Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver

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Abstract The relationship between rates of hepatic sterol synthesis and rates of hepatic low density lipoprotein (LDL) uptake (clearance) was studied in animals with high (rats), low (female hamsters), and very low (male hamsters) basal rates of hepatic sterol synthesis. In rats and female hamsters, rates of hepatic sterol synthesis were varied over a 110-fold range by feeding cholesterol or cholestyramine; nevertheless, rates of hepatic LDL clearance remained essentially unchanged as did plasma LDL-cholesterol concentrations. In contrast, in male hamsters, which have a very limited capacity to synthesize cholesterol in the liver, cholestyramine feeding increased rates of hepatic LDL uptake by 2.5-fold and this was associated with a 50% reduction in plasma LDL-cholesterol concentrations. The observed increase in LDL uptake was due to an increase in receptor-dependent LDL transport while receptor-independent lipoprotein uptake remained constant. These studies suggest that rates of hepatic cholesterol synthesis and receptor-dependent LDL uptake are regulated independently. Furthermore, the primary response of the liver to changes in cholesterol availability is regulation of sterol synthesis and only when the capacity of this compensatory mechanism is exceeded is the rate of LDL transport altered. — **Spady, D. K., S. D. Turley, and J. M. Dietschy.** Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* 1985. **26:** 465–472.

Supplementary key words receptor-dependent LDL transport • [^{3}H]water • plasma cholesterol concentration • lipoprotein transport

Circulating low density lipoproteins (LDL) are derived largely from the metabolism of very low density lipoproteins and are removed from the plasma by a combination of receptor-dependent and -independent uptake in many organs of the body. Recent data indicate that the receptor-dependent mechanism is quantitatively the most important of these and accounts for at least 60–75% of LDL turnover in the rat (1, 2), 67% in the rabbit (3), and 56–80% in man (4, 5). While early work established that the liver contained binding sites that resembled the LDL receptor originally described in the fibroblasts (6–8), the contribution of this organ to total and to receptor-dependent LDL degradation was clearly underestimated, largely because most of these studies were carried out using heterologous LDL preparations (9–11). Such LDL prepared from the plasma of one species does not bind well to the LDL receptors of another species and is degraded in the whole animal at a much lower rate than is homologous LDL (1, 2, 12, 13). With the recognition that reliable quantitative data on LDL turnover can only be obtained using homologous preparations, more recent studies have shown that the liver is the single most important site in the body for the uptake of LDL and that the majority of this uptake is receptor-mediated (2, 14–16).

Not only is the liver the principle site for the clearance of LDL from plasma, but it is also one of the major organs in the body for cholesterol synthesis, the only site for the conversion of cholesterol to bile acids, and essentially the only organ for the excretion of cholesterol (and bile acids) from the body (17, 18). Obviously, each of the pathways must be appropriately regulated in order to meet the complex and changing needs of the different tissues if sterol homeostasis is to be maintained in the face of marked fluctuations in rates of sterol acquisition or loss from the body. From the standpoint of the circulating levels of LDL-cholesterol, regulation of receptor-dependent hepatic LDL uptake is particularly important. In studies in which isolated fibroblasts or hepatocytes were exposed to high concentrations of cholesterol or lipoprotein-cholesterol, there was apparently simultaneous suppression of both the rate of cholesterol synthesis and the rate of LDL uptake (19, 20). Whether regulation of these two processes is similarly linked in the liver and other differentiated tissues under in vivo conditions is currently unknown, although there are a variety of observations

Abbreviations: LDL, low density lipoproteins; methyl-hLDL, human LDL reductively methylated; DPS, digitonin-precipitable sterols.

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that now suggest that each of these processes is independently regulated in the intact animal and man.

In order to assess the regulation of these two important processes, the current studies were undertaken to measure directly the rate of hepatic LDL uptake under circumstances where the rate of hepatic cholesterol synthesis was varied over a very large range. Furthermore, these studies were carried out in three different types of experimental animals that are known to vary markedly in their inherent capacities to synthesize sterol in the liver (17, 21). The results indicate that in the liver, as in the intestine (22), LDL uptake and cholesterol synthesis are regulated independently.

