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Lipid mediator serum profiles in asthmatics significantly shift following dietary supplementation with omega-3 fatty acids

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Abstract

Scope—In contrast to well-characterized polyunsaturated fatty acid (PUFA) levels in serum, little is known regarding their downstream metabolic products. However, many of these compounds are lipid mediators with prominent roles during pro- and anti-inflammatory processes.

Methods and Results—In this double blind crossover study on asthmatics, shifts in serum levels of ω -3 and ω -6 PUFA-derived oxidized fatty acids (*e.g.*, eicosanoids, oxylipins) were quantified following dietary fish oil supplementation. Serum was obtained from subjects following fasting at 3 occasions; 1) Prior to supplementation, 2) Following a 3-week supplement intake of either placebo or fish oil, and 3) Following a 3-week washout period with a subsequent 3-week period of either fish oil or placebo supplement. A total of 87 oxylipins representing cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) metabolic pathways were screened via liquid chromatography-tandem mass spectrometry (LC-MS/MS). The primary alterations observed were in CYP- and 15-LOX-derived EPA- and CYP-derived DHA oxylipins.

Conclusion—The results indicate that intake of an ω -3 rich diet alters not only the PUFA ratio, but also the ratio of downstream oxylipins. These data further support that dietary manipulation with ω -3 PUFAs affects not only PUFA levels, but importantly also the downstream metabolic profile.

Keywords

asthma; eicosanoid; lipidomics; omega-3; oxylipin

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INTRODUCTION

The potential impacts upon health and disease of the long-chain omega-3 fatty acids (ω -3 FAs) have become an area of intense investigation [1-3]. The American Heart Association recommends that all adults eat fish at least twice per week, as well as vegetables containing plant-derived ω -3 FAs, and that patients with documented heart disease consume fish oil capsules [4, 5]. To date, the majority of studies have focused on the parent polyunsaturated fatty acid (PUFA) vs. the downstream metabolic products. However, there is a growing interest in exploring the biology associated with ω -6 and ω -3 FA-derived oxidized lipid metabolites (*e.g.*, eicosanoids, oxylipins) [6-8]. It is well known that ω -6 FA-derived oxylipins from in particular the arachidonic acid (AA) cascade (*e.g.*, leukotrienes, prostaglandins) have prominent roles during the inflammatory process [9-12]. In particular, eicosanoids are linked to the incidence of inflammatory events, and correlate to a number of diseases including atherosclerosis, diabetes, coronary heart disease, hypertension and obesity [13-18]. In contrast, data suggest that oxylipins derived from ω -3 FAs (*e.g.*, resolvins and protectins) are involved in the resolution phase of inflammation, providing evidence for their beneficial health effects in the diet [19-21]. Much of the research performed to date has focused on the ω -3 FAs EPA and DHA, the so-called fish oils, although there are other sources of plant-derived ω -3 FAs in the diet (*e.g.*, α -linolenic acid [ALA] from soybean, rapeseed and flaxseed).

The ω -6 and ω -3 FA-derived oxylipins are formed via the same 3 main enzymatic pathways: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP), suggesting that lipid mediator formation can be affected by substrate abundance. It is therefore likely that the balance between ω -6 and ω -3 FAs and their downstream metabolic products are connected to disease etiology. However, to date, only a few studies have investigated how the intake of ω -3 FAs affects the overall profile of downstream oxylipins [8, 22, 23]. Accordingly, additional information is required to establish the baseline levels of ω -3 FA-derived oxylipins in clinical samples from both healthy and diseased groups as well as the effect of dietary changes.

In this double blind, randomized, placebo controlled crossover study we investigated serum levels of ω -3 and ω -6 FA-derived oxylipins in clinically mild to moderate asthmatic individuals at baseline and following placebo- and ω -3 FA-supplementation. An oxylipin metabolic profiling approach was applied in which a broad selection of compounds were quantified, representing multiple components of the relevant biosynthetic pathways (LOX, COX and CYP). Multivariate statistics were used to probe the relationship between oxylipin and PUFA levels. This report provides useful data on oxylipin serum ratios in a mild asthmatic population, and relative intra-individual shifts in ω -6/ ω -3 FA-derived lipid mediators following dietary ω -3 FA supplementation. In addition, it presents one of the few oxylipin metabolic profiling studies performed to date in human serum.

MATERIALS AND METHODS

Clinical data and study design

A total of 25 clinically stable mild-moderate asthmatic patients (age 20-54 years, 11 females, BMI 19-39) were included in the study (Table 1). Inclusion criteria were non-smoking subjects with clinically diagnosed asthma and current asthma symptoms with a forced expiratory volume in one second (FEV₁) >70% of predicted values. Exclusion criteria included significant gastrointestinal, hematological, cardiovascular, cerebrovascular or other system disorders. Additionally, smoking, regular fish or ω -3 FA supplement consumption, as well as intake of leukotriene receptor antagonists 4 weeks prior to study initiation were excluded. Blood sampling following 12-14 h fasting was performed at 3

