Plasma oxylipins respond in a linear dose-response manner with increased intake of eicosapentaenoic and docosahexaenoic acids: results from a randomized controlled trial in healthy humans

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Short running head: Dose-response effect of EPA and DHA on plasma oxylipins

Abbreviations:

ALA	alpha linolenic acid
ARA	arachidonic acid
CI	confidence interval
COX	cyclooxygenase
CYP	cytochrome P450
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DiHDPE	dihydroxy-DHA
DiHETE	dihydroxy-EPA
DiHETrE	dihydroxy-ARA
DiHODE	dihydroxy-ALA
DiHOME	dihydroxy-LA
EPA	eicosapentaenoic acid
EpDPE	epoxy-DHA
EpETE	epoxy-EPA
EpETrE	epoxy-ARA
EpODE	Epoxy-ALA
EpOME	epoxy-LA
HDHA	hydroxy-DHA
HEPE	hydroxyl-EPA
HETE	hydroxyl-ARA
HETrE	hydroxy-DGLA
HODE	hydroxy-LA
HOTrE	hydroxy-ALA
IS	internal standard
IsoP	isoprostane
LA	linoleic acid
LC-MS	liquid chromatography-mass spectrometry
LT	leukotriene
LOX	lipoxygenase
PC	plasma phosphatidylcholine
PG	prostaglandin
PUFA	polyunsaturated fatty acid
QC	quality control samples
RBC	red blood cells
SEM	standard error of the mean
SPM	specialized pro-resolving lipid mediator

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Abstract

Background: The health effects of long chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) are partly mediated by their oxidized metabolites, i.e. eicosanoids and other oxylipins. Some intervention studies demonstrate that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increase systemic concentrations n-3 PUFA-derived oxylipins and moderately decrease arachidonic acid-derived oxylipins. There is no information on the dose-response of oxylipin concentrations following n-3 PUFA intake.

Objective: The objective was to quantify oxylipins in human plasma samples from an intervention study in which participants were randomly assigned to different daily intakes of EPA and DHA for 12 months.

Design: Healthy adult men and women with low habitual fish consumption (*n* = 121) were randomized to receive capsules providing doses of n-3 PUFAs reflecting three patterns of consumption of oily fish (i.e. 1, 2, or 4 portions/week with 3.27 g EPA+DHA (1:1.2, (*wt:wt*)) per portion) or placebo. Oxylipins were quantified in plasma after 3 and 12 months. Relative and absolute changes of individual oxylipins were correlated with the dose and the content of EPA and DHA in blood lipid pools.

Results: 73 oxylipins, mostly hydroxy-, dihydroxy-, and epoxy-PUFAs were quantified in the plasma samples. After 3 and 12 months a linear increase with dose was observed for all EPA- and DHA-derived oxylipins. Cytochrome-P450-derived anti-inflammatory and cardio-protective epoxy-PUFAs increased linearly with n-3 PUFA dose and showed low inter-individual variance (r^2 >0.95). Similarly, 5, 12- and 15-lipoxygenase derived hydroxy-PUFAs as well as those formed autoxidatively increased linearly. These include the precursors of so-called specialized pro-resolving mediators (SPMs), e.g. 17-hydroxy-DHA and 18-hydroxy-EPA.

Conclusions: Plasma concentrations of biologically active oxylipins derived from n-3 PUFAs, including epoxy-PUFAs and SPM-precursors, increase linearly with elevated intake of EPA and DHA. Inter-individual differences in resulting plasma concentrations are low.

This trial was registered at Current Controlled Trials (www.controlled-trials.com) as ISRCTN48398526.

Key words: omega-3 polyunsaturated fatty acids, eicosanoids, oxylipins, fish oil, n-3/n-6-ratio, essential fatty acids, dose-response, DHA, EPA

Introduction

The intake of the long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) is associated with beneficial health effects (1, 2). Some of the physiological effects of n-3 PUFAs can be attributed to their oxidized products, i.e. eicosanoids and other oxylipins. These are formed in a cascade of reactions that is fairly well described for the n-6 PUFA arachidonic acid (20:4n-6, ARA). In these reactions, PUFAs can be converted via three enzymatic pathways (cyclooxygenase (COX), lipoxygenase (LOX) and Cytochrome P450 (CYP)) and via autoxidation (3, 4). n-3 PUFAs compete with ARA for conversion in these pathways which is a common mechanistic explanation of their physiological action. This can reduce the formation of ARA-derived pro-inflammatory eicosanoids such as PGE_2 and LTB_4 (2, 4). Moreover, enzymatic conversion of EPA and DHA yields highly potent lipid mediators. For example, CYP-catalyzed epoxidation leads to anti-arrhythmic acting 17(18)-epoxy eicosatetraenoic acid (EpETE) from EPA and 19(20)-epoxy docosapentaenoic acid (EpDPE) from DHA (5). Multiple hydroxylation leads to highly potent, specialized pro-resolving lipid mediators (SPMs) such as resolvins and protectins (6, 7).

Several human intervention studies describe changes with EPA+DHA supplementation in oxylipins formed across the different branches of the ARA cascade (COX, LOX, CYP and autoxidation) (8-18). As recently reviewed (19) these studies demonstrate strong elevation of (free and esterified) n-3 PUFA-derived oxylipins particularly of the CYP and LOX pathways (17(18)-EpETE, 19(20)-EpDPE, 17-HDHA) (8-10, 12, 13, 15-18) as well as 18-HEPE (8, 17, 20) in response to n3-PUFA supplementation. However, due to methodical differences as well as inter-individual variability these studies are difficult to compare and no information about the response to different doses of EPA+DHA can be deduced from these findings (19).

The dose response relationships regarding the incorporation of n-3 PUFAs into different blood lipids and cells are now fairly well characterized (21, 22). The question of whether the plasma

147 concentrations of oxylipins follow a similar dose-response is not clear. However, because of the
148 high biological activity of n-3 PUFA-derived oxylipins (1, 2, 4), knowing how these relate to
149 intake of EPA and DHA is fundamental for the understanding of n-3 PUFA physiology, health
150 effects and intake recommendations.

151 This study was primarily designed to elucidate the effect of different doses of EPA and DHA on
152 their incorporation into blood and tissue pools in healthy adult humans with low habitual
153 consumption of fatty fish, as reported elsewhere (21). The current research extends this to
154 investigate the dose-response effect of EPA+DHA intake on the plasma oxylipin pattern. Four
155 dose groups were selected to reflect EPA+DHA intake on a Western diet poor in EPA+DHA (no
156 additional EPA+DHA) and that reflecting intake of one, two or four servings of fatty cold water
157 fish per week. Effects on oxylipins were quantified in plasma after 3 and 12 months of
158 supplementation utilizing a state of the art comprehensive targeted oxylipin metabolomics
159 platform.

160

Subjects and Methods

The primary aim of the present study was to identify the dose and time effect of EPA and DHA incorporation into selected blood as well as tissue pools and the main findings have been published elsewhere (21). The new findings reported here (i.e. the investigation of the dose response effect of EPA and DHA supplementation on plasma oxylipin levels) are a (not predeclared) secondary aim of the study.

Subjects and Study Design

The effects of 12 months n-3 PUFA (EPA+DHA) supplementation on the blood oxylipin pattern were investigated using plasma samples derived from a double-blinded, randomized controlled intervention trial in healthy subjects aged 20 to 79 y. Study design, intervention, inclusion and exclusion criteria, randomization process and comprehensive baseline characteristics of the whole study population have been described in detail elsewhere (21). Briefly, the study participants received n-3 PUFA-triglycerides in capsules corresponding to doses of 0, 1, 2 or 4 meals of fatty fish per week, with one serving of fatty fish being equivalent to 3.27 g EPA+DHA (1:1.2, *wt:wt*). The control group received high oleic sunflower oil capsules. All procedures were approved by the Suffolk Local Research Ethics Committee (approval 05/Q0102/181). For the present study, a subset of 121 participants (60 male, 61 female) was selected out of the 128 who completed the study, based upon availability of plasma for all three time points to be investigated. Basic characteristics of the selected subset of subjects of the present study are presented in **Table 1**.

Oxylipin Analysis

Free (i.e. non-esterified) oxylipins in plasma were analyzed at baseline and after 3 and 12 months of supplementation. Analysis of oxylipins in plasma was conducted as described elsewhere (23, 24). In brief, after addition of internal standards (IS) and antioxidants, proteins were precipitated with methanol. Oxylipins were extracted using Bond Elut Certify II Cartridges

(Agilent, Waldbronn, Germany) and analyzed via liquid chromatography-mass spectrometry (LC-MS) following negative electrospray ionization in scheduled selected reaction monitoring (24) (see **Supplemental Table 1** for list of analytes). One sample (2 portions group, baseline, male) was excluded from data evaluation because of >70 fold higher concentrations for 5-LOX products compared to the mean of the groups and quality control samples. Thus, data are presented for 28 subjects (placebo group), 35 subjects (1 portion group), 29/28 subjects (2 portions group) and 29 subjects (4 portions group).

Study samples were prepared in five batches of 55-88 samples over a period of five weeks. Distribution of samples on 12-position-racks was as follows: 3-4 dose-randomized (blinded) study samples from each time point (baseline, 3 months and 12 months) along with one human quality control (QC) plasma which was prepared exactly as the study samples. Samples were analyzed by liquid chromatography-mass spectrometry (LC-MS) in the order they were prepared. Additionally, standards containing selected analytes and IS were measured periodically.

