Dietary Fish Oil Stimulates Hepatic Low Density Lipoprotein Transport in the Rat

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Abstract

These studies were undertaken to examine the effect of fish oil, safflower oil, and hydrogenated coconut oil on the major processes that determine the concentration of low density lipoprotein (LDL) in plasma, i.e., the rate of LDL production and the rates of receptor-dependent and receptor-independent LDL uptake in the various organs of the body. When fed at the 20% level, fish oil reduced plasma LDL-cholesterol levels by 38% primarily by increasing LDL receptor activity in the liver. Dietary safflower oil also increased hepatic LDL receptor activity; however, since the rate of LDL production also increased, plasma LDL-cholesterol levels remained essentially unchanged. Hydrogenated coconut oil had no effect on LDL receptor activity but increased the rate of LDL-cholesterol production causing plasma LDL-cholesterol levels to increase 46%. Dietary fish oil had no effect on the receptor-dependent transport of asialofetuin by the liver, suggesting that the effect of fish oil on hepatic LDL receptor activity was specific and not due to a generalized alteration in the physical properties of hepatic membranes. Finally, dietary fish oil increased hepatic cholesteryl ester levels and suppressed hepatic cholesterol synthesis rates, suggesting that the up-regulation of hepatic LDL receptor activity in these animals was not simply a response to diminished cholesterol availability in the liver.

Introduction

Epidemiologic studies suggest that the consumption of a diet rich in marine lipids is associated with a reduction in the incidence of coronary heart disease (1, 2). The active component of fish oil appears to be the long-chain ω-3 polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid), which are known to affect both platelet function and plasma lipid levels (3–5). Dietary fish oil markedly reduces plasma triglyceride levels in normal and hyperlipidemic subjects and is much more active than equal amounts of polyunsaturated vegetable oil in this regard (6–10). Dietary fish oil has also been shown to lower plasma cholesterol levels in normal and hyperlipidemic subjects and, in several reports, was as effective, if not more effective, than equal amounts of polyunsaturated vegetable oil (6, 7, 11–15). Furthermore, the reduction in plasma cholesterol levels seen with fish oil feeding is due primarily to a decrease in cholesterol carried in the very low and low density lipoprotein (VLDL and LDL) fractions. High density lipoprotein (HDL) cholesterol, in contrast, usually remains unchanged or actually increases (5, 6, 10, 15). Although dietary fish oil usually lowers plasma total and LDL-cholesterol levels, the magnitude of this response varies widely from study to study. The greatest reductions in total and LDL-cholesterol levels have been achieved when the triglyceride normally present in the diet is largely replaced by fish oil. In contrast, when fish oil is simply added to a typical Western diet, plasma cholesterol levels may remain unchanged or actually increase (16). Another important variable in these studies is the source and composition of the marine lipid. The crude fish oil used in many studies contains substantial amounts of cholesterol and saturated fatty acids which, in susceptible individuals, may counteract the cholesterol-lowering activity of the ω-3 polyunsaturated fatty acids. Thus, the response to dietary fish oil in a given individual probably depends on a number of factors including the quantity and source of the marine lipid ingested, the degree to which other dietary fats are replaced by the fish oil, and the underlying lipid phenotype of the individual. There is little doubt, however, that specific fatty acids present in the diet can markedly influence the concentration of LDL in the plasma. Since an elevated concentration of LDL in the plasma is a well-established risk factor for the development of coronary heart disease, it is critically important to understand how these lipids regulate circulating LDL levels.

The concentration of LDL in plasma is determined by the rate at which LDL is introduced into the plasma relative to the rate at which LDL is removed from plasma by receptor-dependent and receptor-independent transport processes in the various tissues of the body. In animals on a low-cholesterol, low-triglyceride diet, receptor-dependent transport accounts for approximately three-fourths of total LDL turnover (17–19). Receptor-dependent LDL transport is saturable and can be described in terms of a maximal transport rate ($*J^m$)[1] and the concentration of LDL in plasma necessary to achieve one-half of this maximal transport rate ($*K_m$) (20). The liver is the most important site of LDL catabolism and accounts for > 80% of whole-body receptor-dependent LDL uptake (20). Receptor-dependent LDL transport in the liver is regulable and it is now apparent that the type and quantity of fat in the diet profoundly influences hepatic LDL receptor activity. For example, in the hamster dietary cholesterol has been shown to suppress receptor-dependent LDL uptake in the liver (21, 22). Responsiveness to dietary cholesterol varies widely, however, among different species and even within the same species (21–25). Dietary triglycerides also appear to regulate hepatic

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Received for publication 4 January 1989 and in revised form 14 March 1989.

J. Clin. Invest.

0021-9738/89/08/0528/10 $2.00
Volume 84, August 1989, 528–537

1. *Abbreviations used in this paper:* $*J^m$, the maximal rate of receptor-dependent LDL uptake in a particular tissue or the whole body; J_t, the total rate of LDL uptake by a particular tissue or the whole body by receptor-dependent and receptor-independent pathways; $*K_m$, the concentration of LDL-cholesterol in plasma necessary to achieve half of the maximal uptake rate; $*P$, the proportionality constant for receptor-independent LDL uptake in a particular tissue or the whole body.

LDL receptor activity although the mechanism(s) involved are poorly understood. In hamsters fed a low-cholesterol diet, polyunsaturated vegetable oil modestly increased hepatic LDL receptor activity whereas saturated fatty acids had no effect (22). Whether or not dietary cholesterol or triglyceride affects receptor-dependent LDL transport in the extrahepatic tissues is not known. In contrast to receptor-dependent LDL transport, receptor-independent LDL transport, which accounts for about one-fourth of total LDL turnover, is widely distributed throughout all tissues of the body (19, 20). Receptor-independent transport is nonsaturable and can be defined by a proportionality constant (*P). Although little is known about the regulation of receptor-independent transport, several experimental manipulations known to alter receptor-dependent LDL uptake in the liver have had no discernible effect on receptor-independent uptake (22).

