

**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**

Annika I. Ostermann, Annette L. West, Kirsten Schoenfeld, Lucy M. Browning, Celia G. Walker,
Susan A. Jebb, Philip C. Calder, Nils Helge Schebb

Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal,
Wuppertal, Germany (AIO, KS, NHS)
Human Development and Health, Faculty of Medicine, University of Southampton, Southampton,
United Kingdom (ALW, PCC)
Medical Research Council, Elsie Widdowson Laboratory, Cambridge, United Kingdom (LMB,
CGW, SAJ)
Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United
Kingdom (SAJ)
NIHR Southampton Biomedical Research Centre, University of Southampton and University
Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom (PCC)

Authors' last names: Ostermann, West, Schoenfeld, Browning, Walker, Jebb, Calder, Schebb

Corresponding author:

Nils Helge Schebb
Chair of Food Chemistry
Faculty of Mathematics and Natural Sciences
University of Wuppertal
Gaußstr. 20
42119 Wuppertal
Germany

schebb@uni-wuppertal.de

Tel: +49-202-439-3457

Source of support:

This study was supported by grants from the German Research Foundation to NHS (Sche
1801), the UK Foods Standards Agency to SAJ and PCC (N05065/66) and the UK Medical
Research Council to LMB, CGW and SAJ (U105960389) and a travel grant of the German
Academic Exchange Service to AIO. SAJ is funded by the NIHR Oxford Biomedical Research
Centre and the NIHR Oxford Collaboration for Leadership in Applied Health Research and Care.
PCC is funded by the NIHR Southampton Biomedical Research Centre.

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

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43 Abbreviations:

44	ALA	alpha linolenic acid	64	HETE	hydroxyl-ARA
45	ARA	arachidonic acid	65	HETrE	hydroxy-DGLA
46	CI	confidence interval	66	HODE	hydroxy-LA
47	COX	cyclooxygenase	67	HOTrE	hydroxy-ALA
48	CYP	cytochrome P450	68	IS	internal standard
49	DGLA	dihomo- γ -linolenic acid	69	IsoP	isoprostane
50	DHA	docosahexaenoic acid	70	LA	linoleic acid
51	DiHDPE	dihydroxy-DHA	71	LC-MS	liquid chromatography-mass
52	DiHETE	dihydroxy-EPA	72		spectrometry
53	DiHETrE	dihydroxy-ARA	73	LT	leukotriene
54	DiHODE	dihydroxy-ALA	74	LOX	lipoxygenase
55	DiHOME	dihydroxy-LA	75	PC	plasma phosphatidylcholine
56	EPA	eicosapentaenoic acid	76	PG	prostaglandin
57	EpDPE	epoxy-DHA	77	PUFA	polyunsaturated fatty acid
58	EpETE	epoxy-EPA	78	QC	quality control samples
59	EpETrE	epoxy-ARA	79	RBC	red blood cells
60	EpODE	Epoxy-ALA	80	SEM	standard error of the mean
61	EpOME	epoxy-LA	81	SPM	specialized pro-resolving lipid
62	HDHA	hydroxy-DHA	82		mediator
63	HEPE	hydroxyl-EPA			

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84 Current Controls Trials Number: [ISRCTN48398526 \(www.controlled-trials.com\)](https://www.controlled-trials.com)

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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 PGE₂ and LTB₄ (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

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

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

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 oxylin 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 oxylin 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**.

Oxylin Analysis

Free (i.e. non-esterified) oxylin in plasma were analyzed at baseline and after 3 and 12 months of supplementation. Analysis of oxylin in plasma was conducted as described elsewhere (23, 24). In brief, after addition of internal standards (IS) and antioxidants, proteins were precipitated with methanol. Oxylin 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 $\geq 65\%$ 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**).

211 **Data Analysis and Statistics**

212 Results are presented as indicated in the table and figure captions calculated with Microsoft
 213 Excel (Office 10, Microsoft, Redmond, WA, USA) or Prism (GraphPad Software, La Jolla, CA,
 214 USA). Concentrations were only calculated if the analyte exceeded the lower limit of
 215 quantification (LLOQ) in $\geq 50\%$ of the samples in the group. Samples with a concentration below
 216 LLOQ were set to the concentration of $\frac{1}{2}$ LLOQ. Relative changes in the study population after 3
 217 and 12 months of supplementation were calculated from the individual data (e.g. **Figure 1**) or
 218 the group means (e.g. **Table 3**) against baseline using the formula $\text{rel change (\%)} =$
 219 $\text{conc(t)}/\text{conc(t}_0\text{)} \times 100$. Linear regressions were calculated using Prism. For each correlation, the
 220 slope with standard error of the mean (SEM), the p value for a slope significantly different from
 221 zero and the regression coefficient r^2 (square of the Pearson correlation coefficient) are
 222 indicated in the figures.

