Role of receptor-independent low density lipoprotein transport in the maintenance of tissue cholesterol balance in the normal and WHHL rabbit

David K. Spady, Manfred Huettinger, David W. Bilheimer, and John M. Dietschy

Departments of Internal Medicine and Molecular Genetics, University of Texas Health Science Center at Dallas, Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235

Abstract These studies were undertaken to determine the role of receptor-independent low density lipoprotein (LDL) transport in cholesterol balance across individual tissues and the whole animal. Homologous LDL, which measures total LDL transport, and methylated heterologous LDL, which measures receptor-independent LDL uptake, were cleared from the plasma at very different rates in the NZ control rabbit (3,900 and 1,010 μl/hr per kg, respectively) whereas in the WHHL rabbit both preparations were cleared at essentially the same rate (approximately 1,070 μl/hr per kg). Receptor-independent LDL clearance was detected in all tissues of the NZ control rabbit and these varied from 32 (spleen) to < 0.5 (skeletal muscle) μl/hr per g. In contrast, receptor-dependent LDL uptake was found in only about half of these same organs. In the WHHL rabbit, the rates of receptor-independent LDL transport were the same as in the NZ control rabbit, but no receptor-dependent uptake was detected. Using these clearance values it was calculated that in the control rabbit nearly 70% of LDL-cholesterol was removed from the plasma by the liver and 89% of this was receptor-mediated. With loss of receptor activity, however, the burden of LDL degradation was shifted away from the liver so that approximately 70% of LDL-cholesterol uptake took place in the extrahepatic tissues of the WHHL rabbit. Thus, in the normal animal, the primary function of receptor-dependent LDL transport is to promote the rapid uptake and disposal of plasma LDL by the liver. In the absence of such receptor activity, cholesterol balance across most individual organs and the whole animal remains essentially normal and is mediated by the receptor-independent process. Because of the much lower absolute clearance rates manifested by this transport mechanism, however, substantial and predictable elevations in the circulating plasma LDL-cholesterol levels are required to maintain this balance. — **Spady, D. K., M. Huettinger, D. W. Bilheimer, and J. M. Dietschy.** Role of receptor-independent low density lipoprotein transport in the maintenance of tissue cholesterol balance in the normal and WHHL rabbit. *J. Lipid Res.* 1987. **28:** 32–41.

Supplementary key words very low density lipoproteins • receptor-dependent low density lipoprotein transport • hepatic cholesterol synthesis • intestinal cholesterol synthesis

In the steady-state, the concentration of plasma cholesterol carried in low density lipoproteins (LDL) is determined by the rate at which LDL is introduced into the plasma relative to the rate at which it is removed and degraded (1). In the normal animal and human, approximately one-third of the LDL that is degraded each day is removed from the plasma by a process that is independent of the LDL receptor pathway (2–6). Recent studies have shown that such receptor-independent LDL transport takes place in nearly every organ in the body, although the highest rates of clearance (per g of tissue) by this mechanism are found in the spleen, liver, small intestinal epithelial cell, and kidney (6–8). When receptor-dependent transport is reduced or absent, either because of a genetic defect (3, 9) or because of dietary suppression (10, 11), the receptor-independent pathway becomes quantitatively much more important and accounts for the degradation of most or all of the LDL that is removed from the plasma in the whole animal or in humans.

Detailed studies have revealed two important differences in the characteristics of the receptor-dependent and receptor-independent LDL transport systems in various organs under in vivo conditions. First, the receptor-dependent transport system in tissues like the liver, small intestine, and endocrine glands manifests saturation kinetics (7, 10, 12). In contrast, the rate of LDL uptake by the receptor-independent system continues to increase linearly as the plasma LDL-cholesterol concentration is raised (6, 12). Secondly, under certain experimental circumstances, the level of receptor-dependent LDL transport can be varied, at least in a few organs (10, 11, 13). Again, in contrast, receptor-independent LDL uptake continues at rates that are unchanged by a variety of metabolic manipulations (6). However, despite these two important differences in the characteristics of LDL uptake

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; WHHL, Watanabe heritable hyperlipidemic; NZ, New Zealand; methyl-hLDL, methylated LDL of human origin; FCR, fractional catabolic rate.

into tissues by the receptor-dependent and -independent systems, once the LDL-cholesterol is delivered into the target organ by either mechanism it appears to be equally effective in regulating the rate of intracellular cholesterol synthesis and in acting as an effective substrate for esterification (6, 14, 15).

