Postprandial metabolism of docosapentaenoic acid (DPA, 22:5n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in humans

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Running title Postprandial metabolism of docosapentaenoic acid

Abstract

The study of the metabolism of docosapentaenoic acid (DPA, 22:5n-3) in humans has been limited by the unavailability of pure DPA and the fact that DPA is found in combination with eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in natural products. In this double blind cross over study, pure DPA and EPA were incorporated in meals served to healthy female volunteers. Mass spectrometric methods were used to study the chylomicron lipidomics. Plasma chylomicronemia was significantly reduced after the meal containing DPA compared with the meal containing EPA or olive oil only. Both EPA and DPA were incorporated into chylomicron TAGs, while there was less incorporation into chylomicron phospholipids. Lipidomic analysis of the chylomicron TAGs revealed the dynamic nature of chylomicron TAGs. The main TAG species that EPA and DPA were incorporated into were EPA/18:1/18:1, DPA/18:1/16:0 and DPA/18:1/18:1. There was very limited conversion of DPA and EPA to DHA and there were no increases in EPA levels during the 5 hour postprandial period after the DPA meal. In conclusion, EPA and DPA showed different metabolic fates, and DPA hindered the digestion, ingestion or incorporation into chylomicrons of the olive oil present in the meal.

Key words

n-3 polyunsaturated fatty acids (PUFA); docosapentaenoic acid (DPA); eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); chylomicron; lipid metabolism

1. Introduction

The essential fatty acid alpha-linoleic acid (ALA, 18:3n-3) can be metabolized *in vivo* by desaturation and elongation enzymes to form a series of polyunsaturated fatty acids (PUFA) of the n-3 series. In addition to potentially being synthesised from ALA, eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and docosapentaenoic acid (DPA, 22:5n-3) are provided from diet, mainly from fish and fish oil products and to a lesser extent from ruminant meats. Currently, there is much information on the metabolism of both EPA and DHA, while less is known about the metabolism of DPA.

In vivo, DPA is formed by chain elongation of EPA by the action of fatty acid elongases 2 and 5, while the conversion of DPA to DHA requires an elongation to 24:5n-3 and desaturation to 24:6n-3 before peroxisomal beta-oxidation to yield DHA. As recently reviewed, ALA supplementation in humans generally leads to an increase in plasma EPA and DPA, but has little or no effect on DHA levels [1].

Previous studies have demonstrated a significant elevation in the level of DPA in the circulating lipid fractions when human subjects have received seal oil [2-4]. However, such effects cannot be directly attributed to the consumption of DPA since it represents approximately 5% of the fatty acids in seal oil with a higher level of EPA that has the potential to generate considerable amounts of DPA via chain elongation.

The previous knowledge on the metabolism of pure DPA in humans is limited to our study [5], where a supplementation of a total of 8 grams of pure DPA or EPA revealed that within four days of supplementation, DPA and EPA demonstrated different and specific incorporation patterns into plasma lipid classes and red blood cell phospholipids.

In rats, short-term supplementation with pure DPA has significantly increased the concentration of DHA in liver and the concentration of EPA in the liver, heart and skeletal muscle, presumably by the process of retroconversion [6]. The retroconversion from DPA to

EPA was especially apparent in the kidney of the rats [7]. The metabolism and the biological effects of DPA have been recently reviewed [8].

Tandem mass spectrometric lipidomic methods enable us to study the composition of lipids as they occur in the human plasma. This information is complimentary to the fatty acid composition that requires the cleavage of the fatty acids from the molecules in which they naturally occur. The lipidomic methods have previously revealed the non-steady state of lipids in the postprandial state [9-11].

We hypothesized that pure DPA and EPA would have different postprandial metabolic fates. To test this, a cross over study with healthy female volunteers and meals containing pure EPA and pure DPA was designed. A meal that contained olive oil was used as a control. Molecular level lipidomic analysis methods were used to investigate the structure and composition of the lipids. Special interest was placed on the metabolism of the n-3 PUFA in chylomicron triacylglycerols (TAG) and phospholipids.

2. Materials and Methods

2.1 Study design

Ten healthy normal weight females between the age of 20 to 30 took part in the randomized cross over study with three different breakfast meals. The subjects had a BMI between 20 to 25 kg/m^2 and their habitual total consumption of omega-3 polyunsaturated fatty acids was not more than 0.5 grams per day as assessed from a food frequency questionnaire [12, 13]. The baseline values for EPA and DHA proportions in their erythrocytes were 1.0±0.1 and 6.7±0.6 per cent (mean±standard error of mean), respectively. Subjects with any form of cardiovascular disease based on self-reported medical status and family history were excluded from the study.

