Regulatory effects of individual n–6 and n–3 polyunsaturated fatty acids on LDL transport in the rat

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Abstract Dietary triglycerides containing predominantly polyunsaturated fatty acids (PUFAs) are known to reduce plasma total and low density lipoprotein (LDL) cholesterol concentrations relative to triglycerides containing predominantly saturated fatty acids. However, there is little information regarding the independent effects of individual n-6 and n-3 PUFAs on LDL metabolism. The present studies were therefore undertaken to examine the effects of individual n-6 (linoleic acid) and n-3 (α-linolenic, eicosapentaenoic, and docosahexaenoic acid) PUFAs on plasma lipid levels and on the major transport processes that determine plasma LDL concentrations. Rats were fed a semisynthetic cholesterol-free diet supplemented with 4% (by wt) linoleic, α-linolenic, eicosapentaenoic, or docosahexaenoic acid for 2 weeks. Dietary eicosapentaenoic and docosahexaenoic acids lowered plasma triglyceride concentrations by 62% and 52%, respectively, and lowered plasma cholesterol concentrations by 54% and 43%, respectively. In contrast, dietary linoleic and α-linolenic acids had relatively little effect on plasma triglyceride or cholesterol concentrations. Dietary eicosapentaenoic and docosahexaenoic acids increased hepatic LDL receptor activity by 72% and 58%, respectively, and reduced the rate of LDL cholesterol entry into plasma by 36% and 30%, respectively. As a consequence plasma LDL cholesterol concentrations fell by 60% in animals fed eicosapentaenoic acid and 54% in animals fed docosahexaenoic acid. In contrast, these parameters of LDL metabolism were not significantly altered by dietary linoleic or α-linolenic acids. Thus, eicosapentaenoic acid and docosahexaenoic acid (the two major n-3 PUFAs present in fish oil) were equally effective in reducing the rate of LDL formation and stimulating hepatic LDL receptor activity, and were much more active in this regard than their parent compound (α-linoleic acid) or linoleic acid.—**Spady, D. K.** Regulatory effects of individual n-6 and n-3 polyunsaturated fatty acids on LDL transport in the rat. *J. Lipid Res.* 1993. **34:** 1337–1346.

Supplementary key words LDL transport • liver • n-3 polyunsaturated fatty acids

The apparent low incidence of coronary heart disease in populations consuming a diet rich in marine lipids (1, 2) has stimulated considerable interest in the use of these lipids in the prevention and treatment of atherosclerosis (3–7). Fish oil is unique in that it contains large amounts of the long-chain n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). These fatty acids are incorporated into cell membranes and modify prostaglandin and leukotriene formation. As a consequence, they have a variety of biologic activities that include effects on platelet function, inflammation, and plasma lipid levels (3–7). Eicosapentaenoic acid and docosahexaenoic acid in marine lipids are derived ultimately from α-linolenic acid (18:3n-3) present in phytoplankton. α-Linolenic acid (but not eicosapentaenoic acid or docosahexaenoic acid) is also present in some vegetable and seed oils. Isotopic studies indicate that α-linolenic acid can be elongated and desaturated to eicosapentaenoic acid and docosahexaenoic acid in mammalian tissues (8, 9); however, the extent of these conversions appears to be limited in humans and rodents (4, 10, 11).

As to the effects of n-3 PUFAs on plasma lipids, dietary fish oil markedly reduces plasma triglyceride levels in normal and hypertriglyceridemic individuals whereas vegetable oils containing predominantly n-6 PUFAs have little effect (12–15). The effect of dietary fish oil on plasma cholesterol concentrations is much less consistent and appears to depend on a number of factors including study design and underlying lipid phenotype. Fish oil generally does not lower plasma total or low density lipoprotein (LDL) cholesterol concentrations when simply taken as a supplement to a typical Western diet and under these conditions may increase LDL concentrations in hypertri-

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); PUFA, polyunsaturated fatty acid; ACAT, acyl-coenzyme A:cholesterol acyltransferase; J^m, the maximal rate of receptor-dependent LDL uptake; K_m, the concentration of LDL in plasma necessary to achieve one-half of the maximal uptake rate; P, the proportionality constant for receptor-independent LDL uptake; J_t, the rate of total LDL uptake by receptor-dependent and receptor-independent pathways.

glyceridemic subjects (13, 14, 16–18). On the other hand, plasma total and LDL cholesterol concentrations do fall when saturated fat is replaced by fish oil or polyunsaturated vegetable oil and indirect (12, 14, 19–24) and, to a limited extent, direct (25) evidence suggests that the n-3 PUFAs present in fish oil may be more effective (on a g/g basis) than the n-6 PUFAs present in vegetable oils.

Since the risk of developing atherosclerosis and coronary heart disease is strongly correlated with the concentration of LDL in plasma, there is considerable interest in understanding how various dietary lipids influence the concentration of this lipoprotein in plasma. The concentration of LDL in plasma is determined by the rate at which LDL enters the plasma relative to the rate at which LDL is cleared from plasma by the various tissues of the body. LDL are formed in plasma during the metabolism of very low density lipoproteins (VLDL) which, in turn are secreted by the liver (26). Tissues take up LDL from plasma by at least two mechanisms. One of these, termed receptor-dependent transport, involves the interaction of LDL particles with cell surface receptors followed by endocytosis and catabolism of the LDL particle in the lysosomal compartment (27, 28). Tissues also take up LDL by a nonsaturable receptor-independent process that is thought to represent bulk fluid-phase endocytosis (29). In normal animals and humans, receptor-dependent mechanisms account for 70–80% of total LDL turnover (30–32) and the vast majority of receptor-dependent LDL uptake occurs in the liver (33). Thus, changes in plasma LDL concentrations are generally due to alterations in the rate of LDL formation or to alterations in receptor-dependent LDL uptake by the liver.

In the rat, plasma total and LDL cholesterol concentrations fall when dietary saturated triglyceride is replaced by fish oil. We previously found that the decrease in plasma LDL cholesterol concentrations under these conditions was due both to the removal of the saturated triglyceride, which leads to a reduction in the rate of LDL formation, and to addition of the fish oil, which accelerates LDL clearance by the liver (34, 35). However, fish oil is a heterogeneous mixture of n-3 polyunsaturated, monounsaturated, and saturated fatty acids, making it difficult to dissect out the effects of the individual fatty acids present in the oil. Thus, it has not been established in humans or in animal models whether the lipid-lowering effects of fish oil are due to eicosapentaenoic acid, docosahexaenoic acid, or a synergistic interaction of the two. Similarly, there is little information as to the activity of these two long-chain n-3 PUFAs relative to their parent compound, α-linolenic acid. The present studies were therefore undertaken to examine the effects of individual n-3 and n-6 PUFAs on the major transport processes that control plasma LDL concentrations.

