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Dependence on dietary cholesterol for n–3 polyunsaturated fatty acid-induced changes in plasma cholesterol in the Syrian hamster

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Abstract Male Syrian hamsters consumed diets containing incremental increases in dietary n–3 fatty acids from fish oil with either low (0.015% w/w) or moderate (0.1% w/w) dietary cholesterol content. Animals consuming diets containing moderate cholesterol, but not animals consuming diets containing low cholesterol, had increased plasma very low (VLDL)- and low density lipoprotein (LDL)-cholesterol levels with increasing fish oil consumption. The plasma concentration of high density lipoprotein (HDL)-cholesterol decreased by 43 and 32% with the consumption of the highest fish oil diets in the low and moderate dietary cholesterol groups, respectively. Hepatic LDL-receptor binding activity did not change with the consumption of low cholesterol diets, but gradually decreased with fish oil consumption in animals consuming the moderate cholesterol diets. Hepatic LDL-receptor binding and plasma LDL-cholesterol levels of the different dietary fish oil groups were highly correlated ($r = -0.91$). Fish oil consumption also caused an increase in hepatic free cholesterol but a decreased cholesteryl ester content. Therefore, in the Syrian hamster, the consumption of n–3 fatty acids increases LDL-cholesterol levels which can be partially explained by decreased hepatic LDL-receptor binding and this response to dietary n–3 fatty acids is dependent on the dietary cholesterol content. However, the effects of dietary n–3 fatty acids on HDL-cholesterol are independent of dietary cholesterol content.—**Surette, M. E., J. Whelan, G-P. Lu, K. S. Broughton, and J. E. Kinsella.** Dependence on dietary cholesterol for n–3 polyunsaturated fatty acid-induced changes in plasma cholesterol in the Syrian hamster. *J. Lipid Res.* 1992. **33:** 263–271.

Supplementary key words LDL-receptor • low density lipoprotein • n–3 fatty acids • high density lipoprotein

Epidemiologic studies (1, 2) as well as feeding trials (3–5) suggest that dietary long chain n–3 polyunsaturated fatty acids (PUFA) from fish oils may reduce the risk of cardiovascular disease (CVD). The underlying effects may involve many mechanisms (6, 7). Eicosanoid production and thus platelet and leukocyte responsiveness may be altered, potentially affecting atherosclerotic lesion development. Additionally, circulating lipid levels that are important risk factors for CVD are affected by dietary fish oils (8). Thus, it is not only important to determine the effects of n–3 PUFA-rich fats on CVD risk parameters, but also to determine the mechanisms by which these risk factors are altered and what interactive effect other dietary lipid components may have on these processes.

The effects of n–3 PUFA on circulating levels of plasma lipoproteins have been shown to be variable (8–11). No consistent changes have been observed in LDL or HDL cholesterol concentrations with n–3 PUFA consumption, much of this variability being attributed to a lack of control of confounding factors such as dietary cholesterol, fat level, and fatty acid composition (8, 9, 11). In addition, little is known about the interactions of dietary n–3 PUFA and cholesterol and their effects on circulating plasma cholesterol levels. This is of importance considering that the effects of dietary saturated and n–6 PUFA on plasma cholesterol concentrations are dependent on the presence of dietary cholesterol (12, 13).

Few reports have elucidated mechanisms by which dietary n–3 PUFA may induce changes in plasma LDL-cholesterol levels. In humans, the consumption of n–3 PUFA decreased the absolute rates of LDL synthesis and catabolism but had no effect on the fractional catabolic rate (14). Studies using rats have shown that consumption of fish oils alters hepatic LDL-receptor expression (15–17). However, in these studies the diets

Abbreviations: PUFA, polyunsaturated fatty acids; CVD, cardiovascular disease; CE, cholesteryl ester; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; C, cholesterol.

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were not supplemented with cholesterol, and opposite effects on hepatic receptor binding activity (17) and receptor-mediated uptake of LDL (15) were reported. Furthermore, the rat may not be the model of choice for the study of dietary effects on LDL metabolism. Compared to humans, rats have very high rates of hepatic cholesterol synthesis and low concentrations of circulating LDL-cholesterol, thus making it difficult to quantify diet-induced changes in plasma LDL-cholesterol concentrations and metabolism.

Hamsters have circulating LDL levels and show responses to dietary fats that are comparable to humans (13, 18, 19). Additionally, the interaction of dietary lipids and cholesterol and their effects on the regulation of the hepatic LDL-receptor and circulating LDL-cholesterol have been well studied in the hamster model (13, 18). Thus, the hamster has become an attractive model for the study of cholesterol metabolism. However, there is limited information on the effect of dietary n–3 PUFA on circulating plasma cholesterol in this animal model (20).

The present study investigated changes in circulating plasma cholesterol levels in the Syrian hamster over a range of dietary n–3 PUFA intakes, while controlling for dietary saturated and n–6 polyunsaturated fats. Dietary cholesterol was maintained at low or moderate levels in order to determine its effect in animals consuming n–3 PUFA. Also, the effects of n–3 PUFA and cholesterol consumption on hepatic LDL-receptor binding activity were determined in an attempt to elucidate possible mechanisms by which dietary n–3 PUFA induce changes in circulating LDL-cholesterol levels.

