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review

Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans

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During the past few years, new methods have evolved that allow much more accurate measurements in vivo of the rate constants for the physiological processes that determine sterol balance in the whole animal and that dictate the level of circulating cholesterol carried in low density lipoproteins (LDL-C). The purpose of this review, therefore, is *1)* to summarize the quantitative physiology of the control of cholesterol balance and LDL-C concentrations in all species in which such data are now available; *2)* to point out where commonly held concepts have been proved incorrect by these new measurements; and *3)* to emphasize those specific areas where further experimental data are required to better understand these regulatory processes.

I. INTRODUCTION AND OVERVIEW

As emphasized in **Fig. 1,** the liver plays the central role in two separate, but related, processes that are responsible for maintaining cholesterol balance across individual organs and the whole animal, and for regulating the steady-state concentration of LDL-C in the circulating plasma. Recent data have established that virtually every tissue

Abbreviations: LDL-C, cholesterol carried in low density lipoproteins; HDL-C, cholesterol carried in high density lipoproteins; VLDL-C, cholesterol carried in very low density lipoproteins; CM-C, cholesterol carried in chylomicrons; J^m, maximal rate of receptor-dependent LDL-C transport in an organ or the whole animal; J_l, rate of LDL-C transport or, in the whole animal, the rate of LDL-C production; SD, Sprague-Dawley; NZW, New Zealand White; GS, Golden Syrian; WHHL, Watanabe heritable hyperlipidemic; FH, familial hypercholesterolemia; ACAT, acyl-CoA:cholesterol acyltransferase.

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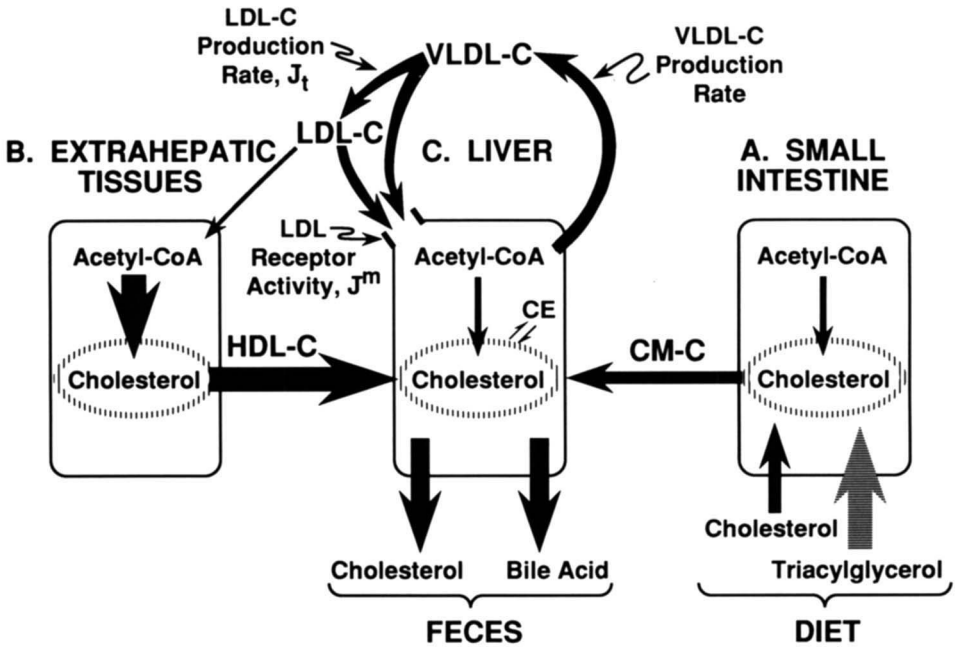

Fig. 1. A model showing the relationship between the absorption and synthesis of cholesterol in an animal and the regulation of the plasma LDL-C concentration. The liver plays a central role in both of these processes as it is the organ principally responsible for the maintenance of cholesterol balance in the body as well as for the production and degradation of LDL-C. The small percentage of sterol that is excreted through the extrahepatic tissues in all species is not shown in this diagram. In this model the body is divided into three functionally distinct tissue groups, the small intestine (A), the other extrahepatic tissues (B), and the liver (C), all of which are capable of synthesizing cholesterol from acetyl-CoA. Dietary cholesterol and the fatty acids consumed in dietary triacylglycerols reach the liver through the intestine. In the adult animal, in the steady-state, the liver must excrete each day an amount of sterol (as cholesterol and bile acid) that equals the amount of cholesterol synthesized in the various organs and absorbed from the diet. The dietary cholesterol and fatty acids reaching the liver significantly alter the concentration of sterol in the cholesteryl ester pool (CE), as well as the rate of LDL-C formation (J_t) and the level of LDL receptor activity in the liver (J^m). These changes, in turn, lead to secondary alterations in the level of circulating LDL-C. The dark arrows are meant to have semi-quantitative significance, as discussed in the text.

synthesizes sterol from acetyl-CoA and, in addition, cholesterol may be absorbed into the body from dietary sources (1–3). In the fetus or immature animal there must be a net accumulation of approximately 1.5–2.0 g of cholesterol for each kg of tissue that is added to the body during growth. In the adult animal that is not growing, however, an amount of cholesterol equal to that which is newly synthesized and absorbed across the small intestine must be excreted each day because the content of sterol in the body remains virtually unchanged over the life of the animal. While small amounts of cholesterol are lost through sloughing of skin and intestinal epithelial cells and by conversion to various steroid hormones, the major excretory pathway involves the secretion of sterols into bile by the hepatic parenchymal cells (4). This process includes both the direct secretion of cholesterol itself as well as the transcanalicular movement of bile acid, the metabolic end-product of cholesterol degradation by the liver (5).

Thus, as illustrated in Fig. 1, an amount of cholesterol equal to that newly synthesized each day in the extrahepatic tissues (B) must be transported through the plasma, presumably carried in high density lipoproteins (HDL-C) (6, 7), to the liver. Similarly, the sterol that is either synthesized or absorbed in the small intestine (A) must also reach the liver, although in this case the chylomicron particle (CM-C) is the primary carrier in the plasma (8). In the steady-state, where the weight of the animal is constant and no changes are occurring in the cholesterol concentration in any tissue compartment, the absolute rate of cholesterol synthesis and absorption must equal the absolute rate of cholesterol and bile acid excretion in the feces (plus the small amounts of sterol lost from the skin or converted to various hormones). Thus, the daily turnover of cholesterol in any species, expressed as the mg of cholesterol entering and leaving the body pool per day per kg body weight, can be quantified by either measuring the absolute rate at which sterol is being synthesized and absorbed in the intact animal or by measuring the absolute rate at which cholesterol and bile acid are being excreted in the feces.

The liver also plays the central role in the metabolism of cholesterol carried in very low density lipoproteins (VLDL-C) and LDL (8). Although the principal function of the VLDL particle appears to be the transport of tria-

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cylglycerol from the liver to the peripheral organs for oxidation or storage, this particle also contains both free and esterified cholesterol; thus, sterol is secreted from the liver in this particle at a velocity defined as the VLDL-C production rate (Fig. 1). During the metabolism of the VLDL particle with removal of a large portion of its triacylglycerol core, a remnant is formed and a portion of the VLDL is also converted to LDL at a velocity defined as the LDL-C production rate (J_t) (9). Both the LDL-C and the VLDL remnants appear to be largely cleared from the plasma by LDL receptors located in the liver (10). The level of hepatic LDL receptor activity (J^m) is markedly influenced by net cholesterol balance across the liver and by the types of fatty acids reaching the hepatocyte from the diet. When hepatic LDL receptor activity is suppressed, less of the VLDL remnants are cleared by the liver and there is a corresponding increase in the LDL-C production rate and an elevation of the circulating LDL-C concentration. Conversely, when LDL receptor activity is increased, the LDL-C production rate is decreased and the LDL-C concentration in the plasma is lowered.

Thus, both net cholesterol balance and the level of circulating LDL-C depend critically upon events taking place in the liver. In order to begin to understand the relationships between these two regulated systems, it is essential to have quantitative data on the importance of the different organs to whole-animal cholesterol turnover and the degradation of LDL-C, and on the physiologic and genetic factors that alter the regulation of these two processes. It is also useful to compare the regulation of cholesterol and lipoprotein metabolism in different species in order to establish whether there are general principles that describe these processes in essentially all animals, or, alternatively, whether there are aspects of cholesterol and lipoprotein metabolism that are unique to humans.

II. CHOLESTEROL SYNTHESIS IN THE ABSENCE OF DIETARY LIPIDS

A. Cholesterol synthesis in the whole animal and humans

The first problem of importance concerns the role of the liver in maintaining sterol homeostasis in the whole animal. In the past, measuring rates of sterol synthesis in vivo in an animal or in humans involved the difficult process of quantifying the rates of cholesterol and bile acid output in the feces each day (11). More recently it has been demonstrated that these rates can be measured directly by administering animals water labeled with either tritium or deuterium. As approximately 22–25 μg-atoms of the hydrogen from water are incorporated into each μmol of sterol, it is possible to calculate the absolute rate of sterol synthesis occurring in vivo from the rates of tritium or deuterium incorporation into cholesterol (12, 13). Utilizing both of these techniques, rates of cholesterol synthesis have now been measured in a number of different species and these are summarized in **Fig. 2.** In nearly all of these studies, the animals were on diets very low in cholesterol and triacylglycerol so that these rates of synthesis essentially equal the rate at which sterol is being turned over each day in vivo in these animals.

As is apparent, the rate of cholesterol synthesis per kg of body weight varies markedly with the size of the animal: humans synthesize about 10 mg/day per kg while the mouse makes approximately 50 mg/day per kg. Many other species, including four nonhuman primates, have intermediate rates of sterol synthesis between these two extreme values. The shaded area in this figure delineates a group of animals where the rate of sterol synthesis decreases by approximately 10 mg/day per kg for each 10-fold increase in body weight. Thus, just as the basal

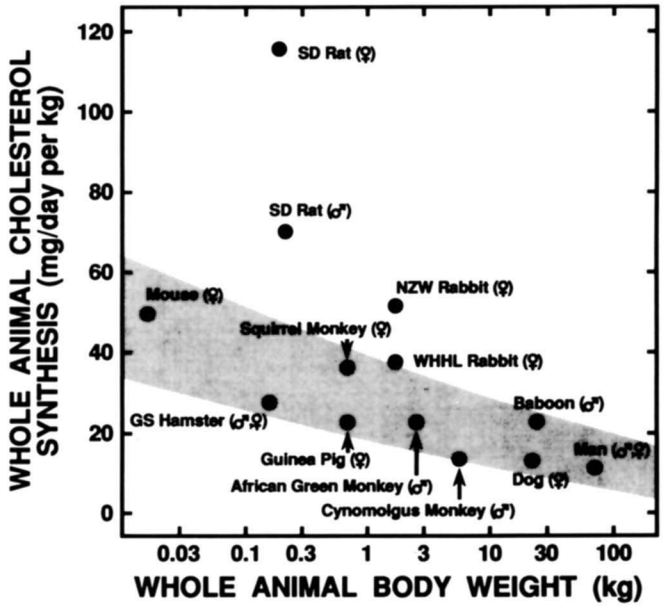

Fig. 2. Representative rates of cholesterol synthesis in the whole animal in vivo as a function of body weight. These rates of sterol synthesis are expressed as the mg of cholesterol synthesized per day per kg of body weight and are plotted against a logarithmic scale of the weight of each species. These data were obtained in vivo by either external sterol balance techniques or by quantitating the rates of incorporation of [^{3}H]water into sterols in the animals and then converting these isotope incorporation data into absolute rates of sterol synthesis (12, 13). In all cases except humans, the animals were on diets low in both cholesterol and triacylglycerol when the measurements were made. SD (Sprague Dawley), NZW (New Zealand White), WHHL (Watanabe heritable hyperlipidemic), and GS (Golden Syrian) designate specific strains of rats, rabbits, and hamsters. These data come from different laboratories (2, 11, 14–22) as well as from unpublished measurements made in this laboratory. The shaded area is drawn in merely to emphasize a group of "main-stream" animals whose rates of synthesis decrease by approximately 10 mg/day per kg for each 10-fold increase in body weight. Most of the data points represent the means of measurements in 6–18 animals.