METHODS

Animals and diets.

Male and female Golden Syrian hamsters were obtained from Charles River Lakeview, Newfield, NJ. Female Sprague-Dawley rats (CD(R)(SD)BR) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. The hamsters and rats were maintained in separate rooms, each with alternating 12-hr periods of light (3:00 PM–3:00 AM) and darkness (3:00 AM–3:00 PM). All animals were maintained in colony cages with free access to water and a control, low cholesterol ground diet (Wayne Laboratory Animal Diet, Allied Mills, Inc., Chicago, IL). After at least 1 week, groups of animals were either continued on the control diet or were switched to a diet containing cholestyramine (2%, wt/wt) or cholesterol (0.5%, wt/wt). The cholestyramine diet was fed for 2 weeks while the cholesterol diet was fed to rats for 2 weeks and to hamsters for 3 days prior to use in specific experiments. All experiments were carried out during the mid-dark phase of the light cycle.

Lipoprotein preparations

The LDL was isolated from human, rat, and hamster plasma by preparative ultracentrifugation in the density range of 1.020–1.055 g/ml as previously described (16). Hamster and human LDL in this density range contained almost exclusively apoprotein B_{100} as judged by polyacrylamide gel electrophoresis and was used without further purification. Rat LDL, however, contained significant amounts of HDL_1 and was further purified by Geon-Pevikon electrophoresis (1). The various LDL preparations were labeled with [^{14}C]sucrose (Amersham, Arlington Heights, IL) as previously described (14, 16). The appropriate homologous LDL preparation was used for studies in the rat and hamster. Since previous experiments have shown that both heterologous LDL and methylated homologous LDL interact poorly with the LDL receptor of a particular species (1, 2, 13, 23), we have used methylated LDL of human origin (methyl-hLDL) to measure rates of receptor-independent LDL metabolism in the two animal species.

Determination of rates of whole-animal LDL turnover

Rates of whole-animal turnover (clearance) of the [^{14}C]sucrose-labeled LDL and methyl-hLDL were measured using a constant infusion technique as previously described (1, 16). Briefly, trace amounts of each labeled lipoprotein were infused at a constant rate until the plasma radioactivity reached steady-state levels. In these experiments, this steady-state concentration was rapidly achieved by administering an appropriate priming dose; nevertheless, the continuous infusions were continued for 14 hr to insure steady-state conditions. Furthermore, the fact that these animals were in a steady state was confirmed by obtaining blood samples 10 and 12 hr into the infusions. The whole-animal clearance rate was then calculated by dividing the hourly infusion rate (dpm/hr) by the steady-state plasma concentration (dpm/μl) and normalizing to 100 g of animal weight. This value expresses the whole-animal turnover in terms of the μl of plasma cleared of a particular lipoprotein molecule per hr per 100 g body wt (μl/hr per 100 g).

Determination of the rate of hepatic [^{14}C]sucrose-LDL uptake

As previously described, animals were administered a priming dose of [^{14}C]sucrose-labeled lipoprotein followed immediately by a constant infusion of the same labeled molecule (16). The radioactivity present in the priming dose relative to the radioactivity subsequently delivered each hour was adjusted so as to maintain a constant specific activity of the lipoprotein in the plasma throughout the experimental period (16). Under such conditions the rates of hepatic LDL uptake are essentially linear between 2 hr and 6 hr in both hamsters and rats (ref. 16 and unpublished observations). Accordingly, groups of five or six animals were killed at 2 hr and 6 hr, and aliquots of plasma and liver were assayed for ^{14}C content. A portion of liver from each of the animals killed at 6 hr was also immediately taken for determination of cholesterol synthesis rates in vitro as described below. The content of radioactivity in the liver at each time point was expressed in terms of the μl of plasma that contained an equivalent amount of radioactivity in that animal. This value, which has the units μl/g, represents the tissue space of the [^{14}C]sucrose-LDL and was calculated by dividing the dpm of ^{14}C per g of liver by the dpm of [^{14}C]sucrose-LDL per μl of plasma (16). Rates of hepatic LDL uptake were determined for each of the animals killed after the 6 hr infusion by calculating the increase in the tissue space (with respect to the mean tissue space found in the animals killed at 2 hr) as a function of the time of infusion. This increase in tissue space represents the μl of

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plasma cleared of its LDL content per hr per g of liver (μl/hr per g). This uptake rate was also multiplied by the plasma LDL cholesterol concentration to give the absolute mass of LDL-cholesterol taken up per hr by each g of liver (μg/hr per g).

