Interaction of dietary saturated and polyunsaturated triglycerides in regulating the processes that determine plasma low density lipoprotein concentrations in the rat

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Abstract These studies were undertaken to determine how polyunsaturated (n-3 and n-6) and saturated triglycerides interact to regulate rates of low density lipoprotein (LDL) production and rates of receptor-dependent and receptor-independent LDL transport. Animals were fed diets containing 20% (by wt) hydrogenated coconut oil or diets in which the coconut oil was progressively removed and replaced with safflower oil or fish oil concentrate. Plasma LDL concentrations fell when either of the polyunsaturated triglycerides was substituted for saturated triglycleride in the diet; however, the reduction in LDL concentrations was greater with fish oil than with safflower oil at all ratios of polyunsaturated to saturated triglyceride that were examined. The lower plasma LDL concentrations when coconut oil was replaced with fish oil could be attributed almost entirely to a much greater increase in hepatic LDL receptor activity when fish oil was used as the substitute than when safflower oil was used as the substitute. To examine the effect of polyunsaturated triglycerides when used to supplement a high saturated fat diet rather than to replace saturated fat in the diet, animals were fed a diet containing 15% coconut oil (by wt) with or without an additional supplement of 5% fish oil or safflower oil. The addition of 15% coconut oil to low fat control diet increased the rate of LDL production causing circulating LDL levels to rise by 40%. The further supplementation of this high saturated fat diet with fish oil concentrate markedly increased hepatic LDL receptor activity causing plasma LDL concentrations to return to control values whereas supplementation with safflower oil had little effect. Thus, at least in the rat, supplementation of a high saturated fat diet with a fish oil concentrate lowers plasma LDL concentrations as effectively as removing the saturated fat from the diet, although in the former case, both the production and the receptor-dependent uptake of LDL are greatly increased. —**Spady, D. K., and L. A. Woollett.** Interaction of dietary saturated and polyunsaturated triglycerides in regulating the processes that determine plasma low density lipoprotein concentrations in the rat. *J. Lipid Res.* 1990. **31:** 1809–1819.

Supplementary key words hepatic LDL transport • dietary triglycerides • fish oil • LDL production

Plasma cholesterol concentrations are strongly influenced by the quantity and quality of fat in the diet. In humans, total and low density lipoprotein (LDL)-cholesterol concentrations fall when saturated triglyceride in the diet is replaced by polyunsaturated vegetable oil (rich in n-6 polyunsaturated fatty acids) or by fish oil (rich in n-3 polyunsaturated fatty acids) (1–7). Since an elevated concentration of LDL in plasma is a major risk ractor in the development of atherosclerosis and coronary heart disease, there currently is considerable interest in understanding how dietary triglycerides regulate the concentration of this lipoprotein in plasma. This is particularly true for the marine lipids since epidemiologic studies suggest that diets rich in fish may reduce the incidence of coronary heart disease (8–10).

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 organs of the body. The uptake of LDL into tissues is mediated by both receptor-dependent and receptor-independent processes. Receptor-dependent LDL transport is saturable and can be described in terms of a maximal transport rate (J^m) and the concentration of LDL in plasma necessary to achieve one-half of this maximal transport rate (K_m) (11). Receptor-independent LDL transport is nonsaturable and can be described by a proportionality constant (P) (11). Receptor-dependent LDL transport accounts for about 75% of total LDL turnover in humans and experimental animals on a low cholesterol diet and is located predominantly in the liver

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); *J^m, the maximal rate of receptor-dependent LDL uptake in an organ or the whole body; *K_m, the concentration of LDL in the plasma necessary to achieve half of the maximal uptake rate; *P, the proportionality constant for receptor-independent LDL uptake in an organ or the whole body; J_t, the rate of total LDL uptake in an organ or the whole body by receptor-dependent and receptor-independent pathways; TLC, thin-layer chromatography.

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(11-16). Receptor-independent transport accounts for about 25% of LDL turnover and is widely distributed throughout all tissues of the body (11, 12, 14, 15). Receptor-independent transport appears not to be regulated in response to a variety of nutritional and pharmacologic manipulations. Thus, changes in circulating LDL concentrations are usually due to changes in the rate of LDL production or to changes in the rate of receptor-dependent LDL uptake by the liver. Since LDL are formed during the metabolism of VLDL, a change in the rate of LDL production may, in turn, be due to a change in the rate of VLDL secretion by the liver or to a change in the proportion of VLDL that is converted to LDL (17).

Although there is relatively little information on how dietary fish oil affects these major transport processes, it is generally believed that the main effect of fish oil is on the production of VLDL and LDL (7, 18-20). In recent studies in the rat, we found that fish oil lowered plasma LDL concentrations when added to a low fat diet and that the reduction of LDL levels was due primarily to an increase in hepatic LDL receptor activity (21). In contrast, saturated triglycerides raised plasma LDL concentrations by increasing the rate of LDL production. Thus, in this model, the decrease in LDL concentrations when saturated triglyceride was completely replaced by fish oil was due both to a decrease in the rate of LDL production and to an increase in hepatic LDL receptor activity. However, typical diets contain a wide variety of saturated and unsaturated triglycerides and it is not known whether these lipids exert the same effects on LDL metabolism when administered in combination as when administered individually. Furthermore, it is not clear whether polyunsaturated triglycerides exert an independent favorable effect on LDL metabolism apart from that of replacing saturated triglycierdes. The present studies were therefore undertaken to determine how polyunsaturated and saturated triglycerides interact in regulating the production and catabolism of LDL and to determine whether polyunsaturated triglycerides exert an independent effect on these processes when added as a supplement to a high saturated fat diet.

