

Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools

(cholesteryl esters/cholesterol synthesis/atherosclerosis/hamster)

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ABSTRACT When the intake of dietary cholesterol in the hamster is constant, feeding the saturated 14:0 fatty acid (*n*-tetradecanoic acid) elevates the plasma low density lipoprotein (LDL) cholesterol concentration from 72 to 204 mg/dl, while the monounsaturated 18:1 fatty acid (*cis*-9-octadecenoic acid) lowers this level to 28 mg/dl. The 14:0 fatty acid lowers the hepatic cholesteryl ester concentration from 12 to 5 mg/g, while the abundance of this fatty acid in the ester fraction is increased 13-fold. Hepatic LDL receptor activity is depressed to 41% of control, while the LDL cholesterol production rate is increased to 132%. These changes account for the 3-fold increase in the plasma LDL cholesterol concentration. In contrast, feeding the 18:1 fatty acid increases hepatic cholesteryl ester concentration to 21 mg/g, and the abundance of this acid in the esters is increased 1.4-fold. Hepatic receptor activity is increased to 145%, while the production rate is suppressed to 68% of control. These changes account for the decrease in plasma LDL cholesterol level to 28 mg/dl. Despite these marked changes in LDL metabolism, however, the 14:0 and 18:1 fatty acids cause no change in net cholesterol balance across the liver. These results suggest that there are two fundamentally different mechanisms regulating hepatic LDL metabolism. One involves changes in net sterol balance across the liver brought about by alterations in the rate of cholesterol or bile acid absorption across the intestine, while the second is articulated through a redistribution of the putative sterol regulatory pool within the hepatocyte that is dictated by the type of long-chain fatty acid that reaches the liver.

While it is clear that both dietary cholesterol and fatty acid reach the liver and expand the various lipid pools, there is little information on how these molecules exert their regulatory effects on the concentration of cholesterol carried in low density lipoprotein (LDL) in the plasma (1–7). Control of the LDL receptor is known to depend on a 42-base-pair element in the 5' flanking region of the LDL receptor gene (8). This region contains three repeat sequences that appear to be essential for feedback regulation of the gene by cellular cholesterol. It has been postulated that either cellular sterol may prevent the interaction of a positive transcriptional factor with one of these regulatory elements or, alternatively, sterol may interact with another protein that, in turn, displaces the positive regulatory factor (9, 10). In either case, the level of manifest receptor activity would be dictated by the amount of sterol in some small, but critical, regulatory pool of cholesterol in the cell. Presumably, the concentration of this putative regulator is in equilibrium with cholesterol sequestered in the ester pool since an inverse relationship is seen between steady-state receptor activity and cellular cholesteryl ester content when net sterol balance across the liver is progressively increased (2). Paradoxically, however,

the addition of saturated fatty acids to the diet markedly lowers the steady-state level of cholesteryl esters while further suppressing receptor activity, and unsaturated lipids increase this pool and restore receptor-dependent LDL transport (1). In the current studies, therefore, net sterol balance was measured in the experimental animals to determine whether the extreme changes in hepatic LDL receptor activity induced by feeding the pure 14:0 (*n*-tetradecanoic acid) and 18:1 (*cis*-9-octadecenoic acid) fatty acids were the result of altered cholesterol balance across the liver or, alternatively, were the consequence of redistribution of cholesterol within the hepatocyte between storage and putative regulatory pools.

MATERIALS AND METHODS

Animals and Diets. Male Golden Syrian hamsters (Charles River Breeding Laboratories) were subjected to light cycling and fed a low cholesterol, ground rodent diet (Allied Mills, Chicago). After 2 wk, groups of these animals were placed on experimental diets containing a constant amount of cholesterol (0.12%; wt/wt), MCT oil (5.0%; Mead Johnson), and a test triacylglycerol (15%) containing a single species of fatty acid. The three test triacylglycerols used in these studies contained exclusively either the 8:0 fatty acid (*n*-octanoic acid; Sigma), the 14:0 fatty acid (*n*-tetradecanoic acid; Fluka), or the 18:1 fatty acid (*cis*-9-octadecenoic acid; Nu Chek Prep, Elysian, MN) (1). These diets were fed ad libitum for 30 days, at which time experimental measurements were made during the mid-dark phase of the light cycle.

