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Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans

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Abstract

The aims of this study were to determine whether age and sex influence both the status and the incorporation of EPA and DHA into blood plasma, cells and tissues. The study was a double-blind, randomised, controlled intervention, providing EPA+DHA equivalent to 0, 1, 2 or 4 portions of oily fish per week, for 12 months. Participants were stratified by age and sex. A linear regression model was used to analyse baseline outcomes, with covariates for age or sex groups, and adjusting for BMI. The change from baseline to 12 months in outcome was analysed with additional adjusting of treatment and average compliance. Fatty acid profiles were determined in plasma phosphatidylcholine (PC), cholesteryl esters (CE), NEFA and TAG, mononuclear cells (MNC), erythrocyte membranes (RBC), platelets (PLAT), buccal cells (BU) and adipose tissue (AT). At baseline, EPA concentration in plasma NEFA and DHA concentration in MNC, BU and AT was higher in females than males (all $P<0.05$). EPA in AT ($P=0.003$) and DHA in plasma TAG ($P<0.01$) and AT ($P<0.001$) were higher with increasing age. Following 12 months supplementation with EPA+DHA, adjusted mean difference for change in EPA in plasma TAG was significantly higher in females than males ($P<0.05$) and was greater with increasing age ($P=0.02$). Adjusted mean difference for change in DHA in AT was significantly smaller with increasing age ($P=0.02$). Although small differences in incorporation with age and sex were identified, these were not of sufficient magnitude to warrant a move away from population-level diet recommendations for n-3 PUFA.

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Keywords

Fish oil; EPA; DHA; n-3 polyunsaturated fatty acids

INTRODUCTION

Oral intake of the n-3 PUFA, EPA and DHA, is the major determinant of the levels of these fatty acids in blood lipids and in cells and tissues, including adipose tissue (AT) [1]. However, other factors, such as age and sex, may also influence the incorporation and levels of EPA and DHA [2, 3]. Fully understanding the different determinants of EPA and DHA status is likely to be important in correlating health benefits of these fatty acids with a particular dietary intake.

There is evidence, predominantly from cross-sectional analyses, to suggest that females have higher plasma DHA levels than males [3, 4, 5]. Tracer studies indicate that females may be more efficient than males at converting precursor n-3 PUFA such as alpha-linolenic acid to the very long chain n-3 PUFA, EPA and DHA [6, 7, 8]. Whether sex influences the incorporation of EPA and DHA across a wide range of plasma lipid fractions and cells in response to different levels of habitual intake has, to our knowledge, not been reported.

There is also evidence to suggest that age may influence the levels of EPA and DHA in plasma fractions, cells and AT. Higher levels of EPA and DHA have been reported in older individuals, which appear independent of differences in dietary intake [3, 9]. In response to supplementation to increase EPA and/or DHA, a greater increase in mononuclear cell EPA in older participants was shown [10], with no effect of age on the increase seen in plasma phosphatidylcholine (PC) [11]. It is not clear whether these conflicting results are due to the different sample types reported in these studies or to the level of n-3 fatty acid at which the population was studied.

No n-3 PUFA supplementation studies have recruited and stratified on the basis of age and sex to explore whether these disparities reflect different intakes or rates of incorporation. Potential differences in EPA and DHA content or incorporation with age and sex have, to date, mostly been examined in post-hoc analyses of plasma fractions and AT and none have reported age and sex effects on EPA and DHA concentrations across a range of sample types. It is not clear whether differences in plasma fractions are reflected by similar differences in cells. Since health benefits of increased EPA and DHA consumption are widely attributed to the levels of these fatty acids present in cell membranes and hence altered cell function [12], it is important to determine whether age and sex similarly affect the incorporation of EPA and DHA into cells as well as into plasma lipids and AT. This will be important in better defining, and tailoring, public health messages regarding consumption of oily fish and intakes of EPA and DHA, for specific age groups and for each sex.

The aim of this analysis was to determine if age and sex influence the concentrations of EPA and DHA found in plasma fractions, cells and AT, and whether age and sex account for differences in the response of plasma fractions and cells to increasing dietary intake of EPA and DHA.

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EXPERIMENTAL METHODS

Study design

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Suffolk Local Research Ethics Committee (approval 05/Q0102/181). Written informed consent was obtained from all participants. The study was registered at www.controlled-trials.com (Trial Registration: ISRCTN48398526).

This study design has been described previously in detail [13]. Briefly, the study was a double-blind, randomised, controlled intervention trial over 12 months, with 5 parallel groups, in 2 centres within the UK. Participants who reported no habitual consumption of oily fish (defined as < 1 portion per month) were recruited in Cambridge and Southampton, UK. Randomisation was stratified by age ('young' 20-39, 'middle' 40-59 and 'old' 60-79 years) and sex (male, female). Exclusion criteria were: diagnosis of diabetes, cancer, cardiovascular disease or other chronic clinical conditions; untreated hypertension; concomitant prescription of anticoagulants, non-steroidal anti-inflammatory drugs, aspirin, steroids or immunosuppressants; allergy or intolerance to fish; consumption of fish oil supplements in the last 3 months; consumption of oily fish more than once a month; smoking status; history of substance abuse or alcoholism; being pregnant, < 1 year postpartum or planning pregnancy; recent weight change (> 2 kg in past 1 month), planning to change dietary habits, increase physical activity, change body weight, move away from the study centre-locality or take a lengthy vacation during the time of the study; BMI < 18 or > 35 kg/m^2.

The intervention was capsule-based to control the delivery of the intervention. Six 0.75g capsules provided a total of 1.5g EPA and 1.77g DHA (i.e. 3.27g EPA plus DHA) as TAG, equivalent to the amount in one portion of oily fish [14]. To simulate habitual consumption patterns, capsules equating to one portion of oily fish were provided on none, one, two or four days of the week, giving so called '0 portions', '1 portion', '2 portions' or '4 portions' groups. To maintain the blinding of the study design, six 0.75g placebo capsules (high oleic sunflower oil) were given on all remaining days of the week. An additional group (not reported here) followed the traditional design of capsule based intervention studies, receiving the equivalent amount of EPA plus DHA provided in the 2 portions of oily fish group, but distributed evenly over the 7 days ('2C portions'). Capsules were blister packed, with day labelling to help increase compliance.

