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Research article

Intake of Camelina Sativa Oil and Fatty Fish Alter the Plasma Lipid Mediator Profile in Subjects with Impaired Glucose Metabolism – A Randomized Controlled Trial

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ABSTRACT

n-3 and n-6 polyunsaturated fatty acids (PUFAs) and their lipid mediator metabolites are associated with inflammation. We investigated the effect of dietary intake of plant- and animal-derived n-3 PUFAs and fish protein on the circulatory concentrations of lipid mediators. Seventy-nine subjects with impaired fasting glucose who completed the controlled dietary intervention after randomization to the fatty fish (FF, n = 20), lean fish (LF, n = 21), *Camelina sativa* oil (CSO, n = 18) or control group (n = 20) for 12 weeks were studied. Lipid mediator profiling from fasting plasma samples before and after the intervention was performed by liquid chromatography-mass spectrometry (LC-MS/MS). The FF diet increased concentrations of 18-hydroxyeicosapentaenoic acid (18-HEPE) and 4- and 17-hydroxydocosahexaenoic acid (4-, 17-HDoHE) derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. Concentrations of lipid mediators derived from α-linolenic acid (ALA) increased and arachidonic acid (AA) derived 5-iso prostaglandin $F_{2\alpha}$-VI decreased in the CSO group. There were no significant changes in lipid mediators in the LF group. The dietary intake of both plant and animal-based n-3 PUFAs increased circulatory concentrations of lipid mediators with potential anti-inflammatory properties.

1. Introduction

Several clinical trials have studied the effects of n-3 polyunsaturated fatty acids (PUFA) on lipid mediators [1-4]. However, these trials have mainly been conducted with either eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as fish oil supplements or with flaxseeds

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; COX, cyclooxygenase; CYP, cytochrome P450; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DiHETE, dihydroxyeicosatetraenoic acid, DiHETrE, dihydroxyeicosatrienoic acid; DiHOME, dihydroxyoctadecenoic acid; HODE, hydroxyoctadecadienoic acid; EKODE, epoxyketooctadecenoic acid; EPA, eicosapentaenoic acid; EpETrE, epoxyeicosatrienoic acid; EpODE, epoxyoctadecadienoic acid; EpOME, epoxyoctadecenoic acid; HDoHE, hydroxydocosahexaenoic acid, HEPE, hydroxyeicosapentaenoic acid; HEDE, hydroxyeicosatrienoic acid; HETE, hydroxytetraenoic acid; HETrE, hydroxyeicosatrienoic aci d; HOTrE, hydroxyoctadecatrienoic acid; LXA, lipoxin A; KODE, oxo-octadecadienoic acid; 5-iP$F_{2\alpha}$-VI, 5-iso prostaglandin $F_{2\alpha}$-VI; DiHDPA, dihydroxy-docosapentaenoic acid, LA, linoleic acid; LOX, lipoxygenase; NE, non-enzymatic auto-oxidation; PGs, prostaglandins; TriHOME, trihydroxyoctadecenoic acid, TXBs, thromboxanes

The study is registered in Clinicaltrials.gov (NCT01768429)

Summary: We performed a 12-week randomized dietary intervention study consisting of a control and three intervention groups with diets enriched with fatty fish, lean fish or *Camelina sativa* oil. Changes in individual circulatory lipid mediator profiles were assessed by liquid chromatography-mass spectrometry (LC-MS/MS). Dietary fatty fish, at a minimum of four meals per week, increased circulating concentrations of hydroxy fatty acid derivatives of EPA and DHA. *Camelina sativa* oil increased concentrations of hydroxy and epoxy fatty acids derived from ALA. Our results indicate that dietary intake of both long and short chain n-3 fatty acids is reflected in the plasma concentrations of lipid mediators derived from these fatty acids.

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or flaxseed oil, which is a rich source of α-linolenic acid (ALA). There are no previous studies comparing the effects of n-3 PUFAs from different dietary sources in parallel, such as fatty fish and *Camelina sativa* oil (CSO), on lipid mediators. The consumption of lean fish has been shown to have beneficial effects on insulin sensitivity [5] and blood pressure [6], but its effects on lipid mediators have not been previously studied. The current project is part of the AlfaFish study [7], in which the effects of different dietary sources of n-3 PUFAs, e.g. from fatty fish (EPA and DHA) and *Camelina sativa* oil (ALA), on serum lipid profile and glucose metabolism were studied. Furthermore, the effects of fish protein were studied using lean fish species with a very low fat content as a source of fish protein.

Dietary linoleic acid (LA) and ALA are enzymatically converted to arachidonic acid (AA) and EPA, respectively [8]. In this pathway, the delta-6 desaturation is the rate-limiting step in the conversion of ALA to EPA, where approximately 8 % and 21 % of ALA is converted to EPA in men and women, respectively [9]. High LA intake likely decreases the delta-6 desaturation of ALA to stearidonic acid (SDA, 18:4n3), and further to EPA, due to competition of ALA and LA and their derivatives for the same enzymes [10]. Due to endogenous conversion of dietary ALA to EPA, the mechanisms of possible anti-inflammatory properties have been a matter of debate, whether ALA itself mediates the anti-inflammatory actions or if the beneficial health effects of ALA are mediated by EPA. The effects of PUFAs are partly mediated by their lipid mediators, which are the products of enzymatic or non-enzymatic conversion of PUFAs. The most common and well-characterized lipid mediators are formed from AA by cyclooxygenase (COX), lipoxygenase (LOX), cytochrome P450 (CYP) and non-enzymatic conversion by e.g. reactive oxygen species (ROS). In addition to AA, these enzymes convert LA, ALA, dihomo-ϒ-linoleic acid (DGLA, 20:3ω6), EPA and DHA to their lipid mediators [11].