Dietary Lipid Absorption. During the last week of the experiments, food intake was quantitated and two 24-h fecal collections were obtained. The total lipid contents of the diet and feces were determined gravimetrically. From these two values, the net absorption of dietary lipids was calculated and was expressed as a percentage of intake (7).

Dietary Cholesterol Absorption. Using a modification of a described method (11), [$1\alpha,2\alpha$ - ^3H]cholesterol dissolved in 0.4 ml of Intralipid (20%) (KabiVitrum, Clayton, NC) was administered intravenously to each animal while [4 - ^{14}C]cholesterol suspended in 0.6 ml of MCT oil was administered intragastrically to animals actively eating their respective experimental diets. The ratio of the two isotopes achieved in the plasma was quantitated 72 h later, and these data were used to calculate net cholesterol absorption expressed as a percentage of dietary intake.

Fatty Acid Abundance in Hepatic Lipids. At the end of the 30-day feeding period, total lipids were extracted from aliquots of liver and the major classes were separated by TLC (7). The fatty acids in each of these samples were then methyl

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Abbreviations: LDL, low density lipoprotein; J^m , maximal achievable rate of receptor-dependent LDL transport in the liver; J_t , LDL cholesterol production rate in the whole animal.
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esterified and the relative abundance of each fatty acid was quantitated by GLC (7, 12).

Tissue Cholesterol Concentrations. The concentration of cholesterol, expressed in mg/dl in whole plasma as well as in the LDL (1.020–1.063 g/ml) and high density lipoprotein (1.063–1.21 g/ml) fractions was determined as described (1). The unesterified and esterified cholesterol in liver was separated on silicic acid/celite columns and quantitated by GLC (13).

Determination of the Hepatic LDL Receptor Activity and LDL Cholesterol Production Rate *in Vivo*. Rates of LDL cholesterol transport into the liver via the receptor-dependent process and into all tissues of the animal by both receptor-dependent and receptor-independent mechanisms were measured *in vivo* as described (5, 14, 15). The first set of data was used to calculate the maximal rate of receptor-dependent LDL cholesterol transport (J^m) into the liver in each experimental group. This parameter was expressed as the amount of LDL cholesterol taken up into the liver by the receptor-dependent process per h per 100 g animal. The second set of data was used to calculate the LDL cholesterol production rate (J_t), which expressed the amount of LDL cholesterol produced per h per 100 g animal. To simplify presentation of these data, the absolute values of J^m and J_t found in the control animals—i.e., those fed the 8:0 fatty acid—were set equal to 100% and the results obtained in the other experimental groups were expressed as percentages of these two values.

Determination of Rates of Sterol Synthesis *in Vivo*. Animals were killed 1 h after intravenous administration of $^3\text{H}_2\text{O}$. Liver, small intestine, and the remaining carcass were then saponified and the digitonin-precipitable sterols were isolated as described (16, 17). The rates of sterol synthesis *in vivo* were expressed as nmol of $^3\text{H}_2\text{O}$ incorporated into sterols per h per organ.

Calculations. The steady-state concentration of LDL cholesterol in the plasma is known to be determined by four separate parameters including J^m , J_t , K_m (the affinity constant of LDL for its receptor), and P (the proportionality constant for the receptor-independent transport process) (18, 19). The values for each of these parameters in the hamster and the equations that define these relationships are described in detail elsewhere (5, 7, 15). Where appropriate, mean values $\pm$ 1 SEM are given for each experimental

Table 1. Dietary intake, lipid and cholesterol absorption, and net weight gain in animals fed three dietary triacylglycerols for 30 days

Experimental measurement	Fatty acid in dietary triacylglycerol		
	8:0	14:0	18:1
Food intake, g/day	9.7 $\pm$ 0.5	9.9 $\pm$ 0.4	9.7 $\pm$ 0.3
Net lipid absorption, % intake	99.0 $\pm$ 0.1	97.0 $\pm$ 0.1	98.0 $\pm$ 0.1
Net cholesterol absorption, % intake	55.6 $\pm$ 2.6	61.1 $\pm$ 4.3	52.6 $\pm$ 4.8
Net body weight gain, g per 30 days	50.0 $\pm$ 4.2	51.0 $\pm$ 5.7	56.0 $\pm$ 4.3