During and right after a batch, sample preparation and LC-MS performance were evaluated regarding retention time shift, areas and concentrations of analytes in standards as well as recovery of IS in study samples and QC (evaluation of IS according to Rund et al. using an IS2 (25) and based on absolute areas). Passing of laboratory internal limits was essential for the next batch to be prepared and analyzed. Analyte concentrations in QC samples were evaluated alongside study samples.

Mean IS recoveries in study samples over all batches were ≥65% and comparable to QC samples with low variability (**Supplemental Figure 1A**). Moreover, concentrations of analytes in QC samples were stable over the whole analysis interval (**Supplemental Figure 1B and Supplemental Table 2**).

Data Analysis and Statistics

Results are presented as indicated in the table and figure captions calculated with Microsoft Excel (Office 10, Microsoft, Redmond, WA, USA) or Prism (GraphPad Software, La Jolla, CA, USA). Concentrations were only calculated if the analyte exceeded the lower limit of quantification (LLOQ) in ≥50% of the samples in the group. Samples with a concentration below LLOQ were set to the concentration of ½ LLOQ. Relative changes in the study population after 3 and 12 months of supplementation were calculated from the individual data (e.g. **Figure 1**) or the group means (e.g. **Table 3**) against baseline using the formula rel change (%) = conc(t)/conc(t_0)*100. Linear regressions were calculated using Prism. For each correlation, the slope with standard error of the mean (SEM), the p value for a slope significantly different from zero and the regression coefficient r^2 (square of the Pearson correlation coefficient) are indicated in the figures.

Data were log transformed prior to statistical analysis in order to achieve normality. A general linear statistical model was used with repeated measures to investigate the change in oxylipin concentrations over the time course of the study, with time set as 'Within-Subject Factor' and n-3 PUFA dose as 'Between Subject Factor'; BMI and baseline plasma phosphatidylcholine (PC) EPA+DHA concentrations were used as covariates. Interactions between BMI and n-3 PUFA dose were set using 'interaction' analysis options within the model. A univariate model was used to investigate the effect of n-3 PUFA dose at each time point using baseline corrected data at 3 months and 12 months for each analyte and using BMI and baseline PC EPA+DHA concentrations as co-variates. Any significant findings for dose were then examined using a Tukey post-hoc test to determine the dose inducing an effect. P values were corrected for multiple analyses using Bonferroni correction analysis. P values ≤ 0.01 indicated statistical significance in the whole data group. Where the data were analysed separately for time point, P values ≤ 0.013 indicated significant differences. These statistics were calculated using SPSS version 24 (IBM, Armonk, NY, USA).

Results

In the present study, a comprehensive LC-MS based quantitative metabolomics platform was used allowing the sensitive, accurate and precise quantitation of 159 oxylipins (Supplemental Table 1). In plasma samples of the study population, 73 oxylipins from the precursor PUFAs linoleic acid (18:2n-6, LA), α-linolenic acid (18:3n-3, ALA), dihomo-γ-linolenic acid (20:3n-6, DGLA), ARA, EPA and DHA exceeded the limit of quantitation in at least one of the study groups in >50% of the samples and were included in the data evaluation. Median concentrations of all quantified oxylipins in all groups are shown in **Table 2**. There were no differences in oxylipin concentrations at baseline between the different treatment groups. Oxylipin concentrations were not related to BMI. Plasma PC EPA+DHA concentrations (reported previously (21)) did not influence the concentration of any oxylipin except 12-HETE ($p = 0.005$; **Supplemental Table 3**).

The plasma oxylipin pattern was modulated in a time- and dose-dependent manner following 3 and 12 months of supplementation with doses of n-3 PUFAs corresponding to 1, 2 and 4 fatty fish meals per week reaching statistical significance for many analytes (**Figures 1-4,** Table 2, **Supplemental Table 4-6**). Following 12 months of supplementation with the equivalent of four weekly servings of EPA and DHA, plasma concentrations of n-6 PUFA-derived hydroxy-PUFAs (from DGLA and ARA) and dihydroxy-PUFAs (from ARA) were decreased from baseline when compared to concentrations seen in the zero and one weekly serving group ($p < 0.001$), while concentrations of EPA- and DHA-derived epoxy-, hydroxy- and dihydroxy-PUFAs were increased from baseline ($p < 0.001$ for most oxylipins; Figure 1; Table 2, Supplemental Table 5-6). Relative and absolute changes in n-3 PUFA-derived oxylipins were higher compared to n-6 PUFA-derived oxylipins (Figure 1, **Table 3**). Regarding changes in n-3 PUFA-derived oxylipins, the relative increase in EPA-derived oxylipins was more pronounced than that of those produced from DHA (Figure 1, Table 3), although the change in (absolute) concentrations was higher for the DHA-derived metabolites (Table 2+3). The decrease/increase in oxylipins was greater in the

first three months of supplementation compared to the change between months 3 and 12 (Figure 2).

The subject's BMI was found to influence n-6 and n-3 dihydroxy-PUFAs to a limited extent; however, the n-6 derived products lost significance with further post-hoc testing while n-3 derived 16,17-DiHDPE and 19,20-DiHDPE were both found to have significantly lower concentrations in obese subjects when compared to normal weight subjects at 3 months (0.7 fold lower for 16,17-DiHDPE ($p = 0.012$); 1.6 fold lower for 19,20-DiHDPE ($p = 0.011$; Supplemental Table 6)) and there was a trend for lower 19,20-DiHDPE at 12 months ($p = 0.023$ after the Bonferroni correction).

After both intervention periods (i.e. 3 months and 12 months), the mean plasma concentrations of EPA- and DHA-derived oxylipins of the LOX and CYP pathways were increased linearly with the supplementation dose (Figure 3, **Supplemental Figure 5**). A higher modulation of the oxylipin profile following 12 months of supplementation is reflected in steeper slopes. Strong correlations were found for the means of n-3 PUFA-derived oxylipins with the relative content of EPA+DHA in plasma PC and red blood cells (reported in (21)) (**Figure 4 II**), although a strong variability in the correlation in individual concentrations was observed (**Figure 4 I**).

Overall, regardless their biochemical route of formation, plasma concentrations of epoxy-, hydroxy- and dihydroxy-PUFAs increased linearly according to the ingested n-3 PUFA dose which was more pronounced following longer supplementation (12 *vs* 3 months). Thus, n-3 PUFA-derived oxylipins followed the linear increase of their precursor fatty acid in plasma lipids (reported in (21)).

Discussion

n-3 PUFAs are important components of the diet and their beneficial health effects are well documented (1, 2). In a previous report we showed that EPA and DHA concentrations in blood lipids (e.g. plasma PC) and in blood cells (e.g. red blood cells) increase in a clear linear fashion with increasing intake of EPA and DHA (using intakes relevant to a Western diet with low, moderate or high EPA+DHA dose replicating different consumption patterns of fatty fish (21)). Using plasma samples from a subset of subjects from that study, we analyzed the oxidized metabolites of n-3 and n-6 PUFAs and showed that these respond to increased intake of EPA and DHA in a linear dose-response manner.

All supplemented n-3 PUFA doses led to an increase in EPA- and DHA-derived oxylipins in plasma (Figure1-4, Supplemental Table 4-6), consistent with earlier reports (19). Because of low initial EPA-oxylipin concentrations, the relative increase was much higher (about 300-500%) compared to DHA-derived oxylipins (about 200-300%) while for absolute concentrations, the trend was the opposite (Table 3).

It was earlier reported that the extent of the individual (relative) increase in EPA- and DHA-derived oxylipins was inversely associated with basal EPA and DHA content in erythrocytes, i.e. the lower the basal n-3 PUFA status, the higher the increase in corresponding oxylipins (9, 13). Similar trends have been observed for changes in the content of EPA+DHA in erythrocytes which has been suggested to be useful in the development of individually adjusted n-3 PUFA doses in the context of a potential therapeutic use (9, 26). In the present study, we found that the plasma n-3 PUFA concentrations in the different supplementation groups were similar between most subjects (Figure 3, Supplemental Figure 5), resulting in low variations in (absolute) concentrations of oxylipins. This indicates that low initial EPA- and DHA-oxylipin concentrations in plasma (resulting from low basal EPA/DHA status) are not predictive for the absolute plasma concentration that results from supplementation. Considering that the concentration – and not the increase – is mediating (dose dependent) physiological effects, the initial n-3 PUFA status

(or oxylipin concentration) seems to be less relevant in the investigation of the long term efficacy of n-3 PUFAs. Except for a few individuals, a similar long-term n-3 PUFA intake (dose) led to similar n-3 PUFA-derived plasma oxylipin concentrations. However, a small number of subjects (1-2 out of >25 per group) showed remarkably higher concentrations, for example in the 15-LOX and CYP metabolites (Figure 3). This might be a result of different activity in the enzymes involved in these subjects. Indeed, genetic variants have been described for the ALOX5 gene which could be associated with higher concentrations of its enzymatic products (27). Moreover, differences in PTGS1 and ALOX12 gene expression were correlated with differences in ARA-derived eicosanoid concentrations in response to n-3 PUFA supplementation (28) and the expression of multiple enzymes involved in ARA metabolism was changed differently in subjects who showed lowered triglycerides following n-3 PUFA supplementation in comparison to non-responders (29). Thus, the investigation of enzyme activities would be an important aspect for future studies to evaluate differences in the response to n-3 PUFA supplementation, for both clinical outcomes (e.g. hypotensive effect) as well as oxylipin concentrations.