METHODS

Animals and diets

All experiments were carried out in female Sprague-Dawley rats (SASCO Inc., Omaha, NE), which were purchased in the weight range of 125-150 g. The animals were subjected to light cycling (dark from 3 AM to 3 PM and light from 3 PM to 3 AM) and were allowed free access to commercial rodent diet (Wayne Lab Blox, Allied Mills, Chicago, IL) for at least 3 weeks before beginning the experimental diets. The experimental diets were prepared by adding the desired lipids to ground Wayne Lab Blox and mixing thoroughly with a commercial food mixer. The Wayne Lab Blox contained 0.023% (by wt) cholesterol and 4.5% (by wt) total lipid. The fatty acid composition of this diet, as determined by capillary gas-liquid chromatography of the fatty acid methyl esters, was 18% of the fatty acids as 16:0, 4% as 18:0, 30% as 18:1, 40% as 18:2, and 5% as 18:3. The fish oil concentrate used in these studies was purchased from Nippon Oil and Fats Company, Ltd., Tokyo, Japan, and contained 28% eicosapentaenoic acid, 13% docosahexaenoic acid, and less than 0.05% (by wt) cholesterol. All diets were enriched with vitamin E (0.05%), BHT (0.02%), and TBHQ (0.02%). The fish oil diets were stored under nitrogen in the dark at 4°C. The various experimental diets were fed ad lib on a daily basis for 1 month. All experiments were carried out during the mid-dark phase of the light cycle.

Lipoprotein preparations

LDL (d 1.020-1.055 g/ml) was isolated from the plasma of normocholesterolemic rat or human donors by preparative ultracentrifugation. The LDL fractions were labeled with [^{125}I]- or [^{131}I]tyramine cellobiose as previously described (22). Rat LDL in this density range does include some apoprotein E-containing HDL_1 particles. However, in preliminary studies we found that rat LDL in this density range was transported at the same rate whether or not it was subjected to further purification by Geon-Pevikon starch block electrophoresis to remove the HDL_1 particles. The homologous LDL preparations were used to measure total LDL transport rates by the various organs of the body. The human LDL was reductively methylated to completely eliminate its recognition by the LDL receptor (14, 23) and was then used to determine the receptor-independent component of total LDL transport. All lipoproteins were used within 48 h of preparation and were filtered through a 0.45-μm filter (Millipore, Bedford, MA) prior to use.

Determination of tissue LDL uptake rates in vivo

Rates of LDL uptake by individual organs and the whole body were determined using primed infusions of [^{125}I]tyramine cellobiose-labeled LDL as previously described (13, 21). Animals were administered a bolus of [^{125}I]tyramine cellobiose-labeled LDL followed by a constant infusion of the same labeled lipoprotein at a rate calculated to maintain a constant specific activity of LDL in plasma over the experimental period. The infusions were continued for 6 h at which time each animal was administered a bolus of [^{131}I]tyramine cellobiose-labeled LDL as a volume marker and killed 10 min later by exsanguination through the abdominal aorta. The organs of interest were removed and multiple samples of these tissues, along with aliquots of plasma, were assayed for

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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 radiolabeled LDL in each tissue at 10 min (^{131}I dpm per gram of tissue divided by the specific activity of ^{131}I in plasma LDL) and at 6 h (^{125}I dpm per gram of tissue divided by the specific activity of ^{125}I in plasma LDL) was then calculated and has the value of micrograms of LDL-protein or LDL-cholesterol per g of tissue. The increase in the tissue content of LDL with time represents the rate of LDL uptake in micrograms of LDL-cholesterol or LDL-protein taken up per hour per gram of tissue (μg/h per g) or per whole organ (μg/h per organ). Since no tissue was discarded in these studies, rates of LDL transport in individual organs as well as the whole body could be determined. Furthermore, this later value could be used as a measure of LDL production since in a steady-state the rate of LDL uptake by the whole body must equal the rate of LDL production. The LDL used to determine rates of LDL transport in these studies was obtained from normocholesterolemic control animals. In preliminary studies, however, we found that LDL preparations from animals fed hydrogenated coconut oil, safflower oil, fish oil concentrate, or control diet were transported at similar rates when infused into control rats (unpublished observation) suggesting that these dietary triglycerides, in contrast to several lipid-lowering drugs (24-26), do not significantly alter the metabolic behavior of LDL in vivo.

Lipid analysis

Total plasma cholesterol and triglyceride concentrations were determined using enzymatic kits (Boehringer Mannheim Biochemical, Indianapolis, IN). Plasma LDL-cholesterol concentrations were determined by simultaneously centrifuging plasma at densities of 1.020 and 1.055 g/ml at 164,905 *g* for 36 h. The cholesterol in the top one-third of eaoch tube was determined colorimetrically (27). Hepatic unesterified and esterified cholesterol were separated using silicic acid/celite columns (27) and quantitated using gas-liquid chromatography. Hepatic triglycerides were isolated by TLC and the fatty acid methyl esters were quantitated by capillary gas-liquid chromatography. The fatty acid profile of the various diets was determined by capillary gas-liquid chromatography of the methyl esters.

Calculations

Since plasma LDL concentrations varied by several fold in animals fed the different experimental diets, and since receptor-dependent LDL transport is a saturable process, it was not possible to directly equate changes in receptor-dependent LDL uptake with changes in LDL receptor activity (11). Thus, a change in receptor-dependent LDL uptake could be due to a change in LDL receptor activity in one or more tissues of the body or to an alteration in the rate of LDL production leading to a change in the number of LDL particles competing for the LDL receptor pathway. In order to relate changes in receptor-dependent LDL uptake to changes in receptor activity, the experimentally obtained uptake rates were superimposed on kinetic curves describing LDL uptake by the various organs of the body as a function of plasma LDL concentrations. These kinetic curves were previously established in control animals by measuring rates of receptor-dependent and receptor-independent LDL transport under conditions where plasma LDL concentrations were acutely varied from normal to 20 times normal by infusing mass amounts of unlabeled LDL (11). The relationship between total LDL uptake (J_t) and the concentration of LDL in plasma (C_1) can be described by the equation $J_t = (^*J^m C_1)/(^*K_m + C_1) + {}^*PC_1$, where $^*J^m$ equals the apparent maximal transport velocity, $^*K_m$ equals the concentration of LDL in plasma necessary to achieve one-half of this maximal transport velocity, and $^*P$ equals the apparent uptake 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 $^*P$ (11). 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 that would be seen in a normal animal at the same LDL concentration) in individual organs or the whole body.