Hamsters, initially weighing 95–110 g, were stabilized for 2 weeks on a low-cholesterol, low-triacylglycerol laboratory diet. The animals were then divided into three groups of similar weight and placed on diets containing 0.12% of added cholesterol, 5% of added MCT oil, and 15% of added test triacylglycerol. The triacylglycerol fed to each of the groups contained only a single fatty acid—i.e., the 8:0 and 14:0 saturated fatty acids and the 18:1 monounsaturated compound. After these diets were eaten for 30 days, food intake and weight gain were measured, and net absorption of total dietary lipids and cholesterol was quantified. Mean values $\pm$ 1 SEM are shown for six animals in each group and none of the experimental values was significantly different from the control (8:0) group.

parameter. The significance of differences from the appropriate control values was usually tested at the $P < 0.05$ level.

RESULTS

Dietary Lipid Absorption. All diets contained 0.12% cholesterol to suppress receptor-dependent LDL transport in the liver $\approx$ 50%, 5% MCT oil to enhance absorption of the 14:0 fatty acid, and 15% ($\approx$ 30% of caloric intake) of the test triacylglycerols containing the 8:0, 14:0, or 18:1 fatty acids. The MCT oil and the 8:0 fatty acid have both been shown to have no regulatory effect on hepatic receptor activity (5, 7); hence, in these studies, the diet containing the 8:0 fatty acid represented the isocaloric, control diet. As shown in Table 1, in the three experimental groups of animals all ate 9.7–9.9 g of diet per day, which contained $\approx$ 1.5 g of the test triacylglycerols. The absorption of the triacylglycerol in these diets was essentially complete (line 2) and net cholesterol absorption in all three groups averaged 52.6–61.1% of intake (line 3).

Abundance of Fatty Acids in the Liver. To evaluate the effects of these diets on hepatic lipid pools, the relative

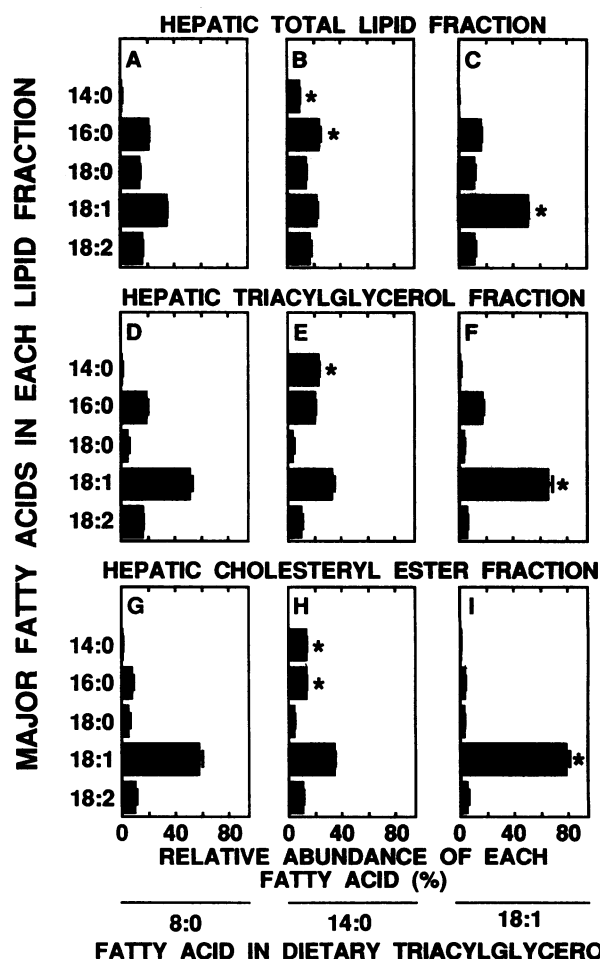


FIG. 1. Relative abundance of major fatty acids in various lipid fractions of the liver of animals fed three different triacylglycerols. After animals received the three different diets for 30 days, liver samples from each animal were extracted and the major classes of lipids were separated by TLC. These specimens were then saponified and the relative abundance of each fatty acid present in the specimens was quantified by GLC. Relative abundance of five fatty acids in the total lipid fraction (A–C), triacylglycerol fraction (D–F), and cholesteryl ester fraction (G–I) in the livers of animals receiving the three different triacylglycerols in their diets is shown. Mean values $\pm$ 1 SEM are shown for six animals in each group; asterisk indicates that the value was significantly higher than the corresponding value found in the control (8:0) group.

