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ORIGINAL CONTRIBUTION

Omega-3 fatty acids and lipoprotein associated phospholipase A_2 in healthy older adult males and females

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Abstract
Purpose Lipoprotein associated phospholipase A_2 (Lp-PLA_2) is a novel inflammatory factor that has been independently associated with stroke and cardiovascular disease (CVD). Omega-3 fats have been implicated in reducing inflammation associated with CVD. The aim of this study was to determine if an 8-week isocaloric diet supplemented with eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) in the form of fish oil or α-linolenic acid (ALA) in the form of flaxseed oil would alter Lp-PLA_2 among healthy adults ages 50 years and older.
Methods Fifty-nine healthy adults (~75% female, average age 61 years) were randomized to one of three groups with equal amounts of total fat intake. All capsules contained ~1 g of fat. The control group ($n = 19$) consumed olive oil capsules (~11 g/day); the ALA group ($n = 20$) consumed flaxseed oil capsules (~11 g/day) and the EPA/DHA group ($n = 20$) consumed fish oil capsules (~2 g/day + 9 g/day of olive oil). Fasting blood samples were obtained before and after the 8-week intervention for determination of Lp-PLA_2 mass and activity as well as lipid values.
Results We did not find any significant changes in Lp-PLA_2 mass or activity after the intervention in any of the groups; however, change in oxidized LDL was associated with change in Lp-PLA_2 mass ($r = 0.37$, $p < 0.01$).
Conclusion Supplementing the diet with omega-3 fatty acids for 8-weeks did not influence Lp-PLA_2 activity or mass among older adults; altering oxidized LDL may be necessary to see changes in Lp-PLA_2 levels.

Keywords Omega-3 fats · Inflammation · Lipids · Lipoprotein-associated phospholipase A2 · Healthy adults

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Introduction

Numerous reports have recently shown both a cross-sectional as well as a longitudinal relationship between inflammatory factors and cardiovascular disease (CVD). Macrophages stimulate the inflammatory response by producing numerous cytokines [1]. What has emerged as a novel inflammatory factor is Lp-PLA_2 which is a macrophage derived enzyme that when released binds to apo B on low-density lipoprotein cholesterol (LDL-C) as well as high-density lipoprotein cholesterol (HDL-C). Lp-PLA_2 remains latent until LDL is oxidized, once this occurs, Lp-PLA_2 cleaves the oxidized phophatidylcholine into two bioactive compounds-lysophosphatidylcholine (LysPC) and oxidized non-esterified free fatty acids (oxNEFA) [2]. These compounds contribute significantly to the inflammation associated with atherosclerosis creating a positive feedback loop whereby lsyoPC and oxNEFA activate monocytes/macrophage to secrete cytokines which promote Lp-PLA_2 release from macrophages [3]. Further, both Lp-PLA_2 mass and activity have been shown to be independent predictors of coronary heart disease and ischemic

stroke in the general population as well as clinical populations [4–11]; and higher levels of circulating Lp-PLA_2 appear to be associated with atherosclerotic burden as assessed by extent of coronary calcification [11], endothelial dysfunction [4] and plaque instability [12].

Numerous studies have considered dietary fatty acids as potential regulators of inflammatory burden. Generally, the omega six polyunsaturated fatty acids (PUFA)s have pro-inflammatory capacities; while the omega -3 PUFAs have anti-inflammatory effects [13]. Omega-3 fatty acids, both α-linolenic acid (ALA) as well as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) have been shown to decrease the synthesis of pro-inflammatory cytokines from monocytes/macrophages as well as lymphocytes [14]. Further, numerous studies have shown reductions in various plasma cytokines with either ALA or EPA/DHA supplementation [15–17]. Because Lp-PLA_2 originates from macrophages [18] and omega-3 fats have shown to decrease inflammatory cytokine synthesis from these cells we hypothesize there may be a direct effect of omega-3s on Lp-PLA_2 levels. Therefore, the purpose of this study was to determine if 8 weeks of dietary supplementation with EPA and DHA in the form of fish oil or ALA in the form of flaxseed oil alters Lp-PLA_2 among healthy adults ages 50 years and older.