Consistent with several previous studies we found that the CYP-derived epoxy-PUFA pathway is strongly affected by n-3 PUFA supplementation (8, 11). Moreover, the observed regio-selectivity, can be explained by the product and substrate specificity of epoxygenating CYP enzymes, such as the CYP2C and CYP2J families (5, 30). These enzymes preferably epoxygenate the n-3-double bond and some isoforms, especially CYP2J2, also show pronounced substrate preference: EPA>>DHA>ARA (5, 30). It should be noted that we recently showed in *in vitro* experiments a similar preference of 12/15-LOX (15-LOX-1) for n-3 PUFAs over ARA which was DHA>EPA>>ARA (31).

Regarding ARA-derived eicosanoids quantified in plasma of the study population, a slight decrease of up to 10-30% compared to baseline was observed for dihydroxy- and hydroxy-PUFAs (Table 2), consistent with previous studies supplementing similar doses of n-3 PUFAs (12, 16). A larger reduction could most likely be achieved by higher daily intakes of EPA+DHA,

as shown in studies using doses of 2.7-6.0 g/d (10, 13, 18), which are not easily achieved through diet or with most supplements. Moreover, a high n-6 PUFA background in the diet, as present in the Western diet (32), might have limited the effect of n-3 PUFA supplementation on ARA-derived eicosanoids (9). With a diet rich in fatty fish, providing similar n-3 PUFA doses as in this study, a larger decrease of ARA-derived eicosaonids could probably be reached by additionally decreasing n-6 PUFA intake through decreased intake of red meat.

In all groups receiving n-3 PUFAs, a shift away from ARA-derived epoxy-PUFAs to DHA- and EPA-derived ones was observed. This could be of physiological relevance because these epoxy-PUFAs share the anti-inflammatory action of the corresponding ARA oxylipins (33). Moreover, 19(20)-EpDPE has been shown to inhibit angiogenesis, while ARA-derived epoxy-PUFAs show opposing effects and thus may promote tumor growth (34). 17(18)EpETE, the main metabolite of CYP2J2 expressed in the heart (30), has high anti-arrhythmic potency (5) and may in part account for the prevention of sudden cardiac death by n-3 PUFAs. Of note, the decrease in the concentrations of epoxy-ARA is lower than the increase in concentrations of the EPA and DHA derived epoxy-PUFAs, resulting in a net increase in the total epoxy-PUFA concentration in the n-3 PUFA supplementation groups (Table 2). The increase in n-3 PUFA-derived oxylipins was much higher in the first three months compared to the following 9 months (Figure 2). Based on this, we assume that maximal plasma oxylipin concentrations for each n-3 PUFA dose are reached after 3 to 6 months of supplementation, following a similar time course as found for n-3 PUFA incorporation in most blood lipid pools (21). However, since only two time points were investigated and the time courses of the oxylipins differ, the details on the time dependent oxylipin modulation following n-3 PUFA supplementation remain to be fully evaluated.

Regarding the response of oxylipin concentrations with n-3 PUFA supplementation doses relevant for nutrition, we found after 3 and 12 months strong linear increases and no indication for saturation up to 13.08 g EPA+DHA/week (corresponding to 1.87 g EPA+DHA/day). This linear increase was observed for all EPA- and DHA-derived oxylipins covered by the analytical

method and which could be quantified in the samples (Figure 3, Supplemental Figure 5). These include oxylipins formed enzymatically, metabolites with no described enzymatic route of formation (e.g. 9-HEPE, 8-HDHA) as well as oxylipins with an unclear formation route (e.g. 18-HEPE). Consequently, the increase in the sum of metabolites from each chemical class (hydroxy-, dihydroxy-, and epoxy-PUFAs) was also linear with the dose of EPA+DHA. We showed a similar relationship between EPA and DHA incorporation in blood lipids (21). Particularly EPA and DHA content in plasma PC increased in a near-perfect linear fashion with doses of EPA+DHA corresponding to 1 to 4 meals fatty fish per week (21). Thus, the mean concentrations of free plasma oxylipins derived from EPA+DHA correlated strongly with the mean concentrations of EPA+DHA in plasma PC in the four supplementation groups (Figure 4). It should be noted that in the same dose range, the incorporation of EPA+DHA in erythrocyte membranes is saturated (quadratic increase with the dose (21), indicating a physiological homeostasis in the regulation of membrane composition and fluidity. Thus, the correlation between erythrocyte membrane content of PUFA and plasma oxylipin pattern – which has been described in previous studies (8, 13) – does not follow a linear trend at high n-3 PUFA supplementation doses as shown for the group receiving EPA+DHA equivalent to 4 portions of fatty fish per week (13.08 g EPA+DHA/week, **Figure 4B II**).

Taken together, the linear dose-response (in an n-3 PUFA dose range relevant for the Western diet) and the uniform increase in n-3 PUFA-derived oxylipins across different chemical classes (hydroxy-, dihydroxy-, and epoxy-PUFA) suggests that the plasma concentrations of free n-3 PUFA-derived oxylipins seem to depend largely on substrate availability. Thus, our findings show that for healthy human subjects on a Western diet the more n-3 PUFAs (i.e. EPA+DHA) consumed with the diet, the higher the plasma concentrations of EPA- and DHA-derived oxylipins.

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Acknowledgments

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The authors' responsibilities were as follows: LMB, SAJ and PCC designed the original study; ALW, LMB and CGW carried out the intervention under the supervision of SAJ and PCC; AIO, ALW, PCC and NHS designed the current research; AIO ALW and KS conducted the current research under the supervision of NHS; AIO and ALW analyzed data or performed statistical analysis; AIO, PCC and NHS wrote the paper; and AIO, PCC and NHS had primary responsibility for final content. All authors read and approved the final manuscript.

Tables

Table 1: Basic characteristics of the study population at baseline.[1]

	n-3 PUFA Equivalent to 0 Portions/Week[2] (n = 28)	n-3 PUFA Equivalent to 1 Portion/Week[2] (n = 35)	n-3 PUFA Equivalent to 2 Portions/Week[2] (n = 29)	n-3 PUFA Equivalent to 4 Portions/Week[2] (n = 29)
Sex (male/female)	14/14	17/18	14/15	15/14
BMI (kg/m^2)	25.8 ± 4.0	26.0 ± 4.2	25.6 ± 4.2	24.6 ± 3.1
Age (y)	51.1 ± 15.4	49.0 ± 16.6	49.9 ± 14.7	50.0 ± 15.4

[1] Data are shown as mean ± SD. Using one-way ANOVA, there were no statistically significant differences between groups for BMI (p = 0.958) or age (p = 0.490).

[2] n-3 PUFA equivalent of one portion fatty fish was 3.27 g EPA+DHA (1:1.2, (*wt:wt*)).

Table 2: Median concentrations (nmol/L) of oxylipins quantified in plasma of the study population at baseline and during the course of supplementation with different doses of EPA+DHA for 3 and 12 months. n-3 PUFA equivalent of one portion fatty fish was 3.27 g EPA+DHA (1:1.2, (*wt:wt*)).[1,2]

