Fish Consumption Shifts Lipoprotein Subfractions to a Less Atherogenic Pattern in Humans[1]

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ABSTRACT The effect of fish consumption on plasma lipoprotein subfraction concentrations was studied in 22 men and women (age > 40 y). Subjects were provided an average American diet (AAD, 35% of energy as fat, 14% as saturated fat, and 35 mg cholesterol/MJ) for 6 wk before being assigned to a National Cholesterol Education Program (NCEP) Step 2 high-fish diet (n = 11, 26% of energy as fat, 4.5% as saturated fat, and 15 mg cholesterol/MJ) or a NCEP Step 2 low-fish diet (n = 11, 26% of energy as fat, 4.0% as saturated fat, and 11 mg cholesterol/MJ) for 24 wk. All food and drink were provided to study participants. Consumption of the high-fish NCEP Step 2 diet was associated with a significant reduction in medium and small VLDL, compared with the AAD diet, whereas the low-fish diet did not affect VLDL subfractions. Both diets significantly reduced LDL cholesterol concentrations, without modifying LDL subfractions. Both diets also lowered HDL cholesterol concentrations. However, the high-fish diet significantly lowered only the HDL fraction containing both apolipoprotein (apo) AI and AII (LpAI:AII) and did not change HDL subfractions assessed by NMR, whereas the low-fish diet significantly lowered the HDL fraction containing only apo AI (LpAI) and the large NMR HDL fractions, resulting in a significant reduction in HDL particle size. Neither diet affected VLDL and LDL particle size. Our data indicate that within the context of a diet restricted in fat and cholesterol, a higher fish content favorably affects VLDL and HDL subspecies. J. Nutr. 134: 1724–1728, 2004.

KEY WORDS: • *lipoprotein subspecies* • *lipoprotein particle size* • *NCEP* • *diet* • *cholesterol* • *triglycerides*

Elevated plasma LDL cholesterol (C)[3] concentrations (≥4.14 mmol/L) and low HDL-C concentrations (<1.03 mmol/L) are independent risk factors for coronary heart disease (1,2). In addition, elevated triglyceride (TG) levels are a risk factor for coronary heart disease, especially in women (3). The major lipoprotein classes, such as VLDL, LDL, and HDL, exist in plasma as families of different lipoprotein subfractions. These lipoprotein subfractions differ in their atherogenic capabilities. Diets restricted in saturated fat and cholesterol were shown to reduce plasma LDL-C concentrations (4–6), but little is known about the effect of such diets on the different subpopulations of LDL, and of VLDL and HDL (7). Fish oil supplements and diets containing fish are enriched with eicosapentaenoic [20:5(n-3)] and docosahexaenoic [22:6(n-3)] acids and have been found to reduce plasma levels of TG, especially in the postprandial state (8), but only a few studies have examined the effect of fish or fish-oil supplements on lipoprotein subpopulations (9–11).

HDL particles, especially those containing apolipoprotein (apo) AI without apo AII (LpAI), are believed to promote cellular free cholesterol efflux more efficiently than particles containing both apo AI and apo AII (LpAI:AII) (12). A number of studies reported that dietary restriction of saturated fat and cholesterol lowers HDL-C (4,8,13). It was reported that low-cholesterol, low-fat diets enriched in fish lower LpAI:AII but not LpAI concentrations (11).

The purpose of this study was to assess the effects of National Cholesterol Education Panel (NCEP) Step 2 diets enriched in fish (high-fish NCEP Step 2) or in lean poultry (low-fish NCEP Step 2) compared with an average American diet (AAD), on lipoprotein particle concentrations and lipoprotein particle sizes in middle-aged and elderly subjects. Our data indicate that these diets lower both LDL-C and HDL-C, but have different effects on VLDL and HDL subspecies.

MATERIALS AND METHODS

Study subjects. A total of 22 subjects (11 men and 11 women) participated in this study. All subjects were >40 y old and were not taking medications known to affect lipoprotein metabolism at the time of screening and throughout the study. All participating women

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[3] Abbreviations used: AAD, average American diet; apo, apolipoprotein; C, cholesterol; IDL, intermediate density lipoprotein; NCEP, National Cholesterol Education Program; TC, total cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoproteins.