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metabolic rate and the turnover of proteins in various species varies inversely with the logarithm of body weight, so also the synthesis and turnover of cholesterol through the cellular membranous structures is markedly influenced by the size of the animal. Also of note in Fig. 2 is that the NZW rabbit, the male SD rat and, particularly, the female SD rat fall well outside this "main-stream" group of animals. In particular, the rate of whole animal sterol synthesis seen in the female SD rat is 3–4 times that expected for an animal weighing 0.2 kg. This unusually high rate is partially explained by the fact that these values are calculated from data determined at the mid-dark phase of the light cycle when hepatic synthesis in this rodent is exceptionally high. Nevertheless, the rates of whole animal synthesis in the rat are well above the "main-stream" group which explains, in part, the anomalous response of this species to a variety of dietary and pharmacological challenges.

While Fig. 2 shows the rates of synthesis in sexually mature members of different species, **Fig. 3** illustrates how these rates vary in the same species when measured in the fetus or newborn, pregnant, obese, and aged animal. Such comparative data are partially available in three species including the SD rat, NZW rabbit, and humans. In the developing fetus, where most of the cholesterol needed for new tissue growth comes from de novo synthesis (26), the rate of whole animal synthesis is 3- to 4-fold higher than in the sexually mature member of that species (SD rat and NZW rabbit). Similarly, the newborn has a rate of synthesis per kg of body weight that is about twice that of the adult (NZW rabbit and human). As is also apparent, the pregnant animal (rat) and obese human have slightly higher rates of whole body synthesis than their respective lean, non-pregnant controls. Of particular importance, Fig. 3 also illustrates the changes in synthesis that occur as the animals age. In the male SD rat and both the male and female GS hamster, for example, the rate of sterol synthesis per kg of body weight decreases by approximately 60% as the animals age from young, sexually mature males and females to older and heavier animals.

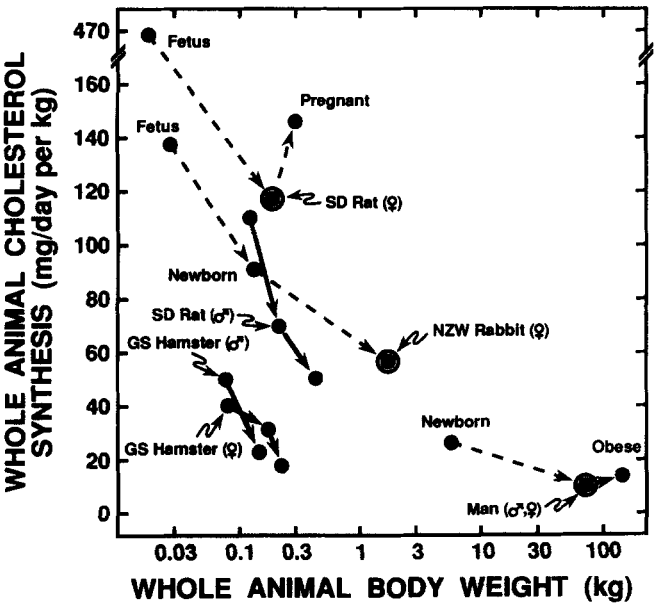

Fig. 3. Cholesterol synthesis in vivo in the fetus and in the newborn, pregnant, and obese animal. The rates of whole animal cholesterol synthesis found in the sexually mature, adult SD rat, NZW rabbit, and human are taken from Fig. 2 and are circled in this diagram. Insofar as data are available, rates of sterol synthesis in the whole fetus and in the newborn, pregnant, and obese members of these same species are also shown. These values are connected to the appropriate adult member of that species by the dashed lines. In addition, the solid lines connect data on rates of synthesis in male SD rats and in male and female GS hamsters of different ages and, therefore, different weights. These data come from both published reports (20–26) and unpublished observations in this laboratory. Most of the data points represent mean values of 6–12 animals in each group.

Thus, these data illustrate the remarkable adaptability of the sterol biosynthetic pathway to meet the changing needs of the organism for cholesterol to support tissue growth and for membrane remodeling. Throughout the life of the rat, for example, there is a nearly 10-fold change in the rate of whole animal synthesis, from approximately 470 mg/day per kg in the rapidly developing fetus to only 50 mg/day per kg in the aged adult. Clearly, the full range of sterol requirements in the body can be met by the biosynthetic pathway from conception to death, even in the total absence of dietary cholesterol.

B. Tissue sites for cholesterol synthesis in the whole animal

It is still commonly believed that the liver is the major site for this biosynthetic activity in the whole animal. This concept arose from early studies where assays of rates of sterol synthesis were performed in vitro using various ^{14}C-labeled precursors such as [^{14}C]acetate. Such studies commonly revealed that the majority of the biosynthetic activity that could be demonstrated in all of the tissues of the body by these in vitro assays was accounted for by the activity observed in the liver (1, 27). However, it became clear that many of these ^{14}C-labeled substrates were poorly taken up and metabolized to [^{14}C]acetyl-CoA in the extrahepatic tissues. Furthermore, the specific activity of the [^{14}C]acetyl CoA pool that is the immediate precursor for sterol biosynthesis was disproportionately (relative to the liver) diluted in many of these tissues by the intracellular generation of large amounts of unlabeled acetyl-CoA (28). As a result of all of these technical problems, it was demonstrated that the rates of synthesis in the extrahepatic organs had been systematically underestimated, in some tissues, by as much as 90–95% (29).

With the advent of techniques that circumvented these artifacts (13, 30), it became possible to measure absolute rates of synthesis not only in the whole animal in vivo

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(Fig. 2), but the contribution of each organ to such whole animal synthesis could also be quantified (12). **Fig. 4** summarizes such data in seven species where rates of sterol synthesis have been measured in vivo under circumstances where the animals had been fed diets low in cholesterol and triacylglycerol. This diagram shows the contributions of the small intestine (A) and liver (C) to whole animal synthesis while the contributions of the remaining extrahepatic organs have been combined into a single value (B). Under these circumstances where dietary cholesterol intake was essentially zero, the liver contributes, at most, 40–50% of the cholesterol synthetic activity found in the female SD rat, mouse, and squirrel monkey. However, this contribution is significantly less in the other species and amounts to < 20% in the NZW rabbit, guinea pig, cynomolgus monkey, and male GS hamster. Estimates of the importance of the liver in humans also suggest that this organ is a relatively minor contributor to whole body synthesis.[2]

Data such as those shown in Fig. 4, however, are very much influenced by the conditions under which the measurements were made, as marked changes in rates of cholesterol synthesis are induced by any condition that alters net sterol balance across a particular organ or across the whole animal. Furthermore, as it is the cholesterol pools in the intestinal epithelial cell and liver that are most influenced by such manipulations, it is the rates of sterol synthesis in these two particular organs that respond to changes in sterol balance. Thus, for example, if net sterol input into the body is increased, e.g., by adding small amounts of cholesterol to the diet, then there is marked suppression of the rate of hepatic cholesterol synthesis, partial suppression of intestinal synthesis, and virtually no change in synthesis in the extrahepatic organs (1, 30, 31). Conversely, if net sterol loss from the body is increased, e.g., by blocking the intestinal absorption of bile acids or cholesterol or by feeding soluble fibers, the rate of cholesterol synthesis in the liver and, to some extent, in the intestine increases to compensate for this loss while the rate of synthesis in extrahepatic organs again remains essentially unchanged (30–33).

Thus, as indicated in Fig. 1, in the steady state the absolute rate of cholesterol synthesis in the liver (C) must always equal the absolute rate of sterol excretion in the feces minus the absolute rate of cholesterol delivered to the liver from the intestine (A) and extrahepatic tissues (B). Hence, hepatic synthesis is necessarily suppressed when

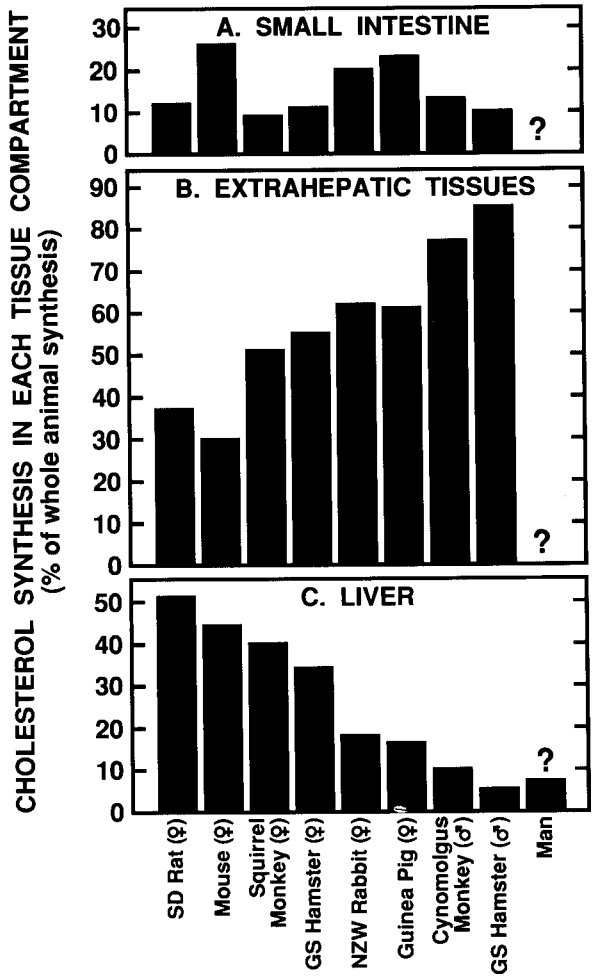

Fig. 4. Relative importance of the small intestine (A), extrahepatic tissues (B), and liver (C) in cholesterol synthesis in vivo in different species. Except for humans, absolute rates of cholesterol synthesis in all organs were measured in vivo using [^{3}H]water in animals on diets low in cholesterol and triacylglycerol (2, 12, 21, 22 and unpublished observations from this laboratory). The percentage of sterol synthesis quantified in the whole animal that was attributable to the small intestine, remaining extrahepatic tissues, and liver was then calculated and is shown in this diagram. The data for humans were calculated indirectly from rates of synthesis measured in liver biopsies.[2] Each value represents the mean of data from 6–18 animals. (Figure modified, with permission, from the *Annual Review of Nutrition,* Vol. 13, 1993 by Annual Reviews Inc.)

net sterol delivery from the intestine to the liver is increased and is markedly elevated when net sterol loss in the feces is enhanced. The data in Fig. 4 reflect the particular situation where all of the animals were fed commercial diets containing constant amounts of dietary fiber and various nutrients, but essentially no cholesterol. If an additional amount of soluble fiber or a bile acid sequestrant were added to the diets of each of these animals to increase fecal sterol loss, then synthesis in the liver, but not in the extrahepatic tissues, would increase and the relative contribution of hepatic cholesterol synthesis to whole animal synthesis would be higher (31). In contrast,

[2]As no data are available in humans on rates of cholesterol synthesis in vivo in the liver, this value was calculated indirectly using in vitro assays. In tissue obtained using a needle biopsy, the rates of incorporation of [^{14}C]acetate into cholesterol are about 10–20% of the rates found when similar needle biopsy specimens are obtained from rat or squirrel monkey liver. After correcting for relative turnover and liver size, these data suggest that the liver of humans contributes about 5–10% of whole body synthesis.