METHODS

Materials

$Na^{125}I$ was obtained from Amersham (Arlington Heights, IL). Bovine serum albumin (BSA) (fatty acid-free), phenylmethylsulfonyl fluoride (PMSF), aprotinin, cholesterol oxidase (EC 1.1.3.6), and cholesterol ester hydrolase(EC 3.1.1.13), were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and diets

Male Syrian hamsters (Charles River Inc., Wilmington, MA) were used in two separate experiments, 36 animals in the first experiment and 40 animals in the second. All hamsters were housed individually. All experimental diets were prepared by adding the desired lipids to a final concentration of 15% (w/w) to fat-free rodent chow (ICN Biochemicals or Dyets Inc.) and mixing thoroughly (**Table 1**). Animals were kept on a 12-h light–dark cycle and fed fresh diet daily for 21 days. After a 12-h fast, blood was collected by cardiac puncture and plasma was obtained by centrifugation at 1500 *g* for 20 min at 4°C. Livers were perfused with ice-cold saline, immediately frozen in liquid nitrogen, and stored at -70°C until further processing.

TABLE 1. The composition of diets consumed by hamsters for 21 days

Diet	Fat-Free Chow	Total Lipid	Cholesterol	n–3 PUFA
	g/100 g	*g/100 g*	*mg/100 g*	*g/100 g*
Experiment 1				
1A	85	15	15	0
2A	85	15	15	0.3
3A	85	15	15	0.8
4A	85	15	15	2.1
5A	85	15	100	0
6A	85	15	100	2.1
Experiment 2				
1B	85	15	100	0
2B	85	15	100	0.3
3B	85	15	100	0.7
4B	85	15	100	1.5
5B	85	15	100	1.9

Values for n–3 polyunsaturated fatty acids (PUFA) represent the contribution of dietary n–3 PUFA from sardine oil.

In the first experiment, animals were divided into six dietary groups (Diets 1A–6A). Diet 1A contained no n–3 PUFA and diets 2A through 4A contained increasing n–3 PUFA as sardine oil (Table 1). The remaining dietary lipid was supplied as a combination of safflower oil, butterfat, and olive oil in ratios that maintained the total n–6 PUFA and saturated fat contents of the diets constant (**Table 2**). These diets were not enriched in cholesterol but were equilibrated to 0.015% (w/w) total cholesterol. Diets 5A and 6A had the same lipid composition as diets 1A and 4A except that they were supplemented with cholesterol to a final concentration of 0.1% (w/w).

In the second experiment, animals were divided into five dietary groups. The control diet (Diet 1B) contained no n–3 PUFA, while diets 2B to 5B contained increasing levels of sardine oil as a source of n–3 fatty acids (Tables 1 and 2). Total dietary n–6 PUFA and saturated fat content were controlled in all diets. All diets were made up to a final cholesterol concentration of 0.1% (w/w). Diets were stored in daily aliquots under an atmosphere of N_2 at -70°C.

Lipid analysis

The fatty acid composition of the diets was determined by capillary gas chromatography of the fatty acyl methyl esters (21). Total plasma cholesterol was determined colorimetrically (22). Plasma was fractionated by centrifugation at densities of 1.019 and 1.063 g/ml (13). The fractionated plasma cholesterol and cholesteryl esters (CE) were determined color-

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TABLE 2. The fatty acid composition of dietary lipids consumed by hamsters for 3 weeks

Fatty Acid	Dietary Groups								
	1A	2A	3A	4A	1B	2B	3B	4B	5B
	Fatty Acid (g/kg diet)								
12:0	1.4	1.7	1.6	1.2	2.5	2.3	2.2	2.1	2.3
14:0	7.2	8.0	8.3	9.9	9.3	9.4	9.7	10.4	11.4
16:0	28.0	28.2	28.6	28.3	31.7	31.9	31.7	32.0	32.4
16:1	1.1	1.8	2.6	4.8	1.5	2.0	2.8	4.2	4.9
18:0	13.2	13.0	13.3	12.7	11.3	11.7	11.8	11.8	11.7
18:1	63.5	58.9	51.4	33.7	60.1	55.5	49.2	37.4	30.4
18:2 n–6	34.6	33.9	35	37.2	32.9	32.9	33.9	35.4	35.9
18:3 n–3	0.6	0.7	0.7	0.9	0.6	0.6	0.7	0.7	0.7
18:4 n–3	ND	0.6	1.4	2.8	ND	0.6	0.5	1.6	1.9
20:5 n–3	ND	1.8	4.5	11.9	ND	1.7	4.2	8.7	11.2
22:5 n–3	ND	0.1	0.3	1.0	ND	ND	0.4	0.7	1.0
22:6 n–3	ND	0.7	1.8	5.0	ND	0.9	2.1	4.1	5.2

Diets 5A and 6A were identical to diets 1A and 4A, respectively, with the exception of the cholesterol content; ND, not detected.

imetrically (22). Hepatic lipids were extracted by the method of Bligh and Dyer (23) and total hepatic cholesterol and CE were determined colorimetrically (22). Hepatic phospholipids were separated from other lipids by thin-layer chromatography and the fatty acid composition was determined by capillary gas chromatography (21).

