Polyunsaturated fatty acids up-regulate hepatic scavenger receptor B1 (SR-BI) expression and HDL cholesteryl ester uptake in the hamster

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Abstract Diets rich in polyunsaturated fatty acids lower plasma HDL cholesterol concentrations when compared to diets rich in saturated fatty acids. We investigated the mechanistic basis for this effect in the hamster and sought to determine whether reduced plasma HDL cholesterol concentrations resulting from a high polyunsaturated fat diet are associated with a decrease in reverse cholesterol transport. Animals were fed semisynthetic diets enriched with polyunsaturated or saturated fatty acids for 6 weeks. We then determined the effect of these diets on the following parameters: *1*) hepatic scavenger receptor B1 (SR-BI) mRNA and protein levels, *2*) the rate of hepatic HDL cholesteryl ester uptake, and *3*) the rate of cholesterol acquisition by the extrahepatic tissues (from de novo synthesis, LDL and HDL) as a measure of the rate of reverse cholesterol transport. Compared to saturated fatty acids, dietary polyunsaturated fatty acids up-regulated hepatic SR-BI expression by ~50% and increased HDL cholesteryl ester transport to the liver; as a consequence, plasma HDL cholesteryl ester concentrations were reduced. Although dietary polyunsaturated fatty acids increased hepatic HDL cholesteryl ester uptake and lowered plasma HDL cholesterol concentrations, there was no change in the cholesterol content or in the rate of cholesterol acquisition (via de novo synthesis and lipoprotein uptake) by the extrahepatic tissues. These studies indicate that substitution of polyunsaturated for saturated fatty acids in the diet increases SR-BI expression and lowers plasma HDL cholesteryl ester concentrations but does not affect reverse cholesterol transport.— Spady, D. K., D. M. Kearney, and H. H. Hobbs. Polyunsaturated fatty acids up-regulate hepatic scavenger receptor B1 (SR-BI) expression and HDL cholesteryl ester uptake in the hamster. *J. Lipid Res.* 1999. **40:** 1384–1394.

Supplementary key words HDL • lipoproteins • cholesterol • cholesteryl esters • liver • reverse cholesterol transport

Epidemiological studies show that the risk of developing clinical coronary heart disease is directly related to the plasma concentration of low density lipoprotein (LDL) cholesterol (1) and inversely related to the plasma concentration of high density lipoprotein (HDL) (2–4). The protective role of HDL is usually attributed to its ability to transport excess cholesterol from peripheral tissues back to the liver in a process that has been termed reverse (5, 6), or centripetal (7), cholesterol transport. Reverse cholesterol transport is initiated in extrahepatic tissues by the transfer of unesterified cholesterol from cell membranes to nascent HDL (8–10). A portion of this cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT) to cholesteryl esters, which by virtue of their hydrophobicity partition into the core of the HDL particle (8). HDL cholesteryl esters are returned to the liver through several pathways including *i*) transfer to lower density lipoproteins with subsequent uptake via the LDL receptor pathway (11), *ii*) uptake of the intact HDL particle (12), and *iii*) selective uptake of HDL cholesteryl esters resulting in an HDL particle of reduced size and cholesteryl ester content (13, 14). The scavenger receptor B1 (SR-BI) plays a major role in selective HDL cholesteryl ester transport (15). SR-BI was shown to mediate selective cholesteryl ester uptake when transfected into cells (16). In mice, overexpressing SR-BI in the liver results in the virtual elimination of HDL cholesteryl ester from plasma (17, 18) whereas disrupting the SR-BI gene by targeted mutation leads to a decrease in HDL cholesteryl ester uptake by the liver and an increase in plasma HDL cholesteryl ester concentrations (19, 20).

The kinetic characteristics of HDL cholesteryl ester transport have been analyzed in the hamster in vivo (21). These studies showed that the majority of HDL cholesteryl ester (~75%) is cleared from plasma by a saturable transport process in the liver and that the transport of HDL cholesteryl ester to the liver closely approximates the rate of cholesterol acquisition by the extrahepatic tissues

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Abbreviations: LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LCAT, lecithin cholesterol acyltransferase; SR-BI, scavenger receptor B1; CETP, cholesteryl ester transfer protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SF-1, steroidogenic factor-1.

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(from de novo synthesis, LDL and HDL). Unexpectedly, the concentration of HDL cholesteryl ester necessary to achieve half-maximal transport (K_m) in the liver was ~14 mg/dl, well below the normal plasma concentration of HDL cholesteryl ester in the hamster. As a consequence, HDL cholesteryl ester uptake by the liver is largely saturated at normal HDL concentrations and it can be predicted that alterations in circulating HDL levels (due to dietary factors or pharmacological agents) will not be accompanied by a change in the absolute flux of HDL cholesteryl ester to the liver unless the activity of the transport mechanism is regulated or the plasma HDL concentration is drastically reduced.

The role of dietary fatty acids in regulating plasma lipoprotein concentrations has been well documented (22). Compared to saturated fatty acids, polyunsaturated fatty acids significantly lower plasma total and LDL cholesterol concentrations in humans (22), nonhuman primates (23, 24), and hamsters (25). High levels of polyunsaturated fatty acids may also lower plasma HDL cholesterol concentrations (23, 24, 26–28). The mechanisms responsible for this effect and the implications with respect to reverse cholesterol transport and atherogenesis are not fully understood. In the hamster (26), as in humans (27, 28) and nonhuman primates (23, 24), plasma HDL cholesterol concentrations fall when a diet rich in saturated fatty acids is replaced by a diet rich in polyunsaturated fatty acids. The present studies were undertaken to examine the mechanistic basis for this effect and to determine whether lower plasma HDL cholesterol concentrations resulting from a high polyunsaturated fat diet are associated with a decrease in the rate of reverse cholesterol transport.

