Trans-9-octadecenoic acid is biologically neutral and does not regulate the low density lipoprotein receptor as the *cis* isomer does in the hamster

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Abstract The concentration of cholesterol carried in low density lipoproteins (LDL-C) is primarily determined by the rate at which LDL-C is produced (J_t) and the rate at which the liver takes up this particle through receptor-dependent transport (J^m). The accumulation of specific dietary fatty acids in the liver profoundly alters these kinetic parameters and will either increase hepatic receptor activity or further suppress J^m, depending upon the particular fatty acid that enriches the various lipid pools. This study tests the thesis that the cellular effects of each fatty acid are determined by the ability of that lipid to act as an effective substrate for cholesteryl ester formation by examining the metabolic effects of either *cis*-9-octadecenoic acid (18:1(9*c*)), the preferred substrate for esterification, or *trans*-9-octadecenoic acid (18:1(9*t*)), a poor substrate for this reaction. When fed to hamsters for 30 days, the steady-state concentration of cholesteryl esters was markedly increased by the 18:1(9*c*), as compared to the 18:1(9*t*), compound. In animals receiving the 18:1(9*c*) fatty acid, hepatic receptor activity was significantly increased, LDL-C production was suppressed, and the steady-state LDL-C concentration was reduced. In contrast, the 18:1(9*t*) fatty acid did not significantly alter J^m, J_t, or the plasma LDL-C level from those values found in the control animals fed an isocaloric amount of a biologically neutral fatty acid, octanoic acid. Despite these different effects on the parameters of LDL metabolism, neither the *cis* nor *trans* fatty acid altered net cholesterol delivery to the liver from de novo sterol synthesis in any tissue in the body or from uptake of dietary cholesterol across the intestine. Therefore, these studies provide strong support for the thesis that fatty acids exert regulatory effects on hepatic LDL receptor activity by altering the distribution of cholesterol in the hepatocyte between a putative regulatory pool and the inert pool of cholesteryl esters. The direction and magnitude of the effects of specific fatty acids on receptor-dependent LDL transport appear to relate directly to the capacity of specific fatty acids to either promote or inhibit cholesteryl ester formation.—**Woollett, L. A., C. M. Daumerie, and J. M. Dietschy.** *Trans*-9-octadecenoic acid is biologically neutral and does not regulate the low density lipoprotein receptor as the *cis* isomer does in the hamster. *J. Lipid Res.* 1994. **35:** 1661–1673.

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The concentration of cholesterol carried in the plasma in low density lipoproteins (LDL-C) is directly related to the development of atherosclerosis in experimental animals and humans (1–3). Unlike the concentration of many other metabolic substrates and products in plasma, however, the level of LDL-C can vary from the low values of 10–20 mg/dl seen in many normal experimental animals to values of 300–600 mg/dl found in humans and experimental animals genetically lacking LDL receptor activity or on diets high in cholesterol and certain triacylglycerols (4–7). In general, the steady-state concentration of LDL-C in any of these situations is determined by four variables. These include the rate at which LDL-C is formed and appears in the plasma compartment, i.e., the LDL-C production rate (J_t); the maximal achievable rate of receptor-dependent LDL-C removal from the plasma (J^m); the functional affinity of the LDL particle for its receptor (K_m); and the rate of receptor-independent LDL-C removal from the plasma (P) (8, 9).

In normal humans and experimental animals the marked changes in LDL-C concentrations that can be induced by alterations in the diet are attributable primarily

Abbreviations: LDL-C, cholesterol carried in low density lipoproteins; VLDL-C, cholesterol carried in very low density lipoproteins; J^m, maximal rate of LDL-C uptake into the liver by the receptor-dependent transport process; K_m, the functional affinity of the LDL particle for its receptor; P, the rate constant for the receptor-independent uptake of LDL-C; J_t, the rate of LDL-C production in the whole animal; C_1, the steady-state concentration of LDL-C in the plasma; ACAT, acyl-CoA:cholesterol acyltransferase; 8:0, *n*-octanoic acid; 14:0, *n*-tetradecanoic acid; 16:0, *n*-hexadecanoic acid; 18:0, *n*-octadecanoic acid; 18:1(9*c*), *cis*-9-octadecenoic acid; 18:1(9*t*), *trans*-9-octadecenoic acid; 18:2, *cis,cis*-9,12-octadecadienoic acid; TAG, triacylglycerol; J_L, rate of LDL-C uptake into the liver by the receptor-dependent and -independent transport processes; J_i, rate of LDL-C uptake into the liver by the receptor-independent process.

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to changes in only two of these four variables, i.e., the maximal achievable rate of receptor-dependent LDL-C transport and the LDL-C production rate (7, 10, 11). Furthermore, there is increasing evidence that the production rate term is determined, in large part, by the level of LDL receptor activity in the whole animal. As J^m is reduced, either through genetic or environmental factors, increased amounts of very low density lipoproteins (VLDL) are converted to LDL and, consequently, the LDL-C production rate increases (10, 12, 13). Not uncommonly, therefore, J_t increases as J^m is reduced, and these two events largely account for the observed increases in the steady-state LDL-C concentration. Thus, in order to understand the regulation of plasma LDL-C levels, it is critically important to understand the regulation of receptor-dependent LDL-C transport in the whole animal or human.

Recent data have localized the site of receptor-dependent LDL-C transport in the whole animal primarily to the liver (14–17). In the mouse, hamster, rat, rabbit, cynomolgus monkey, and, probably, human, approximately 60–80% of the LDL-C that is removed from the plasma is taken up by this organ, and the great majority of this uptake occurs through the receptor-dependent process (18). Furthermore, the mechanism of environmentally induced changes in steady-state plasma LDL-C concentrations can usually be traced to changes in this hepatic receptor-dependent transport step (7, 10, 18–20). Feeding cholesterol alone or cholesterol in combination with saturated fatty acids invariably reduces hepatic receptor activity, increases the LDL-C production rate, and raises the plasma LDL-C concentration. In contrast, when certain unsaturated fatty acids are substituted for these saturated lipids in human or animal diets, hepatic receptor activity is restored and plasma LDL-C levels are reduced (7, 10, 21–23).

Recent studies have suggested that this regulation of hepatic receptor activity is controlled at the cellular level by two fundamentally different, physiological mechanisms acting through the enzyme acyl-CoA:cholesterol acyltransferase (ACAT) (23, 24). The first involves changes in net sterol delivery to the liver. When cholesterol is added to the diet, there is a dose-dependent, parallel expansion of both a putative regulatory pool of cholesterol and the cholesteryl ester pool that, presumably, is mediated by ACAT (21, 24). As the ratio of the size of these two pools remains constant at different dietary cholesterol loads, in this situation the level of hepatic receptor activity (and receptor mRNA levels) varies inversely with the steady-state cholesteryl ester concentration in the hepatocyte (24, 25). The second mechanism of regulation involves shifts in this ratio of cholesterol between the regulatory and storage pools that are brought about by enrichment of the liver cell with specific fatty acids that either augment or inhibit ACAT activity and cholesterol esterification. Under circumstances where net cholesterol balance across the liver is kept constant, enrichment of the liver with *cis*-9-octadecenoic acid (18:1(9*c*)) shifts this equilibrium towards the ester pool, while fatty acids such as *n*-hexadecanoic acid (16:0) inhibit ACAT activity (24, 26). Thus, under these conditions, hepatic receptor activity will be either increased or decreased, depending upon the fatty acid, and the level of receptor-dependent LDL-C transport will vary directly, not inversely, with the steady-state cholesteryl ester concentration in the liver (24).