Following the postprandial study, the subjects consumed the study oil (olive, EPA or DPA) as a one gram daily supplement for the subsequent six days after which the fasting blood lipids were studied as described elsewhere [5]. After the end of the supplementation study, there was a two-week wash out period prior to the next postprandial study. Throughout the study weeks and the washout periods, the subjects were requested to refrain from consuming products rich in long chain omega-3 PUFA including fish, red meat and omega-3 fortified products (<2 marine and/or 2 red meat meals/week and <2 omega-3 fortified products/week.

All subjects provided written informed consent. Ethics approval was obtained from the Deakin University Human Research Ethics Committee (EC2011-023).

The night before each of the three study days, the participants consumed a standardized dinner meal (containing pasta (dry 200 grams), tomato stir-through sauce (70 grams) and a packet pudding) and were given instructions to fast overnight for 10 hours after the dinner.

The study breakfast consisted of 180 grams of instant mashed potato (Continental Deb™, Unilever, Australasia) mixed with 70 milliliters boiled water and 20 grams of oil. In each of the three meals, 18 grams of the 20 grams of oil consisted of olive oil (La Espanola Pure

Olive Oil, Seville, Spain). Additionally, the DPA breakfast included 2 grams of DPA (Equateq Ltd, Breasclete, Callanish, Scotland), the EPA breakfast 2 grams of EPA (Equateq Ltd, Breasclete, Callanish, Scotland) and the control (olive oil) meal an additional 2 grams of olive oil. EPA and DPA were included in the olive oil as free fatty acids. The subjects could use salt, pepper or chicken flavoured salt with the meal, which was consumed within 15minutes; water was provided ad libitum throughout the five hour study period.

After the DPA meal, there were two cases of diarrhea and one case of upset stomach but no diarrhea. One case of diarrhea was reported after the EPA meal, and there were no complaints after the olive oil meal. All complaints occurred 2 to 3 hours after the breakfast.

2.2 Isolation of plasma and chylomicrons

Venous blood was drawn at the fasting state and thereafter hourly between one and five hours postprandially. EDTA blood samples were immediately centrifuged for fifteen minutes at 591 x g to isolate the plasma.

A chylomicron-rich fraction (Svedberg flotation unit (Sf) > 400), later abbreviated to "chylomicrons", was isolated from plasma by ultracentrifugation using a Beckman ultra centrifuge and TLA 100.4 rotor (Beckman instruments, Palo Alto, CA, USA) as previously described [14]. Briefly 1.8 milliliters of EDTA plasma was overlaid with saline solution (density = 1.006 kg/l) in ultracentrifuge tubes and centrifuged at 35,000 x g for 30 minutes at 23°C. The top 1 millilitre was aspirated to remove the chylomicron-rich fraction. All samples were frozen at -80°C prior analysis.

2.3 TAG concentration analysis

TAG concentrations in plasma and the isolated chylomicrons were measured on a Roche Cobas Integra 400 plus autoanalyser (Roche, Lavel, Quebec, Canada) by enzymatic colorimetric method using commercially available kits (TRIGL) as per the manufacturer's instructions (Roche, Lavel, Quebec, Canada).

2.4 Fatty acid analysis

An internal standard mixture of triheptadecanoin (Sigma-Aldrich, St.Louis, MO, USA), dinonadecanoylphosphatidylcholine (Sigma-Aldrich, St.Louis, MO, USA) and cholesterylpentadecanoate (Nu-Chek Prep. Inc., Elysian, MN, USA) was added to the isolated chylomicrons. Then 1.5 milliliters of methanol, 3 milliliters of chloroform and 0.8 milliliters of 0.88 % KCl in water were added and the blend was thoroughly vortexed after each addition. The tubes were centrifuged 2000 x g for 3 minutes to separate the layers, and the chloroform rich layer was removed and evaporated to dryness [15]. TAGs and phospholipids were isolated from the extracted lipid mixture with solid phase extraction based on silica columns [16].

Fatty acid methyl esters (FAME) were prepared with a sodium methoxide method. In short, the lipids were suspended to 1 milliliters of dry diethylether; then 25 microliters of methylacetate and 25 microliters of sodium methoxide were added and the blend was incubated for 5 minutes while shaken at times. The reaction was stopped with 6 microliters of acetic acid. The tubes were centrifuged 2000 x g for 5 minutes, after which the supernatant was removed and gently evaporated to dryness. The resulting FAME were transferred to 100 microliter inserts in hexane [17]. The FAME were analysed with gas chromatography (Shimadzu GC-2010 equipped with AOC-20i auto injector, flame ionization detector (Shimadzu corporation, Kyoto, Japan) and wall coated open tubular column DB-23 (60 m x 0.25 mm i.d., liquid film 0.25 μm, Agilent technologies, J.W. Scientific, Santa Clara, CA, USA). Splitless/split injection was used and the split was opened after 1 minute. Supelco 37 Component FAME Mix (Supelco, St. Louis, MO, USA), 68D (Nu-Check-Prep, Elysian, MN, USA) and GLC-490 (Nu-Check-Prep, Elysian, MN, USA) were used as external standards.