Methods

Participants

Participants were recruited from the Fort Collins community through flyers, and advertisements in local papers and publications. Participants included males and females aged 50 years and older who were free of diabetes, known heart disease or other inflammatory condition including asthma, arthritis or other major medical problem as determined by a health history questionnaire. Other exclusion criteria included regular vigorous physical activity (>3 days/week), women who were premenopausal or using hormone replacement therapy or oral contraceptives, and tobacco users. In addition, subjects were weight stable for a minimum of 3 months prior to the study. We included subjects taking aspirin, statins and ibuprofen; however, they were not to alter the dose while participating in the study.

Experimental design

The study design was approved by the Institutional Review Board at Colorado State University and the subjects gave their informed consent. Upon inclusion, subjects underwent baseline testing including determination of percent body fat by dual x-ray absorptiometry (DEXA; Lunar DPX-IQ, v.4.5c), fasting blood sample and anthropometric measurements. Each subject recorded 3 days (2 weekdays and 1 weekend day) of detailed food records for assessment of baseline dietary intake. The 3-day diet record was entered into the Food Intake and Analysis System (FIAS v. 3.0, University of Texas School of Public Health, 1998) to determine total energy intake.

Participants were then randomly assigned to the isocaloric control, ALA or EPA/DHA supplementation groups. All subjects were blinded to their treatment group. Based on the calculation of total energy from the baseline 3-day food records, subjects were assigned a supplement dosage that represented 5.3% of average caloric intake, resulting in a range from 6 to 16 g/day of the provided oil/s. Each capsule contained 1 g of fat so average intakes were between 6 and 16 capsules per day. Participants were told to take the capsules as suited them, they were told it may be easiest to take with each meal. Because we wanted to compare a similar amount of omega-3 fatty acids using fish oil and flaxseed oil (e.g. EPA/DHA vs. ALA) we used the studies of Emken et al. [19] showing that in a typical U.S. diet ~300 mg of omega-3 (EPA/DHA) would be synthesized from ~2 g of ALA; therefore we estimated that ~7 g of ALA = ~1 g of EPA/DHA. To ensure similar intakes of fat between the groups we supplemented the fish oil group with olive oil. Specifically, the ALA group was supplemented with flaxseed oil (Spectrum Naturals, Inc. Boulder, CO, 0.57 g alpha-linolenic acid/capsule) where 3% of their calories came from ALA and the other 2.3% from other fats contained in the flax oil capsule (~11/g day per person). The EPA/DHA group was supplemented with fish oil (Spectrum Naturals, Inc. Boulder, CO, 180 mg eicosapentaenoic acid/capsule and 120 mg docosahexaenoic/capsule) where 0.45% of their calories came from EPA/DHA, 0.65% from other fats in the fish oil (~2 g/day per person) and the other 4.2% of their calories came from olive oil (Seagate, Inc San Diego, CA 800 mg MUFAs/capsule). The control group used olive oil, (Seagate, Inc. San Diego, CA 800 mg MUFAs/capsule) where 5.3% of the calories came from olive oil. To maintain the isocaloric nature of the study, all groups were provided instruction on how to reduce their dietary fat intake by a similar amount as that provided by the capsules. Subjects were advised to decrease their intake of high fat foods, whole and 2% milk products, and added fats. Subjects were also provided lists of lower fat alternatives to these foods. Diet records of all groups were assessed about every other week together with weigh-ins to ensure compliance to the diet prescriptions and weight stability. The groups received supplements at this time in unlabeled containers. Compliance was determined by diet records, capsule counts as well as assessment of erythrocyte membrane fatty acid composition using gas chromatography.

Anthropometrics

Waist circumference was measured at the level of the umbilicus using a Gulick spring-loaded measuring tape. Height and weight were obtained from subjects dressed in lightweight clothes with their shoes removed. BMI was calculated as weight (kg) divided by height squared (m^2). In order to assess percent body fat, dual energy x-ray absorptiometry (DEXA) scans were obtained using a Lunar DPX-L total body scanner (DEXA; Lunar DPX-IQ, v.4.5c).

Blood collection

Following an overnight fast, a 4 ml blood sample was collected in a purple-top tube containing 54 μL EDTA and centrifuged for 15 min at 2,800 rpm and 0 °C (Beckman GS-15R Centrifuge). Following removal and storage of the plasma, red blood cells were isolated after three wash cycles with EDTA and 10 min centrifugation. Plasma and red blood cell (with equal part K_3-EDTA) samples were stored at −80 °C until analysis.