occasions; 1) Prior to supplementation (baseline), 2) Following a 3 week supplement intake of **A**: placebo or **B**: ω -3 FA, and 3) Following a 3 week washout period (normal diet) with a subsequent 3 week period of **A**: ω -3 FA or **B**: placebo supplement. The 3-week washout period was chosen based upon previously reported studies for DHA and EPA [24-27] as well as the normal washout time between successive provocations in order to regain baseline airway responsiveness [28]. Case **A** and **B** were randomized. ω -3 FA (400 mg of EPA and 200 mg DHA/capsule; 40/20EE capsules; product code 4020PB1000CT; QC Lot # QC48781 [Ocean Nutrition, Nova Scotia, Canada]; equated to a daily dose of 4.0g EPA and 2.0g DHA) and placebo (50:50 mix of soybean and corn oil; product code PLACEBO1000; QC Lot # QC48748; Ocean Nutrition) supplements were administered daily in the form of 10 capsules during the supplement periods. Five capsules were taken in the morning and 5 capsules in the evening, with 12h between evening capsule consumption and blood draw. Blood was collected immediately upon patient arrival in the clinic. Samples were collected in standard non-heparinized tubes (Vacutainer, Becton Dickinson), allowed to sit for 1 hr and spun at room temperature at 1000g for 15 min. Serum was aliquoted in eppendorf tubes via a micropipette and stored at -80°C until analysis. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Review Board of St Joseph's Healthcare, Hamilton (R.P. #06-2750) and by Health Canada (Approval No. 120532). Written informed consent was obtained from all subjects. More details of the study design and primary endpoint are available from The Clinical Trial Registration (<http://www.clinicaltrials.gov>; Identifier Number NCT00526357) [29].

Oxylipin extraction and analysis

External standards, deuterated internal standards and the technical standard N-cyclohexyl-N'-dodecanoic acid urea (CUDA) were obtained from Cayman Chemical (Ann Arbor, MI, USA), Larodan Fine Chemicals AB (Malmö, Sweden), Biomol International (Plymouth Meeting, PA, USA) or synthesized in-house [30]. Off-line SPE-extraction was performed on 220 μL serum aliquots using Waters Oasis-HBL 60 mg cartridge columns (Milford, MA, USA) as previously described [30, 31]. A detailed description of the instrument method is given elsewhere [30]. An Agilent 1200 SL separation module (Santa Clara, CA, USA) coupled to an ABI QTRAP® 4000 hybrid triple quadrupole/linear ion trap mass spectrometer (Foster City, CA, USA) was used for analyses and separation was performed via a 2.1×150 mm Eclipse Plus C18 column with a $1.8 \mu\text{m}$ particle size (Agilent, Santa Clara, CA, USA). Oxylipins were quantified using stable isotope internal standard methods as previously described [30].

PUFA extraction and analysis

Fatty acid compositions of total serum phospholipids were determined in the laboratory of Bruce Holub at the University of Guelph based upon previous methods [32]. Lipids were extracted from the serum samples according to the method of Folch *et al.* [33] and the serum phospholipids were separated from the neutral lipids by thin-layer chromatography [32]. The fatty acid methyl esters were prepared from the isolated phospholipid fraction by the method of Morrison and Smith *et al.* [34] and were analyzed on a Varian 3400 gas-liquid chromatograph (Palo Alto, CA) with a 60 m DB-23 capillary column (0.32 mm internal diameter).

Statistical methods

Univariate statistical analysis was performed using Student's paired t-test. Multivariate analyses by orthogonal projections to latent structures (OPLS) were performed using SIMCA v.13.0 (Umetrics, Umeå, Sweden) following log transformation, mean centering and

UV scaling [35]. Model performance was reported as cumulative correlation coefficients for the model (R^2), predictive performance based on cross validation calculations (Q^2), and cross validated analysis of variance (CV-ANOVA).

RESULTS

Levels of EPA and DHA increased in the serum phospholipids following ω -3 FA supplementation

Levels of FAs in the serum phospholipids are provided on a percent composition basis in Table 2 for placebo and ω -3 FA supplementation (baseline values were not assessed). As expected, supplementation with EPA/DHA resulted in a concomitant increase in these species in serum indicating that all patients were compliant with capsule consumption. The percentage of EPA following supplementation was 5.7% relative to 1.0% for placebo, whereas the DHA levels were 6.2% and 3.7%, respectively (reflecting the higher dosing of EPA in the capsules). The overall percentage of ω -6 FAs was lower following ω -3 FA supplementation, driven primarily by decreases in linoleic and arachidonic acid.

Supplementation with ω -3 FAs increased serum levels of ω -3-derived oxylipin

Of the 87 oxylipins screened in serum, 41 species were detected above the method limit of quantification (mLOQ). The oxylipin levels (from all 3 sampling times combined) ranged over 3 orders of magnitude from ~50 pM to ~40 nM (Table 3). Average concentrations (nM) and coefficients of variance (CVs) are given for the 3 sampling conditions in Table 3 (*i.e.*, Baseline [B], placebo supplement [P] and ω -3 FA supplement [ω -3]). It should be stressed that all oxylipins were quantified as the free fatty acid according to previously published methods [30]. There were no significant changes in the levels of any oxylipin species between baseline and ω -3 FA diet between individuals at time points 1 or 2.