abundance of the major fatty acids in the total lipid, triacylglycerol, and cholesteryl ester fractions of the liver was determined. As shown in Fig. 1, after 30 days of control diet (8:0 fatty acid), the profile of fatty acids in the total lipid fraction consisted primarily of the 16:0 (21%), 18:0 (14%), 18:1 (34%), and 18:2 (16%) compounds (Fig. 1A), while the 18:1 fatty acid was most abundant in the triacylglycerol lipids (Fig. 1D) and accounted for 57% of the fatty acids present in the cholesteryl esters (Fig. 1G). When the diet containing the 14:0 compound was fed, the level of this fatty acid in the total lipid fraction increased 20-fold (from 0.4% to 9%) and there was also a small, but significant, increase in the abundance of the 16:0 compound (Fig. 1B). This latter change presumably reflected the ability of the liver to elongate the 14:0 fatty acid. More strikingly, the sum of the abundances of the 14:0 and 16:0 fatty acids in the cholesteryl ester fraction increased from 9% in the control animals to 26% in the group receiving the 14:0-containing triacylglycerol (Fig. 1H). Similarly, when the 18:1 fatty acid was fed for 30 days, it became relatively enriched in the total lipid (Fig. 1C) and triacylglycerol fractions (Fig. 1F) and this compound now accounted for nearly 80% of the fatty acids in the cholesteryl esters (Fig. 1I).

Effects on Parameters of LDL Metabolism. In animals receiving the control diet for 30 days, J_t averaged 324 $\mu\text{g/h}$ per 100 g animal while J^m equaled 348 $\mu\text{g/h}$ per 100 g. In animals receiving no cholesterol in the diet, J^m usually equals 650–700 $\mu\text{g/h}$ per 100 g (5, 7, 15); thus, as expected, the

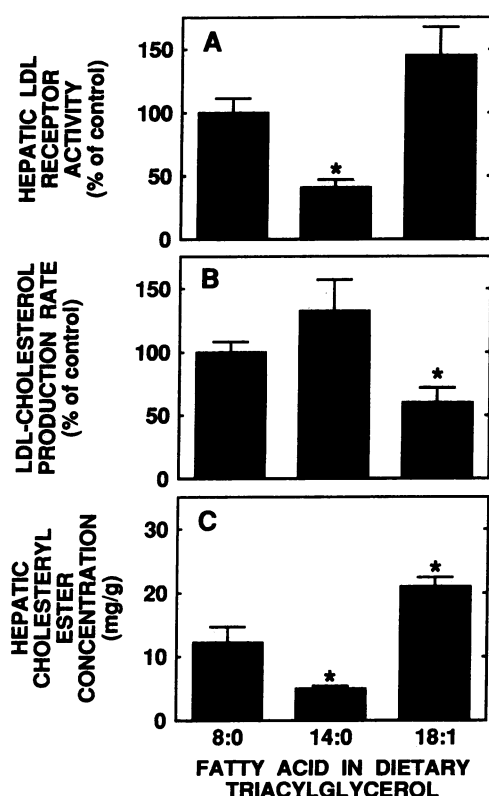


FIG. 2. Hepatic LDL receptor activities, whole animal LDL cholesterol production rates, and hepatic cholesteryl ester concentration in animals fed three different triacylglycerols for 30 days. (A) J^m was measured in each animal and the average of values found in the animals fed control (8:0) triacylglycerol was set equal to 100% hepatic receptor activity. (B) Similarly, J_t was measured in each animal and the mean of values quantified in the animals fed the 8:0 fatty acid was again set equal to 100%. (C) Concentration of cholesteryl esters present in the liver after feeding the three diets is shown. Mean values $\pm$ 1 SEM are shown for six animals in each group; asterisk indicates that the value was significantly different from the corresponding value found in the control (8:0) group.

constant level of 0.12% cholesterol in these diets suppressed hepatic receptor activity by $\approx$ 50%. To simplify presentation of the data, however, these respective absolute values of J^m and J_t found in the control animals were set equal to 100%.