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were postmenopausal. Before enrollment into the study, each subject received a complete medical history and physical examination. No subject had any evidence of thyroid disease, diabetes mellitus, kidney disease, or liver disease as assessed by history and clinical laboratory tests. Subjects who smoked or consumed large amounts of alcohol (>0.340 L/d of beer, or 0.142 L/d of wine, or 0.043 L/d of distilled liquors) were not enrolled into the study.

The subjects and study design were described previously (8). Briefly, all subjects consumed the AAD for 6 wk and then 11 subjects (4 men and 7 women, mean age 66 ± 6.6 y, BMI 26 ± 3 kg/m^2) were assigned to an NCEP Step 2 diet enriched in fish (high-fish NCEP Step 2) and 11 subjects (7 men and 4 women, age 61 ± 13 y, BMI 26 ± 3 kg/m^2) were assigned to an NCEP Step 2 diet low in fish and high in lean turkey and chicken (low-fish NCEP Step 2) for 24 wk. The 2 groups had similar mean plasma lipid levels at screening (data not shown). Three women participated in both the high-fish and low-fish diet studies. The study protocol was approved by the Tufts University-New England Medical Center Investigation Review Board. Informed consent was obtained from all subjects.

Study diets. The NCEP Step 2 diets were designed according to the criteria defined by the NCEP Adult Treatment Panel II (≤30% of energy as total fat, <7% as saturated fat, and <200 mg cholesterol/d) (1). All food and drink for the AAD diet and the NCEP Step 2 diets were prepared by the Metabolic Research Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University and were provided to the participants. Meals consisted of breakfast, lunch, dinner, and snacks, on a 3-d cycle menu. Energy intake was adjusted to keep each subject's body weight constant (±1 kg) throughout the study. The composition of the AAD diet and of the NCEP Step 2 diets, as assessed by chemical analysis of triplicate samples of each diet (Hazleton Laboratories America), is shown in **Table 1**. The types of fish in the high-fish diet included tuna, salmon, and sole fillet.

Lipid measurements. Subjects had blood drawn into 0.15% (wt/v) EDTA tubes after a 12- to 14-h fast. Blood samples for plasma lipid measurements were collected at wk 4, 5, and 6 of the AAD diet, and at wk 4, 8, 12, 16, 20, and 24 of the NCEP Step 2 diets. Also, postprandial plasma samples were collected at the end of each diet phase during a constant feeding period, in which subjects consumed food hourly for a 20-h period, with each meal containing 1/20 of the energy consumed during the day and having the same composition as the diet of that phase. This modified postprandial test was performed as part of a protocol for the study of the kinetics of apolipoproteins with stable isotopes. This protocol requires steady-state lipoprotein metabolism, achieved by consuming food hourly. Blood samples (n = 10) were collected between 5 and 20 h after food consumption had begun.

Plasma was subjected to ultracentrifugation in a Beckman ultracentrifuge at 109,000 × g for 18 h at 4°C for the isolation of VLDL (supernatant) and infranatant. HDL and the HDL_3 fraction were isolated by dextran sulfate-Mg^{2+} methods, as previously described (14,15). Concentrations of plasma total C (TC), TG, HDL-C, HDL_3-C and of C in the infranatant were measured using an Abbott Diagnostics Spectrum ABA-200 bichromatic analyzer with Abbott enzymatic reagents (16). VLDL-C, LDL-C, and HDL_2-C concentrations were calculated as follows: VLDL-C = TC – infranatant C; LDL-C = infranatant C – HDL-C; and HDL_2-C = HDL-C – HDL_3-C. Within and between run CVs for these lipid measurements were <5%.

Apolipoprotein measurements. Plasma concentrations of apo AI and apo B were measured by noncompetitive ELISAs, using affinity purified polyclonal antibodies as previously described (17,18). Within and between run CVs for these assays were <10%.

Measurement of lipoprotein subfractions. Plasma samples at the end of each study period (wk 6 for the AAD and wk 24 for the NCEP Step 2 diets) were subjected to differential electroimmunoassay to determine the concentration of HDL particles containing only apo AI, or LpAI, as previously described (19). CVs were <5%. The plasma concentration of HDL particles containing both apo AI and apo AII, or LpAI:AII, was obtained as the difference between plasma apo AI and LpAI.

