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The effect of camelina sativa oil and fish intakes on fatty acid compositions of blood lipid fractions

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Abstract *Background and aims:* Blood lipid fractions serve as objective biomarkers of dietary fat intake. It is unclear which fatty acid pool most accurately reflects the dietary intakes of different *n*-3 PUFAs. We aimed to investigate the effect of fish and camelina sativa oil (CSO) intakes on fatty acid composition of erythrocyte membranes (EM), plasma phospholipids (PL), cholesteryl esters (CE) and triglycerides (TG). We also aimed to identify the most appropriate blood lipid fraction for assessing n-3 PUFA intake.

Methods and results: Altogether 79 volunteers with impaired glucose metabolism were randomly assigned either to CSO, fatty fish, lean fish or control groups for 12 weeks. Fatty acid compositions of lipid pools were measured by gas chromatography. The proportion of alpha-linolenic acid (ALA) increased in all lipid pools in the CSO group (false discovery rate (FDR) $p < 0.001$ for all). Similarly, the proportions of EPA and DHA increased in all lipid fractions in the fatty fish group (FDR $p < 0.001$ for EM, PL and CE; FDR $p = 0.005$ for TG; FDR $p < 0.001$ for EM, PL, CE; FDR $p < 0.007$ for TG, respectively). Changes in the dietary intakes of ALA, EPA and DHA correlated with the changes in their proportions in all lipid pools ($r = 0.3–0.5$, $p < 0.05$).

Conclusion: There is no difference in the ability of blood lipid fractions in reflecting the dietary intake of different *n*-3 PUFAs over a time period of 12 weeks in subjects with high baseline omega-3 index.

This trial was registered in Clinicaltrials.gov (NCT01768429)

Introduction

Fatty acid composition in different tissues and blood lipid fractions serve as objective biomarkers of dietary fat intake [1–3]. Erythrocyte membranes (EM) and plasma and serum phospholipids (PL), cholesteryl esters (CE), triglycerides (TG), for example, are commonly used. Plasma lipid fractions reflect dietary fat intake over the preceding days and weeks, whereas the lifespan of erythrocytes is 120 days [1,2,4]. EM fatty acid composition may therefore

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be a more reliable indicator of long-term dietary fat intake than plasma fatty acids [2,5]. In addition to diet and other lifestyle factors, genes significantly affect the variation of fatty acid composition in different lipid pools [6–8].

Several studies have assessed the intake of major marine *n*-3 PUFAs (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) [2–5,9–12] and the intake of the plant-derived *n*-3 PUFA, alpha-linolenic acid (ALA) [12–16] with different biomarkers. However, only a few studies have investigated the biomarkers of both the marine *n*-3 PUFAs and plant-derived *n*-3 PUFA [12,14] and, to our knowledge, there are no previous dietary intervention studies comparing the effects of the intakes of *n*-3 PUFAs from plant origin and animal origin as food on the fatty acid composition of EM, PL, CE and TG. Furthermore, biomarkers of long-chain *n*-3 PUFA have been mostly investigated in supplementation studies, although the bioavailability of *n*-3 fatty acids has been shown to be better from fish than fish oil [17]. Due to lack of endogenous synthesis in humans the ALA content of various biomarkers tends to correlate well with the dietary intake of ALA [1]. Furthermore, marine *n*-3 PUFA intake and the *n*-3 PUFA contents of different biomarkers are strongly correlated [2,3,5]. It is unclear, however, which fatty acid pool most accurately reflects the dietary intakes of different *n*-3 PUFAs [1,3,18].

In this study we investigate how intakes of fish and camelina sativa oil (CSO), a source of ALA, affect the fatty acid composition of EM and plasma lipid fractions. We also aimed to identify the most appropriate blood lipid fraction for assessing *n*-3 PUFA intake.

Methods

Study population

Details about the recruitment and study subjects have been described previously [19]. Briefly, all recruited subjects had impaired glucose metabolism: fasting plasma glucose concentration 5.6–6.9 mmol/l and the 2-h glucose concentration < 11.0 mmol/l in the oral glucose tolerance test (OGTT). Subjects did not differ in the baseline characteristics among the groups (Supplementary Table 1).

Study design

The study was approved by the Ethical committee of the Hospital District of Northern Savo (55/2012). The subjects received both oral and written information and gave their written informed consent. The intervention has been described previously [19]. Before the intervention, the subjects were instructed discontinue the use of any oil supplements or products enriched in plant stanols or sterols, which were not allowed during the study. After a 4-week run-in period the subjects were randomly assigned into four parallel groups for 12 weeks: CSO, lean fish, fatty fish or control group. The blood samples were drawn after 10-h overnight fasting at baseline (0 wk) and at the end of the study (12 wk). Physical activity, alcohol intake, smoking, body weight and use of medication known to affect the measures of lipid metabolism were kept constant during the study.

The study diets were isocaloric and current nutrient recommendations [20] were followed except for fish and ALA intakes. Subjects in the fish groups were instructed to consume four fish meals per week, e.g. salmon, vendace in the fatty fish group providing around 1 g EPA+DHA per day and e.g. pike and perch in the lean fish group. The CSO group ingested camelina sativa oil (27 g) in order to get 10 g ALA per day. The fatty acid composition of CSO is following: ALA 38.4%, linoleic acid 16.4%, PUFA 57.1%, MUFA 32.7% and SFA 10.2%. The control and CSO groups were allowed to eat one fish meal per week and were instructed to consume lean meat and chicken. Dietary intakes of ALA, EPA, DHA, SFA, MUFA and PUFA have been shown in Supplementary Fig. 1. Other dietary intakes have been reported in detail elsewhere [19]. Food records from four consecutive days, including one weekend day, were collected and checked at return by clinical nutritionists at baseline and during the study in weeks 3, 7 and 11. The food records were analyzed with AivoDiet nutrient calculation software (v. 2.0.2.1, Aivo Finland, Turku, Finland) based on national and international analyses and international food composition tables (fineli.fi).

Fatty acid composition analyses

Fatty acid composition of EM and lipid fractions were analyzed using gas chromatography as previously described [2,21]. Method has been described in Supplementary text (Method 1). The fatty acid composition of EM and lipid fractions is expressed as mol % of total fatty acids. Additionally, serum concentration of DHA was determined using high-throughput proton nuclear magnetic resonance (NMR) spectroscopy. Method has been described in detail previously [22,23].

Statistical analyses

The Kolmogorov–Smirnov test was used to test the normality of the variables and logarithmic transformation was performed for skewed variables. For the comparison of baseline and end point values within the groups, Paired samples t-test or Wilcoxon signed ranks test were used. Changes in the fatty acid composition of different blood lipid fractions among the groups were tested with the analysis of covariance (ANCOVA) followed by multiple comparisons test with Bonferroni correction. Adjustments for baseline values, age and sex were used in ANCOVA models. Analyses were performed using fold changes. Fold changes were calculated by dividing the endpoint values of the variable by their baseline values. $P < 0.05$ was considered statistically significant, and Benjamini-Hochberg false discovery rate (FDR) was calculated by using R Project for Statistical Computing, version 3.2.2 due to multiple comparisons. Associations between dietary intakes of ALA, EPA and DHA and their proportions in different blood lipid fractions were tested using Spearman

rank correlation. IBM SPSS statistical software (v. 21, IBM Corp., Armonk, NY) was used for statistical analyses.