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if cholesterol were added to the diets of each of these species in the small amounts usually present in Western human diets, i.e., an amount equal to 30–60% of the daily sterol turn-over rate, then synthesis in the liver, but not in the extrahepatic organs, would be partially suppressed. In this situation the relative hepatic contribution to whole animal cholesterol synthesis would be very small, even in the rat, mouse, and squirrel monkey. Thus, under dietary conditions equivalent to those found in Western humans, the extrahepatic tissues probably account for > 80% of whole animal sterol synthesis in virtually every species that has been studied.

III. LDL-C TRANSPORT IN THE ABSENCE OF DIETARY LIPIDS

A. LDL-C transport out of the plasma in the whole animal and humans

The second problem of importance concerns the role of the liver in determining the steady-state concentration of LDL-C in the plasma. As illustrated in Fig. 1, LDL-C is formed primarily from the metabolism of VLDL-C. In the past it has been suggested that some LDL-C may also be secreted directly by the liver, although a recent analysis of this possibility suggests that this latter pathway is relatively unimportant or may not exist at all (34). In the steady state, the rate at which LDL-C is removed from the plasma and degraded by all of the tissues of the body must equal the LDL-C production rate (J_t). Thus, one way in which to express the rate of LDL-C uptake is as an absolute rate of transport having the units of mg of LDL-C taken up by the various organs each day per kg of body weight. The amount of LDL-C removed from the plasma can also be expressed as a classical clearance value. The absolute rate of LDL-C removed from the plasma each hour divided by the plasma LDL-C concentration yields the LDL-C clearance rate, which describes the ml of plasma entirely cleared of its LDL-C content per hr per kg of body weight. Finally, either the absolute rate of LDL-C transport out of plasma or the clearance rate can be expressed as a fraction of the LDL-C pool or the plasma volume, respectively, present in 1 kg of body weight. This calculation yields a term called the fractional catabolic rate which describes the fraction of the LDL-C pool removed from the plasma each hour or day. Thus, the rate at which LDL-C is removed from the plasma can be expressed in three different ways, i.e., the absolute rate of LDL-C transport (mg/day per kg), the LDL-C clearance rate (ml/h per kg), and the LDL-C fractional catabolic rate (pools/day). The first two values must be normalized to a constant body weight, e.g., 1 kg, while the third is independent of body weight. Different methods are available for directly quantifying the absolute rate of LDL-C transport in the whole animal (35), the whole animal LDL-C clearance rate (36), and the fractional

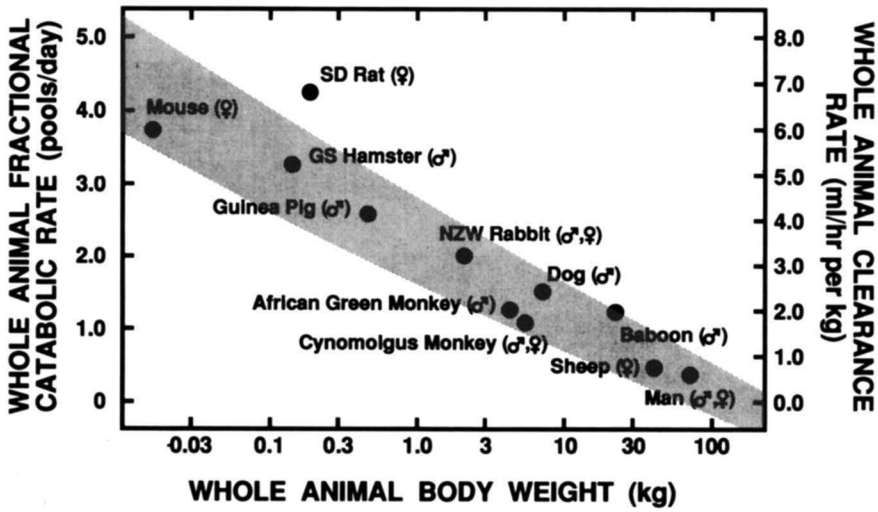

Fig. 5. Representative rates of LDL-C degradation in the whole animal in vivo as a function of body weight. The rates of LDL-C degradation were measured either indirectly from dieaway curves (37) or directly using the steady-state, constant infusion of homologous LDL (35, 36) in animals fed diets low in cholesterol and triacylglycerol. These rates are plotted against a logarithmic scale of the weight of each species and are presented in two ways in this diagram: *1)* as the fractional catabolic rate (left vertical axis) expressed as the pools of LDL-C removed from the plasma space each day (pools/day), and *2)* as the clearance rate (right vertical axis) expressed as the ml of plasma completely cleared of its LDL-C content per h by each kg of body weight (ml/h per kg). These two rate constants are essentially interchangeable. These data come from several different laboratories (35–47) as well as from unpublished observations in this laboratory. The shaded area is drawn in merely to emphasize a group of "main-stream" animals whose fractional catabolic rates (clearance rates) decrease by approximately 1.1 pools/day (1.8 ml/h per kg) for each 10-fold increase in body weight. All data points represent the means of measurements in 6–16 animals.

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catabolic rate (37). However, it should be emphasized that once one of these values has been quantified, the other two can be calculated.

The rate of LDL-C removal from the plasma space has now been measured in at least 11 species and these are summarized in **Fig. 5.** These rates are expressed as both the whole animal fractional catabolic rate and clearance rate, and are plotted against the logarithm of the weight of each species. Most animals, except humans, were on diets low in cholesterol and triacylglycerol. As is apparent, the effect of body size on LDL-C turnover is even greater than on whole animal cholesterol synthesis (Fig. 2). Small animals such as the mouse, GS hamster, and SD rat, for example, degrade about 4 pools of LDL-C per day while humans remove from the plasma only about 0.4 pools/day. Stated differently, one kg of a human subject will clear only about 0.6–0.7 ml of plasma per h of its LDL-C content while one kg of these small animals will clear 6–7 ml/h. The shaded area in Fig. 5 delineates a group of animals where the fractional catabolic rate and the clearance rate decrease by approximately 1.1 pools/day and 1.8 ml/h per kg, respectively, for each 10-fold increase in body weight. Also of note is the fact that the rate of LDL-C clearance in the female SD rat is much closer to the mainstream group of animals than is this species with respect to cholesterol biosynthesis (Fig. 2). Furthermore, it is of interest that this relationship is made up of animals that are omnivorous, carnivorous, and herbivorous. Included within the herbivorous group is at least one species of ruminant.

B. Mechanisms of LDL-C removal from the plasma

The key to understanding LDL-C turnover came with the discovery and characterization of the LDL receptor (48, 49). It was clear that such receptor-dependent LDL-C transport could be identified in vivo and accounted for a significant amount of LDL-C removal from the plasma. Furthermore, it was also found that derivatization of the LDL particle through chemical modifications such as reductive methylation or glycosylation blocked interaction of this lipoprotein with its receptor (46, 50, 51). Such modified LDL-C was cleared from the plasma in vivo at a rate that was similar in the normal animal (or human) and in the animal (or human) genetically lacking LDL receptor activity (45, 52, 53). Thus, by using homologous and derivatized LDL, it became possible to fractionate the rates of LDL-C turnover seen in the whole animal (Fig. 5) into receptor-dependent and receptor-independent components. Representative values for such data are summarized for five species in **Table 1.**

This table gives representative rates of LDL-C removal from the plasma expressed as the absolute transport rate (B), the fractional catabolic rate (C), and the clearance rate (F). It is of interest that the absolute rate of total LDL-C transport out of the plasma varies relatively little among the five normal species (B, from 12 to 30 mg/day per kg) even though there is a 10-fold difference in the total fractional catabolic rate (C, from 0.43 to 4.3 pools/day) and total clearance rate (F, from 0.72 to 7.1 ml/h per kg). The component of the total fractional catabolic and clear-

TABLE 1. Representative values in several species for the parameters that describe the rates of LDL-C removal from the plasma by both the receptor-dependent and receptor-independent transport processes

Species	A. Plasma LDL-C Concentration	B. LDL-C Transport Out of the Plasma	Whole Animal Fractional Catabolic Rate			Whole Animal Clearance Rate			I. Receptor-Dependent LDL-C Transport Out of the plasma
			C. Total	D. Receptor-Dependent	E. Receptor-Independent	F. Total	G. Receptor-Dependent	H. Receptor-Independent	
	mg/dl	*mg/day per kg*		*pools/day*			*ml/h per kg*		%
SD rat	7	12	4.3	3.2	1.1	7.1	5.3	1.8	75
GS hamster	21	28	3.3	2.4	0.9	5.5	4.0	1.5	73
Guinea pig	30	30	2.5	1.9	0.56	4.2	3.3	0.9	78
NZW rabbit	20	18	2.3	1.6	0.66	3.9	2.8	1.1	72
WHHL rabbit	400	106	0.66	0.0	0.66	1.1	0.0	1.1	0
Human	75	13	0.43	0.25	0.18	0.72	0.42	0.30	58
FH human	560	40	0.18	0.0	0.18	0.30	0.0	0.30	0

This table shows representative values for the concentration of LDL-C in different species (column A) and for the absolute amount of LDL-C that is transported out of the plasma space each day per kg body weight (B). These absolute rates of removal are also expressed as whole animal fractional catabolic rates (C) and clearance rates (F), and each of these values, in turn, is subdivided into the receptor-dependent (D, G) and receptor-independent (E, H) components. The final column (I) shows the percentage of whole animal LDL-C turnover that is receptor-dependent. These data are calculated from measurements made in normal animals on diets low in cholesterol and triacylglycerol (36, 45–47) except for those made in normal humans (38) where the subjects were on the usual lipid-rich human diets. In addition to normal animals and humans, this table also contains equivalent measurements made in subjects that genetically lacked functional LDL receptor activity, i.e., the WHHL rabbit and FH humans (38, 45). The interconversions of the three kinetic parameters were done by assuming that the plasma volumes were approximately equal in the various species.

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ance rates that is receptor-dependent is shown in columns D and G, respectively, and this component is expressed as a percentage of the total LDL-C turnover in column I. It is evident that in the SD rat, GS hamster, guinea pig, and NZW rabbit, studied while on a diet low in cholesterol and triacylglycerol, receptor-dependent LDL-C transport accounts for 72–78% of the degradation of LDL-C taking place in the whole animal (36, 45–47). In the young, healthy human subject, this figure is only 58%, but these subjects were on the usual human diets containing cholesterol and fatty acids known to partially suppress receptor activity (38).

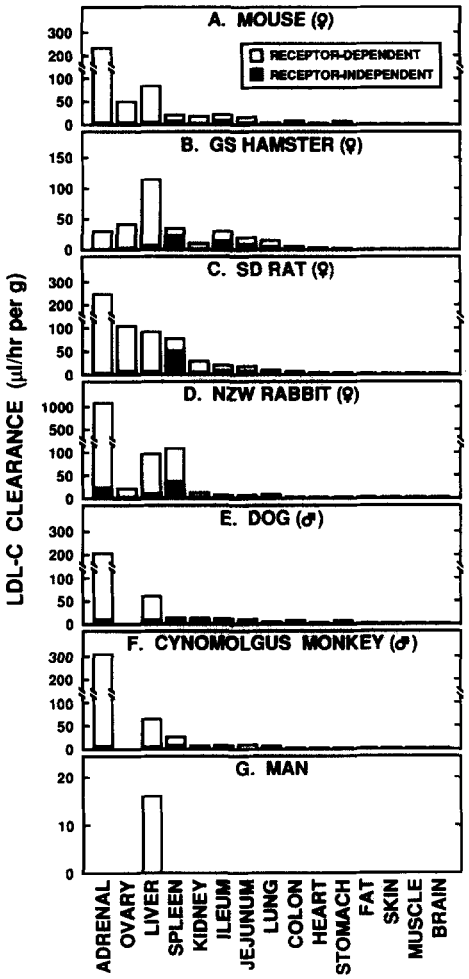

Fig. 6. Rates of LDL-C clearance in 15 tissues of different species. Using steady-state infusions of homologous LDL-C or derivatized heterologous LDL-C (35, 36), absolute rates of LDL-C clearance by the receptor-dependent and receptor-independent transport processes in the individual organs of these species were quantified in vivo. These rates, measured in animals on diets low in cholesterol and triacylglycerol, are expressed as the μl of plasma entirely cleared of LDL-C per h per g of each organ (μl/h per g). Each value represents the mean of data in 3–12 animals (35, 36, 45, 56 and unpublished observations from this laboratory). The data for the liver of humans were calculated indirectly[3] and show only the receptor-dependent component. No transport data are available for the other tissues of man in vivo.