The series of experiments reported here further test this model by taking advantage of the fact that the two Δ^9 geometrical isomers of the 18:1 fatty acid are handled very differently by the hepatic ACAT enzyme. Under in vitro conditions it has been shown that ACAT uses the 18:1(9*c*) compound to esterify cholesterol at very high rates, while various saturated and polyunsaturated fatty acids are poor substrates for this reaction (27). This is true regardless of whether the reaction is carried out with the free fatty acids or with the acyl CoA derivatives (27, 28). When hepatic microsomes are incubated with either the 18:1(9*c*) or 18:1(9*t*) compound, > 90% of the fatty acids in the newly synthesized cholesteryl esters are the *cis* or *trans* isomer, respectively. However, despite the fact that both isomers can be used by the enzyme, the velocity of the esterification reaction with the 18:1(9*t*) compound is only a fraction of that seen with the 18:1(9*c*) fatty acid (28). Furthermore, when increasing amounts of the *trans* fatty acid are added in vitro to a system containing the 18:1(9*c*) compound, cholesteryl ester formation from the *cis* fatty acid is actually inhibited (28). Thus, if the proposed model is correct and if these same events take place in vivo when the liver becomes enriched with these two isomers, it would be predicted that the ability to shift cholesterol into the ester pool and up-regulate hepatic LDL receptor activity would be lost when the double bond in the 18:1 fatty acid is shifted from the *cis* to the *trans* configuration. The studies reported here were designed to establish whether such a subtle change in molecular configuration profoundly alters the regulation of hepatic LDL receptor activity by altering the distribution of cholesterol in the hepatocyte between the regulatory and storage pools.

MATERIALS AND METHODS

Materials

The diets used in these studies were constructed so that only one fatty acid differed in the various experimental diets. Triacylglycerols containing *n*-octanoic acid (8:0) and *n*-octadecanoic acid (18:0) were purchased from Sigma Chemical Co. (St. Louis, MO), while those made up of *cis*-9-octadecenoic acid (18:1(9*c*)) and *cis,cis*-9,12-octadecadienoic acid (18:2) came from Nu-Chek Prep,

Inc. (Elysian, MN) and *n*-tetradecanoic acid (14:0) and *n*-hexadecanoic acid (16:0) came from Fluka Chemical Corp. (Ronkonkoma, NY). Unilever Research Laboratorium[2] (Vlaardingen, The Netherlands) provided two triacylglycerol blends that contained a large proportion of the fatty acids as either the *cis*-9-octadecenoic compound (18:1(9*c*)) or the *trans*-9-octadecenoic fatty acid (18:1(9*t*)).[3] [1α,2α-^{3}H]cholesterol, [^{14}C]cholesterol, [^{3}H]water, ^{125}I, and ^{131}I were all purchased from Amersham Corp. (Arlington Heights, IL).

Animals

Male, Golden Syrian hamsters approximately 10 weeks old were purchased from Charles River Lakeview (New Field, NJ) and housed in colony cages for 2 weeks before the experiments were begun. The animals were then placed on specific experimental diets, as described below, for 1 month when a variety of experimental measurements were made. Throughout this entire period the animals were maintained in rooms with controlled humidity and alternating 12-h periods of light and darkness. All experimental measurements were made at essentially the mid-dark phase of the light cycle.

Diets

A major technical problem with these studies was to construct diets that varied only with respect to a single fatty acid. The limiting factor was the availability of a triacylglycerol that contained significant amounts of the 18:1(9*t*) compound. Analysis of the triacylglycerol supplied by Unilever Research Laboratorium revealed that approximately 52% of the fatty acids in this product was the *trans* compound while the remaining lipids consisted primarily of the 16:0, 18:0, 18:1(9*c*), and 18:2 fatty acids. As this was the maximal concentration of the *trans* fatty acid commercially available as a triacylglycerol, all other triacylglycerol blends used in this study were made up to have compositions identical to this product, except that the 52% of the fatty acids that consisted of the 18:1(9*t*) compound was systematically replaced by an equal amount of other specific fatty acids of interest. These various experimental triacylglycerols were formulated by mixing together the required amounts of triacylglycerol containing only a single fatty acid, i.e., the 8:0, 14:0, 18:0, 18:1(9*c*), and 18:2 compounds. Batches of these five mixtures large enough to carry out all of the studies were prepared, and the fatty acids in each of these blends were reanalyzed by gas–liquid chromatography (GLC). As shown in **Table 1,** in the five experimental triacylglycerols prepared in this manner, 52.4% of the fatty acids consisted of a single compound, i.e., either the 8:0, 14:0, 18:0, 18:1(9*c*), or 18:1(9*t*) fatty acid (A). The remaining 47.6% of the fatty acids (B) consisted of a mixture of the 16:0, 18:0, 18:1(9*c*), and 18:2 lipids, and these were present in all five experimental triacylglycerols at the same fixed concentrations. Thus, all studies were performed by adding varying amounts (0 to 20 g/100 g diet) of these experimental triacylglycerol blends and a constant amount of cholesterol (0.12 g/100 g diet) to ground animal diet (Allied Mills, Chicago, IL). These experimental diets were then fed to groups of animals for 30 days at which time all analytic measurements were made. In experiments to be published elsewhere, we have shown that the metabolic effects of individual fatty acids were not influenced by the position of the acid in the TAG molecule.

Cholesterol, cholesteryl ester, and fatty acid concentrations in plasma and tissues

Plasma LDL-C concentrations were determined by simultaneously separating plasma samples at densities of 1.020 and 1.063 g/ml, and then quantitating the cholesterol content in the top and bottom of each centrifuge tube (7, 10). The concentration of unesterified and esterified cholesterol was determined by separating these two components on silicic acid/celite columns and then determining the concentration of cholesterol in each fraction by GLC (29). The total lipid fraction obtained from the liver was separated into the cholesteryl ester, triacylglycerol, and phospholipid fractions by TLC. These fractions were then saponified and methyl esterified, and the relative abundance of the various fatty acids in each of these fractions was quantified by GLC (30).

Dietary triacylglycerol absorption

In some studies the ability of animals to absorb the five experimental triacylglycerols was measured. During the last week of the 30-day feeding period, food intake was quantified daily and two 24-h fecal collections were obtained. Total lipid content in the feces was determined gravimetrically, and net lipid absorption was calculated and expressed as a percentage of intake.

Hepatic LDL receptor activity and LDL-cholesterol production rate in vivo

Rates of receptor-dependent and receptor-independent transport into the liver and into the remaining extrahepatic tissues of the animals were measured in vivo as previously described (7, 14, 31). This method involved the short term, steady-state infusion of LDL-C harvested from hamsters on a low fat diet and labeled with ^{125}I-labeled tyramine cellobiose (32). The first set of data was used to calculate the maximal achievable rate of receptor-

[2]The authors express their appreciation to Dr. Nanneke de Fouw and her colleagues at Unilever Research Laboratorium Vlaardingen for supplying these triacylglycerol blends.

[3]During hydrogenation of the 18:1(9*c*) compound there is some migration of the double bond longitudinally along the fatty acid chain. Thus, in this compound the *trans* double bond was predominantly, although not exclusively, in the 9 position.