On the basis of quantitative experiments carried out in the normal rat and hamster, it is possible to predict what will happen to cholesterol balance across each individual tissue, as well as the whole animal, under circumstances where the amount of receptor-dependent LDL transport activity is systematically reduced. Furthermore, from these calculations it is also possible to predict what the final plasma LDL-cholesterol concentration will be in the new steady-state and to define in quantitative terms the role of the receptor-independent LDL transport system in the maintenance of this new steady-state (12). Unfortunately, these predictions cannot be easily tested in these two species since no model exists where LDL receptor activity is totally absent. For this reason, the current studies were undertaken in the normal (NZ) and receptor-deficient Watanabe heritable hyperlipidemic (WHHL) rabbit (16, 17). Three types of investigations were carried out. First, the magnitude of the receptor-dependent and receptor-independent components of plasma LDL clearance was defined in the normal animal. Second, the distribution of these two transport activities was quantitated in every major organ in the control rabbit. Third, from these measurements predictions were made as to the role of receptor-independent LDL transport in maintaining cholesterol balance in the whole animal and across individual tissues, and these predictions were then tested by making appropriate measurements in the receptor-deficient, WHHL animal.

MATERIALS AND METHODS

Animal preparations

Female New Zealand (NZ) albino rabbits were purchased from Hickory Hill Rabbitry (Flint, TX). Female homozygous WHHL rabbits were raised in Dallas. All animals were maintained on Lab Rabbit Chow (Ralston Purina Co., St. Louis, MO) and were used at 3–4 months of age when they weighed 2.2–2.8 kg.

Lipoprotein preparations

Rabbit LDL was isolated in a density range of 1.030 to 1.050 g/ml from the plasma of NZ rabbits maintained on the standard low-cholesterol diet. Human LDL also was isolated at a density of 1.030 to 1.050 g/ml from the plasma of normolipidemic volunteers. Both preparations showed only apoprotein B_{100} on polyacrylamide gel electrophoresis. The LDL preparations were labeled with either ^{125}I (18) or [^{14}C]sucrose (19). The labeled human LDL was then reductively methylated (methyl-hLDL) (20). All lipoprotein fractions were dialyzed extensively against 0.9% NaCl and then passed through a 0.22 μm Millipore filter just prior to use.

Whole-animal LDL clearance rates

Nonfasted rabbits received a primed-continuous infusion of tracer quantities of radiolabeled LDL preparations through an intravenous catheter secured in a lateral ear vein. The infusion was continued for 14 hr to ensure steady-state plasma radioactivity levels (7, 21). Repeated sampling of plasma obtained from the opposite ear during the terminal 6 hr of the infusion consistently revealed a variation in the specific activity of the plasma (dpm/μl) of $<4\%$. After 14 hr the animals were anesthetized and exsanguinated through the abdominal aorta. The delivery of each pump was calibrated by allowing it to continue to deliver the infusate directly into counting vials for two consecutive 30-min periods. These samples, along with triplicate aliquots of plasma, were then assayed for radioactivity. The clearance rate from the plasma of each labeled compound was then calculated by dividing the amount of radiolabeled LDL infused per hr (dpm/hr) by the steady-state concentration of radiolabeled LDL in the plasma (dpm/μl). This calculation gives the μl of plasma cleared of a particular lipoprotein preparation each hour (μl/hr). This value was then normalized to 1 kg body weight (μl/hr per kg).