2.5 Lipidomics

Lipidomic analysis of the one, three and five hour chylomicron samples was performed by liquid chromatography, electrospray ionisation-tandem mass spectrometry using an Applied Biosystems 4000 QTRAP mass spectrometer running Analyst 1.5 software. Liquid chromatography was performed on a Zorbax C18, 1.8 micrometer, 50 x 2.1 millimeter column (Agilent technologies, Santa Clara CA, USA). The lipids of the chylomicrons were extracted with chloroform:methanol (2:1, 20 volumes), mixed, sonicated (30 minutes) and

allowed to stand for 20 minutes. Samples were centrifuged (16 000 x g, 10 minutes) and the supernatant transferred to a 96 well PPE plate and dried until a stream of nitrogen at 40^{o}C. Immediately before analysis, samples were resuspended in water saturated butanol and methanol containing 10 millimolar ammonium formate. The mobile phase was tetrahydrofuran:methanol:water in a 30:20:50 ratio (A) and 75:20:5 (B) both containing 10 millimolar ammonium formate. TAG were separated with an isocratic flow (100 microliters per minute) of 85% mobile phase B. Phospholipids and cholesteryl esters were separated by a gradient from 0%B and 100%A to 100%B and 0%A over 8 minutes then held at 100%B for 2 minutes before equilibrating to starting conditions. Quantification of individual TAG species was performed using scheduled multiple-reaction monitoring in the positive ion mode [18]. Lipid concentrations (picomoles per milliliter) were calculated by relating the peak area of each species to the peak area of the internal standard of triheptadecanoin (Sigma Aldrich, St Louis MO, USA) for TAGs, cholesteryl ester-18:0-d_6 (CDN isotopes, Quebec, Canada) for cholesteryl esters, phosphatidyl choline-13:0/13:0 (Avanti Polar Lipids, Alabaster AL, USA) for phosphatidyl cholines and phosphatidyl ethanolamine-17:0/17:0 (Avanti Polar Lipids, Alabaster AL, USA) for ethanolamines and phosphatidyl inositols (using Multiquant 1.2 software). As no standards were available for each TAG species, no adjustment was made for different response factors and the relative proportions of different species should be taken as semi-quantitative. The MRM analysis of TAG species provides information on a group of isomeric TAGs for which the sum of the fatty acid chain lengths and number of double bonds is that same rather than the individual species. TAGs that were likely to contain arachidonic acid, EPA, DPA or DHA were subjected to additional MRM analysis monitoring the loss of neutral 16:0, 16:1, 18:1, 18:2, 18:3, 20:4, 20:5, 22:5 and 22:6. Although these losses are known to not be strictly quantitative as the branching ratio between different fragmentation channels is controlled by fatty acid position, chain length and degree of unsaturation, this experiment provides an indication of the predominant molecular species within each isomeric group. The most likely TAG fatty acid combinations were estimated from the results.

2.6 Statistical analysis

Normal distribution of the data was tested with the Shapiro-Wilk test. Depending on the normality of the data, paired samples t-test or Wilcoxon matched-pairs signed ranks test, was used to compare the measured responses to control. ANOVA for repeated measurements was used for multiple comparisons. Paired samples t-test or Wilcoxon matched-pairs signed ranks test with Bonferroni correction was used for post hoc comparisons. Statistical significance was indicated by $p < 0.05$. Statistical analyses were performed with SPSS 18.0 software (SPSS Inc, Chicago, IL, USA).

3. Results

Chylomicron TAGs remained at almost fasting level after the DPA breakfast. The incremental area under the chylomicron TAG curve after the DPA meal was significantly reduced when compared with the corresponding area after the olive oil meal ($p=0.021$) or the area after the EPA meal ($p=0.034$). In plasma, there was no significant difference between the TAG areas under the curve after DPA meal and the olive oil control meal ($p=0.078$). Of the individual time points, the TAG concentration was lower after the DPA breakfast at one and two hours ($p=0.024$ and $p=0.014$ respectively, for plasma and $p=0.017$ and $p=0.068$ respectively, for chylomicrons) compared with the control meal (Figure 1).