TABLE 1. Fatty acid composition of the triacylglycerols added to the various experimental diets

Experimental Triacylglycerol	Relative Abundance of Each Fatty Acid (%)						
	8:0	14:0	16:0	18:0	18:1(9*c*)	18:1(9*t*)	18:2
A. Variable fatty acids							
8:0-TAG	52.4	0.0	0.0	0.0	0.0	0.0	0.0
14:0-TAG	0.0	52.4	0.0	0.0	0.0	0.0	0.0
18:0-TAG	0.0	0.0	0.0	52.4	0.0	0.0	0.0
18:1(9*c*)-TAG	0.0	0.0	0.0	0.0	52.4	0.0	0.0
18:1(9*t*)-TAG	0.0	0.0	0.0	0.0	0.0	52.4	0.0
B. Constant fatty acids							
All-TAG	0.0	0.0	3.7	5.2	32.0	0.0	6.8

The fatty acid composition of the triacylglycerol (TAG) blends added to the experimental diets was determined by GLC. All compositions were within 1% of their predicted values. Section A shows the amount of the variable fatty acid present in each of the five experimental triacylglycerols. Section B lists the fatty acids that were present in constant amounts in these same five triacylglycerols. Different amounts of each of these five blends were added to experimental diets, as described in the Results section.

dependent LDL transport into the liver (J^m) in each animal. This parameter is a function of receptor number, but gives the maximal rate of LDL-C uptake each hour by the receptor-dependent process when all receptors are fully occupied (μg/h per liver per 100 g animal). The second set of data was used to calculate the LDL-C production rate (J_t) which denotes the amount of LDL-C that is produced and delivered into the plasma space each hour (μg/h per 100 g animal). To simplify the presentation of these data, the absolute values of J^m and J_t found in the control animals (those fed the 8:0-TAG) were set equal to 100% and the results obtained in the other experimental groups are presented as a percentage of these control values.

Dietary cholesterol absorption

Cholesterol absorption was determined by a modification of the dual isotope method previously described (33). Animals were dosed in the mid-light phase while being maintained in the fed state. [1α,2α-^{3}H]cholesterol in Intralipid 20% (KabiVitrum, Inc., Clayton, NC) was injected into the femoral vein of the animal. Immediately after this, [^{14}C]cholesterol dissolved in MCT oil (Mead Johnson & Co., Evansville, IN), was given to the animal through a tube in the stomach. Seventy-two hours later, animals were exsanguinated, and the ratio of the two isotopes in the plasma was determined (34).

Cholesterol synthesis

As previously described (6, 35), hamsters were given intravenously 100 mCi of [^{3}H]water and placed in individual restraining cages. One hour later the animals were anesthetized and exsanguinated. Aliquots of plasma were taken for measurement of the specific activity of the pool of body water. The liver and remaining carcass were saponified, and digitonin-precipitable sterols were isolated and the radioactivity was quantified. Rates of sterol synthesis are expressed as the nmol of [^{3}H]water incorporated into digitonin-precipitable sterols per hour per whole organ (nmol/h per whole tissue).

Calculations

Where appropriate, mean values ± 1 SEM are given in the tables and figures. The Student's *t*-test was used to compare two experimental variables for statistical significance ($P \leq 0.05$). The rate of LDL-C uptake into the liver (J_L) is known to be dictated by the maximal velocity of the receptor-dependent transport process (J^m), the affinity of the LDL particle for its receptor (K_m), and the rate of the receptor-independent transport process (P) (8, 9). In some cases, the kinetic curves defining the relationship between J_L and the concentration of LDL-C in the plasma (C_1) were calculated using the following equation:

$$J_L = \frac{J^m C_1 + P C_1 K_m + P C_1^2}{K_m + C_1} \qquad \textit{Eq. 1)}$$

RESULTS

Initial experiments were performed to determine whether the 18:1(9*c*) and 18:1(9*t*) fatty acids did have differential effects on the parameters of LDL-C metabolism and to explore the dose-dependency of these effects. Animals were fed diets containing a constant amount of cholesterol (0.12 g/100 g diet) but increasing amounts of the 18:1(9*c*)-TAG and 18:1(9*t*)-TAG equal to 0, 5, 10, 15, and 20 g/100 g diet. Thus the concentration of the two variable fatty acids in these diets equaled 0, 2.6, 5.2, 7.8, and 10.5 g/100 g (Table 1). These diets were fed for 30 days as we previously have shown that approximately 120 pools of LDL-C

are turned-over during this time, and the parameters that dictate cholesterol and LDL-C homeostasis across the liver have essentially reached new steady-state values (18).

To verify that these two geometrical isomers of the 18:1 fatty acid were absorbed, incorporated into the chylomicron, and reached the liver, the relative abundance of these two compounds in the hepatic lipid pools was quantified in the animals fed these different levels of triacylglycerols for 30 days. As is evident in **Fig. 1,** in animals fed cholesterol, but no triacylglycerol, the 18:1(9*c*) compound accounted for 36% of the fatty acids in the hepatic total lipid fraction (A) but 69% of the fatty acids esterified to cholesterol (C). This latter finding is consistent with the known specificity of the esterification reaction (27, 28) and the fact that the 18:1(9*c*) compound can be synthesized endogenously. When increasing amounts

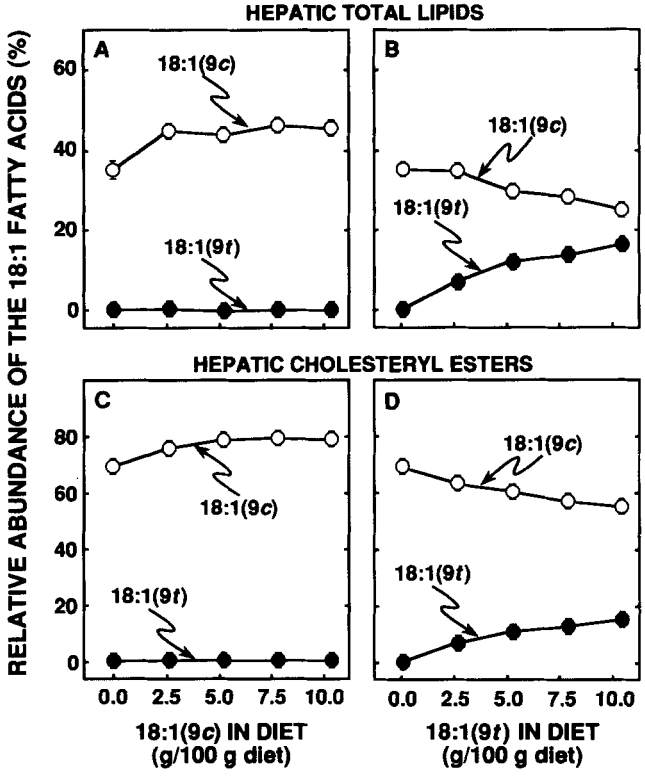

Fig. 1. Relative abundance of the 18:1 fatty acids in the liver of animals fed increasing amounts of the 18:1(9*c*) or 18:1(9*t*) compound. Animals were fed diets containing 0, 5, 10, 15, and 20 g of triacylglycerol per 100 g of diet for 30 days. These diets contained 0, 2.6, 5.2, 7.8, and 10.5 g/100 g, respectively, of either the 18:1(9*c*) or 18:1(9*t*) fatty acid. The other fatty acids in the triacylglycerols were the same in all diets as was the concentration of cholesterol (0.12 g/100 g). At the end of the feeding period, the total lipids were extracted from liver samples from each animal, and the major classes of lipids were separated by TLC. After saponification, the fatty acids in the total lipids and cholesteryl esters were methyl esterified, and the relative abundance of each fatty acid was quantified by GLC. The relative abundance of the 18:1(9*c*) and 18:1(9*t*) fatty acids in the total lipid fraction (A and B) and in the cholesteryl esters (C and D) of animals fed the two isomers of the 18:1 fatty acid is shown. Mean values ± 1 SEM are given for six animals in each group.

