

## Original Research

# Responses of Plasma Lipoproteins and Sex Hormones to the Consumption of Lean Fish Incorporated in a Prudent-Type Diet in Normolipidemic Men

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**Key words:** fish, lipoproteins, lipases, sex hormones, prudent diet, men

**Objective:** The effects of lean fish on plasma lipoproteins, postheparin plasma lipolytic activities and sex hormones were examined in 11 normolipidemic male subjects.

**Methods:** This study was a randomized crossover trial of two isoenergetic prudent-type diets, lean fish diet and beef, pork, veal, eggs and milk (nonfish) diet. Experimental diets provided approximately 11800 kJ—18% as proteins, 50% as carbohydrates, 32% as lipids [ratio of polyunsaturated to saturated fatty acids (P:S) of 1:1 compared with 0.5:1 in preexperimental diet], and 260 mg cholesterol/day.

**Results:** Compared with the nonfish diet, the lean fish diet induced higher plasma total and LDL apolipoprotein (apo) B and apo B:apo A-1 ratio, indicating that the substitution of lean fish for beef, veal, pork, eggs and milk provides little benefits with regard to plasma apo B concentrations in a low-fat high P:S diet. Moreover, triglycerides:apo B and cholesterol:apo B ratios of VLDL were lower following the lean fish diet than the nonfish diet, suggesting the presence of smaller very low-density lipoprotein (VLDL) particles following the consumption of lean fish. Higher plasma concentrations of sex hormone-binding globulin (SHBG), HDL<sub>2</sub> cholesterol and HDL<sub>2</sub>:HDL<sub>3</sub> cholesterol ratio were found with the lean fish diet compared with the nonfish diet. Negative correlations between plasma postheparin lipoprotein lipase (LPL) activity and VLDL triglycerides (n = 11, r = -0.53, p = 0.02), and between plasma postheparin LPL activity and VLDL triglycerides:apo B ratio (n = 11, r = -0.64, p = 0.02) were also observed following the lean fish diet.

**Conclusion:** These results suggest that the effects of substituting lean fish for beef, veal, pork, eggs and milk on plasma lipoproteins may be partly associated with variations in plasma sex hormone status and plasma LPL activity in normolipidemic men.

## INTRODUCTION

Atherosclerosis is a multifactorial disorder associated with the development of coronary heart disease (CHD), which has become the commonest cause of disability and death in industrialized countries. Dietary recommendations have been proposed in order to prevent or reduce the development of atherosclerosis in the general population [1,2]. According to the

recommendations for a prudent diet from the 1994 National Cholesterol Education Program (NCEP) [2], the proportion of lipids consumed should be limited to 30% or less of total energy, consumption of saturated fatty acids should be 8% to 10% of total energy, consumption of omega-6 (n-6) polyunsaturated fatty acids (PUFA) up to 10% of total energy, and cholesterol consumption should be limited to less than 300 mg per day. Low-fat high polyunsaturated/saturated fat ratio (P:S)

Abbreviations: apo = apolipoprotein, BMI = body mass index, nonfish = beef, pork, veal, eggs and milk, CHD = coronary heart disease, HDL = high-density lipoproteins, HTGL = hepatic triglyceride lipase, LDL = low-density lipoproteins, LPL = lipoprotein lipase, lean fish = lean white fish, n-3 = omega -3, n-6 = omega-6, NCEP = National Cholesterol Education Program, P:S ratio = polyunsaturated to saturated fatty acid ratio, P:M:S ratio = polyunsaturated to monounsaturated to saturated fatty acid ratio, PUFA = polyunsaturated fatty acids.

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diets can contribute to reduce the incidence of CHD risk factors, such as elevated concentrations of cholesterol and apolipoprotein (apo) B in total plasma and low-density lipoproteins (LDL) and reduced concentrations of cholesterol in high-density lipoproteins (HDL). The NCEP [2] also recommends including fish in the diet because it is relatively low in saturated fat and is a natural source of omega-3 (n-3) polyunsaturated fatty acids (PUFA) known to reduce blood clotting and serum triglycerides [3]. In this respect, it has been suggested that fish oil lowers triglyceride concentrations by inhibiting VLDL triglyceride synthesis [4]. More recently, it has been also shown that n-3 fatty acids can stimulate endogenous activities of lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) in normolipidemic subjects [5], but not plasma postheparin LPL or HTGL [6,7].

