Effect of dietary corn, coconut, and menhaden oils on lipoprotein, liver, and heart membrane composition in the hypercholesterolemic rabbit

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The relative capacities of n-3 and n-6 polyunsaturated fatty acid-containing diets (PUFA) to modify chemical composition in plasma lipoproteins, liver membrane, and heart phospholipids and to thereby modulate lipoprotein and membrane fluidity were studied in the rabbit. Stock diet-fed New Zealand rabbits were made hypercholesterolemic by feeding a casein-based, semi-purified diet containing coconut oil. Subsequent replacement of the coconut oil with corn or menhaden oil caused reduction or elevation, respectively, of blood cholesterol levels. Very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) became progressively less fluid following transfer from the PUFA containing low fat stock diet to the coconut oil diet. Subsequent corn oil feeding induced significant increases in VLDL and LDL, but not HDL, fluidities. Menhaden oil feeding did not affect lipoprotein fluidity. Liver membranes were less fluid in corn oil-fed and menhaden oil-fed than in coconut oil-fed rabbits. Despite these differences in lipoprotein and membrane fluidity the amount of rabbit ^{125}I-LDL capable of binding to liver membranes from the animals was not affected by the dietary fat modifications. Fatty acyl compositions were affected by the diets. Heart and liver phospholipids showed extensive incorporation of n-3 fatty acids and deletion of linoleate in the menhaden oil-fed animals. Dietary effects on lipoprotein phospholipid fatty acids were statistically significant but not as pronounced as in these tissues.

Keywords: cholesterol; LDL receptor; n-3 fatty acid; n-6 fatty acid; PUFA

Introduction

The hypocholesterolemic nature of diets containing polyunsaturated fatty acids of the n-6 type has been well established in humans and animals, however uncertainties remain with respect to those with fatty acids of the n-3 type, as in marine lipids.[1,2] The underlying mechanism for the potential effectiveness of fish oil supplements for the prevention of heart disease is not known yet but has been related[3] to effects on arachidonate metabolism, monocytes with respect to proliferation of smooth muscle cells, and triacylglycerol-rich very low density lipoprotein (VLDL).

Dietary n-3 fatty acids are potent reducers of plasma triacylglycerols,[4] but reports conflict with respect to their effects on low density (LDL) and high density (HDL) lipoproteins.[5-7] Nestel[6] reported that habitual consumption of fish oils effectively lowered cholesterol and triacylglycerol levels in plasma, VLDL, LDL, and HDL in humans. Thiery and Seidel[8] reported that fish oil feeding enhanced cholesterol in-

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duced atherosclerosis in the rabbit, however Field et al.[9] reported that dietary cholesterol was less hypercholesterolemic when rabbits were fed 1% cholesterol diets including menhaden oil than 1% cholesterol diets including cocoa butter. Field et al.[9] demonstrated that the level of lipoprotein cholesterol was controlled by the activities of hepatic hydroxymethylglutaryl coenzyme A reductase and intestinal acyl coenzyme A:cholesterol acyl transferase. They associated the dietary cholesterolemia with modulation of these enzyme activities through dietary influences on the fatty acyl compositions of the appropriate membranes. Blood triacylglycerol and VLDL levels are also modulated by choice of dietary fat via control of enzyme activity. Herzberg and Rogerson[10] reported that menhaden oil feeding lowered hepatic fatty acid synthesis and triacylglycerol secretion in rats.

Investigation of the extent of dietary fatty acid incorporation into lipoproteins and tissues may provide further understanding of the roles of n-3 and n-6 polyunsaturated fatty acid-containing diets (PUFA) in modulating enzyme activities and physiologic processes. Fish oil feeding induces replacement of n-6 fatty acids with n-3 fatty acids in cell membranes in various human and animal tissues.[11-16] Vas Dias et al.[12] reported increased eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids in platelet, liver, adipose tissue, and aortic lipids of fish oil-fed rabbits. The present study, using the hypercholesterolemic rabbit as a model, was undertaken to assay lipoprotein and tissue incorporation of dietary fatty acids during feeding regimens selected to sustain or reduce the hypercholesterolemia and to determine if dietary fatty acid control of blood cholesterol level is mechanistically related to modulation of lipoprotein receptor activity via diet induced modification of lipoprotein and tissue lipid composition and membrane fluidity.

This study is part of our continuing investigations of the effects of various natural fat diets on the physico-chemical properties of plasma lipoproteins. Studies[17-19] with fats of vegetable (corn, cocoa, and coconut) origin and animal (milkfat) origin demonstrated that the natural fats have unique effects on lipoproteins which may not be predicted from dietary fatty acid composition alone. The animals were not fed cholesterol in this study nor in two prior studies,[17,18] but with soy protein-based diets to avoid the extreme hypercholesterolemia that often occurs in cholesterol-fed rabbits.[20] Such hypercholesterolemia is probably not related to human atherosclerosis. In this study the rabbits were fed a casein-based, cholesterol-free, semi-purified diet that is reported to increase blood cholesterol only three- to four-fold from a baseline of 40-70 mg/dL.[21,22] The casein-based diet is well suited for studying dietary fat effects, as the development of hypercholesterolemia in casein-fed rabbits is affected by the type of dietary fat.[23] Saturated fats, i.e., cocoa butter, palm kernel, and coconut oils, elevate serum cholesterol, while corn oil maintains the concentration of cholesterol at a low level.

We have now studied the effects of menhaden and corn oils on lipoprotein and liver membrane composition and fluidity when these fats are fed to hypercholesterolemic rabbits previously fed a coconut oil-containing diet. Binding of radioiodinated rabbit LDL to liver membranes from these rabbits was measured to assess compositional and fluidity effects on the binding. Fatty acyl compositions of heart phospholipids in these rabbits are also reported.