Assays

Lp-PLA$_2$

Lp-PLA_2 concentration was measured by an enzyme linked immunosorbant assay (ELISA) and activity determined by a colorimetric activity method, both performed by diaDexus, Inc (mass, intra-assay coefficient of variation (CV)% <0.1%; activity, intra-assay CV% <2%).

Lipids

Commercially available kits were used to measure total cholesterol (intra-assay CV% <0.87%), triglycerides (intra-assay CV% <0.60%), glucose (intra-assay CV% <1.6%), HDL-C (intra-assay CV% <1.15%) (Wako Chemicals USA Inc., Virginia) as well as insulin (intra-assay CV% <2.6%) (Mercodia, Inc., North Carolina). An automated microplate reader was used for quantitative assessment for each assay (Biotek Instruments Inc., Vermont). LDL-C was calculated by using the Friedewald equation, as determined by Friedewald et al. [20]. Erythrocyte membrane fatty acid composition was determined by gas chromatography using standard procedures [21, 22]. The detection limit was 0.25% of total fatty acids. Some fatty acids were not detectable as noted in the tables.

Oxidized LDL-C

Oxidized LDL-C (intra-assay CV% <6.7%) (Mercodia, Inc., North Carolina). Mercodia oxidized LDL competitive ELISA is based on the monoclonal antibody 4E6. Oxidized LDL in the sample competes with a fixed amount of oxidized LDL bound to the micro titer well for the binding of the biotin-labeled specific antibodies. After a washing step that removes unreactive sample components, the biotin-labeled antibody bound to the well is detected by HRP-conjugated streptavidin. After a second incubation and an additional washing step, the bound conjugate is detected by reaction with 3,3′5,5′-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically. These are the same methods used by Holvert et al. [23–25] see references.

Dietary data

Food records were analyzed using the Food Intake and Analysis System (FIAS v. 3.0, University of Texas School of Public Health, 1998), which utilizes the United States Department of Agriculture (USDA) database of food composition information (including individual fatty acids) for over 7,300 foods.

Statistical analysis

Descriptive statistics (means and standard deviations) were performed as well as a one-way ANOVA for comparison of baseline values for age, anthropometrics, percent body fat, Lp-PLA_2 levels, LDL-C, HDL-C, triglycerides, cholesterol, cholesterol/HDL ratio, oxidized LDL, oxidized LDL/LDL ratio and mediation use between controls and the ALA and EPA/DHA intervention groups. A Chi-square test was used to compare gender between the groups. Univariate spearman correlation coefficients were also determined for the relationship of baseline Lp-PLA_2 mass and activity with the variables listed above as well erythrocyte membrane percentage of EPA, DHA and ALA. Change scores were also calculated for each of these variables comparing pre to post values and spearman correlation coefficients were determined for changes in Lp-PLA_2 mass and activity and the above listed variables.

Because the dietary data and erythrocyte membrane fatty acid data were not normally distributed even after log transformations, a comparison across groups was calculated for the change scores using the Kruskal–Wallis test. The n-6/n-3 ratio was calculated using the sum of arachidonic acid and linoleic acid divided by the sum of ALA, EPA and DHA. A repeated measures general linear model (GLM) was used to determine time x group interactions for BMI, WC, percent fat, Lp-PLA_2 levels, LDL-C, HDL-C, triglycerides, cholesterol/HDL-C ratio, oxidized LDL and oxidized LDL/LDL ratio between the baseline and post-test control, ALA and EPA/DHA groups. Age, gender, and medication use were controlled for in these analyses.

Statistical significance was accepted at $p < 0.05$. All analyses were run using SPSS 13.0 (Statistical Package for Social Science, SPSS Inc., Chicago, IL) for Windows.

Results

Table 1 shows descriptive statistics for these subjects. There were 19 subjects in the control group, 20 subjects in the flaxseed oil group and 20 subjects in the fish oil group. The mean Lp-PLA_2 levels were low to borderline risk. Lipid levels were considered low/moderate risk with ~17% taking statins. Almost 40% of these participants were taking anti-inflammatory medications, primarily aspirin on a regular basis.

