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Twice weekly intake of farmed Atlantic salmon (*Salmo salar*) positively influences lipoprotein concentration and particle size in overweight men and women

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ABSTRACT

The US Dietary Guidelines for Americans recommend twice weekly fish intake. Farmed Atlantic salmon is a good source of omega-3 (n-3) fatty acids which have positive lipid modifying effects; however, it is unknown whether these responses are dose-dependent. Our primary research objective was to determine the effect of dose-dependent intake of farmed Atlantic salmon on lipoprotein particle (P) size and concentration. We hypothesized that low-density lipoprotein (LDL)–P and high-density lipoprotein (HDL)–P size and concentration would increase with salmon intake in a dose-dependent manner. Overweight, adult participants (n = 19) were enrolled in a cross-over designed clinical trial evaluating intake of farmed Atlantic salmon. In random order, participants were assigned to 90, 180, or 270 g of salmon twice weekly for 4-week dietary treatments. Following a 4- to 8-week washout, participants crossed over to another dose of fish intake until all treatments were completed. Plasma lipid concentrations were determined and serum lipoprotein concentrations and particle size were determined by nuclear magnetic resonance. Intake of salmon reduced plasma and serum triglyceride (TG) concentrations and increased plasma HDL-C concentrations. The concentrations of large very low-density lipoprotein (VLDL)–P and chylomicron (CM)–P were reduced. Large LDL-P concentrations were increased in a dose-dependent manner. The mean size of VLDL-P was reduced and that of LDL was increased. Total TG was reduced as was the TG content of VLDL-P and CM-P. Twice weekly intake of farmed Atlantic salmon portions influences lipoprotein particle size and concentration in a manner associated with cardiovascular disease risk reduction.

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Abbreviations: C, cholesterol; CM, chylomicron; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFHNRC, Grand Forks Human Nutrition Research Center; HDL, high density lipoprotein; LCn-3, long chain omega-3; LDL, low density lipoprotein; NMR, nuclear magnetic resonance; P, particle; TG, triglyceride; VLDL, very low density lipoprotein.

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1. Introduction

Clinically, lipoprotein concentrations are evaluated to determine the risk of atherosclerosis [1]. Elevated concentrations of high-density lipoprotein cholesterol (HDL-C) and low concentrations of low-density lipoprotein cholesterol (LDL-C) are associated with reduced risk. Elevations in fasting and/or postprandial TG concentrations are seen to be atherogenic [2]. The lipoproteins responsible for transport of lipids include chylomicron (CM), very low-density lipoprotein (VLDL), LDL, and HDL with the classifications based upon the relative content of lipid and protein in each. The composition and physical structure of lipoprotein molecules is in constant flux and changes as the core contents are taken up by peripheral tissues The cholesterol and TG composition within the lipoprotein classes vary among individuals as a result of genetics [3,4], lifestyle [5,6], including diet [7,8], and drug therapy [9,10].

Elevated total and LDL-C and TG are associated with cardiovascular disease (CVD) risk; however, disease occurs among people with normal lipid levels [1]. Variation in the concentration and size of lipoprotein particles (P), particularly LDL-P and HDL-P, has an impact on their function and relationship to atherosclerosis development [11,12]. Individuals who have normal concentrations of cholesterol that are distributed in small, dense LDL-P may be at increased risk of coronary heart disease [13]. LDL-P size is an important CVD risk factor that correlates inversely with sub-clinical atherosclerosis as measured by intima-media thickening [14]. Although the total HDL-C concentration is associated with reduced CVD [15], it has been shown that, like LDL-P, small dense HDL-Ps are positively associated with increased risk of CVD [16] while an increased concentration of large HDL-Ps is considered protective [17,18]. VLDL-Ps are positively associated with CVD risk but a lower concentration of large VLDL-P is associated with reduced risk [16].

Consuming fatty fish and fish oil is associated with reduction of fatal coronary events [19]. The protective effect of fatty fish intake is ascribed to their content of the long-chain (LC) omega-3 (n-3) fatty acids [20] eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3). Supplementation with LCn-3 is an accepted therapy for the reduction of elevated blood TG levels [21,22] although it typically also results in increases in total cholesterol, HDL-C and LDL-C concentrations [23]. Controlled feeding studies show that fish intake increases large HDL-P concentrations [24,25]. Fish oil supplementation increases the content of large HDL-P and large LDL-P and reduces the size and concentration of VLDL-P [26,27], all changes that are associated with reduced risk of CVD [12].