At one to five hours postprandially, EPA was significantly higher in the chylomicron TAGs after the breakfast containing EPA than after the breakfast containing olive oil only. The difference in EPA was significant between the EPA meal and the DPA meal at 1 hour and three to five hours (Figure 2). Correspondingly the DPA content was significantly higher after the DPA breakfast than after the olive oil meal (2-5 hours, p value for the 3 hour difference being 0.06) or after the EPA meal (3-5 hours). DPA did not raise the proportion of EPA in chylomicron TAGs. DHA was increased after the DPA breakfast compared with the olive oil breakfast at 2 hours and 3 hours, and increased after the EPA breakfast compared with the olive oil breakfast at 5 hours (Fig 2). The largest difference in the CM TAG concentrations after the three meals was at the one hour time point in which there was proportionally more 16:0, 18:0 and 18:2n-6 and proportionally less 18:1n-9 in the chylomicron TAGs after the DPA meal compared with the olive oil control meal (data not shown).

Chylomicron phospholipid fatty acid compositions were less affected by the meal compared with chylomicron TAGs. At 2 hours, the proportion of EPA was increased after the EPA breakfast compared with the two other breakfasts, and at 2 hours, the EPA breakfast also increased the amount DPA and DHA compared to the olive oil breakfast (Fig 3). There were no differences in the prevalences of the polyunsaturated fatty acids at other time points.

There were significant differences in the concentrations of TAGs containing PUFA between the breakfast groups (Figure 4). The predominant species contributing to these groups of TAGs were estimated through the use of more extensive multiple-reaction monitoring experiments monitoring the neutral losses of fatty acids. The major species that contained EPA after the EPA breakfast included 20:5/18:1/18:1 and 20:5/18:1/16:0. The overall presence of DPA was lower than that of EPA as seen also from the TAG concentration and fatty acid composition data. The major TAGs containing PUFA after the DPA breakfast were 22:5/18:1/16:0, 22:5/18:2/18:1 and 22:5/18:1/18:1. TAG 54:5, mostly 20:4/18:1/16:0, was detected in equal amounts after all meals.

Although very modest in the overall response, some conversion to DHA was apparent in the TAG 58:9 (mostly 22:6/18:2/18:1) as there was significantly more of this TAG after the EPA and DPA breakfasts compared with the olive oil breakfast at the 3 and 5 hour time points. Apart from the PUFA containing TAGs presented in Figure 4, TAGs 18:1/18:1/16:0, 18:1/18:1/18:1 and 18:2/18:1/16:0 were abundant TAGs after all meals (data not shown).

DPA containing TAGs were less abundant at one hour postprandially compared with the three and five hour time points indicating that their digestion was delayed compared with the EPA containing TAGs. This was seen also in the fatty acid composition data.

Of the phospholipid species measured, phosphatidyl cholines were the most abundant phospholipid species in chylomicrons followed by inositols, ethanolamines and serines as measured with HPLC-MS/MS (data not shown). There were no between-breakfast differences in the individual phospholipids or clear increasing or decreasing trends within the measured time points.

No differences were found in chylomicron cholesteryl esters species between breakfasts or between the three measured time points (1, 3 and 5 hours) (data not shown). The most abundant fatty acid in chylomicron cholesteryl esters was 18:2 followed by 16:0, 18:1 and 20:4 in about equal amounts and then by 16:1, 18:3, 20:5 and 22:6.

4. Discussion and Conclusions

DPA is an elongated metabolite of EPA and it is one of the intermediate products between EPA and DHA. The present study investigated the postprandial metabolism of pure DPA and EPA in an olive oil containing meal.

The major finding in this study is that the addition of 2 grams of DPA to the 18 grams of olive oil almost completely eliminates the incorporation of fatty acids in chylomicrons within five hours. In contrast, this effect was not seen with the addition of EPA.

One of the possible potential mechanisms that can explain the decreased chylomicronemia caused by DPA could be that DPA was acting as a pancreatic lipase inhibitor. If DPA did hinder the action of the lipase, the result would be a reduced or slower chylomicronemia and there would be reduced levels of chylomicron TAGs, particularly those with oleic acid (from the 18 gram of fed olive oil). Both of these effects were observed in this study. Furthermore, if some of the fat ingested was not thoroughly or efficiently digested by the lipase, some of the fat would be malabsorbed and lost in the feaces. This hypothesis is supported by the recorded observation that three out of the ten subjects reported diarrhea or upset stomach in the three hours following the DPA breakfast.

