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Changes in relative and absolute concentrations of plasma phospholipid fatty acids observed in a randomized trial of Omega-3 fatty acids supplementation in Uganda

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Abstract

Expressing circulating phospholipid fatty acids (PLFAs) in relative concentrations has some limitations: the total of all fatty acids are summed to 100%; therefore, the values of individual fatty acid are not independent. In this study we examined if both relative and absolute metrics could effectively measure changes in circulating PLFA concentrations in an intervention trial. 66 HIV and HHV8 infected patients in Uganda were randomized to take 3g/d of either long-chain omega-3 fatty acids (1,856 mg EPA and 1,232 mg DHA) or high—oleic safflower oil in a 12-week double-blind trial. Plasma samples were collected at baseline and end of trial. Relative weight percentage and absolute concentrations of 41 plasma PLFAs were measured using gas chromatography. Total cholesterol was also measured. Intervention-effect changes in concentrations were calculated as differences between end of 12-week trial and baseline. Pearson correlations of relative and absolute concentration changes in individual PLFAs were high (>0.6) for 37 of the 41 PLFAs analyzed. In the intervention arm, 17 PLFAs changed significantly in relative concentration and 16 in absolute concentration, 15 of which were identical. Absolute concentration of total PLFAs decreased 95.1 mg/L (95% CI: 26.0, 164.2; $P = 0.0085$), but total cholesterol did not change significantly in the intervention arm. No significant change was observed in any of the measurements in the placebo arm. Both relative weight percentage and absolute concentrations could effectively measure changes in plasma PLFA concentrations. EPA and DHA supplementation changes the concentrations of multiple plasma PLFAs besides EPA and DHA.

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Keywords

Phospholipid fatty acid; Relative concentration; Weight percentage concentration; Absolute concentration; EPA; DHA

1. Introduction

Epidemiologic studies of the relationship between circulating phospholipid fatty acids (PLFA) and disease outcomes have expressed each fatty acid as either weight or molar percentage of total fatty acids analyzed [1-3]. Using such relative concentrations, the total of all fatty acids are summed to 100%; therefore, the values of individual fatty acid are not independent. This has led to debate, along with the suggestion to use absolute concentrations of fatty acids [4-8]. The interdependent nature of the relative metric could be especially problematic when studying changes in concentrations: when certain fatty acids maintain their absolute concentrations while others increase, their relative concentrations would decrease because all fatty acids have to add up to 100%.

In the present study, we used samples from an EPA (eicosapentaenoic acid, 20:5n3) plus DHA (docosahexaenoic acid, 22:6n3) supplementation trial in human immunodeficiency virus (HIV) infected patients and measured changes of individual plasma PLFA concentration in both relative (weight percentage) and absolute terms. The hypothesized intervention effects were that plasma phospholipid EPA and DHA concentrations would increase and that EPA and DHA supplementation may influence both the relative and absolute concentrations of other plasma PLFAs. In this unique study population, we had an opportunity to test whether one metric vs. the other may be more sensitive at picking up intervention-effect changes in plasma PLFAs.

2. Materials and methods

2.1. Study population and study samples

The Uganda Omega-3 trial was a 12-week randomized, double-blind, placebo-controlled trial among men and women infected with both human immunodeficiency virus (HIV) and human herpesvirus 8 (HHV8). Participants were recruited between June and November 2012 from the Uganda Cancer Institute (UCI)/Hutchinson Center Cancer Alliance in Kampala, Uganda. Individuals with Kaposi's sarcoma (KS) were also recruited by collaborating physicians during routine clinic visits to the UCI. Exclusions criteria included pregnancy, age of less than 18, and active disease, i.e. KS-positive individuals receiving chemotherapy and KS-negative individuals receiving antiretroviral therapy. Participants were block randomized by KS status to receive either the omega-3 supplement or placebo. The intervention group consumed 8 capsules/day of a fish oil preparation, which contained 3.1g of EPA (1.86g) and DHA (1.23g), plus 2.5g other fatty acids naturally present in fish oil; while the placebo group consumed 5.6 g high—oleic safflower oil (HOSO) in identical packaging and appearance. Participants were instructed to take 4 capsules with meal twice a day. The EPA+DHA dose used in the study was considered safe to consume without physician supervision [9]. HOSO, which contains about 78% of oleic acid [10], was used as