When we correlated Lp-PLA_2 mass and activity with the descriptive variables, including erythrocyte membrane concentrations of EPA, DHA and ALA; Lp-PLA_2 activity was significantly associated with oxidized LDL ($r = 0.39$, $p < 0.01$) and oxidized LDL/LDL ratio ($r = 0.26, p < 0.05$); while Lp-PLA_2 mass was significantly inversely associated with erythrocyte DHA levels ($r = -0.27, p < 0.05$).

Table 2 shows dietary intake at baseline and the average intake for weeks 1–8 in the control, ALA group, and EPA/DHA group. There were not any changes between weeks 1–8 for the dietary data and therefore the average of the 8 week intervention is presented. As expected, there were significant changes between groups for all variables except total calories, total fat, and saturated fat intake.

Importantly we also assessed compliance to this intervention by examining changes in fatty acid content of the erythrocyte membranes from baseline to post intervention. Table 3 shows that most of the fatty acids did not significantly change; however, EPA, 20:5 went from being non-detectable in the EPA/DHA group to being 0.6%, while DHA, 22:6 significantly increased in the EPA/DHA group compared to the control group and the n-6/n-3 ratio in the erythrocyte membrane significantly decreased in the EPA/DHA group. Table 4 shows the results of GLM repeated measures analysis, and as expected BMI, WC and percent fat did not change from baseline to post intervention due to the isocaloric nature of the study. Lp-PLA_2 mass and activity also did not change from pre to post testing; nor did any of the lipids including oxidized LDL or the oxidized LDL/LDL ratio. We also considered spearman correlations for the changes scores and found change in Lp-PLA_2 activity was significantly inversely associated with change in waist circumference; while change in Lp-PLA_2 mass was significantly associated with change in oxidized LDL and inversely associated with change in BMI (Table 5).

Discussion

Overall we did not find any changes in Lp-PLA_2 activity or mass after supplementing healthy older adults with omega-3 fatty acids, 5.3% of calories (~11 g/day) from ALA or (~2 g/day) EPA/DHA. Further oxidized LDL levels did

Table 1 Descriptive statistics at baseline for the control, ALA and EPA/DHA groups

	Control group ($N = 19$) Mean ± SD	ALA group ($N = 20$) Mean ± SD	EPA/DHA group ($N = 20$) Mean ± SD	p value across groups
Age	58.6 ± 6.3	63.4 ± 8.2	62.1 ± 7.7	0.13
Gender (% female)	74%	65%	85%	0.35
BMI (kg/m^2)	25.5 ± 3.6	25.2 ± 3.1	26.3 ± 4.9	0.66
WC (cm)	86.6 ± 9.8	85.6 ± 10.6	85.0 ± 13.9	0.90
Percent fat (%)	32.3 ± 8.3	31.3 ± 7.9	35.9 ± 6.1	0.20
Lp-PLA_2 mass (ng/mL)	204.1 ± 58.8	219.9 ± 64.0	222.9 ± 56.8	0.58
Lp-PLA_2 activity (nmol/min/mL)	142.4 ± 28.9	145.8 ± 29.1	140.1 ± 36.6	0.85
LDL-C (mmol/L)	3.52 ± 1.1	3.03 ± 0.7	3.33 ± 0.7	0.20
HDL-C (mmol/L)	1.11 ± 0.3	1.14 ± 0.3	1.02 ± 0.3	0.50
Triglycerides (mmol/L)	1.06 ± 0.7	0.91 ± 0.4	1.02 ± 0.4	0.65
Total cholesterol (mmol/L)	5.12 ± 1.1	4.60 ± 0.8	4.80 ± 0.8	0.19
Total cholesterol/HDL	5.1 ± 2.0	4.3 ± 1.3	5.1 ± 1.5	0.28
Oxidized LDL (U/L)	70.6 ± 30.9	64.2 ± 23.6	66.7 ± 22.9	0.75
Ox. LDL/LDL ratio	0.53 ± 0.22	0.56 ± 0.21	0.53 ± 0.21	0.79
Medications				
Statins	21%	15%	15%	0.85
Anti-inflammatory	32%	60%	25%	0.06
Blood sugar	0%	0%	5%	0.37

BMI, body mass index, *WC* waist circumference, *Lp-PLA_2* lipoprotein associated phosphlipase A_2, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol

Table 2 Dietary intake presented as means and percentage of total calories at baseline and average intake for weeks 1–8 based on self- reported daily diet records

	Control group		ALA group		EPA/DHA group		*p* value across groups
	$N = 19$		$N = 20$		$N = 20$		
	Baseline Mean (%) ± SD	Average intake weeks 1–8 Mean (%) ± SD	Baseline Mean (%) ± SD	Average intake weeks 1–8 Mean (%) ± SD	Mean (%) ± SD	Average intake weeks 1–8 Mean (%) ± SD	
Total calories	1,888.1 ± 453.0	1,985.4 ± 394.4	1,782.0 ± 432.0	1,858.5 ± 441.9	1,753.9 ± 380.2	1,792.2 ± 318.9	0.99
Total fat (g, %)	75.7 (36%) ± 25.3	85.3 (39%) ± 17.9	72.8 (36%) ± 25.2	81.5 (38%) ± 22.7	70.4 (36%) ± 24.2	83.0 (41%) ± 19.5	0.75
PUFA (g, %)	14.9 (7%) ± 5.4	15.4 (7%) ± 3.6	15.3 (7%) ± 7.1	22.5 (10%) ± 7.4	16.1 (8%) ± 8.1	17.0 (8%) ± 5.0	<0.001[a]
MUFA (g, %)	29.8 (14%) ± 11.0	37.6 (17%) ± 8.7	27.9 (14%) ± 11.1	29.7 (14%) ± 8.5	28.1 (14%) ± 11.0	35.4 (17%) ± 9.3	0.02[b]
SFA (g, %)	25.4 (12%) ± 10.5	24.5 (11%) ± 6.8	24.3 (12%) ± 7.9	23.9(10.2%) ± 7.0	20.9 (11%) ± 7.0	23.9 (12%) ± 6.3	0.36
ALA (g, %)	1.1 (0.6%) ± 0.4	1.3 (0.6%) ± 0.3	1.4 (0.7%) ± 0.5	7.2 (3%) ± 1.7	1.7 (0.8%) ± 2.0	1.6 (0.8%) ± 0.7	<0.001[a]
EPA (g, %)	0.01 (0.01%) ± 0.02	0.03 (0.01%) ± 0.04	0.05 (0.03%) ± 0.09	0.03 (0.02%) ± 0.04	0.05 (0.03%) ± 0.83	0.41 (0.22%) ± 0.07	<0.001[c]
DHA (g,%)	0.03 (0.01%) ± 0.04	0.05 (0.02%) ± 0.06	0.09 (0.05%) ± 0.10	0.06 (0.03%) ± 0.04	0.06 (0.04%) ± 0.11	0.31 (0.16%) ± 0.05	<0.001[c]
n6/n3 ratio	12.0 ± 4.1	10.5 ± 2.1	8.9 ± 3.4	2.0 ± 0.5	10.1 ± 4.4	6.3 ± 1.4	<0.001[c]

SD standard deviation, *PUFA* polyunsaturated fatty acid, *MUFA* monounsaturated fatty, *SFA* saturated fatty acid; *ALA* α-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *n6* omega 6 fatty acid, *n3* omega 3 fatty acid

[a] ALA group is significantly different from the control and the EPA/DHA group is significantly different from the ALA group

[b] ALA group is significantly different from the control group

[c] The EPA/DHA group is significantly different than the control group and the ALA group

not change and the other lipids decreased but not significantly. Given our study design and statistical tests, we could detect an effect of 14% on our outcomes with power >80%. These findings are similar to those of Pedersen et al. [26] who also did not show a significant decrease in Lp-PLA_2 mass with fish oil supplementation. The study by Pedersen et al. is the only study we are aware of that has considered the effects of an omega-3 fatty acid intervention on Lp-PLA_2 levels. Specifically, their study included a population of healthy males and females with a mean age of 38 years. Subjects were studied for 12 weeks and were given either 6.6 g of fish oil, 2.0 g of fish oil plus 4.9 g of olive oil, or 7 g of olive oil per day. In comparison to our study where we gave either ~2 g of fish oil plus ~9 g of olive oil, ~11 g of flaxseed oil, or ~11 g of olive oil per day for 8 weeks. The corroboration of these two studies given that the interventions included very different doses of fish oil as well as age groups suggests that omega-3 fatty acids may have no effect on altering Lp-PLA_2 levels; however, neither study followed participants for longer than 3 months, which may be indicated based on the recent findings of Schmidt et al. [27]. They found Lp-PLA_2 mass to be significantly inversely ($r = -0.18$, $p < 0.01$) associated with adipose levels of EPA among patients undergoing elective coronary angiography. Because adipose tissue represents dietary intake over 6–12 months perhaps a longer intervention with omega-3 fats could influence Lp-PLA_2 levels. Important to note though, our study did not find any significant correlation between Lp-PLA_2 levels and EPA or DHA in the erythrocyte membrane nor did the Pedersen et al. [26] study find any significant correlation with the EPA/DHA content in platelets or granulocytes.