Lipid mediators derived from PUFAs function as a part of signaling cascades. Several biological processes, including e.g. platelet aggregation, regulation of blood pressure and regulation of inflammation, are mediated by these lipids [11]. Both n-3 and n-6 PUFAs compete for the activity of the enzymes converting each of them to their respective lipid mediator(s) [12]. Both n-6 AA and n-3 EPA/DHA are precursors for potentially pro- or anti-inflammatory lipid mediators. The shift from n-6 to n-3 lipid mediators may potentially affect inflammation, due to differences in the affinity of analogous n-3 or n-6 derived lipid mediators on the receptors [13].

Because some of the biological actions of n-3 PUFAs are mediated by their lipid mediators, it is important to describe changes in lipid mediator profiles resulting from dietary changes in addition to dietary supplements. Within the field of inflammation and inflammatory diseases, a growing interest is aimed towards the use of n-3 PUFAs to ameliorate inflammation. Therefore, the aim of this study was to examine the effects of increased dietary intake of n-3 PUFAs and fish protein on lipid mediator profiles in subjects with impaired fasting glucose. In the present study, the effects of fatty fish (dietary source of EPA, DHA and fish protein), lean fish (source of fish protein) and CSO (source of ALA) were studied in a controlled dietary intervention study. Changes in plasma lipid mediator concentrations were also compared with the changes in the proportions of their parent PUFAs in plasma phospholipids (PL) and with the dietary intake of PUFAs.

2. Patients and methods

2.2. Study design, subjects and diets

The AlfaFish study was a 12-week randomized parallel controlled dietary intervention including 4 groups. Altogether 79 participants completed the intervention (fatty fish (FF) n = 20, lean fish (LF) n = 21, *Camelina Sativa* oil (CSO) n = 18, control (CTRL) n = 20). There was a 4-week run-in period before the intervention when participants consumed their habitual diets but discontinued the possible use of oil supplementations or products enriched in plant stanols/sterols. The baseline characteristics of the study subjects have been reported earlier [7]. In brief, the study population consisted of 79 (39/40 female/male) Caucasian subjects with impaired fasting glucose and a mean age of 58.9 ± 6.5 years. Participants in the both fish groups were instructed to consume four fish meals per week, e.g. salmon and rainbow trout in the FF group and saithe, cod, pike and perch in the LF group. The FF consumption provided approximately 1 g EPA + DHA per day in the FF group. In the CSO group the amount of CSO was 30 ml/day, containing approximately 10 g of ALA. Subjects were free living and consumed fish meals and CSO dose freely during the week or day, respectively. The CTRL and CSO groups consumed mainly lean meat and poultry and were allowed to eat one fish meal per week. The average dietary intakes of nutrients during the intervention are calculated based on the mean of 4-day food records collected at weeks 3, 7 and 11. Full description of experimental setup and dietary intake are reported in the original study article [7]. Blood samples were drawn at the beginning (week 0) and at the end of the intervention (week 12). Fasting blood samples collected in EDTA were centrifuged for 10 min, 2400 *g*, +4°C. Separated plasma fraction was frozen immediately in liquid nitrogen and stored in -80°C.

2.3. Biochemical analyses

Lipid mediators were measured with two different liquid chromatography–mass spectrometry (LC-MS/MS) methods as described earlier [14]: a general lipid mediator profiling method and a chiral separation of the monohydroxy fatty acids. Plasma PL and erythrocyte membrane (EM) phospholipid fatty acid (FA) compositions were measured by gas chromatography as previously described [15, 16]. FA composition of PL and EM are presented as mol % of total FAs.

2.4. Statistical analyses

The normality of variables within a group at each time point were tested with Shapiro-Wilk's test for both raw and log10 transformed values. Differences between the study groups were tested with ANCOVA with the following co-variates: age, gender and baseline value of the variable. In the case when both baseline and week 12 measurements were normally distributed, the paired sample t-test was used, otherwise Wilcoxon signed-ranks test was used to evaluate significance levels of changes within the groups. Normality of fold changes, raw and log10 transformed values, were tested with Kolmogorov-Smirnov test. For non-normally distributed variables, Kruskall-Wallis test was used instead of ANCOVA. Average dietary intake of LA, EPA, ALA and DHA were adjusted to body weight at the screening phase to gain information about daily dose of PUFAs (Table 1). There were no significant changes in the body weight during the intervention [17]. The log10-transformed lipid mediator concentration fold change values were correlated with average dietary intake adjusted with the baseline concentration of the corresponding lipid mediator. Statistical analyses were calculated with the SPSS statistical software (version 24, IBM Corp., Armonk, NY). Benjamini-Hochberg false discovery rate (FDR) was calculated by using RStudio (Version 1.1.463, RStudio Team, Inc., Boston, MA). An FDR-corrected p-value < 0.05 was considered as statistically significant. All the presented correlations were calculated by Spearman's rank correlation and a p-value < 0.05 was considered statistically significant. The R-package complex Heatmap [18] were used to draw the heatmaps. Cohens D values were calculated as reported previously [19]. Lipid mediators on the rows were clustered using Euclidean distances and Ward.D2 grouping method.