control because changes in PLFA profile after intake of 5.6g/d of HOSO were expected to be small. Both active and placebo capsules were supplied by Capsugel Liquids Group (Greenwood, South Carolina). A flavorant was added to the omega-3 fatty acid capsules to mask the fish scent. All capsules contained mixed tocopherols (to prevent oxidation) and other excipients necessary for the stability of the supplement. Non-fasting plasma samples were collected at baseline and at the end of the 12-week trial, stored locally at -80°C , and shipped on dry ice to the Fred Hutchinson Cancer Research Center for lab analyses. Non-fasting blood does not affect the analytes in the present study—plasma phospholipid fatty acids reflect the intake of past 1-2 weeks [11] and circulating total cholesterol changes minimally in response to food intake [12, 13]. The study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center in Seattle, USA; the School of Medicine Research and Ethics Committee at Makerere College of Health Sciences in Kampala, Uganda; the Uganda National Council for Science and Technology; and the Uganda National Drug Authority. All participants provided written, informed consent.

2.2. Plasma phospholipid fatty acids (PLFA) analysis

The plasma PLFA assay was described previously [14]. Briefly, total lipids were extracted from plasma using the method of Folch [15]. An internal standard, 1,2-dihexarachidoyl-sn-glycero-3-phosphocholine (21:0 PC, Avanti Polar Lipids, Inc., Alabaster, Alabama), was added to each plasma sample before lipid extraction. Phospholipids were separated from other lipids by one dimensional thin layer chromatography [16]. Fatty acid methyl esters of the phospholipids were prepared by direct transesterification [17] and separated using gas chromatography (Agilent 5890 Gas Chromatograph with flame ionization detector, Supelco fused silica 100m capillary column SP-2560). This GC method quantified 41 known fatty acids for this study. Relative concentration of each fatty acid was expressed as a weight percentage of total PLFAs analyzed. Absolute concentration (mg/L) of each fatty acid was calculated by comparing its peak area to that of the internal standard: $\text{mg/L}_{21:0} * (\text{peak area count}_{\text{fatty acid of interest}} / \text{peak area count}_{21:0})$. A lab quality control (QC) sample (a pooled plasma) was included with each batch of study samples. When measured in weight percentage, coefficients of variation (CVs) of the 41 PLFAs in the QC samples ranged from 0.3% to 20.9%; when measured in mg/L, from 2.2% to 21.8%. All 32 relatively abundant PLFAs ($>0.1\%$) in the QC sample had CVs less than 6% whether measured in weight percentage or mg/L except for 18:1n7c, which had CV of 13.6% measured in weight percentage and 14.6% in mg/L.

2.3. Other laboratory assays

Total plasma cholesterol was measured on a Roche Cobas Mira Plus Chemistry Analyzer, using reagents from Sekisui Diagnostics (Lexington, MA) and following the manufacturer's instructions.

2.4. Statistical analyses

Baseline means and standard deviations in the intervention arm and in the placebo arm were calculated for each plasma PLFA, measured as relative weight percentage and absolute concentrations. Intervention-effect changes in concentrations were calculated as differences between end of 12-week trial and baseline. Pearson correlations were used to measure

agreement between relative and absolute changes for each fatty acid; Spearman correlations were nearly identical to Pearson correlations and are therefore not given in results. One-sample t-tests were used to evaluate whether change in each fatty acid concentration differed significantly from zero. Paired t-tests were used to compare baseline vs. end of trial (12-week) total cholesterol, and absolute concentration of total PLFAs analyzed. Bonferroni correction was used to account for multiple comparisons of 41 fatty acids; otherwise statistical tests are two-sided with significance level set at $P < 0.05$. Analyses were performed using SciPy Stack version 0.12.0 downloaded from SciPy.org or StataSE 13 (StataCorp LP, College Station, TX).

3. Results

Participants in the intervention and placebo arms were well balanced in terms of age, sex, BMI, KS status, total cholesterol, and total plasma PLFAs analyzed (**Table 1**). Concentrations of individual PLFAs at baseline expressed as both relative weight percentage and absolute concentrations were not significantly different between the two arms after correction for multiple comparisons (**Table 2**; P values not shown).