The research objective of this study was to determine whether the intake of farmed Atlantic salmon (*Salmo salar*) would modify lipoprotein particle size and concentration in a manner associated with reduced CVD risk. Specifically, we hypothesized that LDL-P and HDL-P concentration and size would increase with salmon intake in dose-dependent manner. To test this hypothesis we performed an analysis of lipoprotein particle size and concentrations in a randomized, crossover-designed trial in which participants were fed 90 g, 180 g, and 270 g of farmed Atlantic salmon twice weekly in 4-week treatments.

2. Methods and materials

2.1. Study design and intervention

This investigation is an ancillary evaluation of a study which evaluated 19 participants in a cross-over designed clinical trial of farmed Atlantic salmon over three 4 week treatment periods. Complete details of the trial are provided elsewhere [28]. Here we report the plasma lipid concentrations and the serum lipoprotein particle concentration and size responses to the fish consumption. All study visits were at the US Department of Agriculture (USDA), Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND. The study was approved by the institutional review board at the University of North Dakota. Informed consent was obtained from all study participants prior to initiation of the study. The trial was registered at www.clinicaltrials.gov as NCT01183520.

2.2. Study population

Participants were recruited from the Greater Grand Forks Area, Grand Forks, ND. Sixty-one volunteers were screened for study participation. Twenty-two volunteers eligible for participation were initially randomized to treatment, although 3 withdrew prior to study initiation. Complete details of the study participant flow through the project are presented elsewhere [28]. The 19 participants who completed all aspects of the trial were men (n = 8) and women (n = 11) who were 51.6 ± 1.5 years (mean ± SE) and had an average body mass index of 29.2 ± 0.6 kg/m^2.

2.3. Dietary intervention

Dietary treatments consisted of farmed Atlantic salmon portions of 90 g, 180 g, or 270 g fed twice weekly. These treatments resulted in daily EPA and DHA intakes of 158 mg and 149 mg, 317 mg and 299 mg, and 475 mg and 448 mg, respectively. The fish fillets were served as prepared entrees on brown rice that were reheated by participants before consumption. Each participant completed all 3 of the 4-week dietary treatments in random order. Treatments were separated by 4- to 8-week washout periods. Throughout the trial participants consumed their habitual diets with the exclusion of fish and high n-3 foods, except for the fish provided.

2.4. Laboratory methods

Fasting blood samples were collected by venipuncture at Day 1 and 29 of each treatment. Serum was collected in tubes with no additive, allowed to clot at room temperature and then centrifuged at 3000 rpm at 4°C. Plasma was collected in tubes containing EDTA and prepared by centrifugation at 3000 rpm at 4°C. Plasma and serum samples were stored at –80°C until analysis.

2.4.1. *Plasma phospholipid fatty acids*

Phospholipid fatty acid analysis was performed using gas chromatography as described [29]. Briefly, lipids were extracted from the plasma samples using chloroform: methanol (2:1, by vol) [30]. Phospholipid was isolated from plasma by thin layer chromatography (silica gel 60, EMD Chemicals, Darmstadt, Germany) using a solvent system of heptane: isopropyl ether: glacial acetic acid (60:40:4, by vol). Bands corresponding to phospholipid standards were transferred to a glass test tube, methyl triheptadecanoin was added as an internal standard, then samples were allowed to dry at 110°C for 45 minutes. Esterified fatty acids in the dry sample were converted to their methylated derivatives in 2.5% sodium methoxide at 40°C for 60 minutes. At 60 minutes, the reaction was stopped with methyl formate and the fatty acid methyl esters were extracted with n-hexane. Fatty acid methyl ester content was quantified using a Shimadzu 2010 gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector and a capillary column (SP 2330; 30 m × 0.32 mm ID, Supelco, Bellefonte, PA). Prior to analysis the gas chromatograph was calibrated using known fatty acid methyl ester standards (NuChekPrep, Elysian, MN, USA). The internal standard, methyl heptadecanoate, and the individual fatty acids were quantified by peak area analysis (Shimadzu Class VP 7.2.1 Datasystem, Kyoto, Japan).