The data are presented as mean values $\pm$ 1 SD. Where appropriate, statistical comparisons between groups were made using the Student's *t*-test.

RESULTS

Plasma LDL concentrations are known to fall when saturated fat in the diet is replaced by polyunsaturated fat. The first group of experiments was therefore undertaken to determine how the major processes that control plasma LDL concentrations are altered when saturated triglyceride is progressively removed from the diet and replaced with n-3 or n-6 polyunsaturated triglycerides. Groups of animals were fed control rodent diet supplemented with 20% triglyceride (by wt) at saturated to polyunsaturated ratios of 20%:0%, 15%:5%, 10%:10%, 5%:15%, and 0%:20% for 1 month. Although not shown, weight gain in the different experimental groups over the 1-month period of time was essentially identical. **Table 1** shows liver weights and cholesterol and triglyceride con-

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TABLE 1. Effect on cholesterol and triglyceride concentrations in the liver and plasma of substituting safflower oil or fish oil concentrate for hydrogenated coconut oil in the diet

Dietary Triglyceride			Hepatic Cholesterol				
Coconut Oil	Fish (FO) or Safflower (SO) oil	Liver Weight	Unesterified	Esterified	Hepatic Triglyceride	Plasma Cholesterol	Plasma Triglyceride
%		g/100 g body wt	mg/g		mg/g	mg/dl	
20	0%	3.2 ± 0.8	2.2 ± 0.2	0.4 ± 0.1	3.9 ± 0.6	72 ± 10	60 ± 8
15	5% FO	3.5 ± 0.6	2.2 ± 0.3	0.6 ± 0.2	3.1 ± 0.7	58 ± 8	44 ± 5[a]
15	5% SO	3.3 ± 0.7	2.3 ± 0.2	0.7 ± 0.1	3.5 ± 0.4	68 ± 10	58 ± 8
10	10% FO	3.7 ± 0.6	2.2 ± 0.2	0.5 ± 0.1[a]	2.7 ± 0.5	51 ± 9	40 ± 6[a]
10	10% SO	3.5 ± 0.5	2.2 ± 0.1	0.8 ± 0.2	3.6 ± 0.8	64 ± 11	64 ± 10
5	15% FO	3.8 ± 0.9	2.1 ± 0.3	0.5 ± 0.1[a]	2.8 ± 0.3	47 ± 6[a]	38 ± 7[a]
5	15% SO	3.4 ± 0.4	2.3 ± 0.2	1.0 ± 0.3	3.3 ± 0.6	64 ± 10	59 ± 9
0	20% FO	3.8 ± 0.6	2.2 ± 0.2	0.4 ± 0.1[a]	2.6 ± 0.5	43 ± 7[a]	34 ± 5[a]
0	20% SO	3.4 ± 0.7	2.2 ± 0.2	1.1 ± 0.3	3.2 ± 0.5	60 ± 9	58 ± 7

Groups of animals were fed diets supplemented with varying proportions of saturated and polyunsaturated triglycerides for 1 month. Each value represents the mean ± 1 SD for data obtained in 12 animals.
[a]Significantly different from the corresponding safflower oil group at $P < 0.001$.

centrations in the liver and plasma of animals fed the nine experimental diets. Liver weight tended to increase as dietary coconut oil was replaced by fish oil but remained unchanged when coconut oil was replaced by safflower oil. The content of unesterified cholesterol in the liver was not significantly altered by any of the experimental diets. Hepatic cholesterol ester levels increased by more than 2-fold as coconut oil was replaced by safflower oil but remained essentially unchanged when coconut oil was replaced by fish oil. Hepatic triglyceride levels tended to decrease as coconut oil was replaced by fish oil or, to a lesser extent, by safflower oil. Plasma cholesterol concentrations fell by up to 40% as coconut oil was replaced by fish oil and by up to 17% as coconut oil was replaced by safflower oil. Plasma triglyceride levels also decreased markedly (by up to 45%) when coconut oil was replaced by fish oil but remained unchanged when coconut oil was replaced by safflower oil.

Fig. 1 shows the changes in plasma LDL-cholesterol concentrations as saturated triglycerides were removed from the diet and replaced with polyunsaturated triglycerides. The plasma LDL-cholesterol concentration equaled 17.5 mg/dl in animals fed 20% coconut oil and progressively fell (by up to 31%) as dietary coconut oil was replaced by safflower oil (open circles). However, the decrease in plasma LDL-cholesterol concentrations was much greater (by up to 54%) when coconut oil was replaced by fish oil (closed circles) and much of this decrease occurred in going from 20% coconut oil to 15% coconut oil:5% fish oil. Thus, plasma LDL-cholesterol concentrations were significantly lower in animals fed n-3 polyunsaturated triglycerides than in animals fed n-6 polyunsaturated triglycerides at every ratio of saturated to polyunsaturated triglyceride that was examined. In these experiments, the cholesterol to protein ratio in plasma LDL varied by 6% or less among animals fed the various diets, indicating that the changes in plasma LDL-cholesterol concentrations shown in Fig. 1 largely reflect changes in the number of LDL particles in plasma.