Materials and methods

Animals, diets, and experimental protocol

Thirty-two 2-month-old male New Zealand White rabbits (Hare Marland, Hewitt, NJ) were selected for this study. The animals were housed individually in wire-bottom cages in controlled temperature and humidity rooms at the Howard University Animal Resources Facility. The animals were fed a stock diet (Purina Rabbit Chow, Purina Mills, Richmond, IN) for a 2-week acclimation period when they weighed 1.5–1.8 kg. Blood samples were taken weekly by ear venipuncture to monitor the progress of change in blood cholesterol level and lipoprotein fluidity throughout the study. At the end of the stock diet period, three animals had developed severe ear necrosis from the ear venipunctures and were sacrificed by euthanasia. Twenty-one of the rabbits were then fed a casein-based, semi-purified diet containing coconut oil for 4 weeks to promote hypercholesterolemia, while the others were kept on the stock diet. One rabbit was found dead during the coconut oil feeding period. The remaining hypercholesterolemic animals were then divided into three groups. Six rabbits were kept on the coconut oil diet, while the others (seven rabbits in each group) were fed similar diets with either corn oil or menhaden oil (Gift of Zapata Hayne, Reedville, VA). The rabbits were fed these diets for an additional 5–6 weeks until hypercholesterolemia had dissipated in the corn oil-fed rabbits. All seven of the menhaden oil-fed rabbits survived to the end of the study, but one rabbit from each of the other groups was found dead. All surviving animals exhibited similar growth patterns with no outward appearances of any disease. The hearts and livers of the animals were similar in appearance, size, and texture. After completing the final feeding phase, blood was drawn via cardiac puncture, using EDTA as anticoagulant (2 mg/mL), and the animals were sacrificed by total exsanguination. Liver samples were taken for lipid analysis and assay of liver membrane binding capacity for ^{125}I-LDL. Hearts were excised immediately after exsanguination, and a sample of tissue was taken and frozen for subsequent phospholipid fatty acyl composition analysis.

Formulations of the semipurified diets were identical (*Table 1*) except for the fat source. All diets included 1% corn oil, to insure essential fatty acid sufficiency, in addition to 14% corn, coconut, or menhaden oil. Fatty acid compositions of the diets, determined by gas chromatography, (*Table 2*) were as expected. The semipurified diets (Teklad, Madison, WI) were

Table 1 Formulation of the experimental diets[a]

Component	Amount, g/kg
Casein, high protein	250.0
DL-Methionine	3.0
Corn starch	200.0
Dextrose, monohydrate	140.1
Cellulose (fiber)	150.0
Molasses	30.0
Fat[b]	140.0
Corn oil	10.0
Mineral mix[c]	66.5212
Vitamin mix, Teklad #40060[d]	10.0
Ethoxyquin (antioxidant)	0.25
Feed dye (to color-code pellets)	0.15

[a] Formulations for the diets were identical except for the source of fat.
[b] The rabbit diets contained 14% coconut oil, corn oil, or "specially processed" menhaden oil (Zapata Hayne Corp.) + 1% corn oil, included to maintain essential fatty acid sufficiency.
[c] Mineral mix consisted of (g/kg) calcium carbonate, 12.5; potassium bicarbonate, 10.0; sodium bicarbonate, 8.0; calcium phosphate, dibasic, 10.0; potassium phosphate, dibasic, 10.0; sodium chloride, 5.0; magnesium sulfate, 10.0; ferric citrate, 0.7063; manganese sulfate, 0.0811; zinc sulfate, anhydrous, 0.169; cupric sulfate, 0.0346; cobalt chloride, 0.0035; potassium iodate, 0.004; ammonium molybdate, 0.0227.
[d] Vitamin mix consisted of (g/kg) p-aminobenzoic acid, 11.0132; ascorbic acid, coated (97.5%), 101.6604; biotin, 0.0441; vitamin B_{12} (0.1% trituration in mannitol), 2.9736; calcium pantothenate, 6.6079; choline dihydrogen citrate, 349.6916; folic acid, 0.1982; inositol, 11.0132; menadione, 4.9559; niacin, 9.9119; pyridoxine HCl, 2.2026; riboflavin, 2.2026; thiamin HCl, 2.2026; dry vitamin A palmitate (500,000 U/g), 3.9648; dry vitamin D_2 (500,000 U/g), 0.4405; dry vitamin E acetate (500 U/g), 24.2291; corn starch, 466.6878.

custom prepared, color coded, pelleted, cooled rapidly, and refrigerated, then shipped to the laboratory by air transport. Upon receipt from the manufacturer, daily feed allotments were repackaged under N_2 in sealed plastic packs and stored under refrigeration until used. Animals were provided with 70 g of fresh diet daily and distilled water ad libitum.

Lipoprotein isolation

Plasma was separated from whole blood by centrifugation at 1,500*g* for 30 min at 4° C. Chylomicrons were removed from the plasma and VLDL ($d \leq 1.006$ g/mL), LDL (1.006 g/mL $\leq d \leq$ 1.063 g/mL) and HDL (1.063 g/mL $\leq d \leq$ 1.210 g/mL) were isolated by ultracentrifugal sequential flotation as described.[24]

LDL for radioiodination and binding studies was isolated from pooled plasma, obtained from Purina Chow-fed rabbits, by density gradient ultracentrifugation using a Beckman 70 Ti rotor (Beckman Instruments, Palo Alto, CA) as described by Chung et al.[25] After recovery at 1.02 g/mL $\leq d \leq$ 1.05 g/mL, the LDL was washed and concentrated at the upper density value by flotation at 114,000*g* for 24 hr at 10° C. After exhaustive dialysis in 150 mM NaCl, 0.3 mM EDTA, 20 mM Tris-HCl, pH 7.4, at 4° C, the LDL was radioiodinated with $Na^{125}I$, carrier-free, specific activity 17.4 Ci/mg (New England Nuclear, Boston, MA), and the product isolated and processed as described by Gianturco and Bradley.[26] The specific activity of the ^{125}I-LDL was 206 cpm/ng protein.