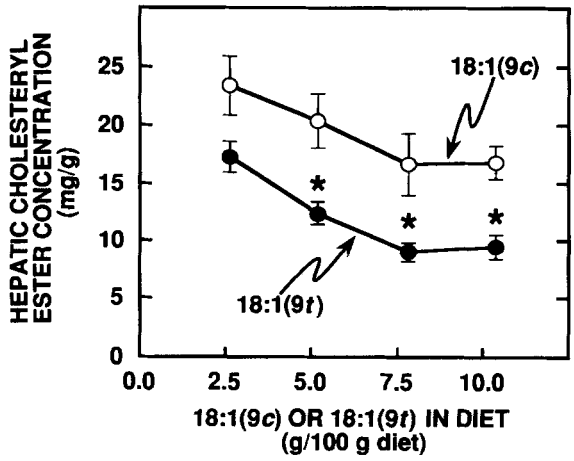

Fig. 2. Steady-state hepatic cholesteryl ester concentrations in animals fed increasing amounts of the 18:1(9*c*) or 18:1(9*t*) fatty acid for 30 days. Diets were fed for 30 days as described in Fig. 1. Mean values ± 1 SEM are shown for 12 animals in each group. Asterisks indicate significant differences ($P \leq 0.05$) between animals fed the same amount of the 18:1(9*c*) or 18:1(9*t*) compound.

of this *cis* monounsaturated acid were added to diet, the contribution of this fatty acid to the total lipid and cholesteryl ester pools increased to 46% (A) and 79% (C), respectively, at the highest dose of dietary fat. There was virtually no 18:1(9*t*) fatty acid detectable in the hepatic lipids of these animals. However, when increasing amounts of this *trans* lipid were fed for 30 days there was a dose-dependent increase in the content of this compound in both the total lipid (B) and cholesteryl ester (D) fractions until approximately 15–18% of the fatty acids in these two lipid pools consisted of the 18:1(9*t*) compound. It is noteworthy that *1)* these increases in the concentration of the *trans* monounsaturated fatty acid were associated with a reciprocal reduction of the abundance of the 18:1(9*c*) compound, and *2)* the preference of the ACAT reaction for the *cis* monounsaturated fatty acid was lost so that the relative abundance of the 18:1(9*t*) fatty acid was the same in the cholesteryl ester (D) as in the total lipid fraction (B).

This progressive enrichment of the hepatic lipid pools with these two fatty acids was clearly associated with significant changes in several parameters of sterol metabolism in the liver. **Figure 2,** for example, shows the steady-state concentration of cholesteryl esters in the livers of these same animals. As is apparent, the concentration of cholesteryl esters decreased as the amount of either type of triacylglycerol in the diet was increased. This came about because the caloric density of the diets increased as the triacylglycerol content was increased. As a consequence, the animals ate less of the diet each day and, therefore, took in less dietary cholesterol. The important observation, however, is that at any level of triacylglycerol feeding, the steady-state concentration of cholesteryl esters in the liver was significantly lower in the animals fed

the 18:1(9*t*) compound than in those receiving the *cis* monounsaturated fatty acid. In contrast, the concentration of unesterified cholesterol was essentially constant at 1.9 to 2.1 mg/g in all groups. Furthermore, there were significant differences in the steady-state plasma LDL-C concentration in these same animals. In the hamsters fed the highest level of the 18:1(9*c*) fatty acid, for example, the plasma LDL-C concentration equaled 43 ± 3 mg/dl while this concentration was significantly higher, 60 ± 4 mg/dl, in those animals fed the *trans* fatty acid. Thus, these initial investigations demonstrated that the *cis* and *trans* isomers must have significantly different effects on both the metabolism of sterol within the liver and on one or more of the parameters that dictate steady-state LDL-C levels.

Based on these results, all subsequent experiments were undertaken using diets that contained a constant concentration of cholesterol (0.12 g/100 g) and triacylglycerol (20 g/100 g), and where the variable fatty acid made up 52.4% (Table 1) of the total fatty acids in the triacylglycerol. Furthermore, in addition to the diets containing 18:1(9*c*)-TAG and 18:1(9*t*)-TAG, all experiments also included a neutral control diet where the variable fatty acid was the 8:0 compound and a positive control diet having the 14:0 compound as the variable fatty acid. The 8:0 fatty acid has been shown to have no effect on LDL-C metabolism or on the plasma LDL-C concentration (26, 36) while the 14:0 saturated fatty acid suppresses hepatic receptor activity when fed along with cholesterol (24, 26). Thus, all studies were carried out under circumstances where cholesterol intake, total triacylglycerol intake, the background level of dietary fatty acids, and weight gain over the 30-day feeding period were all constant. The only variable was that the four experimental diets contained 10.5 g/100 g of either the 8:0, 14:0, 18:1(9*c*), or 18:1(9*t*) fatty acid.

Before beginning the metabolic studies, net fat absorption was measured in animals receiving these four experimental diets to establish that the four variable fatty acids were equally well absorbed. This proved to be the case in that fecal fat output in all four groups equaled only 1–3% of dietary fat intake. Furthermore, after 30 days of feeding these diets, the liver total lipid pool (**Table 2A**) became enriched with each of the experimental fatty acids. After feeding the 8:0-TAG, the profile of fatty acids in the liver did not differ significantly from that seen in animals receiving no dietary triacylglycerol where the predominant fatty acids were the 18:1(9*c*) (37.6%), 18:2 (19.8%), 16:0 (14.8%) and 18:0 (15.0%) compounds. As the 8:0 fatty acid is known to be rapidly taken up by the liver and oxi-

TABLE 2. Relative abundance of each fatty acid in total lipid extracts, cholesteryl esters, triacylglycerols, and phospholipids of liver

Dietary Experimental Triacylglycerol	Relative Abundance of Each Fatty Acid (%)					
	14:0	16:0	18:0	18:1(9*c*)	18:1(9*t*)	18:2
A. Total lipids						
8:0-TAG	0.2 ± 0.0	14.8 ± 0.4	15.0 ± 0.2	37.6 ± 0.6	0.3 ± 0.1	19.8 ± 0.5
14:0-TAG	4.8 ± 0.2[a]	17.7 ± 0.2[a]	14.8 ± 0.4	31.8 ± 0.9[a]	0.2 ± 0.1	19.1 ± 0.5
18:1(9*c*)-TAG	0.2 ± 0.0	13.5 ± 0.4	13.9 ± 0.4	44.6 ± 1.0[a]	0.4 ± 0.1	15.0 ± 0.3[a]
18:1(9*t*)-TAG	0.0 ± 0.0	13.6 ± 0.6	13.7 ± 0.2	23.0 ± 0.9[a]	15.3 ± 0.2[a]	21.7 ± 0.4
B. Cholesteryl esters						
8:0-TAG	0.2 ± 0.0	5.7 ± 0.2	4.6 ± 0.2	72.7 ± 0.3	ND[b]	10.7 ± 0.4
14:0-TAG	5.7 ± 0.2[a]	9.6 ± 0.2[a]	6.5 ± 0.2[a]	59.7 ± 0.7[a]	ND	13.2 ± 0.5[a]
18:1(9*c*)-TAG	0.1 ± 0.0	3.7 ± 0.1[a]	3.1 ± 0.1[a]	80.8 ± 0.2[a]	ND	7.0 ± 0.2[a]
18:1(9*t*)-TAG	0.1 ± 0.0	6.6 ± 0.3	6.8 ± 0.3[a]	56.0 ± 0.8[a]	13.6 ± 0.3[a]	11.6 ± 0.6
C. Triacylglycerols						
8:0-TAG	1.5 ± 1.6	17.7 ± 0.4	6.7 ± 0.3	53.9 ± 0.7	ND	12.9 ± 0.5
14:0-TAG	13.0 ± 1.2[a]	19.1 ± 0.9	6.1 ± 0.4	45.0 ± 0.6[a]	ND	11.1 ± 0.5
18:1(9*c*)-TAG	1.3 ± 1.2	19.1 ± 0.8	5.5 ± 0.2	60.0 ± 0.0[a]	ND	8.2 ± 0.2[a]
18:1(9*t*)-TAG	0.9 ± 0.1	17.4 ± 0.7	6.3 ± 0.3	35.9 ± 0.8[a]	22.9 ± 0.4[a]	11.6 ± 0.3
D. Phospholipids						
8:0-TAG	ND	23.5 ± 0.4	27.8 ± 0.3	19.0 ± 0.7	0.2 ± 0.1	19.2 ± 0.6
14:0-TAG	1.1 ± 0.2[a]	27.6 ± 0.5[a]	28.8 ± 0.3	14.2 ± 0.2[a]	0.4 ± 0.1	18.7 ± 0.7
18:1(9*c*)-TAG	ND	23.6 ± 0.2	29.1 ± 0.5	19.9 ± 1.7	0.2 ± 0.1	16.1 ± 0.7[a]
18:1(9*t*)-TAG	ND	19.6 ± 0.2[a]	22.2 ± 0.2[a]	12.6 ± 0.5[a]	16.2 ± 0.3[a]	20.1 ± 0.5