METHODS

Animals and diets

Male Golden Syrian hamsters (Sasco, Inc., Omaha, NE) were housed in colony cages and subjected to alternating 12-h periods of light and darkness for at least 3 weeks prior to introduction of the experimental diets. The control semisynthetic diet used in these studies contained 20% soy protein, 0.3% dl-methionine, 10% cellulose, 8.5% salt mix, 1% vitamin mix, 0.2% choline bitartrate, 2% corn oil, and 58% corn starch. The experimental diets were prepared by replacing corn starch with the desired amount of triglyceride on a cal/cal basis assuming 4 cal/g of corn starch and 9 cal/g of triglyceride. The triglycerides used in these studies were safflower oil and hydrogenated coconut oil. The fatty acid composition of the safflower oil, as determined by capillary gas–liquid chromatography, was 7% as 16:0, 2% as 18:0, 12% as 18:1, and 78% as 18:2. The hydrogenated coconut oil contained 9% as 8:0, 6% as 10:0, 51% as 12:0, 17% as 14:0, 8% as 16:0, and 8% as 18:0. The diets were fed ad lib for 6 weeks and all studies were carried out during the mid-dark phase of the light cycle. All experiments were approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center at Dallas.

Determination of HDL cholesteryl ether transport

Plasma was obtained from hamsters maintained on a standard low-cholesterol, low-fat rodent diet and HDL was isolated in the density range of 1.070–1.21 g/ml by sequential ultracentrifugation (29). The HDL was labeled with either the intracellularly trapped [1α,2α(n)-³H]cholesteryl oleyl ether (30, 31) or with [cholesteryl-4-¹⁴C]oleate by exchange from donor liposomes (13, 21, 32) and used within 24 h. Animals were administered a priming dose of [³H]cholesteryl ether-labeled HDL through the femoral vein followed by a continuous infusion of the same radiolabeled lipoprotein so as to maintain a constant plasma specific activity (21). The infusions of [³H]cholesteryl ether-labeled HDL were continued for 4 h at which time each animal was administered [¹⁴C]cholesteryl ester-labeled HDL intravenously (as a marker of the volume of plasma in each tissue) and killed 10 min later. Plasma and tissue samples were saponified in alcoholic KOH (33) and the sterols were quantitatively extracted (33, 34) and assayed for ³H and ¹⁴C as previously described (21). The tissue spaces achieved by the labeled HDL at 10 min (¹⁴C dpm per gram of tissue divided by the ¹⁴C dpm per microliter of plasma) and at 4 h and 10 min (³H dpm per gram of tissue divided by the steady-state ³H dpm per microliter of plasma) were then calculated and have the units of μl/g. The increase in tissue space over the 4-h experimental time period equals the rate of radiolabeled HDL cholesteryl ether movement into each organ and is expressed as the microliters of plasma cleared of its HDL cholesteryl ether content per hour per gram of tissue or per whole organ (21). Clearance values were multiplied by the plasma HDL cholesteryl ester concentration to obtain the absolute rates of HDL cholesteryl ester uptake expressed as the micrograms of HDL cholesteryl ester taken up per hour per gram of tissue or per whole organ. This assumes that the labeled cholesteryl ether accurately traces cholesteryl ester in the HDL particle as previously demonstrated in the rat by Glass et al. (31, 35). Preliminary studies in the hamster showed that the tissue spaces at 10 min and the clearance rate of HDL from plasma were the same whether the particles were labeled with [³H]cholesteryl oleyl ether or [¹⁴C]cholesteryl oleate. In some experiments the relationship between the rate of HDL cholesteryl ester transport and the concentration of HDL cholesteryl ester in plasma was determined by adding mass amounts of unlabeled hamster HDL (d 1.07–1.21 g/ml) to the primed infusions of trace-labeled HDL.

Determination of LDL cholesterol transport

Rates of LDL cholesterol uptake were measured using primed infusions of [¹²⁵I]tyramine cellobiose-labeled hamster LDL as described previously (36). The infusions were continued for 4 h at which time each animal was administered a bolus of ¹³¹I-labeled hamster LDL and killed 10 min later. Tissue samples and plasma were assayed for radioactivity and rates of LDL cholesterol uptake were calculated as described (36).

Determination of cholesterol synthesis rates

Hamsters were administered ~100 mCi of [³H]water intravenously (33). One hour later 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 liver and the entire remaining carcass were taken for the isolation of digitonin-precipitable sterols. Tissues were saponified and the digitonin-precipitable sterols were isolated and assayed for tritium content. Rates of sterol synthesis are expressed as the μg of sterol formed per hour assuming that 22 ³H may be incorporated into the cholesterol molecule during synthesis from acetyl CoA in the presence of [³H]water in vivo (33).

Immunoblot analysis

Membrane fractions were prepared from pulverized hamster tissue that had been frozen in liquid N_2 immediately after har-

vesting and stored at −80°C. Immunoblot analysis was performed under reducing conditions using polyclonal antibodies raised against the C-terminal 14 amino acids of the mouse SR-BI (37).

Determination of mRNA levels

Hepatic SR-BI and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were determined using a nuclease protection assay as previously described (36). GAPDH was used as an invariant control as levels of this transcript were not altered by the experimental diets. The SR-BI probe used in these studies detects both splice forms of SR-BI (38). ^{32}P-labeled probes were synthesized as previously described using 0.5 μm [^{32}P]dCTP and 1 μm (SR-BI) or 300 μm (GAPDH) unlabeled dCTP.

Samples of liver were homogenized in ULTRASPEC-II (Biotecx Laboratories, Houston, TX). Total RNA (40 μg) was hybridized with the ^{32}P-labeled cDNA probes simultaneously at 48°C overnight. Unhybridized probe, present in excess relative to the amount of specific mRNA, was then digested with 40 units of mung bean nuclease (GIBCO BRL/Life technologies, Gaithersburg, MD). The mRNA-protected ^{32}P-labeled probes were separated on 7 m urea, 6% polyacrylamide gels together with ^{32}P-labeled MspI-digested pBR322 size standards. The radioactivity in each band, as well as background radioactivity, was quantified using an isotopic imaging system (Ambis, Inc., San Diego, CA).

Determination of plasma, liver, and carcass cholesterol concentrations

Blood samples were obtained by exsanguination through the abdominal aorta. The liver was removed and saponified. The entire remaining carcass was also saponified. The cholesterol concentration in the liver and carcass (extrahepatic tissues) was determined by gas–liquid chromatography and is expressed as mg per wet weight of tissue. The cholesterol distribution in plasma lipoproteins was determined by FPLC as previously described (21). Cholesterol was assayed using an enzymatic kit (Sigma Chemical Co.).