Sample preparation and fatty acid composition analysis

Fasting blood, buccal cell and AT samples were collected at baseline and 12 months. The following samples were analysed for fatty acid composition: plasma PC, plasma cholesteryl esters (CE), plasma TAG, plasma NEFA, blood mononuclear cells (MNC), erythrocyte membranes (RBC), platelets (PLAT), buccal cells (BU) and adipose tissue (AT).

Total lipid was extracted into chloroform:methanol (2:1 vol/vol) from plasma, MNC, RBC, PLAT, BU and homogenised AT as described previously [13]. Plasma lipids were further separated into the four fractions described above using solid phase extraction (SPE). Fatty

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acid methyl esters (FAME) were formed by incubation with methanol containing 2% (vol/vol) H_2SO_4 at 50°C for 2 hr. After allowing the tubes to cool, samples were neutralised with a solution of 0.25 M $KHCO_3$ and 0.5 M K_2CO_3.FAME were extracted into hexane, dried down, redissolved in a small volume of hexane, and separated by on a Hewlett Packard 6890 gas chromatograph fitted with a BPX-70 column (30 m × 0.22 mm × 0.25 μm) [13]. The instrument was controlled by, and data collected using, HPChemStation software. FAME were identified by comparison of retention times with those of authentic standards run previously.

Statistics

Data were analysed for participants completing the 12-month intervention. Comparisons were made between age groups (‘Middle’ vs. ‘Young’ and ‘Old’ vs. ‘Young’) and between sexes (male vs. female). Baseline data were analysed in a linear regression model to determine age or sex differences, adjusted for BMI and change data (12-months – baseline) were analysed using a linear regression model adjusted for study centre, treatment group (number of ‘portions per week’), BMI and average compliance (%) over the 12 months. We defined significance as a P 0.05; and for this analysis no multiplicity correction was considered. All analyses were performed with Stata Version 11 (StataCorp LP, Texas, USA).

RESULTS

Compliance and dietary intake

Compliance and dietary intake data have been previously reported [13]. Briefly, capsule count data showed compliance to be high (98.1%, IQR 2.2, range 87.4-100%), with 4-day un-weighed food diaries showing no significant differences in dietary variables between intervention groups or timepoints [13]. Dietary data were further analysed for sex (Table 1) and age (Table 2) effects. MUFA intake (as a percentage of total dietary energy) was significantly higher in males compared to females but there were no differences with age. There were no significant differences in reported intake of any of the fat variables, when analysed for change between baseline and 12 months.

Sex differences in baseline EPA and DHA in plasma fractions, cells and AT

The concentrations of EPA and DHA in the different fractions at baseline are shown for males and females in Table 3. Females had a higher concentration of EPA in plasma NEFA than males. There were no other sex differences for EPA at baseline. Females had higher DHA in MNC, BU and AT than males, with non-significant trends for higher DHA in plasma CE (P=0.07) and PLAT (P=0.09).

Age differences in baseline EPA and DHA in plasma fractions, cells and AT

The concentrations of EPA and DHA in the different fractions at baseline are shown for the three age categories in Table 4. Overall EPA in AT and DHA in plasma TAG and AT increased significantly with increasing age (i.e. across the three age categories), with similar trends for EPA in MNC and DHA in BU (P = 0.07). EPA in MNC and DHA in AT were

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significantly higher in middle vs. young aged participants. EPA in MNC and AT and DHA in BU and AT were significantly higher in old vs. young aged participants.

Sex differences in response to 12 months EPA and DHA supplementation

Sex differences in EPA and DHA concentrations following 12-months supplementation in the 0, 1, 2 and 4 portions groups are shown in Figure 1. There were clear dose-dependent increases in EPA (Figure 1a) and DHA (Figure 1b) in all fractions studied in both males and females, except in RBC and BU for EPA. Adjusted mean differences in EPA and DHA concentrations between sexes for change between baseline and 12 months are shown in Table 5. The increase in EPA was significantly greater in females than males for plasma TAG, with a similar non-significant trend for PLAT ($P = 0.08$). There were no significant sex differences in the increase in DHA.

Age differences in response to 12 months EPA and DHA supplementation

Age differences in EPA and DHA concentrations following 12-months supplementation in the 0, 1, 2 and 4 portions groups are shown in Figure 2. There were clear dose-dependent increases in EPA (Figure 2a) and DHA (Figure 2b) in all fractions studied in all three age groups, except in RBC and BU for EPA. Adjusted mean differences in EPA and DHA concentrations between age categories for change between baseline and 12 months are shown in Table 6. Overall the increase in EPA in plasma TAG was greater with increasing age, while the increase in DHA in AT was smaller with increasing age. The increases in EPA were greater in several fractions for middle vs. young aged group, reaching significance for EPA in plasma NEFA, plasma TAG and PLAT. The increase in concentration of DHA in AT was smaller in the old vs. the young aged group.

DISCUSSION

This study addressed whether the response to n-3 PUFA interventions is related to sex and age. We have previously reported a significant dose response to the EPA+DHA intervention in the same study population [13]. Here we report some significant differences according to sex and age in EPA and DHA in some plasma fractions and cells at both baseline and in the extent of change in response to modest levels of supplementation with EPA and DHA.

Sex

We found significantly higher DHA in MNC, BU and AT in females compared to males at baseline, and a greater increase in EPA in plasma TAG in females in response to supplementation with EPA and DHA for one year. Similar trends in the DHA concentration of plasma fractions and cells at baseline were seen, although these did not reach statistical significance. The dietary data suggests that there are no significant differences in intake of n-6 or n-3 PUFA between males and females to account for these differences suggesting that they reflect biological differences. The difference was not explained by differences in BMI.

Previous studies have reported differences in the DHA content of plasma fractions between sexes similar to those found in the current study. These previous studies include both small cross sectional studies [15, 16, 4] and large-scale population surveillance [3, 17, 18]. However,

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previous studies did not purposively recruit non-consumers of oily fish and so include participants with varying consumption, and one study included high habitual consumers of oily fish [19]. Amongst a population with known high intake of oily fish, women reported significantly lower consumption of n-3 PUFA, yet were found to have significantly higher phospholipid DHA [20]. Where dietary data were collected, differences in dietary intake between men and women did not explain the detected sex differences in DHA content of the plasma lipid fraction studied.