We originally hypothesized that Lp-PLA_2 would be altered by omega-3 fatty acids based on the premise that Lp-PLA_2 originates from macrophages and omega-3 fats have shown to decrease inflammatory cytokine synthesis from these cells [14]. Pedersen et al. [26] originally hypothesized that omega-3's may influence Lp-PLA_2 through their effects on LDL-C, possibly decreasing LDL-C oxidation which would thereby reduce the substrate available for Lp-PLA_2. Although we did not measure macrophage activity in this study, we did measure lipid levels and oxidized LDL but did not find any significant changes in these variables with the intervention. We did not expect LDL-C or HDL-C to significantly change with this intervention and although triglycerides have previously been shown to significantly decrease with an omega-3 fatty acid intervention [28]; the current study did find any significant changes in triglycerides. The EPA/DHA groups triglycerides decreased ~0.095 mmol/L which is what we might expect given that the participants average baseline levels of triglycerides were quite low, ~0.96 mmol/L. The studies showing significant changes in triglycerides have primarily been among

Table 3 Percentage of fatty acids present in erythrocyte cell membranes before and after the intervention

	Control group		ALA group		EPA/DHA group		*p* value across groups
	N = 18		*N* = 19		*N* = 19		
	Baseline Mean % ± SD	8-weeks Mean % ± SD	Baseline Mean % ± SD	8-weeks Mean % ± SD	Baseline Mean % ± SD	8-weeks Mean % ± SD	
SFA							
16:0 palmitic acid	21.0 ± 3.8	20.2 ± 1.2	19.8 ± 1.2	19.7 ± 0.8	20.4 ± 1.0	20.7 ± 1.0	0.57
18:0 stearic acid	18.5 ± 1.3	18.4 ± 0.8	18.5 ± 1.4	18.5 ± 1.1	18.6 ± 1.2	18.5 ± 1.0	0.51
MUFA							
18:1 oleic acid	13.8 ± 1.0	14.0 ± 1.2	12.9 ± 1.1	13.0 ± 1.2	13.1 ± 0.8	13.6 ± 1.0	0.51
PUFA							
18:2 linoleic acid	10.8 ± 1.7	11.0 ± 1.1	10.3 ± 1.8	10.4 ± 1.5	11.0 ± 1.7	11.0 ± 1.8	0.51
18:3 ALA	0.1 ± 0.3	ND	0.1 ± 0.5	0.1 ± 0.4	ND	ND	–
20:4 arachidonic acid	15.4 ± 2.9	16.0 ± 1.1	15.4 ± 1.6	15.1 ± 1.8	16.2 ± 1.6	15.6 ± 1.2	0.11
20:5 EPA	ND	ND	0.1 ± 0.3	0.4 ± 0.6	ND	0.6 ± 0.7	–
22:6 DHA	3.76 ± 1.3	3.81 ± 1.0	4.4 ± 1.0	4.2 ± 1.1	4.4 ± 1.1	5.3 ± 1.0	<0.001[a]
n6/n3 ratio	7.2 ± 1.6	7.5 ± 1.8	5.9 ± 1.7	6.0 ± 2.0	6.7 ± 2.3	4.9 ± 1.5	<0.001[a]

SD standard deviation, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *ALA* α-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *n6* omega 6 fatty acid, *n3* omega 3 fatty acid, *ND* not detectable

[a] The EPA/DHA group is significantly different from the control group and the ALA group

Table 4 Baseline and 8-week values for body composition, Lp-PLA_2 mass and activity, lipids and oxidized LDL