Determination of sterol synthesis rates in vitro

As each of the animals from the 6 hr infusion group was killed, a portion of liver was immediately chilled and sliced. As previously described, aliquots of these slices weighing 300 mg were incubated in Krebs' bicarbonate buffer containing 25 mCi of [^{3}H]water following which the digitonin-precipitable sterols (DPS) were isolated (24, 25). The data are expressed as the nmol of [^{3}H]water incorporated into DPS per hr per g of liver (nmol/hr per g).

Determination of sterol synthesis rates in vivo

Rates of hepatic sterol synthesis also were measured under in vivo conditions in separate groups of animals that had been fed the control and cholestyramine-containing diets described above. As previously described in detail, animals were administered [^{3}H]water as a bolus (~50 mCi) intravenously and thereafter were kept under fume hoods until they were killed 1 hr later (17, 24). Aliquots of plasma were taken for the determination of plasma water specific activity and aliquots of liver were taken for isolation of the DPS. Rates of sterol synthesis (newly synthesized sterol content) were expressed as the nmol of [^{3}H]water incorporated into DPS per hr per g of liver (nmol/hr per g).

Analytic procedures

Pooled plasma was anticoagulated with dry EDTA and equal volumes were adjusted to densities of 1.006, 1.020, 1.055, 1.095, and 1.21 g/ml and centrifuged simultaneously at 165,000 *g* for 36 hr. The cholesterol content of each fraction, as well as the total plasma cholesterol concentration of the individual animals, was measured colorimetrically (17).

The hepatic content of free and esterified cholesterol was measured using previously described methods (26). Sterols were extracted from samples of liver with chloroform–methanol 2:1 (v/v) and the cholesteryl ester fraction was separated on columns (1 × 30 cm) containing silicic acid and celite (1:1, wt/wt), using benzene as the eluting solvent. The cholesteryl esters were then hydrolyzed and the cholesterol was isolated as the digitonide and quantitated using the ferric chloride–sulfuric acid method (27). Radiochemically pure [4-^{14}C]cholesteryl oleate (New England Nuclear, Boston, MA) was added as an internal standard to all tissue samples to correct for procedural losses.

Statistical methods

Where necessary, the best-fit linear regression lines were fitted by the method of least squares. Significance of differences between mean values was tested at the $P < 0.05$ level using the two-tailed Student's *t*-test.

RESULTS

In order to examine the putative relationship between the rates of cholesterol synthesis and LDL uptake in the liver in vivo, these studies took advantage of the fact that the capacity of the liver to synthesize sterols varies markedly among different species (17). Thus, in animals fed a low dietary cholesterol intake, 1 g of liver from the female rat synthesizes about 70 μg of sterol per hr, while this rate equals only 8 μg/hr per g and 1.5 μg/hr per g in the female and male hamster, respectively (21). Furthermore, in these three experimental animals the basal rates of hepatic cholesterol synthesis can be varied over a wide range by feeding either cholesterol or cholestyramine.

The first experiment was undertaken to measure the quantitative importance of receptor-dependent LDL degradation, both in the whole-animal and in the liver, in these two species. As shown in **Table 1**, the turnover (clearance) of homologous LDL in control rats and hamsters maintained on a low cholesterol diet equaled 711 μl/hr per 100 g and 547 μl/hr per 100 g, respectively. In

TABLE 1. Rates of whole-animal LDL turnover and hepatic LDL uptake in vivo

	Whole-Animal LDL Turnover			Hepatic LDL Clearance		
	A. Homologous LDL	B. Methyl-hLDL	C. Receptor-Dependent LDL Turnover	D. Homologous LDL	E. Methyl-hLDL	F. Receptor-Dependent LDL Clearance
	μl/hr per 100 g		%	*μl/hr per g*		%
Rat (female)	711 ± 25	180 ± 3	75	92 ± 4	8 ± 1	91
Hamster (female)	547 ± 19	157 ± 2	71	114 ± 8	9 ± 1	92

Rates of whole-animal LDL turnover (clearance) and hepatic LDL uptake (clearance) were determined using either homologous LDL or methylated human LDL (methyl-hLDL) in rats and hamsters previously maintained on control diet for 2 weeks. The rate of clearance of the methyl-hLDL was taken as a measure of receptor-independent LDL uptake and degradation. The values shown for the hamster were previously reported (16). Mean values ± 1 SEM are shown for six animals in each group.