Differences in fatty acid metabolism (in synthesis, interconversion and degradation of fatty acids) may be responsible for the sex differences in DHA status observed in the current study. Studies using stable isotopes to trace fatty acid metabolism have shown increased DHA synthesis from shorter chain precursors in females [6, 7, 8] and decreased retro-conversion of DHA to DPA and EPA in females [21]. It is hypothesised that female hormones are responsible for these changes in n-3 PUFA metabolism [2]. Indeed, studies have shown differences in DHA status with use of the hormone replacement therapy [22] and that hormone replacement therapy reduces retro-conversion of DHA [23]. A study of the use of raloxifene implicates the oestrogen receptor in the control of n-3 PUFA metabolism [24].

In addition we found significantly higher EPA in plasma NEFA in females than in males, though not in any of the other plasma fractions or cells. While most studies report no significant difference in EPA between males and females, Crowe *et al.* reported significantly higher EPA in plasma phospholipid and CE, but not TAG, in females [3]. They did not report on plasma NEFA. In the fasting state plasma NEFA reflects the fatty acids most recently mobilised from AT. There was no sex difference in the EPA concentration of adipose tissue in the current study. However, we assessed the fatty acid composition of only a single depot (abdominal subcutaneous) and there is some evidence that the fatty acid composition of different depots may be different [25]. Thus, it may be that the different fat distribution in females compared with males and different rates or patterns of release of NEFA from different depots gives rise to a difference in plasma NEFA composition.

The current study found no significant sex differences in the change in DHA with EPA +DHA intervention. This is in contrast to cross-sectional studies which show higher DHA in females than males with high consumption of oily fish [19, 20]. These latter studies suggest that sex differences in DHA status do not only occur in the absence of dietary DHA, where they would rely predominantly on endogenous production, but can occur in the presence of higher intakes of preformed DHA. It is possible that the current study was too small to identify an effect of sex on increase in DHA concentration when preformed DHA is consumed. It is also possible that effects of sex are dependent on age, especially if female sex hormones play a central role.

Age

In keeping with reported literature, we show an age-related increase in EPA and DHA concentration in some of the plasma fractions and cells studied and in AT (except for DHA in AT, where there was a smaller change in DHA with increasing age). Whilst most studies report results from plasma fractions [3, 17, 19, 20, 26] there are also reports of significant age differences in EPA and DHA in RBC [27, 28] and AT [9, 29]. Previous studies attribute age

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differences in EPA and DHA to differences in dietary intake [3, 19, 20, 28] but this cannot explain the findings of the present study or others, and some studies have shown that age remains a significant determinant of EPA and DHA status after adjustment for dietary intake [27, 30, 31] suggesting an age-dependent effect independent of dietary intake. The current study did not recruit habitual oily fish consumers and therefore intakes of preformed EPA and DHA were likely to be low. Therefore, it seems unlikely that the age-related differences in EPA and DHA concentrations at baseline are due to differences in intake of n-3 PUFA with age. Rather they may relate to age-related differences in endogenous production and incorporation of EPA and DHA. This could be due to hormones and hormone sensitivity, body composition, and physical activity, all of which change with age.

In contrast to the observation made at baseline, we saw a smaller increase in DHA concentration in AT with increasing age in response to the EPA and DHA intervention. This would suggest age-related differences in the handling and storage of exogenously supplied DHA. This could relate to impaired insulin sensitivity with ageing or to differences in body composition with ageing. Despite the relatively low contributions of EPA and DHA to total AT fatty acids, because of the bulk of AT, this represents a significant store of EPA and DHA, containing the equivalent of several hundred days of typical intake.

The observed greater incorporation of EPA in relation to increasing age mostly reflected the difference between middle and young aged adults, rather than between old and young aged adults. Crowe *et al.* showed a non-linear trend in EPA content in plasma phospholipids with age, with a bigger between group difference at the lower end [3]. This could be due, in part, to greater differences in female hormone concentrations amongst younger women, again suggesting that effects of age may, in part be due to sex and hence menopausal status is an important consideration. The current study was not large enough to determine the interaction of sex and age upon the EPA and DHA response to the intervention and as such we are unable to determine any menopausal difference in women in this study.

Other Considerations

It is conceivable that factors other than those fully considered here may help to explain the observed differences in EPA and DHA concentrations in a given plasma fraction or cell type and their responses to intervention. Dewailly *et al.* showed no effect of smoking on n-3 PUFA concentrations in Nunavik Inuit population who consume high quantities of oily fish, but that alcohol intake, waist circumference and use of CVD medication were all significant factors [19]. In Quebec participants who also have a high oily fish intake, smoking, alcohol and waist circumference were significant determinants of EPA and DHA status [20]. In the Framingham Heart Study, population factors that were significantly correlated with n-3 PUFA status were age, sex, higher education, use of fish oil supplements, dietary EPA and DHA intake, aspirin use, use of lipid lowering medications and LDL cholesterol concentration (all positive), and heart rate, triglyceride concentration, waist girth and smoking (all negative) [32]. Of these factors, the current study investigated effects of age and sex and excluded users of fish oil supplements and high oily fish consumers. It is possible that some of the other factors may have played a role.

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Relevance to cell function and health

EPA and DHA are known to affect the function of many cell types with actions in many aspects of physiology including lipid metabolism, inflammation, immune function, platelet aggregation, smooth muscle contraction, cardiac rhythm, insulin sensitivity and bone turnover [12]. The effects of EPA and DHA are best described in relation to a beneficial effect on cardiovascular morbidity and mortality [33, 34]. The current study suggests that both sex and age need to be considered when anticipating any potential health advantage of EPA and DHA.

CONCLUSIONS

In this study we report some age- and sex-related differences in EPA and DHA concentrations in some plasma fractions, cells and AT and some age- and sex-related differences in the increase in EPA and DHA when these fatty acids are given preformed. However, the effect sizes of both sex and age are smaller than the effect size of a modest increase in intake of EPA+DHA equivalent to one portion of oily fish per week. Therefore, the findings of this study do not support the development of different dietary guidelines for EPA and DHA intake for adults according to sex or age. A possible interaction between age and sex on EPA and DHA status should be investigated in future well powered studies.

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The author contributions were as follows: LMB, PCC and SAJ designed the study; LMB, CGW, JM and ALW conducted the research; LMB, CGW, and APM analysed data; CGW and LMB wrote the manuscript; all authors read and approved the final manuscript.