Another possible explanation relates to the TAG reservoirs that are known to exist in enterocytes [19]. It might be that the lipids of the DPA breakfast were stored in the enterocytes and released either over a longer time span than the five hours that were followed in this study or after the following meal.

Other possible mechanisms e.g. ones involving bile salts, absorption into mucosal cells, disruption of TAG synthesis or the packaging of chylomicron, and enhancement of

312 chylomicron clearance are also possible. However, the diarrhea observed by some of the
313 subjects supports effects taking place in the gut rather than in the mucosal cells or blood.

314

315 The beneficial effects of long-term fish oil supplementation on lowering plasma TAGs has
316 been well documented, but the underlying mechanisms remain poorly defined [20]. It has
317 been suggested that n-3 fatty acids could enhance postprandial chylomicron clearance
318 through reduced VLDL secretion and by directly stimulating the activity of lipoprotein lipase
319 [21, 22]. The VLDL rich lipoprotein fraction was not collected in this study, however, no
320 significant difference ($p=0.078$) was seen in the areas under the plasma TAG curves, possibly
321 due to higher variation in between-subject plasma TAG concentrations than in chylomicron
322 TAG concentrations. EPA and DHA supplementation have equally effectively decreased
323 chylomicron particle sizes and accelerated chylomicron triacylglycerol clearance [23], while
324 the effect of DPA has not been studied before.

325

326 The bioavailability of EPA or DHA as free fatty acids has not differed from natural TAGs in
327 previous studies [24]. Also, the absorption of EPA and DHA supplementation in plasma
328 appears to be similar to each other but varies with the form in which fatty acids are
329 supplemented. Lawson and Hughes [25] reported that as free acids, both EPA and DHA were
330 well absorbed (up to 95%) while the TAGs form of EPA and DHA were absorbed almost as
331 well as the free acids. The ethyl esters of EPA and DHA on the other hand, were relatively
332 poorly absorbed. However, DPA has not been administered previously in postprandial
333 human studies as a free fatty acid or other form so the potential route of absorption as a
334 free fatty acid bound to albumin for DPA should be looked for in the future studies. We did
335 not find indications of selective loss of certain FA over others. The likely explanation for the
336 differences in the chylomicron TAG FA composition of the one hour time point after the
337 different meals is that the endogenous lipids are represented in greater proportions in the
338 chylomicron TAGs after the DPA meal than after the olive oil meal. However, in further
339 studies the possible selectivity in the loss of different FA should be looked into for example
340 by analyzing the composition of fecal lipids.

Despite differences in the total amount of lipids absorbed or the absorbance rates, both DPA and EPA were transported in the chylomicron TAGs rather than in chylomicron phospholipids over the five hour period followed. Previously it has been found that linoleic acid was proportionally incorporated more into chylomicron phospholipids and cholesteryl esters than oleic or palmitic acids at four and seven hours postprandially [26].

New tools for fighting the growing prevalence of obesity worldwide are needed. Currently orlistat, a lipase inhibitor, is the only available long-term treatment for obesity. In the past years, numerous drugs have been approved for the treatment of obesity; however, most of them like amphetamine, rimonabant and sibutramine have been withdrawn from the market because of their adverse effects [27]. Should DPA prove to have potential as an agent that decreases postprandial lipemia, side effects other than steatorrhea need to be considered. The n-3 PUFA have multiple cardiovascular benefits including inhibition of platelet aggregation [28]. This might pose a side effect of increasing bleeding risk. However, in a recent report of a large cohort of more than 1500 patients with acute myocardial infarction no relation was found between the omega-3 index and bleeding [29].

In population studies DPA has been associated positively with cardiovascular health. A higher level of DPA in the circulation has been associated with a lower risk of coronary heart disease [30], and a higher intake of DPA and a higher level of DPA in circulation have been associated with the protection against carotid atherosclerosis [31]. These findings may in part be mediated via the lowered postprandial chylomicronemia, as an increased postprandial plasma TAG level is a risk factor for cardiovascular diseases [32-34]. However, n-3 PUFA exert their cardioprotective effects through multiple mechanisms, including reducing arrhythmias or altering production of prostaglandins, which reduces inflammation and improves platelet and endothelial function [35]. Indeed, DPA has shown the ability to inhibit human platelet aggregation in vitro and to suppress thromboxane formation [36]. It has been postulated that due to its high DPA content as well as high EPA content, seal oil

may be even more efficient than fish oil at promoting healthy plasma lipid profiles and lowering thrombotic risk [4].

