Annu. Rev. Nutr. 1993. 13:355–81

REGULATION OF PLASMA LDL-CHOLESTEROL LEVELS BY DIETARY CHOLESTEROL AND FATTY ACIDS

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KEY WORDS: liver cholesterol synthesis, LDL receptors, LDL production, cholesteryl esters, atherosclerosis

CONTENTS

Introduction and Scope

Abundant data now support the conclusion that atherosclerotic disease in Western countries is related to a number of factors such as the level of circulating lipids, hypertension, smoking, and glucose intolerance (2, 25). Of these various factors, the concentration of cholesterol carried in low density lipoproteins (LDL-C) is one of the most important. Thus, the incidence of various complications of atherosclerosis appears to be directly related to the level of this specific class of plasma lipids (2, 25, 57). Lowering this level by dietary and/or pharmacological means decreases the incidence of these events

0199-9885/93/0715-0355$02.00

(34, 44) and, in some instances, may even lead to a reduction in the size of the atherosclerotic lesion (8, 10).

Considerable data also support the conclusion that the relatively high levels of LDL-C found in these same countries are associated, in some manner, with the intake of large quantities of dietary lipids including both cholesterol and triacylglycerol. Furthermore, innumerable studies in both experimental animals and humans have elucidated certain general principles that describe the effects of these lipids on the plasma cholesterol levels. First, the concentration of LDL-C usually increases as the level of dietary cholesterol is increased (46). Second, in general, triacylglycerols containing predominantly saturated fatty acids further increase the concentration of cholesterol carried in this lipoprotein fraction while those containing predominantly unsaturated fatty acids lower these levels. Third, when fed at equal levels, saturated fatty acids are more active in increasing the LDL-C concentration than are unsaturated lipids in reducing the level of concentration (33, 41).

While these principles appear to be valid for most species, until very recently relatively little has been known about which specific fatty acids are responsible for these effects, which organ(s) these lipids act in to regulate the plasma LDL-C concentration, and how cholesterol and fatty acids interact biochemically in this (these) tissue(s) to alter plasma cholesterol levels. This chapter reviews recent information on those organs in various species, including humans, that are involved in the synthesis and excretion of cholesterol and in the synthesis and degradation of LDL-C. The quantitative relationships between these processes are also described, and the manner in which dietary cholesterol and triacylglycerol alter these processes is reviewed. Finally, to the extent that data are available, a model is presented that suggests how fatty acids and cholesterol interact biochemically to bring about the marked changes in the steady-state concentration of LDL-C observed in both experimental animals and humans.

General Features of Cholesterol and LDL Metabolism

The general characteristics of cholesterol and LDL-C metabolism have been elucidated over the past few years, and some of the key features of these processes are shown diagrammatically in Figure 1. A portion of the cholesterol that is present in the diet is absorbed by the small intestine (*A*), incorporated into the chylomicron particle, and delivered to the liver (*C*) after partial degradation of the chylomicron to its remnant (31, 69). The arrival of dietary cholesterol in the liver is signaled by an increase in the level of cholesteryl esters (CE) and partial suppression of the rate of hepatic cholesterol synthesis from acetyl-CoA (56). Nearly all of the other extrahepatic tissues (*B*) are also capable of synthesizing cholesterol, although the rates vary markedly among the different organs (62). Each day an amount of cholesterol equal to that

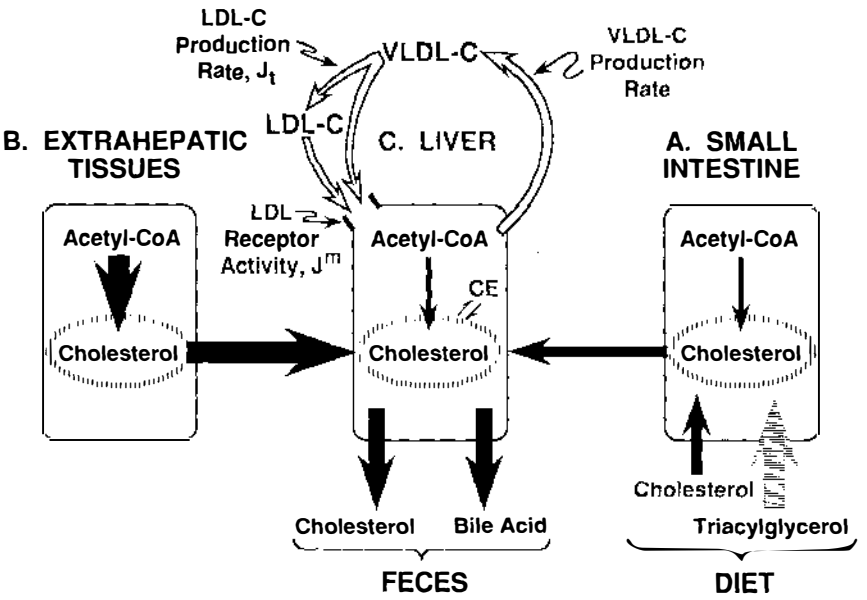

Figure 1 A model showing the central role of the liver in maintaining both net cholesterol balance and plasma low density lipoprotein-cholesterol (LDL-C) levels in animals and humans. The *solid arrows* are meant to show, in a semiquantitative manner, the rates of cholesterol synthesis in the three tissue compartments and the rate of intestinal sterol absorption, as well as the flow of this cholesterol to the liver and out of the body in the feces. The steady-state concentration of cholesterol carried in LDL is also primarily determined by the rate of conversion of very low density lipoprotein-cholesterol (VLDL-C) to LDL-C and the subsequent clearing of this particle from the plasma through the intervention of LDL receptors located primarily in the liver. The exact magnitude of each of these processes in maintaining cholesterol and LDL-C balance in the intact animal and man is discussed in detail in the text. CE represents the inert storage pool of cholesteryl esters in the liver.

which is synthesized in these extrahepatic tissues must be transported to the liver for excretion. This process of "reverse" cholesterol transport presumably involves high density lipoproteins (HDL) (24). Other than small losses of cholesterol through the sloughing of skin and endothelial cells and the synthesis of steroid hormones, most sterol is excreted from the body as biliary cholesterol and bile acids (7, 76). Thus, in the steady state the rate at which sterol is excreted in the feces as cholesterol and bile acid must equal the rate at which cholesterol is synthesized in all of the tissues and absorbed from the diet. The rate of synthesis in the liver, but not in the extrahepatic tissues, will change to accommodate any alteration in net sterol balance in the animal. An increase in fecal sterol excretion, for example, will invariably lead to an

increase in hepatic synthesis (75, 79) while suppression of net fecal sterol loss is associated with inhibition of cholesterol synthesis in the liver (13). Thus, the liver plays the central role in maintaining net sterol balance in the body and rapidly responds to any perturbation of this balance by changing its rate of cholesterol synthesis.