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PCC serves on Scientific Advisory Boards of the Danone Research Centre in Specialised Nutrition, Aker Biomarine, Pronova BioPharma and Smartfish. He acts as a consultant to Mead Johnson Nutritionals, Vifor Pharma and Amarin Corporation. He has received speaking honoraria from Solvay Healthcare, Solvay Pharmaceuticals, Pronova BioPharma, Fresenius Kabi, B. Braun, Abbott Nutrition, Baxter Healthcare, Nestle, Unilever and DSM. He currently receives research funding from Vifor Pharma and Abbott Nutrition. He serves on the executive board of the International Society for the Study of Fatty Acids and Lipids, an organisation that is partly supported by corporate membership fees, mainly the food and supplements industries. He is a member of the Board of Directors of the European Nutraceutical Association and of the Council of the British Nutrition Foundation; these organizations are each supported in part by the food and supplements industries. SAJ previously served on Scientific Advisory Boards for Tanita, PepsicCo, Nestlé, Coca-cola, Californian Almond Board, Heinz and Kellogs. She is currently a member of the Tanita Medical Advisory Board and receives a fee for nutrition articles in the Rosemary Conley Diet and Fitness Magazine.

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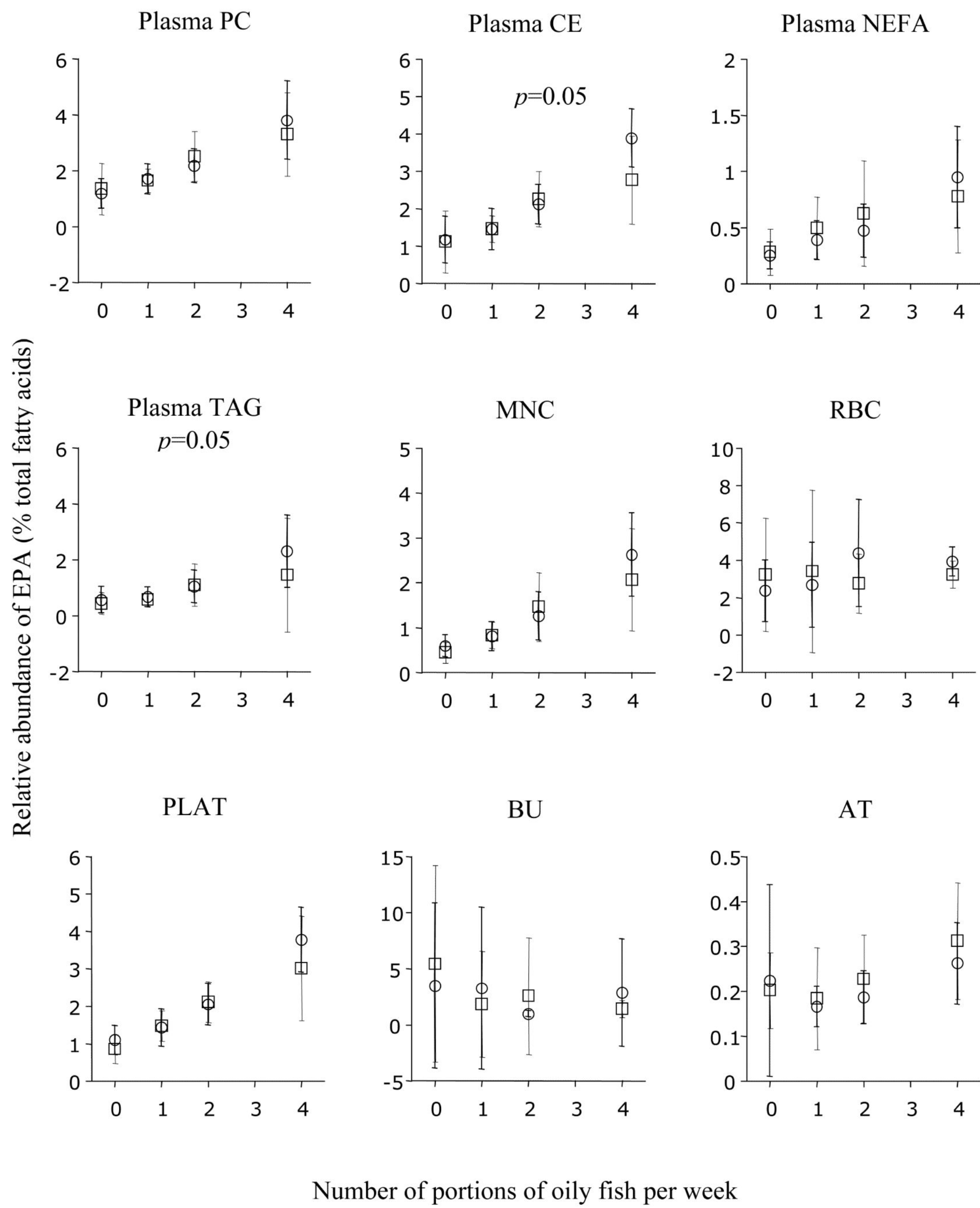