Calculations and statistical analysis

The liver takes up HDL cholesteryl ester and apoA-I via saturable transport processes as recently described (21). Because HDL uptake by the liver is saturable, and because plasma HDL concentrations varied among the experimental groups, changes in HDL transport could not be directly equated with regulation of the activity of the HDL transport process. To determine whether a change in HDL cholesteryl ether transport was due to regulation of the HDL transport mechanism or simply reflected a change in the concentration of particles competing for this transport mechanism, rates of hepatic HDL cholesteryl ether transport were superimposed on kinetic curves (±95% confidence intervals) that describe the relationship between HDL transport and circu-

lating HDL concentrations in the control hamster. The kinetic parameters for HDL cholesteryl ether transport were previously determined by quantifying rates of HDL cholesteryl ether transport in control hamsters under conditions in which circulating HDL concentrations were acutely raised and maintained at various levels during the 4-h period of time over which HDL transport was measured (21). By relating rates of HDL cholesteryl ether transport in the experimental animals to these normal kinetic curves, it was possible to determine how the experimental diets affected transporter activity.

The data are presented as means ± 1 SD. To test for differences among dietary regimens, one-way analysis of variance was performed using the statistical software package StatView 4.5 (Abacus Concepts, Berkeley, CA) for the Macintosh. Significant effects were further analyzed using Sheffe's F procedure for post-hoc comparisons.

RESULTS

Effect of dietary fatty acids on plasma and liver cholesterol levels
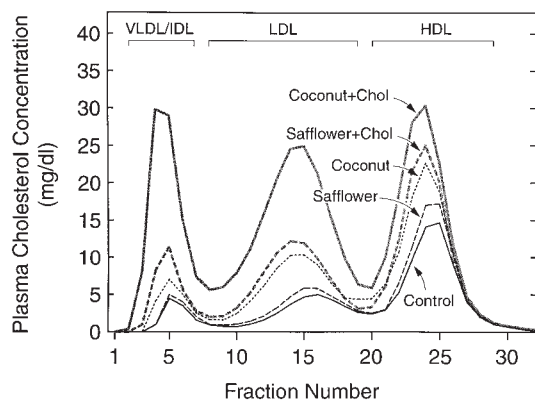
Hamsters were fed a control semisynthetic diet in which safflower oil or coconut oil was substituted (on a calorie/calorie basis) for carbohydrate to provide 30% of energy. The triglyceride-enriched diets contained either 0% or 0.08% (~180 mg/1000 calories) cholesterol. The cholesterol concentration in plasma, liver and the extrahepatic tissues of these animals is shown in **Table 1**. Plasma cholesterol concentrations were lowest in animals fed the cholesterol-free, low-fat diet. Plasma cholesterol concentrations were significantly higher in animals fed coconut oil than in animals fed safflower oil both on the cholesterol-free and cholesterol-enriched diets. In contrast, liver cholesterol concentrations were significantly higher on the safflower oil diets than on the coconut oil diets. The cholesterol concentration in the carcass, which included all extrahepatic tissues, was not affected by the experimental diets. The lipoprotein distribution of plasma cholesterol in animals fed the control and triglyceride-containing diets is shown in **Fig. 1**. The cholesterol content of all lipoprotein fractions was higher in animals fed coconut oil than in animals fed safflower oil both on the cholesterol-free and the cholesterol-enriched diets. Plasma HDL cholesteryl ester concentrations are shown in Table 1. On the cholesterol-free diet, plasma HDL cholesterol concentra-

TABLE 1.   Characteristics of the hamsters used in these studies

	Control	Safflower Oil	Coconut Oil	Safflower Oil + Cholesterol, 0.08%	Coconut Oil + Cholesterol, 0.08%
Animal wt (g)	139 ± 14	151 ± 13	149 ± 9	148 ± 13	146 ± 11
Liver wt (g)	4.8 ± 0.4	5.3 ± 0.6	5.1 ± 0.6	6.1 ± 0.9[b]	5.7 ± 0.4[b]
Plasma cholesterol (mg/dl)	108 ± 19	119 ± 18	168 ± 31[ab]	182 ± 27[b]	357 ± 82[ab]
HDL cholesteryl ester (mg/dl)	56 ± 6	64 ± 7[b]	90 ± 12[ab]	97 ± 12[b]	115 ± 20[b]
Liver cholesterol (mg/g)	2.8 ± 0.4	4.3 ± 0.6[b]	2.7 ± 0.3[a]	18 ± 3[b]	7 ± 3[ab]
Extrahepatic tissue cholesterol (mg/g)	1.8 ± 0.2	1.7 ± 0.1	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.2

[a] The mean value for animals fed coconut oil is significantly different from the mean value in the corresponding safflower oil group.

[b] The mean value is significantly different from the mean control value.

Fig. 1. Distribution of plasma lipoprotein cholesterol by particle size in animals consuming saturated or polyunsaturated fatty acids. Hamsters were fed a control semisynthetic diet or the same diet in which safflower oil or coconut oil was substituted (on a cal/cal basis) for carbohydrate to provide 30% of energy. The triglyceride-containing diets contained either 0% or 0.08% cholesterol. After 6 weeks on the experimental diets, animals were killed and equal volumes of plasma from 6 animals were pooled. Lipoproteins were separated by FPLC using a Superose 6 column as described in Methods. Two-ml aliquots were collected and assayed for cholesterol. Each point represents the mean value from 3 experiments in which plasma was pooled from 6 animals per group. The retention times for hamster VLDL (d < 1.006 g/ml), LDL (d 1.02–1.055 g/ml), and HDL (d 1.07–1.21 g/ml) are indicated.

tions were 41% higher in animals fed coconut oil than in animals fed safflower oil ($P < 0.05$). On the cholesterol-supplemented diet the difference was 19% but this did not achieve statistical significance.