The animals used in this study were fed one of four experimental diets for 30 days. Each of these diets contained one of the variable fatty acids listed in the left column (10.5 g/100 g diet) and a constant amount of cholesterol (0.12 g/100 g diet). At the end of this feeding period the liver of each animal was extracted and the relative abundance of the major fatty acids in the total lipid extract, as well as in the cholesteryl ester, triacylglycerol, and phospholipid fractions, was determined by GLC.

[a] $P \leq 0.05$ when compared to the group fed the 8:0-TAG.

[b] ND, not detectable.

dized to acetyl-CoA, it is not surprising that this lipid did not alter the pattern of fatty acids in the liver. In contrast, feeding the 14:0-TAG significantly increased the abundance of the 14:0 and 16:0, but not the 18:0, fatty acids. This finding presumably reflects the limited capacity of the liver to elongate the 14:0 compound. Even though the 18:1(9*c*) fatty acid is normally the most abundant compound in hepatic lipids, feeding the 18:1(9*c*)-TAG further increased the relative abundance of this acid to 44.6% while feeding the 18:1(9*t*)-TAG markedly decreased the abundance of this *cis* isomer to only 23.0%. At the same time, the abundance of the 18:1(9*t*) compound reached 15.3% in the liver. Clearly the dietary fatty acids fed in these experiments were reaching the liver and, after 30 days, had established new profiles of fatty acid abundance within the hepatic total lipid pool.

These profiles, however, varied in the subpools of lipids where the specificity of the biosynthetic pathways apparently limited the utilization of certain of these fatty acids. For example, the abundance of the various fatty acids in the triacylglycerol fraction of the liver (Table 2C) faithfully reflected those fed in the diet, whereas the relative abundance of those in the phospholipid fraction (D) were less affected by feeding the 14:0-TAG and 18:1(9*c*)-TAG. Significant amounts of the 18:1(9*t*) compound, however, were incorporated into this fraction at the expense of the 16:0, 18:0, and 18:1(9*c*) acids. Of particular importance, all three of these dietary fatty acids became incorporated into the cholesteryl ester fraction (B) in significant amounts. Thus, the abundance of the 14:0 plus 16:0 fatty acids in these esters increased from 5.9% to 15.3% with 14:0-TAG feeding. The amount of the 18:1(9*c*) compound increased from 72.7% to 80.8% with 18:1(9*c*)-TAG feeding, and the relative amount of the 18:1(9*t*) fatty acid increased from essentially 0% to 13.6% when this compound was fed. It was clear from these studies, therefore, that each of these experimental dietary lipids was absorbed and resulted in characteristic changes in the fatty acid profile of the liver.

Studies were next performed to characterize the changes in the parameters of LDL metabolism that were induced by these dietary fatty acids, and these included the rate of LDL-C transport into the liver (J_L), the maximal rate of LDL-C transport into the liver by the receptor-dependent process (J^m), the LDL-C production rate (J_t), and the steady-state concentration of LDL-C in the plasma (C_1). **Figure 3** shows the steady-state rate of hepatic LDL-C transport and the plasma LDL-C concentration attained at 30 days in the four experimental groups. In addition, the transport curve for the receptor-independent process (J_i) is shown as the dashed line, while the relationships appropriate for the receptor-dependent process are illustrated by the series of solid curves. These latter kinetic curves were calculated using equation 1 and systematically varying receptor activity in

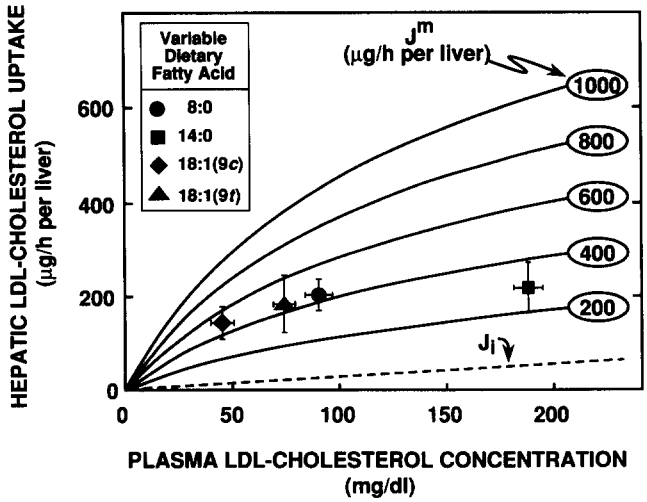

Fig. 3. Rate of hepatic LDL-cholesterol uptake as a function of the plasma LDL-cholesterol concentration in hamsters fed four different fatty acids for 30 days. Groups of animals were fed diets containing 20 g of triacylglycerol per 100 g of diet. Each of the four experimental diets contained a variable fatty acid, i.e., the 8:0, 14:0, 18:1(9*c*), and 18:1(9*t*) compounds, at a constant level of 10.5 g/100 g and cholesterol at a concentration of 0.12 g/100 g. Each of the data points shows the mean rate of LDL-C transport into the liver and the steady-state plasma LDL-C concentration achieved after 30 days on such diets. The dashed line shows the rate of receptor-independent LDL-C uptake (J_i) into the liver while the five solid curves illustrate the kinetic relationships for receptor-dependent transport under circumstances where receptor activity (J^m) was varied from 200 to 1000 μg/h per liver. These theoretical curves were calculated from equation 1 using the following transport parameters: J^m of 200, 400, 600, 800, and 1000 μg/h per liver, K_m of 109 mg/dl, C_1 varying from 0 to 200 mg/dl, and P of 0.30 μg/h per mg/dl. All values are normalized to a 100 g animal. Data points represent mean values ± 1 SEM for six animals.

the liver, J^m, from 1,000 μg/h to only 200 μg/h.