Hepatic LDL-receptor binding assays

Human LDL (d 1.025–1.055 g/ml) was prepared by sequential ultracentrifugation and radioiodinated by the iodine monochloride method (24). Binding of ^{125}I-labeled LDL to hepatic membranes was determined using a modification of the method of Nanjee and Miller (25). All tissue preparation was conducted at 4°C. Liver tissue was quickly thawed and homogenized using 10 strokes of a Dounce homogenizer in 10 volumes of 50 mM Tris-HCl, 25 mM NaCl, 2 mM $CaCl_2$, 1 mM PMSF, and 100 kallikrein inhibitor units/ml as aprotinin (pH 7.4) (Buffer A). The homogenate was centrifuged at 1500 *g* for 10 min and the resulting supernatant was centrifuged at 10,000 *g* for 15 min. The pellet was resuspended in 1.5 volumes of buffer A. Binding assays were carried out in polyethylene microcentrifuge tubes that were preincubated overnight at 37°C with 2% BSA in buffer A to saturate nonspecific binding sites. Binding assays contained the following reactants: 100 µl of membrane preparation (150–250 µg protein), 100 µl ^{125}I-labeled LDL (15 µg protein) in buffer A containing 10% (w/v) bovine serum albumin, and 100 µl of buffer A or 100 µl buffer A containing 30 mM Na_2-EDTA, for 1 h at 37°C. The tubes were then centrifuged at 12,000 *g* for 10 min at 4°C in a Microspin 24S microcentrifuge (Sorval Instruments). The supernatants were aspirated, and the pellets were washed with 200 µl buffer A and recentrifuged at 12,000 *g* at 4°C for 5 min. The supernatant was aspirated and the pellets were counted in a Beckman 4000 gamma counter. EDTA-suppressible binding as a measure of LDL-receptor binding was taken as the difference in counts associated with pellets from reactions carried out in the absence of EDTA and counts obtained from reactions in the presence of EDTA.

Protein was determined colorimetrically (26) using bovine serum albumin as the standard.

Statistical analysis

The effects of dietary treatments were determined by analysis of variance procedures. Comparison between individual diets was made using Fisher's Protected Least Significant Difference method.

RESULTS

Hepatic lipids

In order to study the effects of dietary n–3 PUFA on hamster circulating lipoprotein levels, hamsters were fed diets with increasing n–3 PUFA content while dietary saturated fats and linoleic acid levels were maintained constant (Table 2). There were no differences in body weight, weight gain, or food consumption in animals consuming the different dietary regimes. The fatty acid composition of hepatic phospholipids reflected the differences in dietary fatty acid composition (**Table 3**). Increasing dietary n–3 fatty acids resulted in an increase in the n–3 fatty acid content of hepatic phospholipids at the expense of oleic acid and the n–6 fatty acids (especially arachidonic acid) even though the dietary n–6 content was constant. The extent of incorporation of n–3 fatty acids into hepatic liver phospholipids was equivalent in both the high and low dietary cholesterol groups (data not shown). Thus, the length of the experimental feeding

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TABLE 3. The fatty acid composition (mole %) of hepatic phospholipids from hamsters consuming the experimental diets for 3 weeks

Fatty Acid	Diet 1B	Diet 2B	Diet 3B	Diet 4B	Diet 5B
14:0	0.2 ± 0.05	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
16:0	18.8 ± 0.4	21.1 ± 0.3	22.8 ± 0.4	23.4 ± 0.6	23.7 ± 0.4
16:1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.03	0.8 ± 0.1
18:0	16.8 ± 0.8	18.0 ± 0.3	17.2 ± 0.4	16.6 ± 0.3	16.3 ± 0.7
18:1	14.4 ± 0.3	11.7 ± 0.3	10.5 ± 0.3	9.4 ± 0.1	8.9 ± 0.1
18:2 n–6	20.0 ± 0.4	20.5 ± 0.3	18.9 ± 0.4	19.0 ± 0.3	17.9 ± 0.4
20:3 n–9	0.6 ± 0.1	0.4 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
20:3 n–6	2.0 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	0.6 ± 0.02	0.7 ± 0.04
20:4 n–6	14.7 ± 0.2	8.8 ± 0.3	7.6 ± 0.6	7.2 ± 0.5	6.6 ± 0.4
22:4 n–6	0.4 ± 0.04	0.2 ± 0.01	0.1 ± 0.02	ND	ND
22:5 n–6	1.6 ± 0.2	0.2 ± 0.01	0.1 ± 0.02	ND	ND
20:5 n–3	0.1 ± 0.02	1.9 ± 0.3	3.1 ± 0.3	3.1 ± 0.3	4.2 ± 0.3
22:5 n–3	0.4 ± 0.04	1.8 ± 0.2	2.0 ± 0.2	1.7 ± 0.2	1.9 ± 0.1
22:6 n–3	8.8 ± 0.7	13.0 ± 0.2	15.4 ± 0.4	17.7 ± 0.6	18.8 ± 0.6

Values given as means ± SEM (n = 8); ND, not detected.

period was sufficient to induce significant changes in the fatty acid composition of the tissues and these changes reflected dietary lipid intake.