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contrast, methyl-hLDL, which does not interact with the LDL receptor in either species, was cleared from the plasma at the much lower rates of 180 μl/hr per 100 g and 157 μl/hr per 100 g, respectively. Hence, in the rat and hamster, approximately three-fourths of homologous LDL turnover in the whole animal is receptor-dependent. This uptake process is quantitatively even more important in the liver, as also shown in Table 1. Methyl-hLDL is taken up by the liver at rates of only 8-9% of the rates observed with the appropriate homologous LDL preparation. Thus, even though rates of hepatic sterol synthesis vary significantly between the rat and hamster, in both species whole-animal LDL turnover is predominantly receptor-dependent; the liver is the primary site for the clearance of LDL from the plasma (55% in the rat (2) and 73% in the hamster(16)); and hepatic LDL uptake is >90% mediated by the LDL receptor.

In the next series of experiments the availability of cholesterol in the liver was either reduced by feeding cholestyramine or increased by feeding cholesterol, and the effect of these manipulations on rates of hepatic sterol synthesis and hepatic and whole-animal LDL clearance was determined in these different groups of experimental animals. As is evident in **Table 2** (column A), cholestyramine feeding enhanced the rate of hepatic cholesterol synthesis, as measured in vivo, by only 2.2-fold in the female rat but by 8.9-fold in the female hamster and 17.1-fold in the male hamster. However, because of the much lower rates of sterol synthesis in the control hamsters, the absolute values achieved in this species after cholestyramine feeding just equaled (female) or were much lower (males) than the absolute rates of sterol synthesis found in the livers of the control rats. Under these conditions cholestyramine feeding did not significantly change the rate of hepatic LDL uptake in the female rats or female hamsters (column B). Furthermore, there were no changes in the rates of whole-animal LDL turnover in these same two groups of animals, and no decline in the plasma LDL-cholesterol (column C) concentration was observed. Thus, in the female animals, hepatic cholesterol synthesis apparently increased enough to fully compensate for the accelerated loss of sterols induced by cholestyramine feeding and, hence, there was no change in either the hepatic or whole-animal metabolism of LDL.

In contrast, in the male hamsters, which were able to increase hepatic cholesterol synthesis to only 855 nmol/hr per g (column A), cholestyramine feeding enhanced hepatic LDL uptake by 2.6-fold (from 124 to 319 μl/hr per g) (column B), and this was associated with a near doubling of whole-animal LDL turnover and a reduction of nearly 50% in the circulating plasma LDL-cholesterol levels (column C). When the plasma LDL level in these animals was acutely restored to normal during the experimental period by adding unlabeled homologous LDL to the primed-continuous infusion, the rate of hepatic LDL clearance was still elevated 2-fold (246 versus 124 μl/hr per g). Thus, the marked acceleration of hepatic LDL clearance observed in male hamsters cannot be attributed simply to diminished competition for transport sites, but, rather, represents an increase in the number or affinity of hepatic LDL receptors. Furthermore, since the rates of hepatic sterol synthesis and hepatic and whole-animal LDL clearance are essentially identical in male and female rats (D. K. Spady, S. D. Turley, and J. M.