As is also illustrated in Figure 1, recent data support the concept that the liver is similarly important in the metabolism of LDL-C. In order to move triacylglycerol from the hepatocyte to the peripheral organs of utilization (muscle) or storage (adipose tissue) (31), the liver synthesizes the very low density lipoprotein (VLDL) particle. As this particle is metabolized in the extrahepatic tissues, a remnant is also formed (31). A portion of these remnants is taken up directly by the liver, apparently through the intervention of the LDL receptor (LDL-R), while the remainder is converted to LDL (42, 84, 85). This latter particle, in turn, is then cleared from the plasma by LDL receptors located primarily in the liver, but also in some extrahepatic tissues (not shown in Figure 1). It is clear from this formulation that the steady-state concentration of LDL-C in the plasma is profoundly influenced by both the rate at which LDL-C is formed, i.e. the LDL-C production rate, and the level of activity of LDL receptors in the liver. However, it should also be noted that the flow of cholesterol out of the liver, through VLDL and LDL, and back to the liver does not alter net sterol balance in this organ. Only if there is net transfer of some of the sterol newly synthesized in the extrahepatic tissues to LDL or VLDL (24, 38) will the uptake of these particles make a net contribution to the hepatic cholesterol pools.

From these general considerations, it is clear that the liver is uniquely situated to integrate the metabolic effects of dietary lipids on both net sterol balance in the body and the level of circulating LDL-C. Much of the dietary cholesterol and at least a portion of the dietary triacylglycerol is delivered to this organ. These lipids alter the pools of cholesterol in the hepatocyte and even change the distribution of fatty acids in the phospholipids, triacylglycerols, and cholesteryl esters that comprise the membranous and bulk lipid compartments of the liver cell. These changes, in turn, are associated with marked alterations in both LDL-R activity and the LDL-C production rate.

Types of Lipids Present in the Western Diet

The diet of the average individual in Western countries typically contains several hundred milligrams of cholesterol and 80–130 g of triacylglycerol. Even though the great majority of the sterol in the diet is unesterified, it is, nevertheless, very poorly absorbed. Because cholesterol contains only a single hydrophilic group, the 3β-hydroxyl group, its absorption is very sensitive to the size of the bile acid pool in the small intestine, the relative hydrophobicity of this pool, the resistance of the intestinal diffusion barrier, the relative

hydrophobicity of the microvillus membrane, and the rate at which cholesterol is esterified once it reaches the interior of the intestinal epithelial cell (69). Hence, in many species, including man, only about 40–60% of the mass of cholesterol reaching the intestinal lumen is absorbed. However, it is equally clear that this percentage is highly variable among individual members of any species (30) so that the same dietary challenge may elicit a wide range of LDL-C concentrations in different individuals (4).

The physiological behavior of fatty acids in the diet is much more complicated. Because of the complex chemistry of these compounds, individual fatty acids vary markedly in their physical characteristics, absorbability, metabolic fate, and regulatory effects. The first variable is the length of the hydrocarbon chain. Whereas most biological tissues contain fatty acids with 16 or 18 carbon atoms, saturated fatty acids containing virtually any number of carbon atoms can be chemically synthesized. In general, those with less than 10–12 carbon atoms, e.g. the 4:0, 6:0, 8:0, and 10:0 fatty acids, have low melting points, are readily absorbed across the gastrointestinal tract, and are not incorporated into the chylomicron particle. They are carried directly to the liver through the portal vein and rapidly oxidized to acetyl-CoA. In contrast, the longer chain saturated fatty acids, e.g. the 16:0 and 18:0 fatty acids, have higher melting points, may be less well absorbed, and are incorporated into the chylomicron particle. They eventually reach the liver, through uptake of the chylomicron remnant and plasma free fatty acids, where they enrich the lipids present in the various metabolic pools (82).

The second variable in the chemistry of these compounds concerns the number of double bonds present in the hydrocarbon chain. Again, while an almost infinite number of isomers is possible, dietary lipids most commonly contain fatty acids with one, two, or three double bonds in the *cis* configuration at the 6, 9, or 12 positions. In general, the addition of a double bond to a saturated fatty acid lowers the melting point of that compound and increases its absorbability. These unsaturated, long chain fatty acids, e.g. the 18:1(*c*9) and 18:2(*c*9, *c*12) compounds, are also incorporated into the chylomicron particle after absorption and eventually reach the liver where they also can enrich the various metabolic pools (16).

The third variable in fatty acid structure is introduced largely during the commercial preparation of edible oils. During the process of hydrogenation the double bonds in the fatty acids may migrate longitudinally along the hydrocarbon chain, and, in addition, may be rotated from their normal *cis* configuration to the *trans* configuration. This chemical change raises the melting point of the lipid (23). Nevertheless, these long chain *trans* fatty acids are absorbed and incorporated into the chylomicron and reach the liver where they can be identified within the various lipid pools (23).

Finally, these various fatty acids may be esterified to the glycerol molecule

in a highly specific manner. Not only does the stereospecific structure of the triacylglycerol molecule affect its physical characteristics and the absorbability of the constituent fatty acids (59), but, in addition, the location of a given fatty acid on the triacylglycerol molecule may determine whether it is predominantly delivered to the liver or to the extrahepatic tissues. Thus, the stereospecific structure of a fat could markedy influence whether a particular triacylglycerol influences LDL-C metabolism or not.

While the types of fatty acids in the diet and their location on the triacylglycerol molecule can, in theory, vary enormously, in practice the great majority of dietary fats contain predominantly two saturated fatty acids, i.e. the 16:0 and 18:0 compounds, and two unsaturated fatty acids, i.e. the 18:1(*c*9) and 18:2(*c*9, *c*12) compounds. In addition, processed and specialty fats may contain *trans* long chain fatty acids as well as the 12:0 and 14:0 compounds. Triacylglycerols containing these various fatty acids are essentially completely absorbed and reach the metabolic pools of lipids in the liver where they potentially can regulate those processes dictating sterol and LDL-C balance across the liver.

Tissue Sites for Cholesterol Synthesis in Different

To begin to understand these regulatory processes, one must first review the quantitative importance of the liver to sterol synthesis in the whole animal and man. The rates of cholesterol synthesis in whole animals have now been measured in at least 15 species, including humans, under circumstances where there is essentially no intake of dietary cholesterol. In general, the rate of whole animal synthesis markedly decreases as body weight increases. Thus, for example, the mouse synthesizes approximately 50 mg of cholesterol per day per kg of body weight. In other species such as the hamster, rabbit, dog, and various nonhuman primates, this rate decreases by about 10 mg/day per kg for each 10-fold increase in body weight. Thus, the typical 70-kg human synthesizes approximately 9 mg/day per kg or about 600–800 mg/day (76).