Effect of dietary fatty acids on HDL cholesteryl ester transport

Rates of HDL cholesteryl ester transport were quantified in vivo using homologous HDL labeled with [3H]cholesteryl oleyl ether, a nondegradable tracer of cholesteryl esters that remains intracellularly trapped after uptake (21). As previously noted (21, 39), very little HDL cholesteryl ether accumulated in the lower density lipoprotein fractions during the 4-h infusions (data not shown) consistent with the known low level of CETP in the hamster (40, 41). **Table 2** shows rates of tissue HDL cholesteryl ether clearance (top) and cholesteryl ester uptake (bottom) in animals consuming the control or experimental diets. As shown in column a, clearance of HDL cholesteryl ether by the liver equaled 301 ± 44 μl/h (77% of whole body clearance) in animals fed the control cholesterol-free, low-fat diet. On the cholesterol-free diet, hepatic HDL cholesteryl ether clearance rates were >2-fold higher in animals fed safflower oil than in animals fed coconut oil. Similarly, on the cholesterol-supplemented diets, hepatic HDL cholesteryl ether clearance rates were 67% higher in animals fed safflower oil than in animals fed coconut oil. To obtain the mass of HDL cholesteryl ester transported per hour, the tissue HDL cholesteryl ether clearance rates in each animal were multiplied by the plasma HDL cholesterol ester concentration in that animal and these data

TABLE 2. Effect of dietary saturated or unsaturated triglyceride without or with cholesterol on HDL cholesteryl ester transport

Diet	Liver	Extrahepatic Tissues	Whole Body
	μl/h per whole tissue per 100 g body wt		
Clearance	(a)	(b)	(c)
Control	301 ± 44	88 ± 12	389 ± 61
Safflower	410 ± 62]a	91 ± 13]b	501 ± 69]a
Coconut	201 ± 36]	75 ± 10]	276 ± 44]
Safflower + cholesterol	280 ± 41]a	72 ± 10	352 ± 49]a
Coconut + cholesterol	168 ± 29]	69 ± 11	237 ± 43]
	μg/h per whole tissue per 100 g body wt		
Uptake (flux)	(d)	(e)	(f)
Control	169 ± 31	49 ± 7	218 ± 35
Safflower	262 ± 35]a	58 ± 4]a	321 ± 46]a
Coconut	181 ± 26]	68 ± 6]	248 ± 31]
Safflower + cholesterol	271 ± 37]a	70 ± 8	341 ± 41]b
Coconut + cholesterol	193 ± 31]	79 ± 9	272 ± 39]

Each value represents the mean ± 1 SD for data obtained in 6 animals. Rates of HDL cholesteryl ether clearance were quantified as described in Methods. Tissue clearance rates were multiplied by the plasma concentration of HDL cholesteryl ester in the same animal to yield the absolute rate of HDL cholesteryl ester uptake (flux). Mean plasma HDL cholesteryl ester concentrations equaled 56 ± 6, 64 ± 7, 90 ± 12, 97 ± 12, and 115 ± 20 mg/dl in the control, safflower, coconut, safflower + cholesterol, and coconut + cholesterol groups, respectively.
a $P < 0.01$.
b $P < 0.05$.

are summarized in Table 2 (bottom). As shown in column d, on the cholesterol-free diet the absolute rate of HDL cholesteryl ester uptake by the liver was significantly higher in animals fed safflower oil than in animals fed coconut oil (262 ± 35 vs. 181 ± 26 μg/h). Similarly, on the cholesterol-supplemented diet, hepatic HDL cholesteryl ester uptake was significantly higher in animals consuming safflower oil than in animals fed coconut oil (271 ± 37 vs. 193 ± 31 μg/h).

Because the liver takes up HDL cholesteryl ester via a high affinity receptor-dependent (saturable) transport process, the diet-related changes in clearance rates shown in Table 2 cannot be equated directly with regulation of transporter activity. To quantify changes in transporter activity in vivo, rates of HDL cholesteryl ester transport in the experimental animals were related to kinetic curves describing the relationship between HDL cholesteryl ester transport and circulating HDL cholesteryl ester concentrations in control animals. **Figure 2** shows the kinetic curves for hepatic HDL cholesteryl ester transport in the control hamster. These kinetic curves (±95% confidence intervals) were previously determined by quantifying rates of HDL cholesteryl ester uptake in control hamsters under conditions in which circulating HDL concentrations were acutely raised and maintained at various levels during the 4-h experimental period. Rates of hepatic HDL cholesteryl ether clearance (top) and HDL cholesteryl ester uptake (bottom) in animals fed the experimental diets are superimposed on these normal kinetic curves.

In the control animals, HDL cholesteryl ether clearance equaled 84 ± 9 μl/h per g liver (top) and the absolute rate of HDL cholesteryl ester uptake equaled 45 ± 5 μg/h
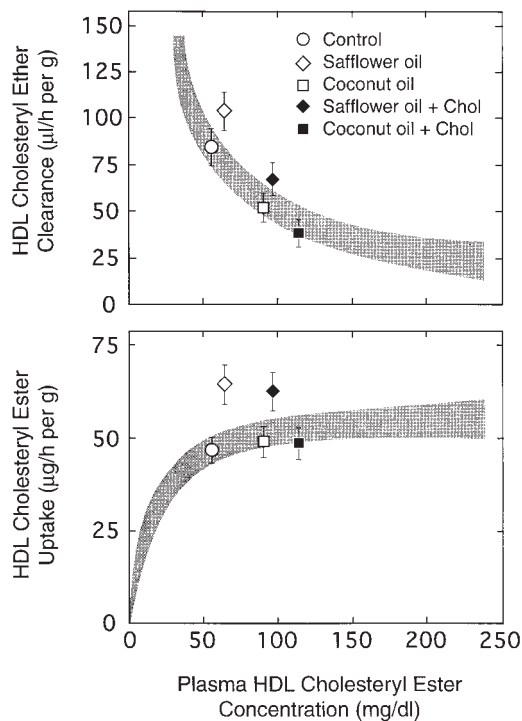
Fig. 2. Effect of dietary saturated and polyunsaturated fatty acids on HDL cholesteryl ester transport in the liver. Groups of animals were fed the experimental diets described in the legend to Fig. 1 for 6 weeks. Clearance rates for [³H]cholesteryl ether-labeled homologous HDL were measured in vivo as described in Methods. These clearance rates were multiplied by the plasma HDL cholesteryl ester concentration in each animal to obtain the absolute rate of HDL cholesteryl ester uptake. The shaded areas represent the kinetic curves (±95% confidence intervals) for HDL cholesteryl ester transport in control animals. Superimposed on these kinetic curves are the rates of HDL cholesteryl ether clearance (top) and HDL cholesteryl ester transport (bottom) in the experimental animals plotted as a function of the plasma HDL cholesteryl ester concentration in the same animals. Each point represents the mean for data obtained in 6 animals.