3. Results

3.1. Dietary intake of PUFAs and their proportions in PL and EM fractions

The average dietary intake of ALA was significantly higher in the

Table 1
Calculated average dietary intakes (mg of PUFA / kg bodyweight) of PUFAs during the 12 week intervention based on three 4-day food records.

	FF Mean ± SD	LF Mean ± SD	CSO Mean ± SD	CTRL Mean ± SD	P	FDR
Average ALA, mg/kg	32.5 ± 10.3	33.7 ± 11.9	149.5 ± 18.9	25.3 ± 9.4	1.00E-09	4.0E-09[a,b,c]
Average DHA, mg/kg	15.1 ± 8.3	2.3 ± 2.2	3.4 ± 2.1	3.2 ± 2.8	5.06E-09	6.75E-09[a,d,e]
Average EPA, mg/kg	6.4 ± 2.9	1.0 ± 1.5	1.3 ± 0.8	1.1 ± 0.9	2.75E-09	5.50E-09[a,d,e]
Average LA, mg/kg	133.4 ± 51.3	134.9 ± 41.5	162.6 ± 35.1	106.7 ± 34.6	4.40E-04	4.40E-04[c]

$P < 0.05$ for the difference between
[a] CSO and FF,
[b] CSO and LF,
[c] CSO and CTRL,
[d] FF and LF,
[e] FF and CTRL

CSO group than in the other groups. Similarly, average dietary intakes of EPA and DHA were significantly higher in the FF group than in the other groups (Table 1). In the current study the calculated daily dietary intakes of EPA and DHA varied considerably in the FF group, 526 ± 248 mg and 1235 ± 695 mg, respectively [7].

The n-3 and n-6 PUFA compositions in PL and EM fractions are shown in the supporting information (Supporting Information Table S1-2). Proportions of ALA increased significantly in PL and EM fractions in the CSO group as compared with other groups. The FF diet increased proportions of DHA in PL and EM fraction as compared with other groups. The difference in EPA in both fractions between FF and CSO was not significant. Proportions of EPA did not increase in response to CSO. Due to LA content of CSO, the proportions of LA in both fractions increased. Proportions of AA decreased in PL fraction in FF and CSO groups, in FF AA decreased also in EM. FF and CSO decreased DGLA proportions in both fractions.

3.2. Concentrations of lipid mediators at baseline

DGLA- and eicosadienoic acid (EDA, 20:2n6) -derived lipid mediators had low baseline concentrations (Supporting Information Table S5). Hydroxydocosahexaenoic acids (HDoHE) with a more distal hydroxy group group (11-, 14- and 17-HDoHEs) had higher concentrations than the ones with a more proximal hydroxy group (4- and 8-HDoHEs) (Supporting Information Table S3).

3.3. CSO and FF increased concentrations of lipid mediators derived from n-3 PUFAs

There were significant changes in the lipid mediator concentrations, especially within the CSO and FF groups (Fig. 1). The increased concentrations of ALA-derived hydroxyoctadecatrienoic acids (9- and 13-HOTrE) and 12(13)-epoxy-octadecadienoic acid (12(13)-EpODE) (Fig. 1 A-C) were significantly different in the CSO group compared with the changes in other groups. EDA-derived 15-hydroxyeicosadienoic acid (15-HEDE) increased in the CSO group (Fig. 1 D). In the FF group the concentrations of DHA-derived 4- and 17-HDoHE and 19,20- dihydroxy-docosapentaenoic acid (19,20-DiHDPA) (Fig. 1 G-I) and EPA-derived hydroxydocosahexaenoic acids (5- and 18-HEPE) increased. Concentrations and statistics of all lipid mediators are presented in supplementary materials (Supporting Information Table S3-8).

In addition to significant differences in changes between baseline and 12-week time points among the study groups, figure 2 illustrates overall changes in the plasma concentrations of each individual lipid mediator within the study groups. There was a strong increase in the concentrations of ALA-derived lipid mediators in the CSO group. Concentrations of lipid mediators derived from end products of the conversion of LA, namely DGLA and AA but not EDA, showed a tendency towards lower week 12 concentrations in the CSO group. Concentrations of EPA- and DHA-derived lipid mediators decreased in the CTRL group. Concentrations of various AA-derived lipid mediators decreased within the CSO group, but the change did not reach the level of significance when compared to other groups.

3.4. CSO decreased the concentration of AA derived 5-iPF$_{2\alpha}$-VI isoprostane

Consumption of fatty fish at a minimum of four meals per week decreased AA proportions in PL and EM fractions within the FF group (Supporting Information Table S2). Overall, the changes in AA-derived lipid mediators were minor in the FF group (Fig. 2, Supporting Information Table S8). There was a significant difference in the changes of 5-iso prostaglandin F$_{2\alpha}$-VI (5-iPF$_{2\alpha}$-VI) concentrations between the FF and CSO group (Fig. 1 J). The concentration of 5-iPF$_{2\alpha}$-VI increased in the FF group and decreased in the ALA group. Concentrations of 5-iPF$_{2\alpha}$-VI were positively correlated with the proportion of EPA in the PL fraction (Supporting Information Fig. S1A-C).