When either the 14:0 or the 18:1 fatty acid was substituted for the 8:0 compound in the control diet, there were marked changes in these parameters (Fig. 2). In the face of the constant cholesterol intake, the saturated fatty acid suppressed hepatic receptor activity to 41% of control (Fig. 2A) while increasing the production rate to 132% (Fig. 2B). In contrast, the 18:1 compound enhanced J^m to 145% (Fig. 2A) and lowered J_t to only 68% of control (Fig. 2B). Also of note was the fact that the steady-state cholesteryl ester pool was markedly suppressed by the 14:0 fatty acid but was nearly doubled by feeding the 18:1 compound (Fig. 2C). There was no significant difference, however, in the concentration of unesterified cholesterol in any of the groups (2.3–2.6 mg/g). Thus, compared to the 14:0 fatty acid, the monounsaturated compound increased the cholesteryl ester pool by 420% ($P < 0.001$), increased receptor activity by 104% ($P = 0.005$), and suppressed the production rate by 64% ($P = 0.028$).

These changes in the parameters of LDL transport were reflected in the steady-state LDL cholesterol levels in the plasma (Table 2). The plasma LDL cholesterol concentration in the control animals averaged 72 mg/dl, which was consistent with the effect of the small amount of cholesterol in the diet (2). This value increased to 204 mg/dl after feeding the 14:0 fatty acid but was reduced to only 28 mg/dl after feeding the monounsaturated compound.

Effects on Net Sterol Balance Across the Liver. While net cholesterol absorption from the diet was not altered by these dietary fatty acids (Table 1), the possibility still existed that they did alter the rate of cholesterol synthesis within some compartment of the body. Therefore, absolute rates of sterol synthesis were measured *in vivo* in all tissues of the animals fed these three triacylglycerols. As illustrated in Fig. 3, sterol synthesis was essentially identical in the liver (Fig. 3A) and remaining tissues of the carcass (Fig. 3C) in the three experimental groups. The two long-chain fatty acids did increase synthesis in the intestine (Fig. 3B), as observed (20). Nevertheless, there was no differential effect of the 14:0 and 18:1 fatty acids on synthesis in any organ (Fig. 3A–C) or in the whole animal (Fig. 3D).

DISCUSSION

In these studies, advantage was taken of our recent observations that the 14:0 fatty acid maximally suppressed hepatic receptor activity when fed along with dietary cholesterol (7) while the 18:1 lipid maximally restored this activity. Thus, under circumstances in which the quantities of both sterol and triacylglycerol absorbed from the diet were essentially identical (Table 1), it was possible to create new steady states in the animals where there was a 7-fold difference in the

Table 2. Cholesterol concentration in different lipoprotein fractions in animals fed three triacylglycerols for 30 days

Lipoprotein fraction	Fatty acid in dietary triacylglycerol		
	8:0	14:0	18:1
LDL, mg/dl	72 $\pm$ 7	204 $\pm$ 16*	28 $\pm$ 2*
HDL, mg/dl	94 $\pm$ 7	84 $\pm$ 2	94 $\pm$ 3
Total, mg/dl	242 $\pm$ 8	549 $\pm$ 51*	166 $\pm$ 9*

After animals were fed diets containing the three different triacylglycerols for 30 days, plasma was obtained in the nonfasting state, and the concentration of cholesterol present in the whole plasma and in the LDL and high density lipoprotein (HDL) fractions was quantified. Each value represents mean $\pm$ 1 SEM for six animals; asterisk indicates that the value was significantly different from the corresponding value in the control (8:0) group.