per g liver (bottom) at a plasma HDL cholesteryl ester concentration of 56 ± 6 mg/dl. These values fall on the kinetic curves describing the relationship between HDL cholesteryl ester transport and plasma HDL cholesteryl ester concentrations in the normal hamster. The values for HDL cholesteryl ester transport in animals consuming the coconut oil diets (with or without added cholesterol) also fall on the normal kinetic curves for HDL cholesteryl ester transport in the hamster. In contrast, the values for HDL cholesteryl ester transport in animals fed the safflower oil diets (with or without added cholesterol) were significantly higher than would be seen in control animals having the same HDL cholesteryl ester concentration. For example, in animals fed the cholesterol-free safflower oil-enriched diet, the liver cleared HDL cholesteryl ether at a rate of 104 ± 12 μl/h per g at a plasma HDL cholesteryl ester concentration of 64 ± 7 mg/dl whereas normal animals clear HDL cholesteryl ether at a rate of 74 μl/h per g at this plasma HDL cholesteryl ester concentration. Assuming that the low rate of receptor-independent (non-

saturable) uptake was not altered, it can be calculated that safflower oil increased HDL cholesteryl ester transporter activity by 47% compared to the control group whereas coconut oil produced a small (nonsignificant) decrease in transporter activity.

The studies outlined above were all carried out using labeled HDL derived from hamsters fed the control low-fat diet. To determine whether the differences in HDL cholesteryl ester clearance in the safflower oil- and coconut oil-fed animals were the result of changes in HDL composition that affect the hepatic clearance of the particles, studies were performed in control hamsters using labeled HDL from animals fed safflower oil or coconut oil diets. As shown in **Fig. 3**, hepatic clearance of trace-labeled HDL cholesteryl ether derived from control animals equaled 81 ± 10 μl/h per g at a plasma HDL cholesteryl ester concentration of 61 mg/dl. Hepatic clearance rates for trace-labeled HDL derived from animals fed safflower oil and coconut oil equaled 84 ± 9 μl/h per g and 77 ± 8 μl/h per g, respectively. When mass amounts of HDL were
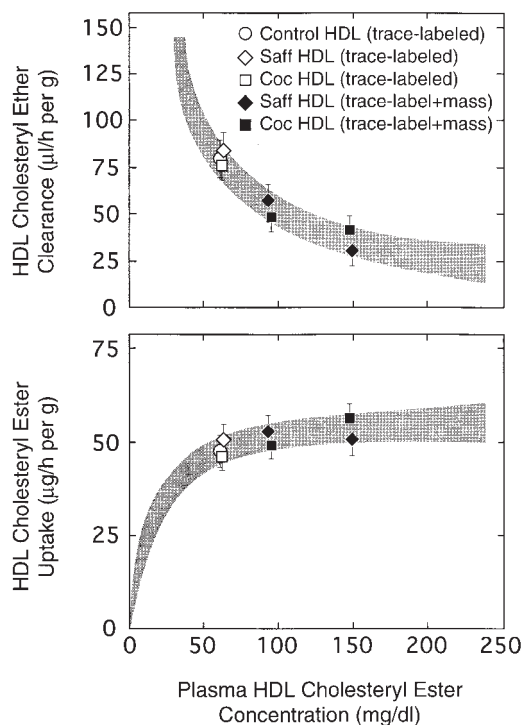
Fig. 3. HDL cholesteryl ester transport in control hamsters using HDL derived from animals fed safflower or coconut oil. HDL (d 1.07–1.21 g/ml) was isolated from hamsters fed cholesterol-free semisynthetic diets containing either safflower oil or coconut oil (30% of energy) and a portion of each preparation was labeled with [³H]cholesteryl oleyl ether as described in Methods. Clearance rates for [³H]cholesteryl ether-labeled HDL were measured in vivo as described in Methods. The shaded areas represent the kinetic curves (±95% confidence intervals) for HDL cholesteryl ester transport in control animals. Superimposed on these kinetic curves are the rates of HDL cholesteryl ether clearance (top) and HDL cholesteryl ester transport (bottom) in the experimental animals plotted as a function of the plasma HDL cholesteryl ester concentration in the same animals. Each value represents the mean ± 1 SD for data obtained in 6 animals.
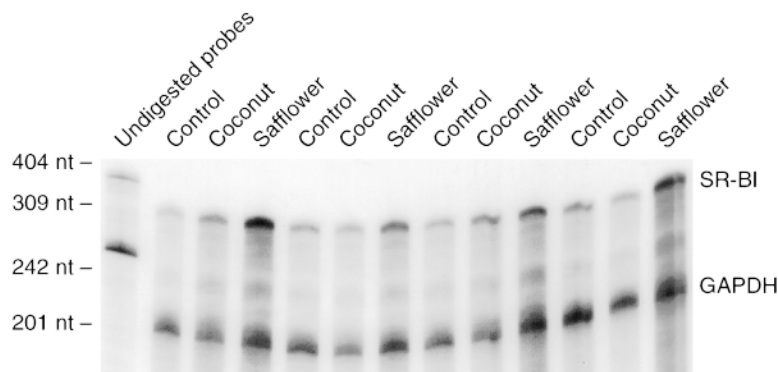
Fig. 4. Hepatic SR-BI mRNA levels in hamsters fed polyunsaturated or saturated fatty acids. Hepatic mRNA was isolated from animals fed a cholesterol-free semisynthetic diet or the same diet containing safflower or coconut oil (30% of energy). Total RNA (40 μg) was hybridized with ^{32}P-labeled single-stranded cDNA probes and the protected bands resistant to mung bean nuclease digestion were analyzed by polyacrylamide gel electrophoresis followed by autoradiography.

added to the primed infusions of trace-labeled HDL, the decrease in hepatic HDL clearance was similar whether the HDL mass was obtained from animals fed safflower oil- or coconut oil-containing diets.