TABLE 2. Rates of cholesterol synthesis and LDL clearance by the liver in vivo

Animal	Diet Treatment	A. Hepatic Sterol Synthesis	B. Hepatic LDL Clearance	C. Plasma LDL-Cholesterol Concentration	D. Hepatic LDL-Cholesterol Uptake
		nmol/hr per g	*μl/hr per g*	*μg/μl*	*μg/hr per g*
Rat (female)	Control	2240 ± 276	91 ± 14	0.07 ± 0.01	6
	Cholestyramine	4900 ± 492[a]	70 ± 11	0.07 ± 0.01	5
	Cholesterol	36 ± 3[a]	99 ± 12	0.07 ± 0.01	7
Hamster (female)	Control	255 ± 44	110 ± 9	0.21 ± 0.02	23
	Cholestyramine	2265 ± 208[a]	114 ± 10	0.20 ± 0.03	23
	Cholesterol	6 ± 1[a]	96 ± 7	0.44 ± 0.04[a]	42
Hamster (male)	Control	50 ± 5	124 ± 12	0.24 ± 0.03	30
	Cholestyramine	855 ± 131[a]	319 ± 30[a]	0.13 ± 0.01[a]	42
	Cholestyramine[b]	–	246 ± 20[a]	0.27 ± 0.02	66
	Cholesterol	4 ± 0.5[a]	98 ± 11	0.61 ± 0.05[a]	60

Animals fed either control, cholesterol-containing, or cholestyramine-containing diets were administered [^{3}H]water and rates of hepatic sterol synthesis were measured 1 hr later (column A). Rates of homologous LDL clearance by the liver were measured in a second, identically treated group of animals (column B). Plasma from this later group was taken for the determination of LDL-cholesterol content (column C). The absolute rate of hepatic LDL-cholesterol uptake (column D) was calculated by multiplying the rate of hepatic LDL clearance by the plasma LDL-cholesterol concentration. Mean values ± 1 SEM are shown for six animals in each group.

[a]Significantly different from the corresponding control value at $P < 0.05$.

[b]Unlabeled homologous LDL was added to the primed-continuous infusion so as to raise and maintain the plasma LDL-cholesterol concentrations in this group to the same level seen in the control animals.

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Dietschy, unpublished observations), the increase in LDL uptake in the liver of the male hamsters fed cholestyramine presumably reflects the very limited sterol synthetic capacity of these particular animals and not just an effect of gender.

The absolute rate of LDL-cholesterol uptake by the liver (μg/hr per g) can be calculated by multiplying each of the clearance rates (μl/hr per g) shown in column B by the corresponding plasma LDL-cholesterol concentrations (μg/μl) shown in column C. Although the rate of hepatic LDL clearance was enhanced 2.5-fold in male hamsters fed cholestyramine, the plasma LDL-cholesterol concentration was reduced 50% so that the absolute rate of hepatic LDL-cholesterol uptake was only modestly increased (42 versus 30 μg/hr per g). However, in the presence of normal plasma LDL levels (accomplished by adding unlabeled homologous LDL to the primed-continuous infusions), the rate of hepatic LDL-cholesterol uptake was more than 2-fold higher in cholestyramine-fed male hamsters than in controls.

Also shown in Table 2 is the effect of cholesterol feeding on rates of hepatic sterol synthesis and LDL clearance. In all experimental groups the rate of hepatic LDL clearance remained essentially unchanged with cholesterol feeding despite a >90% suppression of the rate of sterol synthesis and a marked accumulation of cholesteryl esters. Since plasma LDL levels increased 2- to 3-fold in the male and female hamsters, the absolute rates of hepatic LDL-cholesterol uptake also increased 2- to 3-fold.

The distribution of plasma cholesterol among five lipoprotein density fractions and the content of free and esterified cholesterol in the liver were next measured in groups of animals treated identically to those described in Table 2. These data are summarized in **Table 3.** Cholestyramine feeding reduced total plasma cholesterol levels in male hamsters but not in female hamsters or rats. In male hamsters fed cholestyramine the cholesterol content of all lipoprotein fractions was reduced; however, the relative decrease was greatest in the density fractions between 1.006 and 1.095 g/ml. Cholestyramine feeding had no significant effect on the hepatic free or esterified cholesterol content in any of the animals. Cholesterol feeding had no effect on plasma cholesterol concentrations in the rat but produced a 60% increase (111 to 178 mg/dl) and a 96% increase (113 to 221 mg/dl) in plasma cholesterol levels in the female hamster and male hamster, respectively. The increase in plasma cholesterol levels in hamsters fed cholesterol was confined entirely to the fractions having a density < 1.055 g/ml. The hepatic cholesteryl ester content increased markedly (13- to 24-fold) in all animals fed cholesterol.