3.5. Higher dietary intake of parent PUFA was associated with the higher lipid mediator concentrations

The fold changes of all three ALA metabolites showed positive correlation with dietary intake of ALA (mg per body weight) during the intervention adjusted with baseline lipid mediator concentration (Fig. 3A). The dietary intakes of EPA and DHA (Fig. 3B-F) and LA (Fig. 3G, H) correlated with the changes in majority of their lipid mediator concentration changes. These findings suggest that subjects with lower baseline lipid mediator concentrations and higher dietary intake of its parent PUFA during the intervention had a larger increase in lipid mediator concentrations.

3.6. Correlations of PUFA proportions in PL and EM with lipid mediator concentrations

There was a positive correlation between parent PUFA proportions in PL and EM and the majority of the EPA- and DHA-derived lipid mediators at the baseline (Supporting Information Fig. S1A). Lipid mediators derived from n-6 PUFAs LA and AA were only weakly correlated to their precursor PUFAs in both fractions. At week 12, positive correlations between EPA and DHA and lipid mediators derived from these PUFAs were mainly lost in the FF group (Supporting Information Fig. S1B).

3.7. Substrate ratios associated with the ratios of formed lipid mediators by selected enzymes

The lipid mediators with homologous structures from the different pathways were analyzed as the ratios of lipid mediators derived from n-6 to n-3 PUFA derived lipid mediators. The PL AA:EPA ratio decreased in the FF group. Similarly, ratios of AA-derived lipid mediators,

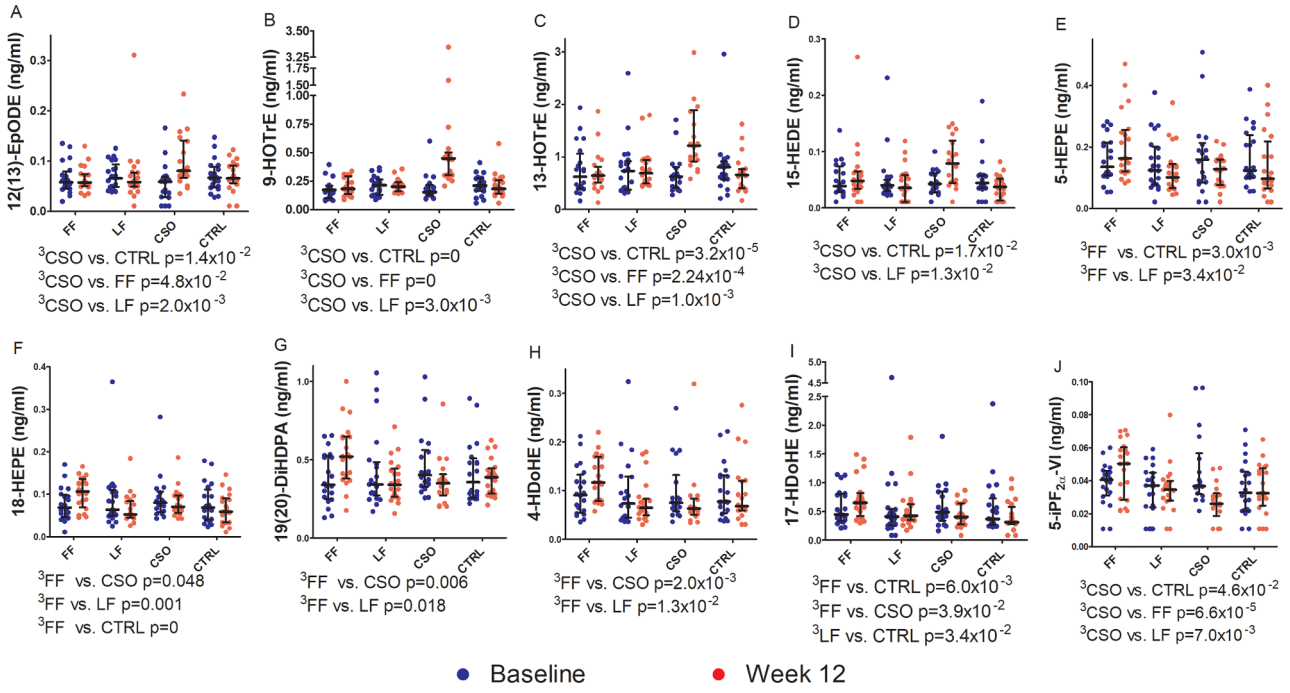

Figure 1. Median concentrations of A) 12(13)-EpODE, B) 9-HOTrE, C) 13-HOTrE, D) 15-HEDE, E) 5-HEPE, F)18-HEPE, G) 19(20)-DiHDPA, H) 4-HDoHE, I) 17-HDoHE, and J) 5-iPF$_{2\alpha}$-VI in plasma at 0 and 12 weeks. [1]ANCOVA (co-variates: age, gender, baseline concentration), [2]Kruskall-Wallis and [3]post hoc test. HDoHE; hydroxydocosahexaenoic acid, HOTrE; hydroxyoctadecatrienoic acid, HEDE; hydroxyeicosatrienoic acid, HEPE; hydroxyeicosapentaenoic acid, DiHDPA, hydroxydocosapentaenoic acid; 5-iPF$_{2\alpha}$-VI, 5-iso prostaglandin F$_{2\alpha}$-VI

hydroxyeicosatetraenoic acid (HETE), to EPA derived lipid mediators (5-HETE:5-HEPE, 11-HETE:11-HEPE and 12-HETE:12-HEPE ratios), decreased in the FF group as compared with the LF group (Supporting Information Table S10). These ratios also correlated with the AA:EPA ratio in plasma PL (Supporting Information Table S11). Ratios of the LA lipid mediator (hydroxyoctadecadienoic acid (HODE) and epoxyoctadecenoic acid (EpOME)) to ALA lipid mediators (9-HODE:9-HOTrE, 13-HODE:13-HOTrE, 12(13)-EpOME:12(13)-EpODE) decreased in the CSO group, and this change differed from other groups. Ratios of LA:ALA derived lipid mediators were positively correlated to LA:ALA ratio in plasma PL.