Baseline

			0 Portions	1 Portion	2 Portions	4 Portions
n-6 PUFA						
LA	Epoxy-PUFA	9(10)-EpOME	6.0 (4.8, 6.6)	5.3 (4.2, 6.7)	5.4 (4.7, 7.0)	5.3 (4.4, 7.1)
		12(13)-EpOME	8.6 (6.5, 10)	7.7 (6.2, 8.4)	8.1 (7, 9.6)	8.4 (7.3, 10)
	vic Dihydroxy-PUFA	9,10-DiHOME	3.8 (2.7, 5.3)	2.8 (2.5, 3.2)	2.9 (2.5, 4.4)	3.2 (2.6, 4)
		12,13-DiHOME	4.9 (3.9, 6.6)	4.5 (3.8, 4.8)	5.2 (3.9, 6.6)	5.4 (4, 6.7)
	Hydroxy-PUFA	9-HODE	15 (13, 17)	13 (11, 15)	15 (13, 16)	16 (14, 17)
		13-HODE	13 (11, 16)	11 (10, 13)	13 (9.8, 14)	12 (11, 17)
DGLA	Prostaglandins	13,14-dihydro-15-keto-PGE_1	< 0.05	< 0.05	< 0.05	< 0.05
	Hydroxy-PUFA	5-HETrE	0.092 (0.071, 0.13)	0.086 (0.064, 0.12)	0.097 (0.075, 0.13)	0.094 (0.06, 0.14)
		15-HETrE	0.37 (0.35, 0.43)	0.41 (0.35, 0.46)	0.4 (0.29, 0.5)	0.38 (0.33, 0.5)
ARA	Prostaglandins	13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.13 (0.11, 0.17)	0.12 (0.11, 0.14)	0.13 (0.098, 0.16)	0.13 (0.11, 0.15)
	Isoprostanes	5(*R,S*)-5-F_{2t}-IsoP	0.13 (0.11 0.14)	0.12 (0.10, 0.14)	0.13 (0.092, 0.15)	0.12 (0.097, 0.13)
	Epoxy-PUFA	5(6)-EpETrE	0.75 (0.65, 0.94)	0.90 (0.73, 1.1)	0.88 (0.60, 1.1)	0.74 (0.62, 1.1)
		8(9)-EpETrE	0.17 (0.15, 0.21)	0.19 (0.14, 0.22)	0.18 (0.11, 0.23)	0.19 (0.15, 0.22)
		11(12)-EpETrE	0.20 (0.16, 0.24)	0.21 (0.16, 0.23)	0.19 (0.14, 0.25)	0.18 (0.15, 0.21)
		14(15)-EpETrE	0.43 (0.36, 0.51)	0.44 (0.38, 0.53)	0.44 (0.30, 0.53)	0.42 (0.34, 0.45)
	vic Dihydroxy-PUFA	5,6-DiHETrE	0.28 (0.22, 0.34)	0.28 (0.20, 0.33)	0.30 (0.21, 0.38)	0.30 (0.21, 0.38)
		8,9-DiHETrE	0.20 (0.18, 0.25)	0.23 (0.20, 0.26)	0.23 (0.17, 0.30)	0.22 (0.17, 0.24)
		11,12-DiHETrE	0.50 (0.46, 0.63)	0.54 (0.52, 0.64)	0.52 (0.46, 0.69)	0.54 (0.47, 0.60)
		14,15-DiHETrE	0.60 (0.52 , 0.73)	0.66 (0.61, 0.73)	0.59 (0.53, 0.75)	0.60 (0.57, 0.72)
	Hydroxy-PUFA	5-HETE	1.2 (0.98 , 1.5)	1.2 (1.1, 1.4)	1.4 (1.2, 1.7)	1.3 (1.0, 1.7)
		8-HETE	0.29 (0.26, 0.32)	0.31 (0.25, 0.38)	0.30 (0.25, 0.35)	0.30 (0.27, 0.35)
		11-HETE	0.25 (0.22, 0.31)	0.28 (0.21, 0.31)	0.25 (0.20, 0.29)	0.25 (0.20, 0.30)
		12-HETE	1.5 (1.0, 2.0)	1.7 (1.2, 2.4)	1.8 (1.5, 3.1)	1.6 (1.1, 2.2)
		15-HETE	0.95 (0.87, 1.2)	1.0 (0.89, 1.1)	0.97 (0.75, 1.1)	0.96 (0.73, 1.3)
		19-HETE	< 1.0	1.2 (0.50, 1.4)	1.1 (0.50, 1.3)	< 1.0
		20-HETE	0.81 (0.66, 1.1)	0.84 (0.72, 1.1)	0.84 (0.68, 1.2)	0.75 (0.71, 1.0)
		12-HHTrE	0.11 (0.093, 0.15)	0.14 (0.10, 1.17)	0.16 (0.096, 0.33)	0.12 (0.083, 0.19)
n-3 PUFA						
ALA	Epoxy-PUFA	9(10)-EpODE	0.59 (0.44, 0.73)	0.51 (0.40, 0.67)	0.52 (0.40, 0.75)	0.57 (0.41, 0.69)
		12(13)-EpODE	0.35 (0.28, 0.47)	0.31 (0.27, 0.36)	0.32 (0.23, 0.44)	0.33 (0.29, 0.40)
		15(16)-EpODE	3.8 (2.7, 4.1)	2.4 (2.0, 3.1)	3.3 (2.2, 4.5)	3.9 (3.1, 4.8)
	vic Dihydroxy-PUFA	9,10-DiHODE	0.25 (0.22, 0.29)	0.22 (0.19, 0.25)	0.27 (0.23, 0.33)	0.27 (0.22 0.34)
		12,13-DiHODE	< 0.1	< 0.1	< 0.1	0.12 (0.050, 0.14)
		15,16-DiHODE	8.7 (7.7, 9.8)	8.1 (6.5, 8.5)	9.1 (6.7, 9.8)	10 (6.9, 12)
	Hydroxy-PUFA	9-HOTrE	0.64 (0.57, 0.71)	0.51 (0.45, 0.62)	0.65 (0.55, 0.66)	0.66 (0.56, 0.76)
		13-HOTrE	0.53 (0.45, 0.68)	0.5 (0.43, 0.58)	0.53 (0.41, 0.59)	0.57 (0.48, 0.63)
EPA	Epoxy-PUFA	11(12)-EpETE	< 0.05	< 0.05	< 0.05	< 0.05
		14(15)-EpETE	0.061 (0.051, 0.089)	0.060 (0.050, 0.089)	0.069 (0.052, 0.082)	0.068 (0.051, 0.082)
		17(18)-EpETE	< 0.1	< 0.1	< 0.1	< 0.1
	vic Dihydroxy-PUFA	8,9-DiHETE	0.081 (0.053, 0.093)	0.086 (0.073, 0.095)	0.083 (0.066, 0.10)	0.083 (0.065, 0.10)
		11,12-DiHETE	0.052 (0.039, 0.064)	0.057 (0.046, 0.066)	0.054 (0.043, 0.060)	0.050 (0.042, 0.062)
		14,15-DiHETE	0.087 (0.067, 0.10)	0.096 (0.085, 0.11)	0.086 (0.068, 0.10)	0.090 (0.077, 0.11)
		17,18-DiHETE	0.58 (0.39, 0.71)	0.59 (0.55, 0.69)	0.57 (0.48, 0.81)	0.61 (0.56, 0.71)
	Hydroxy-PUFA	5-HEPE	0.35 (0.22, 0.46)	0.32 (0.24, 0.36)	0.36 (0.30, 0.39)	0.33 (0.26, 0.44)
		8-HEPE	0.079 (0.064, 0.11)	0.079 (0.068, 0.086)	0.076 (0.07, 0.085)	0.084 (0.069, 0.10)
		9-HEPE	0.19 (0.17, 0.30)	0.21 (0.18, 0.24)	0.19 (0.16, 0.24)	0.23 (0.18, 0.27)
		11-HEPE	0.11 (0.08, 0.14)	0.082 (0.064, 0.12)	0.097 (0.075, 0.13)	0.098 (0.078, 0.14)
		12-HEPE	0.21 (0.13, 0.32)	0.21 (0.15, 0.36)	0.25 (0.15, 0.35)	0.26 (0.16, 0.46)
		15-HEPE	0.13 (0.063, 0.16)	0.13 (0.063, 0.17)	< 0.13	0.13 (0.063, 0.20)
		18-HEPE	0.19 (0.14, 0.22)	0.17 (0.16, 0.21)	0.18 (0.15, 0.21)	0.19 (0.15, 0.25)
		19-HEPE	0.84 (0.65, 1.0)	0.93 (0.67, 1.1)	0.93 (0.61, 1.2)	0.97 (0.62, 1.3)
		20-HEPE	0.59 (0.37, 0.82)	0.54 (0.40, 0.74)	0.52 (0.43, 0.71)	0.54 (0.43, 0.72)
DHA	Epoxy-PUFA	10(11)-EpDPE	0.26 (0.23, 0.32)	0.29 (0.22, 0.39)	0.24 (0.22, 0.34)	0.30 (0.23, 0.35)
		13(14)-EpDPE	0.21 (0.19, 0.32)	0.23 (0.19, 0.29)	0.23 (0.18, 0.26)	0.23 (0.18, 0.29)

		16(17)-EpDPE	0.24 (0.21, 0.33)	0.25 (0.21, 0.31)	0.25 (0.21, 0.28)	0.24 (0.20, 0.28)
		19(20)-EpDPE	0.41 (0.34, 0.50)	0.42 (0.36, 0.58)	0.41 (0.33, 0.56)	0.45 (0.34, 0.55)
	vic Dihydroxy-PUFA	4,5-DiHDPE	1.2 (0.87, 1.5)	1.3 (1.2, 1.5)	1.3 (1.1, 1.6)	1.3 (1.1, 1.7)
		7,8-DiHDPE	< 0.1	< 0.1	< 0.1	0.10 (0.05, 0.13)
		10,11-DiHDPE	0.19 (0.15, 0.28)	0.21 (0.18, 0.25)	0.19 (0.16, 0.23)	0.23 (0.15, 0.29)
		13,14-DiHDPE	0.21 (0.18, 0.29)	0.24 (0.22, 0.28)	0.23 (0.19, 0.24)	0.25 (0.22, 0.30)
		16,17-DiHDPE	0.28 (0.22, 0.33)	0.31 (0.27 , 0.36)	0.29 (0.25, 0.33)	0.32 (0.27, 0.36)
		19,20-DiHDPE	2.4 (2.1, 3.4)	3.0 (2.5, 3.3)	2.9 (2.3, 3.7)	3.1 (2.5, 3.5)
	Hydroxy-PUFA	4-HDHA	0.43 (0.29, 0.52)	0.38 (0.32, 0.47)	0.43 (0.34, 0.52)	0.47 (0.38, 0.53)
		7-HDHA	< 0.1	< 0.1	< 0.1	< 0.1
		8-HDHA	0.48 (0.31, 0.66)	0.45 (0.36, 0.52)	0.42 (0.36, 0.47)	0.48 (0.36, 0.58)
		10-HDHA	0.11 (0.091, 0.15)	0.12 (0.093, 0.14)	0.10 (0.086, 0.14)	0.11 (0.088, 0.13)
		11-HDHA	0.19 (0.16, 0.24)	0.23 (0.17, 0.24)	0.19 (0.17, 0.23)	0.22 (0.16, 0.29)
		13-HDHA	0.091 (0.086, 0.12)	0.10 (0.075, 0.12)	0.089 (0.076, 0.12)	0.10 (0.080, 0.12)
		14-HDHA	0.88 (0.51, 1.1)	0.91 (0.49, 1.5)	0.91 (0.58, 1.3)	1.1 (0.60, 1.6)
		16-HDHA	0.16 (0.12, 0.20)	0.15 (0.14, 0.18)	0.14 (0.13, 0.18)	0.15 (0.14, 0.19)
		17-HDHA	0.87 (0.75, 1.0)	0.91 (0.76, 1.1)	0.75 (0.68, 0.93)	0.93 (0.62, 1.2)
		20-HDHA	0.42 (0.30, 0.52)	0.42 (0.38, 0.47)	0.38 (0.34, 0.43)	0.41 (0.35, 0.53)
		21-HDHA	2.8 (2.1, 3.6)	2.8 (2.5, 3.7)	2.9 (2.4, 3.4)	3.2 (2.1, 4.0)
		22-HDHA	2.4 (1.8, 3.4)	2.3 (2.0, 3.2)	2.4 (1.8, 2.8)	2.4 (1.7, 3.3)