Sample fatty acid content was computed using an internal standard calculation based on established calibration curves and mole percentages were calculated by dividing the computed concentration of individual fatty acids by total computed sample fatty acid content then multiplying this number by 100. The detector response was linear for all fatty acid standards measured having correlation coefficients of 0.998 or greater.

2.4.2. *Plasma lipids*

Plasma lipids were measured in the clinical laboratory of the Grand Forks Human Nutrition Research Center. Cholesterol, HDL, and TG were measured by COBAS Integra 400 Plus with corresponding COBAS test kits (Cat #, 03 039 773, 20 767 107, and 03 039 773, Roche Diagnostics, Indianapolis, IN, USA; CVs for these kits are 1.4%, 1.9%, and 2.1% respectively). LDL concentration was determined by subtraction [31].

2.4.3. *Lipoprotein particles*

Lipoprotein particle size and concentration were measured from serum with nuclear magnetic resonance (NMR) spectroscopy at LabCorp Inc (Burlington, NC, USA) using the NMR *LipoProfile* test (http://www.labcorp.com). Briefly, the analysis measures individual lipoprotein subfractions and calculates the cholesterol contained within each lipoprotein; full details of this methodology are presented elsewhere [4].

2.5. *Statistical analyses*

The sample size estimate was based on expected mean differences for DHA reported by Calzada, et al. [30]. Because they only reported group (between subject) error estimates, data from our prior work [31] was used to estimate that the ratio of within- to between-subject variability to be approximately 0.5. Using this ratio and the between-subject standard deviation reported by Calzada et al, we estimated that the within-subject standard deviation would be approximately 0.175 mol%. Using a repeated measures analysis of variance, an SD of 0.175 and $\alpha = .05$, 17 subjects provided 80% power to detect a mean difference in DHA of 0.2 mol%.

Data are reported as means ± SEM. Primary analysis was conducted to determine the differences of fatty acids, lipids and lipoprotein variable concentration and particle size before and after each dietary treatment. For each study outcome, data were analyzed by using repeated measures analysis of variance (ANOVA), in which portion (90 g, 180 g, or 270 g of farmed Atlantic salmon), time (pre- or post-treatment), portion by time interaction, feeding sequence and time periods were fixed effects and subjects were random effects. Feeding sequence and time period were not significant for any of the outcomes. In Tables 1, 2 and 3, the ANOVA *P* value for portion indicates whether the effects of the 3 doses of salmon differed at both pre- and post-treatment; the *P* value for time indicates if there was a difference between the pre- and post-treatment means regardless of dose, and the portion × time interaction *P* value indicates whether the response from pre- to post-treatment differed depending upon the dose. Tukey's contrasts were used for post-hoc comparisons. All analyses were conducted using SAS, version 9.4 (SAS Institute, Inc, Cary, NC, USA), with $P < .05$ considered statistically significant.

3. Results

Nineteen participants completed each of the assigned treatments. Participants reported >99% compliance with the fish provided. Complete details of compliance monitoring are presented elsewhere [28]. No changes were seen in body weight throughout the study [28].

Plasma PLFA concentrations pre- and post-treatment are presented in Table 1. Parts of these data were previously published but are presented again for the reader [28]. Baseline values were not different between treatment periods for any of the fatty acids. At 4 weeks, palmitic (16:0), stearic (18:0), and oleic (18:1n-9) concentrations were reduced by all fish treatments (*P* for portion <.001, <.001, and .005, respectively). Fatty acid concentrations of n-6 fatty acids were generally reduced by fish intake with the largest reductions observed in arachidonic acid (20:4n-6) ($P < .001$ for portion and time). EPA, DHA and total LCn-3 concentrations were increased after each treatment ($P < .0001$ for portion) and the increases in EPA and total LCn-3 (sum of EPA and DHA) were dose-dependent ($P < .0001$ for portion × time).

Study results for plasma lipid levels are shown in Table 2. Total cholesterol concentration did not change with any treatment. HDL-C concentrations were increased by all treatments ($P = .01$). LDL-C levels were not significantly altered. Plasma TG concentrations were significantly reduced by all portions ($P = .02$). No significant difference was observed in the total cholesterol to HDL-C ratio by fish intake and is not presented here.