There is generally little information on the effect of fish oil on these three basic processes that determine circulating LDL levels. The present studies were therefore undertaken to quantitate and compare the effects of fish oil (rich in ω-3 polyunsaturated fatty acids), safflower oil (rich in ω-6 polyunsaturated fatty acids), and hydrogenated coconut oil (saturated fatty acids only) on the rate of LDL production and on the rates of receptor-dependent and receptor-independent LDL uptake in all of the organs of the rat. Studies were also carried out to determine the effects of dietary fish oil, safflower oil and coconut oil on rates of cholesterol synthesis in the various tissues of the body. These studies provide the first quantitative data on the mechanisms by which these three dietary triglycerides alter plasma LDL-cholesterol concentrations in the rat.

Methods

Animals and diets. All studies were performed in female Sprague-Dawley rats that were obtained from SASCO Inc., Omaha, NE in the weight range of 125–150 g. The animals were housed in an isolation room with light cycling (dark from 3 a.m. to 3 p.m. and light from 3 p.m. to 3 a.m.) and allowed free access to water and standard rodent diet for at least 2 wk before beginning the experimental diets. The control diet used in these studies, ground Wayne Lab Blox (Allied Mills, Chicago, IL), contained only 0.025% (wt/wt) cholesterol and < 5% (wt/wt) triglyceride. The fatty acid composition of this diet, as determined by gas-liquid chromatography of the methyl esters, was 16% of the fatty acids as 16:0, 4% as 18:0, 28% as 18:1, 44% as 18:2, and 6% as 18:3. The experimental diets were prepared by mixing the various triglycerides into the control diet using a commercial food mixer. The addition of the triglyceride necessarily diluted out the other components of the control diet. Preliminary studies showed that the addition of up to 20% glucose (wt/wt) to the control diet had no effect on receptor-dependent or receptor-independent LDL uptake in any organ. Nevertheless, from a strict nutritional standpoint, the most proper comparisons are between diets with equal amounts of added triglyceride.

The fish oil concentrate used in these studies (Sanomega) was purchased from Nippon Oil and Fats Company, Ltd., Tokyo, Japan, and contained 28.4% eicosapentaenoic acid, 13.7% docosahexaenoic acid, and virtually no cholesterol (< 0.05%). The fish oil diets were stored in sealed containers under nitrogen at 4°C. All diets were enriched with vitamin E (1 g/kg of diet). Preliminary studies showed that this amount of vitamin E had no effect on the parameters of cholesterol and lipoprotein metabolism that were being studied. Animals were fed the various experimental diets ad lib. and weight gain was monitored. All studies were carried out during the mid–dark phase of the light cycle.

Determination of tissue LDL uptake rates in vivo. Plasma was obtained from normocholesterolemic rat or human donors. The LDL fraction was isolated in the density range of 1.020–1.055 g/ml, washed and concentrated, and labeled with [^{125}I]tyramine cellobiose (26) or ^{131}I (27) as previously described (19). The human LDL was also subjected to reductive methylation to completely eliminate its recognition by the LDL receptor (28). Total LDL transport in the organs of the rat was measured using the homologous LDL preparations while the receptor-independent component of total LDL transport was determined using the methylated human LDL preparations. In either case, lipoproteins were used within 48 h of preparation and were filtered through a 0.45-μM filter (Millipore/Continental Water Systems, Bedford, MA) immediately before use. As previously described, rates of tissue LDL uptake were determined using a primed-continuous infusion of [^{125}I]tyramine cellobiose-labeled LDL (22, 29). The radioactivity present in the priming dose relative to the radioactivity infused each hour was adjusted so as to maintain a constant specific activity of LDL in plasma over the experimental period. The infusions of [^{125}I]tyramine cellobiose-labeled LDL were continued for 6 h at which time each animal was administered a bolus of ^{131}I-labeled LDL and killed 10 min later by exsanguination through the abdominal aorta. Samples of various organs were quickly rinsed and weighed, and along with aliquots of plasma were assayed for radioactivity in a gamma counter (Packard Instrument Co., Inc., Downers Grove, IL). The organs sampled included the liver, small intestine, spleen, kidney, adrenal gland, heart, lung, adipose tissue, and skeletal muscle. The entire remaining carcass was then homogenized and aliquots were assayed for radioactivity. Since no tissue was discarded, rates of LDL uptake in individual organs, as well as the whole animal, could be determined. The amount of labeled LDL in each organ at 10 min (^{131}I disintegrations per minute per gram of tissue divided by the specific activity of ^{131}I in the plasma) and at 6 h (^{125}I disintegrations per minute per gram of tissue divided by the specific activity of ^{125}I in the plasma) was then calculated and has the units of micrograms of LDL-cholesterol per gram of tissue. The increase in the tissue content of LDL-cholesterol with time represents the rate of LDL uptake in micrograms of LDL-cholesterol taken up per hour per gram of tissue (μg/h per g) or per whole organ (μg/h per organ). In some studies hepatic asialofetuin transport was determined using ^{131}I- and [^{125}I]tyramine cellobiose-labeled asialofetuin.

In these studies total LDL uptake was measured in the experimental animals using LDL derived from donor rats fed standard rodent diet. In preliminary studies, however, we found that LDL preparations obtained from animals fed fish oil, safflower oil, or coconut oil were transported at similar rates when infused into control animals (Spady, D. K., unpublished observation).

Determination of cholesterol synthesis rates in vivo. As previously described (30, 31), the animals were administered ~ 50 mCi of [^{3}H]H_2O i.v. The animals were then returned to individual cages under a fume hood. 1 h after the injection of [^{3}H]H_2O the animals were anesthetized and exsanguinated through the abdominal aorta. Aliquots of plasma were taken for the determination of body water specific activity, and samples of the various organs were taken for the isolation of digitonin-precipitable sterols and fatty acids. In the case of the small intestine and the remaining carcass, the entire tissue was saponified and brought to an exact volume. Rates of sterol synthesis are expressed as the micromoles of [^{3}H]H_2O incorporated into digitonin-precipitable sterols per hour per gram of tissue (μmol/h per g) or per whole organ (μmol/h per organ).