METHODS

Animals and diets

Female Sprague-Dawley rats (Sasco Inc., Omaha, NE) were housed in colony cages and subjected to light cycling for at least 3 weeks prior to introduction of the experimental diets. At the time of the experiments, all animals were in the 250–300 g weight range. The control semisynthetic diet used in these studies contained 20% soy protein, 0.3% DL-methionine, 10% cellulose, 8.5% salt mix, 1% vitamin mix, 0.2% choline bitartrate, 2% corn oil, and 58% corn starch. The experimental diets were prepared by replacing corn starch with the desired amount of unesterified fatty acids on a cal/cal basis assuming 4 kcal/g of corn starch and 9 kcal/g of lipid. The fatty acids used in these studies were generously provided by Hidehiko Hibino, senior chemist, Biotechnology Group 2, Nippon Oil and Fats Company, Ltd., Tokyo, Japan, and included linoleic acid, α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. In all preparations the specified fatty acid represented ≥92% (wt %) of total fatty acids. All diets were supplemented with vitamin E (0.05%), BHT (0.02%), and TBHQ (0.02%) and stored in a freezer under a blanket of nitrogen. The diets were fed ad lib on a daily basis for 2 weeks.

Determination of hepatic LDL uptake rates in vivo

Plasma was obtained from normocholesterolemic rat and human donors. The LDL was isolated from plasma by preparative ultracentrifugation in the density range of 1.020–1.055 g/ml and labeled with ^{125}I-labeled or ^{131}I-labeled-tyramine cellobiose as previously described (36). The human LDL was also reductively methylated to completely eliminate its recognition by the LDL receptor (32, 37). Rates of total and receptor-independent LDL uptake were measured using primed-infusions of ^{125}I-labeled-tyramine cellobiose-labeled homologous and methylated human LDL, respectively (34, 38). The infusions of ^{125}I-labeled-tyramine cellobiose-labeled LDL were continued for 6 h at which time each animal was administered a bolus of ^{131}I-labeled-tyramine cellobiose-labeled LDL as a volume marker and killed 10 min later by exsanguination through the abdominal aorta. Samples of the liver along with aliquots of plasma were assayed for radioactivity in a gamma counter (Packard Instrument Co., Inc., Downers Grove, IL). The entire remaining carcass was also homogenized and aliquots were assayed for radioactivity. The amount of labeled LDL in the liver and carcass at 10 min (^{131}I disintegrations per min per g of liver divided by the specific activity of ^{131}I in plasma) and at 6 h (^{125}I disintegrations per min per g of liver divided by the specific activity of ^{125}I in plasma) was then calculated

and has the units of micrograms of LDL cholesterol or LDL protein per g of tissue. The increase in the tissue content of LDL cholesterol or LDL protein with time represents the rate of LDL uptake in micrograms of LDL cholesterol or LDL protein taken up per h per g of tissue or per whole organ (μg/h per g or per organ). Since no tissue was discarded, the rate of LDL uptake by the whole body could be determined. This value was used as a measure of LDL production because in a steady-state the rate of LDL uptake by the whole body must equal the rate of LDL entry into plasma.

As receptor-dependent LDL uptake by the liver is saturable and as plasma LDL concentrations varied considerably among the different experimental groups, changes in receptor-dependent LDL uptake could not be directly equated with changes in LDL receptor activity (33). In order to relate changes in receptor-dependent LDL uptake to changes in LDL receptor activity, the experimentally determined uptake rates were superimposed on kinetic curves describing the relationship between hepatic LDL uptake and circulating LDL concentrations in normal animals. These kinetic curves were established in control rats maintained on a low-fat low-cholesterol diet by measuring rates of total and receptor-independent LDL transport under conditions where plasma LDL concentrations were acutely varied over a wide range by infusing mass amounts of unlabeled LDL. The relationship between total LDL uptake (J_t) and the concentration of LDL in plasma (C) can be described by the equation $J_t = (J^mC)/(K_m + C) + PC$, where J^m equals the maximal uptake velocity by way of the receptor dependent pathway, K_m equals the concentration of LDL in plasma necessary to achieve one-half of this maximal uptake rate, and P equals the proportionality constant for receptor-independent transport. The kinetic curves for normal LDL uptake used in these studies were constructed using previously published values for J^m, K_m, and P (33). By relating the rates of receptor-dependent and receptor-independent LDL uptake in the experimental animals to these normal kinetic curves, it was possible to determine how the various dietary manipulations affected LDL receptor activity (defined as the rate of receptor-dependent LDL uptake in an experimental animal relative to the rate of receptor-dependent LDL uptake seen in control animals at the same plasma LDL concentration).

Determination of the cholesterol distribution of liver and plasma and the fatty acid profile of liver lipids

Hepatic esterified and unesterified cholesterol were separated using silicic acid/celite columns and quantified by capillary gas–liquid chromatography (31). Total plasma cholesterol and triglyceride concentrations were determined using enzymatic kits from Boehringer Diagnostics (Indianapolis, IN) and Sigma (St. Louis, MO), respectively. The cholesterol distribution in plasma was determined by simultaneously centrifuging plasma at densities of 1.020, 1.055, and 1.095 g/ml at 164,905 *g* for 36 h (29). The cholesterol in the top one-third of each tube was determined colorimetrically. Liver lipids were separated by TLC, transesterified (39), and the fatty acid methyl esters were separated by capillary gas chromatography.

RESULTS

Dietary fish oil is known to reduce plasma cholesterol and triglyceride concentrations in the rat. Initial studies were therefore performed to determine the changes in plasma lipid levels when varying amounts of individual n-3 PUFAs were added to the diet. The n-3 PUFAs introduced into the diet replaced carbohydrate on a kcal/kcal basis. Dietary eicosapentaenoic acid and docosahexaenoic acid lowered plasma triglyceride and cholesterol levels in a dose-dependent manner with significant reductions observed when these fatty acids were present in the diet at the 1% (wt/wt) level. At dietary intakes greater than 4% (wt/wt), weight gain in animals ingesting α-linolenic acid or docosahexaenoic acid began to fall below that in the control animals. Thus, all further studies were carried out in animals ingesting PUFAs at the 4% by wt level (~11% of total energy as test fatty acid).

Table 1 shows the effect of linoleic acid and the three n-3 PUFAs on the fatty acid profile of liver triglycerides and phospholipids. As shown in the top panel, liver triglycerides were enriched with the fed fatty acid in all experimental groups. As shown in the bottom panel, dietary linoleic acid had relatively little effect on the fatty acid profile of liver phospholipids when fed at the 4% (wt/wt) level. Dietary α-linolenic acid had little effect on the α-linolenic acid content of liver phospholipids but did increase the proportion of eicosapentaenoic acid (from 0.2 to 3.8%) and 22:5n-3 (from 0.3 to 2.4%). Dietary α-linolenic acid did not increase the docosahexaenoic acid content of liver phospholipids. Dietary eicosapentaenoic acid increased the relative proportion of eicosapentaenoic acid in liver phospholipids by 65-fold (from 0.2 to 13%) and also increased 22:5n-3 by 26-fold (from 0.3 to 7.8%) but again had no effect on docosahexaenoic acid. Thus, neither dietary α-linolenic acid nor eicosapentaenoic acid (the two major precursors of docosahexaenoic acid) led to an accumulation of docosahexaenoic acid in liver lipids. Dietary docosahexaenoic acid increased the relative proportion of docosahexaenoic acid in liver phospholipids by 3.3-fold (from 7 to 23%); the proportion of eicosapentaenoic acid in liver lipids also increased (from 0.2 to 4.4%) suggesting some retroconversion of docosahexaenoic acid to eicosapentaenoic acid. In control animals, n-3 polyunsaturated fatty acids represented 7.5% of total fatty acids in liver phospholipids; this value increased to