Serum lipoprotein particle concentration and size determined by NMR are presented in Table 3. The concentrations of large lipoprotein particles were decreased pre- to post-treatment for VLDL and CM ($P < .01$ for time) and increased for LDL-P ($P < .01$ for time). The level of increase in the

Table 1 – Pre- and post-treatment outcomes for plasma phospholipid fatty acid proportions (mol%) by twice-weekly portions of salmon for 4 weeks in 19 overweight men and women [1,2]

	90 g Salmon (n = 19)		180 g Salmon (n = 19)		270 g Salmon (n = 19)		ANOVA P [3]		
Fatty acid	Baseline	4 weeks	Baseline	4 weeks	Baseline	4 weeks	Portion	Time	Portion × Time
14:0	6.9 ± 0.4	7.6 ± 0.3	7.0 ± 0.3	7.1 ± 0.3	7.5 ± 0.2	6.9 ± 0.2	.73	.82	.06
16:0	8.1 ± 0.2	9.5 ± 0.2	8.2 ± 0.2	9.7 ± 0.4	8.0 ± 0.2	9.5 ± 0.4	.78	<.0001	.92
18:0	21.2 ± 0.7	19.9 ± 0.6	20.7 ± 0.5	19.3 ± 0.4	21.2 ± 0.5	19.4 ± 0.4	.44	<.001	.87
18:1n-9	8.4 ± 0.4	9.2 ± 0.4	8.2 ± 0.4	9.4 ± 0.3	8.6 ± 0.5	9.0 ± 0.3	.99	.006	.52
18:2n-6	16.7 ± 0.6	17.7 ± 0.6	17.4 ± 0.7	18.3 ± 0.7	17.3 ± 0.5	17.7 ± 0.7	.55	.10	.85
18:3n-6	0.8 ± 0.02^{ab}	0.7 ± 0.03^{b}	0.8 ± 0.03^{ab}	0.9 ± 0.1^{a}	0.7 ± 0.03^{b}	0.8 ± 0.04^{ab}	.01	.41	.003
20:3n-6	4.9 ± 0.2	4.0 ± 0.2	5.0 ± 0.2	4.2 ± 0.2	5.0 ± 0.2	4.4 ± 0.2	.45	<.0001	.64
20:4n-6	18.8 ± 0.9	17.8 ± 0.3	18.2 ± 0.9	16.4 ± 0.6	17.6 ± 0.8	14.9 ± 0.5	.005	.0002	.33
22:4n-6	2.1 ± 0.1	1.8 ± 0.13	2.3 ± 0.1	1.6 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	<.0001	<.0001	.08
20:5n-3	0.9 ± 0.04^{a}	0.9 ± 0.04^{a}	0.9 ± 0.04^{a}	1.9 ± 0.1^{b}	0.9 ± 0.03^{a}	2.6 ± 0.1^{c}	<.0001	<.0001	<.0001
22:5n-3	1.6 ± 0.2	1.3 ± 0.1	1.7 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.4 ± 0.1	.21	.15	.18
22:6n-3	3.2 ± 0.1	4.7 ± 0.2	3.1 ± 0.2	4.6 ± 0.2	3.4 ± 0.2	5.2 ± 0.3	.03	<.0001	.71
∑ 20:5n-3 + 22:6n-3	4.1 ± 0.1^{a}	5.6 ± 0.2^{b}	4.0 ± 0.2^{a}	6.5 ± 0.2^{c}	4.3 ± 0.2^{a}	7.8 ± 0.2^{d}	<.0001	<.0001	<.0001

[1] Values are means ± SEM.
[2] Means within a row not sharing a common superscript are significantly different ($P < .05$) by Tukey contrasts.
[3] The portion effect tests whether the response to the salmon differed depending on the amount of salmon consumed. The time effect tests whether subjects responded to the salmon, regardless of the amounts consumed. The portion × time effect tests for a differential response (ie, dose-response) to the amount of salmon amounts consumed.

concentration of large LDL-P was dose dependent with greater increases in concentration as the fish dose increased ($P = .03$ for portion × time). No changes were observed in the total or large HDL-P size concentration.