Early epidemiological studies of Greenland Eskimos [8] and Japanese [9] who consume large amounts of fish and marine foods, indicated a very low incidence of ischemic heart disease in these populations. Since then, evidences from studies in human populations generally indicated that the risk of death from CHD is lower among people who consume low to moderate quantity of fish compared with those who avoid it [10,11]. However when fish intake is relatively high, no relationship between fish intake and rates of total or fatal CHD is observed [12,13]. An epidemiological study conducted in the United States [14] also reported that increasing fish intake from 1–2 servings/wk to 5–6 servings/wk does not substantially reduce the risk of CHD in healthy men. The explanation for this lack of increased protection against CHD with high fish intake is not clear. This could be attributed to the type of fish or diet consumed, the subjects studied, the experimental designs or the presence of other components in fish. Other nutrients present in fish may indeed influence plasma lipids and lipoproteins. Factorial experiments conducted in rabbits [15,16] demonstrated that defatted fish consisting of 92% fish protein can maintain unchanged total and high-density-lipoprotein (HDL) cholesterol even in combination with n-6 PUFA, which usually reduce these concentrations. Moreover the higher HDL cholesterol concentration induced by fish protein, compared with soy protein, has been associated with decrease in very low-density lipoprotein (VLDL) triglycerides and increase in postheparin plasma LPL activity [16].

The effects of lean fish, whose major constituent is fish protein containing 1% or less of fish oil (0.45% or less of total energy derived from n-3 PUFA), have also been examined in premenopausal [17] and postmenopausal [18] women fed well-controlled low-fat (30%) high P:S (1:1) ratio diets. In premenopausal [17] and postmenopausal [18] women, lean fish induced higher concentrations of LDL-apo B in plasma than other animal protein sources, resulting perhaps from increased conversion of VLDL to LDL, which is LPL mediated. In postmenopausal women [18], lean fish, compared with other animal protein sources, induced higher concentrations of plasma total and HDL cholesterol as well as sex hormone-binding globulin

(SHBG), referred to as circulating testosterone-estradiol-binding globulin. Interestingly, the testosterone-estradiol balance, mainly regulated by circulating SHBG [19], influences the lipid profile in men. Thus, it was deemed of interest to determine whether lean fish compared to other animal protein sources influences lipid and lipoprotein concentrations through postheparin plasma lipolytic activities and sex hormone variations in men.

## MATERIALS AND METHODS

### Subjects

After physical examination and medical history, 11 normolipidemic French-Canadian men, between 19 and 27 years of age and students at Laval University, were selected. Subjects were encouraged to maintain their normal lifestyles. They practiced regular physical activity (e.g., 2–5 h/wk of muscular training, cycling or jogging) and were nonsmokers. Exclusion criteria included dyslipoproteinemias, LPL gene defects, use of medication known to affect lipid metabolism, important weight loss within the previous six months, chronic, metabolic or acute disease or major surgery within the previous three months, and dietary incompatibility with calcium supplementation and/or fish consumption (allergy, intolerance, dislike). Based on genetic analyses as previously described [20], carriers of LPL gene mutations (G188E, P207L and D250N) were also excluded. All subjects had normal body mass index (BMI) and normal lipid profiles (Table 1). According to data from the third National Health and Nutrition Examination Survey, individual plasma total and LDL cholesterol values were distributed between the 5<sup>th</sup> and 50<sup>th</sup> percentiles of the population for the corresponding group age [2]. These subjects with low levels of circulating total and LDL cholesterol were selected to determine clearly the effects of the lean fish diet in normolipidemic subjects and not in borderline hypercholesterolemic subjects. This study was approved by the Clinical Research Ethical Committee of Laval University. Each participant gave written informed consent and was free to withdraw at any time.

**Table 1.** Physical Characteristics and Lipid Profile of Study Subjects<sup>a</sup>

Body weight (kg)	74.3 ± 2.1
Height (cm)	176.6 ± 1.6
BMI (kg/m <sup>2</sup> ) <sup>b</sup>	24.0 ± 1.0
Age (years)	22.6 ± 0.7
Total cholesterol (mmol/L)	3.96 ± 0.11
LDL cholesterol (mmol/L)	2.56 ± 0.13
HDL cholesterol (mmol/L)	1.03 ± 0.06
Total: HDL cholesterol	3.98 ± 0.24
Triglycerides (mmol/L)	1.17 ± 0.12

<sup>a</sup> Mean ± SEM; n = 11.

<sup>b</sup>Body mass index (weight in kg/m<sup>2</sup> of body surface).

## Experimental design

The study design included two experimental periods. Prior to each experimental period, participants were asked to follow a controlled pre-experimental diet similar to their usual diet for two weeks. Then the subjects were distributed according to a randomized crossover design. Six subjects were randomly assigned to begin the study with the lean fish diet, and the other five subjects to begin with a diet containing beef, pork, veal, eggs, milk and milk products (nonfish). At the end of this first experimental period (experimental period 1) lasting four weeks, participants returned to their usual diet for a wash-out period of five weeks. Because the washout period included the Christmas season, the chosen total length for the washout period was five weeks including the two-week pre-experimental diet, instead of four weeks, to eliminate the residual effects of the first experimental diet on the tested parameters. After the wash-out period, each group received the other diet for an additional four-week period (experimental period 2).