Cholesterol, triglyceride, and fatty acid content in plasma, tissues, and diets. Total plasma cholesterol and triglyceride levels were determined using enzymatic kits obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. The distribution of cholesterol in plasma was determined by simultaneously centrifuging plasma at densities of 1.020, 1.063, 1.095, and 1.21 g/ml. The cholesterol content of the top one-third of each tube was assayed colorimetrically as previously described (32). Hepatic free and esterified cholesterol was separated using silicic acid/celite columns and quantitated using gas chromatography

as previously described (32). The fatty acid profile of plasma, tissues, and diets was determined after extracting total lipids into chloroform/methanol (2:1) (33). The phospholipids, triglycerides, and cholesteryl esters were separated by TLC. Each of these lipid fractions was methyl esterified and the fatty acid profile determined by capillary gas chromatography.

Calculations. Plasma LDL-cholesterol levels varied by more than three-fold in animals ingesting the various experimental diets. Thus, to relate the changes in LDL-cholesterol uptake in the organs of these animals to changes in LDL receptor activity, it was necessary to superimpose the experimentally determined uptake rates on the kinetic curves defining rates of LDL uptake in the various tissues of control animals as a function of plasma LDL-cholesterol concentrations (20, 22, 29). Total LDL-cholesterol uptake by a particular tissue or the whole body is equal to the rates of LDL-cholesterol uptake by receptor-dependent and receptor-independent pathways. The relationship between total LDL-cholesterol uptake (J_t) and plasma LDL-cholesterol concentrations (C_l) in normal animals can be described by the equation: $J_t = (*J^m C_l)/(*K_m + C_l) + *PC_l$, where $*K_m$ and $*J^m$ equal the apparent Michaelis constant and maximal transport velocity, respectively, for the receptor-dependent process, and $*P$ equals the apparent uptake constant for receptor-independent LDL uptake (20). The kinetic curves for normal LDL transport used in these studies were constructed using previously published values for $*J^m$, $*K_m$, and $*P$ obtained in control rats (20). Using these curves, the regulation of LDL receptor activity by the various experimental diets could be determined by comparing rates of receptor-dependent LDL transport in the experimental animals to rates of receptor-dependent LDL transport that would be seen in control animals at the same plasma LDL-cholesterol concentration. Values for LDL receptor activity are presented as percentages of the appropriate control values.

The data are presented as mean values±1 SEM. In most experiments, three different triglycerides were fed at three different levels (5, 10, or 20%). Statistical analysis of the effects of these two factors was carried out using two-way analysis of variance. When a significant triglyceride or dose effect was shown, individual contrasts were performed using Student's t tests at the $P < 0.01$ level.

Results

In preliminary experiments we noted that dietary fish oil markedly lowered plasma cholesterol and triglyceride levels in the rat. As illustrated in Table I, total plasma cholesterol equaled 62 mg/dl in control animals and was reduced by 23, 31, and 29% when fish oil was fed at the 5, 10, and 20% levels, respectively. Most of the reduction in total plasma cholesterol occurred in the $d < 1.095$ g/ml density fractions. For example, when fed at the 20% level, fish oil markedly lowered cholesterol in the $d < 1.020$ g/ml (60% reduction), $d = 1.020$–1.063 g/ml (36% reduction), and $d = 1.063$–1.095 g/ml (42% reduction) fractions but had relatively little effect on the $d > 1.095$ g/ml (18% reduction) density fraction. In the rat, cholesterol in the $d = 1.063$–1.095 g/ml fraction is carried predominantly in apoprotein E–containing HDL_1 particles. Thus, dietary fish oil primarily lowered those lipoproteins which, by virtue of their apoprotein B and/or apoprotein E content, are cleared from plasma by the LDL receptor pathway.

In contrast to the fish oil, safflower oil had relatively little effect on plasma cholesterol levels while hydrogenated coconut oil raised cholesterol in the $d = 1.020$–1.063 g/ml and $d = 1.063$–1.095 g/ml fractions and, to a lesser extent, in the $d > 1.095$ g/ml fraction. As also shown in Table I, dietary fish oil

Table I. Plasma Cholesterol and Triglyceride Concentrations in Animals Fed Fish Oil, Safflower Oil, or Hydrogenated Coconut Oil

	Plasma cholesterol concentration					
		Density fraction				
Diet	Total	$d < 1.020$	$d = 1.020$–1.063	$d = 1.063$–1.095	$d > 1.095$	Plasma triglyceride concentration
		g/ml				
		mg/dl				
Control	62±3	5±1	11±1	12±1	34±2	64±3
Fish oil						
5%	48±5	3±0.5	8±1	8±2	29±2	39±2
10%	43±4	2±0.3	6±1	7±1	28±2	31±2
20%	44±3	2±0.3	7±1	7±1	28±1	27±3
Safflower oil						
5%	62±4	6±1	11±2	13±1	32±2	60±3
10%	58±3	5±1	10±1	10±2	33±3	61±2
20%	58±5	4±1	10±2	10±1	34±2	56±3
Coconut oil						
5%	66±4	5±1	12±1	13±2	36±3	66±6
10%	76±6	5±1	15±1	18±2	38±4	59±3
20%	79±5	4±1	17±2	19±2	39±2	60±4

Groups of animals were fed diets enriched with varying amounts of fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. Each value represents the mean±1 SEM for data obtained in six animals. An analysis of variance showed that plasma cholesterol (total and various density fractions) and triglyceride were significantly affected by the type of triglyceride present in the diet. Individual contrasts showed that total cholesterol and cholesterol carried in the $d = 1.020$–1.063 and $d = 1.063$–1.095 g/ml density fractions were lower with dietary fish oil than with dietary safflower oil and lower with dietary safflower oil than with dietary coconut oil ($P < 0.01$). Plasma triglyceride and cholesterol carried in the $d < 1.020$ and $d > 1.095$ g/ml fractions were lower in animals fed fish oil than in animals fed safflower oil or coconut oil ($P < 0.01$).