In the first experiment, there were no changes in the hepatic cholesterol or cholesteryl ester concentration with increasing n–3 PUFA consumption when animals consumed the diets with low cholesterol (Diets 1A–4A) (**Table 4**). However, in the animals consuming higher dietary cholesterol (Diets 5A and 6A), there was a small increase in hepatic cholesterol content compared to liver of animals consuming low cholesterol diets. The hepatic cholesteryl ester content increased to 78 μg/mg protein in animals consuming diets devoid of n–3 PUFA (Diet 5A), while in the animals consuming n–3 PUFA (Diet 6A) the hepatic cholesteryl ester content was only increased to 28 μg/mg protein.

In the second experiment where all animals consumed diets with higher cholesterol (Diets 1B–5B), there was a small increase in hepatic free cholesterol with n–3 PUFA consumption. However, the hepatic cholesteryl ester content decreased progressively in a dose-dependent fashion as the dietary n–3 PUFA intake increased (**Table 5**).

Plasma cholesterol

In animals consuming the low cholesterol diets (Diets 1A–4A), there were no changes in the plasma cholesterol concentrations of the d < 1.019 g/ml (VLDL) or the d 1.019–1.063 g/ml (LDL) fractions with increasing n–3 PUFA intake (Table 4). However, there was a gradual decrease in the cholesterol content of the d > 1.063 g/ml (HDL) fraction with increasing intake of n–3 PUFA. Upon the introduction of cholesterol in the diets (Diet 5A) total plasma cholesterol increased compared to Diet 1A, from 110 mg/dl to 144 mg/dl, the increase being in the VLDL and LDL fractions. In animals consuming a high cholesterol and high n–3 PUFA diet (diet 6A) there was an increase in total plasma cholesterol to 211 mg/dl. The cholesterol concentrations in both the VLDL and the LDL fractions doubled compared to Diet 5A while the cholesterol content in the HDL fraction decreased from 61 to 41 mg/dl. Comparable decreases in HDL cholesterol with n–3 PUFA consumption were observed in both the high (Diets 5A and 6A) and low dietary cholesterol groups (Diets 1A and 4A).

The second experiment was undertaken to examine the effects of a progressive increase in dietary n–3 PUFA on plasma cholesterol levels in animals consuming the higher dietary cholesterol levels (Diets 1B–5B). As observed in the first experiment, the consumption of n–3 PUFA with the higher dietary cholesterol resulted in an increase in cholesterol in the very low and low density lipoprotein fractions of plasma (Table 5). These increases were significant in all dietary groups when compared to the control diet (Diet 1B). The cholesterol concentration in the HDL fraction gradually declined with increasing consumption of dietary n–3 PUFA. The decrease in cholesterol concentration observed in the high density fraction with n–3 PUFA consumption was very comparable in the groups of animals consuming both the high (Diets 1B–5B) and low (Diets 1A–4A) dietary cholesterol.

Hepatic LDL binding assays

Hepatic LDL-receptor binding activity was assayed in liver membrane preparations using a modification of the method of Nanjee and Miller (25). In animals consuming low dietary cholesterol (Diets 1A–4A), EDTA-suppressible binding of ^{125}I-labeled LDL to hepatic membranes was unchanged with increasing n–3 PUFA consumption (**Fig. 1B**). In animals consuming higher dietary cholesterol (Diet 5A), there was a slight decrease in ^{125}I-labeled LDL binding activity that was further decreased in animals consuming high cholesterol and high n–3 PUFA diets (Diet 6A) (Fig. 1B). In the experimental group of animals consuming higher

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TABLE 4. Plasma and hepatic cholesterol concentrations of animals consuming diets 1A to 6A

Dietary Group			Liver wt (g) per 100 g Body Weight	Liver Cholesterol		Plasma Cholesterol			
	chol	n–3		Free	Ester	Total	VLDL	LDL	HDL
				μg/mg protein		*mg/dl*			
1A	low	0	3.8 ± 0.2^{a}	11.9 ± 1.0^{a}	7.1 ± 2.6^{a}	110 ± 10^{ab}	10 ± 2^{a}	37 ± 12^{a}	62 ± 5^{a}
2A	low	+	4.0 ± 0.2^{ab}	11.3 ± 0.8^{a}	4.0 ± 1.0^{a}	105 ± 10^{ab}	12 ± 2^{a}	41 ± 11^{a}	52 ± 7^{ab}
3A	low	++	3.8 ± 0.1^{a}	12.5 ± 0.6^{a}	3.2 ± 0.8^{a}	112 ± 12^{ab}	15 ± 4^{ab}	43 ± 9^{a}	47 ± 3^{ab}
4A	low	+++	3.7 ± 0.1^{a}	11.3 ± 0.2^{a}	2.2 ± 0.5^{a}	86 ± 11^{a}	12 ± 3^{a}	37 ± 11^{a}	35 ± 8^{b}
5A	mod	0	4.5 ± 0.1^{bc}	15.3 ± 1.0^{b}	77.9 ± 13^{b}	144 ± 10^{b}	28 ± 6^{b}	54 ± 16^{a}	61 ± 13^{a}
6A	mod	+++	4.8 ± 0.2^{c}	16.4 ± 0.9^{b}	32.3 ± 3.6^{c}	211 ± 31^{c}	53 ± 12^{c}	103 ± 16^{b}	41 ± 7^{b}