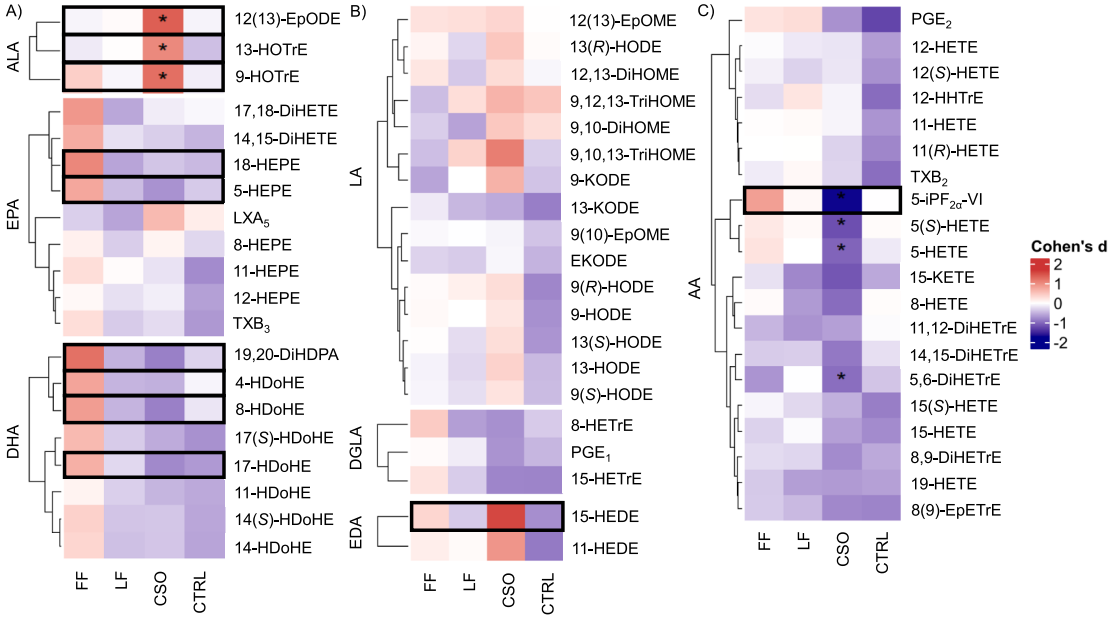

Figure 2. The directions and sizes of the changes between baseline and week 12 concentrations of lipid mediators are presented with Cohen's d values. Columns correspond to the study groups, and rows to lipid mediators derived from the PUFA annotated on the left of the each heatmap. Lipid mediators derived from A) n-3 PUFAs; ALA, EPA and DHA, B) n-6 PUFAs; LA, DGLA and EDA and C) n-6 AA. Lipid mediators are reported from both the general profiling method (e.g., 9-HODE) as well as from the chiral method (e.g., 9(*S*)-HODE, 9(*R*)-HODE). Bolded black squares indicate significant differences between the groups and black asterisk (*) indicates FDR-corrected p-value <0.05 for within group changes.