Binding of ^{125}I-LDL to liver membranes

Immediately after exsanguination of the rabbits, livers were excised and rinsed in ice-cold 150 mM NaCl, 10 mM Tris-HCl, pH 7.5. Liver membranes were prepared and ^{125}I-LDL binding was determined as described by Kovanen et al.[27]

Fluidity measurements

Lipoprotein and liver membrane fluidities were evaluated by measuring 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence polarization. Probe incorporation was accomplished by diluting 2 mM DPH in tetrahydrofuran 500-fold into the isolated lipoprotein or liver membrane suspensions and incubating with shaking at 37° C for 2 hr. Steady state fluorescence polarization was measured with an SLM Model 4800 spectrophotofluorometer (SLM-Aminco, Urbana, IL) equipped with Glan Thompson prism polarizers in the T-optical format. Excitation and emission wavelengths were 366 and 460 nm, respectively.

Chemical analyses

Lipids were extracted with chloroform-methanol by an adaptation[28] of the method of Sperry and Brand.[29] Phospholipids were separated from the neutral lipids by silicic acid column chromatography with Unisil (Clarkson Chemical Co., Williamsport, PA), and the phospholipid fatty acyl composition determined by gas chromatography of the corresponding methyl esters prepared by transesterification with methanolic HCl.[28] Chromatography was performed with a Hewlett-Packard Model 5700A gas chromatograph (Hewlett-Packard, Avondale, PA) coupled to a Model 3885A

Table 2 Fatty acid compositions of the experimental diets

Major diet fat	Menhaden oil	Coconut oil	Corn oil
Acid	mole percent		
12:0	0.26	53.75	—
14:0	15.28	22.85	0.17
15:0	0.85	0.03	—
16:0	24.43	9.91	14.21
16:1	17.15	0.04	0.17
18:0	3.68	2.62	2.22
18:1	11.48	6.20	25.11
18:2	5.06	4.44	56.94
18:3	1.26	—	0.79
20:0	0.42	—	—
20:3	0.13	—	0.13
20:4	1.03	—	—
20:5	12.67	—	—
22:5	2.17	—	—
22:6	3.47	—	—
24:0	0.42	—	0.13
24:1	0.25	—	—

automation system. The instrument was equipped with dual flame ionization detectors and a Model 7671A automatic sampler. Detection was in the dual differential mode. Stainless steel columns (1.8 m) were packed with Supelcoport (Supelco, Bellefonte, PA) coated with a slurry of 10% (by weight) phosphoric acid-modified ethylene glycol succinate.[28]

Cholesterol[30] and triacylglycerol[31] analyses were performed enzymatically and protein was determined colorimetrically.[32]

Statistical analysis

Statistical significance of differences was evaluated using analysis of variance[33] and Duncan's multiple range test applicable to group means with unequal sample sizes.[34]

Results

The casein-based, semi-purified diet containing coconut oil induced hypercholesterolemia, as expected. The mean serum cholesterol level after 4 weeks was 152 ± 5 mg/dL in the coconut oil-fed rabbits (n = 20), compared to initial data of 58 ± 3 mg/dL for all the rabbits when fed Purina Chow (n = 31). At the end of the 4-week feeding period the Purina Chow-fed rabbits (n = 8) exhibited serum cholesterol concentrations of 50 ± 4 mg/dL. Subsequent feeding of either corn or menhaden oil in place of the coconut oil effected changes in serum cholesterol and lipoprotein levels (*Table 3*). Corn oil feeding lowered serum cholesterol 42%, but menhaden oil feeding increased serum cholesterol by 73% at the end of 5-6 weeks. Corn oil feeding did not affect LDL cholesterol but reduced HDL cholesterol. Menhaden oil consumption markedly elevated LDL protein and cholesterol contents but not VLDL and HDL.

Dietary fatty acid incorporation was observed in all fractions but was more pronounced in the tissue phospholipids than in the lipoprotein phospholipids as shown in the mole percentage data in *Tables 4–8*.

Table 3 Effect of dietary fat on plasma cholesterol and lipoprotein concentrations (means ± SEM for n rabbits)

Diet fat	Coconut oil (n = 5)	Corn oil (n = 6)	Menhaden oil (n = 7)
	mg percent		
Plasma cholesterol	136 ± 13[a]	79 ± 5[b]	235 ± 31[c]
VLDL			
Protein	13 ± 1[a]	8 ± 1[a]	12 ± 2[a]
Cholesterol	17 ± 3[a]	6 ± 1[a]	19 ± 6[a]
LDL			
Protein	38 ± 7[a]	30 ± 3[a]	91 ± 12[b]
Cholesterol	40 ± 6[a]	30 ± 3[a]	102 ± 16[b]
HDL			
Protein	171 ± 39[a]	120 ± 26[a]	144 ± 22[a]
Cholesterol	42 ± 4[a]	23 ± 3[b]	42 ± 4[a]

[a-c] Values in a row with different superscripts are significantly different ($P < 0.05$).

Table 4 VLDL phospholipid fatty acyl composition (means ± SEM)

Diet fat	Menhaden oil	Coconut oil	Corn oil
Acid	mole percent		
12:0	0.01 ± 0.01[a]	0.07 ± 0.05[a]	0.01 ± 0.01[a]
14:0	1.32 ± 0.13[b]	2.27 ± 0.31[a]	0.63 ± 0.07[c]
15:0	0.76 ± 0.03[a]	0.50 ± 0.05[b]	0.57 ± 0.09[a,b]
16:0	39.80 ± 1.37[a]	28.57 ± 1.17[b]	30.49 ± 2.47[b]
16:1 (n-7)	3.04 ± 0.38[a]	1.36 ± 0.36[b]	0.20 ± 0.20[c]
18:0	24.09 ± 0.58[a]	25.71 ± 2.02[a]	30.55 ± 2.83[a]
18:1 (n-9)	11.99 ± 0.31[b]	18.49 ± 1.00[a]	13.30 ± 0.78[b]
18:2 (n-6)	5.20 ± 0.39[b]	16.58 ± 3.31[a]	17.24 ± 4.27[a]
18:3 (n-3)	0.77 ± 0.07[a]	0.76 ± 0.06[a]	0.74 ± 0.06[a]
20:0	1.42 ± 0.11[a]	0.93 ± 0.14[a]	0.98 ± 0.24[a]
20:3 (n-6)	1.45 ± 0.11[a]	1.30 ± 0.11[a]	1.19 ± 0.25[a]
20:4 (n-6)	1.49 ± 0.32[a]	0.96 ± 0.28[a]	0.63 ± 0.11[a]
20:5 (n-3)	3.55 ± 0.41[a]	0.10 ± 0.04[b]	0.15 ± 0.15[b]
22:5 (n-3)	0.49 ± 0.07[a]	0.07 ± 0.02[b]	0.04 ± 0.03[b]
22:6 (n-3)	1.01 ± 0.15[a]	0.04 ± 0.02[b]	0.30 ± 0.15[b]
24:0	1.78 ± 0.20[a]	1.29 ± 0.20[a]	2.22 ± 0.53[a]
24:1 (n-9)	1.82 ± 0.16[a]	0.99 ± 0.09[b]	0.89 ± 0.17[b]
U/S[d]	0.45 ± 0.02[a]	0.70 ± 0.09[a]	0.59 ± 0.15[a]
P/S[d]	0.20 ± 0.01[a]	0.35 ± 0.07[a]	0.35 ± 0.11[a]