Pearson correlations of relative and absolute concentration changes in individual PLFAs were high except for 4 saturated fatty acids (16:0, 18:0, 20:0, and 24:0), which had correlation coefficients < 0.6 (**Table 3**). After the 12-week EPA+DHA supplementation, EPA and DHA increased significantly in both relative and absolute concentrations (Table 3, intervention arm): EPA had 5.7 and 5.1 mean fold change in relative and absolute terms, respectively; and DHA 1.6 and 1.5. One other PLFA, 22:5n3 (DPA, docosapentaenoic acid), increased (2.2 and 2.0 fold change in relative and absolute terms, respectively) and 12 PLFAs (18:2n6, 18:3n6, 20:2n6, 20:3n6, 20:4n6, 22:4n6, 22:5n6, 16:1n9c, 18:1n9c, 18:1n10-12t, 18:1n9t, and 18:2n6tc) decreased significantly in both relative and absolute concentrations. The range of mean fold changes for the decreased PLFAs was 0.38 – 0.84 in relative term and 0.36 – 0.79 in absolute term. In addition, two PLFAs (20:0 and 22:0) decreased in relative concentration only and one PLFA (16:1n9t) decrease in absolute concentration only in the intervention arm. None of the PLFAs changed significantly in the placebo arm (Table 3, placebo arm).

In the intervention arm, the mean absolute concentration of total PLFAs (sum of the 41 PLFAs analyzed) decreased significantly from 1264.8mg/L at baseline to 1169.7mg/L at the end of the 12-week EPA+DHA supplementation (mean change $-95.1\mu\text{g/ml}$; 95% CI: $-164.2, -26.0$; $P = 0.0085$). In the placebo arm, the mean absolute concentration of total PLFAs did not change significantly (data not shown). In addition, total plasma cholesterol did not change significantly in either arm (data not shown).

4. Discussion

This study shows that both relative weight percentage and absolute concentrations could be used effectively to measure changes of circulating PLFA concentrations in intervention trials. Changes of individual PLFA concentration measured in relative and absolute terms were highly correlated. EPA and DHA supplementation led to significant changes in both

relative and absolute concentrations of multiple PLFAs besides EPA and DHA; and the fatty acids identified as significantly changed by either metric were nearly identical.

In a previous cross-sectional study using samples from three independent and diverse populations, we showed that relative and absolute concentrations were moderately to highly correlated (Pearson and Spearman correlation coefficients >0.6) for most of the 41 circulating PLFAs examined [14]. The correlation coefficients further increased (by a factor of 1.12, median) after the absolute concentrations were adjusted for total circulating cholesterol concentration. Those results suggested that for PLFAs measured at one time point, except for fatty acids such as 16:0 that have low correlation between the two metrics, choice of relative vs. absolute concentration may not matter in studies of association between individual PLFAs and disease outcomes, especially if a covariate reflecting total lipids, such as total circulating cholesterol, is included in the model. In the present study in the context of an EPA and DHA supplementation trial, we have shown that both relative and absolute concentrations effectively measured the changes of plasma PLFAs.

The pattern of plasma PLFA concentration changes in the intervention arm in our study confirmed previous observations that DPA (22:5n3) also increased after EPA and DHA supplementation and that increase in omega-3 fatty acids was largely at the expense of the omega-6 fatty acids [18-20]. However, since we analyzed more PLFAs than in previous studies and we measured changes in both relative and absolute concentrations, our results provide a more comprehensive picture of the change pattern. Of particular interest is the reduction in some of the trans fatty acids since industrial trans fatty acids have been shown to have detrimental effects on heart health [21]. A limitation of our study was that general diet data were not collected. Even though participants were instructed to take the supplements with their usual meals, we do not know if any participant changed their dietary pattern during the trial period. In addition, EPA and DHA accounted for 55% of the fatty acids in the fish oil preparation taken by the intervention group; the other fatty acids in the supplement were naturally present in fish oil but the exact composition was not provided by the manufacturer. Therefore, we could not completely exclude the possibility that factors other than EPA and DHA supplementation contributed to the concentration change of other PLFAs besides EPA and DHA.

We observed no change in circulating total cholesterol concentration after EPA and DHA supplementation, which is consistent with previous omega-3 fatty acids trials including some that were conducted in HIV-infected populations [22, 23]. Although having no effect on total cholesterol, some fish oil intervention trials showed increased HDL and LDL particle size [24-26]. We did not measure particle size, but observed in the intervention arm a decrease in absolute concentration of total PLFAs. Since phospholipids in circulation are predominantly associated with lipoproteins as a component of the surface monolayer [27, 28], reduction in total PLFAs with no concurrent change of total cholesterol is consistent with increase in lipoprotein particle size.