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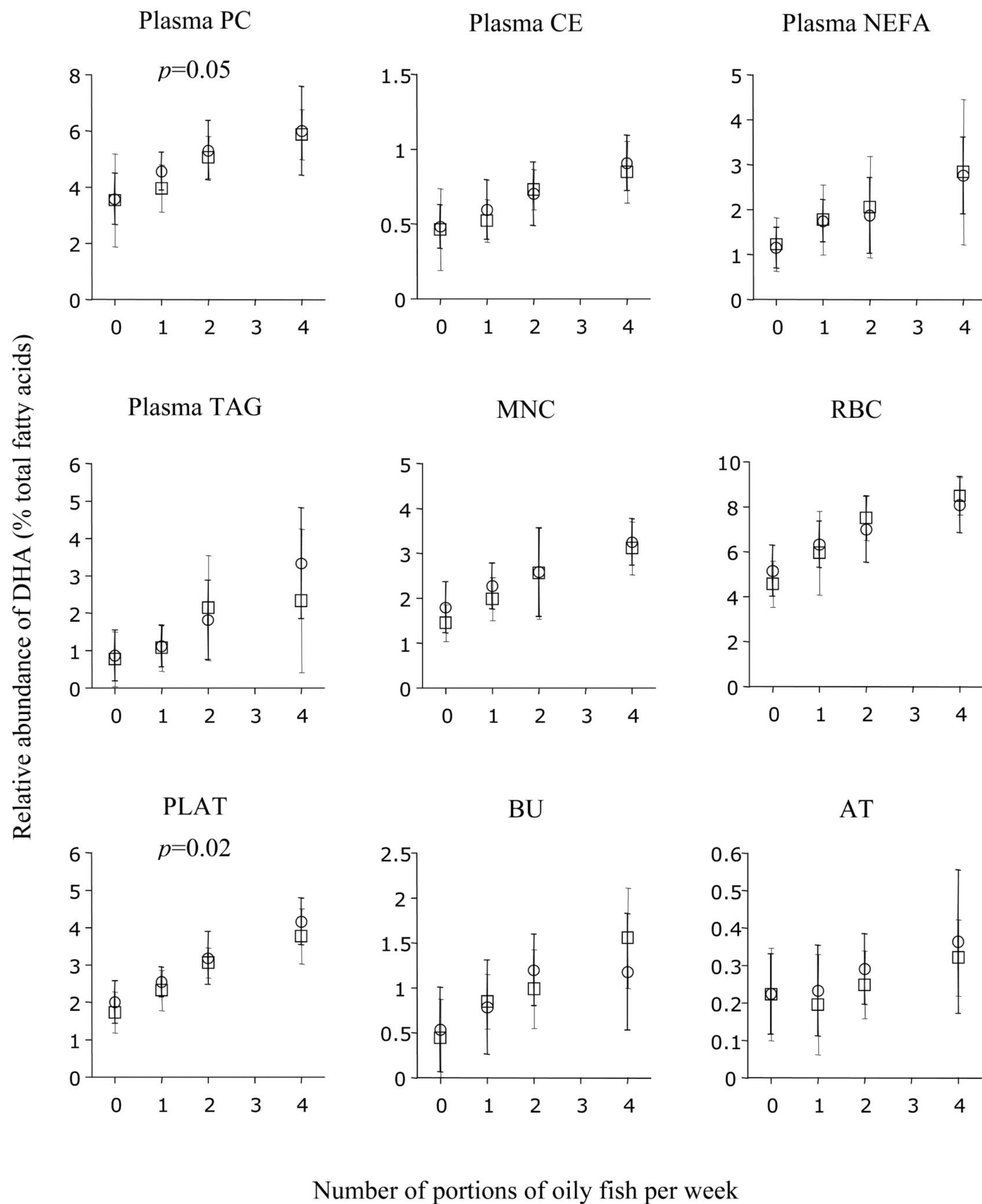

Figure 1.

Sex differences in EPA (a) and DHA (b) concentration in plasma fractions, cells and AT at 12 months following supplementation with EPA and DHA equivalent to 0, 1, 2, and 4 portions of oily fish per week. Data are mean and SD EPA (Figure 1a) or DHA (Figure 1b) as a percentage of total fatty acids at 12 months; Men open squares, women open circles. Mean ±SD DHA (as a percentage of total fatty acids) in plasma fractions and cell membranes at 12 months in men and women receiving EPA and DHA equivalent to 0, 1, 2, and 4 portions of oily fish per week.

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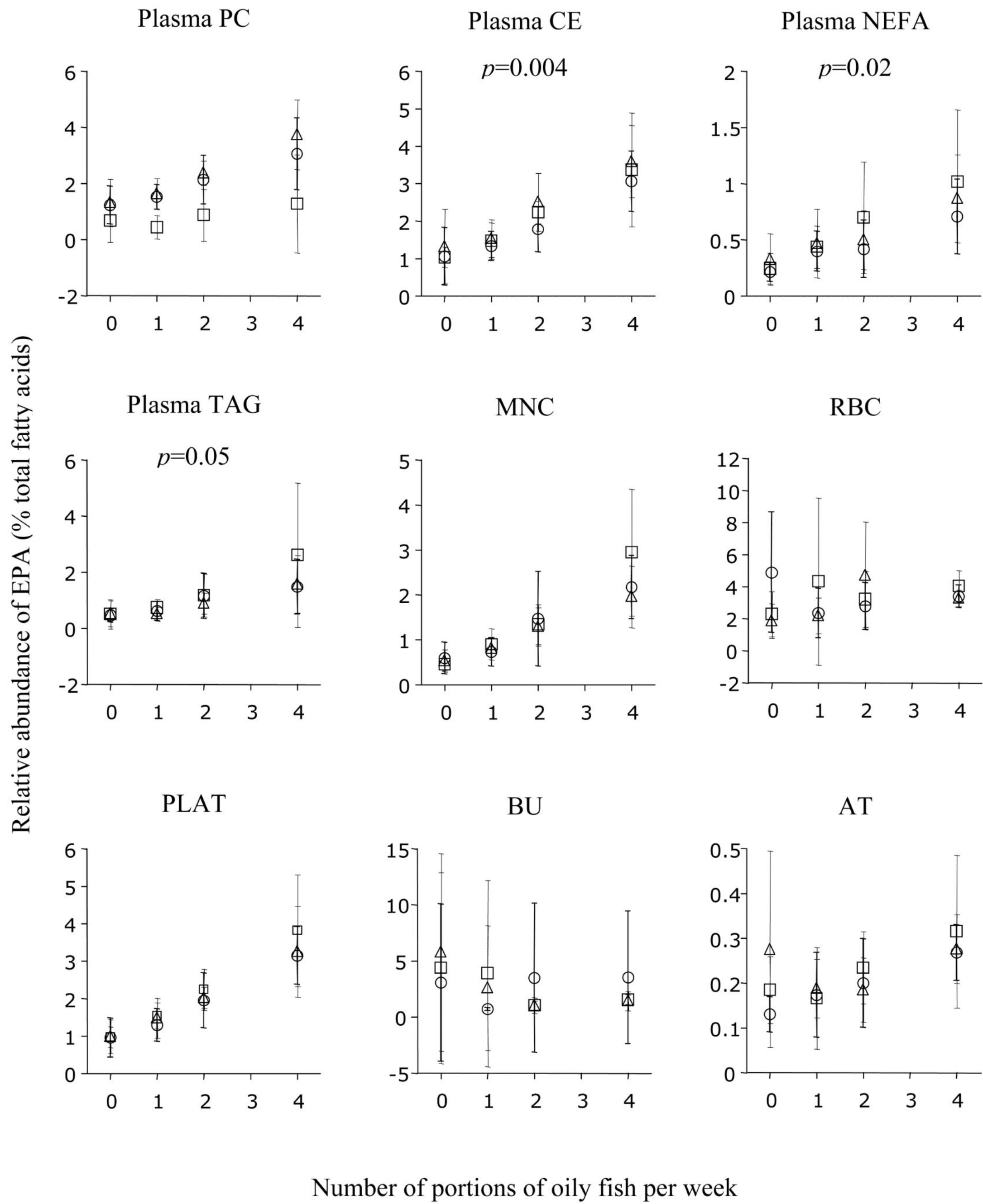