Five EPA-derived and 5 DHA-derived oxylipins were detected above the mLOQ. Of these, the majority were CYP products: EPA: 11,12- 14,15- and 17,18-dihydroxyecosatetraenoic acid (DiHETE), and DHA: 10(11)- epoxydocosapentaenoic acid (EpDPE), 4,5-, 10-11, 13,14, and 16,17-dihydroxydocosapentaenoic acid (DiHDPE). In addition, two 15-LOX-formed EPA products (12- and 15-hydroxyecosapentaenoic acid [HEPE]) were detected. Following ω -3 FA supplementation, almost all of the quantified EPA- and DHA-derived oxylipins were significantly elevated compared to both baseline and placebo serum levels (Table 3). As shown in Figure 1 and Table 3, the relative difference between ω -3 FA and placebo ranged on average from 5-25-fold increases for EPA metabolites and 2-3-fold increases of the DHA metabolites following ω -3 FA supplementation. The largest shifts were observed in the EPA 15-LOX-produced compounds, which on average increased 20-25 fold relative to placebo.

In addition to the EPA and DHA products, metabolites originating from ω -3 FAs: α -linolenic acid (ALA, n=4) and ω -6 FAs: linoleic acid (LA, n=9), dihomo-gamma-linolenic acid (DGLA, n=1) and arachidonic acid (AA, n=17) were detected. Following ω -3 FA supplementation, 2 CYP-formed products from the ALA pathway and 4 CYP-produced compounds from the AA (n=1) and LA (n=3) pathways were significantly decreased compared to concentrations following placebo: 15(16)- epoxyoctadecadienoic acid [EpODE], 15,16-dihydroxyoctadecadienoic acid [DiHODE], 5(6)- dihydroxyecosatrienoic acid [DiHETrE], 9,10-and 12,13-dihydroxyoctadecenoic acid [DiHOME], and 12(13)- epoxydecanoic acid [EpOME], respectively. A similar observation was observed comparing ω -3 FA supplementation with baseline concentrations that showed a significant decrease in 4 CYP-produced AA products (5[6]-DiHETrE, 8[9]-DiHETrE, 11[12]-DiHETrE and 14[15]-DiHETrE) and 2 CYP-produced LA products (9,10-DiHOME and 12(13)-EpOME).

In Figure 2 the overall profile of targeted ω -3 FA-derived (Figure 2A) and ω -6 FA-derived (Figure 2B) oxylipins is presented as the sum of the median values of each fatty acid subgroup. As expected, the sum of EPA and DHA products significantly increased following ω -3 FA supplementation compared to both baseline and placebo (Table 3, Figure 2). In contrast, the sum of the ALA products significantly decreased following ω -3 FA supplementation compared to the placebo (Table 3, Figure 2). The drop in AA-derived compounds from baseline to placebo and ω -3 FA supplementation shown in Figure 2 is primarily due to the variation in the 12-HETE pathway (If 12-HETE and the 12-HETE product 12-oxoeicosatetraenoic acid [KETE] are removed, the p-value shifts from $p=0.047$ to $p=0.10$; Table 3). The sum of LA products significantly decreased following ω -3 supplementation compared to baseline ($p=0.03$) and the DGLA products were not significantly altered between the groups.

Integrated multivariate modeling demonstrated that serum PUFA levels had the largest effect on differentiating ω -3 FA vs. placebo supplementation

Multivariate statistical modeling integrating oxylipin data with relative serum PUFA levels (%) was performed in order to investigate overall trends in the data. An OPLS-DA model examining separation according to placebo *versus* ω -3 FA supplementation was constructed ($R^2[\text{cum}]=0.81$, $Q^2[\text{cum}]=0.78$, CVANOVA $p\text{-value}=2.17\text{E}^{-14}$). The R^2 value indicates how well the model explains the current dataset, whereas the Q^2 is the correlation based on averaging the results from repeated iterations of cross-validation. As such, the Q^2 represents a measure of the predictive power of the model (*i.e.*, how well the model is expected to fit additional samples from the same groups). The model (Figure 3) was built from a single predictive and 1 orthogonal component, resulting in a robust separation between the two test conditions. One placebo subject was located outside of Hotelling's T^2 in the scores plot (Subject 13). However, it was only an outlier in terms of the orthogonal vector, and further examination of the Distance to Model X (DModX) confirmed that this individual was within the 95% confidence interval. Accordingly, this individual was not excluded from the model. The loadings column plot (Figure 3B) showed that the PUFAs as well as both specific ω -3 and ω -6 derived oxylipins are primary variables contributing to subject separation. Subjects $3_{\omega-3}$ and $19_{\omega-3}$ (dark gray), which slightly cluster with the placebo group, are still clearly distinguished from their 3_P and 19_P (light gray) counterparts (the EPA- and DHA-derived oxylipins and PUFA variables were more prominent following ω -3 FA supplementation). The PUFA data are the strongest contributors to the model, with increases in EPA and DHA and decreases in DGLA and LA possessing the largest loadings. No clustering was observed according to age, gender or BMI.

DISCUSSION

Oxylipin composition of serum and plasma evidences similar profiles

Both the human plasma [36, 37] and serum lipidomes [38] have been examined in detail, revealing a significant diversity of lipid species. However, these studies tend to focus on higher abundance structural lipids rather than signaling lipids (*e.g.*, eicosanoids). Earlier studies have reported the baseline oxylipin profiles in plasma [39-41] and a number of studies have examined the effect of ω -3 FA supplementation [8, 22, 23]. However, it is known that the human plasma and serum metabolomes profiles differ [42, 43], which should potentially be reflected in the oxylipin levels. The current study is one of the first to measure the oxylipin component of the human serum lipidome, both at baseline and following dietary supplementation with ω -3 FAs (a recent paper examined the serum oxylipin profiles in immunoglobulin A nephropathy [IgAN] patients [44]).