The changes in the major transport processes that control plasma LDL concentrations were next determined under circumstances where saturated triglyceride was progressively removed from the diet and replaced with n-3 or n-6 polyunsaturated triglyceride. Absolute rates of total and receptor-independent LDL uptake were measured in vivo using homologous and methylated human LDL, respectively. Receptor-dependent LDL uptake was

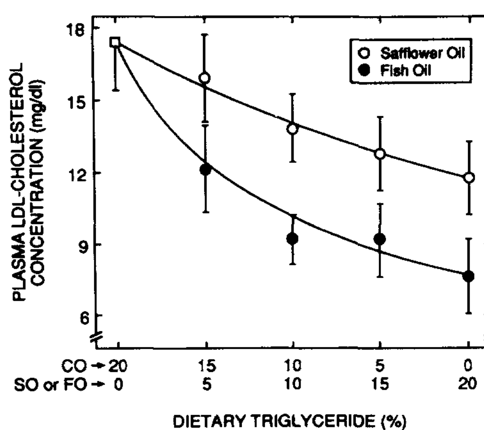

Fig. 1. Effect on plasma LDL-cholesterol concentrations of substituting safflower oil (SO) or fish oil concentrate (FO) for hydrogenated coconut oil (CO) in the diet. Groups of animals were fed control rodent diet supplemented with 20% triglyceride at saturated to polyunsaturated ratios of 20%:0%, 15%:5%, 10%:10%, 5%:15% or 0%:20% for 1 month. Each value represents the mean ± 1 SD for data obtained in 12 animals. The fish oil groups (closed circles) were significantly different from the safflower oil groups (open circles) at each saturated to polyunsaturated triglyceride ratio ($P < 0.001$).

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taken as the difference between total and receptor-independent uptake. Since receptor-dependent LDL transport is saturable and since plasma LDL concentrations varied considerably among animals fed the various experimental diets, changes in the rate of receptor-dependent LDL uptake could not be equated directly with changes in LDL receptor activity. In order to relate the changes in absolute rates of LDL-cholesterol 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-cholesterol uptake and circulating LDL-cholesterol concentrations in control rats fed standard rodent diet. **Fig. 2** shows the normal kinetic curves for LDL transport in the liver, extrahepatic tissues, and whole animal of rats fed control rodent diet. The shaded areas represent the relationship between total (stippled) and receptor-independent (hatched) LDL-cholesterol uptake and circulating LDL-cholesterol concentrations over the range of LDL levels encountered in these studies. Superimposed on these normal kinetic curves are the actual rates of total and receptor-independent LDL uptake for several of the experimental groups shown in Fig. 1. As shown in the top panel, total and receptor-independent LDL uptake rates in animals fed 20% coconut oil (open diamonds) were not displaced significantly from the normal kinetic curves, indicating that the addition of coconut oil to standard rodent diet had no significant effect on the receptor-dependent or receptor-independent transport processes in the liver. When dietary coconut oil was entirely replaced by fish oil concentrate (solid squares), total LDL-cholesterol uptake by the liver equaled 56 μg/h at a plasma LDL-cholesterol concentration of 7.5 mg/dl, whereas normal animals would be expected to transport 29 μg/h at this LDL-cholesterol concentration. Since receptor-independent LDL uptake was normal in these animals, the increase in total LDL-cholesterol uptake was due entirely to an increase in receptor-dependent LDL transport, which could be calculated to be about 2-fold higher than in normal animals at the same LDL-cholesterol concentration. Similarly, when half of the coconut oil in the diet was replaced by fish oil concentrate (solid circles), total LDL-cholesterol uptake in the liver equaled 63 μg/h at a plasma LDL-cholesterol concentration of 9.5 mg/dl, whereas normal animals would be expected to transport 38 μg/h at this LDL-cholesterol concentration. Again the rate of receptor-independent LDL uptake was normal and it could be calculated that the rate of receptor-dependent LDL transport was about 75% higher than would be seen in normal animals at the same LDL-cholesterol concentration. Although the changes were much smaller, rates of receptor-dependent LDL-cholesterol uptake in the liver were also increased (relative to those in normal animals at the same LDL-cholesterol concentration) when coconut oil was replaced by safflower oil. It should be noted that replacing dietary saturated triglyceride with n–3 or n–6 polyunsaturated triglyceride increased hepatic LDL receptor activity but had relatively little effect on absolute rates of total LDL-cholesterol uptake by the liver. This was due to the fact that as hepatic LDL receptor activity increased, plasma LDL-cholesterol concentrations fell and in the new steady-state, the increase in LDL receptor activity enabled the liver to take up LDL-cholesterol at essentially the same rate even though the concentration of LDL-cholesterol in plasma was greatly reduced.

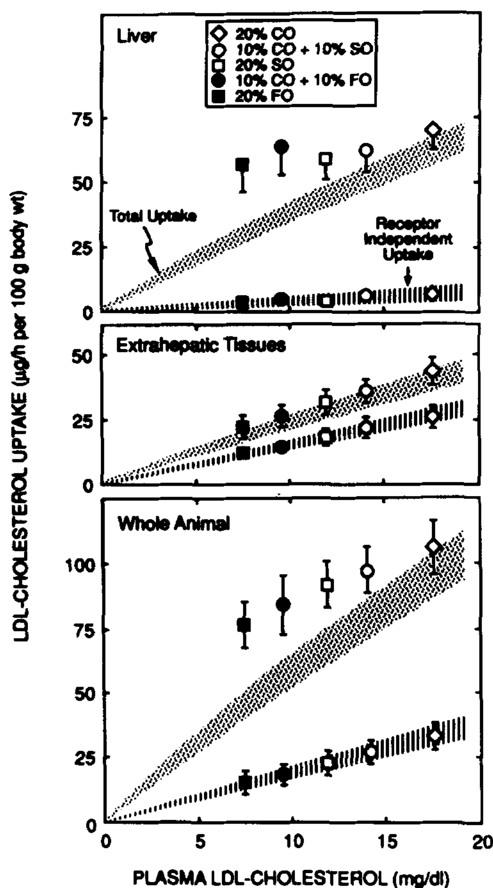

Fig. 2. Effect on LDL-cholesterol uptake in the whole liver (top panel), all of the extrahepatic tissues combined (middle panel), and whole animal (bottom panel) of substituting safflower oil (SO) or fish oil concentrate (FO) for hydrogenated coconut oil (CO) in the diet. Groups of animals were fed control rodent diet supplemented with 20% triglyceride at saturated to polyunsaturated ratios of 20%:0%, 10%:10% or 0%:20% for 1 month. The shaded areas represent the kinetic curves for total (stippled) and receptor-independent (hatched) LDL-cholesterol transport determined in control animals fed standard rodent diet as described in Methods. Superimposed on these normal kinetic curves are the absolute rates of total and receptor-independent LDL-cholesterol uptake in the experimental animals plotted as a function of the plasma LDL-cholesterol concentration in the same animals. Each point represents the mean ± 1 SD for data obtained in 12 animals.