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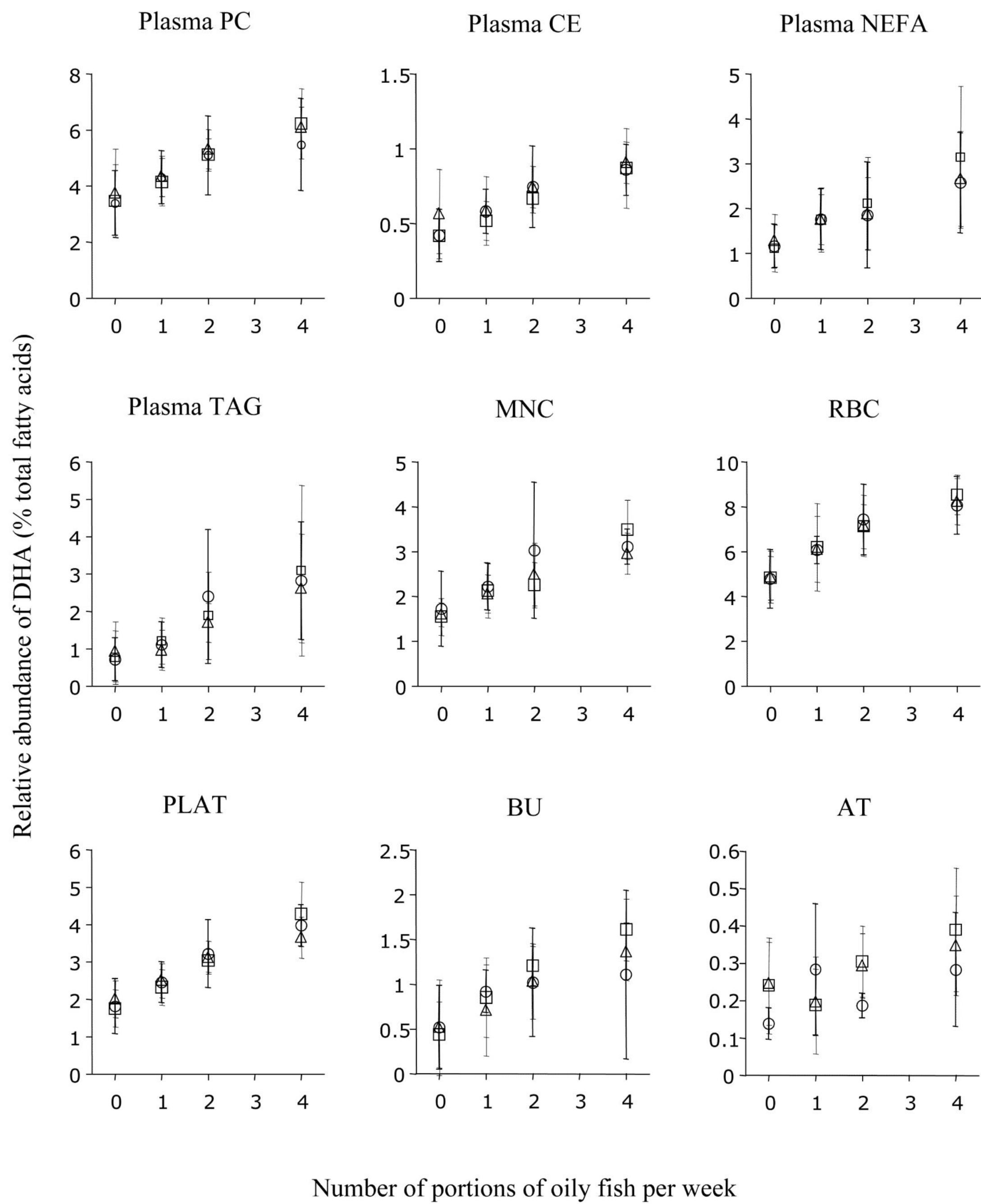

Figure 2.
Age differences in EPA (a) and DHA (b) concentration in plasma fractions, cells and AT at 12 months following supplementation with EPA and DHA equivalent to 0, 1, 2, and 4 portions of oily fish per week.. Data shown are mean and SD EPA (Figure 2a) or DHA (Figure 2b), as a percentage of total fatty acids, at 12 months; 'young' open circles, 'middle' open squares, 'old' open triangles.

Mean ±SD DHA (as a percentage of total fatty acids) in plasma fractions and cell membranes at 12 months in ‘young’, ‘middle’ and ‘old’ age categories receiving EPA and DHA equivalent to 0, 1, 2, and 4 portions of oily fish per week.

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Table 1

Sex differences in macronutrient intake from 4-day un-weighed food diary at baseline and change with 12-months EPA+DHA intervention

	Baseline[1]			Change[2]		
	Female	**Male**	***P*** [3]	**Female**	**Male**	***P*** [4]
n	***101***	***92***		***73***	***76***	
Protein	15.66 (2.65)	16.10 (3.28)	0.49	0.37 (3.46)	−0.12 (3.75)	0.59
Carbohydrate	49.14 (7.14)	46.73 (8.48)	0.04	−0.91 (9.34)	1.81 (12.14)	0.18
Total Fat	33.48 (5.70)	34.69 (5.81)	0.19	0.29 (10.73)	−0.22 (10.74)	0.80
Total SFA	12.65 (3.06)	12.94 (3.20)	0.49	−0.09 (4.88)	−0.43 (4.94)	0.57
Total MUFA	10.97 (2.16)	11.86 (2.27)	0.01	0.42 (3.80)	−0.16 (3.69)	0.44
Total PUFA	5.92 (2.04)	5.77 (1.66)	0.49	0.05 (2.51)	0.32 (2.66)	0.38
Total n-6 PUFA	5.27 (1.91)	5.10 (1.56)	0.41	0.02 (0.27)	0.02 (0.30)	0.35
Total n-3 PUFA	0.75 (0.25)	0.75 (0.26)	0.94	0.03 (2.38)	0.31 (2.57)	0.95

[1] Data are mean (SD) expressed as a percentage of total dietary energy per day.

[2] Data are mean (SD) in change (12-months – baseline) expressed as a percentage of total dietary energy per day.