Treatment specific changes in the medium and small HDL particle concentrations were observed. The medium HDL-P concentration was increased after consuming the lowest dose of salmon and decreased after the highest dose ($P < .01$) while the small HDL-P size concentration was decreased after consuming the lowest dose of salmon and increased after the highest dose. The concentrations of the medium and small particles were not equivalent at baseline suggesting that there may have been some carry-over between treatments for these variables. The mean size of lipoprotein particles was decreased from pre- to post-treatment for VLDL-P and increased for LDL-P across all treatments ($P < .01$ for time for both). No change was observed in HDL-P size.

The NMR calculated lipid concentrations show a significant decrease in total TG ($P < .01$ for time) and for the TG content of both VLDL-P and CM-P ($P < .01$ for time). Total HDL-C values were unchanged across treatments. The plasma and serum HDL-C concentrations were dissimilar, likely due to actual differences in serum and plasma as well as the measurement techniques (NMR calculated values vs. COBAS measurement).

4. Discussion

Salmon intake resulted in marked changes in n-3 PLFAs. DHA was increased by treatments equally and appeared to reach saturation levels at the lowest salmon portion while EPA and total n-3 were increased in a dose-responsive manner. It has been shown that the intake of n-3 supplements resulted in a

Table 2 – Pre- and post-treatment outcomes for plasma lipoproteins by twice-weekly portions of salmon for 4 weeks in 19 overweight men and women [1,2]

		Salmon (90 g)		Salmon (180 g)		Salmon (270 g)		ANOVA P [3]		
Parameter	Units	Pre	Post	Pre	Post	Pre	Post	Portion	Time	Portion × Time
Cholesterol	mmol/L	5.12 ± 0.16	5.17 ± 0.17	5.11 ± 0.15	5.19 ± 0.16	5.2 ± 0.15	5.34 ± 0.17	.26	.20	.83
LDL-C	mmol/L	3.13 ± 0.18	3.14 ± 0.20	3.09 ± 0.18	3.17 ± 0.19	3.17 ± 0.16	3.34 ± 0.20	.16	.13	.56
HDL-C	mmol/L	1.29 ± 0.10	1.33 ± 0.12	1.31 ± 0.11	1.36 ± 0.12	1.28 ± 0.10	1.40 ± 0.12	.65	.01	.43
TG	mmol/L	1.57 ± 0.19	1.53 ± 0.15	1.56 ± 0.22	1.44 ± 0.15	1.64 ± 0.22	1.32 ± 0.14	.76	.02	.21

[1] Values are means ± SEM.
[2] Means within a row not sharing a common superscript are significantly different ($P < .05$) by Tukey contrasts.
[3] The portion effect tests whether the response to the salmon differed depending on the amount of salmon consumed. The time effect tests whether subjects responded to the salmon, regardless of the amounts consumed. The portion × time effect tests for a differential response (ie, dose-response) to the amount of salmon amounts consumed.

Table 3 – Pre- and post-treatment outcomes for serum lipoprotein particle concentration and size by twice-weekly portions of salmon for 4 weeks of treatment in 19 overweight men and women [1,2]