HOSO, used as placebo in our study, consists mostly (~78%) of oleic acid (18:1n9). Oleic acid is abundant in circulating phospholipids and accounted for about 10% of total PLFAs in our study population at baseline. The already high abundance of this fatty acid may have

been the reason no change of its plasma PLFA concentration was observed in the placebo arm, which received only 5.6g of HOSO per day. In feeding studies where high-oleic vegetable oils were used to substitute for other fats and oils [29, 30], oleic acid increased in various blood lipid compartments. However, in those feeding studies, the amount of high-oleic oil consumed was much higher (>10 times) than that in our study, which was a supplement trial without experimentally altering participants' usual diet. A 3-month extra virgin olive oil (>70% oleic acid) supplementation trial showed change of red blood cell fatty acid profile [31]; however, the dose of 25ml oil per day was a little over 4 times that used in our study.

5. Conclusion

Our results demonstrated that both relative weight percentage and absolute concentrations could effectively measure changes in circulating PLFA concentrations and that EPA and DHA supplementation changes the concentrations of multiple plasma PLFAs besides EPA and DHA.

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Abbreviations

CV	coefficient of variation
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
KS	Kaposi's sarcoma
GC	gas chromatography
HOSO	high—oleic safflower oil
PLFA	phospholipid fatty acid
QC	quality control
UCI	Uganda Cancer Institute

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Highlights

Both relative weight percentage and absolute concentrations could effectively measure changes in plasma phospholipid fatty acid (PLFA) concentrations. EPA and DHA supplementation changes the concentrations of multiple plasma PLFAs besides EPA and DHA.

Table 1

Participants characteristics at baseline in the Uganda Omega-3 Trial.

		<u>intervention arm (n = 33)</u>	<u>placebo arm (n = 33)</u>
Age		38.6 ± 9.4	39.8 ± 8.9
BMI		23.2 ± 3.8	23.8 ± 6.3
Sex			
	Male	17 (51.5%)	20 (60.6%)
	Female	16 (48.5%)	13 (39.4%)
KS Status			
	KS Positive	27 (81.8%)	28 (84.8%)
	KS Negative	6 (18.2%)	5 (15.2%)
Total plasma cholesterol (mg/dl)		165.0 ± 46.0	151.9 ± 36.9
Total plasma PLFAs analyzed (mg/L)		1264.8 ± 280.4	1246.0 ± 269.2

Data are means ± SD or frequency (%)

KS, Kaposi's sarcoma

Table 2

Relative (weight percentage) and absolute (mg/L) plasma phospholipid fatty acid concentrations at baseline in the Uganda Omega-3 Trial.