Results

Changes in fatty acid compositions of plasma erythrocyte membranes and lipid fractions

ALA, EPA and DHA

The changes in fatty acid compositions (mol %) of different fractions are presented in Tables 1–4 and changes in the concentrations of fatty acids in PL, CE and TG in Supplementary Tables 4–6. The proportion of ALA (18:3*n*-3) in EM, PL, CE and TG and the concentration of ALA in PL, CE and TG increased in the CSO group as compared with other groups (overall differences among the groups FDR $p < 0.001$ for all). In other groups, there were no significant changes in the concentration or proportion of ALA in any fractions except an increase in CE within the fatty fish group (Table 3). The proportions of EPA (20:5*n*-3) in EM, PL, CE and TG increased in the fatty fish group (overall differences among groups FDR $p < 0.001$ for EM, PL and CE, FDR $p = 0.004$ for TG) (Tables 1–4). In Bonferroni's pairwise comparison the change in EM and CE was significant as compared with all other groups and in PL and TG as compared with the lean fish and control groups. The concentration of EPA increased in PL and CE the fatty fish group (FDR $p < 0.001$ for both) (Supplementary Tables 4 and 5). The change in TG EPA was statistically significant after adjusting for baseline value, sex and age ($p = 0.019$) but not after FDR-adjustment (Supplementary Table 6). The proportion of DHA (22:6*n*-3) in EM, PL, CE and TG increased in the fatty fish group (FDR $p < 0.001$ for EM, PL, CE, $p < 0.007$ for TG) as compared with the other groups but the change within the fatty fish group was statistically significant only in EM and PL (Tables 1–4). The concentration of DHA increased in PL and CE in the fatty fish group (FRD $p < 0.001$ for both) (Supplementary Tables 4 and 5) but not in TG (Supplementary Table 6). Instead, the proportion of DHA decreased in EM and PL (Tables 1 and 2) and the concentration of DHA decreased in PL and CE (Supplementary Tables 4 and 5) in the CSO group. In the lean fish group, the proportion or concentration of DHA did not change significantly in any fraction.

SFA, MUFA and PUFA

There was a decrease in the proportion of SFA in all fractions within the CSO group, but compared with all the other groups the change was statistically significant only in EM (Table 1). In the CE fraction the decrease in SFA in the CSO group was significant as compared with the fatty fish and lean fish groups. Of individual SFAs, palmitic acid (16:0) proportion in all fractions decreased within the CSO group (Tables 1–4). However, as compared with the other groups, the change was significant only in CE. The proportion of stearic acid (18:0) did not change in any fractions among the groups.

The proportion of total MUFA varied in different fractions and among the groups. An increase was found in EM within all the groups and in the CSO group as compared with the other groups (Table 1). In contrast, in CE the proportion of total MUFA decreased in the CSO group as compared with the lean fish and control groups (Table 3) whereas there were no statistically significant changes in TG (Table 4). In PL fraction the proportion of total MUFA increased in the control group as compared with the fatty fish group but the change was no longer statistically significant after adjusting for baseline values, sex and age (Table 2). The proportion of oleic acid (18:1*n*-9) in EM increased in the lean fish and control groups as compared with the CSO group (Table 1).

There were no changes in the proportion of total PUFAs in EM or PL fractions (Tables 1 and 2). However, in CE and TG the proportion of PUFAs increased in the CSO group (Tables 3 and 4). In pairwise comparison using Bonferroni's correction, the CSO group significantly differed from the lean fish and control groups only in the CE fraction.

Correlations between dietary ALA, EPA, DHA and their proportions in erythrocyte membranes and lipid fractions

Baseline associations

Baseline associations of dietary ALA, EPA and DHA with their respective proportions in different lipid pools are presented in Supplementary Table 2. Baseline associations of these fatty acids and their respective concentrations in PL, CE and TG are presented in Supplementary Table 7. For DHA we also had a serum concentration determined by NMR. Dietary ALA intake was associated with the proportion of ALA in blood lipid fractions at baseline ($r = 0.273$, $r = 0.229$, $r = 0.269$, $p < 0.05$ for EM, PL and CE, respectively; $r = 0.304$, $p < 0.01$ for TG) whereas intake of ALA did not correlate with the concentrations of ALA in PL, CE or TG at baseline. Similar baseline associations were also found for dietary EPA and the proportion of EPA in all fractions ($r = 0.244$, $p < 0.05$ for EM; $r = 0.309$, $r = 0.326$, $r = 0.334$, $p < 0.01$ for PL, CE and TG, respectively) as well as the concentration of EPA ($r = 0.292$, $p < 0.01$ for PL; $r = 0.267$, $r = 0.239$, $p < 0.05$ for CE and TG, respectively). Dietary DHA was associated with the proportion of DHA only in TG at baseline ($r = 0.252$, $p < 0.05$) whereas DHA intake did not correlate with the concentration of DHA.

Associations during intervention

Associations of mean dietary ALA, EPA and DHA during intervention (weeks 3, 7, 11) with their respective proportions in different lipid pools after the intervention (week 12) are presented in Supplementary Table 3. Associations of these fatty acids with their respective concentrations in PL, CE and TG are presented in Supplementary Table 8. The mean dietary ALA during the intervention was associated with the proportion of ALA in all fractions ($r = 0.526$, $r = 0.555$, $r = 0.585$, $r = 0.523$, $p < 0.001$ for EM, PL, CE and TG, respectively). Similarly, dietary intake of ALA correlated with the concentration of ALA ($r = 0.512$, $r = 0.581$, $r = 0.478$, $p < 0.001$ for PL, CE and TG,

Table 1 Erythrocyte membrane fatty acid composition at baseline and at the end of the study.