## Diets

Before the beginning of the study, participants filled a three-day dietary record, including two weekdays and a weekend day, in order to estimate their individual energy intake and to build seven-day rotating menus which respected their food preferences as much as possible. The formulation of experimental diets was based on the guidelines for a prudent diet recommended by the 1994 NCEP [2]. Nutrient composition of diets and dietary records were calculated with the computer-assisted analysis of the Canadian Nutrient File database [21] and the US Department of Agriculture's *Agricultural Handbook No. 8* [22].

The lean fish diet was formulated using cod and sole which contained less than 1% fat. The nonfish diet consisted of lean beef, pork and veal, eggs, skim and partially skimmed milk and milk products. Approximately 70% to 75% of dietary protein came from fish protein or beef, pork, veal, eggs and milk proteins, the remaining being of vegetable origin. Table 2 provides a sample one-day menu for both experimental diets. Because no milk products were allowed during the lean fish diet, a calcium (500 mg) and vitamin D (125 I.U.) supplement was given daily to subjects on this diet. Alcohol consumption was strictly prohibited two weeks prior to and during both experimental periods. Table 3 compares the mean nutrient composition of lean fish and nonfish diets to the preexperimental diet. In order to obtain 70% of the daily protein intake from fish or beef, veal, pork, eggs and milk, the protein content of experimental diets was increased by 2% compared to the pre-experimental diet. The major differences between the two experimental diets (lean fish and nonfish) and the preexperimental diet were an increase in P:S ratio from 0.5:1 to 1.1:1 and a 55 mg decrease in dietary cholesterol. The mean fatty acid content of experimental diets is presented in Table 4. The proportions of saturated, monounsaturated and polyunsaturated

fatty acids, and therefore the P:M:S ratio, were similar between the two experimental diets. The main differences between the two experimental diets were the protein source, either from fish or from other animal protein sources, and a slightly higher (1%) content of n-3 long-chain fatty acids in the lean fish diet (Table 4).

Seven different energy levels (9960 kilojoules (kJ), 10920 kJ, 12180 kJ, 13440 kJ, 14700 kJ, 15960 kJ and 17220 kJ) were formulated for each experimental diet. Subjects started the study at the energy level closest to their usual intake. Participants were weighed every two days while wearing light clothing, and their energy intake was adjusted in order to maintain a stable body weight. A maximum variation of 2 kg was allowed during the study and no one exceeded this limit.

All meals, except breakfast, were prepared under the supervision of two qualified dietitians. Breakfasts and snacks were prepared at home by subjects using a predetermined food list. Lunches and dinners were prepared and served by professional dietitians at our experimental nutrition laboratory. Weekend meals were prepared in advance and distributed on Friday evenings.

## Blood analysis

Fasting blood samples were taken at the beginning and at the end of each experimental four-week period. A catheter was inserted in the antecubital vein from which 7 mL of blood were collected in tubes with and without ethylenediamine tetraacetic acid (EDTA) in order to obtain plasma and serum, respectively. Ten minutes after the injection of 60 U of heparin/kg of body weight, postheparin blood samples were collected in EDTA tubes. Centrifugations were performed immediately at 4°C for 10 min at 1500 X g. Plasma samples were stored at 4°C and analysed within five days for lipoproteins or were kept at -80°C until lipases were assayed. Serum samples were stored at -80°C until sex hormones were measured. Lipoprotein fractions (VLDL, LDL and HDL) were separated and assayed for their cholesterol, triglyceride, apo A-I and B contents, according to methods previously described by Gascon *et al.* [17] and Jacques *et al.* [18].

Serum total and free testosterone and SHBG were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA). LPL and HTGL activities were determined in postheparin plasma after preincubation with sodium dodecyl sulfate (SDS) as previously described by Watson *et al.* [23].

## Statistical analysis

Statistical analyses were performed using SAS (Statistical Analysis System Institute, Cary, NC, USA) programs. The results are expressed as mean  $\pm$  standard error of the mean (SEM) and the significance level is  $p < 0.05$  unless otherwise specified. To compare the effects of lean fish and nonfish diets, analysis of variance was performed according to the crossover design as described by Hills and Armitage [24]. Because no significant effect of either experimental period or sequence was