A persistent misconception is that much of this de novo synthesis takes place in the liver. This concept comes from older studies in which rates of sterol synthesis were quantified in vitro using various ^{14}C-labeled substrates (20, 22). Subsequent investigations, however, demonstrated that these in vitro techniques systematically underestimated rates of cholesterol synthesis in the extrahepatic organs and, consequently, overestimated the importance of the liver to whole animal sterol synthesis (1). Newer methods have been developed that obviate these artifacts and allow measurement of absolute rates of cholesterol synthesis in the individual organs of the live animal (1, 37). These new methods have now been applied to a number of species, and the results of these studies are summarized in Figure 2. This figure illustrates the percentage of whole animal cholesterol synthesis that can be accounted for

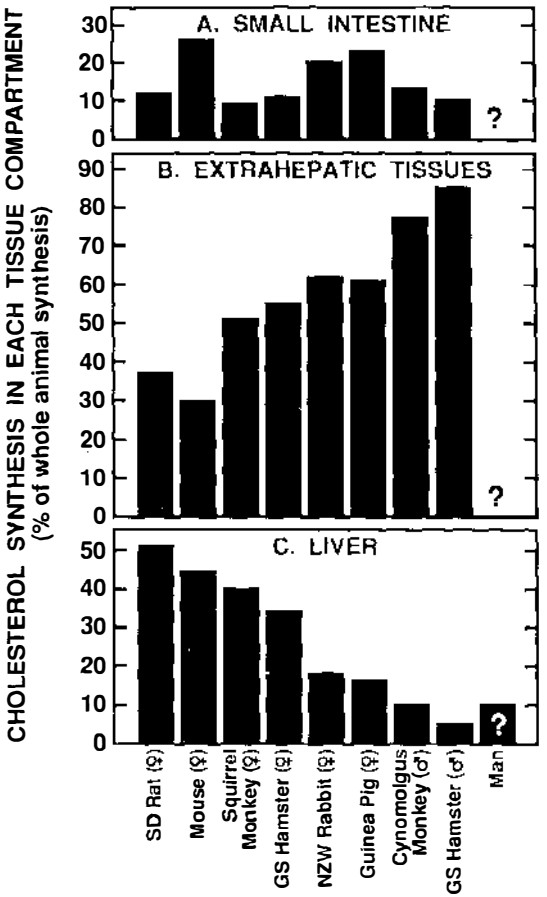

Figure 2 Relative importance of the small intestine, other extrahepatic tissues, and liver as sites for cholesterol synthesis. Rates of cholesterol synthesis were measured in all of the organs of live animals maintained on diets essentially free of cholesterol and triacylglycerol using [^{3}H]water. The data in humans were estimated from rates of synthesis measured in liver biopsy specimens in vitro. SD, GS, and NZW refer to the Sprague-Dawley, Golden Syrian, and New Zealand White strains, respectively, of rats, hamsters, and rabbits. These data come from both published and unpublished observations in this laboratory (18, 19, 62, 68, 77).

by synthesis in the small intestine (*A*), liver (*C*), and remaining extrahepatic organs (*B*) (18, 19, 68, 77).

In some species such as the rat, mouse, and squirrel monkey, cholesterol synthesis in the liver occurs at relatively high rates and so accounts for 40–50% of whole animal sterol synthesis (*C*). In most other species, however, including the rabbit, guinea pig, Cynomolgus monkey, hamster, and apparently, man,

the liver accounts for only about 5–20% of whole animal synthesis. Furthermore, when any of these species is placed on a diet containing the amounts of cholesterol typically present in human diets (100–300 mg per/1,000 kcal intake), synthesis in the liver, but not in the extrahepatic tissues, is suppressed so that in such animals 90% of whole animal sterol synthesis takes place in the extrahepatic tissue compartment.

These quantitative data have important implications when superimposed on the general model shown in Figure 1. The higher the rate of cholesterol synthesis in the liver in the absence of dietary sterol, the more tolerant is that species of cholesterol feeding. Thus, the rat and squirrel monkey, by suppressing hepatic synthesis can adapt to a greater load of dietary cholesterol than can the hamster and Cynomologus monkey before cholesteryl esters begin to accumulate in the liver and alterations in LDL-C metabolism occur. Obviously, the liver of the squirrel monkey that synthesizes cholesterol at 800 nmol/hr per g, can adapt to the inflow of a greater amount of dietary cholesterol than can the liver of the Cynomolgus monkey that normally synthesizes sterol at only 50 nmol/hr per g (62). Nevertheless, in the presence of small amounts of dietary sterol, the net movement of cholesterol into the liver (Figure 1, *C*) from the small intestine (*A*) and the extrahepatic tissues (*B*) probably accounts for 90% of the cholesterol that is moving through the system, so that in most species the liver is only a minor net contributor to whole animal synthesis.

Tissue Sites for LDL-C Transport out of the Plasma in Different

The second aspect of the scheme shown in Figure 1, and for which there is much new quantitative data, concerns the role of the liver in LDL-C metabolism. Although the hepatocyte has long been known to be the site of origin of VLDL and, hence, LDL, the mechanism(s) of degradation of these particles was (were) poorly understood until the seminal studies of Brown & Goldstein in which the LDL receptor was described (11). With the further observation that interaction between the LDL particle and its receptor could be blocked by chemical modification of the lipoprotein (45, 80), a second, receptor-independent mechanism for the removal of LDL from the plasma was defined. The importance of these two transport processes for the removal of LDL-C from the plasma has now been established in a number of different species. Animals on diets that are low in both cholesterol and triacylglycerol have plasma LDL-C concentrations that typically are only 10–30 mg/dl, and receptor-dependent transport usually accounts for 75% of the LDL-C that is removed from the plasma and degraded each day (61, 65, 66, 70). When lipids are added to the diet and the plasma concentration of LDL-C increases to 75–200 mg/dl, this percentage decreases and, in man, equals 65–80% (6,

40). In animals or humans that genetically lack LDL-R activity, essentially 100% of LDL-C removal from the plasma takes place by the receptor-independent mechanism (6, 65).

As methods for measuring the rates of both receptor-dependent and receptor-independent LDL-C uptake into the various tissues of the live animal have been developed (58, 61), the quantitative importance of the different organs for the degradation of LDL-C has been defined in a number of species and is summarized in Figure 3. LDL-C uptake by either mechanism can be

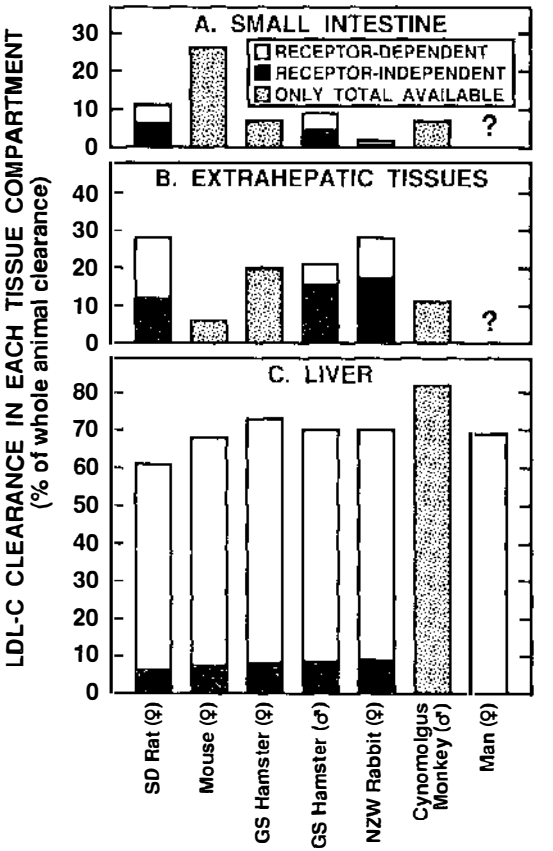

Figure 3 Relative importance of the small intestine, other extrahepatic tissues, and liver in the uptake of LDL-C. Rates of homologous LDL-C uptake were measured in all of the organs of live animals maintained on diets essentially free of cholesterol and triacylglycerol. In some species both the receptor-dependent and receptor-independent components of this LDL-C transport process were quantified and are also shown. The human data were calculated from LDL-C turnover studies in a patient receiving a liver transplant (5). These data come from both published and unpublished observations in this laboratory (61, 65, 66).