Table 1 also shows the transport data in two species, the WHHL rabbit and FH human, that genetically lack receptor-dependent LDL-C transport activity. In both species the magnitude of the receptor-independent component is essentially the same in the normal and receptor-deficient members of that species. Thus, because it is the receptor-dependent component of LDL-C transport that appears to be suppressed by dietary lipids, feeding cholesterol and saturated fatty acids lowers the total fractional catabolic rate towards the value of the receptor-independent component, but never below that value. Table 1 also illustrates the high rates of transport that can be achieved by the receptor-independent component. The absolute rate of LDL-C movement out of the plasma (B) is six times normal in the WHHL rabbit and three times normal in the FH human. These high velocities of receptor-independent transport are only achieved, however, by allowing the steady-state plasma LDL-C concentration to increase into the range of 400–600 mg/dl (A).

C. Rates of LDL-C transport into different tissues

The next question of importance is which tissues in the body account for these rates of receptor-dependent and receptor-independent LDL-C uptake observed in the whole animal. With the development of radiolabeled markers for LDL that are retained within the different organs (54, 55) and short-term, steady-state infusion techniques using both homologous and derivatized LDL-C (35, 36), it has become possible to determine the absolute rates of receptor-dependent and receptor-independent LDL-C transport into every organ in the live animal. **Fig. 6** summarizes the available data of this type and shows the rates of receptor-dependent and receptor-independent transport into 15 different tissues of six species. The calculated rate of receptor-dependent uptake in the human liver is also shown.[3] These data are expressed as clearance rates and describe the μl of plasma cleared of its LDL-C content each h per g of each organ.

As is apparent, the general profile of LDL transport is the same in all species. First, only three organs consistently manifest high rates of LDL-C transport per g of tissue: these include the liver and two endocrine tissues, the

[3]In a typical young, normal adult human, the fractional catabolic rate is about 0.43 pools/day while the receptor-independent component of this turnover equals approximately 0.18 pools/day (38). Thus, such a 70-kg individual has a receptor-dependent clearance rate of about 10 ml/day per kg (0.42 ml/h per kg). If 70% of this clearance occurs in the liver, then the hepatic receptor-dependent clearance rate would equal about 15 μl/h per g. Direct confirmation of this value comes from studies in a young adult with homozygous familial hypercholesterolemia who received a normal liver transplant (53). This transplant increased the LDL-C clearance rate from 5.4 to 13.9 ml/day per kg (from 0.23 to 0.58 ml/h per kg). As this organ was the only source for receptor-dependent LDL-C transport in this individual, the receptor-dependent clearance rate in the transplanted liver must have equaled approximately 17 μl/h per g.

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adrenal gland and ovary. Furthermore, it is clear that these high rates are achieved only because there is a large component of receptor-dependent uptake in these three organs. The magnitude of this component in the adrenal gland and ovary, however, varies markedly among the different species regardless of size (56), while receptor-dependent transport in the liver varies inversely with animal weight. Thus, in small animals such as the mouse, GS hamster, and SD rat, hepatic LDL-C clearance equals approximately 100 μl/h per g, but this value drops to about 60 μl/h per g in the dog and cynomolgus monkey, and to only 15–17 μl/h per g in humans. There is a second group of tissues where receptor-dependent transport can also be clearly identified, yet the overall rates of LDL-C uptake are usually low. This group includes the spleen, kidney, small intestine, lung, and colon. Finally, there is a third group of organs that include adipose tissue, skin, muscle, and brain, where it is essentially impossible to detect any LDL-C uptake, either receptor-dependent or receptor-independent.

Except for the receptor-dependent transport rate calculated for the liver in a young transplant patient (G), no detailed clearance data are available in vivo in humans. Heparin-specific LDL binding to homogenates of various human tissues has, however, been reported (57). To the extent that such binding data reflect receptor-dependent LDL transport, these data generally agree with the actual clearance rates illustrated in Fig. 6. Homogenates of organs such as brain, adipose tissue, muscle, and skin have little specific binding, while tissues such as spleen, kidney, small intestine, and colon manifest significantly greater specific interaction with LDL. The two endocrine tissues, the adrenal gland and ovary, bind the greatest amount of LDL. Unfortunately, heparin-specific LDL binding in hepatic membranes in this study was very low. However, these particular measurements were carried out in tissues obtained from older patients who presumably were maintained on diets containing cholesterol and saturated fatty acids, and many of these subjects had tumors and were subjected to the trauma of surgery. It is to be anticipated, therefore, that hepatic LDL-C clearance in the livers of such subjects would be suppressed and the rates of transport in these individuals would certainly not be comparable to those shown in Fig. 6 which were all obtained in healthy, young, sexually mature animals maintained on diets essentially free of lipids.

D. Importance of each organ for LDL-C transport in the whole animal and humans

The key to understanding LDL-C turnover in the whole animal or humans revolves around the relative importance of each of these tissues for clearing LDL-C from the plasma. The absolute rate of LDL-C uptake into each organ can be calculated by multiplying the rate of receptor-dependent and receptor-independent LDL-C transport per g of tissue (Fig. 6) by the weight of each organ in each species. The sum of the uptake rates in the individual organs equals the rate of LDL-C turnover independently determined in the whole animal (Fig. 5). The relative importance of each organ to whole animal LDL-C degradation can then be calculated and these data are summarized in **Fig. 7** for all species in which such measurements have been made. In five species maintained on diets low in cholesterol and triacylglycerol, it is

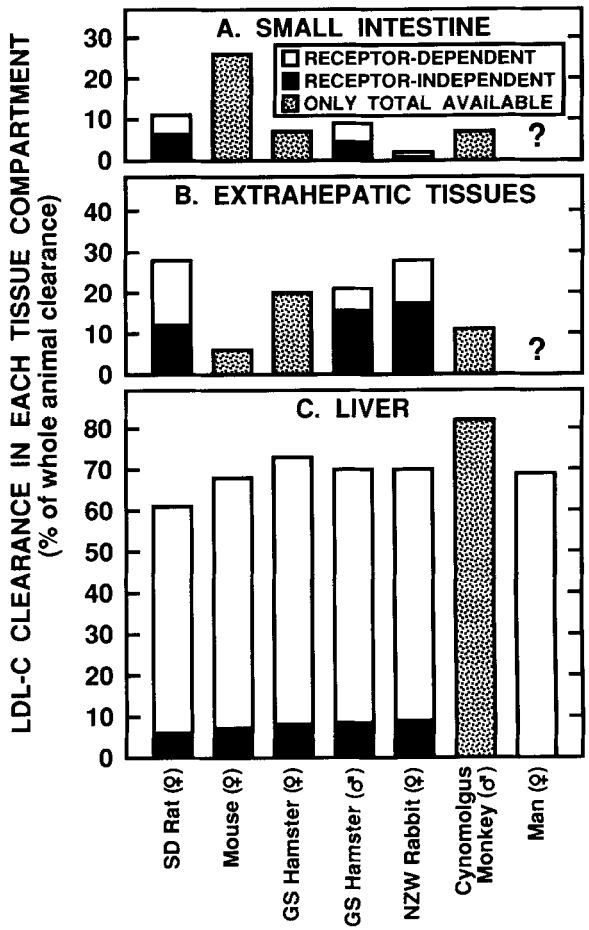

Fig. 7. Relative importance of the small intestine (A), extrahepatic tissues (B), and liver (C) in removing LDL-C from the plasma in vivo in different species. By multiplying the weight of each organ by the rate of LDL-C clearance per g of that organ (Fig. 6), the absolute rates of clearance of LDL-C by each tissue can be calculated. The sum of the clearance rates in all organs equals the clearance rate of LDL-C in the whole animal (Fig. 5, Table 1). This figure shows the percentage of whole animal LDL clearance that can be accounted for by uptake in the small intestine, remaining extrahepatic organs, and liver in animals maintained on diets low in cholesterol and triacylglycerol. These percentages include both the receptor-dependent and receptor-independent components. In the case of the mouse, female GS hamster, and cynomolgus monkey, detailed organ weights were not available so only total clearance rates are shown. The data in humans were calculated indirectly[3] assuming that the liver accounts for 2.0% of body weight. Except for the data in humans, these values represent the means of data from 3–12 animals (35, 36, 45, 47 and unpublished observations from this laboratory). (Figure modified, with permission, from the *Annual Review of Nutrition,* Vol. 13, 1993 by Annual Reviews Inc.)

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clear that the liver accounts for the uptake and degradation of about 70% of the LDL-C that is turned over in the whole animal each day (C). A similar figure can be calculated from the limited data available in humans.[3] The absolute level of hepatic LDL-C uptake in these various animals is dictated by two variables: in going from small animals such as the mouse to the larger primates *1)* the rate of clearance into the liver decreases from ~120 to ~15 μl/h per g (Fig. 6); and *2)* the relative size of the liver decreases from ~5.5 to ~1.5% of body weight. Yet despite these major variables, the liver is the overwhelmingly important site for LDL-C clearance from the plasma in every species in which quantitative data are available. The small intestine (A) and all of the remaining extrahepatic tissues (B) together account for the degradation of the remaining ~30% of LDL-C. Furthermore, within this latter group large and important organs such as muscle and the central nervous system appear to take up virtually no cholesterol from the circulating LDL-C pool. Thus, in the whole animal, and presumably in humans, most cholesterol is synthesized and utilized in the extrahepatic organs (Fig. 4) while most of the cholesterol carried in LDL is taken up into the liver (Fig. 7).

Two other important conclusions come from the data shown in Fig. 7. In the whole animal, 72–78% of LDL-C degradation is receptor-mediated (Table 1). Of this receptor-dependent transport identifiable in the whole animal, ~80% takes place in the liver of all species in which data are available (Fig. 7). In contrast, 22–28% of LDL-C is removed from the plasma in the whole animal by the receptor-independent process (Table 1), and ~70% of this transport activity is located in the intestine (A) and other extrahepatic tissues (B). Thus, any genetic or environmental factor that reduces receptor-dependent LDL-C transport in the liver will necessarily partially shift the burden of LDL-C clearance in the whole animal from the liver to the extrahepatic organs.

As is the case with the data on cholesterol synthesis in the various organs (Fig. 4), the data in Fig. 7 apply only to the particular situation where animals are maintained on commercial diets essentially free of cholesterol and triacylglycerol. Any dietary addition that would enhance net sterol loss from the body may increase receptor-dependent LDL-C transport into the liver and so increase the relative importance of this organ as a site for LDL-C clearance in the whole animal. Conversely, the addition of certain lipids to the diets of these animals may lead to suppression of hepatic receptor-dependent transport and a decrease in the importance of the liver to whole animal LDL-C degradation.

These two sets of data also provide important insights into the possible physiological function of circulating LDL-C. Contrary to earlier assumptions, it now seems evident that LDL does not function in a major way to deliver cholesterol from the liver to the extrahepatic organs. From the data shown in Table 1 and Figs. 4 and 7, it can be calculated that in all of these species, including humans, the amount of cholesterol synthesis taking place in the extrahepatic tissues is 3–5 times greater than the amount of cholesterol being delivered to these organs by LDL. Furthermore, when the LDL-C concentration in the plasma is decreased to near 0 mg/dl, either by blocking VLDL-C secretion (58) or by markedly increasing hepatic receptor-dependent clearance (59), synthesis in these extrahepatic tissues can increase to compensate for even this small contribution of LDL-C to the peripheral sterol pools. Similar findings have been reported in human subjects with little or no circulating LDL-C. Whole body cholesterol synthesis is either normal (60) or slightly increased (61), and there is no evidence of organ dysfunction attributable to a shortage of sterol in any extrahepatic tissue except the adrenal gland under circumstances of extreme ACTH stimulation (62, 63).