Fatty acid (mol%)	CSO (n = 18)		Fatty fish (n = 20)		Lean fish (n = 21)		Control (n = 20)		p values ANCOVA[a]		
	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	Model 1	Model 2	FDR
Myristic acid (14:0)	0.47 ± 0.07	0.42 ± 0.06[b]	0.42 ± 0.05	0.41 ± 0.06	0.47 ± 0.07	0.42 ± 0.08[b]	0.42 ± 0.09	0.42 ± 0.07	**0.006**[c]	0.253	0.285
Pentadecanoic acid (15:0)	0.17 ± 0.03	0.15 ± 0.03[b]	0.15 ± 0.03	0.14 ± 0.02[b]	0.17 ± 0.03	0.15 ± 0.03[b]	0.16 ± 0.07	0.17 ± 0.12	0.539	0.396	0.428
Palmitic acid (16:0)	21.1 ± 0.73	20.70 ± 0.76[b]	20.92 ± 0.71	20.96 ± 0.76	21.26 ± 0.53	21.13 ± 0.70	21.01 ± 0.76	21.15 ± 0.78	**0.009**[d]	**0.017**[d]	**0.021**
Margaric acid (17:0)	0.32 ± 0.04	0.31 ± 0.04	0.29 ± 0.05	0.28 ± 0.04[b]	0.31 ± 0.03	0.30 ± 0.04	0.32 ± 0.15	0.32 ± 0.18	0.959	0.872	0.872
Stearic acid (18:0)	14.94 ± 0.33	14.82 ± 0.40	15.03 ± 0.45	14.81 ± 0.45[b]	14.95 ± 0.41	14.88 ± 0.44	15.05 ± 0.45	14.92 ± 0.42[b]	0.472	0.506	0.525
Arachidic acid (20:0)	0.53 ± 0.06	0.59 ± 0.07[b]	0.53 ± 0.09	0.54 ± 0.08	0.55 ± 0.06	0.55 ± 0.06	0.53 ± 0.08	0.53 ± 0.08	**0.001**[def]	**0.001**[def]	**0.002**
Behenic acid (22:0)	1.67 ± 0.18	1.52 ± 0.16[b]	1.62 ± 0.29	1.59 ± 0.29	1.63 ± 0.17	1.62 ± 0.16	1.68 ± 0.21	1.63 ± 0.20	**0.002**[ef]	**0.003**[ef]	**0.005**
Lignoceric acid (24:0)	4.84 ± 0.46	4.32 ± 0.45[b]	4.70 ± 0.35	4.63 ± 0.40	4.72 ± 0.19	4.67 ± 0.25	4.78 ± 0.31	4.63 ± 0.31[b]	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
Saturated	44.00 ± 0.58	42.81 ± 0.52[b]	43.65 ± 0.66	43.35 ± 0.56[b]	44.05 ± 0.64	43.72 ± 0.60[b]	43.96 ± 0.64	43.77 ± 0.53	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
Palmitoleic acid (16:1n-7)	0.40 ± 0.12	0.34 ± 0.08[b]	0.46 ± 0.17	0.42 ± 0.15[b]	0.42 ± 0.42	0.40 ± 0.10	0.39 ± 0.06	0.41 ± 0.11	**0.012**[d]	**0.016**[d]	**0.021**
Oleic acid (18:1n-9)	12.16 ± 0.84	12.01 ± 0.75	12.03 ± 0.73	12.19 ± 0.76[b]	12.46 ± 0.78	12.63 ± 0.59	11.86 ± 0.68	12.27 ± 0.73[b]	**0.004**[d]	**0.007**[df]	**0.010**
Vaccenic acid (18:1n-7)	1.04 ± 0.11	1.01 ± 0.09	1.09 ± 0.09	1.09 ± 0.08	1.08 ± 0.10	1.11 ± 0.10[b]	1.08 ± 0.07	1.11 ± 0.07	**0.021**[d]	**0.002**[df]	**0.003**
Eicosenoic acid (20:1n-9 + 11)	0.34 ± 0.08	0.68 ± 0.15[b]	0.33 ± 0.04	0.34 ± 0.04	0.32 ± 0.06	0.32 ± 0.04	0.31 ± 0.05	0.33 ± 0.09	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
Nervonic acid (24:1n-9)	5.33 ± 0.47	6.82 ± 0.52[b]	5.42 ± 0.60	5.71 ± 0.50[b]	5.29 ± 0.48	5.46 ± 0.43[b]	5.51 ± 0.51	5.69 ± 0.47	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
Monounsaturated	19.26 ± 1.09	20.86 ± 1.04[b]	19.32 ± 1.01	19.76 ± 0.93[b]	19.57 ± 1.01	19.92 ± 0.73[b]	19.16 ± 0.94	19.81 ± 0.92[b]	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
α-linolenic acid (18:3n-3)	0.18 ± 0.05	0.43 ± 0.13[b]	0.19 ± 0.05	0.20 ± 0.05	0.22 ± 0.06	0.21 ± 0.07	0.18 ± 0.05	0.23 ± 0.14	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
Eicosapentaenoic acid (20:5n-3)	1.65 ± 0.78	1.63 ± 0.55	1.50 ± 0.58	1.90 ± 0.51[b]	1.58 ± 0.42	1.41 ± 0.40	1.43 ± 0.44	1.29 ± 0.35	**< 0.001**[egh]	**< 0.001**[egh]	**< 0.001**
Docosapentaenoic acid (22:5n-3)	3.05 ± 0.41	3.25 ± 0.44	3.09 ± 0.25	3.03 ± 0.29	3.04 ± 0.21	2.87 ± 0.23[b]	3.15 ± 0.31	3.04 ± 0.31[b]	**< 0.001**[def]	**< 0.001**[def]	**< 0.001**
Docosahexaenoic acid (22:6n-3)	7.70 ± 1.03	6.67 ± 0.91[b]	7.42 ± 1.15	8.17 ± 0.93[b]	6.91 ± 1.07	7.22 ± 0.87	7.76 ± 1.18	7.24 ± 0.96[b]	**< 0.001**[cefh]	**< 0.001**[defgh]	**< 0.001**
Sum of n-3	12.57 ± 1.90	11.99 ± 1.46	12.19 ± 1.62	13.30 ± 1.31	11.74 ± 1.27	11.71 ± 1.09	12.53 ± 1.62	11.80 ± 1.25	**< 0.001**[egh]	**< 0.001**[egh]	**< 0.001**
Linoleic acid (18:2n-6)	7.93 ± 0.93	8.37 ± 0.97[b]	8.00 ± 0.78	7.69 ± 0.68[b]	8.17 ± 0.87	8.08 ± 0.86	7.69 ± 0.80	7.77 ± 0.73	**< 0.001**[efg]	**< 0.001**[def]	**< 0.001**
Dihomo-γ-linolenic acid (20:3n-6)	1.52 ± 0.26	1.40 ± 0.29[b]	1.65 ± 0.28	1.54 ± 0.26[b]	1.50 ± 0.28	1.45 ± 0.26	1.53 ± 0.32	1.56 ± 0.33	**0.005**[dg]	**0.009**[dg]	**0.012**
Arachidonic acid (20:4n-6)	12.54 ± 0.99	12.49 ± 0.90	13.01 ± 1.11	12.41 ± 1.01[b]	12.73 ± 0.69	12.87 ± 0.77	12.79 ± 1.51	12.87 ± 1.01	**0.003**[gh]	**< 0.001**[gh]	**< 0.001**
Adrenic acid (22:4n-6)	1.90 ± 0.53	1.82 ± 0.44	1.85 ± 0.46	1.67 ± 0.37[b]	1.95 ± 0.40	1.94 ± 0.38	2.02 ± 0.49	2.09 ± 0.49	**0.012**[g]	**< 0.001**[gh]	**< 0.001**
Osbond acid (22:5n-6)	0.28 ± 0.06	0.25 ± 0.07[b]	0.32 ± 0.07	0.28 ± 0.06[b]	0.29 ± 0.07	0.31 ± 0.06[b]	0.31 ± 0.10	0.32 ± 0.08	**< 0.001**[dfgh]	**< 0.001**[dfgh]	**< 0.001**
Sum of n-6	24.17 ± 1.39	24.34 ± 1.22	24.84 ± 1.55	23.59 ± 1.24	24.63 ± 1.16	24.65 ± 1.09	24.35 ± 1.64	24.62 ± 1.25	**< 0.001**[egh]	**< 0.001**[egh]	**< 0.001**
Polyunsaturated	36.74 ± 1.22	36.32 ± 0.92[b]	37.03 ± 0.88	36.90 ± 1.03	36.38 ± 1.12	36.36 ± 0.74	36.88 ± 0.89	36.42 ± 0.94	0.266	0.243	0.285

Values are means ± SD. ANCOVA, analysis of covariance; CSO, camelina sativa oil.

Post hoc tests: [c] $p < 0.05$ lean fish vs control group; [d] $p < 0.05$ CSO vs. control group; [e] $p < 0.05$ CSO vs. fatty fish group; [f] $p < 0.05$ CSO vs. lean fish group; [g] $p < 0.05$ fatty fish vs. control group; [h] $p < 0.05$ fatty fish vs. lean fish group.

[a] Differences in fold changes among the groups were tested using ANCOVA and Bonferroni's post hoc tests. ANCOVA: Model 1 no adjustments. Model 2 adjusted for baseline value, age and sex. Benjamini-Hochberg false discovery rate (FDR) was used to adjust results for multiple comparisons.

[b] Change within the group was determined by Paired samples t-test or Wilcoxon signed ranks test, $p < 0.05$.