In a final experiment, rates of hepatic cholesterol synthesis were altered over a wide range by feeding varying amounts of cholesterol and cholestyramine to the same three groups of experimental animals. The rate of homologous LDL uptake by the liver of each animal was then measured in vivo following which the rate of hepatic cholesterol synthesis was assayed in vitro in the same liver. As is evident from the data plotted for the individual animals in panel A of **Fig. 1,** hepatic cholesterol synthesis could be varied over a 110-fold range in female rats, yet there was neither suppression of hepatic LDL uptake with cholesterol feeding nor enhancement of LDL uptake with cholestyramine feeding. Similar findings were observed with the female hamsters (panel B), even though these animals synthesized sterol in a much lower range than seen in the rats. The male hamsters, however, with their very limited capacity to synthesize sterol in the liver, markedly increased hepatic LDL uptake when challenged with cholestyramine (panel C).

TABLE 3. Cholesterol content of plasma, five lipoprotein density fractions, and liver

Animal	Diet Treatment	A. Plasma Cholesterol Concentration in Different Density Classes						B. Hepatic Cholesterol Content	
		Total	< 1.006	1.006-1.020	1.020-1.055	1.055-1.095	1.095-1.21	Free	Esterified
		mg/dl						*mg/g*	
Rat (female)	Control	56 ± 4	3	1	8	11	33	2.30 ± 0.05	0.23 ± 0.02
	Cholestyramine	58 ± 4	4	1	8	13	32	2.23 ± 0.06	0.21 ± 0.03
	Cholesterol	55 ± 3	3	1	7	12	32	2.39 ± 0.04	3.21 ± 0.01[a]
Hamster (female)	Control	111 ± 8	8	4	22	19	58	2.01 ± 0.05	0.33 ± 0.05
	Cholestyramine	109 ± 7	9	3	21	18	57	1.94 ± 0.03	0.27 ± 0.03
	Cholesterol	178 ± 16[a]	67	14	46	15	36	2.20 ± 0.06[a]	8.22 ± 0.40[a]
Hamster (male)	Control	113 ± 5	9	4	23	19	58	2.10 ± 0.09	0.86 ± 0.08
	Cholestyramine	73 ± 3[a]	8	2	13	10	40	1.98 ± 0.11	0.78 ± 0.06
	Cholesterol	221 ± 22[a]	86	15	65	18	37	2.30 ± 0.10	9.88 ± 0.50[a]

Groups of animals were treated identically to those described in Table 2. Total plasma cholesterol and hepatic free and esterified cholesterol were determined in individual animals and values ± 1 SEM are given for six animals in each group. The distribution of plasma cholesterol among the five lipoprotein density fractions was determined in a pooled plasma sample from each group.

[a]Significantly different from the corresponding control value at $P < 0.05$.

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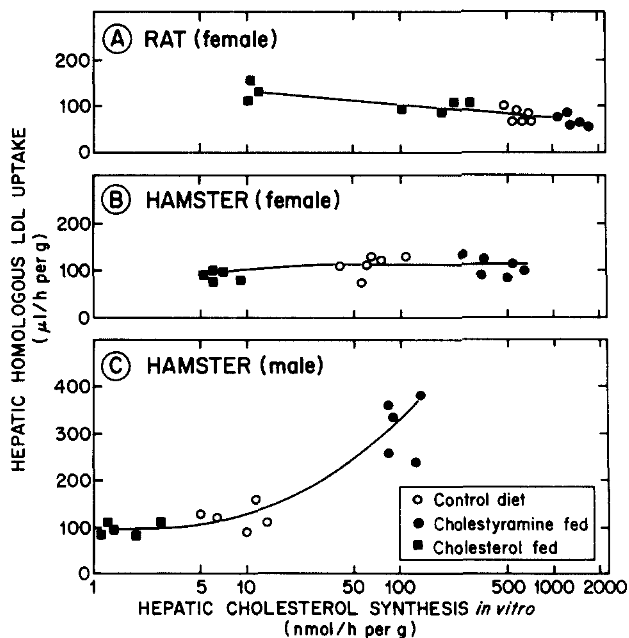