As even this small contribution of cholesterol to the extrahepatic organs is primarily mediated by the receptor-independent transport process (Fig. 7), one would anticipate even less disruption of cholesterol homeostasis in these tissues with loss of receptor-dependent LDL-C uptake. This prediction has been confirmed in the homozygous WHHL rabbit where rates of cholesterol synthesis are virtually unchanged from control animals in every extrahepatic organ except the adrenal gland, where synthesis is increased 5-fold (21). Similarly, in the heterozygous and homozygous FH patient, whole body cholesterol synthesis is essentially normal and there is no evidence of organ dysfunction other than, again, the response of the adrenal gland to extreme ACTH stimulation (64, 65). Thus, when subjected to marked stimulation, the adrenal gland (and, possibly, the ovary) may actually use LDL-C as a second source of cholesterol for hormone production. With this exception, however, there is currently no evidence that the function of any extrahepatic organ is dependent upon LDL-C for maintenance of physiological function.

If this is the case, and there is little or no biological use for LDL-C, then the alternative possibility is simply to consider LDL-C one of the remnants of VLDL metabolism that should be cleared from the plasma as rapidly as possible. Thus, the two classes of apoB-containing lipoproteins would manifest similar functions and behaviors. The chylomicron particle functions primarily to move triacylglycerol from the intestinal epithelial cell to the peripheral sites of utilization and storage, and the remnant of this particle is rapidly cleared from the plasma by the liver, primarily through utilization of the chylomicron remnant receptor. In a similar fashion, VLDL functions primarily to move triacylglycerol from the liver to the same peripheral sites of utilization and storage, and its remnants are also rapidly cleared from the plasma by the liver, principally through intervention of

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the LDL receptor (Fig. 1). Thus, in the absence of dietary lipids, the LDL-C concentration is well below 40 mg/dl in virtually every species in which such data are available and these include the rat, hamster, guinea pig, various nonhuman primates, and the human infant (66). Even this low level is attributable to the fact that hepatic LDL-C clearance occurs at rates of only about 100 μl/h per g (Fig. 6) while the hepatic clearance of chylomicron remnants takes place at rates in excess of 20,000 μl/h per g (67). If hepatic LDL receptor activity is increased only modestly (59, 68, 69), to 500 μl/h per g, the concentration of LDL-C, like the concentration of chylomicron remnants, decreases essentially to zero.

IV. REGULATION OF CHOLESTEROL SYNTHESIS AND LDL-C TRANSPORT BY DIETARY LIPIDS

A. General features of the regulation of LDL-C transport

While the studies reviewed thus far delineate the relative importance of various organs to receptor-dependent and receptor-independent LDL-C transport out of the plasma in a particular dietary circumstance, it is clear that these quantitative relationships are profoundly influenced by both environmental and genetic factors in such a way that the steady-state LDL-C concentration in any species can be varied from virtually 0 mg/dl to over 500 mg/dl. In order to define how these environmental and genetic factors might alter the biology of the liver cell and lead to these changes in LDL-C concentration, it is next necessary to review the quantitative relationships that have been described between hepatic cholesterol metabolism and the rates of LDL-C formation and degradation.

In general terms, the steady-state concentration of LDL-C is determined by only three processes. These include the rate at which LDL-C enters the plasma space, i.e., the LDL-C production rate, and the rates at which LDL-C is transported out of the plasma by the receptor-dependent and receptor-independent processes. The kinetic characteristics of these two transport processes in the whole animal are illustrated by the various curves shown in **Fig. 8.** These curves were constructed by systematically increasing the LDL-C production rate from 0 to a high value so as to produce steady-state plasma LDL-C concentrations varying from 0 to 500 mg/dl. Panel A shows how the absolute rate of LDL-C transport out of the plasma varies as a function of the plasma LDL-C concentration, while panel B shows the same transport data expressed as either a whole animal LDL-C fractional catabolic rate or clearance rate.

The first observation illustrated by these kinetic curves is that in experimental animals and in humans the rate of receptor-independent transport of LDL-C out of the plasma (and into the individual organs) is a linear function of the plasma LDL-C concentration (A) (36, 45, 47). Because of this linear relationship, the fractional catabolic and clearance rates for this receptor-independent process are constant and independent of the plasma LDL-C concentration (B and Table 1) (38, 45, 52). In contrast, the

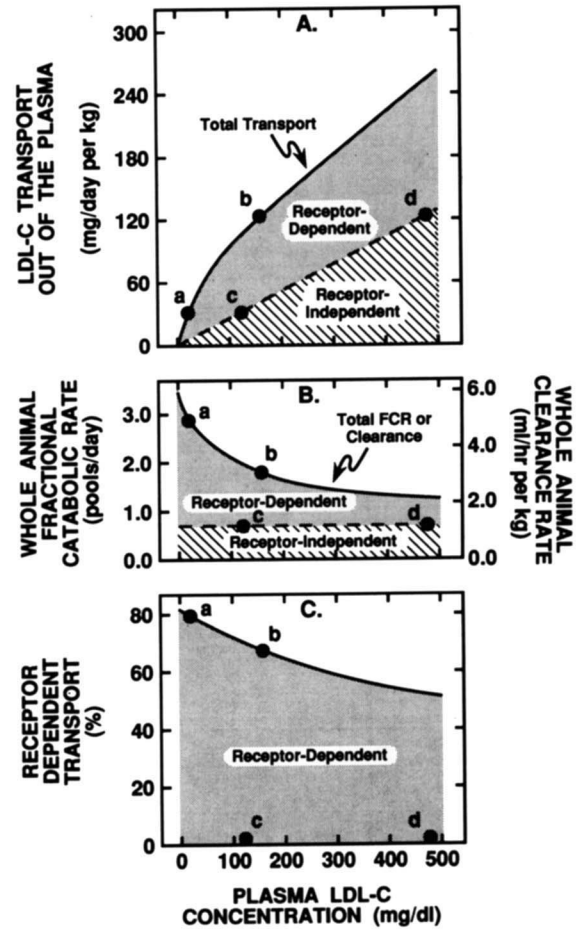

Fig. 8. Representative values for the parameters describing LDL-C turnover as a function of the plasma LDL-C concentration. This figure illustrates the manner in which the absolute and relative magnitudes of receptor-dependent and receptor-independent LDL-C removal from the plasma change at different concentrations of plasma LDL-C. These curves were constructed using rate constants appropriate for a small animal such as the hamster or guinea pig where maximal receptor activity (J^m), receptor affinity (K_m), and the proportionality constant for the receptor-independent process (P) were all kept constant. The formation of LDL-C (J_t) was assumed to progressively increase so as to raise the steady-state plasma LDL-C concentration from 0 to 500 mg/dl. The rates of LDL-C removal from the plasma by the receptor-dependent and receptor-independent processes are presented as either the absolute rates of LDL-C removal (A) or as the fractional catabolic or clearance rates (B). In both of these panels the receptor-dependent component equals the value for total LDL-C removal minus the receptor-independent component (cross-hatched) and is shaded. Panel C shows the percentage of LDL-C removal from the plasma that is receptor-dependent at each plasma LDL-C concentration. The data points labeled a and b illustrate the differences in these parameters at two different concentrations of plasma LDL-C when J^m and P are constant, while the points labeled c and d illustrate the effects of eliminating receptor-dependent LDL-C transport (i.e., J^m becomes zero) in these two instances.

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receptor-dependent transport that is also removing LDL-C from the plasma in the normal animal and human has been shown to be saturable (A) (35, 47). Consequently, the magnitude of this receptor-dependent process, when expressed as a fractional catabolic or clearance rate, decreases as the plasma LDL-C concentration is increased (B). Thus, as illustrated by this set of curves, when the plasma LDL-C level is progressively raised by systematically increasing the LDL-C production rate, the absolute rate of total LDL-C removal from the plasma increases in a curvilinear fashion (A), while the fractional catabolic rate falls progressively (B). Under these circumstances the percentage of LDL-C that is removed from the plasma by the receptor-dependent transport process necessarily also declines (C). These changes occur, it should be emphasized, under circumstances where LDL receptor activity in the whole animal has been kept constant.

The four data points in Fig. 8 also show how these relationships are altered with extreme changes in LDL-C formation or receptor activity. Point a, for example, shows the typical situation in an experimental animal on an essentially lipid-free diet. The rate of LDL-C production, and its rate of transport out of the plasma, are shown equal to about 30 mg/day per kg, and the steady-state concentration of LDL-C in the plasma equals ~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/h 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 now receptor-dependent (C), and the plasma LDL-C concentration would increase 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 was still 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).

Observations such as these on the kinetic characteristics of the receptor-dependent and receptor-independent LDL transport processes in the whole animal and in individual organs provide the basis for describing the regulation of plasma LDL-C concentrations in relatively simple mathematical terms. As the rate of receptor-independent LDL-C transport (J_i) is a linear function of the concentration of LDL-C in the plasma (C_1), the velocity of this transport process at any plasma LDL-C concentration must equal the product PC_1 where P is the proportionality constant that describes the change in the value of J_i for each mg/dl increase in C_1, i.e., the slope of the dashed line in panel A, Fig. 8 (47, 70, 71). Similarly, because the kinetics of the receptor-dependent transport process are saturable, the rate of this process (J_d) can be calculated from the relationship $J^mC_1/K_m + C_1$ where J^m is the maximal rate of receptor-dependent LDL-C transport that can be achieved when all receptors are fully occupied (a function of receptor number) and K_m is the concentration of plasma LDL-C necessary to achieve half of this maximal transport velocity (a function of the affinity of the LDL particle for its receptor under in vivo conditions). In the whole animal the rate of total LDL-C transport out of the plasma (J_t) equals J_i plus J_d. Thus, the rate of total LDL-C transport out of the plasma at any LDL-C concentration can be calculated from the following expression (47, 70, 71):

$$J_t = J_i + J_d = \frac{J^mC_1 + PC_1 K_m + PC_1^2}{K_m + C_i} \qquad \textit{Eq. 1)}$$

Because, in the steady-state, this rate of total LDL-C transport must equal the rate of LDL-C production, this expression takes into account both the rate of LDL-C entry into the plasma and the rate of exit of this particle. Hence, by solving this equation for C_1, the following expression is obtained:

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

This expression allows calculation of the steady-state LDL-C concentration that will be achieved at any value of the LDL-C production rate (J_t, mg/h per kg), maximal receptor-dependent transport activity in the whole animal (J^m, mg/h per kg), LDL-C affinity for its receptor (K_m, mg/dl), and the receptor-independent transport constant (P, mg/h per kg per mg/dl). The values for each of these four transport parameters have now been determined in a number of animals and data from four representative species are shown in **Table 2.**

From equation 2 it is apparent that genetic polymorphisms or environmental factors that raise the plasma LDL-C concentration must do so by decreasing J^m or P, or by elevating J_t or K_m. For example, mutations at many different locations in the LDL receptor have been described that result clinically in FH and are manifest physiologically as decreases in J^m (73). A single amino acid substitution in apoB also has recently been reported to increase K_m and so raise the steady-state concentration of LDL-C in the plasma (74–76). However, the environmental effects on plasma LDL-C levels are mediated

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TABLE 2. Values for the various kinetic parameters of LDL-C metabolism in four species

Species	A. LDL-C Production Rate, J_t	B. Maximal Receptor-Dependent Transport, J^m	C. LDL-C Affinity, K_m	D. Receptor-Independent Transport, P
	mg/h per kg	*mg/h per kg*	*mg/dl*	*mg/h per kg per mg/dl*
SD rat	0.50	4.1	97	0.006
GS hamster	1.17	4.9	91	0.012
NZW rabbit	0.75	2.1	90	0.009
Human	0.54	0.8	90	0.003

This table gives representative values for the four parameters, J_t, J^m, K_m, and P, that dictate plasma LDL-C levels, C_1, in four species (equation 2). These values were determined directly in the SD rat, GS hamster, and NZW rabbit maintained on commercial diets low in cholesterol and triacylglycerol while the values in humans were determined indirectly (72) in subjects on the usual Western diet.

primarily through changes in LDL receptor activity and LDL-C production rates. Thus, while additional investigations need to be undertaken into the subtle alterations in P and K_m that may occur with these dietary challenges, the remainder of this review will deal with the major effects of these environmental factors on J^m and J_t.