Our current understanding of the role of TAGs in human nutrition largely comes from the measurement of either the concentration of TAGs or from the fatty acid composition, which requires the release of the fatty acids from the TAGs. In this study, molecular level tandem mass spectrometric lipidomic analysis method was used to identify and study the TAG species present in chylomicrons. In addition to identifying the most abundant EPA and DPA containing TAGs, the non steady state of the chylomicron lipid molecules over the postprandial period was revealed. Although in the current study the major difference in the TAG composition was between the one hour time point compared with the three and five hour time points, it supports previous studies [9 -11] where the composition of chylomicrons has not been constant during the postprandial state. This indicates that different TAGs may be formed and/or cleared in favour of others.

Whether the individual long chain n-3 PUFA have shared or complimentary effects is not well established due to the challenges in obtaining pure DPA for research purposes. Overall, for many cardiovascular pathways and outcomes, identified studies of individual polyunsaturated fatty acids are limited, especially for DPA [37]. The data presented in this manuscript indicates that EPA and DPA are metabolized differently postprandially. The lipidomic analysis of the postprandial fate of DPA as well as the reduced chylomicronemia observed in this study, invite long term human trials with pure DPA, further postprandial trials with larger lipid loads and different forms of DPA as well as in vivo and *in vitro* studies of the mechanisms involved.

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Conflict of interest

The authors declare no conflict of interest.

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Fig. 1 Triacylglycerol concentrations (mmol/l +/- standard deviation, n=10) in plasma (A) and chylomicron rich fraction (B) after the meals containing olive oil (open circles), or olive oil together with EPA (closed rectangles) or DPA (closed triangles) up to five hours postprandially. The incremental area under the curve differed significantly between the olive oil meal and the DPA meal in chylomicrons (p=0.021), but no such difference was found in plasma (p=0.078). Significant differences ($p < 0.05$) in individual time points between the olive oil breakfast and the DPA breakfast are marked by an asterisk