Fig. 1 Rates of hepatic LDL uptake as a function of the rate of hepatic cholesterol synthesis in control animals and in animals fed either cholesterol or cholestyramine. After the period of dietary treatment, each animal was infused in vivo with radiolabeled homologous LDL and then killed. Aliquots of the liver were taken for determination of the rate of hepatic LDL uptake and for measurement of the rate of cholesterol synthesis under in vitro conditions. This figure shows the rates of hepatic LDL uptake and hepatic cholesterol synthesis in individual female rats and in male and female hamsters. The rates of hepatic cholesterol synthesis are plotted on a logarithmic scale because of the very large range of values. The lines were fitted to the data points visually.

In order to further define the nature of the changes (or lack of changes) in LDL uptake observed in these experiments, the rate of homologous LDL transport was resolved into its receptor-dependent and receptor-independent components. As shown in **Fig. 2,** in the rat the rate of total homologous LDL uptake in the liver was again found to be independent of the rate of hepatic cholesterol synthesis. Furthermore, the rate of receptor-independent LDL uptake was also constant at about 8–10 µl/hr per g at all rates of hepatic cholesterol synthesis.

In the male hamster, the rate of receptor-independent LDL uptake by the liver also remained constant at about 8–9 µl/hr per g, even when cholestyramine feeding increased total LDL transport to approximately 280 µl/hr per g. Thus, these studies specifically established that in the rat receptor-dependent LDL transport remains constant as the rate of hepatic cholesterol synthesis is altered and, further, that it is the receptor-dependent component of hepatic LDL uptake that changes in the cholestyramine-fed hamster.

DISCUSSION

These studies confirm, in both the rat and hamster, that the major site for LDL clearance from the plasma is the liver and that this uptake process is mediated nearly entirely by the LDL receptor mechanism (Table 1). Furthermore, the rate of this receptor-dependent uptake process is regulated independently of the rate at which the hepatocyte is synthesizing sterol. Apparently the primary response to a change in cholesterol flux across the liver cell is an appropriate alteration in the rate of sterol synthesis. Only if this response is inadequate to meet the changing needs for cellular cholesterol does the change in receptor-dependent LDL uptake take place. These findings are analogous to those recently reported in the intestinal mucosa where changes in the flux of sterol across the epithelial cells were associated with changes in rates of cholesterol synthesis while LDL uptake remained essentially constant (22).

There are marked differences in rates of hepatic cholesterol synthesis among different animal species and even between individual members of a given species when studied under similar experimental conditions. On a low cholesterol intake, for example, there is a nearly 40-fold variation (when expressed per g of tissue) in the rate at which the liver synthesizes cholesterol in the rat, squirrel monkey, hamster, rabbit, guinea pig, and man (17, 18). Furthermore, when the flux of cholesterol into or out of the body is changed (by cholesterol or cholestyramine feeding) there is also a marked quantitative difference in the capacity of the liver of the different species to respond appropriately. For example, the absolute rate of cholesterol synthesis in the liver of the rat can be increased from the control value of about 70 µg/hr per g to 150 µg/hr per g by cholestyramine feeding or can be suppressed to nearly 0 µg/hr per g by cholesterol feeding. In contrast, in the male hamster, hepatic synthesis can be increased from the control value of approximately 1.5 µg/hr per g to only 26

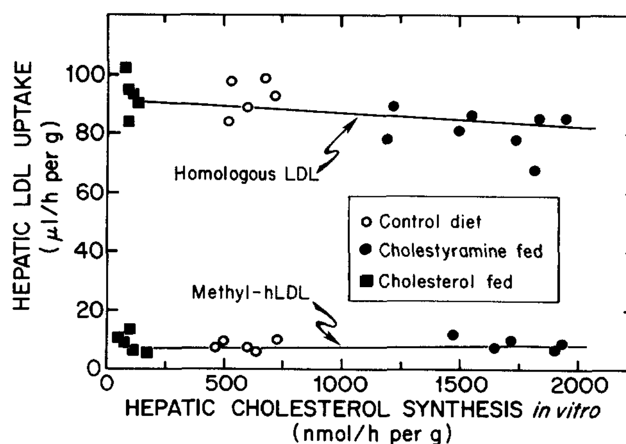

Fig. 2 Rates of total and receptor-independent LDL uptake in the liver of the rat. Animals were fed the same diets described in Fig. 1 and then rates of total LDL and receptor-independent LDL uptake were measured. The rates of receptor-dependent LDL transport can be calculated as the difference between these two values. The lines were fitted to the individual data points visually.