TABLE 1. Effect of individual polyunsaturated fatty acids on the fatty acid profile of liver triglycerides and phospholipids

Diet	Fatty Acid (wt%)								
	16:0	18:0	18:1	18:2	18:3 n-3	20:4	20:5 n-3	22:5 n-3	22:6 n-3
					Triglycerides				
Control	36 ± 2	4 ± 1	29 ± 3	22 ± 1	1 ± 0.1	3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	1.2 ± 0.2
4% 18:2	32 ± 2	3 ± 1	21 ± 2[a]	35 ± 3[a]	1 ± 0.2	4 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 0.2
4% 18:3 n-3	34 ± 3	3 ± 1	34 ± 3	16 ± 2[a]	6 ± 0.4[a]	1 ± 0.2[a]	1.0 ± 0.2[a]	0.3 ± 0.2	0.7 ± 0.1[a]
4% 20:5 n-3	27 ± 2[a]	3 ± 1	24 ± 1[a]	22 ± 2	1 ± 0.1	1 ± 0.3[a]	14 ± 1[a]	3.8 ± 1[a]	1.0 ± 0.1
4% 22:6 n-3	23 ± 3[a]	3 ± 1	20 ± 1[a]	20 ± 1	1 ± 0.1	1 ± 0.2[a]	3.4 ± 0.6[a]	1.8 ± 0.1[a]	24 ± 2[a]
					Phospholipids				
Control	18 ± 1	33 ± 2	5 ± 1	10 ± 1	0.1 ± 0.1	24 ± 1	0.2 ± 0.1	0.3 ± 0.1	7 ± 1
4% 18:2	18 ± 2	31 ± 3	4 ± 1	13 ± 1[a]	0.1 ± 0.1	25 ± 2	0.1 ± 0.1	0.3 ± 0.1	6 ± 1
4% 18:3 n-3	17 ± 1	33 ± 3	6 ± 1	11 ± 2	0.3 ± 0.4	17 ± 3[a]	3.8 ± 0.2[a]	2.4 ± 0.2[a]	7 ± 1
4% 20:5 n-3	17 ± 2	29 ± 3	5 ± 1	9 ± 2	0.1 ± 0.1	11 ± 1[a]	13 ± 1[a]	7.8 ± 1[a]	6 ± 1
4% 22:6 n-3	17 ± 1	30 ± 1	4 ± 1	8 ± 1	0.1 ± 0.1	10 ± 1[a]	4.4 ± 0.6[a]	0.4 ± 0.1	23 ± 2[a]

Groups of animals were fed a semisynthetic diet supplemented with individual polyunsaturated fatty acids at the 4% (wt/wt) level for 2 weeks. Each value represents the mean ± 1 SD for data obtained in six animals.
[a]Significantly different from the control group at $P < 0.01$.

13%, 27%, and 28% in animals fed α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively.

The effect of the four PUFAs on plasma triglyceride and cholesterol concentrations is shown in **Table 2.** Dietary linoleic and α-linolenic acids did not significantly lower plasma triglyceride and cholesterol levels. In contrast, dietary eicosapentaenoic and docosahexaenoic acids lowered plasma triglyceride levels by 62% and 52%, respectively, and lowered plasma cholesterol concentrations by 54% and 43%, respectively. As also shown in Table 2, the greatest reductions in total plasma cholesterol were in the d < 1.095 g/ml fractions. Thus, dietary eicosapentaenoic acid and docosahexaenoic acid markedly reduced the amount of cholesterol carried in the d < 1.020 g/ml fraction (90% and 74% reductions, respectively), d 1.020–1.055 g/ml fraction (64% and 55% reductions, respectively), and d 1.055–1.095 g/ml fraction (71% and 57% reductions, respectively), but had relatively less effect on the amount of cholesterol carried in the d > 1.095 g/ml fraction (40% and 31% reductions, respectively). In the rat, cholesterol in the d 1.055–1.095 g/ml fraction is carried largely in apolipoprotein E-containing HDL_1 particles. Thus, although eicosapentaenoic acid and docosahexaenoic acid reduced the cholesterol content of all lipoprotein fractions, the greatest reductions were in lipoproteins that, because of their content of apolipoprotein B-100 and/or apolipoprotein E, are cleared from plasma via the LDL receptor pathway. Although not shown, the cholesterol to protein ratio in plasma LDL (d 1.020–1.055 g/ml) was reduced by 7% in animals fed eicosapentaenoic acid and 5% in animals fed docosahexaenoic acid, indicating that the changes in plasma LDL cholesterol levels were due predominantly to changes in the concentration of LDL particles in plasma.

Studies were next undertaken to determine the effect of the four PUFAs on the major transport processes that determine the concentration of LDL in plasma. Absolute

TABLE 2. Effect of individual polyunsaturated fatty acids on plasma cholesterol and triglyceride concentrations

Diet	Plasma Triglyceride	Plasma Cholesterol Concentration				
		Total	Density Fraction (g/ml)			
			<1.020	1.020–1.055	1.055–1.095	>1.095
				mg/dl		
Control	52 ± 7	65 ± 7	5 ± 1	11 ± 1	14 ± 1	35 ± 2
4% 18:2	50 ± 6	61 ± 6	5 ± 1	10 ± 1	13 ± 1	33 ± 2
4% 18:3 n-3	48 ± 5	55 ± 7	5 ± 2	9 ± 2	12 ± 2	29 ± 3[a]
4% 20:5 n-3	20 ± 4[a]	30 ± 3[a]	0.5 ± 0.2[a]	4 ± 1[a]	4 ± 1[a]	21 ± 3[a]
4% 22:6 n-3	25 ± 3[a]	37 ± 2[a]	1.3 ± 0.3[a]	5 ± 1[a]	6 ± 1[a]	24 ± 2[a]

Groups of animals were fed a semisynthetic diet supplemented with individual polyunsaturated fatty acids at the 4% (wt/wt) level for 2 weeks. Each value represents the mean ± 1 SD for data obtained in six animals.
[a]Significantly different from the control group at $P < 0.01$.

rates of total and receptor-independent LDL uptake were measured in vivo using homologous and methylated human LDL, respectively. Receptor-dependent LDL uptake was taken as the difference between total and receptor-independent uptake. As receptor-dependent LDL uptake is saturable and as plasma LDL levels varied considerably among the different experimental groups, changes in absolute rates of LDL uptake could not be equated directly with changes in LDL receptor activity. To relate the changes in absolute rates of LDL uptake to changes in receptor activity, the values for total and receptor-independent LDL uptake determined in the experimental animals were superimposed on the kinetic curves that define the relationship between LDL uptake and circulating LDL concentrations in control animals (33, 34).