Values are given as means ± SEM (n = 5 or 6). Values in each column without a common superscript are significantly different, $P < 0.05$, as determined by analysis of variance using Fisher's Protected Least Significant Difference method; chol, dietary cholesterol; mod, moderate; n–3, dietary n–3 PUFA.

dietary cholesterol (Diets 1B–5B), EDTA-suppressible binding of ^{125}I-labeled LDL to hepatic membranes decreased gradually with increasing dietary n–3 PUFA (**Fig. 2B**), and was inversely correlated ($r = -0.91$, $P = 0.029$) with circulating levels of LDL-cholesterol (**Fig. 3**). Thus, the changes in LDL-binding activity inversely reflected the circulating plasma LDL-cholesterol concentrations for the different dietary groups.

In confirmatory studies, EDTA-suppressible binding was linear with increasing hepatic membrane protein concentration within the range of protein used in assays, and was saturable with increasing concentrations of ^{125}I-labeled LDL.

DISCUSSION

Collectively, previous reports on the effects of dietary n–3 fatty acids in the form of fish oils on plasma lipoprotein cholesterol concentrations have been equivocal (9). Much of the variability in the data may be attributed to a lack of control of confounding dietary factors such as cholesterol and saturated fatty acids (8, 11). However, little information exists on whether dietary cholesterol influences the effects of dietary n–3 PUFA on circulating plasma cholesterol.

This study was designed to determine the effects of dietary n–3 fatty acids and cholesterol on plasma cholesterol in hamsters consuming diets with increasing concentrations of n–3 PUFA from fish oil while controlling for the dietary levels of n–6 PUFA and saturated fats. The dietary cholesterol was maintained either at a low level (0.015% w/w) or diets were supplemented with cholesterol to a concentration of 0.1% (w/w) to determine whether a dietary cholesterol supplement alters the response to dietary n–3 PUFA. A 0.1% dietary cholesterol level is effective in establishing the differential effects of dietary saturated and PUFA on cholesterol metabolism in the hamster (13).

The results presented in this paper are the first to demonstrate that increases in plasma LDL- and VLDL-cholesterol with n–3 PUFA consumption are dose-dependent and require dietary cholesterol. In animals consuming low amounts of dietary cholesterol, the increase in dietary n–3 PUFA had no effect on the level of circulating VLDL- or LDL-cholesterol. However, HDL-cholesterol decreased with increasing n–3 PUFA. This parallels several other recent controlled animal and human feeding trials where a decrease in HDL-cholesterol has been observed with fish oil consumption (20, 27–29). The consumption of dietary n–3 PUFA with 0.1% (w/w) cholesterol resulted in an increase in circulating VLDL-C and LDL-C even in the lowest dietary n–3 PUFA group, with the greatest increase being in animals consuming the highest n–3 PUFA diets. This is consistent with a reported 60–70% increase in non-HDL plasma cholesterol in hamsters consuming menhaden oil and 0.1% (w/w) cholesterol (20). The observed response to dietary n–3 PUFA is

TABLE 5. Plasma and hepatic cholesterol concentrations of animals consuming the different experimental diets

Dietary Group			Liver wt (g) per 100 g Body Weight	Liver Cholesterol		Plasma Cholesterol			
	chol	n–3		Free	Ester	Total	VLDL	LDL	HDL
				μg/mg protein		*mg/dl*			
1B	mod	0	4.82 ± 0.2^{a}	15.6 ± 0.4^{a}	115 ± 8^{a}	168 ± 6^{a}	26 ± 3^{a}	30 ± 2^{a}	111 ± 6^{a}
2B	mod	+	5.25 ± 0.1^{ab}	16.3 ± 0.6^{ac}	94 ± 3^{b}	192 ± 11^{a}	43 ± 3^{b}	53 ± 13^{b}	96 ± 1^{ab}
3B	mod	++	5.21 ± 0.2^{ab}	16.1 ± 1.2^{a}	82 ± 10^{b}	203 ± 22^{a}	45 ± 5^{b}	54 ± 8^{b}	107 ± 15^{a}
4B	mod	+++	5.14 ± 0.3^{ab}	18.8 ± 0.9^{b}	84 ± 5^{b}	182 ± 9^{a}	42 ± 3^{b}	50 ± 3^{b}	85 ± 9^{ab}
5B	mod	++++	5.51 ± 0.2^{bc}	18.1 ± 0.7^{bc}	51 ± 6^{c}	197 ± 8^{a}	48 ± 3^{b}	74 ± 6^{c}	75 ± 4^{b}

Values are given as means ± SEM. Liver weights and cholesterol, n = 7 or 8. Plasma cholesterol was determined in each group on four pooled samples of two animals per pool. Values in each column without a common superscript are significantly different, $P < 0.05$, as determined by analysis of variance using Fisher's Protected Least Significant Difference method; chol, dietary cholesterol; mod, moderate; n–3, dietary n–3 PUFA.