[3] Analysis is baseline regressed on male vs. female, adjusted for BMI.

[4] Analysis is change regressed on male vs. female, adjusted for treatment group, compliance and BMI.

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Table 2

Age differences in macronutrient intake from 4-day un-weighed food diary at baseline and change with 12-months EPA+DHA intervention

	Baseline[1]				Change[2]			
	Young	**Middle**	**Old**	***P*** [3]	**Young**	**Middle**	**Old**	***P*** [4]
n	***59***	***66***	***68***		***42***	***50***	***57***	
Protein	16.00 (3.55)	15.90 (2.83)	15.73 (2.56)	0.79	−0.72 (4.63)	0.24 (3.06)	0.63 (3.11)	0.06
Carbohydrate	47.81 (7.86)	47.95 (8.14)	48.17 (7.74)	0.95	−0.52 (9.61)	0.34 (12.90)	1.32 (9.99)	0.67
Fat	33.90 (5.48)	34.01 (5.91)	34.25 (5.95)	0.98	−0.30 (10.22)	−1.54 (10.34)	1.65 (11.30)	0.34
Total SFA	12.55 (2.80)	12.72 (3.00)	13.06 (3.51)	0.69	−0.73 (4.08)	−0.99 (5.27)	0.71 (5.02)	0.20
Total MUFA	11.52 (2.21)	11.23 (2.44)	11.44 (2.12)	0.56	0.43 (3.78)	−0.40 (3.74)	0.36 (3.73)	0.67
Total PUFA	5.89 (1.76)	6.01 (2.20)	5.64 (1.58)	0.45	0.15 (2.73)	−0.03 (2.53)	0.40 (2.54)	0.56
Total n-6 PUFA	5.28 (1.66)	5.33 (2.03)	4.96 (1.53)	0.37	0.01 (0.26)	0.02 (0.28)	0.03 (0.31)	0.55
Total n-3 PUFA	0.71 (0.21)	0.75 (0.33)	0.76 (0.19)	0.52	0.10 (2.61)	−0.02 (2.51)	0.39 (2.37)	0.73

[1] Data are mean (SD) expressed as a percentage of total dietary energy per day.

[2] Data are mean (SD) in change (12-months – baseline) expressed as a percentage of total dietary energy per day.

[3] Analysis is baseline regressed on Middle vs. Young and Old vs. Young with a global test for age group presented, adjusted for BMI.

[4] Analysis is change regressed on Middle vs. Young and Old vs. Young with a global test for age group presented, adjusted for treatment group, compliance and BMI.

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Table 3

Sex differences in baseline EPA and DHA in plasma fractions, cells and AT

	EPA				DHA			
	Female[1]	Male[1]	Adjusted mean difference[2]	P[3]	Female[1]	Male[1]	Adjusted mean difference[2]	P[3]
n	*104*	*99*			*104*	*99*		
Plasma PC	1.17 (0.63)	1.14 (0.75)	−0.07 [−0.23, 0.08]	0.37	3.68 (0.99)	3.53 (1.39)	−0.19 [−0.58, 0.21]	0.36
Plasma CE	1.09 (0.64)	1.07 (0.73)	−0.08 [−0.29, 0.13]	0.46	0.70 (0.21)	0.64 (0.23)	−0.07 [−0.14, 0.00]	0.07
Plasma NEFA	0.48 (0.50)	0.37 (0.27)	−0.14 [−0.27, 0.00]	0.05	1.74 (1.27)	1.44 (0.99)	−0.23 [−0.57, 0.12]	0.20
Plasma TAG	0.31 (0.55)	0.31 (0.70)	−0.01 [−0.09, 0.08]	0.91	0.94 (1.26)	0.83 (0.63)	0.01 [−0.14, 0.16]	0.93
MNC	0.74 (0.51)	0.76 (0.59)	0.00 [−0.18, 0.19]	0.99	1.98 (0.51)	1.81 (0.59)	−0.18 [−0.36, −0.01]	0.04
RBC	2.22 (2.74)	2.02 (1.82)	0.23 [−0.29, 0.76]	0.39	5.41 (1.40)	5.17 (1.50)	−0.38 (−0.84, 0.07]	0.10
PLAT	1.11 (0.48)	1.14 (0.80)	0.03 [−0.17, 0.22]	0.80	2.13 (0.54)	1.93 (0.60)	−0.15 [−0.33, 0.024]	0.09
BU	1.43 (1.74)	1.54 (4.06)	0.34 [−0.69, 1.37]	0.52	0.88 (0.32)	0.75 (0.31)	−0.13 [−0.23, −0.03]	0.01
AT	0.16 (0.06)	0.16 (0.06)	0.00 [−0.02, 0.02]	0.78	0.21 (0.11)	0.18 (0.09)	−0.04 [−0.07, 0.00]	0.05

[1] Data are mean (SD) with EPA or DHA expressed as a percentage of total fatty acids.

[2] Data are mean difference [95% CI] in EPA or DHA expressed as percentage of total fatty acids female compared to male adjusted for BMI.

[3] Analysis is baseline regressed on male vs. female, adjusted for BMI.