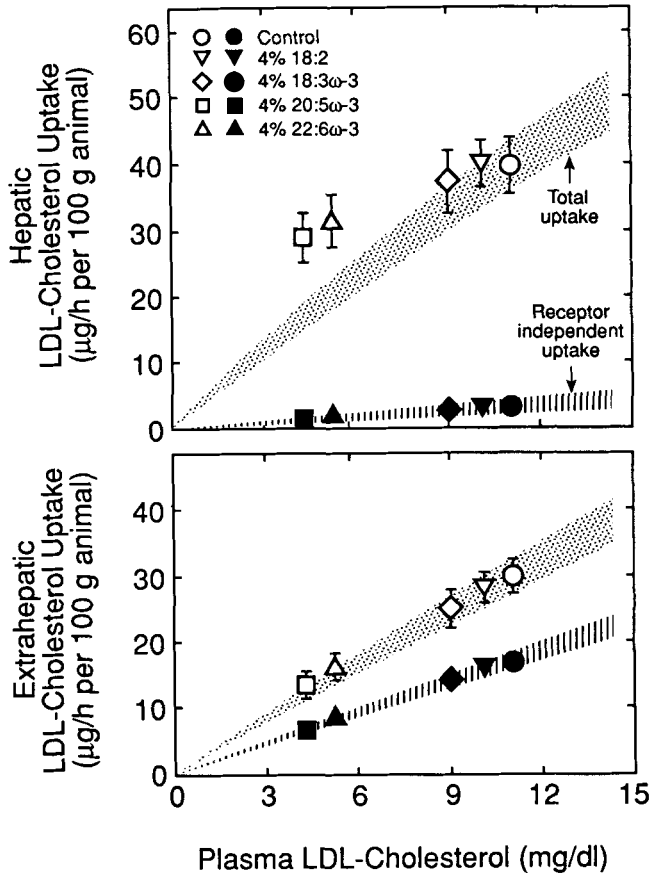

Fig. 1. The effect of dietary linoleic acid, α-linolenic acid, eicosapentaenoic acid, or docosahexaenoic acid on LDL-cholesterol uptake in the whole liver (top panel) and all of the extrahepatic tissues combined (bottom panel). Animals were fed a semisynthetic diet supplemented with individual PUFAs at the 4% (wt/wt) level for 2 weeks. The shaded areas represent the kinetic curves for total (stippled) and receptor-independent (hatched) LDL-cholesterol uptake in control animals. These curves were calculated as described in Methods. The individual points superimposed on these kinetic curves show the rates of total and receptor-independent LDL cholesterol uptake in the experimental animals plotted as a function of the plasma LDL cholesterol concentrations in the same animals. Each point represents the mean ± 1 SD for data obtained in six animals.

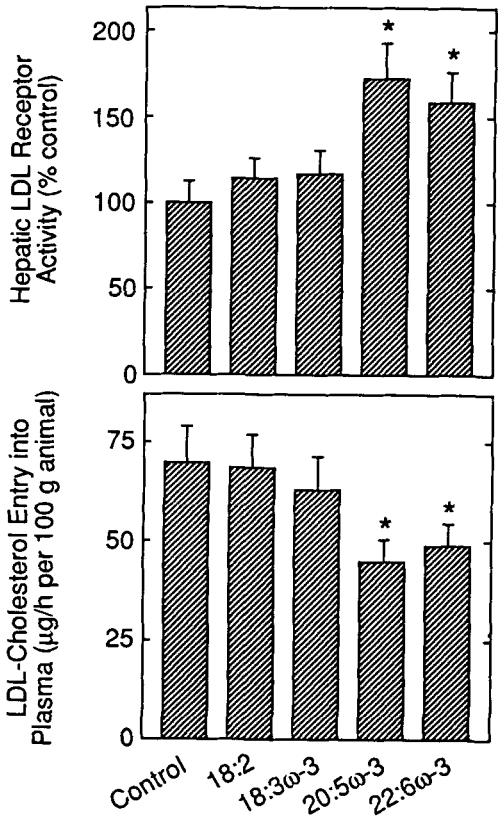

Fig. 2. The effect of dietary linoleic acid, α-linolenic acid, eicosapentaenoic acid, or docosahexaenoic acid on hepatic LDL receptor activity (top panel) and the rate of LDL cholesterol entry into plasma (bottom panel). Animals were fed a semisynthetic diet supplemented with individual PUFAs as described in the legend to Fig. 1. Values for hepatic LDL receptor activity represent the rates of receptor-dependent LDL cholesterol uptake in experimental animals as percentages of the rates of receptor-dependent LDL cholesterol uptake that would occur in control animals at the same LDL cholesterol concentration (as illustrated in Fig. 1). Each value represents the mean ± 1 SD for data obtained in six animals; $^*P < 0.01$.

Fig. 1 shows the kinetic curves for normal LDL transport in the liver and extrahepatic tissues of control rats. The shaded areas represent the relationship between total (stippled) and receptor-independent (hatched) LDL uptake and plasma LDL concentrations over the range of LDL concentrations observed in these studies. The rates of total and receptor-independent uptake measured in the experimental animals have been superimposed on these normal kinetic curves. As shown in the top panel, rates of total and receptor-independent LDL uptake in the control animals used in these studies equaled 39 and 3 μg/h per 100 g body wt, respectively, at a plasma LDL cholesterol concentration of 11 mg/dl. These values fall on the normal kinetic curves previously established for LDL transport in the liver (33). In animals fed linoleic acid, rates of total and receptor-independent LDL cholesterol uptake equaled 40 and 3 μg/h per 100 g body wt, respectively, at a plasma LDL cholesterol concentration of 10 mg/dl. These values were not displaced significantly

from the normal kinetic curves indicating that neither receptor-dependent nor receptor-independent transport was significantly altered by this fatty acid. In contrast, total hepatic LDL cholesterol uptake in animals fed eicosapentaenoic acid equaled 29 μg/h per 100 g body wt at a plasma LDL cholesterol concentration of 4.5 mg/dl whereas normal animals transport only ~17 μg/h per 100 g body wt at this LDL cholesterol concentration. As receptor-independent LDL uptake was normal in these animals, the increase in total LDL cholesterol uptake could be attributed entirely to an increase in receptor-dependent LDL uptake. Similarly, the rate of receptor-dependent LDL uptake in animals fed docosahexaenoic acid was significantly higher than in control animals at the same LDL cholesterol concentration. As shown in the bottom panel of Fig. 1, rates of total and receptor-independent LDL uptake in the extrahepatic tissues were not significantly displaced from the normal kinetic curves by any of the experimental diets.

From the type of analysis illustrated in Fig. 1, the changes in absolute rates of total and receptor-independent LDL uptake could be converted to changes in hepatic LDL receptor activity (defined as the rate of receptor-dependent LDL uptake in experimental animals relative to the rate of receptor-dependent uptake that would be seen in control animals at the same LDL cholesterol concentration). In addition, rates of LDL cholesterol entry into plasma could be calculated because, in a steady-state, the rate of LDL entry into plasma equals the whole body LDL uptake rate (the sum of the uptake rates in the liver and extrahepatic tissues shown in Fig. 1). The effect of the four PUFAs on hepatic LDL receptor activity and the rate of LDL cholesterol entry into plasma are shown in **Fig. 2.** Dietary linoleic acid and α-linolenic acid did not significantly alter hepatic LDL receptor activity or the rate of LDL cholesterol entry into plasma. In contrast to linoleic and α-linolenic acids, the long-chain PUFAs characteristic of marine lipids produced much greater changes in hepatic LDL receptor activity and LDL formation. Thus, dietary eicosapentaenoic acid and docosahexaenoic acid increased hepatic LDL receptor activity by 72% and 58%, respectively, and reduced the rate of LDL cholesterol entry into plasma by 36% and 30%, respectively. These changes in hepatic LDL receptor activity and LDL cholesterol entry into plasma accounted almost entirely for the marked reductions in plasma LDL cholesterol concentrations shown in Table 2.