Table II. Total and Receptor-independent LDL-Cholesterol Uptake Rates and Plasma LDL-Cholesterol Concentrations in Animals Fed Fish Oil, Safflower Oil, or Hydrogenated Coconut Oil

Diet	A. Final body weight	B. Liver weight	Total LDL-cholesterol uptake		Receptor-independent LDL-cholesterol uptake		G. Plasma LDL-cholesterol concentration
			C. Hepatic	D. Extrahepatic	E. Hepatic	F. Extrahepatic	
	g	*g/100 g body wt*	*μg/h per 100 g body wt*				*mg/dl*
Control	218±13	3.2±0.2	48±4	30±3	4.6±0.5	19.6±2	13±1
Fish oil							
5%	220±18	3.4±0.1	56±4	22±2	3.2±0.2	13.6±1	9±2
10%	229±11	3.7±0.3	58±3	20±1	2.9±0.2	12.4±1	8±1
20%	225±14	4.1±0.2	61±4	20±3	2.9±0.2	12.8±2	8±1
Safflower oil							
5%	222±12	3.2±0.3	58±5	28±2	4.2±0.2	18.1±2	12±2
10%	230±16	3.6±0.1	59±4	30±2	4.8±0.4	19.8±1	13±1
20%	234±16	3.7±0.2	61±5	29±3	4.3±0.3	18.4±1	12±1
Coconut oil							
5%	218±10	3.3±0.2	55±5	34±3	5.3±0.4	22.7±2	15±2
10%	222±12	3.4±0.2	68±3	41±3	6.4±0.3	27.0±2	18±1
20%	222±18	3.6±0.3	71±6	43±4	6.6±0.3	28.2±1	19±2

Groups of animals were fed diets enriched with varying amounts of fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. Each value represents the mean±1 SEM for data obtained in six animals.

markedly reduced plasma triglyceride levels whereas safflower oil and hydrogenated coconut oil had relatively little effect.

Studies were next undertaken to examine the effect of fish oil, safflower oil, and hydrogenated coconut oil on those parameters that determine the concentration of LDL in plasma, i.e., the rate of LDL production and the rates of receptor-dependent and receptor-independent LDL uptake by the various tissues of the body. The results of these studies are shown in Table II. In these experiments, the three triglycerides were fed at the 5, 10, or 20% level (wt/wt) for 1 mo. Weight gain was similar among animals fed the various experimental diets (column A). Liver size, however, tended to increase when increasing amounts of triglyceride were added to the diet. Thus, when fed at the 20% level, fish oil, safflower oil, and hydrogenated coconut oil increased liver size by 28, 16, and 13%, respectively (column B). Columns C and D show the absolute rates of total (receptor-dependent plus receptor-independent) LDL-cholesterol uptake in the liver and extrahepatic tissues of animals fed the three triglycerides. All uptake rates are normalized to a body weight of 100 g. Total LDL-cholesterol uptake in the liver equaled 48±4 μg/h per 100-g animal in control animals and increased modestly when increasing amounts of fish oil, safflower oil, or coconut oil were added to the diet (column C). Rates of LDL-cholesterol uptake in individual extrahepatic tissues were generally quite low. Therefore, uptake rates in all of the extrahepatic tissues were combined and these values are presented in column D. In control animals, LDL-cholesterol uptake in the extrahepatic tissues equaled 30±3 μg/h per 100-g animal. Extrahepatic LDL-cholesterol uptake decreased in animals fed fish oil, remained unchanged in animals fed safflower oil, and increased in animals fed coconut oil. It should be noted that total LDL-cholesterol uptake in the liver plus the extrahepatic tissues equals whole-body LDL-cholesterol uptake which, in a steady state, must equal the rate of LDL-cholesterol production. The receptor-independent component of total LDL-cholesterol uptake in the hepatic and extrahepatic tissues is shown in columns E and F. In control animals, receptor-independent LDL-cholesterol uptake equaled 4.6±0.5 and 19.6±2 μg/h per 100-g animal in the liver and extrahepatic tissues, respectively. In both the liver and extrahepatic tissues, receptor-independent LDL-cholesterol uptake decreased in animals fed fish oil, remained unchanged in animals fed safflower oil, and increased in animals fed coconut oil. As shown in column G, the plasma LDL-cholesterol concentration equaled 13 mg/dl in control animals. In animals fed 20% triglyceride, plasma LDL-cholesterol levels decreased 38% with fish oil, remained virtually unchanged with safflower oil, and increased 46% with coconut oil.

From Table II, rates of receptor-dependent LDL uptake can be obtained by subtracting the rate of receptor-independent LDL uptake from the rate of total (receptor-dependent plus receptor-independent) LDL uptake. However, since receptor-dependent LDL transport is saturable and since plasma LDL levels varied widely in animals fed the three triglycerides, changes in the rate of receptor dependent LDL uptake can not be equated directly with changes in LDL receptor activity (20). In order to relate the changes in absolute rates of LDL-cholesterol uptake to changes in receptor activity, each of the values shown in Table II was superimposed on the kinetic curves that define the relationship between LDL-cholesterol uptake and circulating LDL-cholesterol concentrations in control rats fed a standard low-cholesterol, low-triglyceride rodent diet. Fig. 1 shows the normal kinetic curves for 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-cholesterol uptake and plasma LDL-cholesterol concentrations over the range of LDL levels seen in these studies. The area between the two curves repre-