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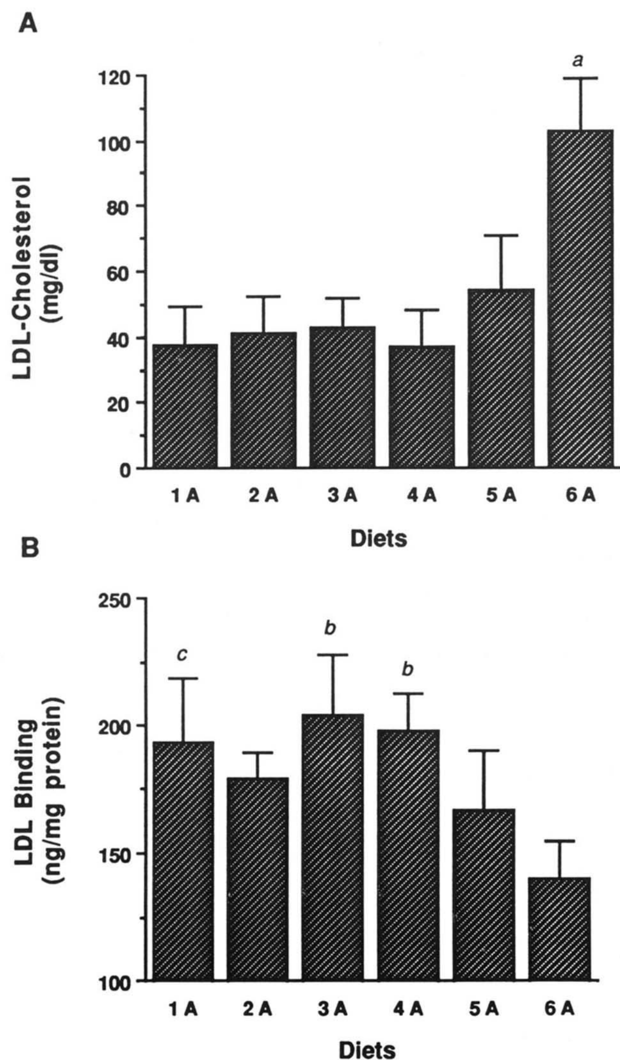

Fig. 1. A: Plasma LDL-cholesterol concentrations of animals consuming the different diets from Experiment 1. Cholesterol values are the total of free and esterified cholesterol expressed as mg free cholesterol. B: EDTA-suppressible ^{125}I-labeled LDL binding to hepatic membranes from animals consuming the different diets. Liver 10,000 *g* membranes (150–250 µg protein) were incubated at 37°C with 15 µg ^{125}I-labeled LDL protein for 1 h in the absence or presence of EDTA (10 mM). EDTA-suppressible binding was taken as the difference in ^{125}I-labeled LDL binding to membranes when incubated with and without EDTA. Diets 1A–4A contained increasing dietary n–3 fatty acids (from 0 to 2.1% w/w) and 0.015% (w/w) cholesterol. Diets 5A and 6A were identical to 1A and 4A, respectively, but contained 0.1% cholesterol; a, significantly different from other dietary groups, $P < 0.05$; b, significantly different from Diet 6A, $P < 0.05$; c, different from Diet 6A, $P = 0.068$.

similar to that in feeding trials using dietary saturated and n–6 PUFA, which only have an important impact on circulating plasma cholesterol when consumed with dietary cholesterol (12, 13). The decrease in plasma HDL-C content with n–3 PUFA intake was of similar magnitude to that observed in animals consuming low dietary cholesterol. Thus, while n–3 PUFA-induced changes in LDL-C and VLDL-C are dependent on dietary cholesterol, changes in HDL-C are not.

The hamster is a very good model for the study of the effects of dietary fat composition and cholesterol on circulating plasma lipoprotein cholesterol and its regulation (13, 18). The attractiveness of this model reflects its similarities to humans in the response to dietary cholesterol and fatty acids, circulating plasma cholesterol levels, and hepatic cholesterol synthesis rates (13, 18, 30, 31). In humans, the circulating LDL-C concentration has been negatively correlated with