A particular issue related to the measure of oxylipins in blood is the potential effects of the blood collection process as well as serum/plasma generation upon the observed oxylipin profile [40, 45, 46]. Accordingly, it is essential that blood collection, processing, and handling methods are reported in detail when providing oxylipin data. For example, higher serum values of TXB₂ relative to plasma are attributed to platelet activation [47-49], and use of heparin in plasma generation is postulated to stimulate lipoprotein lipases, which results in concomitant increases in overall oxylipin levels [45]. In the current study, as expected baseline TXB₂ levels were roughly 3.5-fold greater than previously published values in plasma [39]. However, overall, the serum baseline concentrations presented herein are similar to previously published values for plasma [22, 39, 50]. A comparison of serum oxylipin values with previously published plasma values evidences a few important trends (Table 4). The plasma data are from two distinct cohorts from the Human Metabolome (HM; n=3 individuals) and the Pennington Plasma (n=70 individuals) [39]. The composition of the oxylipin analytical platforms is slightly different, but there is sufficient overlap for comparison purposes. The current study observed 41 oxylipin species in serum relative to 50 in the HM plasma and 76 in the Pennington Plasma. The ratio of the serum/plasma levels for all compounds in common between both analytical platforms was 1.45 and 1.67 for the HM and Pennington Plasma, respectively. As discussed above, the notable outlier was TXB₂, removal of which slightly decreased the ratios (1.38 and 1.62, respectively). The other major outlier in the current study was 12-HETE, which was significantly elevated in serum. It is unclear what is causing the elevated levels of 12-HETE and its downstream product 12-KETE; however, it is well known that platelets can produce 12-HETE [51]. Further removal of 12-HETE reduced the ratios to 1.19 and 1.32, respectively (12-KETE was not observed in the work by Psychogios *et al.* [39]). Accordingly, serum and plasma levels of the majority of oxylipin species appear to be relatively similar, which is in agreement with recently published data [46].

The ratio of [AA]/[EPA+DHA]-derived oxylipins from the 15-LOX and CYP pathways demonstrates inter-study homogeneity

The [AA]/[EPA+DHA] ratio of 15-LOX- and CYP-produced compounds is more consistent than the concentrations as shown in Table 5 for 3 different studies: this study (Lundström *et al.*) and Shearer *et al.* [22], in which oxylipin levels were measured in plasma, and Honstra *et al.* [23], in which oxylipin levels in plasma were measured following platelet activation with collagen. Interestingly, the ratios of the groups having an ω -3 FA or fish supplemented diet are similar between the studies. Additionally, the other groups (baseline pre-study, controls during study and placebo supplement) are also similar between studies, but differ from the ω -3 FA groups. Thus, results indicate that an ω -3 FA rich diet affects downstream metabolic pathways and the intra-individual ratio of ω -3 and ω -6 15-LOX-derived and CYP-derived products in a consistent manner. The shift is mainly due to changes in the concentration of EPA- and DHA-derived oxylipins, but also affects the levels in pathways derived from LA, AA and ALA (Table 3, Figure 2). Decreased levels in AA-derived oxylipins following ω -3 supplementation have previously been reported by Shearer *et al.* [22].

Asthmatics evidence similar serum oxylipin profile to healthy individuals following ω -3 FA supplementation

No obvious differences were observed in the oxylipin shifts following ω -3 FA supplementation that would distinguish the healthy populations characterized by Shearer *et al.* [22, 23] and Honstra *et al.* [22, 23] to the asthmatic population investigated herein. However, we cannot exclude the possibility that there are some differences between the two populations in terms of how they handle ω -3 FAs. It is distinctly possible that differences not visible in the serum signature could be observed by pulmonary examination (*e.g.*, bronchoalveolar lavage). It is clear that a shift to an ω -3 FA rich diet will affect not only the

PUFA ratio, but also the overall downstream oxylipin profile, which can have ramifications for disease. For example, the 15-LOX-derived EPA pathway is involved in the formation of resolvins, which have anti-inflammatory or resolution-like properties [19], whereas the majority of AA-derived mediators have pro-inflammatory properties [9-11]. Accordingly, shifts in downstream ω -3 FA-derived lipid mediators such as resolvins and protectins could affect disease processes. Several studies have indicated positive effects of ω -3 FA supplementation for asthma treatment, whereas ω -6 and *trans*-fatty acids may adversely affect disease [52-55]. However, there are also studies arguing that a high intake of ω -3 FAs will not have protective effects [56, 57]. In addition, the observed intra-individual variability in oxylipin levels following ω -3 treatment could potentially correlate with beneficial response to dietary supplementation [8]. Accordingly, any discussion of the role of ω -3 FAs in the diet should also consider potential effects on the downstream metabolic pathways.