detected in many organs (61, 65, 66). However, because of the high rate of LDL-C transport per unit weight in the liver and because of its large size, hepatic uptake accounts for about 70% of the LDL-C degradation that can be detected in every species in which data are available (*C*) (61, 65, 66). A similar value can be calculated indirectly for humans (5). In most of these species the small intestine accounts for the clearance of approximately 10% of the LDL-C (*A*) while the remaining extrahepatic organs account for only about 20% of LDL-C degradation (*B*). Furthermore, insofar as data are available, the liver is also the tissue site that accounts for the great majority of the receptor-dependent LDL-C transport that can be detected in the whole animal (*C*). In contrast, 60–70% of the receptor-independent transport detected in the whole animal takes place in extrahepatic organs (*A*, *B*). Thus, in the absence of dietary lipids where the circulating LDL-C concentration is low, most cholesterol synthesis takes place in the extrahepatic organs (Figure 2) while the great majority of LDL-C is cleared from the plasma by receptor-dependent transport into the liver (Figure 3).

Quantitative Considerations in the Regulation of LDL-C Levels in Plasma

Unfortunately, this relatively simple scheme has little application when the diet contains cholesterol and triacylglycerol and where there are significant alterations in both the rates of sterol synthesis and receptor-dependent LDL-C clearance. Therefore, we need to define the quantitative, kinetic relationships that exist between the rates of LDL-C formation and these two transport systems that are responsible for LDL removal from the plasma.

Such relationships are illustrated in Figure 4 for a hypothetical animal weighing ~0.5 kg. These same relationships are seen in all species that have been studied, including humans; however, the absolute values for these transport parameters vary from species to species. Panel *A* illustrates how the absolute rate of LDL-C transport out of the plasma space varies as a function of the concentration of LDL-C in the plasma. Panel *B* expresses these same transport data as either the LDL-C fractional catabolic rate (FCR) or the clearance rate. These three transport parameters are all interchangeable, but are measured experimentally using different techniques. The first point illustrated by these curves is that the rate of LDL-C transport out of the plasma by the receptor-independent process is a linear function of the plasma LDL-C concentration (*A*). This relationship has been demonstrated in both animals and humans (6, 65, 66). Because of this linear relationship, the FCR and clearance rate for the receptor-independent process is constant at any level of plasma LDL-C (*B*). In contrast, the receptor-dependent transport process that is occurring simultaneously is saturable (*A*) (65, 66). Consequently, the value of the FCR and clearance rate for this component of LDL-C transport

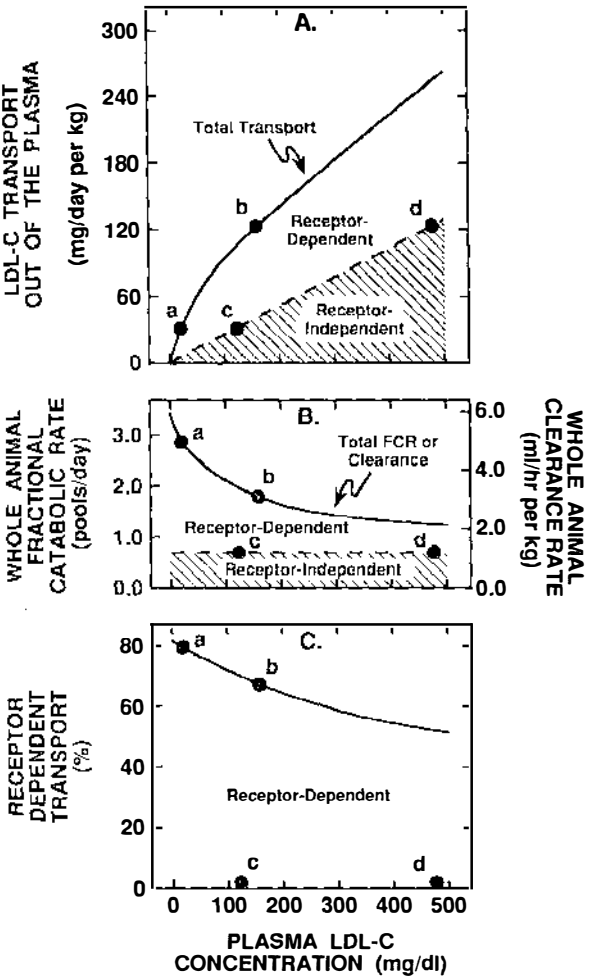

Figure 4 The kinetic relationships between the rates of LDL-C transport out of the plasma by the receptor-dependent and receptor-independent processes in the whole animal and the concentration of LDL-C in the plasma. These rates are expressed in panels *A* and *B* in three ways: (*i*) as the absolute rate of LDL-C taken up by the various tissues each day (mg/day per kg body weight), (*ii*) as the fraction of the plasma LDL-C pool removed each day (pools per day), and (*iii*) as the milliliters of plasma cleared entirely of their LDL-C content each hour (ml/hr per kg). Panel *C* shows the percentage of LDL-C transport out of the plasma that is receptor dependent. In constructing these curves, the level of LDL-R activity in the body was kept constant while the LDL-C production rate was systematically increased to raise the LDL-C concentration in the plasma. The experimental points labeled a, b, c, and d in the three panels represent specific, theoretical situations that may occur in such animals and are discussed in detail in the text. These curves apply to an animal weighing about 0.5 kg but were adapted from curves determined experimentally in the rat, hamster, and man (48, 50, 64, 66).

progressively decreases (*B*) as the plasma LDL-C concentration is increased, even though LDL-R activity in this illustration has been kept constant (50, 64).

The four data points in Figure 4 also show how these relationships are altered with extreme changes in the LDL-C formation or receptor activity. Point *a*, for example, shows the typical situation in an experimental animal on a lipid-free diet. The rate of LDL-C production, and its rate of transport out of the plasma, are shown as about 30 mg/day per kg, and the steady-state concentration of LDL-C in the plasma as 25 mg/dl. This absolute transport rate of 30 mg/day per kg (*A*) can also be expressed as an FCR of 2.8 pools/day or a clearance rate of about 5 ml/hr per kg (*B*). In this case, 80% of the transport is receptor-dependent (*C*). If the LDL-C production rate is increased 4-fold to 120 mg/day per kg (point *b*), the FCR and clearance rate both decline (*B*), about 67% of transport is receptor-dependent (*C*), and the plasma LDL-C concentration increases to about 150 mg/dl. Points *c* and *d*, respectively, illustrate the changes in these relationships that would occur if all receptor-dependent transport were lost but the LDL-C production rate remained either 30 or 120 mg/day per kg. In both cases the FCR and clearance rates would decrease to the same fixed values (*B*), and the steady-state plasma LDL-C concentrations would increase to the high levels necessary to drive receptor-independent LDL-C uptake at the two respective rates of LDL-C production. Thus, for example, the plasma LDL-C concentration would have to reach almost 500 mg/dl to achieve a rate of LDL-C removal through the receptor-independent process equal to 120 mg/day per kg (point *d*).