In these studies, all of the extrahepatic tissues combined accounted for only 15–20% of whole body receptor-dependent LDL uptake. Rates of LDL transport in the

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various extrahepatic organs were therefore summed and these data are shown in the middle panel of Fig. 2. Rates of total and receptor-independent LDL transport were not significantly displaced from the normal kinetic curves, indicating that the receptor-dependent and receptor-independent transport processes were not altered in the extrahepatic tissues by any of the dietary manipulations. Finally, as shown in the bottom panel of Fig. 2, LDL uptake rates in the liver and extrahepatic tissues can be summed to yield whole animal uptake rates. In a steady state, total LDL-cholesterol uptake in the whole animal must equal the rate of LDL-cholesterol production. As is apparent, total LDL-cholesterol uptake, and thus the rate of LDL-cholesterol production, equaled 107 μg/h in animals fed 20% coconut and fell to 90 μg/h and 76 μg/h when coconut oil was completely replaced by fish oil concentrate and safflower oil, respectively.

From the type of analysis illustrated in Fig. 2, the changes in absolute rates of total and receptor-independent LDL transport 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 that would be seen in control animals at the same LDL concentration) and these values, along with the changes in LDL-cholesterol production are shown in **Fig. 3.** As seen in the top panel, hepatic LDL receptor activity increased by nearly 2-fold as dietary coconut oil was replaced by fish oil (closed circles). Again the greatest increase in hepatic LDL receptor activity was seen in going from a 20% coconut oil diet to a 15% coconut oil:5% fish oil diet. Hepatic LDL receptor activity increased only slightly when dietary coconut oil was replaced by safflower oil (open circles). Thus, hepatic LDL receptor activity was significantly greater in animals fed n−3 polyunsaturated triglycerides than in animals fed n−6 polyunsaturated triglycerides at every ratio of saturated to polyunsaturated triglyceride that was studied. In contrast to LDL receptor activity in the liver, receptor activity in the extrahepatic tissues was largely unaffected by the ratio of saturated to polyunsaturated triglyceride or the type of polyunsaturated triglyceride in the diet as shown in the middle panel of Fig. 3. As shown in the bottom panel of Fig. 3, rates of LDL-cholesterol production fell as coconut oil was progressively removed from the diet and replaced by fish oil or, to a lesser extent, by safflower oil.

Thus, in this model, when total triglyceride was kept constant, the decrease in LDL concentrations when saturated fat was replaced by polyunsaturated fat was greater with n−3 than with n−6 polyunsaturated triglycerides. Furthermore, the differences in plasma LDL concentrations were due primarily to the much greater increase in hepatic LDL receptor activity when n−3 fatty acids were used to substitute for saturated triglyceride in the diet.

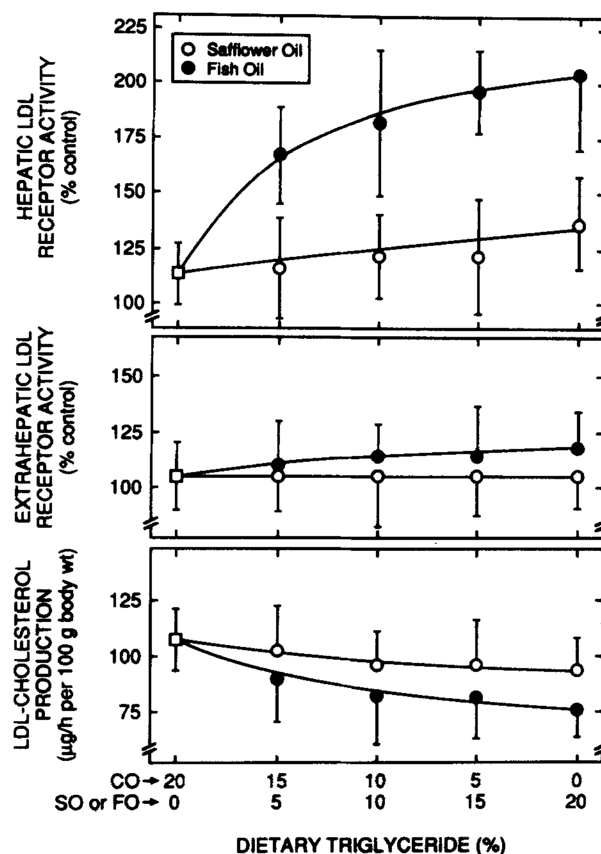

Fig. 3. Effect on hepatic (top panel) and extrahepatic (middle panel) LDL receptor activity and on LDL-cholesterol production (bottom panel) of substituting safflower oil (SO) or fish oil concentrate (FO) for hydrogenated coconut oil (CO) in the diet. Groups of animals were fed control rodent diet supplemented with 20% triglyceride at saturated to polyunsaturated ratios of 20%:0%, 15%:5%, 10%:10%, 5%:15% or 0%:20% for 1 month. Values for receptor activity represent the rates of receptor-dependent LDL uptake in experimental animals as percentages of the rates of receptor-dependent LDL uptake that would occur in control animals at the same LDL concentration. Each value represents the mean ± 1 SD for data obtained in 12 animals. Hepatic LDL receptor activity was significantly greater in the fish oil groups (closed circles) than in the safflower oil groups (open circles) at each saturated to polyunsaturated triglyceride ratio ($P < 0.001$).