In conclusion, dietary supplementation of ω -3 FAs results in concomitant increases in downstream metabolic products. The oxylipin profile in serum and plasma are quite similar, with the exception of eicosanoid products due to platelet activation (*e.g.*, TXB₂). ω -3 FA supplementation appears to have similar effects upon the oxylipin profile in serum from asthmatic and healthy individuals. The major observed shifts in EPA- and DHA-derived mediators, and minor shifts in LA and AA-derived mediators, could potentially contribute to the observed beneficial effects of fish oil supplementation in a healthy population. Accordingly, studies involving ω -3 FA supplementation need to also consider the consequences of shifts in downstream lipid mediator profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

AA	Arachidonic acid
ALA	α -linolenic acid
COX	cyclooxygenase
CUDA	<i>N</i> -cyclohexyl- <i>N'</i> -dodecanoic acid urea
CYP	cytochrome P450
DGLA	dihomogamma linolenic acid
DiHETrE	dihydroxyeicosatrienoic acid
DiHDPE	dihydroxydocosapentaenoic acid
DiHETE	dihydroxyeicosatetraenoic acid
DiHODE	dihydroxyoctadecadienoic acid

DModX	distance to model X
EpETrE	epoxyeicosatrienoic acid
EpDPE	epoxydocosapentaenoic acid
EpODE	epoxyoctadecadienoic acid
HETE	hydroxyeicosatetraenoic acid
HETrE	hydroxyeicosatrienoic acid
HEPE	hydroxy eicosapentaenoic acid
HODE	hydroxyoctadecadienoic acid
KODE	oxooctadecadienoic acid
HOTE	hydroxyoctadecatrienoic acid
LA	linoleic acid
LT	leukotriene
LOX	lipoxygenase
MS/MS	tandem mass spectrometry
mLOQ	method limit of quantification
OPLS-DA	orthogonal projections to latent structures – discriminate analysis
PUFA	polyunsaturated fatty acid
TriHOME	trihydroxyoctadecenoic acid
TX	tromboxane

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Figure 1.

Log_{10} fold change in EPA-derived and DHA-derived oxylipins comparing ω -3 and placebo (P) supplementation. Values ≥ 0 indicate an increase following ω -3 supplement. Oxylipin nomenclature is provided in the Supporting Information Table S1.

Figure 2.

Oxylipin levels group by substrate using the sum of the median values. (A) ω-3 derived and (B) ω-6 derived oxylipins. Significant shifts are indicated as calculated with a Student's t-test. Individual values for averages and CVs are provided in Table 3. Oxylipin nomenclature is as provided in the Supporting Information Table S2. ALA=alpha-linolenic acid; LA=linoleic acid; DGLA=dihomo-gamma-linolenic acid; AA=arachidonic acid.

Figure 3. OPLS-DA model of placebo (light gray) versus ω -3 supplementation (dark gray). (A) Scores plot. The placebo outlier 13 is outside the shown plot range ($t[1]:-4.4$, $to[1]:-14.5$). (B) Loadings column plot. Mediators correlating with 95% confidence are shown. The model was built from a single predictive and 1 orthogonal component ($R^2[\text{cum}]=0.81$, $Q^2[\text{cum}]=0.78$, CVANOVA $p\text{-value}=2.17\text{E}^{-14}$).

Table 1

Clinical data of participating study subjects

Subject	Gender ¹	Age ²	BMI ³	FEV ₁ (%Pred) ⁴
1	M	31	30	75.6
2	M	38	23	88.6
3	F	43	26	84.7
4	F	26	30	80.3
5	M	23	27	102.6
6	M	30	37	76.4
7	F	33	35	88.1
8	M	24	28	98.0
9	F	28	22	72.1
10	F	21	37	71.1
11	M	20	33	84.7
12	M	28	34	89.6
13	M	26	27	110.0
14	F	33	25	81.8
15	M	29	19	82.5
16	F	22	22	99.5
17	F	24	20	81.1
18	M	54	27	76.8
19	M	47	26	76.2
20	M	19	39	70.2
21	F	39	23	103.2
22	M	26	30	79.8
23	M	22	25	74.5
24	F	42	32	87.8
25	F	21	24	87.8

¹Female (F) and Male (M)²Years³Body mass index: [mass(kg)]/[height(m)]²⁴Percent predicted Forced Expiratory Volume in one second. FEV₁% predicted is defined as FEV₁% of the patient divided by the average FEV₁% in the population for any person of similar age, sex and body composition