These considerations clearly indicate that the steady-state plasma LDL-C concentration is dependent upon four variables (49, 50, 64, 66): (*a*) the rate at which LDL-C enters the plasma space, the LDL-C production rate (J_t), (*b*) the maximal achievable rate of receptor-dependent transport (J^m), (*c*) the functional affinity of the LDL particle for its receptor (K_m), and (*d*) the proportionality constant that describes the rate of receptor-independent transport (*P*). Since each of these variables can be measured directly in the experimental animal (21) and they have been estimated in man (48), one can calculate how the steady-state concentration of LDL-C (C_1) will change under circumstances where dietary components have altered one or more of these variables using the following equation:

$$C_1 = \frac{J_t - J^m - PK_m + [(J_t - J^m - PK_m)^2 + 4PK_mJ_t]^{1/2}}{2P}. \quad 1.$$

Although environmental factors such as diet might, in theory, change any one of these four variables, in practice dietary cholesterol and triacylglycerol

primarily alter the level of LDL-R activity in the body (J^m) and the rate of LDL-C production (J_t). Such dietary manipulations have relatively little effect, if any, on the values of K_m or P.

Using this equation, one can calculate how changes in either J^m or J_t, or both, will alter the steady-state concentration of LDL-C. These relationships are illustrated, in the case of the male hamster, by the two solid curves shown in Figure 5. Similar curves may be generated for other species, including humans, by substituting the appropriate values for the four variables into Equation 1 (21). In this figure, the absolute values for J^m and J_t measured in control animals fed a lipid-free diet have both been set equal to 100% (*open circle*). The two *solid curves* show how the plasma LDL-C concentration will

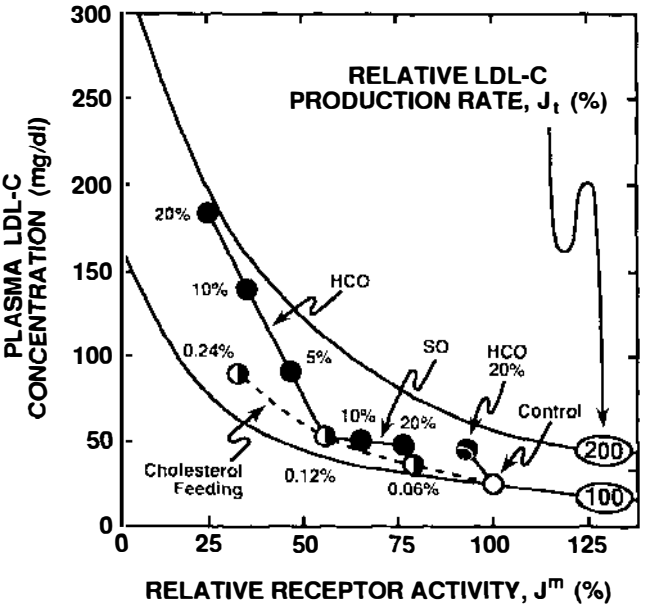

Figure 5 Relationship between the steady-state concentration of LDL-C and whole animal receptor activity and production rates. These curves were constructed by entering into equation #1 the appropriate rate constants for J^m, J_t, K_m, and P as experimentally determined in the male hamster. The lower curve (100% LDL-C production) was then constructed by systematically reducing receptor activity to 0% while keeping the other three parameters constant. The upper curve (200% LDL-C production) was similarly constructed after doubling the value of J_t. To simplify this diagram, the absolute values of the receptor activity and production rate found in the control animals fed a lipid-free diet are each set equal to 100%. Superimposed upon these two theoretical curves are experimental data obtained after feeding different amounts of cholesterol alone *(dashed line)* or a constant level of cholesterol along with different amounts of hydrogenated coconut oil (HCO) or safflower oil (SO) *(solid lines)*. The numbers represent the amounts of cholesterol and triacylglycerol present in each diet expressed as percentages by weight (grams per 100-gram diet).

increase as LDL-R activity is reduced to 0% under circumstances where the LDL-C production rate is set at either 100 or 200% of the control value. Two general principles are evident from these curves. First, the plasma LDL-C concentration does not increase as an inverse, linear function of J^m. Rather, loss of receptor activity has relatively little effect on the circulating cholesterol level until 50% of receptor-dependent transport is suppressed. Second, the level of receptor activity does, however, profoundly affect the response of the steady-state plasma LDL-C concentration to changes in LDL-C production. Doubling J_t, for example, raises the plasma LDL-C level by only about 30 mg/dl if receptor activity is 100%, but by nearly 125 mg/dl if J^m is only 25% of control. Thus, the most profound elevations in plasma LDL-C will be seen with dietary manipulations that suppress J^m and increase J_t.

Effect of Dietary Cholesterol on LDL Receptor Activity and LDL-C Production

Having defined the theoretical relationships between these two parameters and the plasma LDL-C concentration, one can review the metabolic effects of the two dietary lipids, cholesterol and triacylglycerol, on J^m and J_t. The addition of cholesterol alone to a lipid-free diet is associated with major metabolic alterations in the liver of every species, including humans, for which data are available. As the sterol reaches the liver in the chylomicron remnant, hepatic cholesterol synthesis is rapidly suppressed and cholesteryl esters increase to levels that are dependent on the amount of sterol absorbed (Figure 1) (56, 64). If such cholesterol feeding is continued, the circulating LDL-C concentration increases more slowly until a new steady-state level is achieved, the latter is also dependent upon the amount of cholesterol absorbed through the intestine (63, 64). Thus, for example, in the young male hamster, the plasma LDL-C level will increase from ~25 mg/dl to new steady-state values of ~40, 55, and 90 mg/dl when 0.06, 0.12, and 0.24% cholesterol, respectively, is added to the diet. Similarly, the human infant increases its LDL-C level from ~25 to 90 mg/dl as the content of sterol in its milk is progressively raised from 0 to 15 mg/dl (15); the human adult increases its LDL-C concentration from about 50 to 120 mg/dl as the daily intake of cholesterol is raised from 20 to 160 mg (14). Note that these increments in LDL-C concentration in the hamster require the daily intake of amounts of cholesterol (~160 mg/kg) that are 4 times higher than the daily synthesis rate in this species (~40 mg/kg); similar increases in LDL-C can be induced in the human infant and adult at much lower intakes of sterol (~24 and 2 mg/day per kg, respectively) relative to whole animal synthesis rates (~25 and 9 mg/day per kg, respectively) (27, 76). However, even though primates, including man, are more sensitive to dietary cholesterol than many smaller animals, nevertheless, virtually every

species will respond to dietary cholesterol feeding by elevating its LDL-C level.