B. Regulation of the plasma LDL-C level by changes in LDL receptor activity and production rate

The insert in **Fig. 9** restates diagrammatically these four kinetic parameters that dictate the steady-state value of C_1, and it is intuitively apparent that any event that increases J_t or lowers J^m must necessarily increase C_1 under circumstances where both K_m and P are kept constant. The exact relationship between C_1 and changes in either J^m or J_t can be calculated by entering the appropriate values for each of these variables into equation 2. Such calculations yield the three curves shown in this figure which illustrate how the plasma LDL-C concentration will change if receptor activity is reduced from 100 to 0% under circumstances where LDL-C production is set at a constant value equal to 100, 200, or 300% of the control value.

Point a represents the usual control situation seen in the experimental animal fed a lipid-free diet or the newborn human infant where plasma LDL-C concentrations are about 25 mg/dl. For simplicity the relative values of J^m and J_t have both been set at 100% in this situation. The experimental points labeled b, c, and d illustrate how the plasma LDL-C concentration will increase to ~160 mg/dl if J_t is constant at 100% but receptor activity is reduced to 50, 25, and 0%, respectively, of control. Clearly, C_1 does not increase significantly until more than half of the receptor activity in the animal is lost. Similarly, the points labeled e and f show how the plasma LDL-C concentration will increase when the LDL-C production rate is increased to 200 and 300% of control under circumstances where receptor activity is kept constant at 100%. It is also possible for both J^m and J_t to change

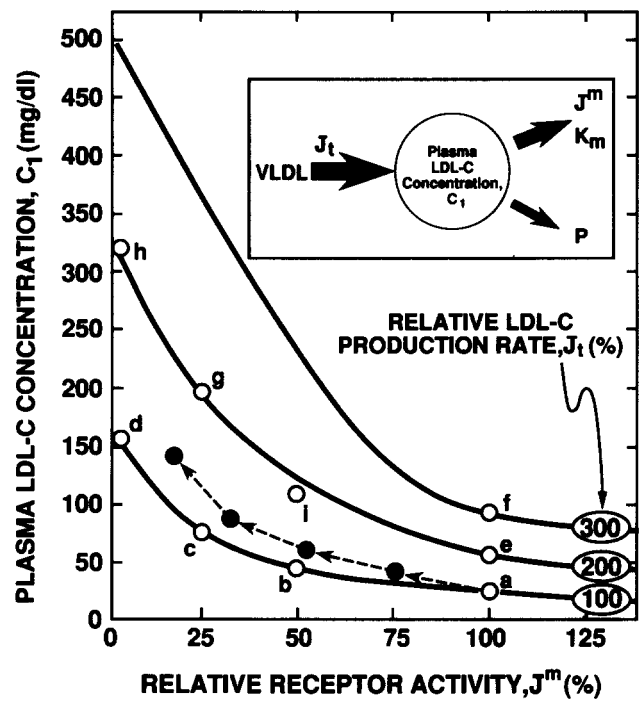

Fig. 9. Theoretical curves showing how the plasma LDL-C concentration would change under circumstances where receptor activity (J^m) in the whole animal is progressively decreased to zero or where the LDL-C production rate (J_t) is set equal to either 200% or 300% of the control value. Point a represents the control animal where the relative values of J^m and J_t are both set equal to 100% and where the steady-state concentration of LDL-C equals ~25 mg/dl. Equation 2 was used to construct these curves assuming that the animal weighed approximately 0.1 kg and using the following kinetic parameters: J_t of 180 μg/h (100%), J^m of 800 μg/h (100%), K_m of 100 mg/dl, and P of 1.08 μg/h per mg/dl. The points labeled a–d show how the plasma LDL-C concentration changes as receptor activity is progressively decreased to zero, while the points labeled a, e, and f illustrate the effect of increasing the LDL-C production rate. The other open circles represent situations that are discussed in the text. Curves similar to these can be constructed for any species, including humans, by using the kinetic parameters in equation 2 that are appropriate for that species. The solid data points show what typically occurs when control animals (a) are fed diets containing progressively greater amounts of cholesterol until new steady-states are reached. The higher the dietary cholesterol content, the more leftward and upward move the data points as J^m is reduced and J_t is increased.

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simultaneously. Point i, for example, represents such a situation where receptor activity is reduced to 50% and the LDL-C production rate is increased to 190% of control.

These sets of theoretical curves, which can be constructed for any species where the four transport parameters are available (Table 2), illustrate four very important principles concerning the regulation of C_1. First, the plasma LDL-C concentration is not related in an inverse, linear fashion to receptor activity. Because of the shape of these curves, the absolute rise in C_1 will always be greater when receptor activity is lost (e.g., point b to d) than will the absolute decrease in the plasma LDL-C concentration when there is a quantitatively similar increase in J^m (e.g., point b to a). One cannot, therefore, make quantitative judgments about whole animal LDL receptor activity based on changes in the plasma LDL-C level. Second, the absolute rise in C_1 produced by a change in the production rate is very dependent upon the amount of receptor activity present in a particular animal. For example, doubling the production rate in the presence of 100% receptor activity increases the absolute concentration of LDL-C by only ~30 mg/dl (points a and e). In contrast, doubling J_t when only 25 or 0% of receptor activity is present in the animal increases C_1 by ~125 mg/dl (points c and g) and ~170 mg/dl (points d and h), respectively. Third, it follows, therefore, that the highest steady-state concentrations of LDL-C will be seen when J^m is reduced simultaneously with an increase in the production rate, and the greatest reduction in this level will follow a dietary or pharmacological manipulation that increases receptor activity while simultaneously decreasing J_t. Fourth, while not shown in Fig. 9, any change in the K_m value that is superimposed on these alterations in J^m and J_t will lead to additional alterations in C_1 that are also predictable from equation 2.

While the curves in Fig. 9 were constructed as if J^m and J_t are independent variables, it is now apparent that this is not the case in either the experimental animal or humans. As illustrated in Fig. 1, the remnants of both VLDL and LDL are thought to be cleared from the plasma largely by the LDL receptor. When receptor activity is suppressed, a greater proportion of the VLDL remnants is converted to LDL, and J_t increases (9, 10, 77, 78). Thus, many examples now exist where changes in LDL-C concentrations brought about by genetic, dietary, or pharmacological means manifest reciprocal changes in receptor activity and production rate. For example, suppression of receptor activity with diets invariably causes a simultaneous increase in J_t (77, 78). In genetic diseases where LDL receptor activity is totally absent, the LDL-C production rate is markedly elevated. In the homozygous FH human and WHHL rabbit this increase equals 3- and 6-fold, respectively (column B, Table 1) (38, 45, 72). On the other hand, the marked reduction in plasma LDL-C concentration seen after administration of HMG-CoA reductase inhibitors or diets containing unsaturated fatty acids is brought about by both an increase in J^m and a reduction in J_t (72, 78).

Thus, these theoretical curves define the relationships that would be anticipated between environmentally induced changes in J^m and J_t and the steady-state LDL-C concentration. Recently, a number of investigations have been reported in which direct measurements of these two transport parameters have been made as varying quantities of the two major dietary lipids, cholesterol and triacylglycerol, have been systematically added to the diet. These studies have provided a rather clear picture of how these lipids alter hepatic metabolism, at least in the hamster, and bring about marked changes in the steady-state levels of cholesterol circulating in the LDL fraction.

C. Effect of changes in net sterol balance across the liver

In virtually every species that has been studied, including humans, the addition of small amounts of cholesterol to an essentially lipid-free diet leads to a series of changes in hepatic sterol metabolism that ultimately results in modest elevations of the plasma LDL-C concentrations (79–81). Cholesterol that is present in the diet is partially absorbed (82, 83) and delivered to the liver in the chylomicron remnant (Fig. 1). The initial metabolic effect of this absorbed sterol is to suppress the rate of cholesterol synthesis to essentially zero. As additional amounts of dietary cholesterol enter the liver they are almost immediately esterified to fatty acids, particularly oleic acid (84, 85), and deposited in the cytosol as biologically inert, cholesteryl esters. The rate of this reaction, which is catalyzed by the enzyme acyl-CoA:cholesterol acyltransferase (ACAT), appears to be driven by the amount of excess cholesterol in the cytosol (85, 86). Thus, the small pool of unesterified sterol that is the putative regulator of hepatic receptor activity appears to vary directly with the steady-state level of cholesteryl esters in the cell. When this steady state is achieved, therefore, the concentration of cholesteryl esters varies directly, and J^m varies inversely, with the amount of cholesterol absorbed from the diet (81, 87, 88). As receptor activity is progressively suppressed, simultaneous increases in LDL-C production are also observed (81).

The net effect of these changes in J^m and J_t on the plasma LDL-C concentration is illustrated by the dashed line in Fig. 9. In most experimental animals, and in the human infant and adult, feeding small amounts of cholesterol in the diet varying from ~20 to 80% of a particular species' daily turnover causes an elevation of the plasma LDL-C concentration in a dose-dependent fashion to levels of ~40 to 150 mg/dl (66, 79, 81, 89). In experimental animals, these elevations result from suppression of J^m (data points move leftward in Fig. 9) and small increases in J_t (data points move upward), and

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the magnitude of these changes is proportional to the amount of cholesterol added to the diet (81). While these changes in the steady-state plasma LDL-C concentration are relatively modest, the partial suppression of J^m brought about by even small amounts of dietary cholesterol makes the animal, or human, vulnerable to further changes in receptor activity and, particularly, LDL-C production that may be induced by other dietary components such as triacylglycerol.

Thus, the effects of cholesterol feeding in the experimental animal or human subject on sterol and LDL-C homeostasis (Fig. 1) can be summarized as follows. First, it is cholesterol itself that very likely is the component of the diet that causes these physiological effects. Second, where this mechanism is operative measurement of net sterol balance in the animal or human, i.e., the total amount of cholesterol synthesized in all of the tissues and absorbed across the small intestine, will always reveal a net increase in sterol delivery to the liver. Third, in response to this net increase in input, both the putative regulatory pool and the esterified pool of cholesterol increase in parallel so that J^m decreases inversely and linearly with respect to the concentration of cholesteryl esters as net delivery of sterol into the liver is progressively increased (81). Fourth, the dose-dependent suppression of J^m, together with small increases in J_t, fully account for the modest increases in the plasma LDL-C concentration observed with cholesterol feeding (Fig. 9). Fifth, because of expansion of this putative regulatory pool, the liver becomes even less important as a site for whole animal sterol synthesis (Fig. 4), and a greater proportion of LDL-C clearance must take place in the extrahepatic organs (Fig. 7). Sixth, these changes, in turn, lead to a decrease in the whole animal LDL-C fractional catabolic and clearance rates, and a decrease in the percentage of LDL-C that is removed from the plasma by receptor-dependent transport (Fig. 8).

All of these changes, it should be noted, come about because of net changes in the delivery of cholesterol carried in the chylomicron to the liver (Fig. 1). A common misconception is that uptake of LDL-C by the liver also regulates these metabolic events. Certainly, when LDL-C is added to a cell preparation in vitro there is a net increase in sterol delivery to the system and down-regulation of J^m. In vivo, however, the cholesterol that is taken up by the liver as LDL-C is largely derived from the hepatocyte itself during VLDL-C secretion. Hence, uptake of LDL-C does not constitute a net contribution of cholesterol to the hepatic sterol pools and so, under these in vivo conditions, does not regulate J^m.