Table 2

Free fatty acid levels (%) in serum phospholipids¹

Free Fatty Acid ²	ω-3 Supplemented		Placebo		p-value
	Average (%)	CV (%)	Average (%)	CV (%)	
C14:0	0.39	29%	0.41	29%	5.22E-01
C14:1	0.00	271%	0.00	304%	9.73E-01
C15:0	0.25	20%	0.27	18%	3.66E-01
C16:0	27.77	4%	27.63	5%	7.15E-01
C16:1	0.33	41%	0.37	33%	3.53E-01
C18:0	14.02	7%	13.84	9%	5.85E-01
C18:1	10.64	9%	11.43	9%	5.30E-03
C18:2n6 (LA)	18.34	17%	22.73	12%	2.62E-06
C18:3n6	0.02	140%	0.07	91%	8.31E-04
C18:3n3 (ALA)	0.21	46%	0.24	42%	1.64E-01
C18:4n3	0.02	234%	0.01	287%	6.46E-01
C20:0	0.14	71%	0.11	103%	2.45E-01
C20:1	0.12	98%	0.14	72%	6.69E-01
C20:2n6	0.19	84%	0.26	65%	1.43E-01
C20:3n6 (DGLA)	2.11	32%	3.28	22%	3.40E-07
C20:4n6 (AA)	9.94	14%	11.58	19%	2.93E-03
C20:3n3	0.01	391%	0.01	414%	6.40E-01
C20:4n3	0.11	95%	0.09	82%	4.67E-01
C20:5n3 (EPA)	5.72	41%	1.01	40%	3.10E-10
C22:0	0.48	39%	0.39	31%	7.54E-02
C22:1	0.00	500%	0.00	500%	6.38E-01
C22:2n6	0.02	199%	0.03	145%	3.95E-01
C22:4n6	0.15	71%	0.34	28%	2.86E-08
C22:5n6	0.02	293%	0.02	340%	8.07E-01
C22:5n3	1.50	21%	0.91	20%	8.44E-10
C22:6n3 (DHA)	6.24	19%	3.72	25%	4.66E-11
C24:0	0.46	46%	0.43	29%	5.27E-01
C24:1	0.78	28%	0.66	26%	3.39E-02
Total	100	0%	100	0%	1.00E+00
Saturated	43.51	2%	43.09	2%	7.76E-02
Monounsaturated	11.88	8%	12.60	8%	1.71E-02
Polyunsaturated	44.61	3%	44.31	3%	3.88E-01
Total ω-3	13.81	23%	5.98	19%	1.21E-12
Total ω-6	30.80	9%	38.33	4%	1.17E-13
EPA + DHA	11.96	25%	4.72	23%	3.08E-12
ω-3/ω-6	0.46	31%	0.16	21%	5.92E-11

Free Fatty Acid ²	ω -3 Supplemented		Placebo		p-value
	Average (%)	CV (%)	Average (%)	CV (%)	
AA/[EPA + DHA] ³	0.83	66%	2.5	38%	3.13E-11

¹ Levels of free fatty acids in serum are shown as percent composition of the total amount of species quantified. P-values in bold represent species that were significantly shifted at the $p < 0.05$ level.

² Free fatty acid species are shown as the number of carbon atoms in the alkyl chain, number of double bonds, and number of unsaturations (*e.g.*, C18:2n6 is linoleic acid with an 18 carbon alkyl chain containing two double bonds and it is an ω -6 fatty acid).

³ Values are given as a ratio of the percentages.

Table 3

Oxylipin concentrations in serum (nM)

Baseline (B), Placebo supplement (P) and ω -3 supplement (ω -3)

Class	PUFA	Oxylipin	Baseline (B)		Placebo supplement (P)		ω-3 supplement (ω-3)		p-values		Fold Change [C _{ω-3}]/[C _P] ^I		
			A _v ²	CV ³	A _v	CV	A _v	CV	B/P	B/ω-3	P/ω-3	A _v	CV
ω-3	ALA	9-HOTrE	0.71	86	0.71	116	0.53	50	9.9E-01	1.7E-01	1.9E-01	1.0	57
		13-HOTrE	0.80	90	0.74	111	0.51	52	8.4E-01	7.8E-02	8.8E-02	0.9	47
		15(16)-EpODE	3.9	83	3.7	115	2.4	75	9.1E-01	6.8E-02	3.2E-02	0.8	52
		15,16-DiHODE	7.1	47	8.7	78	6.2	54	3.4E-01	2.9E-01	1.4E-02	0.8	32
		Sum (n=4)	13	53	14	90	10	54	6.6E-01	1.2E-01	2.2E-02	0.8	33
	EPA	12-HEPE	2.4	291	0.55	87	6.9	188	2.0E-01	1.3E-01	2.2E-02	27	228
		15-HEPE	0.07	129	0.10	114	0.56	88	3.2E-01	1.3E-05	2.4E-05	17	112
		11,12-DiHETE	0.04	83	0.05	103	0.22	59	2.7E-01	1.1E-07	2.6E-07	6.1	68
		14,15-DiHETE	0.07	60	0.09	87	0.36	55	3.7E-01	6.5E-08	2.2E-07	4.9	58
		17,18-DiHETE	0.47	48	0.56	80	2.2	59	3.9E-01	3.9E-07	5.1E-07	4.5	58
DHA	Sum (n=5)	3.1	236	1.3	73	10.2	141	2.6E-01	3.1E-02	5.1E-03	9.0	124	
	10(11)-EpDPE	0.25	74	0.24	111	0.47	77	9.2E-01	4.2E-03	7.2E-03	2.5	58	
	4,5-DiHDPE	0.79	79	0.91	75	2.0	57	5.0E-01	1.5E-05	4.2E-05	2.7	48	
	10,11-DiHDPE	0.19	71	0.24	154	0.38	55	5.2E-01	2.2E-05	8.1E-02	2.4	52	
	13,14-DiHDPE	0.22	63	0.23	76	0.38	41	8.0E-01	9.8E-06	2.6E-04	2.0	41	
	16,17-DiHDPE	0.30	49	0.33	76	0.50	40	6.0E-01	1.6E-05	4.3E-03	1.7	38	
	Sum (n=5)	1.7	65	1.9	85	3.7	49	6.1E-01	4.6E-06	1.8E-04	2.3	39	
	9,12,13-TrHOME	3.5	67	3.3	56	3.2	55	7.5E-01	6.3E-01	8.9E-01	1.2	52	
	9,10,13-TrHOME	1.7	60	1.6	59	1.6	55	7.0E-01	8.0E-01	8.9E-01	1.3	63	
	9-HODE	13	81	13	103	9	43	9.7E-01	1.2E-01	1.1E-01	0.9	46	
ω-6	LA	13-HODE	23	79	23	83	17	49	9.3E-01	5.0E-02	5.8E-02	0.9	42
		9-KODE	2.9	122	2.2	153	1.9	104	5.2E-01	2.0E-01	6.5E-01	1.5	87
		9,10-DiHOME	3.7	73	4.5	102	2.5	52	4.4E-01	3.3E-02	2.2E-02	0.8	70
		12,13-DiHOME	6.8	61	7.6	80	5.0	46	5.9E-01	5.1E-02	1.8E-02	0.8	53
		9(10)-EpOME	4.1	101	3.2	125	2.4	99	4.2E-01	9.1E-02	3.4E-01	1.1	79
		12(13)-EpOME	6.1	93	4.6	91	3.5	76	2.9E-01	5.0E-02	1.8E-02	1.0	67