The mechanisms responsible for this elevation are also illustrated in Figure 5 in the case of the hamster. As the amount of cholesterol in the diet is increased, sterol synthesis in the liver is essentially fully suppressed, a new level of cholesteryl esters is established in the hepatocyte that is proportional to the amount of cholesterol absorbed, and LDL-R activity is reduced (64). Thus, as shown by the *dashed line* in Figure 5, when diets are fed that contain 0.06 to 0.24% cholesterol and new steady states are achieved, J^m at the highest dose is reduced to only about 30% of the activity found in the control animals and J_t increases slightly to approximately 130% of control. These two changes alone fully account for the modest increases in LDL-C levels from ~27 to 90 mg/dl. Thus, the primary effect of cholesterol feeding is to expand the storage pool of cholesteryl esters in the hepatocyte and reduce the level of hepatic receptor activity. These changes, in turn, reduce the percentage of LDL-C cleared by the receptor-dependent process and reduce both the FCR and clearance rate of LDL-C observed in the whole animal (Figure 4). Little or no change is found in the small fraction of receptor-dependent uptake that occurs in the extrahepatic tissues or in receptor-independent LDL-C transport.

Effect of Different Fatty Acids on LDL Receptor Activity and LDL-C Production in Animals

The addition of commercial triacylglycerols to the diets of various experimental animals results in further changes in the steady-state LDL-C concentrations. The magnitude and direction of these changes depend upon the amount of cholesterol also present in the diet and the types of fatty acids in the triacylglycerols. For example, in the hamster, feeding predominantly longer chain saturated fatty acids (~40% of caloric intake) elevates the LDL-C levels by only about 25 mg/dl if no cholesterol is in the diet but by nearly 160 mg/dl if 0.12% cholesterol is present (81). In virtually all species, when the cholesterol content of the diet is kept constant, feeding increasing amounts of triacylglycerol containing mainly long chain saturated fatty acids markedly elevates the plasma LDL-C levels in a dose-dependent manner (32, 39, 83), whereas triacylglycerols containing medium chain-length saturated fatty acids have virtually no effect on the plasma cholesterol level (81). Triacylglycerols containing predominantly long chain unsaturated fatty acids tend to lower the plasma LDL-C levels below those seen with cholesterol feeding alone, although the absolute magnitude of this effect is small compared to the effect of the saturated fatty acids in raising these levels (39, 63, 64, 83).

The mechanisms responsible for these changes are also illustrated in Figure 5. When the cholesterol content of the diet is kept constant at 0.12%, the addition of 5, 10, and 20% (by weight) of a triacylgycerol containing

predominantly long chain saturated fatty acids (HCO) causes further suppression of receptor activity and a near doubling of the LDL-C production rate. This decrease in J^m to only 25% of control and the increase in J_t to nearly 200% of control fully accounts for the rise in the LDL-C concentration from about 55 to 190 mg/dl. Figure 5 also shows that such changes do not occur in the control animal fed a cholesterol-free diet. When the animals fed 0.12% cholesterol are also fed triacylglycerol containing predominantly unsaturated fatty acids (SO), receptor activity is partially restored (83) so that the plasma LDL-C concentration decreases slightly to about 45 mg/dl. Note, however, that if the dietary load of triacylglycerol is kept constant at 20% and the unsaturated triacylglycerol is systematically substituted for the saturated lipid, then the plasma LDL-C concentration falls dramatically, from ~190 to 55 mg/dl (83). This type of substitution is commonly carried out in human studies and obscures the fact that saturated and unsaturated lipids have independent and oppositely directed effects on receptor activity (83). These effects are primarily manifest in the liver, although small changes also occur in receptor-dependent transport in the extrahepatic tissues (81). While cholesterol feeding markedly increases the level of cholesteryl esters in the liver, paradoxically, the suppression of hepatic LDL-R activity seen with saturated lipids is associated with a marked reduction in the cholesteryl ester (CE) content of the hepatocyte (63, 64, 81, 83).

The next question of importance is which specific fatty acids in commercial triacylglycerols are responsible for these regulatory effects. This question has been addressed in animal studies using synthesized triacylglycerols that contain only a single species of fatty acid. Although such studies are not complete, it is becoming clear that fatty acids fall into three different groups. In the first group are short and medium chain-length compounds such as the 4:0, 6:0, 8:0, and 10:0 saturated fatty acids that are rapidly oxidized by the liver to acetyl-CoA. These fatty acids do not alter the composition of the lipid pools in the liver, do not change the concentration of free or esterified cholesterol in the hepatocyte, and do not alter J^m or J_t from those values attributable to the cholesterol also present in the experimental diets (82). Thus, such fatty acids are biologically neutral with respect to regulation of the concentration of LDL-C in the plasma. The long chain 18:0 fatty acid also belongs to this biologically neutral group (82). A second category of lipids includes the 12:0, 14:0, and 16:0 saturated fatty acids that do enrich the hepatic lipid pools, including the pool of fatty acids esterified to cholesterol. When fed with sterols, they suppress steady-state cholesteryl ester levels in the hepatocyte, lower hepatic receptor activity, and markedly increase the rate of LDL-C formation (16, 82). Therefore, these three saturated fatty acids, when fed with cholesterol, are biologically very active and markedly elevate the plasma LDL-C concentration.

The third group of fatty acids is best exemplified by the 18:1(*c*9) compound which, when fed with cholesterol, markedly increases the steady-state cholesteryl ester fraction in the liver, restores hepatic receptor activity, and reduces the rate of LDL-C production (16). Thus, such compounds significantly reduce the circulating level of LDL-C. However, even though the 18:1(*c*9) fatty acid is just as effective in raising J^m as is the 14:0 fatty acid in suppressing this activity nevertheless, when fed with cholesterol the unsaturated fatty acid will only lower the LDL-C concentration modestly from ~70 to 30 mg/dl while the 14:0 fatty acid will raise this level from ~70 to 200 mg/dl (16). This very different quantitative response is due to the nonlinear relationship that exists between receptor activity in the animal and the steady-state LDL-C concentrations (Figure 5). Finally, when the 18:1(*c*9) compound is converted to the 18:1(*t* 9) fatty acid or when a second double bond is introduced into the molecule to form the 18:2(*c*9, *c*12) fatty acid, the new compounds become biologically much less regulatory and essentially lose their ability to markedly increase receptor activity and lower the LDL-C concentration, i.e. they become nearly neutral.

Regardless of whether such studies are carried out with mixed, commercial triacylgycerols or with fats containing a single fatty acid, there usually is a reciprocal relationship between changes in LDL-R activity and LDL-C formation. Thus, the possibility exists that fatty acids may regulate two separate processes that independently dictate the values of J^m and J_t. However, fat feeding does not increase mRNA levels for apo B in the liver (43, 60). Furthermore, there is no increase in apo B secretion from the perfused liver of the fat-fed monkey (60) or the receptor-deficient rabbit (35), even though LDL-C production is elevated in both of these situations. Since the LDL receptor is involved in the clearance of VLDL remnants (42), as well as LDL, the most likely explanation for these findings is that suppression of J^m leads to increased conversion of VLDL remnants to LDL and, hence, to an increase in J_t (Figure 1). Conversely, restoration of receptor activity would be associated with a decrease in LDL-C production. If this explanation is correct, then these findings are consistent with the view that fatty acids, working in conjunction with dietary cholesterol, regulate a single event within the liver, i.e. the level of LDL-R activity. The reciprocal changes observed in LDL-C production rates presumably are only the secondary consequences of these changes in receptor activity.