**Table 2.** Sample 1-Day Menu for the Lean Fish and Nonfish Diets<sup>a</sup>

	Lean Fish Diet	Nonfish Diet
Breakfast	395 g Citrus fruit juice	115 g Banana
	69 g White bread	69 g White bread
	14 g Butter	14 g Margarine
	22 g Peanut butter	90 g Cheese (16% fat)
Lunch	20 g Strawberry jam	259 g Milk (1% fat)
	415 g Vegetable soup	415 g Vegetable soup
	258 g Sole jardiniere	184 g Meat bread
	83 g Roasted potatoes	222 g Mashed potatoes
	97 g Cooked broccoli	97 g Cooked broccoli
	9.3 g Margarine	288 g Strawberry croustade
	9.3 g Butter	130 g Milk (1%)
288 g Strawberry croustade		
Snack		181 g Yogurt
Dinner	184 g Vegetable juice	184 g Vegetable juice
	235 g Sole and 358 g Spinach roll	125 g Lean ground veal
	230 g Vegetable rice	230 g Vegetable rice
	132 g Yellow beans	132 g Yellow beans
	9.3 g Butter	5.7 g Sunflower seeds
	120 g Carrot muffin	150 g Cake
		259 g Milk (1% fat)

<sup>a</sup> First day of the 13440-kJ diet.

observed and no residual effect of the first experimental period over the second was noted for all lipid and sex hormone variables, the data for experimental period, sequence and dietary treatment were pooled. Statistical comparisons of nutrient intake between the lean fish and the nonfish diets were performed using Student's *t* test. The relationships between lipoprotein variations and either LPL or SHBG variations were estimated with Pearson's correlation coefficient (*r*).

## RESULTS

Mean initial body weights were  $75 \pm 2$  kg and  $74 \pm 2$  kg for the lean fish and nonfish groups respectively. Mean initial body mass index (BMI) was  $24 \pm 1$  kg/m<sup>2</sup> for both diet groups. At the end of experimental diets, mean body weight and BMI remained unchanged.

Mean concentrations of plasma lipids, lipoproteins and apolipoproteins measured before and after lean fish and nonfish diets are indicated in Table 5. The nonfish diet decreased total and LDL cholesterol by 10% and 11%, respectively and the lean fish diet by 4% and 1%, respectively. However, no significant differences were observed on plasma total and LDL cholesterol concentrations between the lean fish and nonfish diets. Plasma concentrations of HDL<sub>2</sub> cholesterol increased by 30% after lean fish ingestion and remained unchanged after ingestion of beef, veal, pork, eggs and milk. Likewise, the HDL<sub>2</sub>:HDL<sub>3</sub> cholesterol ratio increased by 59% with the lean fish diet but to a lower extent (19%) with the nonfish diet. Consequently, plasma HDL<sub>2</sub> cholesterol and HDL<sub>2</sub>:HDL<sub>3</sub> cholesterol ratio were significantly ( $p < 0.05$ ) higher following the lean fish diet than the nonfish diet. Although the lean fish diet decreased plasma total and VLDL triglycerides by 27% and 34%, respectively, and the nonfish diet only by 11% and 13%,

**Table 3.** Daily Food Consumption<sup>a</sup>

	Preexperimental Diet <sup>b</sup>	Experimental Diets	
		Lean Fish	Nonfish
Energy (kJ)	11970 ± 538	11672 ± 132	12079 ± 538
Protein (% of energy)	16 ± 1	18 ± 0.05 <sup>c</sup>	18 ± 0.03 <sup>c</sup>
Carbohydrate (% of energy)	51 ± 2	50 ± 0.1	50 ± 0.1
Lipid (% of energy)	32 ± 1	32 ± 0.1	32 ± 0.1
P:M:S <sup>d</sup>	0.5:1.2:1	1:1:1 <sup>e</sup>	1:1:1 <sup>e</sup>
Cholesterol (mg)	316 ± 38	263 ± 12	257 ± 11
Total fiber (g)	22 ± 2	26 ± 1	25 ± 1

<sup>a</sup> Mean ± SEM; n = 11.

<sup>b</sup> From a 3-day food record.

<sup>c,e</sup> Significantly different compared to the preexperimental diet: <sup>c</sup> $p < 0.05$ , <sup>e</sup> $p < 0.01$ .

<sup>d</sup> Proportion of polyunsaturated to monounsaturated to saturated fatty acids.

**Table 4.** Mean Fatty Acid Content of the Experimental Diets

	Lean Fish Diet	Nonfish Diet
Fatty acids	g/100 g of total fatty acids	
Saturated		
Butyric (C4:0)	1.05	0.28
Caproic (C6:0)	0.62	0.19
Caprylic (C8:0)	0.37	0.14
Capric (C10:0)	0.87	0.47
Lauric (C12:0)	1.85	2.64
Myristic (C14:0)	3.93	2.89
Palmitic (C16:0)	17.54	18.84
Stearic (C18:0)	7.26	8.36
Total	33.49	33.81
Monounsaturated		
Palmitoleic (C16:1)	1.23	1.99
Oleic (C18:1)	31.95	31.70
Total	33.18	33.69
Polyunsaturated		
Linoleic (C18:2)	30.59	31.38
Linolenic (C18:3)	1.84	1.69
Arachidonic (C20:4)	0.22	0.21
Eicosapentænoic (C20:5)	0.44	0.06
Docosahehexænoic (C22:6)	0.50	0.01
Total	33.59	33.35
n-6	30.81	31.59
n-3	2.78	1.76

respectively, there was no significant difference in plasma total ( $p = 0.14$ ) and VLDL ( $p = 0.19$ ) triglyceride concentrations between the lean fish and nonfish diets.