3 Months

			0 Portions	1 Portion	2 Portions	4 Portions
n-6 PUFA						
LA	Epoxy-PUFA	9(10)-EpOME	5.8 (4.5, 7.3)	4.7 (3.8, 5.6)	5.7 (4.2, 6.9)	5.3 (4.0, 6.9)
		12(13)-EpOME	9.2 (7.1, 11)	7.1 (5.5, 9.1)	8.3 (6.3, 11)	7.5 (6.0, 9.5)
	vic Dihydroxy-PUFA	9,10-DiHOME	4.0 (2.7, 5.2)	3.2 (2.5, 4.4)	3.8 (2.5, 5.7)	3.2 (2.3, 4.1)
		12,13-DiHOME	5.5 (4.0, 6.1)	4.8 (3.4, 5.9)	4.7 (4.0, 5.6)	4.7 (4.0, 5.8)
	Hydroxy-PUFA	9-HODE	17 (13, 21)	15 (14, 17)	14 (12, 18)	16 (13, 17)
		13-HODE	14 (11, 17)	12 (11, 14)	13 (11, 16)	12 (10, 15)
DGLA	Prostaglandins	13,14-dihydro-15-keto-PGE_1	< 0.05	< 0.05	< 0.05	0.11 (0.025, 0.14)
	Hydroxy-PUFA	5-HETrE	0.07 (0.058, 0.11)	0.00 (0.063, 0.13)	0.068 (0.051, 0.10)	0.048 (0.036, 0.078)
		15-HETrE	0.38 (0.34, 0.41)	0.38 (0.33, 0.44)	0.35 (0.27, 0.48)	0.37 (0.30, 0.42)
ARA	Prostaglandins	13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.13 (0.12, 0.15)	0.13 (0.12, 0.15)	0.12 (0.093, 0.16)	0.12 (0.09, 0.14)
	Isoprostanes	5(*R,S*)-5-F_{2t}-IsoP	0.12 (0.10, 0.14)	0.13 (0.11, 0.14)	0.10 (0.082, 0.13)	0.093 (0.073, 0.12)
	Epoxy-PUFA	5(6)-EpETrE	0.8 (0.66, 0.93)	0.68 (0.59, 0.80)	0.72 (0.59, 0.92)	0.83 (0.70, 1.1)
		8(9)-EpETrE	0.15 (0.12, 0.22)	0.13 (0.12, 0.18)	0.16 (0.14, 0.22)	0.15 (0.12, 0.22)
		11(12)-EpETrE	0.18 (0.13, 0.20)	0.15 (0.13, 0.20)	0.17 (0.15, 0.21)	0.17 (0.14, 0.22)
		14(15)-EpETrE	0.37 (0.27, 0.44)	0.35 (0.30, 0.44)	0.40 (0.35, 0.50)	0.37 (0.31, 0.45)
	vic Dihydroxy-PUFA	5,6-DiHETrE	0.23 (0.20, 0.31)	0.26 (0.20, 0.29)	0.26 (0.20, 0.30)	0.22 (0.20, 0.28)
		8,9-DiHETrE	0.20 (0.15, 0.25)	0.20 (0.17, 0.24)	0.19 (0.16, 0.25)	0.17 (0.15, 0.21)
		11,12-DiHETrE	0.48 (0.44, 0.64)	0.46 (0.43, 0.52)	0.48 (0.36, 0.58)	0.40 (0.37, 0.48)
		14,15-DiHETrE	0.62 (0.53, 0.72)	0.57 (0.51, 0.64)	0.54 (0.45, 0.69)	0.51 (0.43, 0.55)
	Hydroxy-PUFA	5-HETE	1.2 (0.83, 1.3)	1.1 (0.98, 1.4)	1.2 (0.84, 1.4)	1.1 (0.86, 1.2)
		8-HETE	0.28 (0.25, 0.32)	0.29 (0.25, 0.36)	0.29 (0.27, 0.34)	0.28 (0.24, 0.33)
		11-HETE	0.21 (0.18, 0.24)	0.25 (0.21, 0.30)	0.23 (0.20, 0.28)	0.23 (0.20, 0.27)
		12-HETE	1.4 (0.98, 2.0)	1.7 (1.2, 2.9)	2.4 (1.8, 3.6)	1.4 (1.2, 2.1)
		15-HETE	0.89 (0.72, 1.1)	0.94 (0.90, 0.99)	0.79 (0.67, 1.2)	0.86 (0.69, 0.92)
		19-HETE	< 1.0	1.0 (0.50, 1.1)	1.0 (0.50, 1.4)	< 1.0
		20-HETE	0.83 (0.58, 1.1)	0.74 (0.70, 0.80)	0.82 (0.54, 1.0)	0.71 (0.60, 1.1)
		12-HHTrE	0.13 (0.094, 0.21)	0.12 (0.10, 0.19)	0.18 (0.11, 0.26)	0.12 (0.086, 0.19)
n-3 PUFA						
ALA	Epoxy-PUFA	9(10)-EpODE	0.53 (0.39, 0.82)	0.42 (0.33, 0.48)	0.52 (0.31, 0.68)	0.54 (0.41, 0.75)
		12(13)-EpODE	0.38 (0.28, 0.43)	0.26 (0.22, 0.34)	0.34 (0.25, 0.41)	0.36 (0.27, 0.43)
		15(16)-EpODE	3.4 (2.8, 4.0)	2.9 (2.0, 3.4)	3.3 (2.0, 3.8)	3.7 (3.2, 4.2)
	vic Dihydroxy-PUFA	9,10-DiHODE	0.24 (0.22, 0.27)	0.22 (0.19, 0.25)	0.29 (0.21, 0.33)	0.24 (0.22, 0.30)
		12,13-DiHODE	< 0.1	< 0.1	< 0.1	0.11 (0.050, 0.15)
		15,16-DiHODE	8.8 (8.0, 9.7)	8.4 (7.0, 10)	8.1 (6.2, 9.8)	9.0 (7.8, 11)
	Hydroxy-PUFA	9-HOTrE	0.59 (0.48, 0.84)	0.57 (0.51, 0.63)	0.58 (0.45, 0.67)	0.62 (0.55, 0.81)
		13-HOTrE	0.59 (0.46, 0.66)	0.48 (0.42, 0.62)	0.51 (0.44, 0.70)	0.60 (0.47, 0.66)
EPA	Epoxy-PUFA	11(12)-EpETE	< 0.05	< 0.05	0.066 (0.025, 0.088)	0.13 (0.095, 0.15)
		14(15)-EpETE	< 0.05	0.087 (0.071, 0.11)	0.12 (0.094, 0.14)	0.18 (0.15, 0.21
		17(18)-EpETE	< 0.1	< 0.1	0.15 (0.11, 0.20)	0.24 (0.20, 0.29)
	vic Dihydroxy-PUFA	8,9-DiHETE	0.066 (0.052, 0.085)	0.098 (0.086, 0.12)	0.11 (0.085, 0.15)	0.18 (0.15, 0.25)
		11,12-DiHETE	0.041 (0.039, 0.049)	0.068 (0.061, 0.083)	0.081 (0.067, 0.095	0.13 (0.10, 0.15)
		14,15-DiHETE	0.078 (0.067, 0.090)	0.12 (0.10, 0.14)	0.13 (0.12, 0.16)	0.22 (0.19, 0.23)