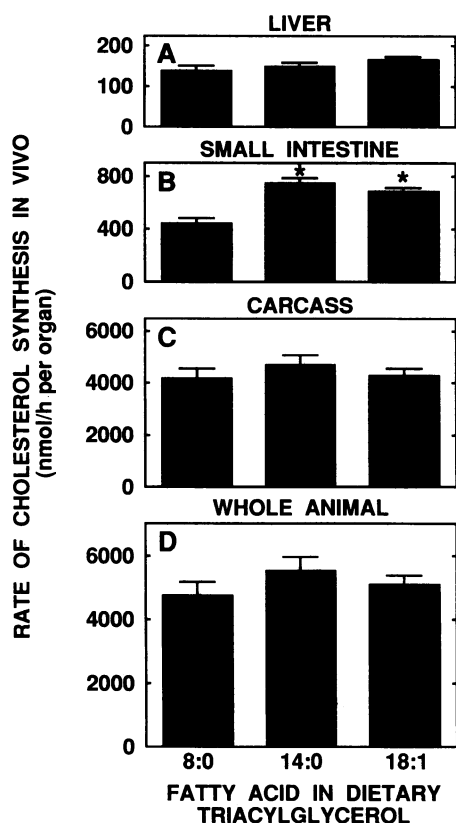


FIG. 3. Rates of sterol synthesis in major tissues and whole animal in groups of hamsters fed the three different triacylglycerols. Vertical axis shows rates of synthesis measured *in vivo* and expressed as nmol of $^3\text{H}_2\text{O}$ incorporated into digitonin-precipitable sterols per h per whole liver (A), small intestine (B), and remaining organs of the carcass (C). Sum of the rates of synthesis in these three groups of tissues equals the rates of synthesis in the whole animal (D). Mean values ± 1 SEM are shown for six animals in each group; asterisk indicates that the value was significantly different from the corresponding value in the control (8:0) group.

plasma LDL cholesterol level (Table 2) brought about by a 104% difference in hepatic receptor activity and a 64% difference in LDL cholesterol production (Fig. 2). The inverse relationship commonly observed between J^m and J_t (Fig. 2; refs. 1, 2, and 5) can probably be attributed to the role of the LDL receptor in clearing partially metabolized very low density lipoprotein particles, as well as LDL, from the plasma (21). Thus, suppression of hepatic receptor activity by either genetic or dietary means is usually associated with a secondary increase in the LDL cholesterol production rate (1, 21, 22). If this is the case, then in the present study the marked effects of the two fatty acids must be attributed to a single regulated event—i.e., the differential effect of the 14:0 and 18:1 fatty acids within the liver cell on regulation of LDL receptor activity.

It has been known since the initial description of the LDL receptor that net sterol balance across the cell is one of the major determinants of receptor-dependent LDL transport (23). Regardless of whether cholesterol is delivered to the cell dissolved in a solvent or carried in LDL or chylomicron remnants, an increase in net cholesterol entry into a tissue is signaled by an increase in the level of cholesteryl esters and suppression of LDL receptor activity. Such a relationship is seen in the liver of the hamster and is illustrated by the solid line in Fig. 4. After feeding different levels of sterol alone in the diet for 30 days, the concentration of cholesteryl esters is increased nearly 50-fold and there is a corresponding inhibition of hepatic receptor activity (2).

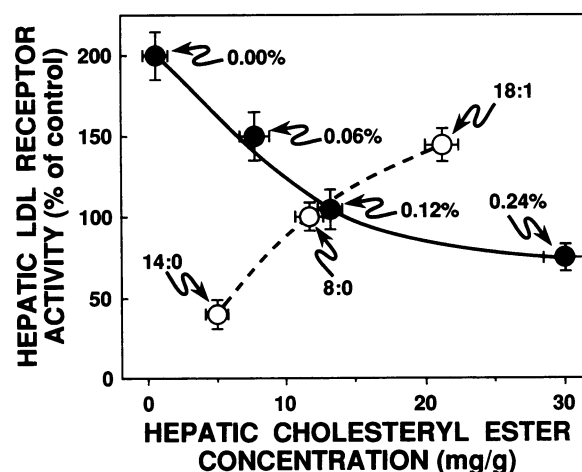


FIG. 4. Relationship between concentration of cholesteryl esters in the liver and hepatic LDL receptor activity when feeding cholesterol alone or in combination with different triacylglycerols. Solid line shows effect of feeding 0%, 0.06%, 0.12%, and 0.24% cholesterol alone in the diet for 30 days. These data are adapted from published studies (2). Dashed line shows the same relationship obtained in the present studies in which dietary cholesterol intake was kept constant at 0.12% and 15% of the triacylglycerols containing either the 8:0, 14:0, or 18:1 fatty acids was fed simultaneously. In this diagram, J^m is expressed relative to the value found in the control group (100%) reported in this study. Mean values ± 1 SEM are shown.