As is apparent, in control animals receiving the 8:0-TAG, J_L equaled 218 ± 20 μg/h giving a J^m value of 440 ± 19 μg/h per liver and a plasma LDL-C level of 94 ± 14 mg/dl. In animals receiving either no lipid or only the 8:0 fatty acid in the diet, J^m is usually 600–650 μg/h per liver and C_1 equals about 22–26 mg/dl. Thus, these control animals had the lower level of hepatic receptor activity and higher concentration of plasma LDL-C that would be expected in hamsters receiving 0.12 g of cholesterol per 100 g of diet (26, 36). Also apparent in Fig. 3 is the fact that feeding the 18:1(9*c*)-TAG lowered C_1 to 40 ± 6 mg/dl while the 14:0-TAG raised this value to 188 ± 15 mg/dl, yet J_L changed relatively little (from 154 to 227 μg/h per liver). Thus, even though J^m was increased to 554 ± 37 μg/h by feeding the 18:1(9*c*) fatty acid and suppressed to 279 ± 53 μg/h when the liver was enriched with the 14:0 compound, transport of LDL-C into the liver remained relatively constant at about 200 μg/h. This finding illustrates the fundamental process that dictates steady-state plasma LDL-C concentrations with triacylglycerol feeding. When hepatic receptor activity is suppressed, as occurs when the 14:0 fatty

acid is enriched in the liver, it is necessary for C_1 to increase markedly to maintain the rate of LDL-C uptake and degradation equal to the rate of LDL-C formation. In contrast, when hepatic receptor activity is relatively increased by feeding the 8:0 or, still better, the 18:1(9*c*) compound, this rate of receptor-dependent LDL-C transport can be maintained at a much lower concentration of LDL-C in the plasma.

In similar studies in a larger group of animals, the two key transport parameters, J^m and J_t, were determined in the four groups of hamsters, and the results are illustrated in **Fig. 4.** As shown in panels A and B, respectively, relative hepatic receptor activity and LDL-C production rates were set at 100% in the animals receiving the 8:0-TAG, and the steady-state LDL-C level in these animals equaled 81 ± 5 mg/dl. Enriching the liver with the 14:0 fatty acid decreased hepatic receptor activity to 63 ± 12% of control and increased J_t to 118 ± 13%. These two alterations fully accounted for the rise in C_1 to 194 ± 9 mg/dl. In contrast, the 18:1(9*c*) fatty acid increased hepatic receptor activity to 126 ± 8% of control, J_t was reduced to 75 ± 6%, and C_1 decreased to only 40 ± 4 mg/dl. Thus, as anticipated, the 14:0 fatty acid actively suppressed hepatic receptor activity while the 18:1(9*c*) compound actively restored J^m. Furthermore, the marked reduction observed in C_1 when the liver was enriched with the monounsaturated fatty acid (40 ± 4 mg/dl) as compared to the saturated compound (194 ± 9 mg/dl) was fully accounted for by the observed 63% increase in receptor activity (A) and 43% reduction in J_t (B) induced by the 18:1(9*c*) compound. However, the ability of this monounsaturated fatty acid to up-regulate hepatic receptor activity was lost entirely when the double bond was converted to the *trans* configuration. Thus, when the liver was enriched with the 18:1(9*t*) fatty acid there was no significant alteration in either relative hepatic receptor activity (A) or LDL-C production (B), and, hence, the steady-state plasma LDL-C concentration did not differ from that observed in the control animals fed the 8:0-TAG (C).

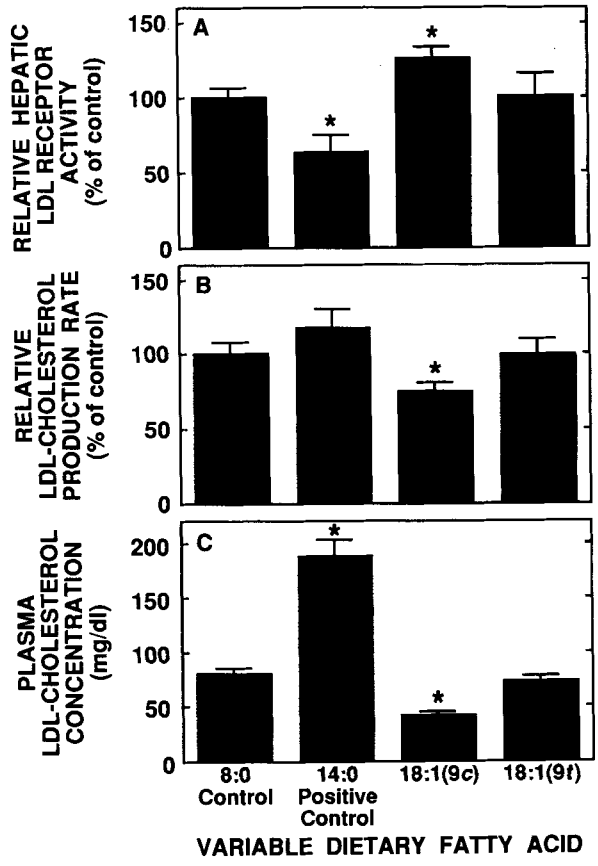

Fig. 4. Relative hepatic LDL receptor activity, relative LDL-cholesterol production rate, and plasma LDL-cholesterol concentration in animals fed different fatty acids for 30 days. The diets fed in this study were the same as those described in Fig. 3. J^m was measured in each animal, and the mean value found in the animals fed the 8:0-TAG was set equal to 100% receptor activity (A). Similarly, J_t was measured in each animal and the mean value quantified in the group fed the 8:0 fatty acid was again set equal to a 100% production rate (B). Mean values ± 1 SEM for six animals in each dietary group are shown. Plasma LDL-C concentrations were also measured in animals fed these same four experimental diets (C) and mean values ± 1 SEM are shown for 18 animals.

To establish whether these marked changes in hepatic receptor activity were associated with changes in net sterol balance across the liver, the magnitude of the three processes that contribute cholesterol to the sterol pools of the hepatocyte was next quantified in vivo. As summarized in **Fig. 5,** feeding the four different variable fatty acids had no effect on the synthesis of cholesterol within the liver itself (A) and, in addition, the rates of sterol synthesis in all of the extrahepatic organs also were unaffected by the type of fatty acid in the diet (B). Furthermore, the percentage of the dietary cholesterol that was absorbed by each group was essentially identical (C). As the average amount of food eaten each day was the same in every experimental group, this finding established that the absolute amount of cholesterol taken up across the gastrointestinal track and delivered to the liver was also unaltered by the type of variable fatty acid present in the diet. Given the fact that 22 μg atoms of hydrogen from water are incorporated into each μmol of cholesterol during sterol synthesis in vivo (37), it can be calculated from these data that net cholesterol flow across the liver from both endogenous synthesis and exogenous dietary sterol totaled ~10.4 mg/day in each animal in all four dietary groups. Thus, the ability of the 18:1(9*c*) fatty acid to increase hepatic receptor activity, and the loss of this ability with the *trans* monounsaturated fatty acid, could not be explained on the basis of a difference in net sterol balance across the liver.

Finally, in previous studies using a similar experimental protocol, we have shown that while the 12:0, 14:0, and 16:0 saturated fatty acids suppressed hepatic receptor ac-