From these types of experiments, however, it was not possible to determine whether the decrease in plasma LDL concentrations when saturated triglyceride was replaced by polyunsaturated triglyceride was due simply to the removal of the saturated triglyceride or to an independent effect of the polyunsaturated triglyceride. Therefore, a group of experiments was undertaken to determine whether polyunsaturated triglycerides exert a favorable effect on LDL metabolism when used to supplement a high saturated fat diet rather than to replace saturated fat in the diet. Groups of animals were fed control rodent diet supplemented with 15% hydrogenated coconut oil (by wt) with or without an additional 5% safflower oil or fish oil concentrate for 1 month. Since the addition of 15% coco-

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nut oil to the control rodent diet diluted out the other components of this diet, a group of animals was fed control rodent diet supplemented with 15% corn starch. Similarly, since the addition of 5% fish oil or safflower oil to the high saturated fat diet diluted out the other components of this diet, an additional group of animals was fed the high saturated fat diet supplemented with 5% corn starch. (Although not shown, identical results were obtained whether corn starch was exchanged for dietary fat on a wt/wt or cal/cal basis.) In these studies, weight gain tended to be higher in animals fed the triglyceride-rich diets although these differences did not achieve statistical significance over the 1 month feeding period. However, when maintained on these diets for several months, weight gain was proportional to the fat content of the diet (unpublished observation from this laboratory). **Table 2** shows liver weights and cholesterol and triglyceride concentrations in the liver and plasma of animals fed the various diets. Liver weights were similar among animals fed the different diets although they tended to be higher in animals fed the fish oil-containing diets. Hepatic unesterified cholesterol levels were essentially identical in all experimental groups. Hepatic esterified cholesterol levels increased in animals fed coconut oil but were not significantly altered by the further addition of polyunsaturated triglyceride. Similarly, hepatic triglyceride levels increased in animals fed coconut oil but were unaffected by the further addition of polyunsaturated triglyceride. Plasma cholesterol concentrations increased in animals fed coconut oil and returned to control values with the further addition of fish oil, whereas the further addition of safflower oil had little effect. Plasma triglyceride concentrations remained unchanged when coconut oil was added to the diet and decreased significantly below control values with the further addition of fish oil, whereas safflower oil had no effect.

The effect of these diets on plasma LDL-cholesterol concentrations is shown in **Fig. 4.** Plasma LDL-cholesterol concentrations equaled 12 mg/dl in animals on ground rodent diet. The addition of 15% hydrogenated coconut oil to this diet increased plasma LDL-cholesterol concentrations to about 17 mg/dl, whereas the addition of 15% corn starch was without effect. The further addition of 5% fish oil to the high saturated fat diet returned plasma LDL-cholesterol concentrations essentially to control values, whereas the further addition of 5% safflower oil or corn starch had little effect. Again, in these experiments the cholesterol to protein ratio of plasma LDL varied by 6% or less, suggesting that the changes in LDL-cholesterol concentrations shown in Fig. 4 reflect a change in the number of LDL particles in plasma.

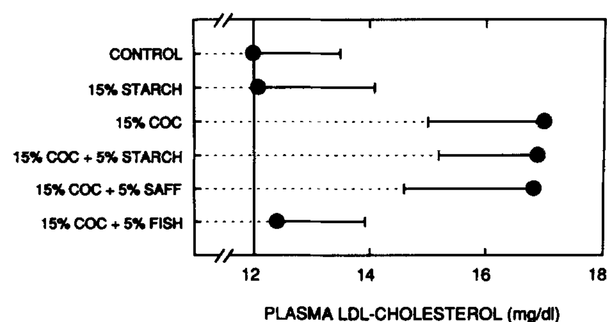

Fig. 4. Effect on plasma LDL-cholesterol concentrations of supplementing a high saturated fat diet with safflower oil or fish oil concentrate. Groups of animals were fed control rodent diet supplemented with 15% hydrogenated coconut oil with or without an additional supplement of 5% corn starch, safflower oil, or fish oil concentrate. Each value represents the mean ± 1 SD for data obtained in 12 animals. Plasma LDL-cholesterol concentrations significantly increased when coconut oil was added to control rodent diet ($P < 0.001$) and significantly decreased when this high saturated fat diet was further supplemented with fish oil concentrate ($P < 0.001$).

The mechanisms responsible for these changes in LDL concentrations are shown in **Fig. 5.** The changes in hepatic and extrahepatic LDL receptor activity and in rates of LDL-cholesterol production are expressed as a percentage of the values obtained in animals fed control

TABLE 2. Effect on cholesterol and triglyceride concentrations in the liver and plasma of supplementing a high saturated fat diet with safflower oil or fish oil concentrate

Diet	Liver Weight	Hepatic Cholesterol: Unesterified	Hepatic Cholesterol: Esterified	Hepatic Triglyceride	Plasma Cholesterol	Plasma Triglyceride
	g/100 g body wt	*mg/g*		*mg/g*	*mg/dl*	
Control	3.1 ± 0.4	2.1 ± 0.3	0.2 ± 0.04	2.3 ± 0.3	61 ± 8	57 ± 6
15% Starch	3.0 ± 0.6	2.2 ± 0.3	0.3 ± 0.05	2.5 ± 0.4	60 ± 10	61 ± 9
15% Coconut oil	3.2 ± 0.6	2.1 ± 0.2	0.5 ± 0.07	3.8 ± 0.6	74 ± 10	55 ± 7
15% Coconut oil + 5% starch	3.2 ± 0.5	2.1 ± 0.4	0.4 ± 0.07	3.7 ± 0.8	76 ± 12	59 ± 7
15% Coconut oil + 5% safflower oil	3.3 ± 0.5	2.2 ± 0.3	0.7 ± 0.1	3.7 ± 0.7	72 ± 8	57 ± 8
15% Coconut oil + 5% fish oil	3.6 ± 0.4	2.1 ± 0.2	0.6 ± 0.1	2.9 ± 0.5	59 ± 7[a]	42 ± 5[a]

Groups of animals were fed control rodent diet supplemented with 15% hydrogenated coconut oil with or without an additional 5% corn starch, safflower oil, or fish oil concentrate for 1 month. Each value represents the mean ± 1 SD for data obtained in 12 animals.
[a]Significantly different from the corresponding safflower oil group at $P < 0.001$.