[a-c] Values for a fatty acid or ratio with different superscripts are significantly different.
[d] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated (S) fatty acids.

VLDL data (*Table 4*) show that consumption of either PUFA in place of coconut oil led to significant reductions in 14:0 and 18:1 fatty acyl content. Menhaden oil feeding resulted in significant differences when compared to either coconut or corn oil feeding. VLDL from the menhaden oil-fed rabbits was significantly higher in 16:0, 20:5, 22:5, 22:6, and 24:1 and lower in 18:2 contents.

LDL data (*Table 5*) show similar effects. The coconut oil-fed rabbits were significantly lower in 16:0 and higher in 18:1 than the other animals. Menhaden oil-fed rabbits were significantly lower than the others in LDL linoleate but higher in LDL content of all fatty acids of 20 or more carbon atoms. The corn oil-fed rabbits showed an elevation in stearate and reduction in myristate.

HDL phospholipid data (*Table 6*) show significant effects on both saturated and unsaturated fatty acids. Menhaden oil-fed rabbits were significantly higher in 16:0 and all fatty acids of 20 or more carbon atoms, but lower in both 18:1 and 18:2 contents. Despite their saturated fat consumption, the coconut oil-fed rabbits were lowest in 16:0 and 18:0 contents. The coconut oil diet contained substantially less palmitate than the other fats (*Table 3*). Corn oil-fed animals were significantly higher than the coconut oil-fed rabbits in HDL stearate.

Liver membrane phospholipid fatty acyl composition data (*Table 7*) show dramatic incorporations of the n-3 fatty acids, 20:5, 22:5, and 22:6, in the menhaden oil-fed rabbits. As in the lipoproteins, liver microsomal phospholipids from these rabbits were higher than the others in palmitate and all acids containing 20

Table 5 LDL phospholipid fatty acyl composition (means ± SEM)

Diet fat	Menhaden oil	Coconut oil	Corn oil
Acid	mole percent		
12:0	0.05 ± 0.02[b]	0.24 ± 0.05[a]	0.08 ± 0.03[b]
14:0	1.54 ± 0.22[a]	2.17 ± 0.20[a]	0.63 ± 0.13[b]
15:0	0.74 ± 0.05[a]	0.44 ± 0.06[a,b]	0.55 ± 0.05[b]
16:0	39.19 ± 1.29[a]	27.67 ± 1.67[c]	35.25 ± 2.00[a,b]
16:1 (n-7)	2.44 ± 0.39[a]	1.42 ± 0.13[a,b]	0.12 ± 0.08[c]
18:0	20.78 ± 0.61[b]	23.99 ± 2.17[b]	31.10 ± 1.87[a]
18:1 (n-9)	10.57 ± 0.50[b]	18.53 ± 0.55[a]	12.35 ± 0.66[b]
18:2 (n-6)	4.34 ± 0.17[c]	19.23 ± 3.58[a]	15.34 ± 3.24[a,b]
18:3 (n-3)	0.56 ± 0.03[a]	0.67 ± 0.04[a]	0.38 ± 0.03[b]
20:0	1.18 ± 0.06[a]	0.74 ± 0.04[c]	0.98 ± 0.03[b]
20:3 (n-6)	1.74 ± 0.13[a]	1.19 ± 0.16[b]	0.77 ± 0.06[b,c]
20:4 (n-6)	3.21 ± 0.54[a]	1.23 ± 0.38[b]	0.25 ± 0.09[c]
20:5 (n-3)	5.44 ± 0.93[a]	0.59 ± 0.26[b]	0.82 ± 0.25[c]
22:5 (n-3)	1.96 ± 0.44[a]	0.07 ± 0.02[b]	0.02 ± 0.01[c]
22:6 (n-3)	2.82 ± 0.67[a]	0.03 ± 0.02[b]	0.13 ± 0.07[c]
24:0	1.24 ± 0.07[a]	0.97 ± 0.07[a,b]	0.57 ± 0.28[c]
24:1 (n-9)	2.20 ± 0.14[a]	0.83 ± 0.22[b]	0.67 ± 0.18[c]
U/S[d]	0.55 ± 0.04[a,b]	0.81 ± 0.11[a]	0.47 ± 0.08[c]
P/S[d]	0.32 ± 0.05[a,b]	0.43 ± 0.09[a]	0.27 ± 0.06[a,b]

[a-c] Values for a fatty acid or ratio with different superscripts are significantly different.
[d] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated (S) fatty acids.