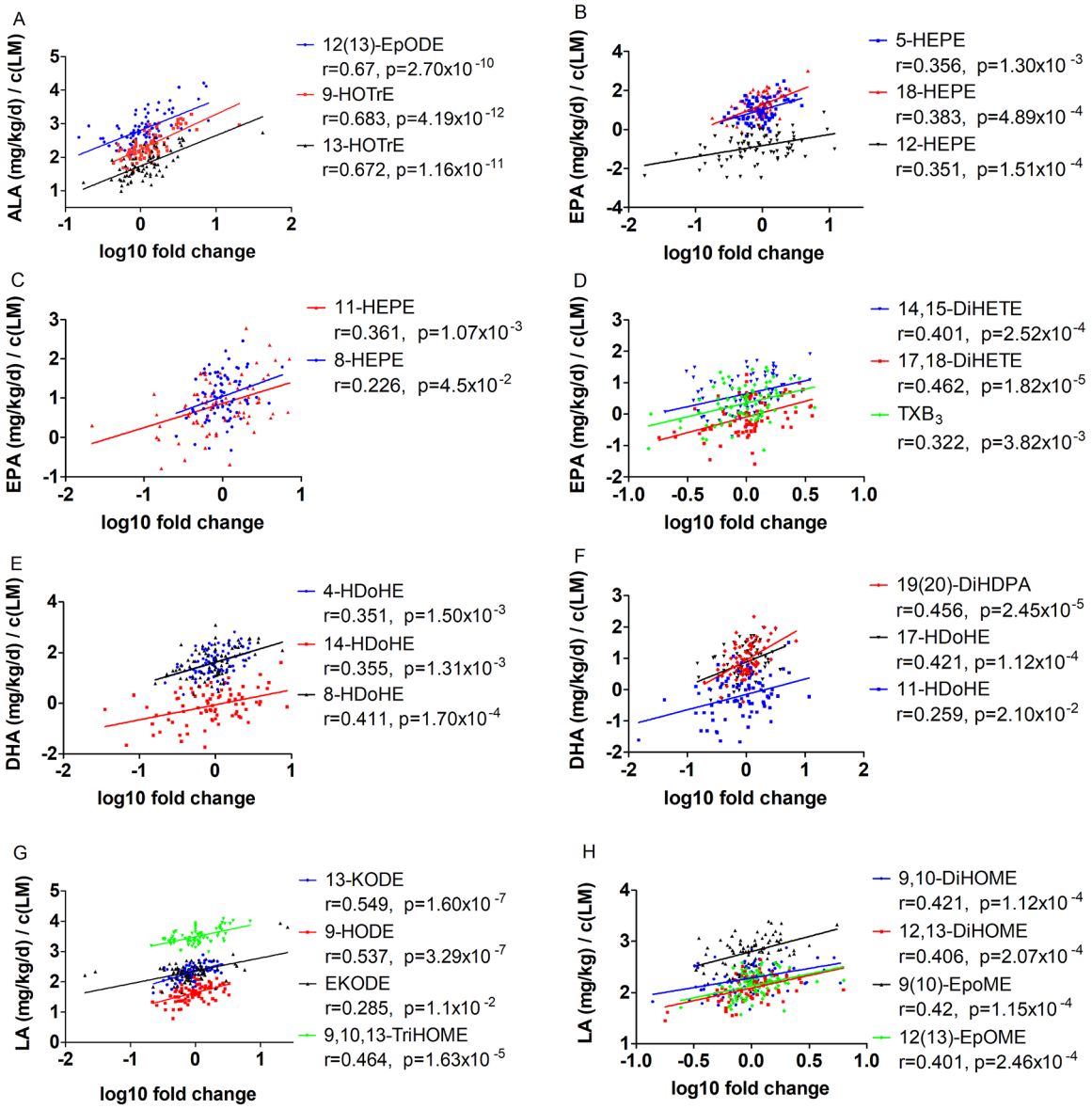

Figure 3. Association between dietary intake of fatty acids during the intervention and changes in lipid mediator concentrations. The average dietary intake, mg (fatty acid)/kg (body weight), of fatty acids (LA, ALA, EPA, DHA) was proportioned with the baseline concentration of lipid mediator and correlated with lipid mediator concentrations fold changes. A) ALA metabolites, B-D) EPA metabolites, E-F) DHA and G-H) LA metabolites (n = 79).

4. Discussion

The effects of the dietary intake of plant- and animal-derived n-3 PUFAs and fish protein on lipid mediator profiles were studied. The daily 30 ml dose of CSO increased the ALA and LA proportions in PL and concentrations of all measured ALA derived lipid mediators. Higher dietary intake of FF was associated with increased concentrations of EPA- and DHA-derived lipid mediators. Both groups with n-3-enriched diets, CSO and FF, also showed decreased proportions of n-6 PUFAs DGLA and AA in PL. The decrease in the plasma concentrations of n-6-derived lipid mediators was greater in the CSO and CTRL groups. There were no changes in lipid mediators due to increased fish protein intake in the LF group.

The increase in the dietary intake of ALA was greater than of LA in the CSO group, which may lead to competition of PUFAs in the enzymes converting ALA and LA to eicosatetraenoic acid/EPA and DGLA/AA, respectively [8]. Our data suggest that the high intake of ALA inhibits the conversion of LA to its longer chain PUFAs, even when the dietary intake of LA simultaneously increases. High dietary intake of ALA has been reported to increase EPA levels when consumed for longer periods, e.g. 42 weeks [20]. CSO consumption, 30 ml/day for 6 weeks, has been shown to increase EPA and docosapentaenoic acid (DPA, 22:5n3) proportions in serum [21]. However, the proportions of EPA did not increase in any measured fractions, namely EM, PL, cholesterol esters and triglycerides, within the CSO group during our 12-week high-ALA diet [22]. Accordingly, we did not see any changes in EPA-derived lipid mediators in the CSO group, which might be related to high baseline omega-3 index [7].

The effects of CSO on lipid mediators have not been previously studied in human subjects. However, studies about the effects of flaxseed (which is not only rich in ALA, but also contains e.g. fiber and phenolic compounds) exist. In a controlled 1-year trial consumption of mixed foods enriched with flaxseeds (30g/d, ~7000 mg/d ALA) decreased plasma concentrations of TXB_2, PGE_2 and 11,12-DiHETrE compared to placebo (wheat and mixed dietary oils) [23]. The reduction of dietary LA has been reported to decrease the circulating plasma levels of LA lipid mediators (9- and 13- HODE) and the corresponding keto-octadecadienoic acids (KODEs) [24]. The dietary intake of LA

increased in the CSO group due to LA content of CSO (LA 16 mol-%, ALA 38 mol-%, Manninen *et al,* unpublished data). However, the competition of ALA and LA for desaturase and elongase enzymes possibly decreased DGLA and AA proportions in PL (Supporting Information Table S1-2).