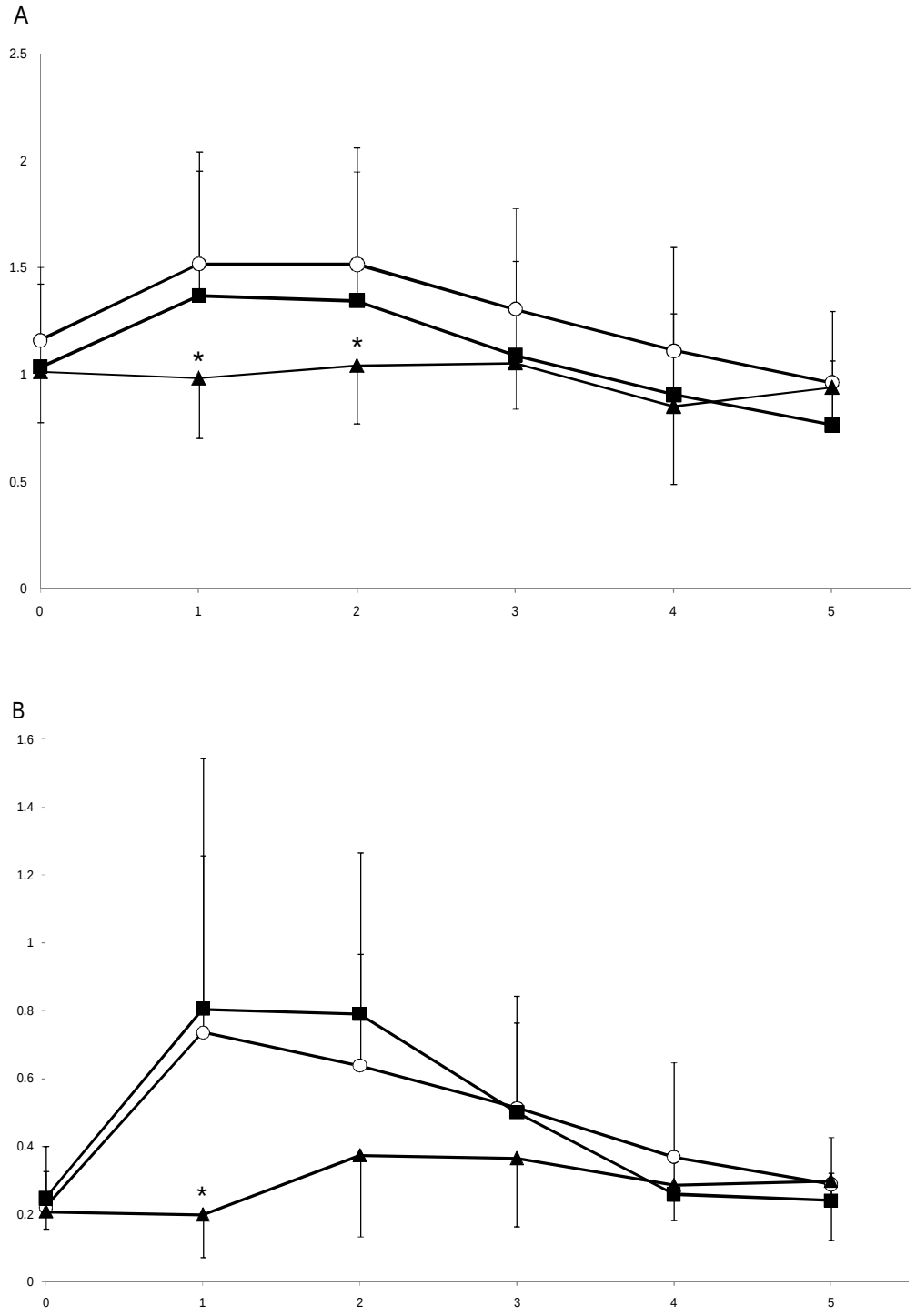

Fig. 2 Chylomicron triacylglycerol fatty acids (20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3) one to five hours postprandially after meals containing olive oil only (olive, white bars) or olive oil mixed with eicosapentaenoic acid (EPA, grey bars) or docosapentaenoic acid (DPA, black bars). Series of bars represent the times at which the blood was drawn (1 to 5 hours) and an asterisk a significant between meal difference in the corresponding time point. Values are mass proportions (mean +/- standard deviation, n=10) of all chylomicron triacylglycerol fatty acids