	Control group (*N* = 19)		ALA group (*N* = 20)		EPA/DHA group (*N* = 20)		*p* value across group
	Baseline Mean ± SD	8-weeks Mean ± SD	Baseline Mean ± SD	8-weeks Mean ± SD	Baseline Mean ± SD	8-weeks Mean ± SD	
BMI (kg/m^2)	25.7 ± 3.9	26.1 ± 3.8	25.2 ± 3.9	25.4 ± 3.9	26.1 ± 3.9	26.1 ± 3.9	0.09
WC (cm)	86.9 ± 8.7	87.1 ± 8.7	85.1 ± 8.9	84.4 ± 8.9	85.1 ± 8.5	84.5 ± 8.9	0.69
Percent fat (%)	33.7 ± 7.0	33.6 ± 7.0	31.3 ± 8.5	31.4 ± 8.5	35.0 ± 7.6	34.4 ± 7.6	0.20
Lp-PLA_2 mass (ng/mL)	201.6 ± 61.9	195.9 ± 63.2	223.3 ± 63.5	213.8 ± 64.4	221.8 ± 62.1	216.1 ± 63.5	0.91
Lp-PLA_2 activity (nmol/min/mL)	145.6 ± 33.1	151.5 ± 38.3	142.3 ± 34.0	139.0 ± 38.9	140.5 ± 35.2	151.3 ± 38.4	0.32
LDL-C (mmol/L)	3.53 ± 0.9	3.14 ± 0.9	3.03 ± 0.9	2.60 ± 0.9	3.34 ± 0.9	3.32 ± 1.0	0.24
HDL-C (mmol/L)	1.08 ± 0.3	1.23 ± 0.4	1.19 ± 0.3	1.19 ± 0.4	1.0 ± 0.3	1.16 ± 0.4	0.23
Triglycerides (mmol/L)	1.08 ± 0.5	1.13 ± 0.5	0.87 ± 0.5	0.82 ± 0.5	1.04 ± 0.5	0.95 ± 0.5	0.19
Total cholesterol (mmol/L)	5.11 ± 0.9	4.89 ± 0.87	4.61 ± 1.0	4.18 ± 0.9	4.80 ± 0.9	4.90 ± 0.9	0.11
Total cholesterol/HDL	5.2 ± 1.7	4.6 ± 1.7	4.2 ± 1.8	4.0 ± 1.8	5.2 ± 1.8	4.7 ± 1.8	0.68
Oxidized LDL (U/L)	72.4 ± 27.4	72.3 ± 27.4	61.9 ± 27.3	61.9 ± 27.3	67.4 ± 26.8	71.5 ± 26.8	0.29
Ox LDL/LDL ratio	0.55 ± 0.22	0.58 ± 0.22	0.54 ± 0.22	0.63 ± 0.22	0.53 ± 0.22	0.58 ± 0.22	0.52

All analyses controlled for age, gender and medication use, adjusted means are shown

SD standard deviation, *Lp-PLA*$_2$ lipoprotein associated phosphlipase A_2, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *ALA* α-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

hypertriglyceridemic subjects [28]. We did however, did find that Lp-PLA_2 activity was significantly associated with oxidized LDL and oxidized LDL/LDL ratio and that change in Lp-PLA_2 mass were associated with change in oxidized LDL. Perhaps a change in oxidized LDL is necessary to see a change in Lp-PLA_2, this hypothesis is based on the knowledge that Lp-PLA_2 remains latent until LDL is oxidized [2] as well as the few studies that have shown a correlation between oxidized LDL and Lp-PLA_2 levels. A study by Vickers et al. found oxidized LDL and Lp-PLA_2 activity to be positively associated within plasma and carotid endarterectomy tissues. This study was conducted

Table 5 Univariate correlation coefficients between change in Lp-PLA_2 mass and activity and change in the other measured variables

	ΔLp-PLA_2 mass *r*	ΔLp-PLA_2 activity *r*
Δ Body mass index	−0.28*	−0.17
Δ Waist circumference	0.02	−0.29*
Δ Percentage body fat	0.09	0.06
Δ LDL-C	0.15	−0.21
Δ HDL-C	0.20	0.23
Δ Triglycerides	0.01	−0.03
Δ Oxidized LDL	0.37**	0.13
Δ Ox LDL/LDL ratio	0.15	0.24
Δ EPA (erythrocytes)	−0.07	−0.06
Δ DHA (erythrocytes)	0.01	0.01
Δ ALA (erythrocytes)	−0.11	0.04