Table 6 HDL phospholipid fatty acyl composition (means ± SEM)

Diet fat	Menhaden oil	Coconut oil	Corn oil
Acid	mole percent		
12:0	0.07 ± 0.03[b]	0.67 ± 0.24[a]	0.08 ± 0.02[b]
14:0	1.44 ± 0.09[b]	2.87 ± 0.38[a]	0.66 ± 0.08[c]
15:0	0.76 ± 0.07[a]	0.36 ± 0.04[b]	0.50 ± 0.05[b]
16:0	42.15 ± 1.51[a]	25.80 ± 0.80[c]	34.42 ± 2.34[b]
16:1 (n-7)	3.07 ± 0.16[a]	1.69 ± 0.15[b]	0.00 ± 0.00[c]
18:0	26.11 ± 0.62[a,b]	21.95 ± 1.66[b]	30.08 ± 1.71[a]
18:1 (n-9)	12.53 ± 0.24[c]	20.14 ± 0.28[a]	14.87 ± 0.67[b]
18:2 (n-6)	3.10 ± 0.48[c]	21.49 ± 1.60[a]	15.92 ± 3.67[a,b]
18:3 (n-3)	0.83 ± 0.09[a,b]	0.73 ± 0.04[b]	0.49 ± 0.03[b]
20:0	1.02 ± 0.11[a]	0.49 ± 0.03[b,c]	0.71 ± 0.04[b]
20:3 (n-6)	1.05 ± 0.11[a]	0.89 ± 0.10[a,b]	0.58 ± 0.04[c]
20:4 (n-6)	0.98 ± 0.18[a,b]	1.26 ± 0.24[a]	0.40 ± 0.08[b,c]
20:5 (n-3)	2.69 ± 0.48[a]	0.31 ± 0.05[b]	0.19 ± 0.04[b]
22:5 (n-3)	0.72 ± 0.21[a]	0.06 ± 0.02[b]	0.05 ± 0.02[b]
22:6 (n-3)	0.89 ± 0.26[a]	0.04 ± 0.01[b]	0.02 ± 0.01[b]
24:0	0.97 ± 0.09[a]	0.44 ± 0.03[b]	0.62 ± 0.08[b]
24:1 (n-9)	1.60 ± 0.22[a]	0.81 ± 0.07[b]	0.44 ± 0.10[b]
U/S[c]	0.38 ± 0.03[b]	0.91 ± 0.07[a]	0.52 ± 0.10[b]
P/S[d]	0.15 ± 0.03[b]	0.48 ± 0.05[a]	0.28 ± 0.08[a,b]

[a-c] Values for a fatty acid or ratio with different superscripts are significantly different.
[d] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated (S) fatty acids.

Table 7 Hepatic microsomal phospholipid fatty acyl composition (means ± SEM)

Diet fat	Menhaden oil	Coconut oil	Corn oil
Acid	mole percent		
12:0	0.12 ± 0.08[a]	0.12 ± 0.12[a]	0.01 ± 0.01[a]
14:0	1.54 ± 0.59[a]	0.98 ± 0.48[a]	0.29 ± 0.08[a]
15:0	0.45 ± 0.10[a]	0.07 ± 0.07[b]	0.19 ± 0.09[a,b]
16:0	24.84 ± 0.81[a]	17.30 ± 0.53[b]	17.44 ± 0.62[b]
16:1 (n-7)	1.44 ± 0.14[a]	1.49 ± 0.20[a]	0.16 ± 0.08[b]
18:0	21.78 ± 2.26[a]	24.00 ± 0.69[a]	26.54 ± 1.19[a]
18:1 (n-9)	6.32 ± 0.64[c]	18.74 ± 0.61[a]	9.82 ± 0.60[b]
18:2 (n-6)	3.16 ± 0.49[c]	23.78 ± 0.96[b]	34.05 ± 1.20[a]
18:3 (n-3)	0.48 ± 0.11[b]	1.53 ± 0.12[a]	0.76 ± 0.07[b]
20:0	0.80 ± 0.17[a]	0.76 ± 0.14[a]	0.65 ± 0.15[a]
20:3 (n-6)	0.47 ± 0.17[a]	0.99 ± 0.13[a]	0.57 ± 0.14[a]
20:4 (n-6)	4.40 ± 0.37[a]	5.50 ± 0.66[a]	5.99 ± 0.46[a]
20:5 (n-3)	6.31 ± 0.64[a]	0.62 ± 0.04[b]	0.37 ± 0.21[b]
22:5 (n-3)	6.56 ± 0.58[a]	0.42 ± 0.03[b]	0.26 ± 0.05[b]
22:6 (n-3)	14.99 ± 1.80[a]	0.34 ± 0.02[b]	0.23 ± 0.03[b]
24:0	6.27 ± 2.11[a]	2.23 ± 0.86[a]	1.80 ± 0.37[a]
24:1 (n-9)	0.03 ± 0.03[b]	1.11 ± 0.43[a]	0.89 ± 0.07[a]
U/S[d]	0.80 ± 0.03[b]	1.06 ± 0.06[a]	1.14 ± 0.06[a]
P/S[d]	0.66 ± 0.03[b]	0.74 ± 0.05[b]	0.91 ± 0.05[a]

[a-c] Values for a fatty acid or ratio with different superscripts are significantly different.
[d] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated (S) fatty acids.

or more carbon atoms except nervonate, but much lower in linoleate content. Differences between the corn oil-fed and coconut oil-fed rabbits were minimal except for the significant elevation of linoleate in the corn oil-fed animals.

The heart phospholipid data (*Table 8*) show similarly significant effects of feeding n-3 fatty acids. The menhaden oil-fed animals were dramatically elevated in 20:5, 22:5, and 22:6 contents, but sharply reduced in linoleate content, particularly in comparison to the corn oil-fed rabbits.

Dietary modulation of unsaturation ratios, U/S or P/S, are noted in some of the data (*Tables 4–8*), but there is no consistent direct relation between these data and dietary P/S and U/S ratios. There were no differences between the diet groups in VLDL unsaturation (*Table 4*). LDL from the coconut oil-fed rabbits was most unsaturated (*Table 5*), though only the U/S ratio for LDL from the coconut oil-fed animals was significantly higher than that of the corn oil-fed animals. HDL unsaturation ratios (*Table 6*) were significantly higher for the coconut oil-fed rabbits than for the others. The heart and liver phospholipids were most unsaturated in the corn oil-fed rabbits (*Tables 6 & 7*).