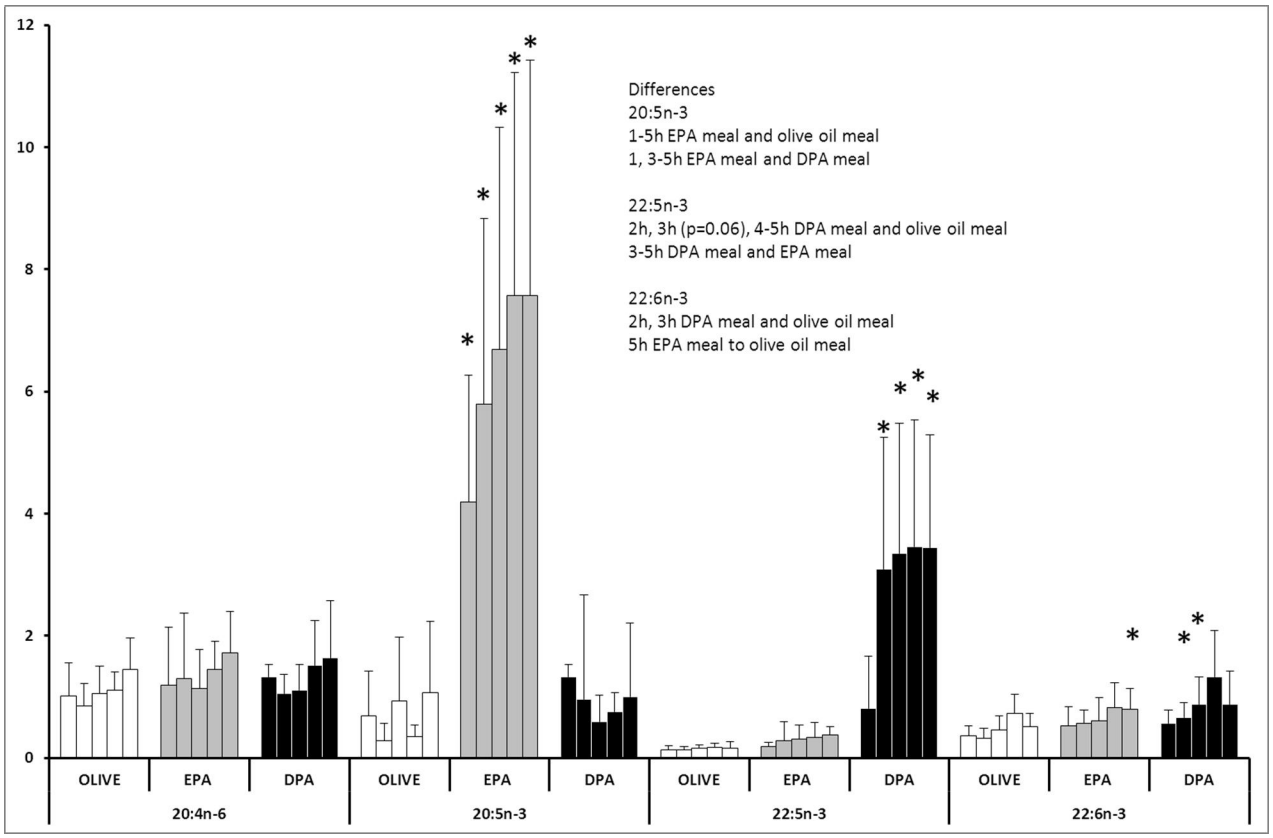

Fig. 3 Chylomicron phospholipid fatty acids (20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3) one to five hours postprandially after meals containing olive oil only (olive, white bars) or olive oil mixed with eicosapentaenoic acid (EPA, grey bars) or docosapentaenoic acid (DPA, black bars). Series of bars represent the times at which the blood was drawn (1 to 5 hours) and an asterisk a significant between meal difference in the corresponding time point. Values are mass proportions (mean +/- standard deviation, n=10) of all chylomicron phospholipid fatty acids

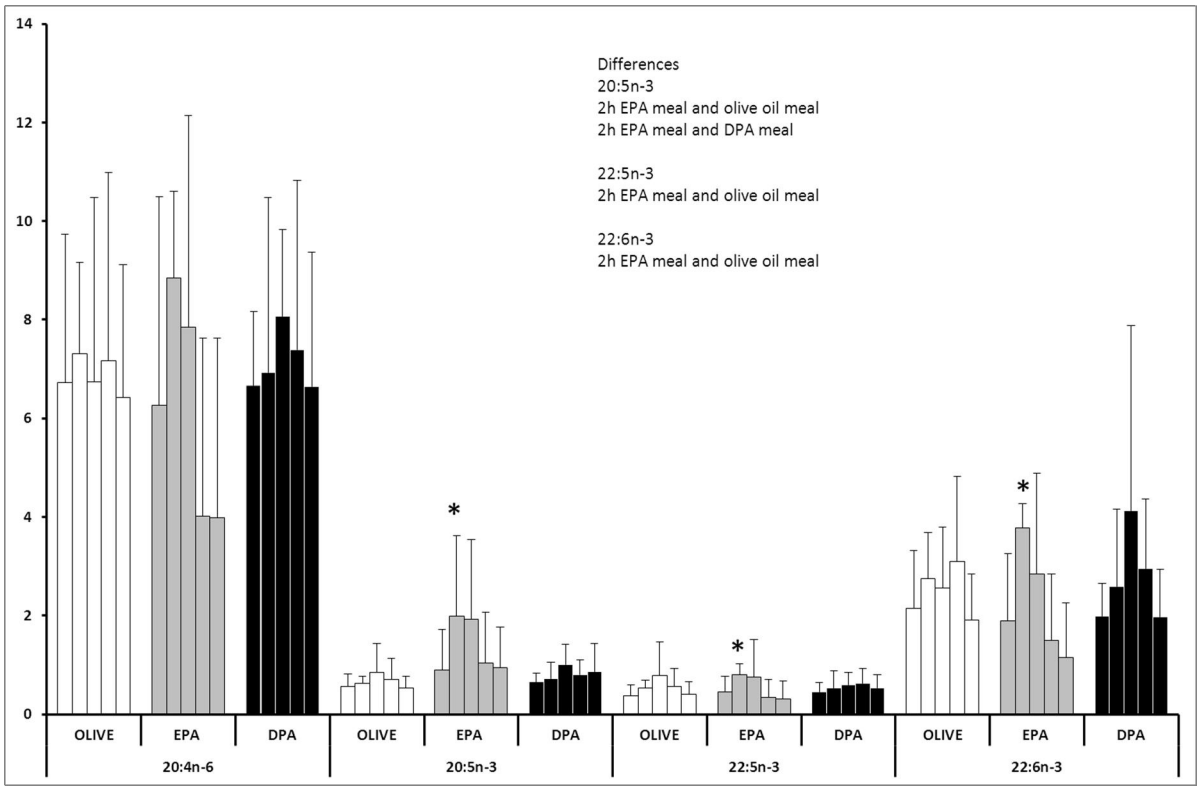

Fig. 4 PUFA containing triacylglycerols (acyl carbon number : number of double bonds) after the breakfasts containing olive oil (white bars), olive oil mixed with eicosapentaenoic acid (EPA, 20:5n-3, grey bars) and olive oil mixed with docosapentaenoic acid (DPA, 22:5n-3, black bars) at one, three and five hours , respectively. Semiquantitative values are expressed as molar percentages (mean +/- standard deviation, n=10) of all chylomicron TAGs. Most prevalent triacylglycerols based on the neutral loss experiments are marked above each group of bars.