Finally, it should also be noted that each one of these alterations can be reversed if net negative sterol balance is induced across the liver by, for example, increasing the content of soluble fiber in the diet or by feeding agents that specifically block bile acid and/or cholesterol absorption. Such maneuvers are invariably signaled by a decrease in the content of hepatic cholesteryl esters and J_t and by an increase in the rate of hepatic cholesterol synthesis and J^m (31). The liver becomes relatively more important as a site for sterol synthesis, and receptor-dependent LDL-C clearance in the whole animal and the fractional catabolic rate increase.

D. Effect of dietary triacylglycerol on hepatic sterol homeostasis

Triacylglycerol is the second major lipid component of human diets and may account for 30–50% of total caloric intake in many individuals. When added to a diet essentially free of cholesterol, triacylglycerols generally have less effect on the plasma LDL-C level than when dietary sterol is present in significant amounts (80, 81). In the presence of dietary cholesterol, however, these lipids profoundly affect hepatic sterol metabolism and the parameters of LDL-C transport. Once absorbed, triacylglycerols are also carried in the chylomicron particle (Fig. 1) (90). The majority of the fatty acids in this lipoprotein are taken up by muscle and adipose tissue as the chylomicron is metabolized in the extrahepatic organs by lipoprotein lipase (8). However, a portion of the triacylglycerol is retained and delivered directly to the liver as the chylomicron remnant is cleared, and, in addition, fatty acids bound to albumin are constantly being circulated from the extrahepatic stores to the liver. Thus, the hepatocyte becomes enriched with the fatty acids present in the triacylglycerol of the diet (87, 91) and this, in turn, may markedly alter the relative activities of the various hepatic metabolic pathways in which the fatty acyl-CoA derivatives are utilized (92–94). In general, the metabolic effects of these lipids depend very much on whether they are saturated fatty acids of medium or long chain length or are unsaturated.

When triacylglycerol made up predominantly of medium chain fatty acids is added to a diet containing cholesterol, there is virtually no additional effect of these lipids on hepatic cholesteryl ester formation or on J^m, J_t, or the plasma LDL-C concentration (77). In contrast, if this triacylglycerol contains predominantly long chain saturated fatty acids, then the elevated level of cholesteryl esters in the liver cell is reduced in proportion to the amount of such triacylglycerol added to the diet (77, 78, 95). Furthermore, as shown in **Fig. 10,** these lipids also markedly alter the major parameters of LDL transport in the liver. Point b, for example, represents an animal fed cholesterol alone in sufficient amounts to raise the hepatic cholesteryl ester content and to suppress J^m to about 50% of the control value. The data points between b and c show the effects of feeding increasing amounts of saturated fatty acids until, at c, the triacylglycerol accounts for about 40% of the dietary caloric intake. It is clear that feeding this lipid, along with cholesterol, leads to further

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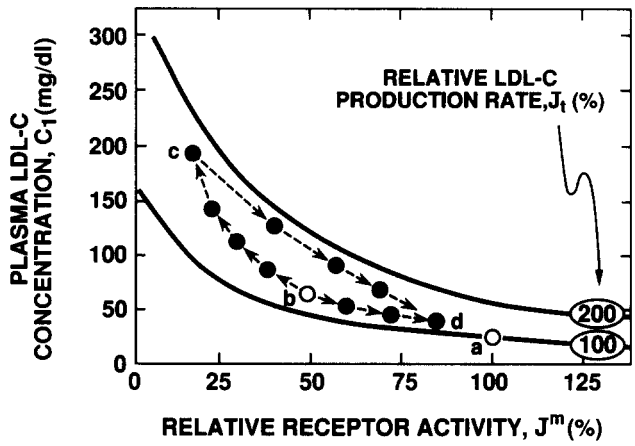

Fig. 10. Effect of naturally occurring triacylglycerols on J^m, J_t, and the plasma LDL-C concentration. These curves are identical to the theoretical curves shown in Fig. 9. The open circle labeled a represents the usual situation in an experimental animal on a lipid-poor diet while the one labeled b shows the effects of adding cholesterol alone to this diet in amounts sufficient to suppress J^m to about 50% of the control value. The data points between b and c illustrate the effects of adding increasing amounts of a triacylglycerol containing predominantly saturated fatty acids to the cholesterol-containing diet until at c about 40% of the calories come from the added lipid. Similarly, the data points between b and d show the effect of adding increasing amounts of a triacylglycerol containing predominantly unsaturated fatty acids to the diet until at d about 40% of the calories come from the added oil. The data points between c and d illustrate what happens when the contribution of triacylglycerol to the diet is kept constant at about 40% of available calories, but unsaturated triacylglycerol is systematically substituted for the saturated oil (78).

suppression of J^m and to marked increases in LDL-C production so that, at the highest level, the plasma LDL-C concentration reaches nearly 200 mg/dl (77, 78, 81, 95). Similar increases in the circulating cholesterol concentration have been reported in a variety of species, including humans, when triacylglycerol containing predominantly saturated fatty acids is added to the diet (96–102).

Feeding cholesterol and triacylglycerol rich in unsaturated fatty acids, on the other hand, increases J^m and modestly reduces C_1 (points between b and d) (78, 87). Because of the shape of the curves in Fig. 10, it is apparent that increasing J^m by, for example, 25% from the cholesterol-fed control value at b produces an absolute decrease in C_1 that is much smaller than the absolute increase in the plasma LDL-C level that follows a similar 25% reduction in J^m. These relationships readily explain the commonly observed finding in human studies that saturated fatty acids are more active in increasing the plasma cholesterol concentration than are unsaturated lipids in reducing this level (96, 97, 102).

Finally, the data points between c and d illustrate the effects on these transport parameters when the level of dietary triacylglycerol is kept constant and unsaturated fatty acids are systematically substituted for saturated ones. There is progressive restoration of J^m, a reduction in J_t, and a decrease in the plasma LDL-C concentration from approximately 200 to 50 mg/dl. This type of study, however, obscures the fact that the saturated and unsaturated lipids have independent effects in regulating J^m (78). In this case, the reduction in the plasma cholesterol level comes about because both the amount of active saturated fatty acid in the diet is being reduced and the level of active unsaturated fatty acid is being increased. It should be also emphasized that these regulatory effects of triacylglycerol are being exerted without any change in the rate of cholesterol absorption from the diet or any change in net sterol delivery to the liver (87, 91).

More recent data have identified which of the specific fatty acids present in mixed triacylglycerols are capable of exerting these regulatory effects in the liver. As summarized in **Fig. 11,** when animals are fed diets containing a fixed amount of cholesterol and triacylglycerol, the changes observed in the parameters of LDL transport in the liver vary markedly with the type of fatty acid making up the triacylglycerol. The saturated fatty acids 6:0, 8:0, and 10:0 are rapidly metabolized to acetyl-CoA by the liver cell and so do not alter the pattern of fatty acids in the various hepatic lipid pools (91). They do not change hepatic cholesteryl ester levels, nor do they alter J^m, J_t, or C_1. Thus, as shown in Fig. 11, these particular lipids are biologically neutral with respect to LDL metabolism. In contrast, when the saturated fatty acids 12:0, 14:0, and 16:0 are fed, they enrich the hepatic lipid pools, suppress cholesteryl ester formation, further reduce J^m, and markedly increase J_t (87, 91). As a result of these changes the plasma LDL-C increases from the level of ~65 mg/dl

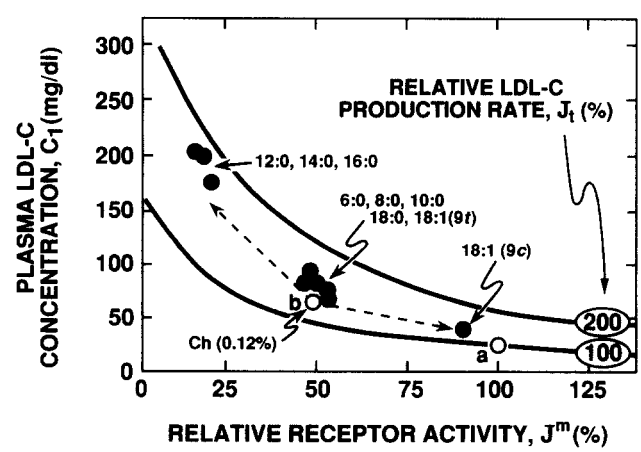

Fig. 11. The effect of specific dietary fatty acids on C_1, J^m, and J_t. Point a illustrates the experimental results obtained in animals receiving neither cholesterol nor triacylglycerol while point b represents the animals receiving 0.12% cholesterol by weight in the diet. The various other data points show the experimental results when a constant 20% of triacylglycerol is added to the cholesterol-containing diets under circumstances where the different triacylglycerols contain a single species of a saturated or unsaturated fatty acid.

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seen with cholesterol feeding alone (b) to nearly 200 mg/dl. The monounsaturated fatty acid 18:1(9*c*), on the other hand, is active in restoring J^m and reducing J_t so that C_1 drops to only about 50 mg/dl. These changes take place under circumstances where the content of cholesteryl esters in the liver cell markedly increases (87). This ability to up-regulate hepatic receptor activity is lost, however, if the double bond in this monounsaturated fatty acid is either converted to the *trans* configuration or is fully saturated (91). Thus, the 18:0 and 18:1(9*t*) fatty acids are biologically neutral and do not alter any parameter of LDL metabolism beyond that attributable to the cholesterol also present in the diet (Fig. 11). Similar results have been reported in human studies where the ability of the 18:1(9*c*) fatty acid to lower the plasma LDL-C level is lost when the lipid is fully saturated or converted to the *trans* configuration (97, 103, 104). It should again be emphasized that all of these regulatory events occur under circumstances where there is no detectable change in the percentage of dietary cholesterol that is absorbed or in net cholesterol delivery to the liver (87).

Thus, fatty acids reaching the liver and enriching the various substrate pools also exert major regulatory effects on hepatic LDL metabolism that can be summarized as follows. First, because the magnitude of the effects of fatty acids is proportional to the amount of cholesterol also present in the diet, regulation of LDL receptor gene transcription by these lipids must be exerted indirectly, probably through an alteration in the size of the putative regulatory pool of cholesterol in the liver (87). Second, in contrast to regulation brought about by cholesterol feeding, these marked changes in J^m caused by fatty acid feeding occur under circumstances where there is no observable change in either cholesterol absorption or synthesis and, hence, no demonstrable change in net sterol balance across the liver or whole animal (87, 91). Third, the active saturated fatty acids that suppress J^m also suppress steady-state cholesteryl ester levels in the liver cell while those that actively restore J^m increase the size of this ester pool. Thus, in contrast to the regulation exerted by dietary cholesterol, with fatty acid feeding J^m varies directly with the steady-state size of the cholesteryl ester pool (3, 87). Fourth, the 12:0, 14:0, and 16:0 fatty acids suppress J^m and increase J_t while the 18:1(9*c*) compound has the opposite effect (Fig. 11). The changes in these two parameters are sufficient to explain the large alterations in C_1 that are induced by these lipids. Many other fatty acids are biologically neutral and exert no regulation on LDL metabolism. Fifth, feeding these fatty acids has no effect on cholesterol synthesis (87, 91) and so does not change the relative importance of the liver for whole animal synthesis (Fig. 4). However, the relative importance of the liver for whole animal LDL clearance (Fig. 7) and the percentage of LDL that is cleared by receptor-dependent transport (Fig. 8) are altered by particular fatty acids, depending upon whether that fatty acid increases or decreases J^m.