Fatty Acid	intervention arm (n = 33)		placebo arm (n = 33)	
	wt % mean ± SD	mg/L mean ± SD	wt % mean ± SD	mg/L mean ± SD
Omega-3 Fatty Acids				
18:3n3	0.15 ± 0.07	1.97 ± 1.21	0.15 ± 0.06	1.95 ± 0.97
20:3n3	0.02 ± 0.01	0.26 ± 0.17	0.02 ± 0.01	0.24 ± 0.10
20:5n3 (EPA)	0.85 ± 0.48	10.80 ± 7.06	0.75 ± 0.31	9.35 ± 4.69
22:5n3	1.09 ± 0.24	13.84 ± 4.48	1.03 ± 0.23	12.74 ± 3.81
22:6n3 (DHA)	4.47 ± 1.33	56.48 ± 19.51	3.79 ± 0.93	46.47 ± 12.65
Omega-6 Fatty Acids				
18:2n6	14.80 ± 2.38	186.05 ± 46.10	15.29 ± 2.51	190.16 ± 50.55
18:3n6	0.10 ± 0.06	1.29 ± 0.91	0.12 ± 0.09	1.61 ± 1.33
20:2n6	0.37 ± 0.06	4.76 ± 1.46	0.38 ± 0.07	4.74 ± 1.11
20:3n6	3.83 ± 0.84	48.63 ± 15.51	3.78 ± 0.77	47.28 ± 14.56
20:4n6	11.01 ± 1.40	138.88 ± 34.06	11.05 ± 1.66	137.48 ± 35.03
22:2n6	0.04 ± 0.01	0.48 ± 0.14	0.04 ± 0.01	0.45 ± 0.12
22:4n6	0.55 ± 0.12	7.06 ± 2.45	0.58 ± 0.12	7.30 ± 2.38
22:5n6	0.49 ± 0.14	6.37 ± 2.94	0.46 ± 0.12	5.76 ± 2.15
Monounsaturated Fatty Acids				
16:1n9c	0.11 ± 0.03	1.46 ± 0.53	0.12 ± 0.02	1.44 ± 0.40
16:1n7c	0.58 ± 0.24	7.48 ± 4.10	0.59 ± 0.23	7.49 ± 3.61
17:1n9c	0.23 ± 0.04	2.86 ± 0.89	0.25 ± 0.07	3.05 ± 0.86
18:1n8c	0.04 ± 0.02	0.54 ± 0.26	0.04 ± 0.02	0.46 ± 0.20
18:1n9c	9.79 ± 1.77	125.50 ± 42.91	10.50 ± 1.63	132.85 ± 43.88
18:1n7c	1.42 ± 0.30	17.65 ± 4.58	1.40 ± 0.27	17.31 ± 4.75
18:1n5c	0.02 ± 0.01	0.29 ± 0.08	0.02 ± 0.01	0.30 ± 0.09
20:1n9	0.14 ± 0.02	1.69 ± 0.42	0.14 ± 0.03	1.73 ± 0.45
24:1n9	2.05 ± 0.56	25.28 ± 6.52	1.99 ± 0.39	24.29 ± 5.20
Saturated Fatty Acids				
14:0	0.22 ± 0.06	2.86 ± 1.25	0.21 ± 0.05	2.57 ± 0.83
15:0	0.12 ± 0.04	1.52 ± 0.62	0.11 ± 0.04	1.40 ± 0.52
16:0	26.46 ± 1.29	334.21 ± 73.43	26.69 ± 1.31	331.51 ± 68.17
17:0	0.42 ± 0.09	5.34 ± 1.62	0.40 ± 0.12	4.97 ± 1.67
18:0	15.06 ± 1.39	191.57 ± 51.12	14.71 ± 1.51	184.23 ± 47.40
20:0	0.50 ± 0.11	6.24 ± 1.85	0.47 ± 0.07	5.83 ± 1.44
22:0	1.51 ± 0.28	19.03 ± 5.03	1.48 ± 0.27	18.40 ± 5.28
23:0	0.51 ± 0.10	6.39 ± 1.95	0.50 ± 0.11	6.23 ± 1.79
24:0	1.57 ± 0.30	19.75 ± 5.46	1.56 ± 0.27	19.33 ± 5.15
Trans Fatty Acids				

Fatty Acid	intervention arm (n = 33)		placebo arm (n = 33)	
	wt %	mg/L	wt %	mg/L
	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD
16:1n9t	0.01 $\pm$ 0.00	0.17 $\pm$ 0.05	0.01 $\pm$ 0.00	0.16 $\pm$ 0.05
16:1n7t	0.16 $\pm$ 0.08	1.94 $\pm$ 0.79	0.13 $\pm$ 0.09	1.60 $\pm$ 1.02
18:1n10-12t	0.03 $\pm$ 0.01	0.35 $\pm$ 0.13	0.03 $\pm$ 0.01	0.34 $\pm$ 0.15
18:1n9t	0.04 $\pm$ 0.01	0.47 $\pm$ 0.20	0.04 $\pm$ 0.01	0.48 $\pm$ 0.18
18:1n8t	0.02 $\pm$ 0.01	0.22 $\pm$ 0.06	0.02 $\pm$ 0.01	0.23 $\pm$ 0.12
18:1n7t	0.15 $\pm$ 0.11	1.85 $\pm$ 1.30	0.11 $\pm$ 0.08	1.39 $\pm$ 1.05
18:1n6t	0.02 $\pm$ 0.01	0.28 $\pm$ 0.12	0.02 $\pm$ 0.01	0.24 $\pm$ 0.10
18:2n6tt	0.06 $\pm$ 0.02	0.71 $\pm$ 0.34	0.06 $\pm$ 0.02	0.71 $\pm$ 0.30
18:2n6ct	0.03 $\pm$ 0.01	0.37 $\pm$ 0.12	0.03 $\pm$ 0.01	0.40 $\pm$ 0.16
18:2n6tc	0.07 $\pm$ 0.03	0.95 $\pm$ 0.46	0.09 $\pm$ 0.04	1.11 $\pm$ 0.51

Table 3

Changes in relative (weight percentage) and absolute (mg/L) plasma phospholipid fatty acid concentrations, and their correlations in the Uganda Omega-3 Trial (12-week intervention).