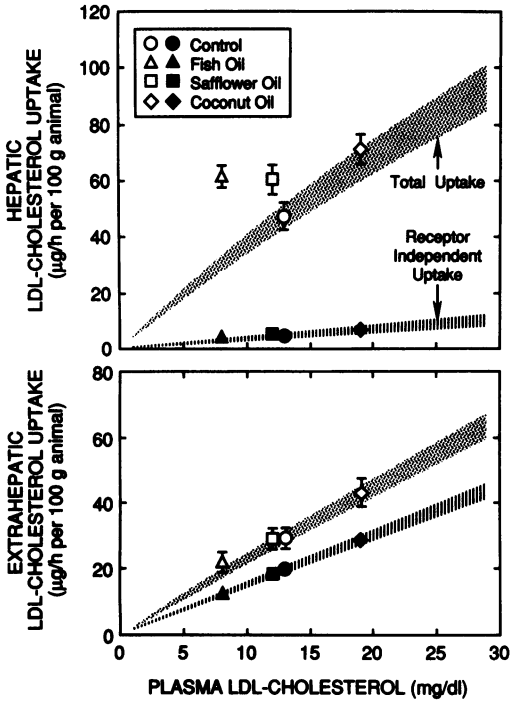

Figure 1. LDL-cholesterol uptake in the whole liver (*top*) and all of the extrahepatic tissues combined (*bottom*) in animals fed 20% fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. The shaded areas represent the kinetic curves for total (*stippled*) and receptor-independent (*hatched*) LDL-cholesterol uptake in control animals fed standard rodent diet. These curves were calculated as described in Methods. The individual points superimposed on these kinetic curves show the rates of total (*open symbols*) and receptor-independent (*solid symbols*) LDL-cholesterol uptake in the experimental animals plotted as a function of the plasma LDL-cholesterol levels in the same animals. Each point represents the mean±1 SEM for data obtained in six animals.

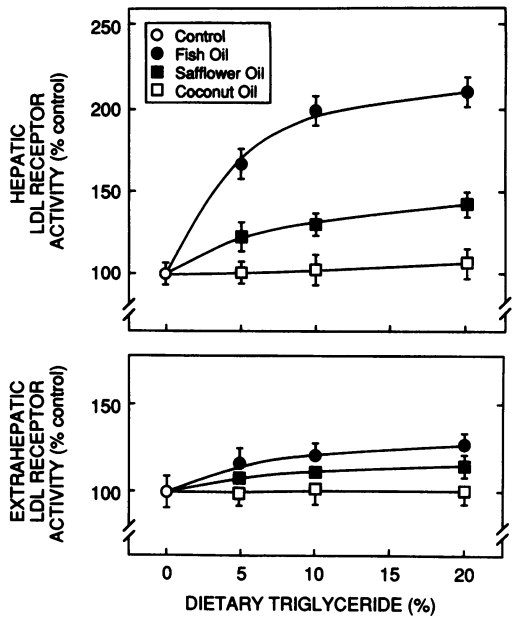

Figure 2. LDL-receptor activity in the whole liver (*top*) and all of the extrahepatic tissues combined (*bottom*) in animals fed varying amounts of fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. The values shown represent the rates of receptor-dependent LDL-cholesterol uptake in experimental 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 point represents the mean±1 SEM for data obtained in six animals. Two-way analysis of variance showed that the type of triglyceride and the amount of triglyceride fed affected hepatic LDL receptor activity. Individual contrasts of the triglyceride effect showed that hepatic LDL receptor activity was higher in animals fed fish oil than in animals fed safflower oil and higher in animals fed safflower oil than in animals fed coconut oil ($P < 0.01$).

sents the receptor-dependent component of total LDL uptake. Superimposed on these normal kinetic curves are the observed rates of total (*open symbols*) and receptor-independent (*closed symbols*) LDL uptake in animals fed the 20% triglyceride diets. As shown in the top panel, the control animals used in these studies manifested rates of total (○) and receptor-independent (●) LDL-cholesterol uptake in the liver of 48±4 and 4.6±0.5 μg/h per 100-g animal, respectively, at a mean plasma LDL-cholesterol concentration of 13 mg/dl. Since these values fell on the normal kinetic curves for LDL transport in the liver, hepatic LDL receptor activity in the control animals was assigned a value of 100%. In contrast, total hepatic LDL-cholesterol uptake in animals fed 20% fish oil (△) equaled 61±4 μg/h at a plasma LDL-cholesterol concentration of 8 mg/dl whereas normal animals would be expected to transport only ~ 30 μg/h at this LDL-cholesterol concentration. Since receptor-independent uptake (▲) was normal in these animals, the increase in total LDL-cholesterol uptake could be attributed entirely to an increase in receptor-dependent LDL transport. Indeed, it could be calculated that hepatic receptor-dependent LDL-cholesterol uptake in animals fed 20% fish oil was 209% of that in normal animals at the same plasma LDL-cholesterol concentration. Similarly, the rate of total LDL-cholesterol uptake in the liver was significantly increased (relative to normal animals at the same LDL-cholesterol level) in animals fed 20% safflower oil. Again, receptor-independent LDL-cholesterol uptake was normal and it could be calculated that receptor-dependent LDL-cholesterol transport was 138% of that in control animals at the same plasma LDL-cholesterol concentration. In animals fed hydrogenated coconut oil, total and receptor-independent uptake rates were not displaced significantly from the normal kinetic curves, indicating that neither transport process was affected by this dietary manipulation. As shown in the bottom panel, rates of total and receptor-independent LDL-cholesterol uptake in the extrahepatic tissues were not displaced significantly 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 LDL uptake shown in Table II could be converted to changes in LDL receptor activity (defined as the rate of receptor-dependent LDL uptake in experimental animals relative to the rate of receptor-dependent uptake in control animals at the same LDL-cholesterol concentration) and these values are presented in Fig. 2. As seen in the top panel, dietary fish oil stimulated hepatic LDL receptor activity by 75, 95, and 105% when fed at the 5, 10, and 20% level, respec-