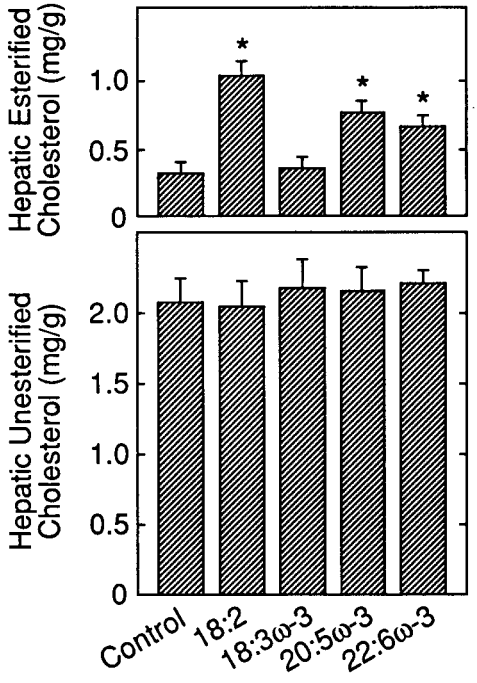

Fig. 3. The effect of dietary linoleic acid, α-linolenic acid, eicosapentaenoic acid, or docosahexaenoic acid on hepatic esterified (top panel) and unesterified (bottom panel) cholesterol levels. Animals were fed a semisynthetic diet supplemented with individual PUFAs as described in the legend to Fig. 1. Each value represents the mean ± 1 SD for data obtained in six animals; $^*P < 0.01$.

The effect of the four PUFAs on the cholesterol content of the liver is shown in **Fig. 3.** As shown in the top panel, hepatic cholesteryl ester levels increased ~3-fold in animals fed linoleic acid and ~2-fold in animals fed eicosapentaenoic acid or docosahexaenoic acid. Dietary α-linolenic acid did not significantly alter the cholesteryl ester content of the liver. As shown in the bottom panel, none of the dietary PUFAs significantly altered the unesterified cholesterol content of the liver.

Finally, unesterified fatty acids were used in all of the experiments described above; however, the same experiments were also carried out using trilinolein, trilinolenin, trieicosapentaenoin, and tridocosahexaenoin. Although the effects of the four PUFAs were similar whether fed as the unesterified fatty acid or the triglyceride, unesterified eicosapentaenoic and docosahexaenoic acids appeared to be slightly more active than the corresponding triglycerides at lowering plasma cholesterol and triglyceride concentrations.

DISCUSSION

These studies were undertaken to evaluate the independent effects of individual n-3 and n-6 PUFAs on serum lipid levels and LDL transport in the rat. When substituted for carbohydrate in a cholesterol-free semisynthetic diet, eicosapentaenoic acid and docosahexaenoic acid markedly lowered plasma triglyceride and cholesterol concentrations whereas the precursor of these two fatty acids, α-linolenic acid, and linoleic acid had relatively little effect. These changes in plasma lipid levels are similar to those previously observed by Worne and Smith (25) in normal human subjects. In their studies 4 g per day of 22:5 n-3 or docosahexaenoate lowered plasma cholesterol concentrations by ~100 mg/dl (~33% reduction)

whereas the same amount of α-linolenate or linoleate had little effect. While few studies have directly evaluated the effects of individual n-3 PUFAs on plasma lipid levels, comparisons of the effects of dietary fish oil and α-linolenate-rich seed oils also suggest that the n-3 PUFAs present in marine lipids are more active than α-linolenic acid at lowering plasma cholesterol and triglyceride concentrations (40, 41).

In the present studies dietary α-linolenic acid did not increase the proportion of α-linolenic acid in liver phospholipids. Moreover, dietary α-linolenic acid only modestly increased the proportion of eicosapentaenoic acid and 22:5-3 and had no effect on docosahexaenoic acid in liver phospholipids. These findings are consistent with previous observations in humans and rats suggesting that desaturation and elongation of α-linolenic acid is quite limited (4, 10, 41, 42). As to the fate of dietary α-linolenic acid, it is clearly well absorbed and is found in triglycerides in the liver and adipose tissue but appears to be preferentially oxidized rather than incorporated into membrane phospholipids (43). In any event, the lipid-lowering activity of individual n-3 fatty acids correlated well with their ability to increase total n-3 PUFAs in liver phospholipids. Thus, dietary α-linolenic acid modestly increased the total n-3 PUFA content of liver phospholipids and had little effect on plasma triglyceride and cholesterol levels. In contrast, dietary eicosapentaenoic acid and docosahexaenoic acid increased the total n-3 PUFA content of liver phospholipids by 3.6-fold and 3.7-fold, respectively, and markedly lowered plasma triglyceride and cholesterol concentrations.

While there is little doubt that long-chain n-3 PUFAs are the major biologically active constituents of fish oil, it is not entirely clear whether eicosapentaenoic acid, docosahexaenoic acid, or a synergistic action of the two is responsible for the effects of fish oil on plasma lipoproteins. In a study in normolipidemic men it was found that plasma triglycerides were markedly lowered by marine lipids enriched either in eicosapentaenoic acid or docosahexaenoic acid, whereas plasma LDL concentrations were reduced only by the docosahexaenoic acid-enriched lipid (44). Data from another study in rats also suggested that dietary eicosapentaenoic acid and docosahexaenoic acid may have differential effects on plasma cholesterol and triglyceride levels (45). Although eicosapentaenoic acid and docosahexaenoic acid clearly differed from α-linolenic acid, we could find no evidence for a differential effect of docosahexaenoic acid and eicosapentaenoic acid on plasma cholesterol or triglyceride levels or on the transport of LDL. This is probably not due to rapid interconversion between the two fatty acids as feeding eicosapentaenoic acid did not increase docosahexaenoic acid in liver lipids and feeding docosahexaenoic acid primarily increased docosahexaenoic acid. Whether eicosapentaenoic acid and docosahexaenoic acid interact synergistically to alter plasma lipoprotein metabolism is not known.