A slight decrease in total plasma (4%) and LDL apo B (4%) was observed following the lean fish diet, and a more pronounced decrease (16% and 14%, respectively) following the nonfish diet. Plasma apo B:apo A-1 ratio increased by 8% during the lean fish diet while decreased by 8% during the nonfish diet. Therefore, total plasma and LDL apo B as well as apo B:apo A-1 ratio were significantly ( $p < 0.05$ ) higher following the lean fish diet than following the nonfish diet.

As shown in Table 6, plasma triglycerides:apo B and cholesterol: apo B ratios of VLDL decreased by 33% and 26%, respectively, during the lean fish diet and increased by 27% and 26%, respectively, during the nonfish diet. Therefore, plasma triglycerides:apo B and cholesterol:apo B ratios of VLDL were significantly ( $p < 0.05$ ) lower when the lean fish diet was compared with the nonfish diet. On the other hand, HDL cholesterol:apo A-1 ratio was significantly ( $p < 0.05$ ) higher after the lean fish diet than after the nonfish diet.

The effects of the lean fish and nonfish diets on plasma postheparin lipase activities are given in Table 7. No significant effect was observed on either plasma postheparin LPL or HTGL activity between lean fish and nonfish diets. Although an increase in LPL activity (26%) after the lean fish diet and a decrease in LPL activity (24%) after the nonfish diet were noted, large intraindividual variations in LPL activity may have precluded the possibility of reaching a significant difference between the two diets. No significant effect was observed on

plasma postheparin HTGL activity between lean fish and nonfish diets because it decreased to a similar extent (17%) for both experimental diets.

Table 8 compares the effects of lean fish and nonfish diets on plasma sex hormones. Plasma SHBG concentrations were significantly ( $p < 0.05$ ) higher following the lean fish diet than following the nonfish diet.

## DISCUSSION

The main finding of this study was that lean fish, incorporated in a prudent-type diet, produced higher total and LDL apo B in addition to higher HDL<sub>2</sub> cholesterol and SHBG concentrations in normolipidemic men. In this study, body weight, physical activity, tobacco smoking and dietary intake, including alcohol consumption, were strictly controlled during both experimental periods. It is unlikely that these factors could have been responsible for the observed lean fish-induced effects on lipids, lipoproteins and sex hormones.

In the present study, all participants showed reductions in plasma total cholesterol, although these varied in magnitude according to the diet consumed. In fact, the lean fish diet induced a 4% reduction from baseline, and the nonfish diet a 10% reduction. The lipid-lowering responses to these diets were close to the 5% to 10% cholesterol reduction expected from the NCEP Step I diet intervention [2]. The observed decreases of plasma total cholesterol concentrations, due to increased P:S ratio and decreased cholesterol content in the nonfish and lean fish diets vs. preexperimental diet, are consistent with those reported in previous studies [25,26]. Reductions in LDL cholesterol values reflected those in total cholesterol, decreasing by 11% when the nonfish diet was followed and by 1% when the lean fish diet was consumed. Despite these apparent divergences, there were no significant differences on plasma total and LDL cholesterol concentrations between subjects fed either lean fish or beef, veal, pork, eggs and milk. Interestingly, NCEP Step 2 diets relatively high or relatively low in fish have also been shown to be both effective in significantly reducing total and LDL cholesterol concentrations under controlled weight-stable conditions in middle-aged and elderly subjects [27].

Although lean fish induced greater reductions (27% and 34%, respectively) in total and VLDL triglyceride concentrations than beef, veal, pork, eggs and milk (11% and 13%, respectively), no differences in total and VLDL triglycerides were observed between the lean fish and nonfish diets. Generally, normal individuals fed marine n-3 fatty acids demonstrate a reduction in plasma triglycerides. But the hypotriglyceridemic effects of n-3 fatty acids are not only dose dependent, but can also be affected by the composition of the diet [28]. In the present study, the lean fish diet provided approximately 2.78 g n-3 fatty acids/day, 1.02 g more n-3 fatty acids than the amount found in the nonfish diet, mainly due to higher content of

**Table 5.** Effects of Experimental Diets on Plasma Lipid, Lipoprotein and Apolipoprotein Concentrations Before (Pre) and After (Post) Lean Fish and Nonfish Diets<sup>a</sup>