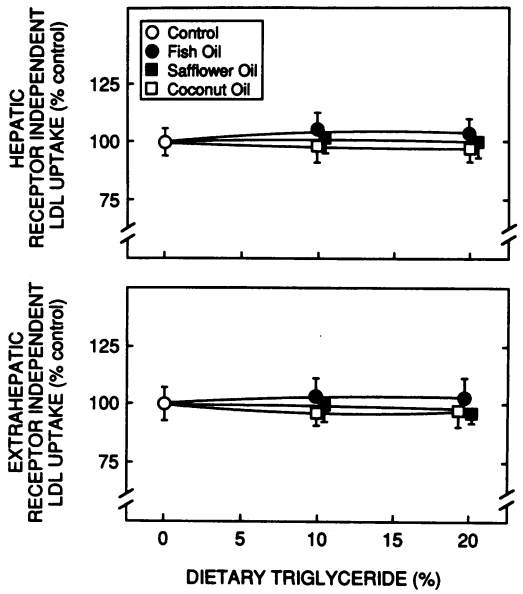

Figure 3. Receptor-independent LDL-cholesterol uptake in the whole liver (*top*) and all of the extrahepatic tissues combined (*bottom*) in animals fed varying amounts of fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. The values shown represent the rates of receptor-independent LDL-cholesterol uptake in experimental animals as percentages of the rates of receptor independent uptake that would occur in control animals at the same LDL-cholesterol concentration (as illustrated in Fig. 1). Each point represents the mean±1 SEM for data obtained in six animals. Two-way analysis of variance showed that neither the type of triglyceride nor the amount of triglyceride fed significantly altered receptor-independent LDL uptake in the liver or extrahepatic tissues.

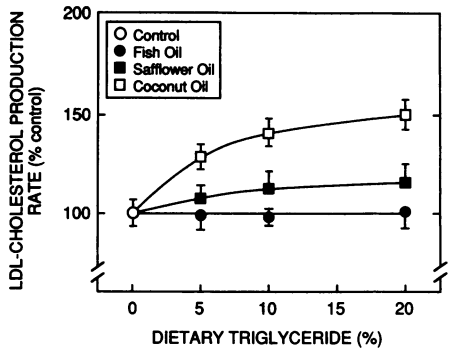

Figure 4. Rates of LDL-cholesterol production in animals fed varying amounts of fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. Each value represents the mean±1 SEM for data obtained in six animals. Two-way analysis of variance showed that both the type and the amount of triglyceride fed significantly affected the rate of LDL-cholesterol production. Individual contrasts of the triglyceride effect showed that the rate of LDL-cholesterol production was significantly higher in animals fed coconut oil than in animals fed fish or safflower oil ($P < 0.01$).

tively. Safflower oil also stimulated hepatic LDL receptor activity but to a much smaller degree while hydrogenated coconut oil had no significant effect on hepatic LDL receptor activity. As shown in the bottom panel, none of the experimental diets had a major effect on receptor activity in the extrahepatic tissues. Fig. 3 shows the effect of the three dietary triglycerides on receptor-independent LDL transport in the liver and extrahepatic tissues. The values shown in this figure were determined by superimposing the absolute rates of receptor-independent LDL-cholesterol uptake shown in Table II on the normal kinetic curves for receptor-independent transport (as illustrated in Fig. 1). Receptor-independent transport in the experimental animals was then expressed as a percentage of the rate of receptor independent transport that would occur in control animals at the same LDL-cholesterol concentration. As is apparent, the three dietary lipids tested had no significant effect on receptor-independent LDL transport in the liver or in the extrahepatic tissues.

The effect of the three dietary triglycerides on LDL-cholesterol production is shown in Fig. 4. Rates of LDL-cholesterol production increased in a dose-dependent fashion when coconut oil was added to the diet. Although not statistically significant at the $P < 0.01$ level, dietary safflower oil also tended to increase the rate of LDL-cholesterol production. In contrast, the addition of fish oil to the diet had no effect on LDL-cholesterol production. It should be noted, however, that dietary fish oil actually reduced the rate of LDL-cholesterol production when compared with equal amounts of the other dietary triglycerides.

To investigate the mechanism by which fish oil regulates hepatic LDL receptor activity, the effect of fish oil on hepatic asialofetuin transport was measured. Asialofetuin is a plasma glycoprotein rapidly cleared by the liver via a receptor pathway similar to the LDL receptor pathway (34, 35). As shown in Fig. 5, hepatic asialofetuin transport was not significantly altered by any of the three dietary lipids used in these studies.

The final group of experiments was undertaken to see if the changes in hepatic LDL receptor activity described above were accompanied by alterations in other aspects of cholesterol metabolism in the liver. Fig. 6 shows the effect of the three dietary triglycerides on free and esterified cholesterol levels and *de novo* cholesterol synthesis rates in the liver. As shown in the top panel, none of the three dietary triglycerides significantly altered the free cholesterol content of the liver. In contrast, hepatic cholesteryl esters, which equaled 0.2 mg/g in control animals, increased by 2.5-, 6.5-, and 2.5-fold in animals fed fish oil, safflower oil, or coconut oil, respectively (middle panel). As shown in the bottom panel, rates of hepatic cholesterol synthesis, when expressed per gram of liver, were sup-

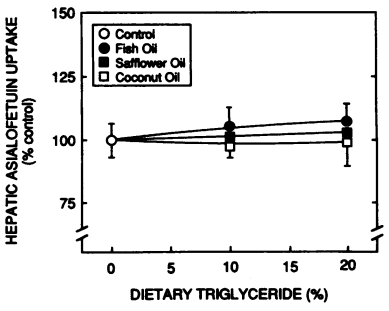

Figure 5. Whole-liver asialofetuin uptake in animals fed varying amounts of fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. Each value represents the mean±1 SEM for data obtained in six animals. Two-way analysis of variance revealed no significant effect of the type of triglyceride or the amount of triglyceride fed on hepatic asialofetuin uptake.

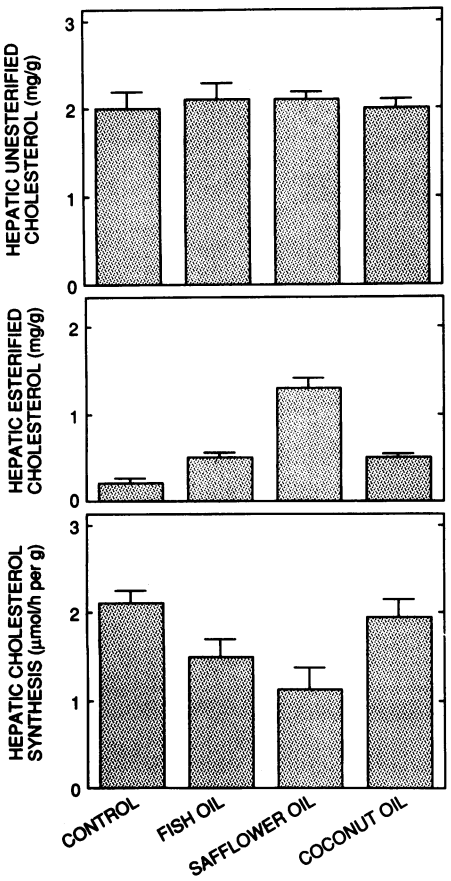

Figure 6. Hepatic unesterified and esterified cholesterol levels and hepatic cholesterol synthesis rates in animals fed 20% fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. All values are expressed per gram of wet weight liver and represent the mean±1 SEM for data obtained in six animals.