Table 2 Fatty acid composition in plasma phospholipids at baseline and at the end of the study.

Fatty acid (mol%)	CSO (n = 18)		Fatty fish (n = 20)		Lean fish (n = 21)		Control (n = 20)		p values ANCOVA[a]		
	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	Model 1	Model 2	FDR
Myristic acid (14:0)	0.53 ± 0.13	0.47 ± 0.08[b]	0.45 ± 0.10	0.43 ± 0.09	0.53 ± 0.15	0.46 ± 0.12[b]	0.43 ± 0.10	0.44 ± 0.11	0.186	0.933	0.933
Pentadecanoic acid (15:0)	0.23 ± 0.06	0.22 ± 0.04	0.20 ± 0.05	0.19 ± 0.04	0.23 ± 0.05	0.20 ± 0.05[b]	0.20 ± 0.04	0.19 ± 0.05	0.471	0.447	0.503
Palmitic acid (16:0)	29.01 ± 1.24	28.42 ± 1.15[b]	29.21 ± 1.54	29.12 ± 1.44	29.17 ± 1.09	28.84 ± 0.93	29.34 ± 1.48	29.12 ± 1.20	0.592	0.277	0.394
Margaric acid (17:0)	0.37 ± 0.07	0.38 ± 0.06	0.36 ± 0.11	0.34 ± 0.07	0.36 ± 0.06	0.35 ± 0.04	0.36 ± 0.07	0.32 ± 0.07[b]	**0.038**[c]	**0.011**[c]	**0.025**
Stearic acid (18:0)	12.38 ± 0.97	12.42 ± 1.13	12.10 ± 1.13	12.10 ± 1.00	12.10 ± 0.82	12.19 ± 0.84	12.56 ± 0.94	12.13 ± 0.95[b]	0.186	0.394	0.484
Arachidic acid (20:0)	0.42 ± 0.11	0.49 ± 0.11[b]	0.41 ± 0.09	0.41 ± 0.08	0.44 ± 0.08	0.44 ± 0.09	0.42 ± 0.13	0.43 ± 0.11	0.053	**0.020**[d]	**0.039**
Behenic acid (22:0)	0.85 ± 0.17	0.80 ± 0.18	0.75 ± 0.18	0.75 ± 0.17	0.82 ± 0.12	0.86 ± 0.20	0.81 ± 0.15	0.76 ± 0.20	0.276	0.347	0.446
Lignoceric acid (24:0)	0.76 ± 0.18	0.63 ± 0.17[b]	0.64 ± 0.15	0.64 ± 0.17	0.71 ± 0.13	0.72 ± 0.18	0.70 ± 0.12	0.65 ± 0.17	**0.014**[de]	0.050	0.083
Saturated	44.55 ± 0.83	43.83 ± 1.10[b]	44.13 ± 0.86	43.99 ± 1.04	44.35 ± 0.90	44.06 ± 0.93	44.81 ± 1.74	44.05 ± 0.77[b]	0.237	0.667	0.720
Palmitoleic acid (16:1n-7)	0.64 ± 0.19	0.60 ± 0.22	0.80 ± 0.33	0.71 ± 0.27[b]	0.71 ± 0.23	0.68 ± 0.25	0.63 ± 0.11	0.72 ± 0.26	**0.016**[f]	**0.038**	0.068
Oleic acid (18:1n-9)	10.40 ± 1.77	10.44 ± 1.46	10.88 ± 1.73	10.70 ± 1.31	10.31 ± 1.36	10.82 ± 0.93	9.73 ± 0.84	10.74 ± 1.52[b]	**0.018**[f]	0.176	0.264
Vaccenic acid (18:1n-7)	1.40 ± 0.21	1.36 ± 0.19	1.48 ± 0.20	1.49 ± 0.20	1.50 ± 0.18	1.49 ± 0.24	1.45 ± 0.16	1.48 ± 0.14	0.617	0.428	0.502
Eicosenoic acid (20:1n-9 + 11)	0.32 ± 0.07	0.56 ± 0.12[b]	0.33 ± 0.05	0.36 ± 0.10	0.31 ± 0.03	0.32 ± 0.04	0.31 ± 0.06	0.32 ± 0.05	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Nervonic acid (24:1n-9)	1.60 ± 0.26	2.10 ± 0.21[b]	1.63 ± 0.41	1.76 ± 0.31	1.68 ± 0.26	1.74 ± 0.39	1.68 ± 0.35	1.64 ± 0.38	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Monounsaturated	14.35 ± 1.70	15.05 ± 1.60[b]	15.12 ± 1.91	15.02 ± 1.41	14.51 ± 1.60	15.05 ± 1.17	13.80 ± 1.00	14.90 ± 1.48[b]	**0.027**[f]	0.313	0.423
α-linolenic acid (18:3n-3)	0.38 ± 0.12	0.80 ± 0.23[b]	0.36 ± 0.11	0.41 ± 0.15	0.43 ± 0.11	0.42 ± 0.11	0.32 ± 0.10	0.35 ± 0.09	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Eicosapentaenoic acid (20:5n-3)	2.22 ± 1.18	2.56 ± 1.08	2.00 ± 1.19	2.72 ± 0.61[b]	2.24 ± 1.11	1.94 ± 0.82	2.04 ± 0.78	1.96 ± 0.84	**< 0.001**[fg]	**< 0.001**[fg]	**< 0.001**
Docosapentaenoic acid (22:5n-3)	1.32 ± 0.22	1.35 ± 0.20	1.34 ± 0.19	1.34 ± 0.17	1.30 ± 0.13	1.22 ± 0.14[b]	1.33 ± 0.16	1.29 ± 0.17[b]	0.068	**0.018**[e]	**0.037**
Docosahexaenoic acid (22:6n-3)	5.87 ± 1.23	5.20 ± 1.23[b]	5.78 ± 1.30	6.48 ± 1.04[b]	5.40 ± 1.27	5.42 ± 1.05	6.16 ± 1.05	5.63 ± 1.03	**< 0.001**[df]	**< 0.001**[dfg]	**< 0.001**
Sum of n-3	9.80 ± 2.29	9.91 ± 2.23	9.48 ± 2.29	10.94 ± 1.31	9.37 ± 2.17	9.01 ± 1.71	9.85 ± 1.60	9.22 ± 1.65	**< 0.001**[dfg]	**< 0.001**[dfg]	**< 0.001**
Linoleic acid (18:2n-6)	19.61 ± 2.50	20.61 ± 2.58[b]	18.96 ± 2.65	18.65 ± 2.07	19.75 ± 2.45	20.04 ± 2.33	18.46 ± 2.67	18.51 ± 2.61	0.373	0.052	0.083
Dihomo-γ-linolenic acid (20:3n-6)	3.03 ± 0.73	2.46 ± 0.54[b]	3.02 ± 0.80	2.79 ± 0.65[b]	2.89 ± 0.55	2.77 ± 0.57	2.96 ± 0.54	2.95 ± 0.52	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Arachidonic acid (20:4n-6)	8.24 ± 1.34	7.79 ± 1.25[b]	8.80 ± 1.90	8.18 ± 1.51[b]	8.69 ± 1.32	8.61 ± 1.33	9.63 ± 2.22	9.85 ± 2.46	**0.029**[f]	**0.007**[cf]	**0.017**
Adrenic acid (22:4n-6)	0.27 ± 0.06	0.23 ± 0.05[b]	0.31 ± 0.08	0.26 ± 0.06[b]	0.28 ± 0.06	0.29 ± 0.07	0.30 ± 0.07	0.33 ± 0.09[b]	**0.001**[cf]	**< 0.001**[cf]	**< 0.001**
Osbond acid (22:5n-6)	0.15 ± 0.06	0.12 ± 0.03[b]	0.19 ± 0.06	0.16 ± 0.05[b]	0.16 ± 0.05	0.17 ± 0.05	0.19 ± 0.06	0.19 ± 0.09	**0.003**[eg]	**0.003**[eg]	**0.008**
Sum of n-6	31.30 ± 2.17	31.21 ± 1.66	31.27 ± 2.78	30.04 ± 1.53	31.77 ± 2.26	31.88 ± 1.90	31.54 ± 2.01	31.84 ± 2.03	0.124	**0.002**[fg]	**0.006**
Polyunsaturated	41.10 ± 1.92	41.11 ± 1.85	40.75 ± 2.33	40.98 ± 1.74	41.13 ± 1.98	40.88 ± 1.41	41.39 ± 1.26	41.06 ± 1.69	0.693	0.870	0.903