		17,18-DiHETE	0.49 (0.44, 0.68)	0.78 (0.70, 0.94)	0.90 (0.78, 1.3)	1.4 (1.1, 1.5)
	Hydroxy-PUFA	5-HEPE	0.21 (0.19, 0.29)	0.42 (0.34, 0.52)	0.48 (0.37, 0.59)	0.73 (0.54, 0.94)
		8-HEPE	0.068 (0.031, 0.081)	0.11 (0.097, 0.13)	0.12 (0.10, 0.15)	0.22 (0.16, 0.29)
		9-HEPE	0.17 (0.14, 0.21)	0.27 (0.25, 0.32)	0.34 (0.26, 0.43)	0.56 (0.48, 0.74)
		11-HEPE	0.078 (0.052, 0.12)	0.16 (0.13, 0.19)	0.18 (0.13, 0.24)	0.27 (0.22, 0.37)
		12-HEPE	0.15 (0.11, 0.26)	0.32 (0.23, 0.54)	0.54 (0.42, 0.73)	0.58 (0.46, 0.79)
		15-HEPE	< 0.13	0.17 (0.14, 0.20)	0.22 (0.14, 0.26)	0.32 (0.23, 0.36)
		18-HEPE	0.15 (0.13, 0.19)	0.28 (0.22, 0.35)	0.31 (0.26, 0.47)	0.52 (0.48, 0.73)
		19-HEPE	0.68 (0.55, 1.0)	1.2 (1.0, 1.4)	1.4 (1.1, 2.0)	2.1 (1.6, 2.8)
		20-HEPE	0.56 (0.35, 0.72)	0.68 (0.56, 0.87)	0.90 (0.60, 1.2)	1.4 (1.3, 1.7)
DHA	Epoxy-PUFA	10(11)-EpDPE	0.20 (0.14, 0.30)	0.27 (0.23, 0.33)	0.40 (0.27, 0.46)	0.49 (0.36, 0.62)
		13(14)-EpDPE	0.18 (0.12, 0.23)	0.24 (0.18, 0.27)	0.32 (0.25, 0.39)	0.47 (0.33, 0.50)
		16(17)-EpDPE	0.20 (0.13, 0.23)	0.26 (0.21, 0.29)	0.33 (0.27, 0.41)	0.44 (0.34, 0.51)
		19(20)-EpDPE	0.33 (0.25, 0.45)	0.42 (0.37, 0.52)	0.60 (0.41, 0.73)	0.78 (0.54, 0.89)
	vic Dihydroxy-PUFA	4,5-DiHDPE	0.94 (0.70, 1.3)	1.5 (1.2, 1.7)	1.6 (1.3, 2.2)	2.2 (1.8, 2.5)
		7,8-DiHDPE	< 0.1	0.11 (0.050, 0.13)	0.13 (0.050, 0.18)	0.16 (0.12, 0.20)
		10,11-DiHDPE	0.17 (0.12, 0.24)	0.21 (0.18, 0.28)	0.24 (0.20, 0.32)	0.34 (0.26, 0.42)
		13,14-DiHDPE	0.22 (0.16, 0.24)	0.28 (0.23, 0.30)	0.33 (0.23, 0.37)	0.38 (0.32, 0.43)
		16,17-DiHDPE	0.28 (0.20, 0.33)	0.36 (0.28, 0.39)	0.40 (0.30, 0.46)	0.45 (0.42, 0.51)
		19,20-DiHDPE	2.8 (1.7, 3.2)	3.2 (2.7, 3.8)	3.6 (3.2, 4.6)	4.1 (3.6, 5.3)
	Hydroxy-PUFA	4-HDHA	0.30 (0.26, 0.41)	0.45 (0.42, 0.51)	0.54 (0.44, 0.64)	0.69 (0.61, 0.88)
		7-HDHA	< 0.1	0.11 (0.05, 0.12)	0.12 (0.05, 0.15)	0.17 (0.14, 0.21)
		8-HDHA	0.30 (0.25, 0.50)	0.56 (0.54, 0.61)	0.64 (0.51, 0.81)	0.99 (0.80, 1.1)
		10-HDHA	0.086 (0.071, 0.11)	0.13 (0.12, 0.16)	0.17 (0.13, 0.21)	0.25 (0.17, 0.28)
		11-HDHA	0.17 (0.12, 0.22)	0.22 (0.20, 0.3)	0.31 (0.24, 0.40)	0.36 (0.28, 0.38)
		13-HDHA	0.078 (0.061, 0.11)	0.11 (0.10, 0.13)	0.14 (0.12, 0.18)	0.19 (0.15, 0.23)
		14-HDHA	0.60 (0.41, 1.2)	1.1 (0.81, 1.8)	1.9 (0.95, 2.3)	1.5 (1.2, 2.1)
		16-HDHA	0.14 (0.11, 0.18)	0.18 (0.16, 0.23)	0.22 (0.19, 0.27)	0.28 (0.24, 0.38)
		17-HDHA	0.70 (0.54, 0.85)	0.96 (0.83, 1.1)	1.1 (0.81, 1.5)	1.5 (1.2, 1.7)
		20-HDHA	0.34 (0.28, 0.44)	0.44 (0.42, 0.51)	0.59 (0.47, 0.71)	0.69 (0.55, 0.82)
		21-HDHA	2.5 (1.9, 3.4)	3.5 (2.6, 4.0)	4.2 (3.3, 5.2)	4.7 (4.1, 6.2)
		22-HDHA	2.0 (1.5, 2.6)	2.8 (2.2, 3.2)	3.4 (2.7, 4.8)	4.2 (3.7, 5.4)

12 Months

			0 Portions	1 Portion	2 Portions	4 Portions
n-6 PUFA						
LA	Epoxy-PUFA	9(10)-EpOME	5.7 (4.2, 6.3)	5.6 (4.6, 6.9)	6.1 (4.6, 6.8)	5.9 (4.6, 6.9)
		12(13)-EpOME	8.0 (7.1, 10)	8.8 (7.0, 10)	8.9 (6.9, 10)	8.7 (7.2, 11)
	vic Dihydroxy-PUFA	9,10-DiHOME	4 (3.2, 4.7)		3.5 (2.8, 4.6)	3.5 (2.6, 4.9)
		12,13-DiHOME	5.2 (4.2, 6.8)	4.8 (4.2, 5.5)	5.2 (4.2, 6.4)	5.3 (4.3, 6.5)
	Hydroxy-PUFA	9-HODE	16 (15, 19)	15 (14, 17)	17 (14, 19)	15 (14, 16)
		13-HODE	14 (13, 17)	12 (11, 15)	13 (11, 15)	12 (11, 14)
DGLA	Prostaglandins	13,14-dihydro-15-keto-PGE_1	< 0.05	< 0.05	< 0.05	0.075 (0.051, 0.12)
	Hydroxy-PUFA	5-HETrE	0.075 (0.048, 0.093)	0.076 (0.058, 0.092)	0.070 (0.043, 0.090)	0.046 (0.038, 0.052)
		15-HETrE	0.35 (0.31, 0.36)	0.40 (0.34, 0.45)	0.34 (0.30, 0.43)	0.34 (0.29, 0.43)
ARA	Prostaglandins	13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.13 (0.11, 0.14)	0.13 (0.10, 0.15)	0.11 (0.092, 0.15)	0.11 (0.076, 0.13)
	Isoprostanes	5(*R,S*)-5-F_{2t}-IsoP	0.11 (0.09, 0.12)	0.10 (0.097, 0.12)	0.096 (0.079, 0.11)	0.098 (0.081, 0.11)
	Epoxy-PUFA	5(6)-EpETrE	0.84 (0.62, 1.0)	0.77 (0.62, 0.97)	0.70 (0.62, 0.95)	0.74 (0.60, 0.94)
		8(9)-EpETrE	0.18 (0.13, 0.21)	0.18 (0.14, 0.21)	0.17 (0.13, 0.19)	0.17 (0.15, 0.20)
		11(12)-EpETrE	0.19 (0.16, 0.21)	0.16 (0.14, 0.20)	0.18 (0.15, 0.21)	0.18 (0.16, 0.20)
		14(15)-EpETrE	0.40 (0.35, 0.50)	0.37 (0.33, 0.41)	0.40 (0.36, 0.47)	0.41 (0.34, 0.47)
	vic Dihydroxy-PUFA	5,6-DiHETrE	0.26 (0.21, 0.32)	0.24 (0.22, 0.30)	0.28 (0.16, 0.34)	0.23 (0.17, 0.27)
		8,9-DiHETrE	0.20 (0.16, 0.25)	0.19 (0.18, 0.22)	0.19 (0.14, 0.25)	0.17 (0.14, 0.19)
		11,12-DiHETrE	0.49 (0.44, 0.56)	0.45 (0.44, 0.53)	0.43 (0.36, 0.55)	0.40 (0.35, 0.45)
		14,15-DiHETrE	0.60 (0.54, 0.67)	0.56 (0.53, 0.63)	0.54 (0.43, 0.66)	0.48 (0.45, 0.53)
	Hydroxy-PUFA	5-HETE	1.0 (0.84, 1.3)	1.1 (0.95, 1.2)	1.1 (0.9,0 1.3)	1.0 (0.83, 1.2)
		8-HETE	0.29 (0.26, 0.34)	0.29 (0.26, 0.33)	0.27 (0.24, 0.32)	0.27 (0.25, 0.32)
		11-HETE	0.22 (0.21, 0.26)	0.24 (0.20, 0.32)	0.23 (0.19, 0.26)	0.20 (0.18, 0.24)
		12-HETE	1.2 (0.87, 2.0)	1.6 (1.1, 2.3)	1.6 (1.2, 2.6)	1.1 (0.91, 1.3)
		15-HETE	0.8 (0.77, 0.90)	0.87 (0.78, 0.98)	0.77 (0.67, 1.0)	0.73 (0.60, 0.79)
		19-HETE	1.0 (0.50, 1.3)	1.0 (0.50, 1.2)	< 1.0	< 1.0
		20-HETE	0.78 (0.70, 1.0)	0.75 (0.67, 0.84)	0.75 (0.58, 1.0)	0.79 (0.63, 0.88)
		12-HHTrE	0.084 (0.072, 0.12)	0.095 (0.06, 0.13)	0.075 (0.065, 0.11)	0.077 (0.025, 0.11)
n-3 PUFA						
ALA	Epoxy-PUFA	9(10)-EpODE	0.58 (0.48, 0.66)	0.59 (0.50, 0.71)	0.54 (0.43, 0.77)	0.62 (0.47, 0.73)
		12(13)-EpODE	0.38 (0.29, 0.47)	0.36 (0.29, 0.45)	0.33 (0.28, 0.46)	0.41 (0.34, 0.47)