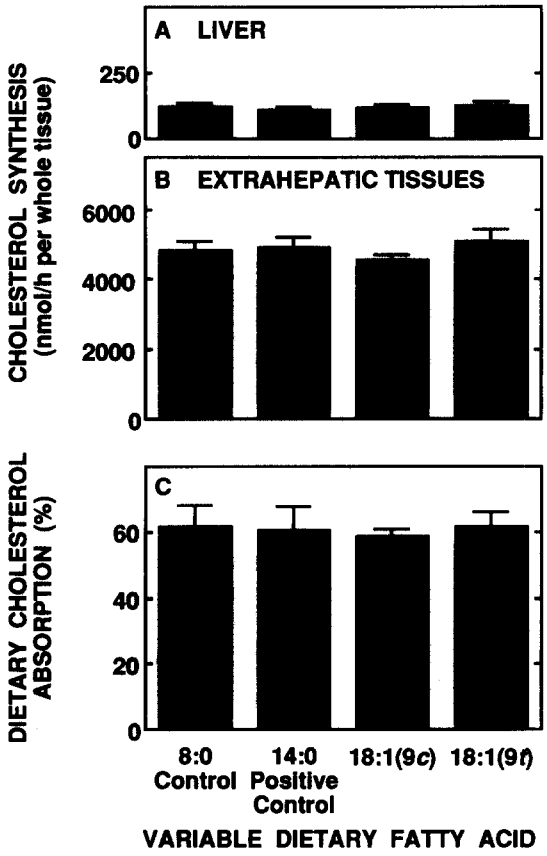

Fig. 5. Absolute rates of sterol synthesis in the liver and extrahepatic organs and cholesterol absorption from the intestine in animals fed different fatty acids for 30 days. The diets fed in these four experimental groups are described in Fig. 3. At the end of the 30-day feeding period absolute rates of cholesterol synthesis were measured in vivo and are expressed as the nmol of [^{3}H]water incorporated into digitonin-precipitable sterols per h per whole liver (A) and in the remaining tissues of the body (B). Absorption was determined in fed animals using the dual isotope method (C). Mean values ± 1 SEM are presented for six animals.

tivity, the 18:0 compound apparently was inactive in this regard (26). It was of considerable importance, therefore, to compare the metabolic effects of the 18:0 and 18:1(9*t*) fatty acids in parallel experiments using identical protocols to determine whether, in fact, the liver could discriminate between these two fatty acids. Using diets containing identical amounts of these two fatty acids (Table 1), the hepatic lipids became similarly enriched with the 18:0 and 18:1(9*t*) fatty acids after 30 days of feeding. Nevertheless, these two groups of animals did not manifest any significant alteration in LDL-C metabolism, as illustrated in **Fig. 6.** Plasma LDL-C concentrations (A) and relative hepatic LDL receptor activity (B) were the same in the two dietary groups and, further, these were not significantly different from the respective values found in animals fed the 8:0-TAG control diet. In addition, steady-state cholesteryl ester levels were the same in the liver of the two groups (C). Thus, the major regulatory effects of the 18:1(9*c*) fatty acid were entirely lost when the double bond in this compound was either saturated or converted to the *trans* configuration.

DISCUSSION

These studies provide strong support for our model in which it is postulated that the size of the putative regulatory pool of sterol in the hepatocyte is dictated, in part, by the fatty acids that are available in the liver for esterification of excess cytosolic cholesterol through the ACAT reaction (24). The ability of the 18:1(9*c*) fatty acid to shift cholesterol from the putative regulatory pool to the cholesteryl ester pool is lost when the double bond in this compound is converted to the *trans* configuration.

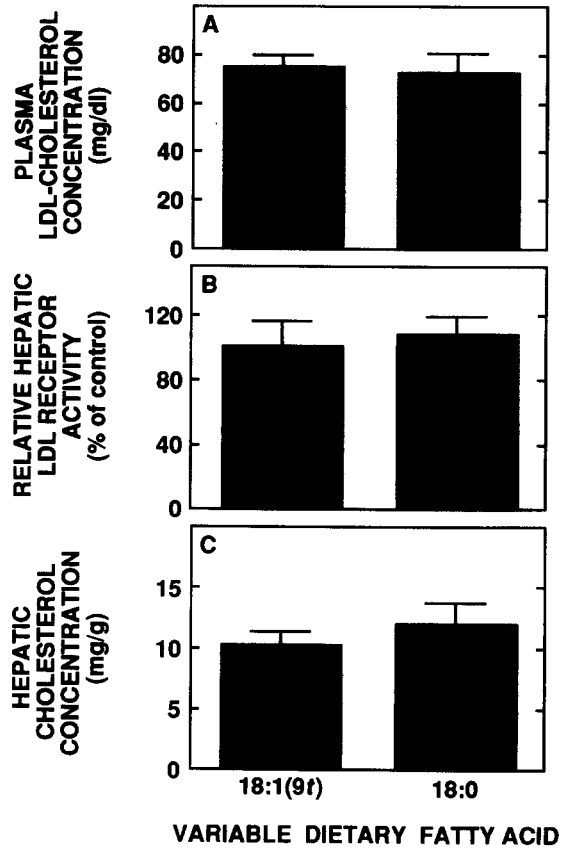

Fig. 6. Plasma LDL-cholesterol concentration, relative hepatic LDL receptor activity, and hepatic cholesterol concentration in animals fed the 18:1(9*t*) and 18:0 fatty acids for 30 days. These animals were fed experimental diets containing 20 g of triacylglycerol per 100 g of diet for 30 days. Each of these diets contained 10.5 g/100 g of the two variable fatty acids, 18:1(9*t*) and 18:0. The diets also contained a constant concentration of cholesterol of 0.12 g/100 g. At the end of the 30-day feeding period the plasma LDL-cholesterol concentration (A), relative hepatic receptor activity (B), and hepatic total cholesterol concentration (C) were measured. Mean values ± 1 SEM are shown for six animals in each group.

Presumably, this effect is solely attributable to the fact that the 18:1(9*t*) compound is a poor substrate for the ACAT reaction (28).

The steady-state concentration of cholesteryl esters in the hepatocyte is determined by the level of ACAT activity relative to the rate of cholesterol ester hydrolysis. The primary assumption in this model is that the level of ACAT protein in the liver is essentially constant, but the activity of this enzyme is determined in a major way by the relative concentrations of the two substrates for the reaction, i.e., the pool of excess cholesterol and the pool of available free fatty acids in the cell (23, 24). Thus, in the steady state, the distribution of cholesterol in the liver cell between a putative regulatory pool and the ester pool would be determined by both the size of the excess cholesterol pool and the fatty acids present in these substrate pools. To the extent that cellular cholesterol is shifted to the ester pool, hepatic LDL receptor activity (J^m) would be relatively increased and the plasma LDL-C concentration would be lowered.

There is considerable support for this assumption. For example, microsomal ACAT activity, assayed as the rate of incorporation of radiolabeled fatty acid CoA into cholesteryl esters (38), is typically elevated in any situation where there is excess cholesterol present in the liver. This can be accomplished by feeding exogenous, dietary cholesterol or by driving endogenous sterol synthesis by the administration of mevalanolactone (39, 40). However, this same high rate of esterification can be achieved in microsomes from animals fed low cholesterol diets simply by providing the microsomes with additional amounts of the sterol substrate (40–42). Indeed, the level of ACAT activity in microsomes from any source appears to be a linear function of the amount of unesterified cholesterol in those membranes (39). Thus, there is little doubt that the level of excess free cholesterol in the cell is a major determinant of the rate of cholesterol ester formation. It should be noted, however, that the molecular structure of this enzyme has just been reported (43). Once the appropriate molecular probes are available, it will be possible to determine whether changes in the level of enzyme protein, in addition to these changes in substrate availability, also play a role in the regulation of steady-state cholesteryl ester levels in the liver.

Similarly, when cholesterol availability is kept constant, the rate of cholesteryl ester formation is also profoundly influenced by the fatty acids or fatty acid CoA derivatives available for the reaction. In general, the 18:1(9*c*) fatty acid gives a very high rate of esterification in vitro while saturated fatty acids such as the 14:0 and 16:0 compounds, the *trans* fatty acid 18:1(9*t*) and the polyunsaturated fatty acid 18:2(9*c*, 12*c*) manifest much lower rates (27, 28). This hierarchy of activity is also supported by in vivo studies in animals fed triacylglycerols containing a single fatty acid. In hamsters fed only the 18:1(9*c*) fatty acid for 30 days, the steady-state cholesteryl ester concentration is much higher than in those animals fed any of the saturated fatty acids (6:0 through 18:0) or the 18:2(9*c*, 12*c*) compound (24, 26). Thus, it is likely that when cholesterol balance across the liver is constant, the availability of specific fatty acids in the hepatocyte also markedly influences the rate of cholesterol ester formation and, hence, the steady-state level of cholesteryl esters in the liver.