Recently a model has been proposed to explain these interactions of dietary cholesterol and fatty acid in regulating hepatic receptor-dependent LDL-C transport (87). The essential features of this model are that *1)* in the steady state, equilibrium between the small pool of sterol that regulates LDL receptor transcription and the biologically inert pool of cholesteryl esters is dictated by ACAT; and *2)* the relative activity of this enzyme is determined by the concentrations of both of the reactants, i.e., the pools of excess cellular cholesterol and fatty acids. When net sterol delivery to the liver is progressively increased, both the putative regulatory and ester pools of sterol increase in parallel so that J^m varies inversely with the level of cholesteryl esters in the liver cell. On the other hand, when net sterol delivery to the liver is kept constant but the cell is enriched with fatty acids that are poor substrates for ACAT, e.g., the 14:0 and 16:0 compounds, cholesteryl ester formation is reduced, more sterol is forced into the putative regulatory pool, and J^m is further suppressed. This same increased sensitivity to the cellular uptake of cholesterol has been reported in isolated cells where ACAT activity was inhibited pharmacologically (105). Contrariwise, if the liver is enriched with fatty acids such as 18:1(9*c*), the preferred substrate for ACAT (84, 85), cellular sterol is shifted out of the regulatory compartment and into the ester pool, and J^m is increased. Hence, with fatty acid feeding J^m varies directly with the steady-state concentration of cholesteryl esters in the cell (87). Whether this model is correct must await new methodology for actually identifying and quantitating the size of the putative regulatory pool of sterol in the cell.

E. Effect of dietary cholesterol and triacylglycerol on absolute cholesterol flux across the liver and extrahepatic tissues

Finally, this regulation of J^m and J_t by dietary cholesterol and triacylglycerol has important implications with respect to absolute rates of cholesterol flux across the liver and extrahepatic organs. These implications are very different under the conditions that exist in the live animal compared to in vitro studies performed in isolated cell systems. **Fig. 12,** for example, illustrates how the absolute rate of LDL-C uptake varies as receptor activity in the liver is progressively suppressed. The four theoretical curves show how the rates of LDL-C transport would vary with changes in the plasma LDL-C concentration under circumstances where J^m in the liver is reduced from 100% to 75, 50, and 25% of the control value. The data points labeled a–f represent actual experimental measurements of LDL-C uptake where J^m was suppressed by cholesterol and saturated fatty acid feeding (77, 78, 81). In the control situation (a) J^m equals 100%, about 130 μg of LDL-C is transported into the liver each hour, and this

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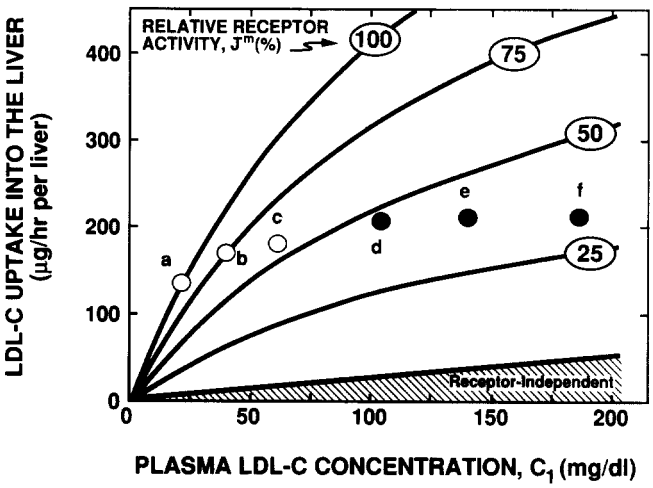

Fig. 12. The rate of LDL-C uptake into the liver plotted as a function of the plasma LDL-C concentration. This graph illustrates the effect of feeding cholesterol alone or cholesterol with triacylglycerols containing saturated fatty acids on the hepatic uptake of LDL-C by both the receptor-dependent and receptor-independent transport processes in the liver. The receptor-independent component of this uptake process is shown by the cross-hatched area and is based on a value of P in the liver of 0.30 µg/h per mg/dl. The four other saturable transport curves represent the kinetic relationships where receptor-dependent transport in the liver is 100, 75, 50, and 25% of the value found in the control animals fed neither cholesterol nor triacylglycerol (point a). The data points b and c come from animals fed 0.06 or 0.12% cholesterol by weight, respectively, in the diet until a new steady-state is achieved. Points d, e, and f represent the results obtained after feeding diets containing, respectively, 5, 10, and 20% saturated triacylglycerol in addition to a constant level of 0.12% cholesterol. These results are derived from experimental data presented in references 77, 78, and 81.

velocity is achieved at a plasma LDL-C concentration of ~20 mg/dl. If these hepatocytes were studied under in vitro conditions where C_1 was kept constant at 20 mg/dl and J^m was progressively suppressed, then the uptake of LDL-C would progressively decrease in direct proportion to the reduction in receptor activity, i.e., under these in vitro conditions where C_1 is constant the rate of LDL-C transport across the liver would be determined directly by J^m.

This is never the case, however, in the live animal or humans. Under such in vivo conditions the rate at which LDL-C is being formed remains constant, or actually increases, as receptor activity in the liver is suppressed by dietary lipids (Figs. 10, 11). Thus, as hepatic receptor activity is suppressed, the plasma LDL-C concentration necessarily increases until a velocity of transport equal to or greater than that seen in the control animals is achieved. Point d (Fig. 12), for example, illustrates the experimental result when cholesterol and a small amount of saturated lipid is fed until a new steady state is achieved. As is evident, even though J^m is decreased to ~45% of control, the velocity of LDL-C uptake actually increased slightly to ~200 µg/h. This occurred because C_1 progressively increased until a velocity of LDL-C transport equal to the rate of LDL-C production was achieved in the new steady-state, even though only ~45% of the LDL receptors were present in the liver. In the control situation (a) this velocity was achieved at a value of C_1 equal to only about 20% of K_m whereas it was necessary for C_1 to increase to a value essentially equaling the K_m (Table 2) of the receptor-dependent transport process when J^m was partially suppressed. As is apparent from the other data points in Fig. 12, these same adaptations take place at all other levels of lipid feeding and suppression of J^m: in all cases C_1 will increase to maintain the flux of LDL-C across the liver at a nearly constant value. Thus, in contrast to the in vitro situation where C_1 is usually kept constant, under in vivo conditions the rate of LDL-C transport across the liver is determined directly by the LDL-C production rate, J_t, and not by hepatic receptor activity, J^m.

These observations have very important implications with respect to the relationship between the plasma cholesterol level and sterol balance across various tissues. As J^m in the liver is progressively suppressed by feeding greater and greater amounts of cholesterol and saturated lipids, the plasma LDL-C concentration increases dramatically while LDL-C flux across the liver (Fig. 12) and the extrahepatic tissues that also possess receptor-dependent transport (Fig. 6) remains nearly constant (77). Viewed differently, if such an animal or human is treated with a pharmaceutical or dietary manipulation that lowers the plasma LDL-C level, for example, from 200 to 50 mg/dl, there is essentially no change in net LDL-C transport across the various organs of the body (Fig. 12). Thus, changing the dietary intake of cholesterol, fatty acid, or soluble fiber markedly alters the plasma LDL-C concentration but, for practical purposes, does not change cholesterol homeostasis across any tissue. Only the endothelial lining of the vascular system, therefore, is subject to the detrimental effects of high plasma cholesterol levels and only the physiology of these cells is influenced when the plasma LDL-C level is dramatically lowered (106, 107).

V. SUMMARY OF THE NORMAL MECHANISMS OF CHOLESTEROL AND LDL-C HOMEOSTASIS IN THE PRESENCE OF DIETARY LIPIDS

Based on the various studies carried out in a number of different species and reviewed here, the role of the liver in the maintenance of cholesterol and LDL-C homeostasis is now fairly well understood. In the absence of dietary lipids the extrahepatic organs synthesize most of the cholesterol that they require for daily membrane turnover (Fig. 4), and the liver synthesizes only enough sterol to balance the amount being synthesized in the extrahepatic organs with the amount being secreted into the gastrointestinal tract as biliary cholesterol and bile acids (Fig. 1).

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Under these conditions the size of the regulatory and ester pools of sterol in the hepatocyte is small, and LDL receptor activity in the liver is high. Thus, the liver rapidly clears both the remnants of VLDL-C, so that the production of LDL-C is low, and LDL-C itself. As J^m is high and J_t is low in this situation, the plasma LDL-C concentration in the steady state is very low and, in most species, is well below 50 mg/dl (Table 1).

When cholesterol is added to the diet the regulatory and ester pools of sterol in the liver are expanded in parallel and receptor activity is suppressed. If, in addition, triacylglycerol containing fatty acids such as myristate and palmitate are fed, cholesterol in the liver is shifted from the ester pool into the putative regulatory pool, and receptor activity is further suppressed (Fig. 10). While such dietary additions apparently do not affect the rate of apoB formation and secretion as VLDL (108–110), less of the metabolized VLDL-C is removed by the liver through receptor-dependent transport and, therefore, the LDL-C production rate increases (Fig. 10). As a result of these changes in J^m and J_t dictated by the diet-induced metabolic alterations in the liver, the plasma LDL-C concentration must increase in order to maintain LDL-C clearance from the plasma at the normal, or even an elevated, level (Fig. 12). This exposes the endothelial lining of the arterial system to the potential detrimental effects of a high LDL-C level but has little effect on cholesterol balance across the extrahepatic tissues or liver (Fig. 12). If triacylglycerol containing fatty acids such as oleic acid are substituted for the saturated lipids, cellular cholesterol in the liver is shifted from the putative regulatory to the ester pool, J^m is partially restored, and the plasma LDL-C level is reduced (Fig. 11). Thus, in all of these situations the plasma LDL-C concentration achieved in the steady state is the passive consequence of changes in J^m and J_t induced as the liver becomes relatively enriched with cholesterol and the various fatty acids.

Although not discussed in this review, these changes in LDL metabolism also produce quantitative, and predictable, alterations in the absolute amount of cholesterol that must be returned to the liver from the extrahepatic organs through intervention of HDL (Fig. 1). In the absence of dietary lipids, this mass must equal the large amount of cholesterol synthesized in the extrahepatic tissues (Fig. 4) plus that derived from the ~30% of the LDL-C pool that is normally taken up and degraded by these organs each day (Fig. 7). When cholesterol and triacylglycerol are added to the diet, there is little change in de novo synthesis in these organs but a greater percentage of the LDL-C pool is degraded in the periphery. As a consequence, the total amount of cholesterol that must be returned to the liver is increased. It is noteworthy, therefore, that feeding lipid-rich diets increases the rate of production of both apoA-I (111) and cholesteryl ester transfer protein (112) and the plasma concentration of HDL-C increases (113). Presumably, these physiological responses meet this need for greater cholesterol transport from the periphery to the liver and, at the same time, the increase in the concentration of HDL may partially militate the potential toxicity of the elevated LDL-C levels for the vascular endothelium.

VI. GENETIC VARIABILITY IN THESE NORMAL MECHANISMS OF STEROL HOMEOSTASIS

This review has dealt primarily with the various physiological processes that normally maintain cholesterol balance and regulate LDL-C metabolism in virtually all species, insofar as data are available. Clearly, however, genetic variation in the dozens of proteins that catalyze these various reactions can alter the magnitude of each of these physiological responses. A number of examples exist, for example, where specific mutations markedly alter J^m (73), J_t (114), and K_m (75). However, in the majority of individuals a far more subtle polymorphism exists that is manifest as a variable response of the LDL-C concentration to a uniform challenge by dietary lipid administration. Thus, in virtually every species there are always individuals that respond to dietary cholesterol challenge with an exaggerated elevation of the plasma cholesterol level while others have little response (115–118). Furthermore, in humans, those individuals who are hyperresponsive to cholesterol are also probably hyperresponsive to saturated fatty acids (119). The experimental means are now available for isolating and identifying which specific transport protein or enzyme is responsible for these polymorphic responses so that considerable progress in this area is to be anticipated in the next few years.

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