Individual tissue LDL clearance rates

The experimental animals received a primed-continuous infusion of [^{14}C]sucrose-labeled LDL through an intravenous catheter placed in a lateral ear vein. The amount of labeled LDL in the initial and hourly infusions was adjusted so that the specific activity of the plasma LDL was constant over the 6-hr experimental period (5, 7). Groups of three animals were then killed at 10 min, 2 hr, 4 hr, and 6 hr, and 15 tissues were quickly removed. Samples of each of these tissues, along with aliquots of plasma, were solubilized and assayed for radioactivity as previously described (22). The radioactivity per g of tissue (dpm/g) was then divided by the steady-state plasma radioactivity (dpm/μl) to give the tissue space (μl/g) achieved by the radiolabeled LDL in each organ at each time point. The clearance rate of LDL by each organ was then calculated from the linear regression curves fitted to the individual data points. The slope of such curves represents the μl of plasma cleared of its LDL content per hr per g of tissue (μl/hr per g). When multiplied by the weight of a particular organ, this value gave the rate of LDL clearance per whole organ (μl/hr per organ). When either of these clearance values was multiplied by the plasma LDL-cholesterol concentration, the rate of LDL-cholesterol uptake per g of tissue or per organ was obtained.

Determination of plasma LDL levels

Plasma from each animal was anticoagulated with dry EDTA and equal volumes were adjusted to densities of 1.020 and 1.063 g/ml using KBr and centrifuged simultaneously at 165,000 *g* for 30 hr. The cholesterol content in the top one-third of each tube, as well as the total plasma cholesterol concentration of individual animals, was measured colorimetrically (23).

RESULTS

In the animals used in these studies the plasma LDL-cholesterol concentration ranged from 18–24 mg/dl in the NZ control rabbits and from 380–440 mg/dl in the WHHL animals. Before performing studies to quantitate rates of receptor-dependent and receptor-independent LDL transport in the normal and receptor-deficient rabbits, preliminary studies were carried out to confirm that in this species lipoprotein preparations labeled with [^{14}C]sucrose behaved quantitatively like LDL labeled more conventionally with ^{125}I. Using both homologous LDL and methyl-hLDL, a direct comparison was made in the NZ control and WHHL animals and, as is evident in **Table 1,** the rates of whole-animal clearance of the respective LDL preparations were indistinguishable regardless of whether they were labeled with [^{14}C]sucrose or ^{125}I. This result was somewhat different from findings in the rat where [^{14}C]sucrose-LDL was cleared from the plasma at a rate that was approximately 10% less than the rate of clearance observed with ^{125}I-labeled LDL (6) and indicated that in the rabbit the procedure of [^{14}C]sucrose labeling did not alter the turnover characteristics of the lipoprotein preparation.

Previous studies carried out in vitro have shown that both heterologous LDL and methylated homologous LDL interact poorly with the LDL receptor (20, 24). Furthermore, under in vivo conditions methylated heterologous LDL was found to have lost all specific binding to the LDL receptor and to be turned-over in the whole animal at the lowest rate found with any LDL preparation (6). Such methylated heterologous LDL, therefore, was used in the present study to quantitate the magnitude of receptor-independent LDL transport in the various organs. As is also apparent in Table 1, the rate of clearance of homologous LDL and methyl-hLDL were both approximately 1,000 μl/hr per kg body weight in the WHHL rabbit which lacks nearly all LDL receptor activity. The methylated derivative was degraded at essentially the same rate in the NZ control animals but the homologous LDL was cleared at a rate of 3,840 μl/hr per kg, indicating that in this species about 73% of LDL turnover in the whole animal was mediated by the receptor-dependent transport process. This figure, derived from whole-animal clearance data, is very similar to previously reported values measured using data on the turnover of ^{125}I-labeled lipoproteins (3).