Transferring Purina Chow-fed rabbits to the casein-based, coconut oil diet yielded increased steady state DPH fluorescence anisotropies in the major plasma lipoproteins. At the end of the 4-week diet period steady state anisotropies for VLDL, LDL, and HDL from Purina Chow-fed rabbits were 0.097, 0.152, and 0.169, respectively, while the corresponding values for the coconut oil-fed animals were 0.210, 0.220, and 0.190. The changes in fluidity, which occurred rapidly and were essentially complete within 1–2 weeks, paralleled corresponding increases in serum cholesterol concentrations.

Lipoprotein and liver membrane fluidity data are shown in *Table 9*. Corn oil feeding yielded significantly more fluid VLDL and LDL, but not HDL, than the

Table 8 Heart phospholipid fatty acyl composition (means ± SEM)

Diet fat	Menhaden oil	Coconut oil	Corn oil
Acid	mole percent		
12:0	0.65 ± 0.24[b]	3.02 ± 0.45[a]	1.27 ± 0.21[b]
14:0	5.21 ± 0.73[b]	10.35 ± 0.61[a]	3.33 ± 0.28[c]
15:0	0.65 ± 0.07[a]	0.52 ± 0.04[a,b]	0.45 ± 0.05[b]
16:0	16.85 ± 0.97[a]	16.13 ± 0.28[a,b]	14.23 ± 0.61[b]
16:1 (n-7)	4.95 ± 0.42[a]	2.68 ± 0.24[b]	0.28 ± 0.28[c]
18:0	16.19 ± 2.04[a]	11.43 ± 0.68[b]	12.26 ± 0.44[a,b]
18:1 (n-9)	13.86 ± 0.49[b]	15.99 ± 0.80[a]	14.28 ± 0.52[a,b]
18:2 (n-6)	9.25 ± 0.56[c]	24.19 ± 0.43[b]	40.90 ± 1.02[a]
18:3 (n-3)	0.70 ± 0.10[a]	0.69 ± 0.07[a]	0.73 ± 0.07[a]
20:0	0.82 ± 0.11[a]	0.58 ± 0.02[b]	0.86 ± 0.03[a]
20:3 (n-6)	0.42 ± 0.13[b]	0.73 ± 0.02[a]	0.20 ± 0.05[b]
20:4 (n-6)	8.87 ± 0.58[b]	11.54 ± 0.56[a]	9.12 ± 0.68[b]
20:5 (n-3)	9.22 ± 0.65[a]	0.19 ± 0.01[b]	0.09 ± 0.03[b]
22:5 (n-3)	2.93 ± 0.78[a]	1.01 ± 0.26[b]	0.50 ± 0.10[b]
22:6 (n-3)	8.59 ± 1.32[a]	0.29 ± 0.03[b]	0.32 ± 0.09[b]
24:0	0.49 ± 0.16[a]	0.27 ± 0.06[a]	0.60 ± 0.22[a]
24:1 (n-9)	0.31 ± 0.06[b]	0.42 ± 0.03[b]	0.55 ± 0.03[a]
U/S[d]	1.50 ± 0.17[b]	1.37 ± 0.03[b]	2.04 ± 0.09[a]
P/S[d]	1.02 ± 0.16[b]	0.91 ± 0.03[b]	1.58 ± 0.08[a]

[a-c] Values for a fatty acid or ratio with different superscripts are significantly different.
[d] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated (S) fatty acids.

other fats. Coconut oil and menhaden oil feeding yielded fractions that did not differ significantly in fluidity. In contrast to the lipoprotein data, liver membrane preparations from the corn oil-fed rabbits were significantly less fluid than preparations from the other animals. Lipoprotein fluidities were related to the triacylglycerol and cholesteryl ester contents of those fractions. DPH anisotropies showed an inverse linear relation to the triacylglycerol:cholesteryl ester ratio in each lipoprotein fraction. The VLDL steady state anisotropy, r_s, data, shown as a function of the molar triacylglycerol:cholesteryl ester ratio, n_{TG}/n_{CE}, in *Figure 1*, follow the linear equation: $r_s = 0.205 - 4.1 \times 10^{-3} (n_{TG}/n_{CE})$; with $r = 0.551$. Regression analysis yielded similar equations for the other fractions. For LDL, $r_s = 0.236 - 5.5 \times 10^{-3} (n_{TG}/n_{CE})$; with $r = 0.655$, and for HDL, $r_s = 0.205 - 2.3 \times 10^{-3} (n_{TG}/n_{CE})$; with $r = 0.348$.

Finally, as shown in *Table 10*, substituting either corn oil or menhaden oil into the diet of rabbits previously rendered hypercholesterolemic by coconut-oil feeding did not affect the binding of rabbit ^{125}I-LDL to liver membranes from the animals.

Table 9 DPH steady state anisotropy data at 37° C (means ± SEM)

Diet	Corn Oil (n = 6)	Menhaden Oil (n = 7)	Coconut Oil (n = 5)
VLDL	0.133 ± .007[b]	0.160 ± .012[a,b]	0.190 ± .009[a]
LDL	0.179 ± .005[b]	0.215 ± .005[a]	0.205 ± .008[a]
HDL	0.183 ± .006[a]	0.199 ± .007[a]	0.196 ± .008[a]
Liver membrane	0.184 ± .008[a]	0.173 ± .006[b,c]	0.153 ± .004[c]

a-c

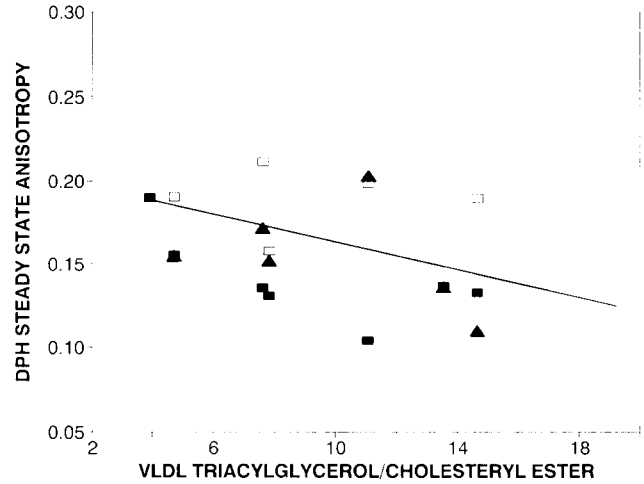

Figure 1 Effect of VLDL triacylglycerol content, as the molar triacylglycerol:cholesteryl ester ratio, on VLDL fluidity, as r_s, DPH steady state anisotropy. Data points for: ▲ menhaden oil-fed rabbits, ■ corn oil-fed rabbits, and □ coconut oil-fed rabbits. The data follow the linear equation: $r_s = 0.205 - 4.1 \times 10^{-3} (n_{TG}/n_{CE})$; with $r = 0.551$.