Values are means ± SD. ANCOVA, analysis of covariance; CSO, camelina sativa oil.
Post hoc tests: [c] $p < 0.05$ CSO vs. control group; [d] $p < 0.05$ CSO vs. fatty fish group; [e] $p < 0.05$ CSO vs. lean fish group; [f] $p < 0.05$ fatty fish vs. control group; [g] $p < 0.05$ fatty fish vs. lean fish group.
[a] Differences in fold changes among the groups were tested using ANCOVA and Bonferroni's post hoc tests. ANCOVA: Model 1 no adjustments. Model 2 adjusted for baseline value, age and sex. Benjamini-Hochberg false discovery rate (FDR) was used to adjust results for multiple comparisons.
[b] Change within the group was determined by Paired samples t-test or Wilcoxon signed ranks test, $p < 0.05$.

Table 3 Fatty acid composition in plasma cholesteryl esters at baseline and at the end of the study.

Fatty acid (mol%)	CSO (n = 18)		Fatty fish (n = 20)		Lean fish (n = 21)		Control (n = 20)		p values ANCOVA[a]		
	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	Model 1	Model 2	FDR
Myristic acid (14:0)	1.07 ± 0.17	0.85 ± 0.13[b]	0.92 ± 0.17	0.87 ± 0.18	1.05 ± 0.21	0.95 ± 0.28	0.89 ± 0.20	0.87 ± 0.21	0.102	0.621	0.658
Palmitic acid (16:0)	11.59 ± 0.77	10.93 ± 0.66[b]	11.46 ± 0.61	11.40 ± 0.62	11.64 ± 0.81	11.52 ± 0.92	11.84 ± 0.78	11.59 ± 0.71	**0.010**[cd]	**0.005**[cde]	**0.011**
Stearic acid (18:0)	0.75 ± 0.18	0.71 ± 0.13	0.71 ± 0.13	0.66 ± 0.12	0.75 ± 0.27	0.73 ± 0.30	0.83 ± 0.33	0.71 ± 0.16	0.567	0.797	0.797
Saturated	13.41 ± 0.85	12.49 ± 0.80[b]	13.09 ± 0.74	12.93 ± 0.76	13.45 ± 0.97	13.20 ± 1.23	13.55 ± 1.03	13.16 ± 0.90	**0.017**[cd]	**0.017**[cd]	**0.028**
Palmitoleic acid (16:1n-7)	3.4 ± 1.33	2.74 ± 1.08[b]	4.16 ± 2.00	3.65 ± 1.63[b]	3.52 ± 1.47	3.28 ± 1.24	3.35 ± 0.88	3.40 ± 1.22	**0.024**[e]	**0.024**[e]	**0.036**
Oleic acid (18:1n-9)	19.86 ± 2.48	19.13 ± 2.44[b]	20.70 ± 2.62	20.74 ± 2.33	19.21 ± 2.38	19.91 ± 1.49	19.41 ± 1.36	20.38 ± 1.95[b]	**0.008**[de]	**0.016**[de]	**0.028**
Vaccenic acid (18:1n-7)	1.11 ± 0.19	1.00 ± 0.18[b]	1.16 ± 0.16	1.18 ± 0.16	1.11 ± 0.17	1.14 ± 0.19	1.19 ± 0.16	1.19 ± 0.15	**0.009**[cd]	**0.002**[cde]	**0.006**
Monounsaturated	24.37 ± 2.87	22.87 ± 3.01[b]	26.02 ± 4.45	25.56 ± 3.87	23.84 ± 3.76	24.33 ± 2.53	23.95 ± 1.97	24.96 ± 2.98	**0.004**[de]	**0.005**[de]	**0.011**
α-linolenic acid (18:3n-3)	1.08 ± 0.27	2.35 ± 0.58[b]	1.04 ± 0.27	1.21 ± 0.46[b]	1.22 ± 0.29	1.15 ± 0.27	0.99 ± 0.29	1.01 ± 0.24	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Eicosapentaenoic acid (20:5n-3)	2.24 ± 1.04	2.42 ± 0.93	1.97 ± 1.11	2.87 ± 0.79[b]	2.33 ± 1.06	2.10 ± 0.95	2.17 ± 0.80	1.89 ± 0.67	**< 0.001**[cfg]	**< 0.001**[cfg]	**< 0.001**
Docosahexaenoic acid (22:6n-3)	1.07 ± 0.22	0.92 ± 0.20	1.01 ± 0.29	1.17 ± 0.25	0.98 ± 0.24	0.99 ± 0.17	1.13 ± 0.21	1.06 ± 0.23	**< 0.001**[cf]	**< 0.001**[cfg]	**< 0.001**
Sum of n-3	4.38 ± 1.36	5.69 ± 1.42	4.01 ± 1.26	5.24 ± 0.94	4.53 ± 1.28	4.24 ± 1.09	4.29 ± 0.93	3.96 ± 0.83	**< 0.001**[defg]	**< 0.001**[defg]	**< 0.001**
Linoleic acid (18:2n-6)	50.05 ± 4.15	51.90 ± 3.96[b]	48.74 ± 4.89	48.60 ± 4.24	50.20 ± 4.95	50.33 ± 4.60	49.10 ± 3.66	48.85 ± 3.92	0.181	0.063	0.087
γ-linolenic acid (18:3n-6)	0.93 ± 0.39	0.78 ± 0.29[b]	0.87 ± 0.40	0.79 ± 0.34	0.85 ± 0.37	0.83 ± 0.40	0.87 ± 0.38	0.92 ± 0.36	0.100	0.116	0.139
Dihomo-γ-linolenic acid (20:3n-6)	0.82 ± 0.25	0.64 ± 0.17[b]	0.81 ± 0.22	0.76 ± 0.20[b]	0.77 ± 0.14	0.74 ± 0.15	0.84 ± 0.16	0.82 ± 0.16	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Arachidonic acid (20:4n-6)	6.03 ± 1.14	5.63 ± 1.29[b]	6.46 ± 1.63	6.12 ± 1.35[b]	6.36 ± 1.33	6.32 ± 1.28	7.40 ± 2.20	7.32 ± 2.20	0.223	0.078	0.100
Sum of n-6	57.84 ± 3.38	58.95 ± 2.98	56.88 ± 4.85	56.26 ± 3.84	58.18 ± 4.22	58.23 ± 3.78	58.21 ± 2.32	57.91 ± 3.37	0.322	0.138	0.155
Polyunsaturated	62.22 ± 3.27	64.64 ± 3.23[b]	60.89 ± 4.65	61.51 ± 4.10[b]	62.71 ± 3.99	62.47 ± 3.22	62.51 ± 2.26	61.87 ± 3.44	**0.008**[de]	**0.006**[de]	**0.012**

Values are means ± SD. ANCOVA, analysis of covariance; CSO, camelina sativa oil.
Post hoc tests: [c] $p < 0.05$ CSO vs. fatty fish group; [d] $p < 0.05$ CSO vs. lean fish group; [e] $p < 0.05$ CSO vs. control group; [f] $p < 0.05$ fatty vs. control group; [g] $p < 0.05$ fatty fish vs. lean fish group.
[a] Differences in fold changes among the groups were tested using ANCOVA and Bonferroni's post hoc tests. ANCOVA: Model 1 no adjustments. Model 2 adjusted for baseline value, age and sex. Benjamini-Hochberg false discovery rate (FDR) was used to adjust results for multiple comparisons.
[b] Change within the group was determined by Paired samples t-test or Wilcoxon signed ranks test, $p < 0.05$.