The level of acyl-CoA cholesterol acyltransferase activity, the enzyme that catalyzes cholesteryl ester formation, is driven by the intracellular availability of sterol (2, 24). Any physiological situation—e.g., cholesterol or bile acid feeding (2, 25) or inappropriately high rates of cholesterol synthesis (26)—that leads to a net increase in intrahepatic cholesterol raises the cholesteryl ester level and suppresses receptor activity. Similarly, any manipulation—e.g., sequestering bile acids (27), ileectomy, or blocking cholesterol absorption (28)—that reduces the net balance of cholesterol across the liver lowers cellular cholesteryl ester concentrations and increases receptor activity. This relationship presumably reflects the fact that the putative regulator that interacts with the receptor gene and the pool of sterol that is substrate for cholesterol acyltransferase are in equilibrium. Hence, in any situation in which net sterol balance across the liver is the physiological variable, the concentration of cholesterol in the ester pool in the hepatocyte is essentially a measure, albeit an indirect one, of the concentration of cholesterol in the putative regulatory pool.

The situation is very different when the liver is flooded with specific long-chain fatty acids, as shown in the present study. The 8:0 fatty acid fed in the control diet is known to be rapidly metabolized to acetyl-CoA in the liver and used for synthesis of a variety of products. Hence, there is no change in the relative abundance of fatty acids in the hepatic lipid pools (Fig. 2), and there is no alteration in either the cholesteryl ester concentration or receptor activity from those levels attributable to the cholesterol (0.12%) also present in the diet (Fig. 4). However, when the animals are fed the 14:0 fatty acid, the hepatic and, particularly, the cholesteryl ester lipid fractions become enriched with this saturated fatty acid (Fig. 2), and there is suppression of receptor activity coupled with a marked reduction in the cholesteryl ester concentration (Fig. 4). In contrast, when the monounsaturated fatty acid is fed, the cholesteryl ester lipids become enriched with the 18:1 compound (Fig. 2), and there is restoration of hepatic receptor activity under circumstances where the concentration of cholesteryl esters in the liver is markedly increased (Fig. 4). Furthermore, in all three experimental groups, the absolute rate of cholesterol absorption averaged 6.1–7.3 mg/day (Ta-

ble 1) and the absolute rate of cholesterol synthesis in the whole animal could be calculated to equal 3.6–4.1 mg/day (Fig. 3) [assuming that 1.45 carbon atoms were incorporated into cholesterol for each tritium atom (17, 29)]. Thus, these marked changes in receptor activity were induced by the two fatty acids under circumstances in which there was no significant change in net sterol balance across the liver.

Under *in vitro* conditions, the level of acyl-CoA cholesterol acyltransferase activity is also very sensitive to the types of fatty acid available for the reaction. For example, the 18:1 fatty acid drives ester formation at much higher rates than do the saturated long-chain fatty acids, regardless of whether these compounds are supplied as the free acids or as the acyl-CoA derivatives (30). These results are consistent with the findings in this study, and suggest that when cholesterol flux across the liver is kept constant, the distribution of sterol between the putative regulatory pool and the pool of cholesteryl esters is dictated by the types of fatty acid present in the liver cell. When the liver is rich in the 18:1 compound, the rate of esterification is high and the equilibrium for intracellular cholesterol is shifted from the putative regulatory pool into the biologically inactive ester pool. In contrast, when the substrate pool of lipid becomes enriched with the saturated fatty acids, ester formation occurs at much lower rates and a greater proportion of the excess cholesterol reaching the liver remains in the regulatory pool.

In summary, these studies suggest that hepatic receptor activity and, therefore, plasma LDL cholesterol concentrations are regulated through two fundamentally different mechanisms. One of these is related to the amount of excess cholesterol within the hepatocyte and is determined by net cholesterol balance across the liver. The second is a function of the distribution of this excess intracellular sterol between the putative regulatory pool and the cholesteryl ester pool and is determined by the types of fatty acid available within the liver. Both of these mechanisms may be explained in terms of the known sensitivity of acyl-CoA cholesterol acyltransferase activity in the liver to the mass of cholesterol available for esterification and the fatty acid specificity of this enzyme. Furthermore, this model is consistent with the observations that the effect of dietary triacylglycerol is dependent on the simultaneous intake of cholesterol (2) and that triacylglycerol containing mixtures of saturated fatty acids markedly lowers cholesteryl ester concentrations in the liver while suppressing receptor activity (1). While there are alternative, more complex, explanations for these observations, these studies do demonstrate unequivocally that fatty acids carried in the triacylglycerol component of the diet can exert major regulatory effects on hepatic receptor activity, and the plasma LDL cholesterol level, under circumstances where there is no change in net cholesterol balance across the liver.

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