The exact mechanism of HDL cholesteryl ester uptake is under active investigation. SR-BI is an HDL receptor that mediates selective HDL cholesteryl ester uptake when transfected into cells (16) and plays a major role in HDL cholesteryl ester transport in vivo (17–19, 20). We therefore compared hepatic SR-BI expression in hamsters fed safflower oil or coconut oil. SR-BI mRNA levels were measured by nuclease protection using probes specific for the Syrian hamster. **Figure 4** is an example of an autoradiogram from a nuclease protection analysis of hepatic SR-BI mRNA levels in animals fed safflower oil or coconut oil. When the data from 12 animals per group were quantified using an isotopic image analysis system as described in Methods, hepatic SR-BI mRNA levels were significantly higher (50 ± 9%) in animals fed safflower oil than in animals fed coconut oil. Similarly, on the cholesterol-containing diets, hepatic SR-BI mRNA levels were significantly higher (43 ± 7%) in animals fed safflower oil than in animals fed coconut oil. SR-BI protein levels were determined by immunoblotting using antiserum raised against the C-terminal 14 amino acids of SR-BI, which detects the major form of SR-BI in the liver (38). As illustrated in **Fig. 5**, hepatic SR-BI protein levels were also increased in animals fed safflower oil compared to animals fed control or coconut oil diets.

A change in the plasma HDL cholesteryl ester concentration can be the result of a change in either the rate at which cholesteryl esters enter plasma HDL (HDL cholesteryl ester flux) or the rate at which cholesteryl esters are removed from the HDL fraction. The effect of saturated and unsaturated fatty acids on whole body HDL cholesteryl ester uptake, which in a steady state equals the rate of cholesteryl ester entry into the HDL fraction (HDL cholesteryl ester flux), is shown in Table 2 (column f). Whole body HDL cholesteryl ester flux equaled 218 ± 35 μg/h per 100 g body wt in control animals. On the cholesterol-free diet, whole body HDL cholesteryl ester flux was significantly higher in animals fed safflower oil than in animals fed coconut oil (321 ± 46 vs. 248 ± 31 μg/h per 100 g body wt). Similarly, on the cholesterol-supplemented diet, whole body HDL cholesteryl ester flux was higher with safflower oil than with coconut oil (341 ± 41 vs. 272 ± 39 μg/h per 100 g body wt).

As HDL cholesteryl ester uptake by the liver was increased in animals fed safflower oil it was of interest to determine whether the total flux of cholesterol from extrahepatic tissues to the liver (reverse cholesterol transport) was also increased in these animals. In a steady state, the movement of cholesterol from extrahepatic tissues into plasma is balanced by the acquisition of cholesterol from de novo synthesis, LDL and HDL. Because the cholesterol concentration in extrahepatic tissues was not affected by the experimental diets, total cholesterol acquisition by the extrahepatic tissues (from de novo synthesis, LDL and HDL) could be taken as a measure of reverse cholesterol transport and these data are summarized in **Table 3**. In control animals, the extrahepatic tissues acquired 23 ± 3
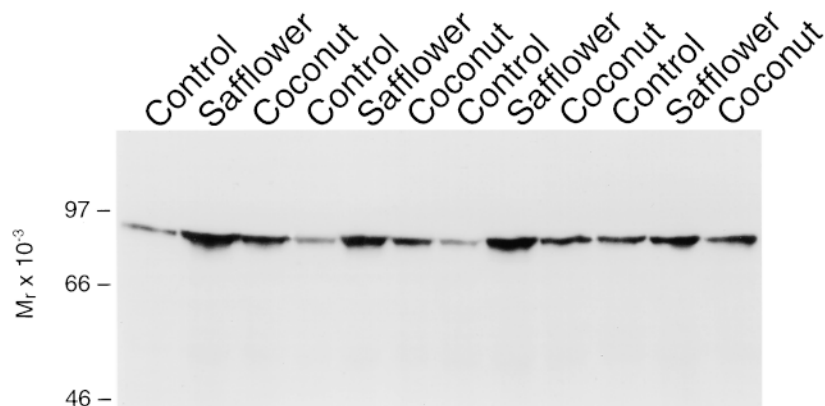
Fig. 5. Immunoblot analysis of hepatic SR-BI protein in hamsters fed polyunsaturated or saturated fatty acids. Hepatic membrane fractions were prepared from animals fed the diets described in the legend to Fig. 4. Immunoblot analysis was performed using antibodies raised against the C-terminal 14 amino acids of mouse SR-BI.

TABLE 3. Effect of dietary triglyceride without or with cholesterol on rates of cholesterol acquisition in the extrahepatic tissues

Diet	LDL Cholesterol Uptake	De Novo Synthesis	HDL Cholesteryl Ester Uptake	Total Cholesterol Acquistion
		μg/h per whole tissue per 100 g body wt		
Control	23 ± 3	105 ± 15	49 ± 7	177
Safflower	27 ± 3	96 ± 11	58 ± 4	181
Coconut	28 ± 5	101 ± 9	68 ± 6	197
Safflower + Ch	35 ± 4	92 ± 10	70 ± 8	197
Coconut + Ch	36 ± 6	87 ± 11	79 ± 9	202

Values represent the mean ± 1 SD for data obtained in 6 animals.

μg/h cholesterol from LDL, 105 ± 15 μg/h from de novo synthesis and 49 ± 7 μg/h from HDL for a total of 177 μg/h. Total cholesterol acquisition by the extrahepatic tissue compartment (taken as a measure of reverse cholesterol transport) was not altered by the experimental diets.

DISCUSSION

The main finding of these studies is that substitution of polyunsaturated for saturated fatty acids in the diet results in up-regulation of hepatic SR-BI mRNA and protein and increased HDL cholesteryl ester transport to the liver; as a consequence, plasma HDL cholesteryl ester concentrations are reduced. Although polyunsaturated fatty acids increased HDL cholesteryl ester uptake by the liver and lowered plasma HDL cholesterol concentrations, there was no change in the cholesterol content or in the rate of cholesterol acquisition (from de novo synthesis, LDL and HDL) by the extrahepatic tissue compartment. Under steady-state conditions, the rate of cholesterol acquisition by the extrahepatic tissues is matched by an equal rate of cholesterol movement out of the extrahepatic tissues. Although some cholesterol is converted to steroid hormones or lost when cells are sloughed from the skin or gastrointestinal tract, the vast bulk of cholesterol that is acquired by the extrahepatic tissues is returned to the liver for excretion in a process termed reverse (5) or centripetal (7) transport. We did not quantify steroid hormone production or the loss of sterol from gut epithelium and skin; however, assuming that these relatively minor pathways of cholesterol loss from the extrahepatic tissue compartment were not altered, it can be concluded that net cholesterol transport from extrahepatic tissues to the liver, i.e., the rate of reverse cholesterol transport, was not affected by the experimental diets.