Eicosapentaenoic acid and docosahexaenoic acid lowered plasma LDL cholesterol levels by more than 50%. As there was little change in the cholesterol to protein ratio of LDL, the decrease in plasma LDL cholesterol levels was due predominantly to a decrease in the number of LDL particles. The concentration of LDL in plasma is determined by the rate at which LDL are formed in plasma relative to the rate at which these particles are removed from plasma by receptor-dependent and receptor-independent transport processes in the various organs of the body. Approximately 70-80% of total LDL turnover is mediated by LDL receptors (30-32), the vast majority of which are located in the liver (33). Thus, changes in circulating LDL concentrations are generally due to changes in the rate of LDL formation or to changes in receptor-dependent LDL catabolism by the liver. We previously found that the addition of saturated fatty acids to a low cholesterol diet raised plasma LDL concentrations by increasing the rate of LDL production whereas fish oil lowered plasma LDL levels by enhancing hepatic LDL receptor activity (34). Fish oil contains a heterogeneous mixture of fatty acids (including n-3 PUFAs and various chain-length monounsaturated and saturated fatty acids). Some of these fatty acids have unknown effects on LDL transport or may be involved in complex interactions (35) making it difficult to predict the independent effects of individual n-3 fatty acids. In the present studies dietary eicosapentaenoic acid and docosahexaenoic acid increased hepatic LDL receptor activity as did fish oil in the previous studies (34). In addition, however, dietary eicosapentaenoic acid and docosahexaenoic acid also significantly reduced the rate of LDL cholesterol entry into plasma, leading to marked reductions in plasma LDL cholesterol concentrations of 64% and 55%, respectively. The failure of fish oil to reduce the rate of LDL formation in previous studies (34) is probably due to the fact that fish oil contains both n-3 PUFAs, which reduce the rate of LDL entry into plasma, and saturated fatty acids, which increase the rate of LDL entry into plasma. Together these two effects apparently result in an intermediate rate of LDL formation that is not different from that observed in the absence of either fatty acid, i.e., with carbohydrate.

As saturated and n-3 polyunsaturated fatty acids have opposite effects on the rate of LDL entry into plasma, the replacement of one by the other in the diet can markedly alter the rate of LDL formation as previously reported (23). The mechanism whereby n-3 fatty acids reduce the rate of LDL formation is not known. LDL arise in plasma during the metabolism of VLDL (26). Thus, a decrease in the rate of LDL entry into plasma may result from a reduction in the number of VLDL particles secreted by the liver or to a decrease in the proportion of VLDL that is metabolized to LDL. Dietary eicosapentaenoic acid and docosahexaenoic acid markedly lowered VLDL cholesterol and triglycerides in these studies. This appears to be due to the secretion of VLDL particles that are

depleted of cholesterol and triglycerides (46, 47) and possibly to a reduction in the number of VLDL particles secreted by the liver (48). In addition, however, n-3 PUFAs appear to enhance VLDL turnover (48, 49). VLDL are converted to VLDL remnants which may be rapidly cleared by LDL receptors in the liver or further metabolized to LDL. Thus, an increase in hepatic LDL receptor activity may, in addition to increasing the clearance of LDL from plasma, also accelerate the uptake of VLDL remnants by the liver, allowing fewer of these particles to be converted to LDL and thereby reducing the rate of LDL formation.

The mechanism whereby n-3 fatty acids increase receptor-dependent LDL uptake by the liver is unknown. Roach et al. (50) reported that fish oil reduced plasma LDL levels in the rat but did not increase the binding of LDL to solubilized liver membranes. Similarly, in preliminary studies in the rat, we found that fish oil increased receptor-dependent LDL uptake by the liver but did not increase hepatic LDL receptor protein or mRNA levels (J. D. Horton, J. A. Cuthbert, and D. K. Spady, unpublished results). An increase in receptor-dependent LDL uptake with no corresponding change in the number of LDL receptors suggests an alteration in receptor function. Thus, it is possible that n-3 fatty acids alter the affinity of LDL receptors for the LDL particle (51) or alter the distribution or recycling rate of LDL receptors in the hepatocyte (52, 53).

In these studies hepatic cholesteryl ester levels increased by about 2-fold in animals fed eicosapentaenoic acid or docosahexaenoic acid and by about 3-fold in animals fed linoleic acid. Dietary fatty acids are known to alter hepatic acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity and cholesteryl ester levels and in many cases these effects appear to be independent of changes in sterol balance across the liver. Dietary fatty acid-induced changes in liver cholesteryl ester levels are accentuated when cholesterol is included in the diet. Studies in rats fed 20% triglyceride and varying amounts of cholesterol showed that hepatic cholesteryl ester levels are ordered as follows: olive oil (mainly oleic acid) > safflower oil (mainly linoleic acid) > fish oil concentrate ≥ hydrogenated coconut oil (D. K. Spady, unpublished observation). These findings are consistent with studies in cultured rat hepatocytes where eicosapentaenoic acid and docosahexaenoic acid were found to promote lower levels of cholesterol esterification than oleic acid (54). Similarly, hepatic microsomal ACAT activity was found to be higher in rats fed fish oil (55) or sunflower seed oil (56) than in animals fed saturated triglycerides. How dietary fatty acids regulate cholesterol esterification has not been fully established. However, various fatty acids appear to differ substantially in their ability to serve as a substrate for the ACAT reaction (54, 57). In addition, by modifying the fatty acid composition of microsomal phospholipids, dietary fatty acids may alter the microenvironment of membrane-associated enzymes and thereby influence enzyme activity (54-56). In the present studies there was no obvious correlation between cholesterol levels (esterified or unesterified) and receptor-dependent LDL uptake by the liver making it unlikely that changes in receptor-dependent uptake were triggered by changes in the free or esterified cholesterol content of the liver.

Although dietary n-3 PUFAs uniformly lower serum triglyceride levels in humans, studies dealing with the effects of marine lipids on serum total and LDL cholesterol levels have often yielded inconsistent and, at times, seemingly contradictory results. A number of factors may contribute to the reported variability in responsiveness to dietary n-3 PUFAs including differences in study design, differences in the underlying lipid phenotype of experimental subjects, and differences in the quantity and quality of the marine lipids used. With respect to study design, plasma total and LDL cholesterol levels consistently fall in normal individuals when large amounts of saturated triglyceride are replaced by marine lipids (12, 15, 19-23). However, from a more practical standpoint, plasma LDL levels generally do not fall when modest amounts of marine lipids are taken as a supplement by individuals consuming a Western diet. Moreover, the hypotriglyceridemic effect of fish oil supplements is frequently accompanied by a significant rise in plasma LDL concentrations in subjects with primary hypertriglyceridemia or familial combined hyperlipidemia (16-18, 58). The failure of fish oil supplements to lower plasma LDL concentrations raises serious doubts about the potential usefulness of these supplements in the prevention and treatment of atherosclerotic complications; however, studies in animals fed atherogenic diets have shown that fish oil supplements can markedly inhibit the development of atherosclerosis despite minimal or even unfavorable effects on plasma LDL and HDL concentrations (59-61). Finally, information regarding the effects of dietary n-3 PUFAs has been derived largely from studies using fish oil, which, in addition to n-3 PUFAs may contain considerable amounts of saturated fatty acids and cholesterol. The few studies that have examined the effects of purified n-3 polyunsaturated fatty acids or esters on plasma cholesterol and triglyceride levels suggest that supplements of these purified compounds may significantly lower cholesterol levels in normal and hypercholesterolemic individuals (25).

The response to dietary marine lipids also appears to vary considerably among different animal species. As in humans, plasma triglyceride and cholesterol concentrations fall in species such as the rat (34) and pig (62) when dietary saturated triglyceride is replaced by fish oil. Plasma cholesterol concentrations also fall in rhesus (63) and African green monkeys (47) when dietary saturated triglyceride is replaced by fish oil; however, unlike the situation in humans, plasma triglyceride levels remain unchanged (63) or

even rise (47). Finally, dietary fish oil tends to raise plasma triglyceride and cholesterol levels in species such as the hamster (64) and cynomolgus monkey (65). For example, addition of crude fish oil to a low fat, low cholesterol diet markedly increases plasma cholesterol and triglyceride concentrations in the hamster. Much of this increase is apparently due to the cholesterol and saturated fatty acid content of crude fish oil; nevertheless, even purified n-3 PUFAs fail to lower plasma triglyceride or LDL cholesterol concentrations in this species (D. K. Spady, unpublished observation). An examination of the mechanisms underlying these species differences in response to dietary n-3 fatty acids may provide insights into how these fatty acids influence plasma lipoprotein levels.