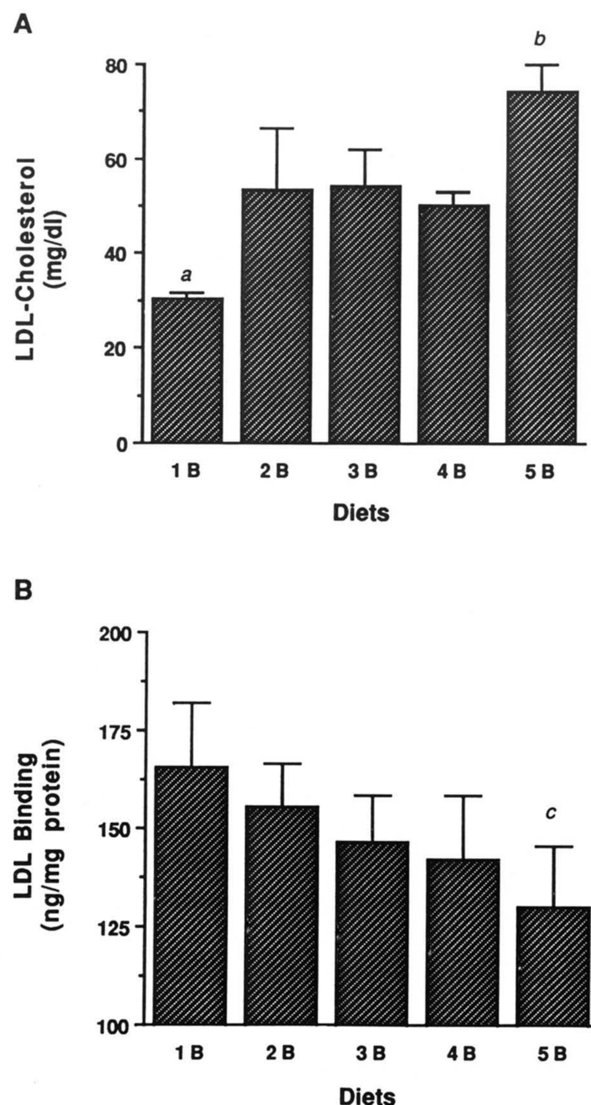

Fig. 2. A: Plasma LDL-cholesterol concentrations of animals consuming the different diets from Experiment 2. Cholesterol values are the total of free and esterified cholesterol expressed as mg free cholesterol. B: EDTA-suppressible ^{125}I-labeled LDL binding to hepatic membranes from animals consuming the different diets. Liver 10,000 *g* membranes (150–250 µg protein) were incubated at 37°C with 15 µg ^{125}I-labeled LDL protein for 1 h in the absence or presence of EDTA (10 mM). EDTA-suppressible binding was taken as the difference in ^{125}I-labeled LDL binding to membranes when incubated with and without EDTA. Diets 1B–5B contained increasing dietary n–3 fatty acids (from 0 to 1.9% w/w) and 0.1% dietary cholesterol; a,b, significantly different from other dietary groups, $P < 0.05$; c, different from Diet 1B, $P = 0.092$.

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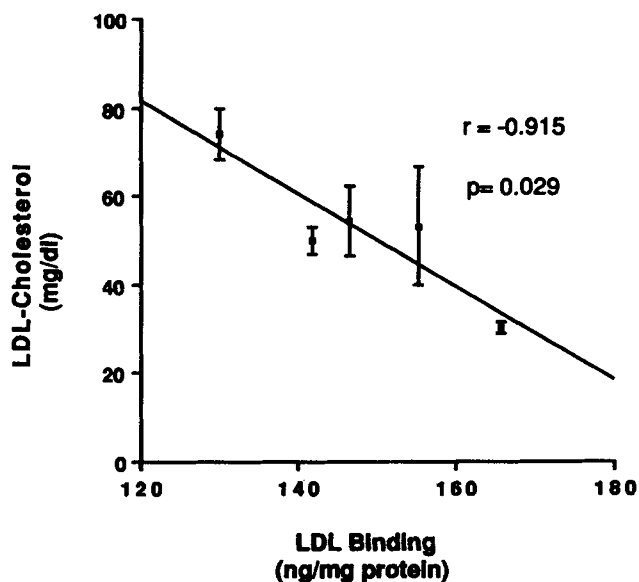

Fig. 3. The relationship between plasma LDL-cholesterol concentrations and EDTA-suppressible binding of ^{125}I-labeled LDL to hepatic membranes from animals consuming the diets from Experiment 2. Each point represents the mean for each dietary group ± SEM.

hepatic LDL-receptor binding activity (25, 32). Similarly, hamsters respond to increased dietary cholesterol or saturated fats by reducing hepatic cholesterol synthesis and receptor-mediated hepatic uptake of LDL (13, 18). Thus, the liver is the main regulatory organ for circulating LDL-C and hepatic LDL-receptor expression appears to be important in LDL-C regulation after dietary interventions that may cause changes in circulating LDL-C.

In an attempt to explain the increased circulating plasma LDL-C concentration observed with n–3 PUFA and cholesterol consumption, the binding activity of the hepatic LDL-receptor was determined as the EDTA-suppressible binding of LDL to hepatic membranes (33). While there was no change in hepatic LDL-receptor binding activity in any of the groups of animals fed low cholesterol diets, as dietary n–3 PUFA increased in animals consuming the higher cholesterol diets, there was a gradual decrease in hepatic LDL-receptor binding activity which corresponded to elevated circulating LDL-C concentrations. Indeed, the receptor-binding activity in the different dietary groups was negatively and significantly correlated to the circulating LDL-C ($r = -0.91$) (Fig. 3). Therefore, the increase in LDL-C observed with dietary n–3 PUFA consumption can be at least partially explained by a reduced hepatic receptor-mediated uptake of LDL.