		15(16)-EpODE	3.6 (3.0, 4.1)	3.1 (2.7, 4.1)	3.8 (2.4, 4.7)	4.6 (3.3, 5.1)
	vic Dihydroxy-PUFA	9,10-DiHODE	0.26 (0.24, 0.29)	0.27 (0.22, 0.30)	0.28 (0.24, 0.35)	0.27 (0.24, 0.33)
		12,13-DiHODE	0.11 (0.050, 0.14)	< 0.1	< 0.1	0.12 (0.050, 0.15)
		15,16-DiHODE	9.7 (8.2, 13)	9.2 (8.0, 12)	8.7 (7.0, 11)	10 (9.0, 12)
	Hydroxy-PUFA	9-HOTrE	0.67 (0.54, 0.78)	0.57 (0.50, 0.73)	0.65 (0.48, 0.82)	0.70 (0.58, 0.75)
		13-HOTrE	0.51 (0.49, 0.60)	0.53 (0.44, 0.63)	0.55 (0.43, 0.64)	0.52 (0.47, 0.63)
EPA	Epoxy-PUFA	11(12)-EpETE	< 0.05	0.052 (0.025, 0.069)	0.088 (0.07, 0.11)	0.17 (0.13, 0.19)
		14(15)-EpETE	0.063 (0.053, 0.080)	0.088 (0.082, 0.11)	0.14 (0.12, 0.17)	0.25 (0.21, 0.32)
		17(18)-EpETE	< 0.1	< 0.1	0.18 (0.15, 0.23)	0.32 (0.26, 0.41)
	vic Dihydroxy-PUFA	8,9-DiHETE	0.064 (0.056, 0.072)	0.099 (0.085, 0.12)	0.13 (0.11, 0.14)	0.19 (0.15, 0.26)
		11,12-DiHETE	0.045 (0.036, 0.052)	0.066 (0.059, 0.079)	0.097 (0.082, 0.10)	0.15 (0.12, 0.21)
		14,15-DiHETE	0.077 (0.068, 0.091)	0.12 (0.099, 0.13)	0.17 (0.13, 0.19)	0.23 (0.20, 0.30)
		17,18-DiHETE	0.53 (0.42, 0.72)	0.76 (0.66, 0.91)	1.1 (0.83, 1.4)	1.4 (1.2, 1.9)
	Hydroxy-PUFA	5-HEPE	0.24 (0.20, 0.31)	0.39 (0.35, 0.49)	0.61 (0.46, 0.81)	0.97 (0.77, 1.4)
		8-HEPE	0.068 (0.031, 0.083)	0.12 (0.10, 0.14)	0.16 (0.14, 0.24)	0.27 (0.23, 0.70)
		9-HEPE	0.18 (0.14, 0.23)	0.30 (0.26, 0.34)	0.48 (0.35, 0.58)	0.77 (0.61, 1.1)
		11-HEPE	0.088 (0.025, 0.12)	0.17 (0.11, 0.21)	0.22 (0.18, 0.28)	0.35 (0.32, 0.66)
		12-HEPE	0.17 (0.098, 0.26)	0.28 (0.21, 0.42)	0.59 (0.38, 1.0)	0.62 (0.42, 0.80)
		15-HEPE	< 0.13		0.17 (0.16, 0.21)	0.21 (0.17, 0.30)
		18-HEPE	0.15 (0.12, 0.20)	0.26 (0.22, 0.33)	0.38 (0.30, 0.47)	0.68 (0.59, 0.90)
		19-HEPE	0.70 (0.54, 1.0)	1.2 (0.91, 1.4)	1.5 (1.2, 2.2)	2.3 (2.0, 2.6)
		20-HEPE	0.46 (0.32, 0.64)	0.74 (0.59, 0.84)	1.1 (0.84, 1.4)	1.7 (1.3, 1.9)
DHA	Epoxy-PUFA	10(11)-EpDPE	0.21 (0.17, 0.27)	0.29 (0.23, 0.37)	0.39 (0.33, 0.47)	0.65 (0.51, 0.72)
		13(14)-EpDPE	0.19 (0.12, 0.27)	0.26 (0.20, 0.31)	0.38 (0.27, 0.43)	0.56 (0.42, 0.65)
		16(17)-EpDPE	0.22 (0.14, 0.28)	0.26 (0.21, 0.34)	0.36 (0.30, 0.46)	0.55 (0.44, 0.69)
		19(20)-EpDPE	0.33 (0.28, 0.47)	0.46 (0.40, 0.53)	0.63 (0.52, 0.72)	1.0 (0.87, 1.1)
	vic Dihydroxy-PUFA	4,5-DiHDPE	0.93 (0.75, 1.3)	1.4 (1.2, 1.6)	1.9 (1.8, 2.3)	2.4 (1.8, 2.9)
		7,8-DiHDPE	< 0.1	< 0.1	0.14 (0.05, 0.17)	0.19 (0.15, 0.21)
		10,11-DiHDPE	0.17 (0.12, 0.22)	0.21 (0.18, 0.28)	0.27 (0.23, 0.32)	0.37 (0.33, 0.41)
		13,14-DiHDPE	0.20 (0.16, 0.22)	0.26 (0.22, 0.33)	0.33 (0.29, 0.39)	0.42 (0.37, 0.50)
		16,17-DiHDPE	0.26 (0.23, 0.30)	0.32 (0.29, 0.36)	0.41 (0.37, 0.52)	0.49 (0.44, 0.57)
		19,20-DiHDPE	2.4 (1.9, 2.6)	3.1 (2.7, 3.8)	4.2 (3.3, 5.3)	4.9 (4.2, 5.2)
	Hydroxy-PUFA	4-HDHA	0.32 (0.28, 0.42)	0.48 (0.43, 0.52)	0.63 (0.55, 0.86)	0.95 (0.78, 1.1)
		7-HDHA	< 0.1	0.11 (0.05, 0.14)	0.14 (0.12, 0.19)	0.25 (0.20, 0.31)
		8-HDHA	0.40 (0.30, 0.52)	0.56 (0.49, 0.75)	0.82 (0.68, 0.94)	1.3 (1.1, 1.8)
		10-HDHA	0.10 (0.086, 0.13)	0.14 (0.12, 0.17)	0.2 (0.17, 0.23)	0.32 (0.26, 0.42)
		11-HDHA	0.18 (0.13, 0.21)	0.25 (0.22, 0.30)	0.31 (0.26, 0.41)	0.45 (0.38, 0.53)
		13-HDHA	0.087 (0.063, 0.11)	0.13 (0.11, 0.16)	0.16 (0.14, 0.21)	0.27 (0.21, 0.32)
		14-HDHA	0.69 (0.43, 1.1)	1.0 (0.67, 1.7)	1.6 (1.1, 3.4)	1.5 (1.1, 1.9)
		16-HDHA	0.15 (0.12, 0.19)	0.20 (0.18, 0.22)	0.25 (0.22, 0.30)	0.38 (0.31, 0.47)
		17-HDHA	0.83 (0.66, 0.99)	0.98 (0.82, 1.3)	1.1 (1.0, 1.4)	1.6 (1.2, 2.0)
		20-HDHA	0.36 (0.29, 0.46)	0.49 (0.42, 0.57)	0.61 (0.53, 0.77)	0.92 (0.75, 1.1)
		21-HDHA	2.4 (2.1, 3.0)	3.3 (2.7, 3.7)	4.4 (3.7, 5.7)	6.5 (5.3, 7.8)
		22-HDHA	1.9 (1.8, 2.4)	2.8 (2.2, 3.1)	3.6 (3.0, 5.0)	5.7 (4.0, 6.9)

[1] Data are median with lower and upper limit of 95% CI (n = 28 for 0 portions group; n = 35 for 1 portion group; n = 29/28 for 2 portions group; n = 29 for 4 portions group)

[2] Results of the statistical analysis can be found in **Supplemental Tables 3-6.**

ALA: alpha-linolenic acid (C18:3 n3); ARA: arachidonic acid (C20:4 n6); DHA: docosahexaenoic acid (C22:6, n3); DGLA: dihomo-γ-linolenic acid (C20:2 n6); DiHDPE: dihydroxy-DHA; DiHETE: dihydroxy-EPA; DiHETrE: dihydroxy-ARA; DiHODE: dihydroxy-ALA; DiHOME: dihydroxy-LA; EPA: eicosapentaenoic acid (C20:5 n3); EpDPE: epoxy-DHA; EpETE: epoxy-EPA; EpETrE: epoxy-ARA; EpODE: Epoxy-ALA; EpOME: epoxy-LA; HDHA: hydroxy-DHA; HEPE: hydroxy-EPA; HETE: hydroxy-ARA; HETrE: Hydroxy-DGLA; HODE: hydroxy-LA, HOTrE: hydroxy-ALA; IsoP: isoprostane; LA: linoleic acid (C18:2 n6); PG: prostaglandin; PUFA: polyunsaturated fatty acid