Parameter	Units	Salmon (90 g)		Salmon (180 g)		Salmon (270 g)		ANOVA P [3]		
		Pre	Post	Pre	Post	Pre	Post	Portion	Time	Portion × time
Particle concentrations										
Total VLDL & CM	nmol/L	68 ± 6.7	70.2 ± 7.1	63 ± 6.6	67.1 ± 7.7	67.1 ± 7.7	62.6 ± 7.3	.23	.64	.61
Large VLDL & CM	nmol/L	6.4 ± 1.1	5.5 ± 1.0	6.6 ± 1.4	5.5 ± 1.0	7.1 ± 1.3	4.4 ± 1.1	.88	<.01	.21
Medium VLDL	nmol/L	22.8 ± 3.5	24 ± 3.7	21.6 ± 3.0	23.9 ± 3.7	21.5 ± 3.7	20.2 ± 2.7	.42	.73	.65
Small VLDL	nmol/L	38.8 ± 4.0	40.7 ± 5.2	34.8 ± 4.5	37.6 ± 4.4	36.1 ± 4.3	38.0 ± 5.2	.41	.34	.98
Total LDL	nmol/L	1161 ± 79	1210 ± 92	1195 ± 79	1216 ± 78	1206 ± 70	1258 ± 94	.22	.08	.81
IDL	nmol/L	254 ± 23	286 ± 24	251 ± 32	273 ± 25	283 ± 25	241 ± 31	.88	.77	.14
Large LDL	nmol/L	359 ± 41	373 ± 50	402 ± 41	461 ± 41	356 ± 44	535 ± 48	.03	<.01	.03
Small LDL	nmol/L	549 ± 90	551 ± 89	542 ± 77	482 ± 73	567 ± 87	483 ± 97	.56	.09	.42
Total HDL	nmol/L	35.1 ± 1.1	35 ± 1.2	34.7 ± 1.2	34.4 ± 1.3	34.3 ± 1.1	33.9 ± 1.5	.33	.66	.97
Large HDL	nmol/L	7.1 ± 1.2	7.2 ± 1.1	6.9 ± 1.1	7.4 ± 1.2	7.1 ± 1.0	7.4 ± 1.3	.99	.13	.78
Medium HDL	nmol/L	8.2 ± 1.1	11.1 ± 1.7	9.7 ± 1.1	9.0 ± 1.1	11.9 ± 1.8	8.1 ± 1.1	.78	.48	<.01
Small HDL	nmol/L	19.8 ± 1.4	16.7 ± 1.8	18.0 ± 1.3	18.0 ± 1.4	15.3 ± 2.1	18.5 ± 1.5	.40	.98	.01
Particle Size										
VLDL Size	nm	50.6 ± 1.8	48.3 ± 1.3	51.8 ± 1.8	49.3 ± 1.2	51.7 ± 1.9	48.5 ± 1.6	.55	<.01	.82
LDL Size	nm	20.9 ± 0.2	21.0 ± 0.2	20.9 ± 0.2	21.0 ± 0.1	20.8 ± 0.2	21.2 ± 0.1	.93	<.01	.15
HDL Size	nm	9.2 ± 0.2	9.2 ± 0.2	9.2 ± 0.2	9.3 ± 0.2	9.3 ± 0.2	9.3 ± 0.2	.53	.75	.71
NMR Calculated Lipids										
TG	mmol/L	1.84 ± 0.2	1.75 ± 0.2	1.79 ± 0.2	1.66 ± 0.2	1.88 ± 0.2	1.47 ± 0.2	.40	<.01	.12
VLDL & CM TG	mmol/L	1.24 ± 0.2	1.16 ± 0.1	1.21 ± 0.2	1.12 ± 0.1	1.26 ± 0.2	1.00 ± 0.1	.53	<.01	.22
HDL Cholesterol	mmol/L	1.46 ± 0.1	1.47 ± 0.1	1.44 ± 0.1	1.48 ± 0.1	1.43 ± 0.1	1.00 ± 0.2	.89	.11	.72

[1] Values are means ± SEM.
[2] Means within a row not sharing a common superscript are significantly different ($P < .05$) by Tukey contrasts.
[3] The portion effect tests whether the response to the salmon differed depending on the amount of salmon consumed. The time effect tests whether subjects responded to the salmon, regardless of the amounts consumed. The portion × time effect tests for a differential response (ie, dose–response) to the amount of salmon amounts consumed.

similar response [32]. It is possible that the EPA provided by the 90 g portion was converted to DHA but reached saturation levels so that increasing intake resulted in EPA increases; however the retroconversion of DHA to EPA appears to be minimal [33]. The incorporation of EPA and DHA into various plasma pools is variable with more EPA going into phospholipid and cholesterol ester and DHA into phospholipid and triglyceride lipid fractions [34]. As we did not examine lipid pools other than PLFA, the observed data indicate that PLFA pools of EPA and DHA behave differently and that this EPA pool is labile.

The present study evaluated plasma lipoproteins and serum lipoprotein particle concentration and size responses of overweight adult men and women to three doses of farmed Atlantic salmon consumed twice weekly for 4 weeks in a randomized, controlled cross-over designed trial. It is well recognized that LCn-3 fatty acids are effective in reducing TG concentrations [21,35]. Recent clinical trial data shows that fish intake or supplementation with fish oil results in modification of lipoprotein particle size and concentration. A shift toward less atherogenic, larger and more buoyant LDL particle composition occurs with both fish intake (both lean and fatty fish) and supplementation with fish oil, EPA and/or DHA [24-27,35,36]. Purified EPA ethyl ester supplementation (1.8 g daily) reduced the concentration of small-dense LDL particles [37] while krill oil supplementation over 28 days at (832.5 mg EPA and DHA daily) resulted in reduced concentrations of plasma TG, and very large VLDL and CM particles [32]. Taken together, these results are similar to that provided by our twice weekly 270 g portions of farmed Atlantic salmon with the exception of a reduction in LDL particle size and concentration. Our data indicate that serum lipoprotein particle concentration and size were modified in response to salmon intake in a manner associate with reduced CVD risk. The intake of salmon twice weekly reduced plasma TG concentrations and increased HDL-C concentrations. These changes were accompanied by reduced concentrations of large VLDL-P and CM-P, a reduction in the mean size of the VLDL-P, and a decrease in the TG content of the VLDL and CM particles.