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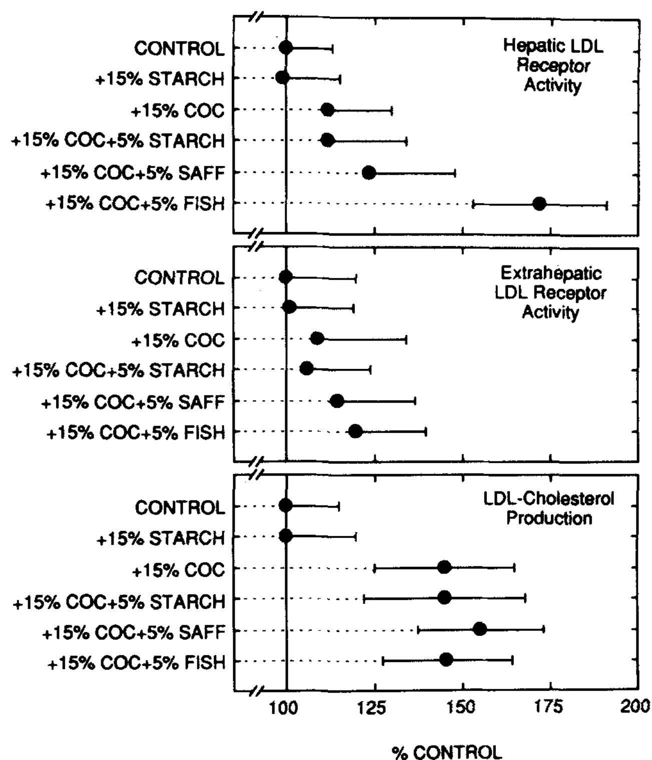

Fig. 5. Effect on hepatic (top panel) and extrahepatic (middle panel) LDL receptor activity and on LDL-cholesterol production (bottom panel) of supplementing a high saturated fat diet with safflower oil or fish oil concentrate. Groups of animals were fed control rodent diet supplemented with 15% hydrogenated coconut oil with or without an additional supplement of 5% corn starch, safflower oil, or fish oil concentrate. Values for receptor activity represent the rates of receptor-dependent LDL uptake in experimental animals as percentages of the rates of receptor-dependent LDL uptake that would occur in control animals at the same LDL concentration. Each value represents the mean ± 1 SD for data obtained in 12 animals. LDL-cholesterol production significantly increased when coconut oil was added to control rodent diet ($P < 0.001$) and hepatic LDL receptor significantly increased when this high saturated fat diet was further supplemented with fish oil concentrate ($P < 0.001$).

rodent diet. As shown in the top panel, hepatic LDL receptor activity increased only slightly when the diet was supplemented with 15% hydrogenated coconut oil. The further addition of 5% fish oil to this high saturated fat diet markedly increased hepatic LDL receptor activity (by nearly 75%) whereas the addition of 5% safflower oil or corn starch had little effect. As shown in the middle panel, extrahepatic LDL receptor activity, which accounted for only about 20% of total body LDL receptor activity in these experiments, was not greatly altered by the addition of hydrogenated coconut oil to the diet or by the further supplementation with safflower or fish oil. As shown in the bottom panel, rates of LDL-cholesterol production increased by about 45% when 15% hydrogenated coconut oil was added to the diet and remained elevated at this level despite further supplementation with fish oil or safflower oil. Thus, addition of hydrogenated coconut oil to a low fat diet raised plasma LDL concentrations by increasing the rate of LDL production. The further supplementation of this high saturated fat diet with fish oil reduced plasma LDL concentrations essentially to control values by increasing hepatic LDL receptor activity, whereas supplementation with safflower oil had little effect on plasma LDL concentrations.

DISCUSSION

The concentration of LDL-cholesterol in plasma is determined by the rate at which LDL-cholesterol is produced relative to the rate at which LDL is cleared from plasma by receptor-dependent and receptor-independent transport processes. The present studies provide quantitative information on how polyunsaturated triglycerides interact with saturated triglycerides to regulate these major determinants of circulating LDL-cholesterol concentrations. When the total fat content of the diet was kept constant, plasma LDL-cholesterol concentrations fell as saturated fat was progressively removed from the diet and replaced by fish oil concentrate or safflower oil. In either case, the fall in plasma LDL-cholesterol concentrations was due to an increase in hepatic LDL receptor activity and a decrease in the rate of LDL production. However, the increase in hepatic LDL receptor activity, and thus the decrease in plasma LDL-cholesterol concentrations, was much greater when fish oil concentrate was used as the substitute than when safflower oil was used as the substitute. Differences in hepatic LDL receptor activity and plasma LDL-cholesterol concentrations between the fish oil and safflower oil groups became apparent when these polyunsaturated triglycerides were used to replace 25% or more of the saturated fat in the diet. Similar changes in plasma total and LDL cholesterol concentrations have been reported in normal human subjects when large amounts of saturated fat have been replaced by polyunsaturated fats (1-7). Thus, when used to replace saturated fat in the diet, n-3 fatty acids appear to be more effective than n-6 fatty acids in lowering total and LDL-cholesterol concentrations and, at least in the rat, the mechanism is a greater increase in hepatic LDL receptor activity when n-3 fatty acids are substituted for saturated fat. Since the n-6 fatty acid content of the safflower oil was nearly twice the n-3 fatty acid content of the fish oil concentrate, the differences between these two oils may even be greater when equal amounts of n-3 and n-6 fatty acids are compared.