Although decreased hepatic LDL-receptor binding activity with n–3 PUFA and cholesterol consumption correlates well with circulating LDL-C, the possibility that there may also be an increased production of LDL-C cannot be excluded. The increased concentration of circulating VLDL-C with n–3 PUFA consumption is consistent with a greater hepatic output, and the proportion of VLDL converted to LDL may be increased with the consumption of n–3 PUFA (34). Therefore, it is plausible that LDL production is also increased in addition to the decrease in receptor-mediated removal from the blood. Moreover, a decreased LDL-receptor activity in itself could lead to a greater production of LDL-C as apoB-containing IDL and VLDL-remnant particles are also cleared from the circulation via the LDL-receptor (35). Thus, a decrease in their clearance could result in an increased residence time in the circulation and greater conversion to LDL.

The increase in LDL-C observed with n–3 PUFA consumption is analogous to that observed in animals where saturated fats have replaced monounsaturates or n–6 PUFA. There have been no irrefutable explanations why saturated dietary fats affect plasma cholesterol concentrations and hepatic cholesterol metabolism as they do.

One theory is that saturated fat consumption could increase the putative regulatory pool of cellular cholesterol, which down-regulates the LDL-receptor (10, 13, 36). The distribution of hepatic cellular cholesterol is markedly affected by the consumption of saturated lipids which results in a large decrease in hepatic cholesteryl ester content (13, 19, 20). Similarly, the consumption of long chain n–3 fatty acids leads to a decrease in hepatic cholesteryl esters (20, 37) (Tables 4 and 5). This may be a result of their being poor substrates for acyl-CoA:cholesterol acyl transferase (ACAT) (38). Also, n–3 fatty acids inhibit the acylation of cholesterol in hepatocyte cell culture and isolated microsomes (38), possibly explaining why the consumption of n–3 PUFA with cholesterol results in a decreased hepatic CE content. The combination of higher dietary cholesterol delivery to the liver and decreased hepatic ester formation may reveal the underlying cause for the decreased LDL-receptor activity in animals consuming n–3 PUFA and cholesterol. When the human hepatoma cell line, HepG2, is incubated with LDL, the selective inhibition of ACAT with compound 58-035 results in an enhanced down-regulation of LDL-receptor activity (39) suggesting that blocking cholesterol esterification in hepatocytes may redirect cellular cholesterol to a putative regulatory pool. Thus, ACAT inhibition by n–3 fatty acids may have similar consequences (38). Indeed, incubation of HepG2 cells with eicosapentaenoic acid results in a decrease in LDL-receptor binding activity (40). In the absence of excess dietary cholesterol, the liver stores relatively little CE and cholesterol flux is not influenced to the same extent by the quality of dietary fatty acids.

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While other dietary studies with fish oils have generally observed slight increases in circulating LDL-C, few have reported increases as great as those reported in the present study. One reason for this is that in many animal feeding trials diets are not supplemented with cholesterol, resulting in little change in circulating LDL-C levels, as was found in the present study with animals consuming low dietary cholesterol. A second reason is that hamsters have a low hepatic cholesterol synthesis rate compared to other species such as the rat or rabbit (30), and thus do not maintain hepatic cholesterol balance in the presence of increased delivery of dietary cholesterol and fats by simply decreasing do novo synthesis (13). Where other species are capable of dealing with a dietary challenge by decreasing cholesterol synthesis, the male hamster will alter rates of LDL transport, resulting in increased circulating levels (41, 42). However, one of the reasons that the hamster is an attractive animal model for the study of cholesterol metabolism is because of this low hepatic rate of cholesterol synthesis which is similar to that of humans (30). Yet, only small increases in LDL-C are usually observed in humans consuming dietary fish oils even though dietary cholesterol is usually present in dietary trials. The efficient absorption of dietary cholesterol by rodents compared to humans (30) may account for the differences in the extent of the increases in LDL-C observed between this study and human trials. Therefore, the limited but rather consistent increases report in LDL-C in humans consuming fish oils may be due to the relatively small proportion of dietary cholesterol being delivered to the liver compared to the amount delivered to the livers of hamsters consuming 0.1% cholesterol diets.

The changes in circulating plasma lipoproteins with n–3 PUFA consumption described in this study are not consistent with the observations that fish oil consumption leads to a decreased incidence of cardiovascular disease. Taken collectively, studies on the effects of fish oils on plasma cholesterol have, if anything, indicated that LDL-C tends to increase with fish oil consumption (8). The decreased rates of cardiovascular disease observed in populations consuming fish, and decreased pathologies observed in animals consuming fish oils (3, 4), may be due to factors other than plasma cholesterol levels (6, 7). Therefore, it is important to understand the effects of dietary n–3 PUFA on cholesterol metabolism and circulating lipid levels, and their interrelation with other dietary components so that the beneficial effects of n–3 PUFA are not compromised. The present study using the hamster model lends some insight into these processes. ■

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