Fatty Acid	intervention arm (n = 33)				placebo arm (n = 33)			
	wt % change		mg/L change		wt % change		mg/L change	
	mean ± SD	p – value ^a	mean ± SD	p – value ^a	mean ± SD	p – value ^a	mean ± SD	p – value ^a
Omega-3 Fatty Acids								
18:3n3	-0.01 ± 0.08	0.58	-0.29 ± 1.12	0.15	-0.03 ± 0.07	0.01	-0.44 ± 1.02	0.02
20:3n3	0.00 ± 0.01	0.04	0.02 ± 0.14	0.45	0.00 ± 0.01	0.16	-0.01 ± 0.07	0.29
20:5n3 (EPA)	4.02 ± 2.89	4.2E-09	44.47 ± 32.62	6.2E-09 *	-0.09 ± 0.26	0.05	-1.06 ± 3.75	0.11
22:5n3	1.26 ± 0.92	6.7E-09	13.65 ± 11.07	5.0E-08 *	0.02 ± 0.22	0.57	0.46 ± 2.84	0.36
22:6n3 (DHA)	2.79 ± 1.88	9.4E-10	28.10 ± 20.25	4.2E-09 *	0.08 ± 0.91	0.60	1.95 ± 8.82	0.21
Omega-6 Fatty Acids								
18:2n6	-2.37 ± 2.64	1.3E-05	-39.64 ± 42.66	7.4E-06 *	0.18 ± 2.92	0.73	6.23 ± 48.51	0.47
18:3n6	-0.05 ± 0.07	4.6E-05	-0.77 ± 0.91	3.0E-05 *	-0.02 ± 0.09	0.31	-0.20 ± 1.32	0.39
20:2n6	-0.07 ± 0.08	3.1E-05	-1.19 ± 1.40	3.1E-05 *	-0.02 ± 0.06	0.04	-0.11 ± 0.85	0.45
20:3n6	-1.68 ± 1.09	3.8E-10	-23.02 ± 17.00	7.1E-09 *	-0.03 ± 0.65	0.81	0.65 ± 9.80	0.71
20:4n6	-2.57 ± 1.94	1.1E-08	-39.67 ± 36.70	5.9E-07 *	0.36 ± 1.31	0.12	6.50 ± 23.47	0.12
22:2n6	0.00 ± 0.01	0.13	-0.07 ± 0.13	0.01	0.00 ± 0.01	0.39	0.00 ± 0.09	0.86
22:4n6	-0.26 ± 0.17	5.3E-10	-3.60 ± 2.70	9.8E-09 *	0.00 ± 0.10	0.98	0.15 ± 1.75	0.64
22:5n6	-0.30 ± 0.19	1.2E-10	-4.06 ± 3.15	2.1E-08 *	0.01 ± 0.08	0.63	0.18 ± 1.15	0.38
Monounsaturated Fatty Acids								
16:1n9c	-0.02 ± 0.03	6.3E-05	-0.37 ± 0.48	1.1E-04 *	-0.01 ± 0.02	0.05	-0.03 ± 0.30	0.57
16:1n7c	-0.11 ± 0.20	0.00	-1.88 ± 3.41	0.00	-0.03 ± 0.21	0.40	-0.22 ± 3.25	0.69
17:1n9c	0.00 ± 0.05	0.83	-0.25 ± 0.87	0.12	-0.01 ± 0.04	0.15	-0.06 ± 0.64	0.59
18:1n8c	-0.01 ± 0.02	0.07	-0.12 ± 0.30	0.03	0.00 ± 0.01	0.61	0.02 ± 0.17	0.52
18:1n9c	-1.63 ± 1.94	3.4E-05	-29.27 ± 40.48	2.3E-04 *	-0.32 ± 1.37	0.18	-2.06 ± 27.50	0.67
								0.82