Although polyunsaturated triglycerides clearly lowered plasma LDL concentrations when used to replace saturated fat in the diet, these studies provide evidence that n-3 fatty acids may also be effective when used to supplement a high saturated fat diet. The addition of 15% saturated fat to control rodent diet raised plasma LDL con-

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centrations by increasing the rate of LDL production. The further addition of 5% fish oil concentrate returned plasma LDL concentrations to control values by stimulating hepatic LDL receptor activity, whereas safflower oil had little effect. Thus, 5% fish oil concentrate offset the LDL raising effect of 15% saturated fat although rates of LDL production and catabolism were greatly increased in animals on the high fat diet compared to animals on the low fat diet. These findings differ from the experience in humans where supplementation of Western diets with fish oil has had relatively small and inconsistent effects on plasma LDL concentrations (28-31). The amount of n-3 fatty acids administered as a supplement in many of these studies was much smaller than that used in the present studies and was often provided in the form of crude fish oil, which, in addition to n-3 fatty acids, contains considerable amounts of saturated fatty acids and cholesterol. In addition, however, responsiveness to n-3 fatty acid supplementation may vary somewhat from species to species and in humans may differ depending on the underlying lipid phenotype (28-31).

In these studies, the changes in plasma LDL-cholesterol concentrations in animals fed the various triglycerides were due almost entirely to changes in the rate of LDL-cholesterol production and to changes in the level of LDL receptor activity in the liver. In contrast, the various dietary triglycerides had little effect on receptor activity in the extrahepatic tissues and had no effect on receptor-independent LDL transport in any organ of the body. In addition, LDL preparations from animals fed coconut oil, safflower oil, or fish oil concentrate were cleared from the plasma at similar rates when infused into control animals, suggesting that these dietary triglycerides do not alter the affinity of the LDL particle for its receptor.

The mechanism by which dietary fatty acids regulate the rate of LDL production is not known. The rate of LDL-cholesterol production fell by about 40% as dietary saturated fat was replaced by fish oil. Since the cholesterol to protein ratio of LDL decreased by less than 6% in these animals, the decrease in LDL-cholesterol production presumably represents a decrease in the number of LDL particles being formed. LDL are formed during the metabolism of VLDL (17, 32-34), although direct secretion of LDL from the liver may also occur under certain circumstances (35-37). Thus, a change in the rate of LDL production usually reflects a change in the rate of VLDL secretion by the liver or a change in the extent to which VLDL are converted to LDL. Studies in cultured hepatocytes and in perfused liver suggest that n-3 polyunsaturated fatty acids reduce triglyceride and cholesterol output into VLDL, apparently by inhibiting triglyceride synthesis and cholesterol esterification in the liver (18, 19, 38, 39). On the other hand, n-3 fatty acids appear to have relatively little effect on the output of VLDL apoprotein B, suggesting that these fatty acids predominantly affect the composition of VLDL rather than the number of VLDL particles secreted by the liver (19, 39). We have noted a similar situation in the rat where dietary fish oil reduces the rate at which VLDL triglyceride, but not VLDL protein, accumulates in plasma after the injection of triton WR-1339 (L. A. Woollett and D. K. Spady, unpublished observation).

Little is known about the effect of dietary fatty acids on the conversion of VLDL to LDL. In the rat, the majority of VLDL are cleared by the liver as VLDL remnants while only a minority are metabolized to LDL (17, 34). Since the hepatic uptake of VLDL remnants is mediated by LDL receptors (17), an increase in hepatic LDL receptor activity, as occurs when fish oil is substituted for saturated fat, may increase VLDL remnant clearance by the liver thus allowing fewer of these particles to be metabolized to LDL. However, a simple reciprocal relationship between rates of LDL production and hepatic LDL receptor activity was not always observed in these studies (Fig. 4). In addition, dietary fatty acids may alter the activities of hepatic and lipoprotein lipase (40), two enzymes thought to play an important role in the conversion of VLDL to LDL. Finally, the effects of n-3 fatty acids may differ somewhat from species to species. For example, in miniature pigs the decrease in LDL concentrations in animals fed fish oil is apparently due to a decrease in the amount of LDL directly secreted by the liver while the proportion of VLDL converted to LDL actually increases (41). The situation is also different in cholesterol-fed monkeys where replacement of saturated fat with fish oil decreases plasma LDL-cholesterol levels mainly by decreasing the cholesterol content of LDL (19). Some of these differences in response to dietary fish oil may be due to the fact that, in some species, dietary fatty acids may have remarkably different effects on LDL metabolism depending on the amount of cholesterol also added to the diet (42).

How dietary fatty acids regulate receptor-dependent LDL transport in the liver is also unknown. The greatest increase in hepatic LDL receptor activity was seen when saturated fat was replaced by fish oil concentrate. Fish oil may reduce cholesterol absorption from the small intestine (43); however, the diets used in these studies contained very little cholesterol and, in the rat, even large amounts of dietary cholesterol have little effect on hepatic LDL receptor activity or plasma lipoprotein levels (44). Furthermore, there was no clear inverse relationship between hepatic LDL receptor activity and hepatic cholesterol ester levels as would be anticipated if the various dietary triglycerides were simply altering the amount of cholesterol delivered to the liver. Dietary fish oil apparently does not increase the binding of LDL to liver membranes, suggesting that the number of LDL receptors is not increased (45). An increase in receptor-dependent LDL transport in the absence of an increase in the number of LDL receptors suggests an alteration in receptor

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function. In some studies, dietary fat-induced changes in LDL receptor activity have correlated with changes in the composition and physical properties of cell membranes (46). Further studies will be necessary to determine whether n-3 fatty acids actually alter the distribution or the recycling rate of LDL receptors in the hepatocyte (47, 48).

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