A Model for the Interaction of Dietary Cholesterol and Triacylglycerol

All of these observations of the seemingly contradictory effects of cholesterol and fatty acids on hepatic cholesterol levels and LDL-R activity can be incorporated into a single, unified model for the regulation of plasma LDL-C

levels. The major assumptions of this model are illustrated in the insert in Figure 6. At any level of cholesterol flux through the liver cell, presumably an equilibrium is established between the sterol that acts as a regulator of hepatic receptor activity (C^R) and the inert storage pool of cholesteryl esters (CE). The size of this putative regulatory pool of cholesterol or a metabolite of cholesterol, acting through the sterol regulatory elements on the LDL-R gene (17, 73, 74), presumably dictates the level of LDL-R mRNA in the cell and, ultimately, the activity of receptor-dependent LDL-C transport in the liver. C^R must represent only a very small part of the unesterified pool of cholesterol in the cell and cannot be identified or measured at this time.

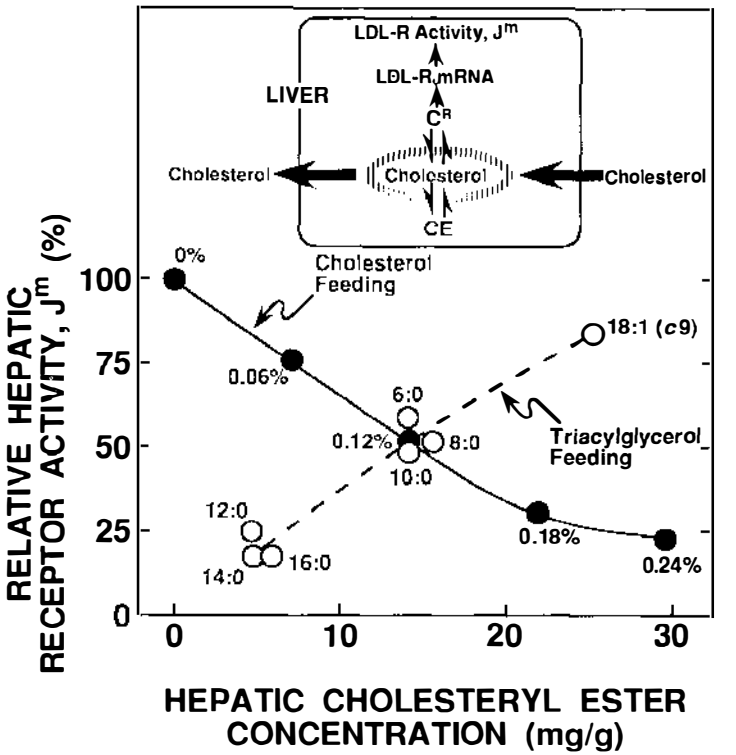

Figure 6 A model illustrating the possible mechanisms of interaction of dietary cholesterol and fatty acids in regulating LDL-R activity in the liver. The insert shows the specific steps involved in this regulation: CE refers to the steady-state concentration of cholesteryl esters in the cell, and C^R is the small component of unesterified cholesterol that is the putative regulator of LDL-R mRNA transcription in the cell nucleus. The *solid curve* illustrates the relationship between J^m and CE when increasing amounts of cholesterol alone are added to the diet for prolonged periods of time. The *dashed line* illustrates this same relationship when the cholesterol content of the diet is kept constant and a constant level of triacylglycerols containing different fatty acids is also fed. The concepts and data presented in this figure are derived from Reference 16.

However, all other parameters of this model, including net cholesterol balance across the liver and the steady-state level of cholesteryl esters, LDL-R mRNA, and receptor-dependent transport, can be measured under circumstances in which the experimental animals have been fed varying amounts of sterol and triacylglycerol.

The first major mechanism that alters the parameters of this system is a change in net sterol balance across the liver. When a particular level of cholesterol is fed in the diet for a prolonged period of time, a new steady state is achieved in which cellular cholesterol levels are elevated and the LDL-R mRNA level and activity are partially suppressed (36, 64). In this case, the equilibrium between C^R and CE is presumably constant so that LDL-R activity varies inversely with the concentration of CE (and, presumably, with C^R) as the level of sterol fed in the diet is progressively increased (*solid line,* Figure 6) (16). This inverse relationship between J^m and CE is invariably seen where there is a demonstrable change in net sterol balance across the liver such as can be induced by feeding cholesterol (64, 81) or bile acids (67), or by feeding agents that block the intestinal absorption of either of these two sterols.

The second mechanism that alters this system is the flow of specific fatty acids into the lipid pools of the liver. When the amount of sterol in the diet is constant, feeding the saturated fatty acids apparently shifts the equilibrium of the excess cholesterol in the liver cell out of the ester pool and into the putative regulatory pool, which, in turn, markedly suppresses the LDL-R mRNA level and activity (16, 36). In contrast, unsaturated fatty acids like the 18:1(*c*9) compound shift this equilibrium out of the regulatory pool towards the cholesteryl ester pool so that receptor-dependent transport is markedly increased (16). Thus, as illustrated by the *dashed line* in Figure 6, when sterol intake is constant and the type of fatty acid in the diet is varied, a direct relationship is found between the steady-state concentration of cholesteryl esters and LDL-R activity in the liver. The magnitude of this effect is markedly diminished when there is little cholesterol in the diet. It should be emphasized that not only is this relationship between CE and J^m diametrically opposed to that observed with cholesterol feeding, but, in addition, these marked changes in LDL-R activity seen with fatty acid feeding occur under circumstances where no measurable change in net sterol balance across the liver has taken place (16). Thus, the most detrimental change in the plasma LDL-C concentration occurs when the liver cell is loaded with cholesterol by feeding sterol in the diet, and this cholesterol is then shifted into the putative regulatory pool by also feeding one of the long chain saturated fatty acids. This combination leads to marked suppression of LDL-R activity, a large increase in the LDL-C production rate, and a marked rise in the LDL-C concentration in the plasma.

One of the key questions raised by this model is what enzyme(s) is (are)

responsible for determining the equilibrium between C^R and CE. Several lines of evidence suggest that this key role is played by acylCoA:cholesterol acyltransferase (ACAT), the enzyme that catalyzes the esterification of cholesterol to a variety of fatty acids (26). The rate of this reaction is clearly influenced by both the mass of cholesterol and the types of fatty acids that are available in the liver cell. Apparently, this enzyme is usually present in excess so that when only cholesterol is entering the cell, the level of apparent ACAT activity varies directly with the mass of sterol available for esterification (71, 72). In the absence of dietary triacylglycerol, this cholesterol is esterified to the 18:1(*c*9) and other fatty acids that are synthesized endogenously (16). Thus, in this situation both C^R and CE presumably increase in proportion to the amount of cholesterol absorbed from the intestine, and the ratio of C^R to CE remains relatively constant. However, this reaction is also exquisitely sensitive to the types of fatty acid that enter the liver during triacylglycerol feeding. For example, feeding the 14:0 fatty acid, which is a poor substrate for ACAT (26), markedly reduces the level of CE in the liver cell, and these esters become very enriched with the 14:0 and 16:0 fatty acids (16). Apparently, these particular fatty acids inhibit ACAT activity by entering the substrate pool of fatty acids used by this enzyme. In contrast, feeding the 18:1(*c*9) fatty acid, which is the preferred substrate for this enzyme (26), markedly increases the level of CE in the cell, and these esters become even more enriched with the 18:1(*c*9) compound. Thus, these data are consistent with the view that flooding the liver with fatty acids such as the 14:0 compound inhibits cholesterol esterification and elevates the C^R/CE ratio, whereas the 18:1(*c*9) fatty acid shifts this equilibrium in the opposite direction so that C^R is relatively reduced. This model, therefore, suggests that all of the observed consequences of dietary cholesterol and triacylglycerol can be explained by the effects of these lipids on ACAT activity; these effects, in turn, alter the distribution of excess cellular cholesterol in the liver between the storage pool of cholesteryl esters and the metabolically active, regulatory pool. Whether this concept is correct must await new methods that allow direct quantification of C^R in all of these experimental situations.