	Lean Fish Diet	Nonfish Diet
Cholesterol (mmol/L)		
Total		
Pre	4.03 ± 0.11	4.08 ± 0.16
Post	3.85 ± 0.14	3.68 ± 0.10
VLDL		
Pre	0.36 ± 0.04	0.36 ± 0.05
Post	0.27 ± 0.04	0.31 ± 0.05
LDL		
Pre	2.65 ± 0.11	2.72 ± 0.17
Post	2.63 ± 0.12	2.43 ± 0.10
HDL		
Pre	1.02 ± 0.06	1.00 ± 0.06
Post	0.95 ± 0.05	0.94 ± 0.05
HDL <sub>2</sub>		
Pre	0.27 ± 0.04	0.30 ± 0.04
Post	0.35 ± 0.04 <sup>b</sup>	0.31 ± 0.04
HDL <sub>3</sub>		
Pre	0.75 ± 0.05	0.71 ± 0.03
Post	0.59 ± 0.02	0.62 ± 0.02
HDL <sub>2</sub> :HDL <sub>3</sub> cholesterol		
Pre	0.37 ± 0.06	0.42 ± 0.04
Post	0.59 ± 0.06 <sup>b</sup>	0.50 ± 0.06
Triglycerides (mmol/L)		
Total		
Pre	1.15 ± 0.08	1.09 ± 0.11
Post	0.84 ± 0.08	0.97 ± 0.13
VLDL		
Pre	0.71 ± 0.07	0.69 ± 0.09
Post	0.47 ± 0.07	0.60 ± 0.11
LDL		
Pre	0.18 ± 0.01	0.17 ± 0.02
Post	0.18 ± 0.01	0.16 ± 0.02
HDL		
Pre	0.26 ± 0.04	0.23 ± 0.02
Post	0.20 ± 0.01	0.20 ± 0.01
Apo B (g/L)		
Total		
Pre	0.77 ± 0.04	0.79 ± 0.04
Post	0.74 ± 0.03 <sup>b</sup>	0.66 ± 0.03
VLDL		
Pre	0.06 ± 0.01	0.07 ± 0.01
Post	0.05 ± 0.01	0.05 ± 0.01
LDL		
Pre	0.72 ± 0.04	0.72 ± 0.04
Post	0.69 ± 0.03 <sup>b</sup>	0.62 ± 0.02
Apo A-1 (g/L)		
HDL		
Pre	1.23 ± 0.04	1.18 ± 0.03
Post	1.04 ± 0.02	1.07 ± 0.02
Apo B:apo A-1		
Pre	0.63 ± 0.04	0.68 ± 0.05
Post	0.71 ± 0.03 <sup>b</sup>	0.63 ± 0.03

<sup>a</sup> Mean ± SEM; n = 11. Apo = apolipoprotein.<sup>b</sup> Significantly different from nonfish diet: <sup>b</sup> *p* < 0.05.

eicosapentænoic and docosahexænoic acids. Layne *et al.* [28] recently demonstrated that a dietary supplementation of 35 mg of eicosapentænoic and docosahexænoic acids/kg of body weight (approximately 2.5 g n-3 fatty acids/day) does not reduce plasma triglycerides in normal subjects consuming a high P:S ratio diet (0.87), whereas it does in subjects consuming a low P:S ratio diet (0.48). Indeed, high levels of linoleate present in high P:S ratio diet would compete more with n-3 long-chain fatty acids for incorporation into plasma lipid and lipoprotein fractions.

Based on significant (*p* < 0.05) lower VLDL triglycerides: apo B and VLDL cholesterol:apo B ratios (Table 4) with lean fish, the present study however suggests that, lean fish may have produced smaller and denser VLDL particles compared with beef, pork, veal, eggs and milk. Inagaki and Harris [29] found that fish oil can reduce the pool of large triglyceride-rich VLDL to a greater extent than small VLDL in hypertriglyceridemic subjects, resulting in smaller VLDL particles. Small VLDL particles are distinguished metabolically from large VLDL particles in that either they can bind to the LDL receptor

**Table 6.** Ratios of Triglycerides and Cholesterol to Apolipoproteins and Ratios of Triglycerides to Cholesterol in Lipoprotein Fractions Before (Pre) and After (Post) Lean Fish and Nonfish Diets<sup>a</sup>