Table 4 Fatty acid composition in plasma triglycerides at baseline and at the end of the study.

Fatty acid (mol%)	CSO (n = 18)		Fatty fish (n = 20)		Lean fish (n = 21)		Control (n = 20)		p values ANCOVA[a]		
	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk	Model 1	Model 2	FDR
Myristic acid (14:0)	2.93 ± 0.66	2.29 ± 0.43[b]	2.50 ± 0.79	2.45 ± 0.74	2.77 ± 0.68	2.55 ± 0.89	2.47 ± 0.82	2.46 ± 0.60	0.144	0.816	0.816
Palmitic acid (16:0)	26.57 ± 2.39	24.56 ± 2.88[b]	27.25 ± 4.19	26.81 ± 2.92	26.78 ± 2.44	26.50 ± 2.98	27.22 ± 2.58	26.10 ± 2.57	0.224	0.066	0.132
Stearic acid (18:0)	3.36 ± 0.41	3.47 ± 0.83	3.57 ± 0.89	3.56 ± 0.62	3.44 ± 0.49	3.65 ± 0.73	3.44 ± 0.66	3.19 ± 0.62	0.226	0.113	0.161
Saturated	32.86 ± 2.88	30.32 ± 3.49[b]	33.32 ± 5.51	32.82 ± 3.84	32.99 ± 3.01	32.71 ± 4.01	33.14 ± 3.53	31.74 ± 3.27	0.267	0.096	0.160
Palmitoleic acid (16:1n-7)	4.08 ± 1.23	3.55 ± 1.39[b]	4.62 ± 1.35	4.31 ± 1.12	4.29 ± 1.05	3.97 ± 1.08	4.32 ± 1.02	4.36 ± 1.14	0.171	0.108	0.161
Oleic acid (18:1n-9)	39.44 ± 3.25	39.67 ± 4.08	39.55 ± 2.80	39.38 ± 2.52	39.02 ± 3.52	40.50 ± 3.47	39.05 ± 3.17	40.02 ± 2.66	0.362	0.447	0.513
Vaccenic acid (18:1n-7)	2.31 ± 0.42	2.14 ± 0.39[b]	2.61 ± 0.44	2.50 ± 0.37	2.56 ± 0.38	2.46 ± 0.37	2.46 ± 0.28	2.57 ± 0.31	**0.019**[c]	**0.005**[c]	**0.013**
Eicosenoic acid (20:1n-9 + 11)	0.62 ± 0.14	0.88 ± 0.24[b]	0.63 ± 0.14	0.67 ± 0.15	0.63 ± 0.10	0.64 ± 0.11	0.58 ± 0.14	0.62 ± 0.12	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Monounsaturated	46.44 ± 3.31	46.23 ± 4.08	47.41 ± 3.01	46.87 ± 2.94	46.50 ± 4.11	47.58 ± 3.66	46.42 ± 3.60	47.57 ± 3.30	0.268	0.330	0.426
α-linolenic acid (18:3n-3)	2.13 ± 0.72	4.16 ± 1.43[b]	1.78 ± 0.57	2.02 ± 0.80	2.06 ± 0.50	2.01 ± 0.51	1.95 ± 0.55	2.04 ± 0.66	**< 0.001**[cde]	**< 0.001**[cde]	**< 0.001**
Eicosapentaenoic acid (20:5n-3)	0.66 ± 0.54	0.73 ± 0.37	0.71 ± 0.80	0.85 ± 0.37[b]	0.71 ± 0.42	0.60 ± 0.42	0.61 ± 0.29	0.66 ± 0.53	**0.010**[g]	**0.001**[gh]	**0.004**
Docosapentaenoic acid (22:5n-3)	0.70 ± 0.33	0.74 ± 0.24	0.66 ± 0.28	0.76 ± 0.18[b]	0.77 ± 0.39	0.63 ± 0.20	0.70 ± 0.21	0.68 ± 0.26	**0.005**[g]	**0.005**[g]	**0.013**
Docosahexaenoic acid (22:6n-3)	2.00 ± 1.67	1.79 ± 1.40	2.05 ± 1.99	2.64 ± 1.53	1.94 ± 1.46	1.63 ± 0.72	1.95 ± 1.05	1.97 ± 1.44	**0.026**	**0.002**[dgh]	**0.007**
Sum of n-3	5.49 ± 2.89	7.42 ± 2.78	5.19 ± 3.31	6.27 ± 2.10	5.48 ± 2.27	4.86 ± 1.31	5.21 ± 1.42	5.35 ± 2.48	**0.002**[ceg]	**< 0.001**[cegh]	**< 0.001**
Linoleic acid (18:2n-6)	13.51 ± 2.53	14.43 ± 3.08	12.35 ± 3.19	12.39 ± 2.53	13.17 ± 2.66	13.04 ± 2.26	13.34 ± 1.57	13.37 ± 2.67	0.576	0.341	0.426
γ-linolenic acid (18:3n-6)	0.33 ± 0.13	0.31 ± 0.15	0.26 ± 0.11	0.23 ± 0.11	0.32 ± 0.17	0.32 ± 0.18	0.35 ± 0.15	0.38 ± 0.21	0.139	0.090	0.160
Dihomo-γ-linolenic acid (20:3n-6)	0.27 ± 0.08	0.23 ± 0.06[b]	0.27 ± 0.08	0.26 ± 0.08	0.28 ± 0.07	0.26 ± 0.08[b]	0.28 ± 0.07	0.29 ± 0.05	**0.008**[c]	**0.001**[cf]	**0.004**
Arachidonic acid (20:4n-6)	1.09 ± 0.25	1.06 ± 0.24	1.21 ± 0.41	1.17 ± 0.32	1.27 ± 0.37	1.24 ± 0.35	1.27 ± 0.50	1.30 ± 0.50	0.933	0.487	0.513
Sum of n-6	15.21 ± 2.79	16.03 ± 3.21	14.08 ± 3.44	14.05 ± 2.71	15.03 ± 2.83	14.85 ± 2.39	15.23 ± 1.46	15.34 ± 2.97	0.686	0.474	0.513
Polyunsaturated	20.70 ± 4.92	23.45 ± 5.10[b]	19.27 ± 5.67	20.32 ± 3.90	20.51 ± 3.93	19.71 ± 2.94	20.44 ± 2.14	20.68 ± 4.91	0.086	**0.049**	0.109

Values are means ± SD. ANCOVA, analysis of covariance; CSO, camelina sativa oil.

Post hoc tests: [c] $p < 0.05$ CSO vs. control group; [d] $p < 0.05$ CSO vs. fatty fish group; [e] $p < 0.05$ CSO vs. lean fish group; [f] $p < 0.05$ lean fish vs. control group; [g] $p < 0.05$ fatty fish vs. lean fish group; [h] $p < 0.05$ fatty fish vs. control group.