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Table 4

Age differences in baseline EPA and DHA in plasma fractions, cells and AT

	Young[1]	Middle[1]	Old[1]	Adjusted mean difference Middle vs. Young[2]	Adjusted mean difference Old vs. Young[2]	P [3]
n	*66*	*68*	*69*			
				EPA		
Plasma PC	1.15 (0.99)	1.08 (0.37)	1.22 (0.58)	0.09 [−0.10, 0.29]	0.20 [0.00, 0.39]	0.13
Plasma CE	0.97 (0.79)	1.07 (0.68)	1.20 (0.56)	0.18 [−0.09, 0.45]	0.21 [−0.06, 0.48]	0.27
Plasma NEFA	0.47 (0.58)	0.38 (0.26)	0.42 (0.32)	−0.07 [−0.24, 0.10]	−0.04 [−0.21, 0.13]	0.73
Plasma TAG	0.42 (1.01)	0.19 (0.20)	0.33 (0.35)	−0.03 [−0.14, 0.08]	0.09 [−0.02, 0.19]	0.08
MNC	0.65 (0.47)	0.75 (0.55)	0.85 (0.61)	0.21 [0.02, 0.43]	0.26 [0.03, 0.48]	0.07
RBC	1.86 (1.35)	1.89 (1.66)	2.60 (3.36)	0.49 [−0.17, 1.14]	0.56 [−0.09, 1.21]	0.21
PLAT	1.11 (0.77)	1.06 (0.36)	1.20 (0.75)	−0.03 [−0.28, 0.22]	0.12 [−0.12, 0.37]	0.39
BU	1.96 (4.91)	1.30 (1.66)	1.21 (1.49)	−0.92 [−2.20, 0.37]	−0.77 [−2.05, 0.50]	0.37
AT	0.14 (0.06)	0.16 (0.05)	0.19 (0.06)	0.01 [−0.02, 0.03]	0.04 [0.02, 0.06]	**0.003**
				DHA		
Plasma PC	3.67 (1.04)	3.46 (0.99)	3.70 (1.49)	−0.05 [−0.54, 0.45]	0.17 [−0.33, 0.66]	0.64
Plasma CE	0.63 (0.22)	0.67 (0.22)	0.71 (0.22)	0.04 [−0.05, 0.13]	0.08 [−0.01, 0.16]	0.25
Plasma NEFA	1.67 (1.36)	1.55 (1.08)	1.57 (1.01)	0.05 [−0.38, 0.49]	0.05 [−0.39, 0.48]	0.97
Plasma TAG	1.07 (1.63)	0.68 (0.32)	0.93 0.57)	−0.04 [−0.22, 0.14]	0.20 [0.02, 0.14]	**0.01**
MNC	1.88 (0.61)	1.81 (0.43)	1.99 (0.60)	−0.06 [−0.28, 0.16]	0.09 [−0.13, 0.31]	0.37
RBC	5.24 (1.28)	5.20 (1.39)	5.43 (1.66)	−0.03 [−0.61, 0.54]	0.20 [−0.37, 0.77]	0.65
PLAT	2.05 (0.58)	1.92 (0.51)	2.13 (0.62)	−0.01 [−0.23, 0.21]	0.15 [−0.07, 0.37]	0.23
BU	0.76 (0.33)	0.80 (0.27)	0.88 (0.35)	0.10 [−0.03, 0.22]	0.15 [0.02, 0.27]	0.07
AT	0.15 (0.07)	0.18 (0.09)	0.25 (0.12)	0.06 [0.02, 0.10]	0.12 [0.08, 0.16]	<**0.001**

[1] Data are mean (SD) with EPA or DHA expressed as a percentage of total fatty acids.

[2] Data are mean difference [95% CI] in EPA or DHA expressed as percentage of total fatty acids between different age categories adjusted for BMI.

[3] Analysis is baseline regressed on Middle vs. Young and Old vs. Young with a global test for age group presented, adjusted for BMI.

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Table 5

Sex differences in change in EPA and DHA in plasma fractions, cells and AT following 12-months supplementation with EPA+DHA

	EPA[1]	P[2]	DHA[1]	P[2]
Plasma PC	−0.07 [−0.37, 0.22]	0.63	−0.16 [−0.62, 0.30]	0.50
Plasma CE	−0.16 [−0.43, 0.11]	0.25	0.08 [−0.02, 0.12]	0.10
Plasma NEFA	0.04 [−0.06, 0.15]	0.42	0.23 [−0.22, 0.69]	0.79
Plasma TAG	−0.30 [−0.60, −0.01]	**0.05**	−0.21 [−0.56, 0.14]	0.25
MNC	−0.16 [−0.42, 0.11]	0.26	0.03 [−0.22, 0.29]	0.82
RBC	−0.51 [−1.40, 0.37]	0.26	0.36 [−0.18, 0.90]	0.19
PLAT	−0.25 [−0.53, 0.03]	0.08	−0.09 [−0.29, 0.11]	0.38
BU	−0.10 [−2.01, 1.82]	0.93	0.07 [−0.11, 0.26]	0.44
AT	0.02 [−0.02, 0.06]	0.25	0.00 [−0.05, 0.05]	0.99

[1] Data are mean difference [95% CI] in the change in EPA or DHA expressed as percentage of total fatty acids female compared to male adjusted for treatment group, compliance and BMI.

[2] Analysis is change regressed on male vs. female, adjusted for treatment group, compliance and BMI.

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Table 6

Age differences in change in EPA and DHA in plasma fractions, cells and AT following 12-months supplementation with EPA + DHA

	EPA			DHA		
	Middle vs. Young[1]	Old vs. Young[1]	*P*[2]	Middle vs. Young[1]	Old vs. Young[1]	*P*[2]
Plasma PC	0.27 [–0.10, 0.64]	0.14 [–0.23, 0.51]	0.35	0.30 [–0.28, 0.87]	0.22 [–0.35, 0.79]	0.59
Plasma CE	0.14 [–0.20, 0.47]	0.28 [–0.06, 0.61]	0.26	0.05 [–0.07, 0.17]	0.00 [–0.12, 0.12]	0.68
Plasma NEFA	0.25 [0.04, 0.47]	0.19 [–0.02, 0.41]	0.07	0.20 [–0.37, 0.76]	0.09 [–0.47, 0.66]	0.79
Plasma TAG	0.42 [0.05, 0.78]	–0.05 [–0.42, 0.32]	**0.02**	0.15 [–0.28, 0.59]	–0.20 [–0.64, 0.23]	0.23
MNC	–0.01 [–0.34, 0.32]	–0.18 [–0.51, 0.15]	0.46	0.09 [–0.22, 0.41]	–0.01 [–0.33, 0.31]	0.76
RBC	–0.46 [–1.56, 0.64]	–0.96 [–2.06, 0.14]	0.23	0.14 [–0.53, 0.81]	–0.06 [–0.74, 0.61]	0.82
PLAT	0.40 [0.05, 0.75]	0.13 [–0.22, 0.47]	0.07	0.13 [–0.12, 0.37]	–0.01 [–0.26, 0.24]	0.45
BU	1.89 [–0.49, 4.27]	1.45 [–0.92, 3.82]	0.28	–0.02 [–0.24, 0.21]	–0.19 [–0.42, 0.03]	0.16
AT	0.00 [–0.05, 0.05]	–0.01 [–0.06, 0.04]	0.91	–0.03 [–0.09, 0.03]	–0.08 [–0.14, –0.02]	**0.02**

[1] Data are mean difference [95% CI] in the change in EPA or DHA expressed as percentage of total fatty acids between different age categories, adjusted for treatment group, compliance and BMI.

[2] Analysis is change regressed on Middle vs. Young and Old vs. Young with a global test for age group presented, adjusted for treatment group, compliance and BMI.