In humans (27, 28) as in the hamster, diet modification aimed at lowering LDL concentrations frequently lowers HDL concentrations as well, and there is concern that the beneficial effect of reducing the plasma concentration of atherogenic LDL particles may be offset by the accompanying reduction in protective HDL. Here we show that substituting polyunsaturated for saturated fatty acids in the diet lowers plasma HDL cholesterol concentrations but does not reduce the rate of cholesterol transport from extrahepatic tissues to the liver. We previously showed that switching hamsters from a Western-type diet (enriched with saturated fat and cholesterol) to a low-fat, high-fiber diet reduced HDL cholesteryl ester concentrations by nearly 50% but did not reduce the transport of HDL cholesteryl ester to the liver (39). Together, these studies in the hamster demonstrate that diet modifications aimed at lowering LDL concentrations do not reduce the flux of HDL cholesteryl esters to the liver or the rate of reverse cholesterol transport even though HDL cholesterol levels fall. These observations are consistent with studies in the CETP transgenic mouse, where a reduction in the plasma concentration of HDL cholesterol was not accompanied by a change in the rate at which the extrahepatic tissues acquired cholesterol from de novo synthesis or LDL uptake (7).

Exactly how HDL protects against atherosclerosis has not been established. Although, emphasis has been placed on the role of HDL in transporting cholesterol from peripheral tissues back to the liver it is possible that HDL could protect against atherosclerosis through mechanisms independent of cholesterol transport (42, 43). If the protective effect of HDL is due to its role in reverse cholesterol transport, the reduction in plasma HDL cholesterol that is seen when saturated fat is replaced by polyunsaturated fat should not adversely affect atherogenesis. Supporting this conclusion is the observation that African green monkeys fed a diet rich in polyunsaturated fatty acids have less coronary artery atherosclerosis than animals fed a saturated fat diet despite a 36% reduction in plasma HDL-cholesterol concentrations on the polyunsaturated fat diet (24). Thus, there is little evidence that the decrease in plasma HDL concentrations that frequently accompanies a cholesterol-lowering diet adversely affects atherogenesis.

While these studies indicate that there is no direct relationship between plasma HDL cholesterol concentrations and reverse cholesterol transport, they also point out the complicated relationship between HDL cholesteryl ester uptake by the liver and reverse cholesterol transport. Although the source of cholesteryl esters entering HDL is not known with certainty, most is thought to originate in extrahepatic tissues. Cholesterol that is acquired by extrahepatic tissues must be returned to the liver for excretion (with the exception of cholesterol that is converted to steroid hormones or lost when cells are sloughed from the skin and gut). In control hamsters the rate of HDL cholesteryl ester uptake by the liver approximates the rate of cholesterol acquisition (from de novo synthesis, LDL and HDL) by the extrahepatic tissues. Here we show that compared to saturated fatty acids, polyunsaturated fatty acids increase the transport of HDL cholesteryl esters to the liver but do not increase the rate of total cholesterol acquisition in the extrahepatic tissues. Thus, in animals fed polyunsaturated fatty acids, the transport of HDL cholesteryl esters to the liver exceeds that necessary to account for reverse cholesterol transport. The most likely explanation is that there is some net movement of cholesterol from the liver into the HDL fraction and that safflower oil

increases this flux while at the same time increasing HDL cholesteryl ester uptake by the liver. The liver is a major source of nascent HDL and these particles do contain some cholesterol; however, the cholesterol content of liver-derived nascent HDL particles was not higher in monkeys fed polyunsaturated fatty acids than in animals fed saturated fatty acids (44). During the metabolism of triglyceride-rich lipoproteins, excess surface material (phospholipid, soluble apoproteins and cholesterol) is generated that forms nascent HDL particles or is transferred to pre-existing HDL (45, 46), and it is possible that safflower oil affects this process. Additional studies will be necessary to determine which, if any, of these potential mechanisms accounts for the increased flux of cholesteryl esters into the HDL fraction in animals fed polyunsaturated fatty acids.

The plasma concentration of HDL cholesteryl esters is determined by the rate at which cholesteryl esters enter the HDL fraction relative to the rate at which cholesteryl esters are removed from HDL. HDL cholesteryl esters are removed from plasma via receptor-dependent (saturable) and receptor-independent (nonsaturable) mechanisms. The transport parameters for receptor-dependent (J^m and K_m) and receptor-independent (P) HDL cholesteryl ester transport have been determined in the hamster in vivo. These studies showed that the bulk of HDL cholesteryl esters are cleared from plasma via a high affinity transport mechanism (K_m ~14 mg/dl) that is located primarily in the liver and is saturated at normal plasma HDL cholesteryl ester concentrations. Knowing the kinetic characteristics of HDL cholesteryl ester transport in vivo, it is possible to calculate how changes in cholesteryl ester entry into HDL (HDL cholesteryl ester flux) or the activity of the HDL cholesterol ester transport mechanism alter steady-state plasma concentrations of HDL cholesteryl ester. The relationship among these three parameters is illustrated in **Fig. 6**, which shows plasma HDL cholesteryl ester concentrations as a function of whole body HDL cholesteryl ester transporter activity and the rate of HDL cholesteryl ester entry into plasma (HDL cholesteryl ester flux). Due to the high affinity of the transport mechanism and the fact that the transport mechanism is saturated at normal HDL concentrations, small changes in either transporter activity or the rate of cholesteryl ester entry into HDL can produce relatively large changes in circulating HDL cholesterol concentrations. For example, a 50% decrease in whole body HDL cholesteryl ester transporter activity will result in a 2.8-fold increase in plasma HDL cholesteryl ester levels if cholesteryl ester entry into HDL is maintained at a constant level. On the other hand, a 50% increase in the rate of cholesteryl ester entry into the HDL fraction will result in a 3-fold increase in plasma HDL cholesteryl ester concentrations if HDL cholesteryl ester transporter activity is kept constant. Data from animals fed the triglyceride-containing diets are superimposed on these kinetic curves. Replacing saturated fat with polyunsaturated fat results in up-regulation of HDL cholesteryl ester transporter activity, which in turn, leads to a fall in plasma HDL cholesteryl ester concentrations. Indeed the fall in plasma HDL cholesteryl ester concentrations would have been even greater
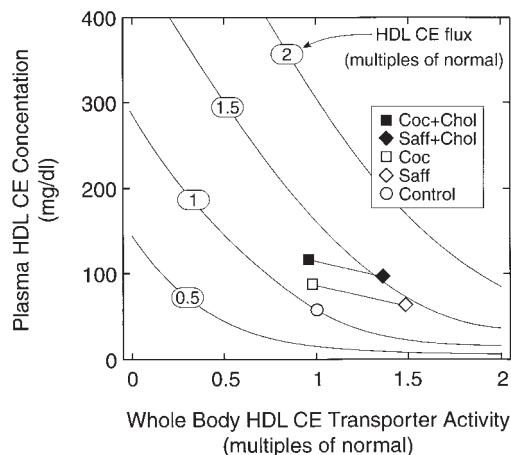
Fig. 6. Steady-state concentration of HDL cholesteryl esters in plasma as a function of whole body HDL cholesteryl ester transporter activity and HDL cholesteryl ester flux. These curves were generated using transport constants that were experimentally determined in control hamsters (21) and show the changes in plasma HDL cholesteryl ester concentrations that will occur as the activity of the HDL cholesteryl ester transport mechanism is varied from 0% to 200% of the normal value at HDL cholesteryl ester fluxes of 50%, 100%, 150%, or 200% of the control value. Superimposed on these curves are the experimental values obtained in animals fed safflower oil or coconut oil diets with or without cholesterol.