Since these preliminary studies confirmed that [^{14}C]sucrose-labeled homologous and methylated heterologous LDL accurately reflected the quantitative handling of LDL in the rabbit by the receptor-dependent and -independent transport processes, these two probe molecules were next used to quantitate the rates of the two transport processes in the individual organs of this species. The basic technique employed to make these measurements involved the use of a primed-continuous infusion to maintain the specific activity of the plasma LDL constant over a 6-hr period. Under these circumstances, the [^{14}C]sucrose-labeled LDL accumulated in the various organs as a linear function of time, as illustrated by the representative data for the adrenal gland, liver, and skeletal muscle shown in **Fig. 1.** The slope of the linear regression curves fitted to the data gives the rate at which the specific LDL preparation is cleared or taken up by that particular tissue, and this rate is expressed as the μl of plasma cleared entirely of its LDL content per hr per g.

From the data shown for the NZ control rabbit in Fig. 1, it is apparent that the rates of homologous LDL uptake (total LDL clearance) varied markedly in the different organs and that the rates of methyl-hLDL clearance (receptor-independent LDL clearance) were significantly lower in some tissues than those rates observed with

TABLE 1. Plasma clearance of homologous LDL and methyl-hLDL labeled with either ^{125}I or [^{14}C]sucrose

	NZ Control Rabbits		WHHL Rabbits	
Radiolabel	Homologous LDL	Methyl-hLDL	Homologous LDL	Methyl-hLDL
	μl/hr per kg			
^{125}I	3,900 ± 350	1,010 ± 200	1,110 ± 190	1,030 ± 110
[^{14}C]Sucrose	3,840 ± 410	1,020 ± 125	1,050 ± 200	1,000 ± 215

Single batches of homologous LDL and methyl-hLDL were labeled with either ^{125}I or [^{14}C]sucrose. Whole-animal clearance rates were then determined for homologous LDL and methyl-hLDL (labeled with either ^{125}I or [^{14}C]sucrose) in groups of NZ control and WHHL rabbits using the primed-continuous infusion technique. Each value represents the mean ± 1 SEM for results obtained in three animals.