Effect of Cholesterol and Different Fatty Acids on Plasma LDL-C Levels in Man

Virtually all of the effects of cholesterol and triacylglycerol feeding that have been seen in experimental animals have also been observed in man. Obvious limitations in studying these regulatory processes in human populations, however, make it difficult to interpret the results of such studies in quantitative terms. For example, many such studies use subjects of different sex, age, ethnicity, and responsiveness to dietary lipids. Such variation in genetic background adds considerable variability to the experimental results obtained

in such groups. Second, in studying triacylglycerol effects, the background level of dietary cholesterol sometimes has not been controlled or else varies markedly in different studies. Third, the quantitative alterations that can be made in the lipid content of human diets are relatively limited. Thus, it is often difficult to discriminate between the effects of two lipids that have subtle differences in metabolic effects. Fourth, comparisons are usually carried out by substituting one active triacylglycerol for another, rather than by substituting an active triacylglycerol for a biologically neutral fat. This often obscures the true effect of a particular fatty acid because such experiments do not make clear whether a change in the LDL-C level was brought about by removal of the active fatty acid from the diet or by the addition of the second triacylglycerol. Fifth, most human studies are necessarily carried out over a period of only 4–8 weeks. In one month, a hamster will turn over approximately 120 pools of LDL-C and come into a new steady state, while a human will metabolize only about 12 pools of LDL-C during this same period. Thus, the results obtained after such relatively short experiments can only indicate the trends in serum lipid levels and not new steady-state values. Sixth, human studies usually yield only very limited experimental data, i.e. changes in the LDL-C concentration. As illustrated in Figure 5, however, the LDL-C level is a poor measure of what is happening with respect to the LDL-C receptor activity and production rate. Finally, one of the most serious limitations in human studies is failure to define the "neutral" LDL-C concentration, i.e. the level of LDL-C dictated by the content of cholesterol and other nutrients in a particular diet. Thus, it becomes nearly impossible to distinguish whether the substitution of a particular triacylglycerol into the diet lowers or raises the LDL-C level because that lipid actively increases or decreases J^m or, alternatively, whether that lipid is biologically inactive and the LDL-C level is merely drifting upwards or downwards towards the neutral value dictated by the level of cholesterol in that experimental diet.

In spite of these limitations, however, several generalizations can be made concerning the effects of various lipids on LDL-C levels in man based on data that have recently been extensively reviewed (29, 55). In the presence of large quantities of dietary triacylglycerol, the plasma cholesterol level in man increases about 10 mg/dl for every 250 mg of cholesterol that is added to the diet (29). This amount of sterol corresponds to a daily intake of ~3–4 mg/kg or about 35% of the amount of sterol synthesized in the body each day. To obtain a similar increase in the plasma cholesterol concentration in a small animal such as the triacylglycerol-fed hamster would require a cholesterol intake of ~10–15 mg/kg or, again, about 35% of the amount of cholesterol synthesized each day. Saturated fatty acids added to these cholesterol-containing diets invariably elevate the plasma cholesterol (and LDL-C) concentration in humans and, as in animals, the active compounds appear to be the 12:0,

14:0, and 16:0 fatty acids while the 18:0 compound is almost certainly biologically neutral with respect to the regulation of J^m and J_t (9, 47, 55).

Triacylglycerols containing predominately unsaturated fatty acids are quantitatively less effective in changing the plasma total and LDL-C levels, although these reductions are clearly evident when comparisons are made to a neutral control diet in which carbohydrate has been isocalorically substituted for the lipid (3, 12, 28, 51–53, 78). The implication in humans, as in animals (Figure 5), is that these lipids actively increase LDL-R activity. Several studies also suggest, but do not prove, that the 18:1(*c*9) monounsaturated compound is more active than the 18:2(*c*9, *c*12) polyunsaturated fatty acid in this regard (53, 78). In contrast to the effect of these two unsaturated fatty acids, the 18:1(*t*9) monounsaturated fatty acid appears to be biologically neutral and results in plasma LDL-C levels indistinguishable from those observed after feeding the 18:0 saturated compound but higher than those observed after feeding the 18:1(*c*9) fatty acid (54, 86). Based on data reported in experimental animals, the 18:1(*t*9) and 18:0 fatty acids are not likely to actively raise the LDL-C concentration in humans, but, rather, are biologically neutral in the regulation of J^m. It is the 18:1(*c*9) compound that is actively increasing J^m and so reducing the LDL-C level below that observed after feeding the 18:1(*t*9) and 18:0 lipids.

Summary

Extensive data obtained in both experimental animals and humans demonstrate that steady-state plasma LDL-C concentrations are determined largely by the rate of LDL-C formation, J_t, and the level of LDL-R activity, J^m, located primarily in the liver. An increase in net cholesterol delivery to the liver suppresses J^m, slightly elevates J_t, and modestly raises the LDL-C level. Feeding lipids such as the 12:0, 14:0, and 16:0 saturated fatty acids further suppresses J^m, increases J_t, and markedly elevates the plasma LDL-C concentration. Feeding triacylglycerols containing the 18:1(*c*9) fatty acid restores hepatic receptor activity, decreases J_t, and modestly reduces the concentration of LDL-C in the plasma. The 18:2(*c*9, *c*12) compound has similar effects, although it is quantitatively less active than the monounsaturated fatty acid in restoring J^m. In contrast to these fatty acids that actively raise or lower hepatic receptor activity, a large group of compounds including the 4:0, 6:0, 8:0, 10:0, 18:0, and 18:1(*t*9) fatty acids have no demonstrable effect on any parameter of LDL-C metabolism. These fatty acids, therefore, can be added to animal and human diets with relative impunity. They will alter plasma LDL-C levels only to the extent that they replace the active saturated fatty acids (in which case they lower the LDL-C concentration) or unsaturated compounds (in which case they raise the plasma cholesterol level). All of these effects of cholesterol and the various fatty acids can be explained

by the effects of these lipids in altering the size of the regulatory pool of cholesterol in the hepatocyte. However, many aspects of the cellular and molecular biology of these regulatory processes require additional investigation. In particular, new studies should focus on how the genetic background of an individual animal or human alters the quantitative response of its plasma LDL-C concentration to the dietary challenge of each of these types of lipids.

ACKNOWLEDGMENTS

The work reviewed in this manuscript from the authors' laboratories was supported by National Institutes of Health Research Grants HL-09610 and HL-38409. Additional support was provided by the Moss Heart Fund, the Institute of Shortening and Edible Oils, and the National Livestock and Meat Board.

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