NMR spectroscopy methodology was used to determine lipoprotein particle subpopulation concentrations (20). Measurement of lipoprotein subfractions by NMR was performed within 1 y of sample collection. The NMR method employs the characteristic methyl group signals broadcast by lipoprotein subclasses of different size as the basis of their quantification. Each measurement includes the concentrations of 6 subclasses of VLDL (V1–V6; larger numbers denoting larger subclasses), 4 subclasses of LDL [L1–L3 and intermediate density lipoproteins (IDL)], and 5 subclasses of HDL (H1–H5), as well as calculated mean particle sizes of VLDL, LDL, and HDL.

Statistical analyses. The SPSS statistical package was used to analyze the data. Logarithmic transformations were applied to variables that were not normally distributed to approximate a normal distribution before testing for significant differences. Paired Student's t tests were used to test for differences between Step 2 diet and the corresponding AAD diet means. P-values < 0.05 were assumed to be significant. The percentage changes were calculated as: (NCEP Step 2 diet value – AAD value)/AAD value × 100. For lipoprotein subfractions, the difference between phases was calculated instead. Differences in lipoprotein concentration and lipoprotein subfraction response between the 2 NCEP Step 2 diets were assessed by Student's t test when the variables were normally distributed and by Mann-Whitney U test when the variables were skewed.

TABLE 1

Composition of the AAD and the NCEP Step 2 diets, as assessed by chemical analysis of food[1]

	AAD	High-fish NCEP Step 2	Low-fish NCEP Step 2
		% energy	
Carbohydrates	49.4 ± 2.2	56.1 ± 2.9	58.2 ± 1.8
Protein	15.0 ± 1.2	17.2 ± 0.9	16.3 ± 0.7
Total fat	35.4 ± 2.3	26.4 ± 2.0	25.5 ± 1.8
SFA	14.1 ± 2.2	4.5 ± 0.7	4.0 ± 0.4
14:0	1.6 ± 0.3	0.2 ± 0.1	0.1 ± 0.0
16:0	7.1 ± 0.5	2.9 ± 0.6	2.2 ± 0.3
18:0	2.9 ± 0.2	1.3 ± 0.4	1.0 ± 0.1
MUFA	14.5 ± 1.0	11.6 ± 1.4	10.8 ± 0.9
18:1 (n-9)	12.6 ± 1.1	10.5 ± 2.2	10.7 ± 2.7
PUFA	6.9 ± 1.2	10.3 ± 0.2	10.5 ± 0.2
18:2 (n-6)[2]	4.1 ± 0.2	7.0 ± 0.4	7.1 ± 0.8
18:3 (n-3)[2]	0.7 ± 0.2	1.9 ± 0.6	2.0 ± 0.2
20:4 (n-6)[2]	<0.01	0.1 ± 0.1	<0.02
20:5 (n-3)[2]	<0.01	0.2 ± 0.1	<0.02
22:6 (n-3)[2]	<0.01	0.5 ± 0.2	0.1 ± 0.1
Cholesterol, *mg/MJ*	35 ± 6	15 ± 4	11 ± 4

[1] Values are means ± SD, n = 3.
[2] Values derived from food composition tables.

RESULTS

Both the high-fish and the low-fish NCEP Step 2 diets significantly lowered plasma TC and LDL-C concentrations (**Table 2**). The diets did not affect fasting plasma TG concentrations, but consumption of the high-fish diet significantly reduced postprandial TG concentrations (Table 2). In addition, the change in postprandial TG levels in subjects that consumed the high-fish diet was significantly greater (P < 0.05) than in those that consumed the low-fish diet.