Table 10 Effect of replacing dietary coconut oil with corn or menhaden oil on rabbit ^{125}I-LDL binding to liver membranes

Experimental diet group	ng ^{125}I-LDL bound/tube		
	Membrane protein content, μg		
	100	200	300
Coconut oil (n = 5)	13.88 ± 0.74	21.11 ± 1.26	27.96 ± 2.05
Corn oil (n = 6)	15.03 ± 0.66	22.81 ± 0.79	29.14 ± 1.35
Menhaden oil (n = 7)	14.25 ± 0.67	20.23 ± 0.99	26.33 ± 0.59

Values are means ± SEM for the indicated numbers of animals in each group. No significant differences exist between the groups for each protein level ($P \leq 0.05$).

Discussion

The hypercholesterolemic nature of casein-based, semi-purified diets to rabbits has been established.[21-23] Casein and other proteins from animal sources were hypercholesterolemic while soy protein isolate or other plant proteins were not.[22] Hypercholesteremia induced by a casein diet is, however, modulated by the type of fat included in the feed. Kritchevsky et al.[23] demonstrated that casein-based diets containing coconut oil, cocoa butter, or palm kernel oil, but not corn oil, were hypercholesterolemic to the rabbit. In the present study with casein-fed rabbits, corn oil lowered plasma cholesterol, but menhaden oil elevated blood cholesterol to levels greater than that of the coconut oil group. Field et al.[9] demonstrated that menhaden oil was less hypercholesterolemic than cocoa butter in the cholesterol-fed rabbit. Evidently, casein-induced hypercholesterolemia is not comparable to

that induced by cholesterol feeding with respect to the effects of the accompanying fats. Kritchevsky et al.[23] showed that cocoa butter did not raise blood cholesterol in the casein-fed rabbit to the same degree as coconut or palm kernel oil. Work in our laboratory[19] with soy protein-fed rabbits showed identical blood cholesterol levels for corn oil-fed and cocoa butter-fed rabbits, however when the soy protein diet was supplemented with 0.2% cholesterol, greater hypercholesterolemia followed in cocoa butter-fed rabbits than in corn oil-fed rabbits.

Thus, the selection of a dietary fat may have a significant effect on cholesterolemia. However the mechanism by which dietary fats modulate blood cholesterol levels is not known. It has been hypothesized that the incorporation of dietary fatty acids may influence lipoprotein structure and thereby affect their removal from the circulation.[35] Fatty acyl chain unsaturation is an important determinant of lipid fluidity,[36] hence it is expected that lipoprotein fluidity would be related to the extent of fatty acyl unsaturation in the constituent phospholipids. Lipoprotein lipid domain fluidity could influence apoprotein conformation and physical orientation in the phospholipid monolayer on the lipoprotein surface and thereby modulate LDL interactions with hepatic cell surface receptors and/or aortic endothelium.

The relationship between fatty acyl chain unsaturation in the lipid domains of lipoproteins and lipoprotein fluidity is not entirely clear. Studies[37-40] with human subjects have shown that dietary fats can have significant effects on lipoprotein fatty acyl composition. Lipoprotein fluidity differences were noted in several of these studies.[37-39] Pownall et al.[39] reported an increase in the unsaturated fatty acyl content of all lipid classes of LDL when individuals consumed a highly polyunsaturated fat diet. LDL from these subjects was more fluid than LDL from subjects consuming a saturated fat diet. Moreover, it was reported that VLDL and HDL fluidities were lowered by a saturated fat diet. Studies in our laboratory with healthy men[41] showed that increasing dietary linoleate resulted in more fluid LDL but not VLDL or HDL. Fluidity was associated with the LDL linoleate content. Myher et al.[40] showed that dietary PUFA led to increased linoleate in major phospholipids of VLDL, LDL, and HDL, however they did not report any fluidity data. Shepherd et al.[37] observed linoleate increase and simultaneous palmitate and stearate decreases in HDL lipids which they associated with an increase in lipoprotein fluidity.

Previous studies[17,18] with rabbits fed a soy protein-based diet showed that dietary fatty acid unsaturation and presumably the degree of fatty acyl chain unsaturation in lipoprotein lipids may not reliably predict lipoprotein fluidity. In the first study[17] VLDL and LDL from rabbits fed a diet containing 5% cocoa butter were more fluid than VLDL and LDL from rabbits fed a 5% corn-oil diet for periods of 42-175 days. However, it was later found[18] with a longer feeding period (379 ± 29 days) that VLDL, LDL, and HDL, from corn oil-fed rabbits tended to be more fluid, though not significantly so, than the respective lipoproteins from cocoa butter-fed rabbits. The apparent differences in fluidity in these earlier studies cannot be attributed to temporal effects on fatty acyl composition, as fatty acyl chain modification can occur within 7 days, at least in humans.[38]

Feeding studies with women[42] and men[43] indicated that increases in lipoprotein fluidity were more dependent on lowering lipoprotein cholesterol content than on increasing lipoprotein unsaturation. In our studies, as well as others reported,[44,45] fluidity was directly related to the lipoprotein triacylglycerol level and inversely related to the cholesteryl ester content. Recently we compared the effects of dietary saturation and cholesterol feeding on lipoprotein fluidity in soy protein-fed rabbits.[19] Animals were fed either corn oil or cocoa butter at the 20% level with or without 0.2% added cholesterol. The cholesterol-fed rabbits showed substantial reductions in VLDL and LDL, but not HDL, fluidities. Cocoa butter feeding had no effect on fluidity, whether or not the animals were fed cholesterol, thus suggesting that lipoprotein fatty acid unsaturation is probably not the major factor in control of lipoprotein fluidity. It should be noted that blood cholesterol was elevated in the rabbits fed these cholesterol-containing soy protein diets with the cholesterol level higher in the animals fed cocoa butter + cholesterol than in those fed corn oil + cholesterol.