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REFERENCES

1. Kromann, N., and A. Green. 1980. Epidemiological studies in the Upernavik District, Greenland. *Acta Med. Scand.* **208:** 401–406.
2. Kromhout, D., E. B. Bosschieter, and C. D. L. Coulander. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N. Engl. J. Med.* **312:** 1205–1209.
3. Goodnight, S. H., Jr., W. S. Harris, W. E. Connor, and D. R. Illingworth. 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis.* **2:** 87–113.
4. Dyerberg, J. 1986. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr. Rev.* **44:** 125–134.
5. Von Schacky, C. 1987. Prophylaxis of atherosclerosis with marine omega-3 fatty acids. *Ann. Int. Med.* **107:** 890–899.
6. Leaf, A., and P. C. Weber. 1988. Cardiovascular effects of n-3 fatty acids. *N. Engl. J. Med.* **318:** 549–557.
7. Kinsella, J. E., B. Lokesh, and R. A. Stone. 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am. J. Clin. Nutr.* **52:** 1–28.
8. Poovaiah, B. P., J. Tinoco, and R. L. Lyman. 1976. Influence of diet on conversion of $^{14}C_1$-linolenic acid to docosahexaenoic acid in the rat. *Lipids.* **11:** 194–202.
9. Yamazaki, K., M. Fujikawa, T., Hamazaki, S. Yano, and T. Shono. 1992. Comparison of the conversion rates of α-linolenic acid (18:3(n-3)) and stearidonic acid (18:4(n-3)) to longer polyunsaturated fatty acids in rats. *Biochim. Biophys. Acta.* **1123:** 18–26.
10. Sanders, T. A. B., and K. M. Younger. 1981. The effect of dietary supplements of ω3 polyunsaturated fatty acids on the fatty acid composition of platelets and plasma choline phosphoglycerides. *Br. J. Nutr.* **45:** 613–616.
11. Garg, M. L., E. Sebokova, A. Wierzbicki, A. B. R. Thomson, and M. T. Clandinin. 1988. Differential effects of dietary linoleic and α-linolenic acid on lipid metabolism in rat tissues. *Lipids.* **23:** 847–852.
12. Harris, W. S., W. E. Connor, and M. P. McMurry. 1983. The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. *Metabolism.* **32:** 179–184.
13. Phillipson, B. E., D. W. Rothrock, W. E. Connor, W. S. Harris, and D. R. Illingworth. 1985. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N. Engl. J. Med.* **312:** 1210–1216.
14. Connor, W. E. 1988. Effects of omega-3 fatty acids in hypertriglyceridemic states. *Semin. Thromb. Hemostasis.* **14:** 271–284.
15. Harris, W. S. 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *J. Lipid Res.* **30:** 785–807.
16. Harris, W. S., C. A. Dujovne, M. Zucker, and B. Johnson. 1988. Effects of a low saturated fat, low cholesterol fish oil supplement in hypertriglyceridemic patients. A placebo-controlled trial. *Ann. Int. Med.* **109:** 465–470.
17. Failor, R. A., M. T. Childs, and E. L. Bierman. 1988. The effects of ω3 and ω6 fatty acid-enriched diets on plasma lipoproteins and apoproteins in familial combined hyperlipidemia. *Metabolism.* **37:** 1021–1028.
18. Nozaki, S., A. Garg, G. L. Vega, and S. M. Grundy. 1991. Postheparin lipolytic activity and plasma lipoprotein response to ω-3 polyunsaturated fatty acids in patients with primary hypertriglyceridemia. *Am. J. Clin. Nutr.* **53:** 638–642.
19. Keys, A., J. T. Anderson, and F. Grande. 1957. "Essential" fatty acids, degree of unsaturation, and effect of corn (maize) oil on the serum-cholesterol level in man. *Lancet.* **ii:** 66–68.
20. Ahrens, E. H., Jr., W. Insull, Jr., J. Hirsch, W. Stoffel, M. L. Peterson, J. W. Farquhar, T. Miller, and H. J. Thomasson. 1959. The effect on human serum-lipids of a dietary fat, highly unsaturated, but poor in essential fatty acids. *Lancet.* **i:** 115–119.
21. Kinsell, L. W., G. D. Michaels, G. Walker, and R. E. Visintine. 1961. The effect of a fish-oil fraction on plasma lipids. *Diabetes.* **10:** 316–319.
22. Kingsbury, K. J., D. M. Morgan, C. Aylott, and R. Emmerson. 1961. Effects of ethyl arachidonate, cod-liver oil, and corn oil on the plasma-cholesterol level. A comparison in normal volunteers. *Lancet.* **i:** 739–744.
23. Illingworth, D. R., W. S. Harris, and W. E. Connor. 1984. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. *Arteriosclerosis.* **4:** 270–275.
24. Nestel, P. J. 1986. Fish oil attenuates the cholesterol-induced rise in lipoprotein cholesterol. *Am. J. Clin. Nutr.* **43:** 752–757.
25. Worne, H. E., and L. W. Smith. 1959. Effects of certain pure long chain polyunsaturated fatty acid esters on the blood lipids of man. *Am. J. Med. Sci.* **237:** 72/710–83/721.
26. Havel, R. J. 1984. The formation of LDL: mechanisms and regulation. *J. Lipid Res.* **25:** 1570–1576.
27. Goldstein, J. L., R. G. W. Anderson, and M. S. Brown. 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature.* **21:** 679–685.
28. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
29. Scharschmidt, B. F., J. R. Lake, E. L. Renner, V. Licko, and R. W. Van Dyke. 1986. Fluid phase endocytosis by cultured rat hepatocytes and perfused rat liver: implications for plasma membrane turnover and vesicular trafficking of fluid phase markers. *Proc. Natl. Acad. Sci. USA.* **83:** 9488–9492.
30. Steinbrecher, U. P., J. L. Witztum, Y. A. Kesaniemi, and R. L. Elam. 1983. Comparison of glucosylated low density lipoprotein with methylated or cyclohexanedione-treated low density lipoprotein in the measurement of receptor-independent low density lipoprotein catabolism. *J. Clin. Invest.* **71:** 960–964.
31. Bilheimer, D. W., Y. Watanabe, and T. Kita. 1982. Impaired receptor-mediated catabolism of low density lipoprotein in the WHHL rabbit, an animal model of familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **79:** 3305–3309.
32. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Receptor-