Class	PUFA	Oxylipin	Baseline (B)		Placebo supplement (P)		ω -3 supplement (ω -3)		p-values			Fold Change $[C_{\omega-3}]/[C_P]^I$	
			A_V^2	C_V^3	A_V	C_V	A_V	C_V	B/P	B/ ω -3	P/ ω -3	A_V	C_V
DGLA		Sum (n=9)	65	68	63	86	46	40	8.8E-01	3.6E-02	7.8E-02	0.9	44
		15-HETrE	0.67	100	0.54	62	0.46	45	3.8E-01	7.5E-02	1.4E-01	0.9	43
AA		Sum (n=1)	0.67	100	0.54	62	0.46	45	3.8E-01	7.5E-02	1.4E-01	0.9	42
		PGE2	0.34	251	0.06	108	0.16	156	1.2E-01	3.1E-01	9.0E-02	6.2	233
		TXB2	3.1	139	1.1	69	1.4	127	3.1E-02	7.7E-02	3.8E-01	2.1	156
		5-HETE	1.7	103	1.2	78	1.1	45	2.6E-01	7.1E-02	6.5E-01	1.2	50
		8-HETE	0.55	235	0.23	154	0.31	87	2.5E-01	3.3E-01	3.3E-01	9.1	179
		11-HETE	0.77	200	0.29	100	0.38	87	1.4E-01	1.7E-01	2.9E-01	3.0	162
		12-HETE	43	168	11	93	17	144	4.1E-02	9.3E-02	2.9E-01	2.8	163
		15-HETE	2.0	129	1.3	52	1.3	45	2.0E-01	1.3E-01	7.4E-01	1.1	44
		12-KETE	4.0	154	1.7	81	3.3	152	8.7E-02	2.9E-01	1.5E-01	6.1	318
		15-KETE	0.39	230	0.15	130	0.25	102	2.1E-01	4.2E-01	8.7E-02	2.9	93
		5(6)-EpETrE	0.51	89	0.38	50	0.34	85	1.7E-01	8.7E-02	5.5E-01	1.0	82
		8(9)-EpETrE	0.37	94	0.27	90	0.22	101	2.2E-01	7.3E-02	4.8E-01	1.2	97
		11(12)-EpETrE	0.43	134	0.29	97	0.29	130	2.5E-01	2.6E-01	9.6E-01	1.5	103
		14(15)-EpETrE	0.48	140	0.32	82	0.38	105	2.8E-01	5.0E-01	5.8E-01	1.5	81
		5(6)-DiHETrE	0.29	65	0.26	63	0.19	40	5.1E-01	2.4E-03	3.3E-02	0.8	38
		8(9)-DiHETrE	0.31	74	0.33	101	0.22	45	8.2E-01	1.7E-02	9.3E-02	0.8	38
		11(12)-DiHETrE	0.75	62	0.70	70	0.51	30	7.3E-01	4.0E-03	6.3E-02	0.9	33
		14(15)-DiHETrE	0.91	67	0.91	81	0.64	26	9.8E-01	1.3E-02	6.6E-02	0.8	29
		Sum (n=17)	60	153	20	64	28	106	4.7E-02	8.7E-02	2.8E-01	1.8	121
		Sum (n=15) ⁴	13	109	7.8	63	7.7	45	1.1E-01	5.1E-02	9.4E-01	1.2	53

^I The fold change in oxylipin serum concentrations (C) comparing subjects following ω -3 supplementation (ω -3) and placebo supplementation (P), i.e., $[C_{\omega-3}]/[C_P]$

² Average

³ Coefficient of variance

⁴ Excluding 12-HETE and 12-KETE

Table 4

Oxylipin levels in serum vs. plasma (nM).