The increase in the concentrations of the EPA metabolites 5- and 18-HEPE and DHA metabolites 4- and 17-HDoHE and 19(20)-DiHDPA in the FF group was different from the other groups. These lipid mediators also showed a response to dietary intake of their precursor PUFA. A recent study [1] also showed increased concentrations of EPA and DHA lipid mediators after n-3 supplementation (840 mg and 1680 mg n-3 PUFA). The above-mentioned study with n-3 supplementation did not show significant changes in AA lipid mediator levels. Similarly, our results indicate that higher dietary intake of EPA and DHA does not significantly decrease plasma concentrations of AA lipid mediators.

An omega-3 index, the sum of EPA and DHA fractions in EM [25], over 8 % has been shown to be cardioprotective. The participants of the AlfaFish study had high baseline omega-3 indices: CSO: 9.3 ± 1.7, FF: 8.9 ± 1.5, LF: 8.5 ± 1.3, CTRL: 9.2 ± 1.5, p = 0.289 [7]. The dietary intake of EPA and DHA varied considerably in the FF group. Previous research carried out in the subjects whose basal omega-3 index was < 6 % [3] after an 8-week dose of 980 mg EPA and 760 mg DHA supplementation found a decrease in the EM AA levels and AA derived CYP-epoxygenase lipid mediators. The stronger effect in this study [3] than in our study was most probably due to a lower baseline omega-3 index, higher proportions of AA or more stable dosing of EPA/DHA by supplementation. At the baseline EPA- and DHA-derived lipid mediators were strongly correlated to their precursors PUFA proportions in PL and EM fractions (Supporting Information Fig. S1A). These findings regarding n-3 PUFAs may explain the low number of significant changes in EPA and DHA lipid mediators in the FF group. With lower baseline omega-3 indices a high dietary fatty fish consumption could be expected to lead to a stronger increase in EPA and DHA derived lipid mediators.

In the FF group, the proportions of AA significantly decreased in both EM and PL. In the CSO group there was tendency towards decreased AA lipid mediators, even if the decrease in AA proportions was smaller than in the FF group. Based on these observations, the higher dietary intake of ALA and EPA/DHA may decrease also the production of other lipid mediators, mainly from AA, by competition. Our findings suggest that enzymes that convert n-3 PUFAs to their lipid mediators are dependent on substrate availability. The n-3 PUFA-derived lipid mediators correlated positively with their parent PUFA level, whereas the n-6-derived lipid mediators were not tightly correlated with their parent PUFA levels. This is in line with the recent finding that correlations between n-6-derived lipid mediators and their parent PUFAs are modified by the *FADS1* genotype [26]. Nevertheless, changes in LA lipid mediator concentrations followed LA intake from dietary sources. Similarly, as Fischer et al.[3] reported, the effect of PUFA ratios on the ratios of produced lipid mediators from these PUFAs by 12- and 15-LOX, we showed that 5/8/11/12-HETE:5/8/11/12-HEPE ratios were positively correlated with the AA:EPA -ratio in PL. These results suggest that the formation of lipid mediators is partly dependent on the availability and ratios of different PUFAs.

We showed an association between dietary intakes of n-3 fatty acids and concentrations of lipid mediator derived from these. Previous results coming from this same trial [22] showed a strong correlation between the dietary intake of omega-3 PUFAs (ALA, EPA and DHA) and their proportions in different blood lipid fractions, including PL and EM. In our study, fold changes in EPA, DHA, ALA and LA derived lipid mediators were positively correlated with the baseline concentrations and dietary intake of their precursor fatty acids during the intervention. This finding is consistent with a previous study where a strong dose response and effect of baseline concentrations on esterified EPA and DHA lipid mediators was reported with n-3 supplementation (EPA + DHA, 11 ± 2 mg/kg/day) [2].

The few observed changes that did not follow the changes in the PL and EM PUFA proportions are most likely to be due to another active components enriched in the study diets. Interestingly, the CTRL group showed a tendency towards decreased AA-derived lipid mediators. Therefore, the changes in lipid mediator profiles may not always follow the changes in the levels of their parent PUFAs. Olive oil, in which n-3/n-6 ratios are low, has been reported to decrease the levels of several LA and AA derived epoxy-, hydroxy- and dihydroxy-FAs [27]. In our study, the CTRL group was instructed to consume low-fat meat and poultry and use olive oil as a salad dressing and cooking oil [7]. These changes in the dietary fat sources potentially affect lipid mediator metabolism.

The concentrations of 5-iPF$_{2\alpha}$-VI were increased in the FF group and decreased in the CSO group. This metabolite is an F2-type isoprostane and is a stable isomer of conventional prostaglandins and is produced from AA via free radical-initiated auto-oxidation [28]. Isoprostanes are often used as markers of oxidative stress and lipid peroxidation [29, 30]. Elevated urine levels of F2 isoprostanes, including 5-iPF$_{2\alpha}$-VI, have been reported in patients with homozygous familiar hypercholesterolemia [31], and they also showed that urine and plasma levels of isoprostanes are comparative. Similarly, apo$^{E-/-}$ mice have been shown to have elevated urine and plasma levels of 5-iPF$_{2\alpha}$-VI [28]. They noticed that vitamin E supplementation significantly decreased plasma and urine levels of 5-iPF$_{2\alpha}$-VI, without changes in cholesterol and triglyceride concentrations. The AlfaFish study found that CSO significantly reduced the total and LDL cholesterol concentrations [7]. The observed decrease in 5-iPF$_{2\alpha}$-VI may be due to decreased total cholesterol, vitamin E or other antioxidative compounds observed in CSO, even though CSO has relatively low content of phenolic compounds [32].

