.06, 0.9	0.8, 0.4
18:1_18:3_22:6	16.8, <0.0001	0.7, 0.5	1.1, 0.3	1.1, 0.3
18:3_18:2_22:6	44.2, <0.0001	1.7, 0.2	0.4, 0.6	0.6, 0.4
18:0_20:4_22:6	10.5, <0.0001	1.6, 0.2	1.4, 0.3	2.0, 0.2
15:0_18:1_22:6	22.1, <0.0001	1.4, 0.3	0.1, 0.8	1.3, 0.3
16:0_22:6_22:6	10.7, <0.001	2.4, 0.1	0.4, 0.6	1.3, 0.3
16:0_12:0_22:6	37.7, <0.0001	1.4, 0.3	0.3, 0.7	0.2, 0.8
18:1_22:6_22:6	1.5, 0.2	0.6, 0.5	1.1, 0.3	1.6, 0.2
12:0_18:2_22:6	53.8, <0.0001	3.3, 0.05	1.0, 0.4	1.2, 0.3
15:0_18:2_22:6	7.4, 0.004	0.5, 0.6	1.3, 0.3	2.2, 0.1
18:2_22:6_22:6	2.68, 0.1	1.5, 0.2	2.4, 0.1	2.5, 0.1
	Molecular species containing 20:5n-5 and 22:6n-3			
16:0_20:5_22:6	45.2, <0.0001	2.3, 0.1	0.1, 0.9	2.0, 0.2
18:1_20:5_22:6	13.7, <0.0001	1.5, 0.2	0.8, 0.4	1.1, 0.3
20:5_18:2_22:6	15.1, <0.0001	2.6, 0.1	0.7, 0.1	2.0, 0.2

Data were analysed by ANOVA with type of oil and sex as fixed factors and time as a repeated measure. Tukey's correction for multiple comparisons were applied *post hoc*. The order of fatty acids does not represent their *sn* positions.

Table 5

Results of statistical analysis of phosphatidylcholine molecular species concentrations during the postprandial period.

	ANOVA			
	Time	Sex*Time	Oil*Time	Oil*Time*Sex
Molecular species	F, P	F, P	F, P	F, P
		Molecular species containing 20:5n-5		
16:0/20:5	67.5, <0.0001	1.9, 0.6	0.27, 0.2	0.52, 0.4
16:1A/20:5	6.6, 0.008	0.7, 0.5	1.0, 0.4	1.6, 0.2
18:0/20:5	28.7, <0.0001	2.6, 0.06	1.4, 0.3	0.5, 0.6
18:1A/20:5	3.3, 0.001	0.07, 0.4	0.4, 0.4	0.4, 0.6
		Molecular species containing 22:6n-3		
14:0/22:6	31.6, <0.0001	0.002, 0.9	0.87, 0.4	1.0, 0.3
15:0/22:6	23.5, < 0.0001	0.97, 0.4	0.6, 0.6	0.07, 0.9
16:0/22:6	25.8, <0.0001	0.35, 0.7	1.0, 0.4	0.95, 0.4
16:1A/22:6	0.02, 0.9	0.04, 0.9	0.89, 0.4	0.67, 0.5
17:0/22:6	6.9, 0.005	0.61, 0.5	0.63, 0.5	0.88, 0.4
18:0/22:6	16.43, <0.0001	0.87, 0.4	0.9, 0.4	1.3, 0.3
18:0A/22:6	8.9, 0.001	0.022, 0.9	0.09,0.9	0.27, 0.7
18:1/22:6	2.5, <0.0001	0.18, 0.8	0.69, 0.5	0.95, 0.4

Data were analysed by ANOVA with type of oil and sex as fixed factors and time as a repeated measure. Tukey's correction for multiple comparisons were applied *post hoc*. For plasma PC, the order of fatty acids indicates their *sn* positions.