	Lean Fish Diet	Nonfish Diet
VLDL		
Triglycerides:apo B		
Pre	8056 ± 820	5668 ± 663
Post	5428 ± 653 <sup>c</sup>	7208 ± 1163
Cholesterol:apo B		
Pre	4208 ± 489	2872 ± 354
Post	3098 ± 338 <sup>c</sup>	3613 ± 481
Triglycerides:cholesterol		
Pre	2.0 ± 0.1	2.0 ± 0.1
Post	1.9 ± 0.2	2.0 ± 0.1
LDL		
Triglycerides:apo B		
Pre	145 ± 12	137 ± 18
Post	141 ± 10	146 ± 13
Cholesterol:apo B		
Pre	2040 ± 42	2073 ± 50
Post	2086 ± 47	2177 ± 60
Triglycerides:cholesterol		
Pre	0.07 ± 0.01	0.07 ± 0.01
Post	0.07 ± 0.01	0.07 ± 0.01
HDL		
Triglycerides:apo A-1		
Pre	5.8 ± 0.8	5.4 ± 0.4
Post	5.4 ± 0.3	5.4 ± 0.3
Cholesterol:apo A-1		
Pre	23.0 ± 0.9	23.7 ± 1.1
Post	25.4 ± 1.3 <sup>b</sup>	24.4 ± 1.1
Triglycerides:cholesterol		
Pre	0.25 ± 0.03	0.24 ± 0.02
Post	0.22 ± 0.02	0.23 ± 0.02

<sup>a</sup> Mean ± SEM; n = 11. Apo = apolipoprotein.<sup>b,c</sup> Significantly different from nonfish diet: <sup>b</sup> *p* < 0.05, <sup>c</sup> *p* < 0.01.

**Table 7.** Plasma Postheparin Lipase Activities Before (Pre) and After (Post) Lean Fish and Nonfish Diets<sup>a</sup>

	Lean Fish Diet	Nonfish Diet
Lipoprotein Lipase Activity ( $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$ )		
Pre	22 ± 3	36 ± 10
Post	29 ± 6	27 ± 4
Hepatic Triglyceride Lipase Activity ( $\mu \cdot \text{L}^{-1} \cdot \text{min}^{-1}$ )		
Pre	267 ± 24	248 ± 21
Post	222 ± 25	207 ± 21

<sup>a</sup> Mean ± SEM; n = 9–11.

[30] or they can progress down the lipolytic cascade, ultimately to form normal LDL. Indeed, Packard *et al.* [31] demonstrated that LDL-apo B is derived from small VLDL and very slightly from large VLDL particles. The presence of smaller VLDL particles could be the explanation for the higher LDL-apo B concentrations observed with lean fish as compared to beef, veal, pork, eggs and milk in the present study as well as in studies on pre- and post-menopausal women [17,18].

There is scientific evidence that n-3 long-chain fatty acids decrease serum triglycerides primarily by reducing the synthesis of triglycerides in the liver [4]. In the present study, n-3 long-chain fatty acids were present in slightly higher amounts (1.02 g n-3 fatty acids) in the lean fish diet than in the nonfish diet (Table 4). Although reduced triglyceride production would play an important mechanistic role, it does not exclude a contribution from enhanced clearance by LPL, an insulin responsive enzyme. Indeed, although neither plasma total triglycerides nor postheparin LPL activity were significantly different between the lean fish and nonfish diets, we found significant negative correlations between variations in plasma VLDL triglyceride concentrations and those in plasma postheparin LPL activity ( $r = -0.53$ ;  $n = 11$ ;  $p = 0.04$ ), and variations in plasma VLDL triglyceride:apo B ratio and those in plasma postheparin LPL activity ( $r = -0.64$ ;  $n = 11$ ;  $p = 0.04$ ) in subjects fed the lean fish diet, suggesting the possibility that repeated exposure to modest increases in LPL activity, although not significant, over extended periods of time could long-term contribute to the production of smaller VLDL following the lean fish diet compared with the nonfish diet. Further studies are however needed to verify this assumption.

We observed in the present study a rise in the HDL<sub>2</sub> cholesterol concentrations in subjects fed the lean fish diet. There has been some published evidence in a previous n-3 fatty acid trial [32] that an increase in HDL<sub>2</sub> cholesterol concentrations may be mediated by an inhibition of lipid transfer protein activity. Such inhibition could slow the exchange of HDL cholesterol esters for triglycerides, resulting in higher HDL<sub>2</sub> cholesterol concentrations. The eventual presence of small and dense VLDL particles could also reduce the substrate available for transfer mediated by lipid transfer protein and thereby allow HDL<sub>2</sub> cholesterol concentrations to increase. Alternatively, the LPL-mediated hydrolysis of triglyceride-rich lipoproteins and transfer of their surface components to HDL precursors is a major pathway for the assembly of circulating HDL particles,

particularly HDL<sub>2</sub> [33]. Yet, a close relationship between postheparin LPL activity, levels of HDL cholesterol and VLDL triglycerides already observed in rabbits fed fish protein [16] suggest that the presence of fish protein in lean fish could also have contributed to increase the formation of circulating HDL<sub>2</sub> particles when the lean fish diet was consumed.