Class	PUFA	Oxylipin	Serum (B ¹)		Plasma (HM ²)		Plasma (PP ³)	
			Av ⁴	SD ⁵	Av	SD	Av	SD
ω-3	ALA	9-HOTrE	0.71	0.61	1.98	0.12	1.19	0.91
		13-HOTrE	0.80	0.72	1.9	0.21	1.11	0.74
		15(16)-EpODE	3.9	3.2	3.27	0.23	2.77	2.1
		15,16-DiHODE	7.1	3.3	14.5	1	5.93	2.4
	EPA	12-HEPE	2.4	7.0	3.19	0.35	0.195	0.11
		15-HEPE	0.07	0.09	0.28	1.63	1.63	1.6
		11,12-DiHETE	0.04	0.03	NR ⁶	- ⁷	NR	-
		14,15-DiHETE	0.07	0.03	ND ⁸	-	0.304	0.1
	DHA	17,18-DiHETE	0.47	0.23	14.4	1.1	2.08	0.85
		10(11)-EpDPE	0.25	0.19	NR	-	NR	-
		4,5-DiHDPE	0.79	0.62	NR	-	NR	-
		10,11-DiHDPE	0.19	0.13	NR	-	NR	-
		13,14-DiHDPE	0.22	0.14	NR	-	NR	-
		16,17-DiHDPE	0.30	0.15	NR	-	NR	-
ω-6	LA	9,12,13-TriHOME	3.5	2.3	0.827	21	4.11	2.2
		9,10,13-TriHOME	1.7	1.0	0.513	0.083	1.16	0.64
		9-HODE	13	10	11.7	0.23	11	6.1
		13-HODE	23	18	47.3	0.53	58.2	28
		9-KODE	2.9	3.5	2.41	0.29	5.3	2.7
		9,10-DiHOME	3.7	2.7	60.5	3.8	29.7	11
		12,13-DiHOME	6.8	4.1	7.69	0.59	5.82	3
		9(10)-EpOME	4.1	4.1	2.17	0.23	5.47	7.4
		12(13)-EpOME	6.1	5.7	4.88	0.34	7.21	8.8
	DGLA	15-HETrE	0.67	0.67	0.437	0.028	0.732	0.45
	AA	PGE2	0.34	0.85	0.0967	0.012	0.172	0.13
		TXB2	3.1	4.3	0.865	0.18	0.919	1.6
		5-HETE	1.7	1.8	0.901	0.029	1.02	0.79
		8-HETE	0.55	1.3	2.09	0.16	0.536	0.4
		11-HETE	0.77	1.5	0.425	0.0095	0.401	0.36
		12-HETE	43	72	6.42	0.74	3.95	3.3
		15-HETE	2.0	2.6	1.8	0.098	2.04	1.2
		12-KETE	4.0	6.2	<0.1	-	<0.1	-
		15-KETE	0.39	0.90	0.749	0.08	0.682	0.76
		5(6)-EpETrE	0.51	0.45	NR	-	NR	-
		8(9)-EpETrE	0.37	0.35	<0.2	-	0.627	0.71
		11(12)-EpETrE	0.43	0.58	0.303	0.028	1.02	1.4

Class	PUFA	Oxylipin	Serum (B ¹)		Plasma (HM ²)		Plasma (PP ³)	
			Av ⁴	SD ⁵	Av	SD	Av	SD
		14(15)-EpETrE	0.48	0.67	1.77	0.05	0.442	0.59
		5,6-DiHETrE	0.29	0.19	0.264	0.025	0.189	0.092
		8,9-DiHETrE	0.31	0.23	0.294	0.056	0.244	0.078
		11,12-DiHETrE	0.75	0.47	0.779	0.037	0.566	0.2
		14,15-DiHETrE	0.91	0.61	0.714	0.031	0.603	0.18

¹Baseline values (prior to ω -3 supplementation)

²Human Metabolome (n=3 individuals). Data are from Psychogios *et al.* [39].

³Pennington Plasma (n=70 individuals). Data are from Psychogios *et al.* [39].

⁴Average

⁵Standard deviation

⁶Not reported

⁷No value

⁸Not detected

Table 5

The average [AA]/[EPA+DHA] ratios in 15-LOX- and CYP-derived oxylipins from 3 studies (Lundström *et al.* [this study], Honstra *et al.* [23] and Shearer *et al.* [22], respectively) comparing baseline, control and placebo groups to ω -3 or fish supplemented diet groups.¹

Pathway	Type ²	Cohort 1 ³	Cohort 2	Cohort 3	Study
15-LOX	Baseline	34	-	-	Lundström <i>et al.</i>
		40	16	40	Honstra <i>et al.</i>
		28	20	47	
		20	-	-	Shearer <i>et al.</i>
	Placebo	26	-	-	Lundström <i>et al.</i>
	Control	37	39	37	Honstra <i>et al.</i>
	ω -3	4	-	-	Lundström <i>et al.</i>
		3	-	-	Shearer <i>et al.</i>
	Mackerel	4	4	6	Honstra <i>et al.</i>
CYP	Baseline	1.9	-	-	Lundström <i>et al.</i>
		3.0	-	-	Shearer <i>et al.</i>
	Placebo	1.5	-	-	Lundström <i>et al.</i>
	ω -3	0.5	-	-	Lundström <i>et al.</i>
		0.8	-	-	Shearer <i>et al.</i>

¹ AA=arachidonic acid; 15-LOX=15-lipoxygenase; CYP=cytochrome P450.

² Grouped according to sampling times. Baseline: prior study; Control: following control supplement during study; Placebo: following blind control supplement during study; ω -3: following blind EPA and DHA supplement during study; and Mackerel: following mackerel paste supplement during study.

³ The study by Honstra *et al.* [23] was divided into 3 different cohorts from Tromsø, Maastricht and Zeist.