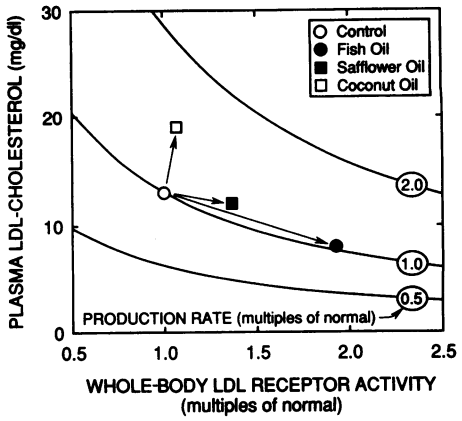

Figure 7. Plasma LDL-cholesterol concentrations as a function of whole-body LDL receptor activity and LDL-cholesterol production rates in animals fed 20% fish oil, safflower oil, or hydrogenated coconut oil for 1 mo. The solid curves show the plasma LDL-cholesterol concentrations that will occur in control animals fed standard rodent diet under circumstances where LDL receptor activity is varied from half normal to two and one-half times normal at LDL-cholesterol production rates of half normal, normal, or twice normal. The individual data points represent the mean±1 SEM for data obtained in six animals.

pressed by 28% in animals fed fish oil and by 46% in animals fed safflower oil but were unaffected by coconut oil. Although the data are not shown, cholesterol synthesis rates and free and esterified cholesterol levels in the extrahepatic tissues were not affected by the three dietary lipids.

Discussion

These studies provide the first quantitative description of the mechanisms by which dietary polyunsaturated (ω-3 and ω-6) and saturated fatty acids regulate plasma LDL levels in the whole animal. The concentration of LDL in plasma is determined by the rate at which LDL is produced relative to the rate at which LDL is removed from plasma by receptor-dependent and receptor-independent transport processes located in the various tissues of the body. In the present investigations, none of the triglycerides studied significantly altered receptor-independent LDL transport in any tissue. Thus, the changes in plasma LDL levels induced by triglyceride feeding were due entirely to changes in the rate of LDL production or changes in whole-body LDL receptor activity. These relationships are illustrated in Fig. 7, where plasma LDL-cholesterol levels are plotted as a function of whole-body receptor activity and LDL production rates. The solid curves show the steady-state LDL-cholesterol concentrations that will occur under circumstances where whole-body LDL receptor activity is varied from half normal to two and one-half times normal at LDL production rates of half normal, normal, or twice normal. These kinetic curves for normal LDL transport in the rat were constructed using previously published values for the transport parameters that describe receptor-dependent ($*J^m$, $*K_m$) and receptor-independent ($*P$) LDL-cholesterol uptake in control animals fed a standard low-cholesterol, low-triglyceride rodent diet. Superimposed on these curves are the data for animals fed the three triglycerides at the 20% level. The control animals in these studies (○), with a normal production rate and normal receptor activity had a plasma LDL-cholesterol concentration of 13 mg/dl. When fish oil was added to the diet, the production rate remained essentially unchanged while whole-body LDL receptor activity increased approximately twofold causing LDL-cholesterol levels to fall 38% to 8 mg/dl. Whole-body LDL receptor activity also increased (~ 40%) in animals fed safflower oil. In these animals, however, the enhanced receptor activity was largely offset by an increase in the rate of LDL-cholesterol production so that plasma LDL-cholesterol levels remained essentially unchanged. Finally, hydrogenated coconut oil significantly increased the rate of LDL-cholesterol production while having no effect on LDL receptor activity and as a result plasma LDL-cholesterol levels rose ~ 50% to 19 mg/dl. Thus, when compared with equal amounts of safflower oil or coconut oil, dietary fish oil reduced plasma LDL-cholesterol levels both by reducing the rate of LDL-cholesterol production and by increasing the rate of receptor dependent LDL transport.

In animals fed fish oil or safflower oil, the increase in whole-body LDL receptor activity was due almost entirely to an increase in receptor activity in the liver. In contrast, these dietary lipids had no major effect on receptor activity in the extrahepatic tissues, which included the small intestine,

spleen, kidney, adrenal gland, heart, lung, adipose tissue, and skeletal muscle as well as the entire residual carcass. Why fish oil and safflower oil altered receptor activity only in the liver is not clear. It should be noted, however, that the liver accounts for ~ 80% of total-body LDL receptor activity in the rat, as it does in all other species that have been examined (20, 36).