The results of the present study support the concept that the cholesterol content of the lipoprotein particles has a major impact in modulating lipoprotein fluidity whether saturated or polyunsaturated fats are consumed. Regression analyses (see *Figure 1*, for VLDL) showed that the DPH steady state anisotropies for the lipoproteins were inversely related to the molar triacylglcerol : cholesteryl ester ratios in the several fractions regardless of fatty acyl composition. Lipoprotein fluidity was thus directly related to the molar triacylglcerol : cholesteryl ester ratio.

Few studies are available concerning the effects of feeding fish oils on lipoprotein fluidity. Belcher et al.[46] reported that the HDL outer phospholipid monolayer was less fluid in rats fed a casein-based diet with menhaden oil than in rats fed the same basal diet with coconut oil. LDL fluidity was not affected. It was assumed that eicosapentaenoic (20 : 5) and docosahexaenoic (22 : 6) acids were incorporated into the phospholipids, however no fatty acid data were provided. In the present rabbit study, substituting menhaden oil for coconut oil in the diet of hypercholesterolemic rabbits resulted in a statistically significant elevation of the 20 : 5 and 22 : 6 fatty acids in VLDL, LDL, and HDL phosopholipids (*Tables 4–6*).

The tissue fatty acid data (*Tables 7 & 8*) clearly show substantial dietary effects on phospholipid fatty acyl composition. Extensive incorporations of eicosapentaenoic and docosahexaenoic acids were observed in both tissues studied with the n-3 fatty acyl incorporation generally occurring in the menhaden oil-fed rabbits at the expense of arachidonic acid. The results

indicate similar total incorporations of n-3 fatty acids in liver and heart phospholipids, but the distributions were somewhat different with 20:5 higher in the heart and 22:6 higher in the liver. Swanson et al.[47,48] and Gudbjarnason and Oskarsdottir[49] reported preferential acylation of 22:6 in cardiac phospholipids, and Neuringer and Conner[50] and Salem et al.[51] reported higher 22:6 acylation in brain and retina. Recently Barzanti et al.[52] reported higher 20:5 and 22:6 contents and lower 20:4 contents in liver microsomes than in brain microsomes of fish oil-fed rats.

The effects on composition in heart phospholipids as well as membrane phospholipids from other cells may have a role in the protection against heart disease that is provided by dietary n-3 fatty acids. The presence of n-3 rather than n-6 fatty acids in the phospholipids, which serve as phospholipase substrates, may modulate prostaglandin metabolism. Effects of altered cell membrane fatty acyl composition on membrane order or fluidity may be involved in the fish oil protection against heart disease. Such effects have been reported with respect to platelet membranes[53] and aggregability; liver membranes and phopholipase A_2 activity;[54] and cardiac membranes and β-adrenergic receptor activity.[55]

Modification of cell membrane structural order might also be implicated in explaining the capacity of dietary saturated and polyunsaturated fats to modulate blood cholesterol levels. Field et al.[9] demonstrated that the level of lipoprotein cholesterol in rabbits was controlled by the activities of hepatic HMG-CoA reductase and intestinal acyl-CoA : cholesterol acyltransferase, activities which were modulated by the fatty acyl compositions of the appropriate membranes as modified by diet. Perhaps the LDL receptor of the cell surface is similarly affected by dietary fat-induced changes in membrane fluidity. Evidence[56,57] indicates that cell membrane phospholipid composition and degree of unsaturation may be important for binding, uptake, and catabolism of LDL. Fibroblasts grown in linoleate supplemented culture medium degraded LDL at a faster rate than fibroblasts grown in palmitate supplemented culture mdedium.[56] LDL receptor number was not affected by the supplementation. Studies[57] with human subjects have shown that LDL receptor activity of blood mononuclear cells decreased as the daily intake of palmitate and stearate was increased. These authors[56,57] suggested that the fatty acids may affect LDL receptor activity by influencing the fluidity of the phospholipid bilayer anchoring the receptor, thereby affecting receptor endocytosis and insertion of the receptor back into the plasma membrane after LDL delivery into the cell.

In the present study, significant incorporation of dietary fatty acids into liver membrane phospholipids was observed (*Table 7*), but unexpected membrane fluidity changes occurred. Furthermore, LDL binding to hepatic membranes (*Table 10*) could not be related to these changes in membrane fluidity. Although corn oil feeding resulted in a significant increase in linoleate and total unsaturated fatty acyl content of liver membrane phospholipids, these membranes were the least fluid. Membranes from coconut oil-fed rabbits were the most fluid, yet the least unsaturated. Substituting corn oil or menhanden oil for coconut oil in the rabbit diet did not affect liver membrane binding of ^{125}I-LDL, hence ^{125}I-LDL binding to hepatic membranes appears unrelated to membrane fluidity.

Substituting corn oil or menhaden oil for coconut oil in the rabbit diet affected blood cholesterol levels independently of any changes in hepatic LDL receptor binding capacity. Thus, the unsaturation of plasma membrane phospholipid fatty acyl chains may not be important in regulating LDL receptor function. Roach et al.[58] reported that feeding fish oil to rats lowered blood cholesterol, but LDL receptor binding activity was reduced in liver membranes from the rats. Thus, it appears more likely that any effects of membrane fatty acyl composition on plasma lipids would result from modulation of membrane localized enzymes. Field et al.[9] described such a mechanism in the control of lipoprotein cholesterol levels. Fish oil lowering of plasma triacylglycerols has also been associated with effects of membrane composition on enzymes involved in their synthesis.[10,59,60]

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