The strength of our study is that we were able to conduct a comprehensive lipid mediator profiling analyses from a well-controlled dietary intervention study. The duration of the study, 12 weeks, was long enough to observe effects of study diets on lipid mediator profiles and on the levels of their parent PUFAs in PL. We observed changes in the 5-iPF2α-VI concentrations, which may reflect the longer-term balance on oxidative stress and lipid peroxidation [33]. The elevated levels of EPA in PL and its lipid mediators have been reported after 6 h with a single dose of n-3 supplementation (1008 mg EPA, 672 mg DHA) [34].

A possible limitation of our study is that the power calculation was based on differences in the serum phospholipid DHA proportion [7]. A larger sample size may have been needed to detect more significant responses. In addition, intestinal absorption of lipids varies according to diurnal rhythms, food and light exposure [35]. Specifically, the production of n-3 derived lipid mediators has been shown to exert diurnal regulation [36]. Accordingly, further studies are needed to resolve any potential timing effects associated with specific meals. In the current study, subjects consumed fatty fish meals and daily doses of *Camelina Sativa* oil according to their daily activities. There is subsequently a potential for variability in the observed lipid mediator profiles, with differences in timing of PUFA rich meals causing differences in the absorption, distribution, and utilization of PUFAs derived from these foods. However, fatty acid levels most likely reached equilibrium following the twelve weeks of dietary intervention. In addition, the observed circulatory profiles from the different treatment groups are directly comparable because blood was always drawn at the same time (morning after 10-h fasting) for each patient. It should also be noted that only subjects with impaired glucose tolerance were recruited into this study and the results cannot be generalized to general public.

In the current study there were no changes in the plasma markers of inflammation or glucose tolerance [7]. Nevertheless, our findings may indicate some anti-inflammatory effect of n-3 enriched diets. We observed an increase in the circulating concentrations of 17-HDoHE and 18-HEPE, precursors for lipid mediators with anti-inflammatory and resolving properties, namely protectins and resolvins, respectively [37]. ALA dampens the polarization of adipose tissue macrophages towards pro-inflammatory phenotype and simultaneously increased

concentrations of ALA derived lipid mediators, including 12(13)-EpODE, 9-HOTrE and potentially anti-inflammatory 13-HOTrE *In vitro* [38, 39]. Due to the 12-week intervention time we cannot estimate the long-term effects of the changes on the observed lipid mediators on cardiovascular health and inflammation.

The study design of the AlfaFish study enabled us to study the effects of dietary fish and CSO on the metabolism of PUFAs and their associated lipid mediators with a comprehensive metabolic profiling approach. The design of controlled intervention enabled the detailed comparison of the effects of short and long chain n-3 PUFAs and fish protein on lipid mediator profiles. Our data indicate that the changes in the lipid mediator concentrations after 12 weeks of high fish consumption are mediated by EPA and DHA. Fish protein alone did not affect the concentrations of measured lipid mediators in this study. Overall, our results are in line, but not fully comparable, with previous research conducted with n-3 supplements. While increased intake of FF resulted in significantly higher concentrations of various EPA- and DHA-derived lipid mediators, dietary ALA intake from CSO increased concentrations of ALA-derived mediators and decreased concentrations of AA-derived lipid mediators.

CRediT authorship contribution statement

Topi Meuronen: Formal analysis, Writing - original draft, Writing - review & editing. **Maria A. Lankinen:** Conceptualization, Writing - original draft, Writing - review & editing, Investigation. **Alexander Fauland:** Methodology, Investigation. **Bun-ichi Shimizu:** Methodology, Investigation. **Vanessa D. de Mello:** Conceptualization. **David E. Laaksonen:** Investigation. **Craig E. Wheelock:** Writing - review & editing, Resources, Methodology. **Arja T. Erkkilä:** Conceptualization, Investigation. **Ursula S. Schwab:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing.

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U.S.S., M.A.L., and A.T.E. formulated research questions, designed and conducted the study. M.A.L., B.S., A.F. and C.E.W. were responsible for lipid mediator profiling. T.M. and M.A.L. analyzed the data and performed statistical analyses. T.M., M.A.L. and U.S.S. wrote the paper. D.E.L. checked the language as a native speaker. All authors have commented the manuscript. The authors gratefully thank Tuomas Onnukka, Erja Kinnunen, Anu Holopainen, and Päivi Turunen for excellent technical assistance. Suomen Kasviöljyt Ltd., Kesko Ltd., and Bunge Finland Ltd. provided oil and fat spreads. The study was financially supported by Finnish Diabetes Research Foundation; Competitive Research Funding of the Northern Savo Hospital District special state subsidy for health research; Juho Vainio Foundation; the North Savo Regional Fund of the Finnish Cultural Foundation; Paavo Nurmi Foundation; Yrjö Jahnsson Foundation (grant number 6437); Academy of Finland (grant number 309311); Matti Uusitupa foundation (Juhlaseminaari rahasto); The Swedish Heart Lung Foundation (HLF 20170734, HLF 20180290), the Swedish Research Council (2016-02798); Tripartite Immunometabolism Consortium–Novo Nordisk Foundation (grant NNF15CC0018486) and the Karin and Sten Mörtstedt Initiative on Anaphylaxis. Funding sources were not involved in the implementation of the intervention, data-analyses or writing of the manuscript. The authors declare no conflict of interest.

Supplementary materials

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