223 Data were log transformed prior to statistical analysis in order to achieve normality. A general
 224 linear statistical model was used with repeated measures to investigate the change in oxylipin
 225 concentrations over the time course of the study, with time set as 'Within-Subject Factor' and n-3
 226 PUFA dose as 'Between Subject Factor'; BMI and baseline plasma phosphatidylcholine (PC)
 227 EPA+DHA concentrations were used as covariates. Interactions between BMI and n-3 PUFA
 228 dose were set using 'interaction' analysis options within the model. A univariate model was used
 229 to investigate the effect of n-3 PUFA dose at each time point using baseline corrected data at 3
 230 months and 12 months for each analyte and using BMI and baseline PC EPA+DHA
 231 concentrations as co-variates. Any significant findings for dose were then examined using a
 232 Tukey post-hoc test to determine the dose inducing an effect. P values were corrected for
 233 multiple analyses using Bonferroni correction analysis. P values ≤ 0.01 indicated statistical
 234 significance in the whole data group. Where the data were analysed separately for time point, P
 235 values ≤ 0.013 indicated significant differences. These statistics were calculated using SPSS
 236 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 (Figure 1-4, 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 eicosanoids 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

None of the authors has any conflict of interest to declare.

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 _{2α}	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)	
	DHA	Epoxy-PUFA	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)
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)	

vic Dihydroxy-PUFA	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)
	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)
Hydroxy-PUFA	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)
ARA	Prostaglandins	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)	
		13,14-dihydro-15-keto-PGF _{2α}	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)
	vic Dihydroxy-PUFA	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)	
		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)	

DHA	Hydroxy-PUFA	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)	
		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)	
	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 _{2α}	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, 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)	

EPA	vic Dihydroxy-PUFA	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)
		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)
	Hydroxy-PUFA	15,16-DiHODE	9.7 (8.2, 13)	9.2 (8.0, 12)	8.7 (7.0, 11)	10 (9.0, 12)
		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)
	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)
	Hydroxy-PUFA	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)
		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)
	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)
		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)
DHA	vic Dihydroxy-PUFA	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: dihomogamma-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 \pm 0.03	720 \pm 140
		14(15)-EpETE	0.20 \pm 0.07	360 \pm 110
		17(18)-EpETE ²	0.32 \pm 0.09	750 \pm 180
	vic Dihydroxy-PUFAs	8,9-DiHETE	0.16 \pm 0.09	280 \pm 100
		11,12-DiHETE	0.12 \pm 0.05	300 \pm 90
		14,15-DiHETE	0.19 \pm 0.08	290 \pm 80
		17,18-DiHETE	1.1 \pm 0.5	260 \pm 70
	Hydroxy-PUFAs	5-HEPE	0.77 \pm 0.35	300 \pm 90
		8-HEPE	0.55 \pm 0.40	750 \pm 470
		9-HEPE	0.79 \pm 0.37	420 \pm 150
		11-HEPE	0.50 \pm 0.28	580 \pm 280
		12-HEPE	0.55 \pm 0.37	280 \pm 120
		15-HEPE	0.30 \pm 0.19	320 \pm 130
		18-HEPE	0.82 \pm 0.42	510 \pm 210
		19-HEPE	1.9 \pm 1.1	270 \pm 100
		20-HEPE	1.3 \pm 0.5	300 \pm 90
DHA	Epoxy-PUFAs	10(11)-EpDPE	0.34 \pm 0.17	210 \pm 50
		13(14)-EpDPE	0.31 \pm 0.14	220 \pm 50
		16(17)-EpDPE	0.34 \pm 0.15	220 \pm 50
		19(20)-EpDPE	0.56 \pm 0.25	210 \pm 50
	vic Dihydroxy-PUFAs	4,5-DiHDPE	1.3 \pm 0.8	190 \pm 50
		7,8-DiHDPE	0.11 \pm 0.11	210 \pm 90
		10,11-DiHDPE	0.20 \pm 0.15	180 \pm 60
		13,14-DiHDPE	0.23 \pm 0.12	190 \pm 40
		16,17-DiHDPE	0.25 \pm 0.15	180 \pm 40
		19,20-DiHDPE	2.1 \pm 1.1	170 \pm 30
	Hydroxy-PUFAs	4-HDHA	0.59 \pm 0.30	230 \pm 60
		7-HDHA ²	0.33 \pm 0.20	760 \pm 410
		8-HDHA	1.2 \pm 0.6	330 \pm 110
		10-HDHA	0.31 \pm 0.19	370 \pm 160
		11-HDHA	0.33 \pm 0.20	240 \pm 80
		13-HDHA	0.29 \pm 0.20	370 \pm 180
		14-HDHA	0.69 \pm 0.97	150 \pm 60
		16-HDHA	0.35 \pm 0.27	300 \pm 150
		17-HDHA	0.90 \pm 0.69	190 \pm 60
		20-HDHA	0.74 \pm 0.51	270 \pm 110
		21-HDHA	3.7 \pm 1.8	220 \pm 50
		22-HDHA	3.2 \pm 1.4	220 \pm 50

¹ Data are mean \pm 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 $\frac{1}{2}$ 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: dihomog- 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 \pm 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 \pm 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 \pm 95%CI of the analytes' concentrations were plotted against means \pm 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 \pm 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)).