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μg/hr per g or suppressed to 0 μg/hr per g by these two manipulations. Hence, the range of alterations in cholesterol flux into or out of the liver that can be accommodated by a change in the rate of cholesterol synthesis equals 0 to 150 μg/hr per g in the rat but only 0 to 26 μg/hr per g in the hamster.

Whether a change in the input or output of cholesterol (or bile acids) in a given species is associated with a change in the amount of receptor-dependent LDL uptake in the liver appears to be determined by the capacity of that species to fully compensate for the induced change in sterol balance. For example, in the male and female rat, several types of monkeys, and a subset of human subjects, the cholesterol content of the diet can be increased several-fold without significantly altering LDL-cholesterol levels in the plasma. In this situation, the increased load of absorbed cholesterol presumably is compensated for by an equivalent decrease in hepatic cholesterol synthesis and there is no change in hepatic LDL receptor activity as judged by direct measurements or by the kinetics of plasma LDL turnover (1, 28–30). Similarly, in the rat, female hamster, dog, and a subset of human subjects, an increased loss of cholesterol from the body also can be fully compensated for so that plasma LDL levels and turnover again remain essentially unchanged (1, 31, 32). Only when these changes in cholesterol balance cannot be fully compensated for by a change in the rate of hepatic synthesis do changes in hepatic LDL receptor activity occur. The female hamster may represent an animal in which the synthetic capacity of the liver is just adequate to compensate for the changes in cholesterol balance induced by cholestyramine feeding. Indeed, an occasional group of female hamsters responds to cholestyramine feeding with a modest (~20–40%) increase in the rate of hepatic LDL clearance in addition to an 8- to 10-fold increase in the rate of hepatic sterol synthesis (16). Taken together these findings suggest that cholestyramine feeding elicits a near maximal response in the rate of hepatic sterol synthesis in female hamsters. Although this response is generally adequate to maintain cholesterol balance, under certain circumstances it may fail to completely offset the enhanced rate of cholesterol degradation, in which case the rate of hepatic LDL transport is also increased. This situation is probably analogous to that in humans where the reduction in plasma cholesterol levels achieved by the administration of cholestyramine is not only variable, but quite modest (33).

In the present studies cholesterol feeding had no significant effect on the rate of hepatic LDL clearance in any of the experimental animals despite a >90% reduction in sterol synthesis and a marked accumulation of cholesteryl esters in the liver. It should be recognized, however, that the period of cholesterol feeding was relatively short. In the rat, even long-term feeding of a 0.5% cholesterol diet fails to suppress rates of hepatic LDL clearance or to alter plasma LDL levels (D. K. Spady, S. D. Turley, and J. M. Dietschy, unpublished observations). This same diet in hamsters, however, is associated with a progressive rise in plasma total and LDL-cholesterol levels that, in the long term, may be associated with a decrease of receptor-dependent LDL transport in the liver.

Finally, these findings have two other important implications. First, since LDL receptor activity on both the enterocyte and hepatocyte appears to vary independently of the rate of sterol synthesis, these two organs readily compensate for hour-to-hour changes in the dietary cholesterol intake while, at the same time, maintaining relatively constant values for the rate of degradation and the plasma concentration of LDL. Thus, the supply of LDL-cholesterol to peripheral organs is maintained constant in the face of marked daily variations in sterol balance across the intestinal-hepatic axis. Second, these studies imply that the intracellular site that "senses" the concentration of cholesterol in the cell and alters sterol synthesis is functionally distinct from the site that is responsible for regulation of LDL-receptor activity.

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