This model, therefore, predicts two, physiologically different types of regulation of hepatic receptor-dependent LDL-C transport. In the first, the fatty acid profile of the liver is kept constant. The delivery of an increased amount of cholesterol to the liver results in a new steady state in which both the putative regulatory pool of unesterified cholesterol and the biologically inert pool of cholesteryl esters are expanded in parallel. There is partial suppression of hepatic receptor activity, an increase in the LDL-C production rate and a modest increase in the LDL-C concentration (21, 36). Thus, in this type of regulation hepatic receptor-dependent transport varies essentially linearly and inversely with the steady-state level of cholesteryl esters in the liver (21, 23), and a change in net cholesterol delivery to the liver can be quantitated by appropriate measurements. In the second, when cholesterol balance across the liver is kept constant, enrichment of the hepatic pool of fatty acids with the 18:1(9*c*) compound presumably shifts cholesterol from a putative regulatory pool to the cholesteryl ester pool while the saturated fatty acids have the opposite affect (24). Thus, in this type of regulation hepatic receptor activity varies essentially linearly and directly with the steady-state level of cholesteryl esters in the liver, and no change in net cholesterol delivery to the liver can be identified (24). Obviously these two regulatory mechanisms may interact. This readily explains why the absolute effect of a dietary fatty acid on plasma LDL-C concentrations is dependent on the amount of cholesterol also present in the diet (21) and why the combination of a saturated fatty acid and cholesterol in the diet leads to greater suppression of hepatic receptor activity and elevation of the plasma LDL-C level than does either lipid alone (36).

In testing this model, the initial observation of importance was to establish that the fatty acid pools of the liver did indeed become enriched with each of the single, variable fatty acids fed in the experimental diets. In the absence of dietary triacylglycerol, the major fatty acids in the liver are synthesized endogenously and in the hamster consist of the 18:1(9*c*), 18:2, 16:0, and 18:0 compounds (26). As the 8:0 fatty acid is rapidly oxidized to acetyl-CoA, feeding the 8:0-TAG does not alter this hepatic lipid profile (Table 2). Feeding the 14:0-TAG, 18:1(9*c*)-TAG, and 18:1(9*t*)-TAG, however, does enrich this pool with these three respective variable fatty acids (Table 2). Furthermore, it is clear that in the steady-state, the various enzymatic pathways have discriminated against the *trans*

fatty acid. When the liver was enriched with the 18:1(9*c*) compound, for example, it accounted for 80.8% of the fatty acids in the cholesteryl esters but only 19.9% in the phospholipid fraction (Table 2). In the animals fed the 18:1(9*t*)-TAG, however, the *trans* compound accounted for only 13.6% of the fatty acids in cholesteryl esters and 16.2% in the phospholipid fraction. Thus, the selectivity of ACAT for the 18:1 compound was lost when the double bond was in the *trans* configuration and, furthermore, the rate of esterification was presumably lowered since the steady-state level of cholesteryl esters in the hepatocyte was significantly reduced (Fig. 2). Hence, in vivo, as in vitro, the presence of the *trans* fatty acid in the liver significantly reduced the level of cellular cholesterol in the ester pool.

This finding, in turn, would imply that the 18:1(9*t*) fatty acid was not capable of shifting cholesterol out of the putative regulatory pool and should not, therefore, regulate receptor-dependent LDL transport. This proved to be the case in that the level of hepatic receptor activity (Fig. 4A) and the LDL-C production rate (B) in the animals fed the 18:1(9*t*)-TAG were the same as in the control animals fed the 8:0-TAG (Fig. 4). Therefore, the plasma LDL-C concentration in these animals was significantly higher than those animals fed the 18:1(9*c*)-TAG and essentially identical to those fed the neutral 8:0-TAG control diet (C). Furthermore, these parameters were also essentially identical in animals fed the 18:0 fatty acid, another biologically neutral lipid, as in those fed the 18:1(9*t*) compound (Fig. 6). Also of note is the fact that the 18:1(9*c*) fatty acid actively increased hepatic receptor activity while the 14:0 compound actively suppressed receptor-dependent transport (Fig. 4) under circumstances where there was no demonstrable change in net cholesterol balance across the liver (Fig. 5). Clearly, this finding is consistent with our model and emphasizes that the effect of the 18:1(9*c*) fatty acid in regulating receptor activity is articulated through its intracellular ability to drive ester formation and shift cellular cholesterol out of the putative regulatory pool. The *trans* isomer, which cannot support this esterification reaction, does not alter hepatic receptor-dependent transport.

Finally, it should be emphasized that the relative changes in LDL-C concentrations seen in humans fed these same isomers of the 18 carbon fatty acids are essentially the same as reported here. The results of five such human studies are summarized in **Fig. 7** (panels B–F) which show the plasma LDL-C concentrations in subjects receiving from 3 to 11% of their energy intake as the 18:1(9*t*), 18:1(9*c*), or 18:0 fatty acid over 3–6 weeks (44–47). These are compared to the results obtained in the hamster in the present study (A). As is apparent, feeding the 18:1(9*t*) and 18:0 compounds resulted in nearly identical plasma LDL-C concentrations in these humans (B), as in the hamster (A). While human studies have apparently

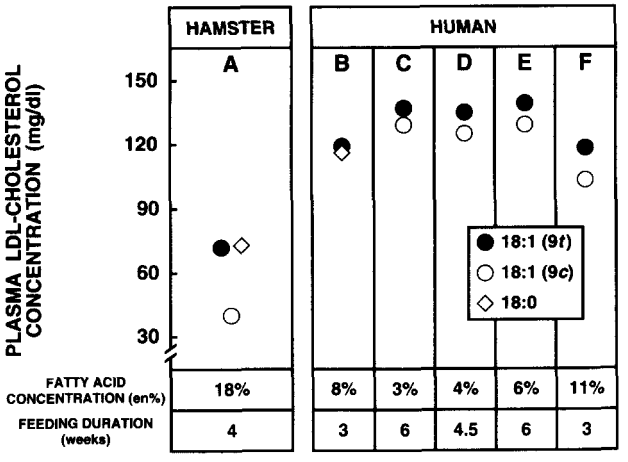

Fig. 7. Plasma LDL-cholesterol concentrations in the hamster and in five human studies in which the subjects were fed varying amounts of the 18:1(9*t*), 18:(9*c*), or 18:0 fatty acids. Also shown are the duration of the experimental feeding periods and the approximate percent of the dietary caloric intake that was comprised of each of the experimental fatty acids. The studies labeled C–F have been arranged in order of increasing percentages of the experimental fatty acid. The data for the human studies come from references 44–47.

never been carried out with a neutral lipid control, it is reasonable to assume that both of these fatty acids, as in the hamster, are also biologically neutral in man. Similarly, in the four other studies where the *cis* and *trans* isomers were directly compared (C–F), plasma LDL-C concentrations were always lower when the human subjects were fed the 18:1(9*c*) compound than the *trans* fatty acid, again as found in the hamster (A). Thus, while the relative dose of each fatty acid was lower and the relative period of feeding was significantly shorter (about 120 pools of LDL-C were turned over in the hamsters while <25 pools were turned over in the humans), these results support the view that in humans, as in the hamster, the 18:1(9*c*) fatty acid actively restores hepatic LDL receptor activity and lowers the plasma LDL-C concentration while the 18:1(9*t*) compound is biologically neutral.

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