Fatty Acid	intervention arm (n = 33)					placebo arm (n = 33)				
	wt % change			mg/L change		wt % change			mg/L change	
	mean ± SD	p - value ^a		mean ± SD	p - value ^a	mean ± SD	p - value ^a		mean ± SD	p - value ^a
			Pearson Coef. ^b							Pearson Coef. ^b
18:1n7c	-0.06 ± 0.33	0.32		-1.76 ± 5.83	0.09	-0.09 ± 0.24	0.04		-0.77 ± 2.86	0.13
18:1n5c	0.00 ± 0.01	0.70		-0.03 ± 0.09	0.13	0.00 ± 0.01	0.04		-0.02 ± 0.07	0.06
20:1n9	0.00 ± 0.03	0.86		-0.11 ± 0.32	0.05	0.00 ± 0.03	0.79		0.04 ± 0.38	0.60
24:1n9	0.13 ± 0.50	0.14		-0.25 ± 5.97	0.81	0.03 ± 0.43	0.72		0.77 ± 4.61	0.34
Saturated Fatty Acids										
14:0	-0.01 ± 0.06	0.38		-0.38 ± 0.84	0.01	0.00 ± 0.05	0.80		0.07 ± 0.80	0.60
15:0	-0.01 ± 0.04	0.44		-0.20 ± 0.58	0.06	0.00 ± 0.03	0.65		-0.01 ± 0.42	0.93
16:0	0.23 ± 1.18	0.28		-22.66 ± 55.59	0.03	-0.07 ± 1.09	0.72		5.70 ± 40.69	0.43
17:0	0.01 ± 0.08	0.56		-0.38 ± 1.46	0.15	-0.02 ± 0.10	0.21		-0.20 ± 1.28	0.39
18:0	0.36 ± 1.08	0.06		-10.71 ± 30.94	0.06	-0.01 ± 0.91	0.93		2.67 ± 26.83	0.57
20:0	0.06 ± 0.09	4.5E-04	*	0.24 ± 1.27	0.28	0.01 ± 0.07	0.63		0.15 ± 0.85	0.32
22:0	0.19 ± 0.22	2.3E-05	*	0.83 ± 2.77	0.09	0.04 ± 0.21	0.33		0.77 ± 2.86	0.13
23:0	0.04 ± 0.10	0.01		-0.04 ± 1.27	0.87	0.00 ± 0.07	0.88		0.09 ± 1.23	0.68
24:0	0.08 ± 0.25	0.06		-0.30 ± 3.05	0.57	-0.03 ± 0.19	0.43		0.06 ± 2.85	0.91
Trans Fatty Acids										
16:1n9t	0.00 ± 0.00	0.00		-0.04 ± 0.04	4.2E-05	0.00 ± 0.00	0.55		0.00 ± 0.04	0.99
16:1n7t	-0.03 ± 0.07	0.03		-0.39 ± 0.84	0.01	0.02 ± 0.06	0.09		0.23 ± 0.61	0.04
18:1n10-12t	-0.01 ± 0.01	6.1E-04	*	-0.10 ± 0.14	1.9E-04	0.00 ± 0.01	0.37		-0.01 ± 0.12	0.54
18:1n9t	-0.01 ± 0.01	3.8E-04	*	-0.15 ± 0.24	1.0E-03	0.00 ± 0.01	0.22		-0.02 ± 0.15	0.55
18:1n8t	0.00 ± 0.01	0.37		-0.03 ± 0.10	0.12	0.00 ± 0.01	0.79		0.00 ± 0.09	0.91
18:1n7t	-0.02 ± 0.11	0.41		-0.30 ± 1.47	0.25	0.03 ± 0.07	0.03		0.32 ± 0.92	0.05
18:1n6t	0.00 ± 0.01	0.93		-0.02 ± 0.14	0.50	0.00 ± 0.01	0.11		0.03 ± 0.09	0.06
18:2n6tt	0.02 ± 0.03	0.00		0.12 ± 0.36	0.06	0.00 ± 0.02	0.27		-0.04 ± 0.29	0.47
18:2n6ct	0.00 ± 0.01	0.04		-0.06 ± 0.11	0.01	0.00 ± 0.01	0.07		-0.03 ± 0.14	0.21
18:2n6ic	-0.02 ± 0.03	8.4E-04	*	-0.31 ± 0.44	3.8E-04	-0.01 ± 0.03	0.04		-0.13 ± 0.47	0.11

^aP-value corresponds to the one-sample t-test of the change in fatty acid concentration from baseline. Bonferroni correction has been applied; $\alpha = 0.05/41 = 0.0012$.

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* indicates significant change.

q Correlation of changes in in plasma phospholipid fatty acids measured as relative (weight percentage) concentration and absolute (mg/L) concentration.