Consumption of both the high-fish and the low-fish NCEP Step 2 diets significantly reduced plasma HDL-C concentrations, due to reductions in both the HDL_2 and the HDL_3 fractions (Table 2). Moreover, plasma apo AI concentrations decreased significantly after consumption of both diets (Table 2). However, the 2 diets differed in the apo AI-containing

TABLE 2

Effects of consuming the AAD diet for 6 wk and the high- or low-fish NCEP Step 2 diets for 24 wk on plasma lipids and lipoproteins in men and women > 40 y old[1]

	AAD	High-fish NCEP Step 2	% change[2]	AAD	Low-fish NCEP Step 2	% change[2]
	mmol/L			*mmol/L*		
TC	5.44 ± 0.83	4.69 ± 0.84*	−14	5.70 ± 0.98	4.64 ± 0.86*	−19
VLDL-C	0.49 ± 0.26	0.46 ± 0.25	0	0.54 ± 0.29	0.52 ± 0.20	+3
LDL-C	3.62 ± 0.73	3.07 ± 0.67*	−15	3.89 ± 0.82	3.10 ± 0.74*	−20
TG	1.20 ± 0.43	1.12 ± 0.38	−4	1.19 ± 0.43	1.25 ± 0.38	+7
Post-TG#	2.14 ± 0.89	1.58 ± 0.63*	−23	1.80 ± 0.69	1.70 ± 0.54	−2
HDL-C	1.33 ± 0.28	1.17 ± 0.24*	−11	1.27 ± 0.24	1.06 ± 0.22*	−17
HDL2-C	0.32 ± 0.15	0.26 ± 0.12*	−19	0.28 ± 0.13	0.20 ± 0.10*	−31
HDL3-C	1.01 ± 0.17	0.91 ± 0.15*	−9	0.99 ± 0.15	0.86 ± 0.14*	−13
	g/L			*g/L*		
Apo AI	1.35 ± 0.25	1.19 ± 0.21*	−12	1.38 ± 0.21	1.18 ± 0.21*	−14
LpAI L	0.43 ± 0.10	0.38 ± 0.10	−9	0.45 ± 0.11	0.32 ± 0.05*	−27
LpAI:AII	0.92 ± 0.17	0.80 ± 0.14*	−13	0.93 ± 0.16	0.87 ± 0.17	−66

[1] Values are means ± SD of the means of wk 4, 5 and 6 of the AAD diet, and of the means of wk 4, 8, 12, 16, 20, and 24 of the NCEP Step 2 diets, $n = 11$. * Different from the AAD diet, $P < 0.05$. # Difference in response between the NCEP Step 2 diets, $P < 0.05$.
[2] % change, mean percentage change from the respective AAD diet.

HDL fraction affected by the diet. In subjects that consumed the high-fish diet, there was a significant reduction in the LpAI:AII fraction, and in those that consumed the low-fish diet, there was a significant reduction of the LpAI fraction.

When the individual lipoprotein subfractions, as assessed by NMR, were examined, the high-fish NCEP Step 2 diet significantly reduced the medium and small VLDL fractions V4 and V2, respectively, without significantly affecting the IDL, LDL, or HDL subfractions (**Table 3**). On the other hand, consumption of the low-fish diet significantly reduced IDL and the concentration of the large HDL subfractions H5 and H4 (Table 3). These changes were accompanied by a significant reduction in HDL mean particle size, as measured by NMR, during consumption of the low-fish but not the high-fish diet (**Table 4**). No significant changes in VLDL and LDL particle size were observed after consumption of the 2 diets.

TABLE 3

Effects of consuming the AAD diet for 6 wk and the high- or low-fish NCEP Step 2 diets for 24 wk on plasma lipids and lipoprotein subfractions as measured by NMR in men and women > 40 y old[1]