Lp-PLA_2 lipoprotein associated phosphlipase A_2, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *ALA* α-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

* $p < 0.05$; ** $p < 0.01$

among males and females average age 69 years undergoing carotid endarterectomy procedures [29]. Similarly Oldgren et al. [30] found significant positive correlations between oxidized LDL and Lp-PLA_2 mass (they did not measure activity) among acute coronary syndrome patients. They did not however, find this association among subjects without acute coronary syndromes. On the other hand, Wootton et al. [31] found that the oxidized LDL/LDL ratio was inversely associated with Lp-PLA_2 activity among type1 and type 2 diabetic males and females average age 62 years; and they found this effect was even stronger among those without CHD. Overall these data suggest positive correlations between oxidized LDL and Lp-PLA_2 among diseased subjects; however this positive association doesn't seem to hold among diabetics. The Pedersen et al. [26] study did not measure Lp-PLA_2 activity or oxidized LDL levels and therefore we cannot compare our results to theirs. We are not aware of any studies that have considered changes in oxidized LDL and the effect on Lp-PLA_2 mass and/or activity as we have done here, certainly more studies need to consider this possibility.

Unlike the Pedersen et al. study we did not find Lp-PLA_2 mass to be significantly correlated with BMI, LDL-C or total cholesterol, we would have expected there to be an association between Lp-PLA_2 and LDL-C since in circulation Lp-PLA_2 is primarily bound to LDL-C; further other studies have found similar correlations [10, 30, 32]. Those studies that attempted to alter Lp-PLA_2 (e.g. statin studies) have consistently found change in LDL-C to be associated with change in Lp-PLA_2; however explaining only ~6–18% of variance [33, 34]. Interestingly none of these statin intervention studies considered oxidized LDL levels, it would be worth determining if change in oxidized LDL is more strongly associated with change in Lp-PLA_2 than LDL-C.

There are several limitations and strengths that should be noted. Almost 20% of the subjects in our study were taking statins. Statins have been shown to decrease Lp-PLA_2 mass and activity as well as LDL-C [33, 35]. Although, the subjects did not change their statin dosage (except one), we reanalyzed our data eliminating these subjects and did not find any differences in our results. Further, ~40% of the participants were taking anti-inflammatory medications, although we controlled for this in the analysis, it could have driven our results toward the null. However, many of these participants on anti-inflammatory were taking aspirin regularly and we are not aware of any studies linking anything other than stains to reductions in Lp-PLA_2. Importantly, our results are very similar to Pederson et al. [26] whose subjects were not taking anti-inflammatory medications.

Another potential limitation was the study population showed increases in total fat consumption; however, we did not see any significant increase in total calories or LDL-C which makes it unlikely this would have influenced our results.

Many of our subjects were taking fish or flax oil before they entered this study (~31%), most of these subjects (67%) went through a wash out period of ~2 months if they were using omega-3 fats. Although, we cannot know if this 2 month period truly washed out the omega-3 fats and/or their potential effects on Lp-PLA_2, this limitation could contribute to a null finding.

Some of the strengths of this study include that it was blinded, participants did not know what type of capsules they were taking; further, we assessed compliance using both dietary records and erythrocyte phospholipid membrane concentrations of the various fatty acids. Self-reported dietary intake was consistent with what we expected as were self-reported intake and capsule counts (~94% of the time they reported adherence); however the erythrocyte membrane data suggests the EPA/DHA group was compliant and that the control and ALA group were not compliant. If we were to conservatively assume this was the case, and only consider the fish oil group, we see that Lp-PLA_2 activity actually goes up and Lp-PLA_2 mass decreases slightly ~5 ng/mL; further oxidized LDL shows no change.

Overall we have corroborated the findings of Pedersen et al. and found no changes in Lp-PLA_2 activity or mass with omega-3 fatty acid supplementation. The significant correlation between Lp-PLA_2 activity and oxidized LDL

may suggest that oxidized LDL needs to be altered before changes in Lp-PLA_2 can be seen.

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