independent low density lipoprotein transport in the rat in vivo. Quantitation, characterization, and metabolic consequences. *J. Clin. Invest.* **76:** 1113–1122.
33. Spady, D. K., J. B. Meddings, and J. M. Dietschy. 1986. Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. *J. Clin. Invest.* **77:** 1474–1481.
34. Ventura, M. A., L. A. Woollett, and D. K. Spady. 1989. Fish oil stimulates hepatic LDL transport in the rat. *J. Clin. Invest.* **84:** 528–537.
35. Spady, D. K., and L. A. Woollett. 1990. Interaction of dietary saturated and polyunsaturated triglycerides in regulating the processes that determine plasma low density lipoprotein concentrations in the rat. *J. Lipid Res.* **31:** 1809–1819.
36. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **258:** 7161–7167.
37. Mahley, R. W., K. H. Weisgraber, T. L. Melchior, T. L. Innerarity, and K. S. Holcombe. 1980. Inhibition of receptor-mediated clearance of lysine and arginine-modified lipoproteins from the plasma of rats and monkeys. *Proc. Natl. Acad. Sci. USA.* **77:** 225–229.
38. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
39. Lepage, G., and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27:** 114–119.
40. Sanders, T. A. B., and F. Roshanai. 1983. The influence of different types of ω3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clin. Sci.* **64:** 91–99.
41. Choi, Y-S., S. Goto, I. Ikeda, and M. Sugano. 1989. Effect of dietary n-3 polyunsaturated fatty acids on cholesterol synthesis and degradation in rats of different ages. *Lipids.* **24:** 45–50.
42. Garg, M. L., A. B. R. Thomson, and M. T. Clandinin. 1988. Effect of dietary cholesterol and/or ω3 fatty acids on lipid composition and 5-desaturase activity of rat liver microsomes. *Lipids.* **118:** 661–668.
43. Leyton, J., P. J. Drury, and M. A. Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* **57:** 383–393.
44. Childs, M. T., I. B. King, and W. K. Yamanaka. 1988. Divergent lipoprotein lipid responses to fish oils enriched in EPA or DHA. *FASEB J.* **2:** A424.
45. Kobatake, Y., K. Kuroda, H. Jinnouchi, E. Nishide, and S. Innami. 1984. Differential effects of dietary eicosapentaenoic and docosahexaenoic fatty acids on lowering of triglyceride and cholesterol levels in the serum of rats on hypercholesterolemic diet. *J. Nutr. Sci. Vitaminol.* **30:** 357–372.
46. Nossen, J. O., A. C. Rustan, S. H. Gloppestad, S. Malbakken, and C. A. Drevon. 1986. Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. *Biochim. Biophys. Acta.* **879:** 56–65.
47. Parks, J. S., M. D. Wilson, F. L. Johnson, and L. L. Rudel. 1989. Fish oil decreases hepatic cholesteryl ester secretion but not apoB secretion in African green monkeys. *J. Lipid Res.* **30:** 1535–1544.
48. Nestel, P. J., W. E. Connor, M. F. Reardon, S. Connor, S. Wong, and R. Boston. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin. Invest.* **74:** 82–89.
49. Harris, W. S., W. E. Connor, D. R. Illingworth, D. W. Rothrock, and D. M. Foster. 1990. Effects of fish oil on VLDL triglyceride kinetics in humans. *J. Lipid Res.* **31:** 1549–1558.
50. Roach, P. D., A. M. Kambouris, R. P. Trimble, D. L. Topping, and P. J. Nestel. 1987. The effects of dietary fish oil on hepatic high density and low density lipoprotein receptor activities in the rat. *FEBS Lett.* **222:** 159–162.
51. Tripodi, A., P. Loria, M. A. Dilengite, and N. Carulli. 1991. Effect of fish oil and coconut oil diet on the LDL receptor activity of rat liver plasma membranes. *Biochim. Biophys. Acta.* **1083:** 298–304.
52. Havel, R. J., and R. L. Hamilton. 1988. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. *Hepatology.* **8:** 1689–1704.
53. Kuo, P., M. Weinfeld, and J. Loscalzo. 1990. Effect of membrane fatty acyl composition on LDL metabolism in HepG2 hepatocytes. *Biochemistry.* **29:** 6626–6632.
54. Rustan, A. C., J. O. Nossen, H. Osmundsen, and C. A. Drevon. 1988. Eicosapentaenoic acid inhibits cholesterol esterification in cultured parenchymal cells and isolated microsomes from rat liver. *J. Biol. Chem.* **17:** 8126–8132.
55. Johnson, M. R., S. N. Mathur, C. Coffman, and A. A. Spector. 1983. Dietary fat saturation and hepatic acylcoenzyme A:cholesterol acyltransferase activity. *Arteriosclerosis.* **3:** 242–248.
56. Spector, A. A., T. L. Kaduce, and R. W. Dane. 1980. Effect of dietary fat saturation on acyl-coenzyme A:cholesterol acyltransferase activity of rat liver microsomes. *J. Lipid Res.* **21:** 169–179.
57. Goodman, D. S., D. Deykin, and T. Shiratori. 1964. The formation of cholesterol esters with rat liver enzymes. *J. Biol. Chem.* **239:** 1335–1345.
58. Davidson, M. H., J. H. Burns, P. V. Subbaiah, M. E. Conn, and K. B. Drennan. 1991. Marine oil capsule therapy for the treatment of hyperlipidemia. *Arch. Intern. Med.* **151:** 1732–1740.
59. Weiner, B. H., I. S. Sckene, P. H. Levine, H. F. Cuenoud, M. Fisher, B. F. Johnson, A. S. Daoud, J. Jarmolych, D. Hosmer, M. H. Johnson, A. Natale, C. Vaudreuil, and J. J. Hoogasian. 1986. Inhibition of atherosclerosis by cod-liver oil in a hyperlipidemic swine model. *N. Engl. J. Med.* **315:** 841–846.
60. Zhu, B-Q., D. A. L. Smith, R. E. Sievers, W. M. Isenberg, and W. W. Parmley. 1988. Inhibition of atherosclerosis by fish oil in cholesterol-fed rabbits. *J. Am. Coll. Cardiol.* **12:** 1073–1078.
61. Kim, D. N., H-T. Ho, D. A. Laurence, J. Schmee, and W. A. Thomas. 1989. Modification of lipoprotein patterns and retardation of atherogenesis by a fish oil supplement to a hyperlipidemic diet for swine. *Atherosclerosis.* **76:** 35–54.
62. Groot, P. H. E., L. M. Scheek, M-L. Dubelaar, P. D. Verdouw, J. M. Hartog, and J. M. J. Lamers. 1989. Effects of diets supplemented with lard fat or mackerel oil on plasma lipoprotein lipid concentrations and lipoprotein lipase activities in domestic swine. *Atherosclerosis.* **77:** 1–6.
63. Davis, H. R., R. T. Bridenstine, D. Vesselinovitch, and R. W. Wissler. 1987. Fish oil inhibits development of atherosclerosis in rhesus monkeys. *Arteriosclerosis.* **7:** 441–449.
64. Surette, M. E., J. Whelan, G-P. Lu, K. S. Broughton, and J. E. Kinsella. 1992. Dependence on dietary cholesterol for n-3 polyunsaturated fatty acid-induced changes in plasma cholesterol in Syrian hamster. *J. Lipid Res.* **33:** 263–271.
65. Manning, J., I. Edwards, W. Wagner, L. Rudel, and J. Parks. 1992. Polyunsaturated dietary fat reduces interaction of low density lipoproteins with arterial proteoglycans. *Circulation.* **86 (supplement I):** I–747.