Although dietary fish oil clearly enhanced hepatic LDL receptor activity in these studies, the precise mechanism by which it did so is not known. In cultured cells as well as in whole organs in vivo, de novo cholesterol synthesis and receptor-dependent LDL uptake are generally regulated by changes in cholesterol homeostasis within the cell or organ (22, 23, 29, 37, 38). Thus, when faced with an alteration in cholesterol balance, the liver responds by appropriately adjusting the rate of de novo cholesterol synthesis and, if necessary, by also regulating the rate of receptor-dependent LDL uptake. These compensatory mechanisms allow the liver to maintain cholesterol balance despite the presence of significant and variable amounts of cholesterol in the diet. It is somewhat surprising that dietary fatty acids also regulate receptor-dependent LDL transport in the liver. This effect of fatty acids, however, may still be mediated by changes in cholesterol homeostasis within the liver. Thus, dietary fatty acids may regulate the LDL receptor pathway indirectly by altering cholesterol balance across the liver or by altering the compartmentation of cholesterol within the liver. Along these lines, dietary fish oil has been reported to decrease cholesterol absorption from the small intestine (5, 39) and to enhance the conversion of cholesterol to bile salts in the liver (5). Both of these effects, by decreasing cholesterol availability in the liver, would be expected to trigger a compensatory rise in cholesterol synthesis and, perhaps, an increase in receptor-dependent LDL-cholesterol uptake. In the present studies, however, hepatic cholesterol synthesis was actually suppressed and hepatic cholesteryl esters increased in animals fed fish oil or safflower oil, suggesting that the availability of cholesterol in the liver was increased rather than decreased. In addition, the rat has an exceptionally high basal rate of cholesterol synthesis in the liver which can be regulated over an extremely wide range in response to changes in cholesterol influx or efflux (23, 30). As a consequence, the liver can usually compensate entirely for changes in cholesterol balance by adjusting the rate of de novo cholesterol synthesis. For example, experimental manipulations that decrease cholesterol availability in the liver such as feeding cholestyramine, which increases bile acid synthesis, or feeding surformer, which specifically blocks cholesterol absorption, are fully compensated for by enhanced cholesterol synthesis while LDL receptor activity remains unchanged (23). Thus, it is difficult to attribute the increase in hepatic LDL receptor activity seen in animals fed fish oil or safflower oil to a gross change in cholesterol balance across the liver. It may be, however, that fish oil alters the distribution of cholesterol within the hepatocyte so as to shift cholesterol out of a critical pool(s) that regulates the LDL receptor pathway.

Alternatively, fish oil could affect the LDL receptor pathway through a mechanism completely unrelated to cholesterol. For example, hepatic membranes become markedly enriched in ω-3 polyunsaturated fatty acids during fish oil feeding. Indeed, in animals fed fish oil at the 20% level, nearly 25% of the fatty acids present in liver phospholipids were ω-3 polyunsaturated fatty acids (Spady, D. K., unpublished observation). Thus, fish oil could affect the LDL receptor pathway by altering the composition and, perhaps, the physical properties of hepatic membranes. Dietary fish oil had little effect, however, on the hepatic transport of asialofetuin, a glycoprotein taken up and degraded in the liver through a receptor pathway similar to that of the LDL receptor pathway (34, 35). Furthermore, fish oil feeding had no detectable effect on receptor-independent LDL transport as might be anticipated if the effect of the ω-3 polyunsaturated fatty acids was simply to induce a nonspecific change in the physical properties of hepatic membranes.

How dietary triglycerides regulate the rate of LDL-cholesterol production is also unknown. LDL are produced in the plasma during the catabolism of VLDL. VLDL, in turn, are triglyceride-rich particles synthesized and secreted by the liver. Several lines of evidence now suggest that, relative to other fatty acids, ω-3 polyunsaturated fatty acids reduce VLDL secretion by the liver (40–43). Since LDL are derived from VLDL, such a decrease in the rate of VLDL production could explain the decrease in LDL production seen in the present studies when fish oil was substituted for safflower or coconut oil in the diet.

These studies are the first to demonstrate an effect of fish oil on receptor-dependent LDL transport in the liver. In contrast, previous studies dealing with the mechanism by which fish oil alters plasma LDL levels have generally suggested that fish oil primarily affects the production of LDL rather than LDL receptor activity. For example, Illingworth et al. (15) reported that the 20% decrease in plasma LDL-cholesterol levels seen in seven normal subjects when they were switched from a control diet to one enriched with ω-3 polyunsaturated fatty acids was due to a reduction in the LDL production rate while the fractional catabolic rate for LDL was unchanged. In these studies, however, the triglyceride content of the control diet equaled 30–40% of total calories and was composed predominantly of saturated and monounsaturated fatty acids. In the present studies, the addition to the control diet of hydrogenated coconut oil or, to a lesser extent, safflower oil increased the rate of LDL-cholesterol production while fish oil had no effect. Thus, relative to the other triglyceride-rich diets, fish oil lowered LDL levels both by increasing hepatic LDL receptor activity and by reducing the rate of LDL production. However, the decrease in LDL production rates when safflower or coconut oil was replaced by fish oil was due not to the addition of the fish oil but rather to the removal of the vegetable oil. More recently, Roach et al. (44) reported that plasma LDL-cholesterol levels fell by 34% in the rat when standard rodent chow was supplemented with fish oil at the 8% level. However, this decrease in plasma LDL levels was associated with a seemingly paradoxical 37% reduction in the binding of LDL to solubilized hepatic membranes. It is difficult to reconcile these findings with the present studies. It is possible, however, that changes in LDL binding to liver membranes in vitro may not always reflect changes in receptor-dependent LDL uptake in vivo.

Finally, various fish oil preparations may differ in their effects on cholesterol and lipoprotein metabolism. The fish oil concentrate used in these studies contained essentially no cholesterol and only small amounts of saturated fatty acids. Crude fish oil, in contrast, contains substantial amounts of both cholesterol and saturated fatty acids. These lipids, which are known to have detrimental effects on LDL metabolism, could

neutralize the potential lipid lowering effects of the ω-3 polyunsaturated fatty acids. In future studies it will be important to compare the cholesterol-lowering activities of highly purified preparations of eicosapentaenoic acid and docosahexaenoic acid and to determine how these fatty acids interact with dietary cholesterol and saturated fatty acids in regulating LDL production and receptor-dependent LDL transport.

Acknowledgments

The authors wish to thank Janet Hainline and Russell Nelson for their excellent technical assistance and Imogene Robison for her assistance in preparing the manuscript.

This work was supported by U. S. Public Health Service research grants HL-38049 and AM-01221 and a grant-in-aid (870653) from the American Heart Association. During the time that these studies were carried out Laura A. Woollett was also supported by U. S. Public Health Service training grant AM-07100 and Mark A. Ventura received support from an American Gastroenterological Association Student Research Fellowship.

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