[a] Differences in fold changes among the groups were tested using ANCOVA and Bonferroni's post hoc tests. ANCOVA: Model 1 no adjustments. Model 2 adjusted for baseline value, age and sex. Benjamini-Hochberg false discovery rate (FDR) was used to adjust results for multiple comparisons.

[b] Change within the group was determined by Paired samples t-test or Wilcoxon signed ranks test, $p < 0.05$.

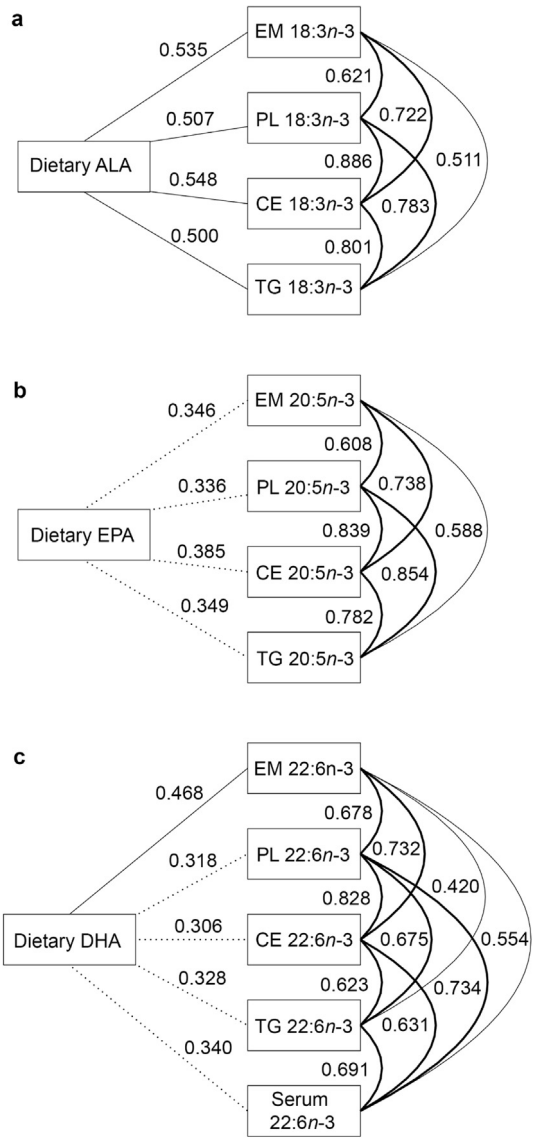

Figure 1 Correlations between fold changes of dietary ALA and proportions of ALA (18:3*n*-3) (**a**), dietary EPA and proportions of EPA (20:5*n*-3) (**b**) and dietary DHA and proportions of DHA (22:6*n*-3) (**c**) in different blood lipid fractions. In addition to plasma blood lipid fractions the correlation between dietary DHA and serum concentration of DHA was tested (**c**). Serum concentration of DHA has been determined by nuclear magnetic resonance (NMR) spectroscopy. Correlation coefficients were calculated using Spearman rank correlation. All correlations are statistically significant ($p < 0.05$). The lines are in three categories according to the strength of the correlation, bolded lines representing the strongest correlations. ALA, alpha-linolenic acid; EM, erythrocyte membrane; PL, phospholipid; CE, cholesteryl ester; TG, triglyceride.

respectively). Proportions of EPA and DHA in EM, PL, CE and TG correlated with their dietary intake during the intervention (Supplementary Table 3). Dietary intake of EPA correlated also with concentration of EPA ($r = 0.362$, $p < 0.01$; $r = 0.441$, $p < 0.001$; $r = 0.289$, $p < 0.05$ for PL, CE and TG, respectively) whereas intake of DHA correlated with concentration of DHA only in PL and CE (r = 0.315, r = 0.331, p < 0.01, respectively). Dietary intake of DHA was also correlated with the serum concentration of DHA determined by NMR ($r = 0.275$, $p < 0.05$).

Associations of the changes

Correlations between the change in dietary ALA, EPA and DHA and the change in their proportion in different lipid fractions did not markedly differ among fatty acids (Fig. 1). The correlations between the change in these fatty acids and the change in their respective concentrations are presented in Supplementary Table 9. The change in dietary ALA intake was associated with the change in the proportion of ALA in all fractions ($r = 0.535$, $r = 0.507$, $r = 0.548$, $r = 0.500$, $p < 0.05$ for EM, PL, CE and TG, respectively). Similarly, the change in the ALA intake was associated with the change in the concentration of ALA (r = 0.479, r = 0.535, r = 0.416, p < 0.001 for PL, CE and TG, respectively). Correlations between the change in dietary EPA with the change in its proportion in different lipid fractions were slightly weaker as compared with ALA ($r = 0.346$, $r = 0.336$, $r = 0.385$, $r = 0.349$, $p < 0.05$ for EM, PL, CE and TG, respectively). Similar correlations were found also between the change in EPA intake and change in concentration of EPA ($r = 0.352$, $r = 0,386$, $r = 0.290$, $p < 0.01$ for PL, CE and TG, respectively). The change in dietary DHA was associated with the change in the proportion of DHA in different lipid fractions, the association with the proportion in EM being the strongest (r = 0.468, r = 0.318, r = 0.306, r = 0.328, $p < 0.05$ for EM, PL, CE and TG, respectively). The change in dietary DHA was also associated with the change in concentrations of DHA ($r = 0.282$, $r = 0.256$, $p < 0.05$ for PL and TG and $r = 0.342$, $p < 0.01$ for CE) as well as the change serum concentration of DHA determined by NMR ($r = 0.340$, $p < 0.05$).

Correlations between changes in the proportions of ALA, EPA and DHA in different lipid pools are presented in Fig. 1. Correlations between CE and PL fraction were the strongest ($r > 0.8$ for each fatty acid, $p < 0.05$). Furthermore, changes in the proportion of DHA in plasma fractions and serum concentration of DHA correlated well (r between 0.50 and 0.80, $p < 0.05$).

Discussion

We investigated and compared the effects of fish and CSO intakes on the fatty acid composition of EM, PL, CE and TG. To the best of our knowledge, no previous intervention studies exist comparing the effects of different dietary *n*-3 PUFA sources on blood lipid fractions. As expected, contents of EPA, DHA and ALA increased in all four fractions – EPA and DHA in the fatty fish group, and ALA in the CSO group. Similar changes have previously been found after fish or fish oil treatments in EM [24–26], plasma PL [24,26,27], CE and TG [10,27] and after an increased ALA intake in EM [12,15,28], CE and TG [29]. In the lean fish group, we found no significant changes in the proportions of ALA, EPA or DHA in any fractions; this finding supported by our earlier findings [27]. Telle-Hansen et al. [30], however, found after a daily cod intake of 150 g for 15 days an increase in DHA content of plasma PL.