This study also showed an elevation of plasma SHBG concentrations with the lean fish diet as compared to the nonfish diet, supporting the observation of Gates *et al.* [34] in Chinese women that fish consumption is positively associated with SHBG concentrations. Jacques *et al.* [18] have demonstrated similar increases in plasma SHBG concentrations in postmenopausal women consuming the lean fish diet. Interestingly, in the present study, a positive correlation between SHBG and HDL<sub>2</sub>-C ( $r = 0.80$ ;  $n = 22$ ;  $p = 0.01$ ) and negative correlations between SHBG and total triacylglycerols ( $r = -0.72$ ;  $n = 22$ ;  $p = 0.01$ ), VLDL-triacylglycerols ( $r = 0.70$ ;  $n = 22$ ;  $p = 0.02$ ) and plasma VLDL triglycerides:apo B ratio ( $r = -0.51$ ;  $n = 22$ ;  $p = 0.02$ ) have been observed, suggesting that the lipoprotein profile induced by experimental diets is closely related to plasma SHBG concentrations. The mechanism by which SHBG is associated with lipoprotein metabolism remains unclear. On one hand, the positive correlation of SHBG with HDL<sub>2</sub> cholesterol levels suggests that SHBG influence the metabolism and/or the production of HDL<sub>2</sub> cholesterol; this effect could be direct or indirect through the equilibrium of the estradiol-testosterone balance and in

**Table 8.** Effects of Lean Fish and Nonfish Diets on Plasma Sex Hormones<sup>a</sup>

	Lean Fish Diet	Nonfish Diet
Free Testosterone (pmol/L)		
Pre	80 ± 6	80 ± 6
Post	71 ± 6	65 ± 5
Total Testosterone (nmol/L)		
Pre	19 ± 1	19 ± 1
Post	19 ± 1	18 ± 1
SHBG (nmol/L)		
Pre	27 ± 2	27 ± 2
Post	31 ± 2 <sup>b</sup>	27 ± 2

<sup>a</sup> Mean ± SEM; n = 11.

<sup>b</sup> Significantly different from nonfish diet; <sup>b</sup>  $p < 0.01$ .

SHBG = sex hormone-binding globulin.

association with the activity of hepatic triglyceride lipase, which is stimulated by androgens and inhibited by estrogens [35]. On the other hand, SHBG, VLDL and immature HDL are synthesised by the liver, and a similar mechanism may be responsible for their hepatic production [36].

The evidence that insulin is an inhibitor of SHBG production *in vitro* [37] and suppresses SHBG production in normal and obese men [38] suggests that low insulin levels may upregulate SHBG and HDL concentrations. According to Haffner *et al.* [39], higher SHBG is associated with both higher total and nonoxidative glucose disposal. Taken together, higher plasma SHBG and HDL<sub>2</sub>-C concentrations, as observed with the lean fish diet compared with the nonfish diet, could be the result of an improvement in insulin sensitivity. In support of this hypothesis, a recent study conducted in our laboratory with rats fed cod protein, soy protein or casein indicated that insulin sensitivity was improved in fasted rats fed cod or soy protein compared with those fed casein [40]. However, further studies are required to confirm the effect of fish protein and lean fish on insulin sensitivity in healthy and non-insulin-dependent diabetic subjects.

Although most studies [29,32] support the evidence that the presence of small quantities of n-3 fatty acids in lean fish would be responsible for the lipid and lipoprotein variations observed in the present study, they do not preclude a contribution from fish protein, another nutrient present in lean fish. Indeed, fish protein has been already shown to reduce plasma triglyceride concentrations, to increase plasma postheparin LPL activity and HDL cholesterol concentrations in rabbits [16] and to improve glucose tolerance and insulin sensitivity in rats [40]. Fish protein is rich in essential amino acids and contains more arginine than casein and more lysine than casein, beef and soy protein [41], two amino acids shown by Vahouny *et al.* [42] to influence serum insulin and lipids in the rat. However, the relative contributions of n-3 fatty acids and fish protein remain unknown, and it could be worthwhile to evaluate the distinct and interactive metabolic effects of fish oil and fish protein on plasma lipids, lipoproteins and insulin sensitivity in future trials.

In conclusion, the present study indicates that lean fish compared with beef, veal, pork, eggs and milk produced higher LDL apo B and HDL<sub>2</sub>-C concentrations when included in a prudent-type diet in normolipidemic men. The present results further suggest that the effects of substituting lean fish for beef, veal, pork, eggs and milk on plasma lipoproteins may be partly associated with variations in VLDL size, sex hormone status and plasma LPL activity. These results were obtained from a group of young men whose plasma lipids are usually less responsive to dietary alterations than those of subjects with elevated plasma lipids. Thus it appears of interest to determine in further studies whether larger effects would be observed in hyperlipidemic patients.

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