	AAD	High-fish NCEP Step 2	Change[2]	AAD	Low-fish NCEP Step 2	Change[2]
	mmol/L			*mmol/L*		
VLDL[3]						
V6	0.07 ± 0.03	0.04 ± 0.01	−0.03	0.03 ± 0.02	0.05 ± 0.01	0.01
V5	0.14 ± 0.06	0.09 ± 0.04	−0.05	0.14 ± 0.06	0.18 ± 0.09	0.04
V4	0.24 ± 0.04	0.17 ± 0.04*	−0.08	0.22 ± 0.02	0.16 ± 0.04	−0.06
V3	0.21 ± 0.03	0.21 ± 0.06	+0.01	0.27 ± 0.06	0.25 ± 0.04	−0.01
V2	0.27 ± 0.03	0.17 ± 0.03*	−0.10	0.24 ± 0.06	0.20 ± 0.03	−0.04
V1	0.03 ± 0.02	0.03 ± 0.01	0.00	0.03 ± 0.02	0.04 ± 0.00	0.00
LDL[3]						
IDL	0.22 ± 0.05	0.14 ± 0.05	−0.08	0.24 ± 0.06	0.08 ± 0.03*	−0.16
L3	2.06 ± 0.33	1.55 ± 0.33	−0.51	1.88 ± 0.34	1.47 ± 0.23	−0.41
L2	0.79 ± 0.16	0.61 ± 0.23	−0.18	0.77 ± 0.12	0.62 ± 0.25	−0.15
L1#	0.34 ± 0.12	0.52 ± 0.10	+0.18	0.72 ± 0.17	0.79 ± 0.14	+0.07
HDL[3]						
H5	0.29 ± 0.05	0.22 ± 0.03	−0.06	0.25 ± 0.05	0.16 ± 0.13*	−0.08
H4	0.26 ± 0.06	0.27 ± 0.05	+0.01	0.25 ± 0.05	0.19 ± 0.05*	−0.06
H3	0.24 ± 0.04	0.19 ± 0.03	−0.05	0.22 ± 0.03	0.20 ± 0.04	−0.01
H2	0.23 ± 0.06	0.19 ± 0.03	−0.04	0.27 ± 0.04	0.25 ± 0.04	−0.02
H1	0.19 ± 0.04	0.20 ± 0.04	+0.01	0.14 ± 0.04	0.15 ± 0.05	+0.01

[1] Data expressed as means ± SE of measurements performed at wk 6 of the AAD diet and wk 24 of the NCEP Step 2 diets, $n = 11$. * Different from the AAD diet, $P < 0.05$. # Difference in response between the NCEP Step 2 diets, $P < 0.05$.
[2] Change, mean difference between Step 2 and AAD diet.
[3] VLDL subfractions are expressed as mmol/L triglycerides; IDL, LDL, and HDL are expressed as mmol/L cholesterol.

TABLE 4

Effects of consuming the AAD diet for 6 wk and the high- or low-fish NCEP Step 2 diets for 24 wk on mean lipoprotein particle size in men and women > 40 y old[1]

	AAD	High-fish NCEP Step 2	% change[2]	AAD	Low-fish NCEP Step 2	% change[2]
	nm			*nm*		
VLDL size	46.2 ± 2.7	44.7 ± 2.4	−1.1	42.8 ± 2.0	46.3 ± 2.4	9.3
LDL size	21.1 ± 0.2	20.8 ± 0.1	−1.2	20.7 ± 0.2	20.6 ± 0.2	−0.5
HDL size	9.3 ± 0.1	9.2 ± 0.1	−0.6	9.2 ± 0.2	9.0 ± 0.1*	−2.2

[1] Data expressed as means ± SE of measurements performed at wk 6 of the AAD diet and wk 24 of the NCEP Step 2 diets, $n = 11$. * Different from the AAD phase, $P < 0.05$.
[2] % change, the percentage change between Step 2 and AAD diet.

DISCUSSION

The National Cholesterol Education Program Panels recommend a diet restricted in saturated fat and cholesterol to lower LDL-C concentrations in patients with increased risk of coronary heart disease (1,2). In this study, we compared 2 diets meeting the criteria for a NCEP Step 2 diet as recommended by the NCEP Adult Treatment Panel II (1). Both diets had ≤30% of total energy as fat, <7% as saturated fat, and <200 mg of cholesterol/d. The 2 NCEP diets were very similar in fat content and fatty acid composition, with the exception that the high-fish diet had a greater content of (n-3) fatty acids than the low-fish diet.

Fish-derived long-chain PUFA of the (n-3) type were shown by many investigators to lower the plasma concentrations of TG, especially in TG-rich lipoproteins (TRL) (21–23). The mechanism for this reduction in TRL involves both a reduction in VLDL synthesis (24) and increased TRL clearance (25,26). Fish oil supplementation was also shown to decrease absorption of dietary C (27) and change HDL and LDL particle compositions (28,29). In addition, long-chain (n-3) fatty acids were shown to decrease the risk of coronary heart disease, partially via an antiarrhythmic effect on myocardial cells (30).