had there not been an increase in the rate at which cholesteryl esters enter HDL in animals fed polyunsaturated fatty acids.

SR-BI appears to play a critical role in HDL cholesteryl ester transport although the exact mechanism has not been established (15). SR-BI is capable of mediating selective HDL cholesteryl ester uptake when transfected into LDL receptor negative CHO cells. Overexpression of SR-BI in the liver markedly reduces plasma HDL cholesteryl ester concentrations (17, 18) whereas targeted mutation of the SR-BI gene resulted in decreased HDL cholesteryl ester uptake by the liver and marked elevation of plasma HDL cholesteryl ester levels (19, 20). SR-BI expression is coordinately regulated with steroidogenesis in the adrenal gland, ovaries, and testes (37, 47). Trophic hormones profoundly increase the expression of this receptor in steroidogenic tissues. The human SR-BI gene contains the consensus site for steroidogenic factor-1 (SF-1) (48) that orchestrates the regulation of many of the genes in the steroidogenic pathway (49). SF-1 is not produced in the liver and little insight has been gleaned as to what regulates SR-BI levels in the liver. In rats, estrogen treatment, which dramatically increases hepatic LDL receptor activity, profoundly inhibits hepatic SR-BI expression (37). A reduction in hepatic SR-BI expression was also seen in rats fed a diet containing 2% cholesterol and 0.5% cholic acid (50). These observations are consistent with the view that intracellular cholesterol levels regulate SR-BI expression as previously suggested (51). However, the results of our studies reveal that this scenario is too simplistic. SR-BI expression and HDL cholesteryl ester transporter activity were increased in animals fed polyunsaturated fatty acids

even though the cholesterol content of the liver was highest in these animals. In previous studies we found no regulation of hepatic SR-BI expression or HDL cholesteryl ester transporter activity under conditions in which the cholesterol content of the liver varied by 10-fold (39).

The mechanism whereby dietary fatty acids regulate hepatic SR-BI expression is unknown. Fatty acids, or their CoA derivatives, alter the transcription of a number of genes involved in the synthesis, transport, interconversion, and catabolism of lipids (52). Both repression (53–55) and induction (56, 57) of gene transcription have been described and fatty acid-response elements have been identified (53, 55, 57). Fatty acid-mediated control of mRNA stability has also been demonstrated (58). Exactly how cells sense specific fatty acids and modulate gene transcription or mRNA stability has not been established in any system. Further work will be required to determine how polyunsaturated fatty acids regulate hepatic SR-BI expression. Dietary fatty acids also regulate hepatic LDL receptor activity at the mRNA level (59); however, here again, the mechanism has not been established.

It is reasonable to ask why cholesterol and fatty acids markedly alter hepatic and plasma cholesterol concentrations while having no significant effect on the extrahepatic tissues. The effects of dietary cholesterol and fatty acids on plasma lipoprotein concentrations primarily reflect the complex interaction of these lipids on sterol metabolism within the hepatocyte (60). The liver is the sole recipient of dietary cholesterol and secretion of cholesterol into bile, either directly or after conversion to bile salts, is the major route for cholesterol elimination from the body. Changes in sterol balance across the liver, resulting from changes in cholesterol absorption or cholesterol excretion into bile are generally associated with parallel changes in the cholesterol concentration of liver and plasma. Dietary fatty acids, on the other hand, appear to alter the intracellular distribution of cholesterol within the liver. Unsaturated fatty acids promote esterification of dietary cholesterol within the hepatocyte, an effect that is associated with derepression of hepatic LDL receptor expression, possibly due to a decrease in the amount of unesterified cholesterol in a critical regulatory pool, and decreased plasma LDL concentrations. Saturated fatty acids have the opposite effect. As a consequence, dietary fatty acids generally produce reciprocal changes in liver and plasma cholesterol concentrations (60). With the exception of the endocrine organs, most extrahepatic tissues derive little cholesterol from lipoproteins (21, 61). Rather, extrahepatic tissues rely largely on de novo synthesis, which is tightly regulated by sterol availability, to supply cholesterol for cell renewal and membrane turnover. Presumably, this is the reason that the cholesterol content of extrahepatic tissues remains constant in the face of large changes in hepatic and plasma cholesterol concentrations. It is important to point out that we did not look at sterol balance across the arterial wall in our studies. Prolonged feeding of a high cholesterol, high saturated fat diet to hamsters clearly increases the cholesterol content of the aorta (62). Apparently, normal reverse cholesterol transport is sufficient to maintain sterol balance in the extrahepatic tissues of hamsters fed hyperlipidemic diets but may not be sufficient to prevent the gradual accumulation of cholesterol in certain regions of the arterial wall.

In summary, these studies show that substitution of polyunsaturated for saturated fatty acids in the diet results in up-regulation of hepatic SR-BI expression and increased HDL cholesteryl ester transport to the liver. As a consequence, plasma HDL cholesterol concentrations fall; however, the rate of reverse cholesterol transport is not affected.

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