Table 3: Change from baseline in plasma concentrations (nmol/L) and relative changes (%) for EPA- and DHA-derived metabolites following 12 months of supplementation with EPA and DHA at intakes equivalent to 4 portions of fatty fish per week (13.09 g EPA+DHA per week).[1]

			Δ 12 months supplementation *vs* baseline	
			Plasma concentration [nmol/L]	**Relative change [%]**
EPA	Epoxy-PUFAs	11(12)-EpETE[2]	0.15 ± 0.03	720 ± 140
		14(15)-EpETE	0.20 ± 0.07	360 ± 110
		17(18)-EpETE[2]	0.32 ± 0.09	750 ± 180
	vic Dihydroxy-PUFAs	8,9-DiHETE	0.16 ± 0.09	280 ± 100
		11,12-DiHETE	0.12 ± 0.05	300 ± 90
		14,15-DiHETE	0.19 ± 0.08	290 ± 80
		17,18-DiHETE	1.1 ± 0.5	260 ± 70
	Hydroxy-PUFAs	5-HEPE	0.77 ± 0.35	300 ± 90
		8-HEPE	0.55 ± 0.40	750 ± 470
		9-HEPE	0.79 ± 0.37	420 ± 150
		11-HEPE	0.50 ± 0.28	580 ± 280
		12-HEPE	0.55 ± 0.37	280 ± 120
		15-HEPE	0.30 ± 0.19	320 ± 130
		18-HEPE	0.82 ± 0.42	510 ± 210
		19-HEPE	1.9 ± 1.1	270 ± 100
		20-HEPE	1.3 ± 0.5	300 ± 90
DHA	Epoxy-PUFAs	10(11)-EpDPE	0.34 ± 0.17	210 ± 50
		13(14)-EpDPE	0.31 ± 0.14	220 ± 50
		16(17)-EpDPE	0.34 ± 0.15	220 ± 50
		19(20)-EpDPE	0.56 ± 0.25	210 ± 50
	vic Dihydroxy-PUFAs	4,5-DiHDPE	1.3 ± 0.8	190 ± 50
		7,8-DiHDPE	0.11 ± 0.11	210 ± 90
		10,11-DiHDPE	0.20 ± 0.15	180 ± 60
		13,14-DiHDPE	0.23 ± 0.12	190 ± 40
		16,17-DiHDPE	0.25 ± 0.15	180 ± 40
		19,20-DiHDPE	2.1 ± 1.1	170 ± 30
	Hydroxy-PUFAs	4-HDHA	0.59 ± 0.30	230 ± 60
		7-HDHA[2]	0.33 ± 0.20	760 ± 410
		8-HDHA	1.2 ± 0.6	330 ± 110
		10-HDHA	0.31 ± 0.19	370 ± 160
		11-HDHA	0.33 ± 0.20	240 ± 80
		13-HDHA	0.29 ± 0.20	370 ± 180
		14-HDHA	0.69 ± 0.97	150 ± 60
		16-HDHA	0.35 ± 0.27	300 ± 150
		17-HDHA	0.90 ± 0.69	190 ± 60
		20-HDHA	0.74 ± 0.51	270 ± 110
		21-HDHA	3.7 ± 1.8	220 ± 50
		22-HDHA	3.2 ± 1.4	220 ± 50

[1] Data are mean ± 95%CI (n = 29). Differences were calculated based on the groups mean at baseline compared to 12 months of supplementation. DiHDPE: dihydroxy-DHA; DiHETE: dihydroxy-EPA; EpETE: epoxy-EPA; EpDPE: epoxy-DHA; HDHA: hydroxy-DHA; HEPE: hydroxy-EPA.

[2] Concentration of analytes were <LLOQ at baseline and changes were calculated against ½

LLOQ.

Legends for Figures

Figure 1: Heatmap of relative changes in oxylipins derived from DGLA, ARA, EPA and DHA following 3 and 12 months of supplementation with EPA+DHA. Mean relative change of all analytes is shown for which the mean could be calculated in more than two groups (n = 28 for 0 portions group; n = 35 for 1 portion group; n = 29/28 for 2 portions group; n = 29 for 4 portions group); ARA: arachidonic acid (C20:4 n6); DHA: docosahexaenoic acid (C22:6, n3); DGLA: dihomo-γ-linolenic acid (C20:2 n6); DiHDPE: dihydroxy-DHA; DiHETE: dihydroxy-EPA; DiHETrE: dihydroxy-ARA; EPA: eicosapentaenoic acid (C20:5 n3); EpDPE: epoxy-DHA; EpETE: epoxy-EPA; EpETrE: epoxy-ARA; HDHA: hydroxy-DHA; HEPE: hydroxy-EPA; HETE: hydroxy-ARA; HETrE: Hydroxy-DGLA; IsoP: isoprostane; PG: prostaglandin; PUFA: polyunsaturated fatty acid

[1] n-3 PUFA equivalent of one portion fatty fish was 3.27 g EPA+DHA (1:1.2, (*wt:wt*)). [2] If the analyte‘s concentration was <LLOQ in more than 50% of the samples in both groups (t_0 or t_3/t_{12}), no mean relative change from the individual data was calculated.

Figure 2: Concentrations of selected oxylipins from the LOX and CYP pathways of the ARA cascade during the course of supplementation with EPA+DHA. Shown are mean with 95%CI (n = 28 for 0 portions group; n = 35 for 1 portion group; n = 29/28 for 2 portions group; n = 29 for 4 portions group). The LLOQ is shown (dashed line) in case the analyte was not quantified in at least one group. Of note, the time course indicated by the lines does not reflect the actual time course of oxylipin concentrations but rather serves as a rough estimate. CYP: cytochrome P450; DiHDPE: dihydroxy-DHA; DiHETE: dihydroxy-EPA; DiHETrE: dihydroxy-ARA; EpDPE: epoxy-DHA; EpETE: epoxy-EPA; EpETrE: epoxy-ARA; HDHA: hydroxy-DHA; HEPE: hydroxy-EPA; HETE: hydroxy-ARA; LOX: lipoxygenase; sEH: soluble epoxide hydrolase. [1] n-3 PUFA equivalent of one portion fatty fish contained was 3.27 g EPA+DHA (1:1.2, (*wt:wt*)).

Figure 3: Correlation of n-3 PUFA dose with the mean plasma oxylipin concentrations (I) and scatter dot plots of individual plasma oxylipin concentrations (II). Shown are the results for selected EPA- and DHA-derived oxylipins and sum parameters following 3 and 12 months of supplementation (n = 28 for 0 portions group; n = 35 for 1 portion group; n = 29/28 for 2 portions group; n = 29 for 4 portions group). The absolute concentrations of all HEPEs, HDHAs, EpETEs and EpDPEs covered by the analytical method were summed from the individual data, i.e. 9xHEPE, 12xHDHA, 4xEpETE and 4xEpDPE. I) Means ± 95%CI of the analytes' concentrations were plotted against the weekly supplementation dose equivalent of EPA+DHA (0-13.08 g EPA+DHA per week). The LLOQ is shown (dashed line) in case the analyte was not calculated in at least one of the groups. Linear regressions were calculated for the data and the slope ± SEM, the p value for a slope significantly different from zero as well as the regression coefficient r^2 are indicated. EpDPE: epoxy-DHA, EpETE: epoxy-EPA; HDHA: hydroxy-DHA; HEPE: hydroxy-EPA. [1] n-3 PUFA equivalent of one portion fatty fish contained was 3.27 g EPA+DHA (1:1.2, (*wt:wt*)).

Figure 4: Correlation of plasma oxylipin concentrations with the relative content of EPA+DHA in A) plasma PC and B) RBC on the basis of I) individuals and II) group means. Shown are the results for selected sums of EPA- and DHA-derived oxylipins following 3 and 12 months of supplementation (n = 28 for 0 portions group; n = 35 for 1 portion group; n = 29/28 for 2 portions group; n = 29 for 4 portions group). The absolute concentrations of all HEPEs, HDHAs, EpETEs and EpDPEs covered by the analytical method were summed from the individual data, i.e. 9xHEPE, 12xHDHA, 4xEpETE and 4xEpDPE. Results for the subjects in the supplementation groups at baseline were assigned to the 'no supplementation' group. II) Means ± 95%CI of the analytes' concentrations were plotted against means ± 95%CI of the content of EPA+DHA in plasma PC (A) and RBC (B). I+II) Linear regressions: for I) the 95%CI of the slope is plotted with the regression and the p value for a slope significantly different from zero as well as the

regression coefficient r^2 are indicated; for II) slope ± SEM, the p value as well as r^2 are indicated. RBC: red blood cells. DHA: docosahexaenoic acid (C22:6, n3); EPA: eicosapentaenoic acid (C20:5 n3); PUFA: polyunsaturated fatty acid; RBC: red blood cells. [1] n-3 PUFA equivalent of one portion fatty fish was 3.27 g EPA+DHA (1:1.2, (wt:wt)).