Typically, changes in the concentration of lipids and lipoproteins are associated with the fatty acid content of the fish; however it is possible that the protein content has positive influences on markers of CVD risk as well. Jensen et al demonstrated that in mice fed cod and salmon, animals had reduced atherosclerotic plaque and reduced LDL cholesterol compared to chicken fed animals [38]. In a clinical trial, the consumption of 60% of dietary protein from lean seafood choices in diets designed to control the LCn-3 intake, resulted in reductions in fasting and postprandial TG, fasting TG content of CM and VLDL, VLDL particle size, and concentration of medium-sized VLDL particles [39].

Reductions of TG in response to fish intake providing more modest levels of EPA and DHA have been shown. An early study evaluating fasting and postprandial lipid responses to fish, fish oil, and a DHA rich oil demonstrated that the daily consumption of 380 mg of EPA and 670 mg of DHA from fish over 15 weeks reduced TG concentrations and increased the HDL_2/HDL_3-C ratio [40]. Our average daily intake ranged from 158 to 475 mg for EPA and 149 mg to 448 mg for DHA depending on the fish portions. Even at the lowest intake levels, we observed significant reductions in TG in healthy participants. Similarly, we observed an increase in plasma HDL-C in response to all treatments; however, the large HDL-P concentration was unchanged.

The concentration of large LDL-P was increased in a dose-dependent manner in response to salmon consumption. Others have reported increases in large LDL-P concentration after intake of fish oil [27] but to our knowledge, this is the first report demonstrating a dose- dependent response to fish intake. Higher numbers of large LDL particles are protective against atherosclerosis by reduction of plaque formation and endothelial dysfunction [41-43].

In our study, the fish entrees served replaced two other main dish food items each week and we have no knowledge of what was replaced. It is possible that the improvements seen in lipoprotein profiles are related to both the LCn-3 fatty acids and other nutritional components of salmon. Some studies have shown increases in large HDL-P concentrations in response to lean fish intake suggesting that it is not only the LCn-3 component of the fish that is bioactive [44,45]. As in any feeding study, one must also consider whether the observed changes are in part due to the replacement of food in the diet as well as the foods given such that the alterations observed in lipoprotein concentrations may be partly due to the replacement of other food items with fish.

The primary strength of our project was the random assignment of participants to treatments and the high reported compliance with the treatments and the instruction to not consume any fish in addition to the treatment and to avoid other sources of n-3 fatty acids while on the treatments [28]. An additional strength is the fact that the baseline fatty acid concentrations at baseline of each treatment period were not different from one another showing that the washout period was adequate between treatments.

We are limited by the small sample size of the study however the trial was performed in a cross-over designed manner which allowed determination of treatment effects. Because of the small sample size, we are unable to evaluate whether the responses are quantitatively similar in men and women. When the effect of dietary components on lipoproteins profiles has been assessed according to sex, variations commonly exist [46,47].

In conclusion, our study demonstrates that twice weekly consumption of farmed Atlantic salmon in doses as low as 90 g modifies serum lipoprotein concentration and particles in a manner related to lower CVD risk. In agreement with our hypothesis, large LDL particle concentration was increased in a dose-dependent manner in response to the consumption of increasing portions of farmed Atlantic salmon. We saw no dose-responsive effect on HDL particle size. The dose-dependent increase in the concentration of large LDL-P observed in this trial is a novel and important finding which will provide direction for recommendations of fish to consume for CVD prevention. Our data indicate that improvements in TG concentrations and lipoprotein particle profiles are possible with whole food intake. Subsequent study is needed to define the extent to which these responses are the result of LCn-3 intake, fish intake, or a combination of both.

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