ALA is a precursor of EPA and DHA synthesis [31]. Therefore, increases in the proportion of these fatty acids could be expected after an increased ALA intake. A recent review summarizes that several studies have found increases in the EPA content in blood lipids or blood cells after increased ALA intake either from supplements or dietary sources [16]. However, some studies have reported no effect of increased ALA intake on the proportion of EPA in EM phospholipids, PL or plasma [15,32,33]. However, the study of James et al. [33] lasted only 6 weeks, and therefore, it may not have been long enough to increase EPA levels in EMPL. In our study, there was no statistically significant increase in the proportion of EPA after increased ALA intake in any lipid fraction (Tables 1–4). After increased ALA intake the proportion of DHA decreased in all fractions, although significant changes were found only in EM and PL (Tables 1–4). Furthermore, we observed similar changes in the concentration of DHA in the CSO group (Supplementary Tables 4–6). These findings are supported by previous studies reporting no change [12,34,35] or a decrease [29] in the proportion of DHA in different lipid pools after increased ALA intake. Furthermore, change in dietary ALA intake was inversely associated with change in the proportions of DHA in PL, CE and EM (data now shown). These results suggest that DHA status cannot be altered by increased ALA intake, which is in alignment with a previous review [36].

The lack of ALA conversion to long-chain *n*-3 PUFAs may be due to downregulation by increased availability of conversion products (EPA + DHA) [37]. In the study by Egert et al. [15], baseline EPA and DHA status were high which was might have downregulated ALA conversion to long-chain *n*-3 PUFAs. Similarly, we found that the long-chain *n*-3 PUFA status was high at baseline (Supplementary Table 1) which may explain the changes regarding the proportions of EPA and DHA observed in the CSO group. Furthermore, ALA conversion to long-chain *n*-3 PUFAs may be most effective during increased ALA intake with a concomitant decrease in linoleic acid intake [36]. CSO also contains linoleic acid, thus, we observed a significant increase in linoleic acid in EM, PL and CE within the CSO group (Tables 1–3), but the change differed significantly from other groups only in EM (Table 1). High linoleic acid intake may reduce the endogenous synthesis of EPA and DHA by competing for the enzymes required for the conversion [36].

Circulating docosapentaenoic acid (DPA *n*-3) levels have been found to be unrelated to the dietary intake and therefore suggested to be mainly derived from endogenous elongation from EPA [5,38]. In this study we found that the change in the dietary EPA intake correlated with the proportion of DPA only in TG (data not shown). We also observed an increase in the proportion of DPA in the fatty fish group in TG (Table 4) which is supported by earlier studies reporting increased plasma DPA levels after fish oil treatment [25,39]. Furthermore, increased ALA intake has been previously reported to increase the proportion of DPA but not DHA in plasma lipid pools [12,34,35]. Competition between ALA and 24:5*n*-3 for ΔD6 desaturase enzyme has been suggested to explain these findings [40]. High level of ALA may inhibit the conversion of 24:5*n*-3 to 24:6*n*-3, and therefore, decrease the amount of precursor for DHA conversion. We did not observe statistically significant increases in the proportion of DPA in the CSO group in any lipid fraction, although the change in the dietary ALA intake correlated with the change in the proportion of DPA in EM (data not shown).

Several non-dietary determinants such as sex and age are known to affect the *n*-3 PUFA composition in tissues [1,41]. Studies have shown that women have lower proportions of EPA [41], but higher proportions of DHA in PL, CE and EM [41,42]. Results regarding the effect of age on fatty acid composition in different lipid pools have been controversial [25,41]. Some studies have reported increased proportions of EPA and DHA in PL and EM with age, from 20-year-olds to over 60-year-olds [41,43] whereas Zulyniak et al. [25] found greater increases in EPA and DHA contents in EM in young men (18–30 years) than in older men (60–74 years). In this study, the changes observed in the fatty acid composition were independent of sex and age.

EPA and DHA in PL and CE have been concluded to be good biomarkers for dietary long-chain *n*-3 PUFA intake, and they respond well also when the baseline status is high [1,3,18]. Furthermore, Stark et al. [44] found that the proportion of EPA + DHA in plasma PL was strongly correlated with the proportion of EPA + DHA in erythrocytes. They also proposed that fatty acids in plasma PL could be used to estimate the EPA + DHA content of erythrocytes. In a recent meta-analysis, it was concluded that diet-induced changes in the fatty acid composition of PL and CE correlate well, especially changes in PUFAs [45]. Consistently with these findings, we found that the changes in plasma EM, PL and CE were similar (Tables 1–3), and the changes in the fatty acid compositions in PL and CE and also in EM and PL correlated well (Fig. 1).

To our knowledge, there are only two previous intervention studies comparing different biomarkers for long-chain *n*-3 PUFA intake [10,46] but none for ALA intake. However, Browning et al. [10] in their study suggested that long-chain *n*-3 PUFA proportions from plasma phosphatidylcholine are good biomarkers for measuring rapid changes in long-chain *n*-3 PUFA intake, whereas measurement from plasma CE or TG may not be as accurate. Furthermore, Hodson et al. [1] concluded that plasma TG may be more useful for investigating the hepatic enzyme activities than to be used as a biomarker for dietary fat intake. The changes we observed in EPA and DHA in the fatty fish group were similar in all lipid pools, although SDs were larger in TG (Tables 1–4). Interestingly, in a recent study of Žáček et al. [46] it was found that long-chain *n*-3 PUFAs incorporate selectively into plasma phosphatidylcholine and TG species after salmon intake. This type of specific approach on *n*-3 PUFA biomarkers may, therefore, be needed when evaluating the associations between *n*-3 PUFAs and diseases.

Previous studies have reported low correlations, mostly $r < 0.40$, between dietary ALA and the proportion in the blood lipid pools and for long-chain *n*-3 PUFA correlations have ranged between $r = 0.40$ and $r = 0.60$ [3]. Similarly,

we found weak associations between dietary ALA, EPA and DHA intake and their contents in lipid pools at baseline (Supplementary Table 2). However, the correlations at the end of the study (Supplementary Table 3) and the correlations between the changes in dietary intake and proportions in lipid fractions (Fig. 1) were stronger than baseline correlations. This may be explained by the difference in the number of days in the food records: at baseline dietary intake data was collected for four consecutive days whereas from the intervention period food records were from 12 days. It has been previously shown that at least 7 days is required for accurate assessment of dietary fat, especially dietary PUFAs [47].

The strengths in the current study include randomized controlled study design and the use of laboratory methods that have been in long-term use and are considered high-quality. Furthermore, the power calculations were based on the differences in the proportion of DHA in PL. The compliance of the study subjects was good based on the changes in the fatty acid composition of lipid pools and consumption records. Dietary intakes were carefully assessed with 4-day food records and fish and camelina oil intakes were also monitored with consumption records. However, baseline food records were collected only from four consecutive days, which is a limitation in this study. Furthermore, subjects in this study were overweight and had impaired glucose metabolism. Therefore, the results of this study may not apply to lean individuals with normal glucose metabolism. It should be also noted, that with only two measurement points (0 and 12 weeks), we could not report the rate of fatty acid incorporation in different lipid pools during the study.

In conclusion, we found a response in EM and plasma lipid fractions to increased ALA, EPA and DHA intakes. Furthermore, correlations between dietary intake of ALA, EPA and DHA did not markedly differ in any lipid pool. Therefore, there is no difference in the ability of blood lipid fractions to reflect the dietary intake of *n*-3 fatty acids with daily intakes of around 1 g of EPA + DHA or 10 g of ALA during a time period of 12 weeks in subjects with high habitual intake of fish.

Author contributions

The authors' responsibilities were as follows: U.S. and A.E. are the principal investigators in the AlfaFish −study. S.M. analyzed the data, wrote the article and had primary responsibility for final content. U.S., A.E. and M.L. helped writing the paper and planned and conducted the study together with V.M. D.L. had the medical charge of the study and checked the language as a native speaker. J.Å. was responsible for the fatty acid analysis. All authors have read and approved the final manuscript.

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Conflicts of interest

None of the authors have a conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2018.10.002.

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