In our study, consumption of the high-fish NCEP Step 2 diet was not associated with a significant reduction in fasting plasma TG concentrations. However, the mean postprandial TG concentration was lowered by 23% when this diet was consumed compared with only a nonsignificant 2% reduction when the low-fish diet was consumed. The lack of a reduction in fasting TG concentrations in those consuming the high-fish diet may be related to the greater carbohydrate content in this diet compared with the AAD diet. It is not uncommon to observe an elevation in TG levels after dietary fat restriction, due to higher hepatic TG synthesis in subjects consuming high glycemic index and high-carbohydrate diets (31).

The focus of our study was to assess the effects of these experimental diets on lipoprotein subfractions and lipoprotein particle sizes. Our data indicate that consumption of the high-fish diet reduced VLDL subfractions of medium and small size, in spite of the lack of a significant effect of this diet on plasma total TG concentrations. No significant changes in VLDL subfractions were noted when the low-fish diet was consumed. Patti et al. (9) studied the effect of long-term fish oil supplementation on VLDL and LDL subfractions in 16 subjects with diabetes mellitus and reported a reduction, although nonsignificant, in the concentration of large, intermediate, and small VLDL. Also, these authors reported a significant increase in the percentage of cholesterol in small LDL, without any change in LDL size, during supplementation with fish oil (9). We also observed an increase, although nonsignificant ($P = 0.34$), in the concentration of small LDL (LDL1) without a change in LDL size when the high-fish diet was consumed. These lipoprotein changes are likely the result of an increased conversion of VLDL to LDL, due to more efficient lipolysis.

In our study, consumption of both the high-fish and the low-fish diets significantly reduced HDL-C, HDL_2-C, and apo A-I concentrations. However, consumption of the low-fish diet reduced these variables more than consumption of the high-fish diet. In addition, consumption of the low-fish diet, but not the high-fish diet, was associated with significant reductions in the large HDL subfractions 5 and 4 as measured by NMR, and in the LpAI subfraction. These changes in HDL subfractions when the low-fish diet was consumed were accompanied by a significant reduction in HDL size. Our results agree in part with those of Beauchesne-Rondeau et al. (10) who studied 18 men consuming lean fish and lean poultry diets. In spite of no changes in total HDL-C levels after consumption of either diet, during consumption of the lean fish diet, but not the poultry diet, there was a significant increase in the HDL_2 fraction. A significant increase in HDL_2 concentrations after consumption of a lean fish diet was also reported in a study of 11 men who consumed a fish and a nonfish diet (32). In our study, the high-fish diet did not cause significant changes in HDL subfractions assessed by NMR, but was associated with a reduction in the LpAI:AII fraction. A lack of effect of fish oil supplementation on HDL subfractions was also observed in a study of 18 hypertriglyceridemic subjects treated with fish oil capsules (33). It was reported that the LpAI particle may be more important for promoting C efflux from cells than the LpAI:AII particle (12). Because we observed a significant reduction in LpAI concentrations with consumption of the low-fish diet, but not the high-fish diet, the latter diet might be preferable for reducing heart disease risk. It should also be noted that dietary intervention studies indicate significant benefits for lowering heart disease death rates with diets that are either high in fish or in (n-3) fatty acids (34–36).

We documented previously that consumption of diets with a reduced content of saturated fat and C results in a reduction in HDL-C and apo A-I concentrations caused by a decrease in the apo A-I secretion rate (37). Moreover, Brinton et al. (38) reported a decrease in apo AI production and an increase in apo AI fractional catabolism in humans consuming a low-fat, high-carbohydrate diet compared with an average U.S. diet; the kinetic parameter that was significantly correlated with the

decrease in HDL cholesterol concentration was a decrease in apo AI production.

In conclusion, our data are consistent with the view that the high-fish and low-fish NCEP Step 2 diets have somewhat different effects on VLDL and HDL subpopulations, i.e., within the context of reduced fat in both NCEP Step 2 diets, the high-fish diet has less of a lowering effect on the large HDL subpopulations and more of a lowering effect on the medium and small VLDL subfractions than the low-fish NCEP Step 2 diet. Although small, these different effects may result in a long-term difference in cardiovascular disease risk.

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