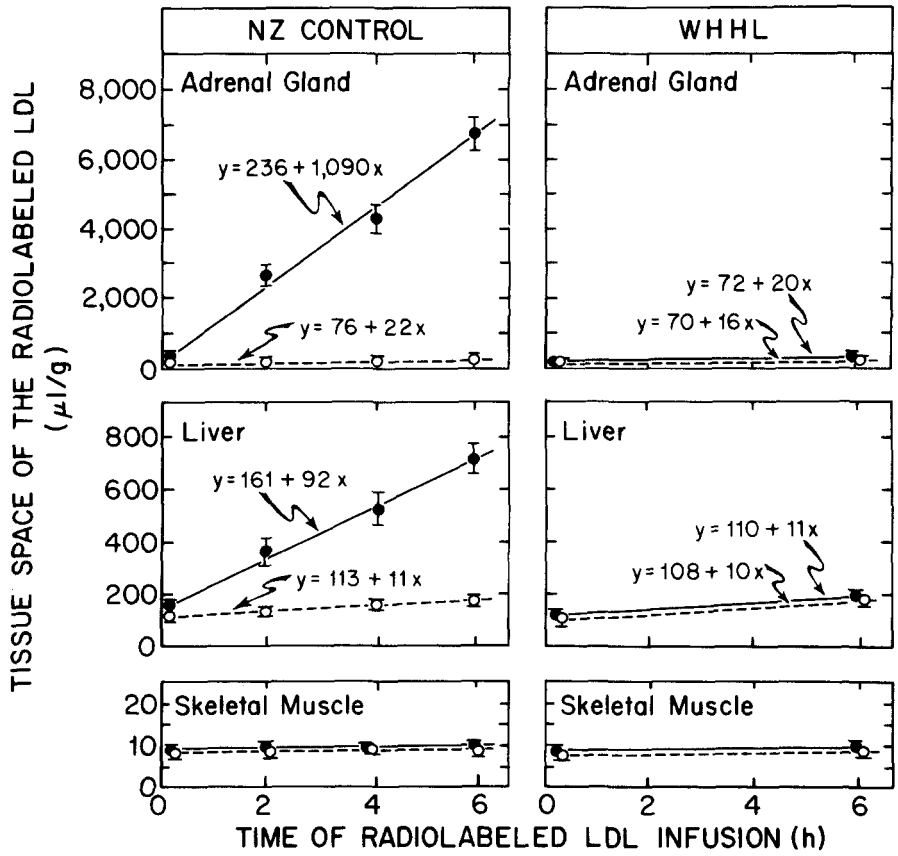

Fig. 1. Representative experimental data for the uptake of homologous LDL and methyl-hLDL by the adrenal gland, liver, and skeletal muscle of NZ control and WHHL rabbits. Groups of animals received a primed-continuous infusion of [^{14}C]sucrose-labeled homologous LDL or methyl-hLDL. Three animals from each group were then killed at 10 min, 2 hr, 4 hr, and 6 hr (NZ control rabbits) or at 10 min and 6 hr (WHHL rabbits), and 15 tissues were removed and assayed for radioactivity. The radioactivity in 1 g of each organ (dpm/g) divided by the steady-state concentration of radiolabeled LDL in the plasma (dpm/μl) equals the space of distribution achieved by LDL in a particular organ at each time point. The slope of the linear regression curve fitted to these values gives the rate of tissue uptake of LDL expressed as the μl of plasma cleared of its LDL content per hr per g of tissue (μl/hr per g).

the homologous preparation. These two sets of clearance rates, expressed per g of tissue, are shown for 15 organs of the NZ control rabbit in **Table 2.** As is apparent, the rates of homologous LDL uptake were highest in the adrenal gland (1,090 μl/hr per g), spleen (142), and liver (92). Rates of methyl-hLDL uptake were much lower in these organs and equaled only 22, 32, and 11 μl/hr per g, respectively. Since the difference between the rate of total LDL uptake (homologous LDL) and the rate of receptor-independent LDL clearance (methyl-hLDL) equals the rate of receptor-dependent LDL transport, it could be calculated that the receptor-dependent mechanism was largely responsible for the very high rates of uptake observed in the adrenal gland (98%), spleen (77%), and liver (88%) of the control animals. Much lower rates of homologous LDL uptake were found in the ovary (15), lung (11) and kidney (11); nevertheless, since rates of methyl-hLDL uptake also were much lower in these organs, receptor-dependent transport still predominated (68–92%). Receptor-dependent LDL transport was also identified in intestine but in the remaining organs not only were the rates of homologous LDL clearance very low, but these rates were also essentially identical to those observed with methyl-hLDL. Thus, the observed very low rates of LDL transport seen in large organs such as skeletal muscle, skin, and central nervous system could be accounted for entirely by uptake of LDL by the receptor-independent process.

In identical experiments, uptake rates for homologous LDL and methyl-hLDL were measured in the various organs of the WHHL rabbit and these results are also shown in Fig. 1 and Table 2. Two points concerning these data warrant emphasis. First, in contrast to the NZ control rabbits, no significant differences were observed in the

TABLE 2. Clearance rates of homologous LDL and methyl-hLDL per g of each organ in NZ control and WHHL rabbits

Organ	NZ Control Rabbits		WHHL Rabbits	
	Homologous LDL	Methyl-hLDL	Homologous LDL	Methyl-hLDL
	μl/hr per g			
Adrenal gland	1,090 ± 121	22 ± 1	20 ± 2	16 ± 2
Spleen	142 ± 8	32 ± 2	36 ± 2	18 ± 3
Liver	92 ± 8	11 ± 1	11 ± 2	10 ± 2
Ovary	15 ± 1	4.8 ± 1	4.0 ± 1	3.2 ± 1
Lung	11 ± 1	0.8 ± 0.2	0.7 ± 0.3	2.1 ± 0.4
Kidney	11 ± 1	3.2 ± 0.5	3.9 ± 0.4	2.8 ± 0.4
Ileum	4.5 ± 1	3.0 ± 0.5	3.3 ± 0.4	2.2 ± 0.3
Jejunum	1.8 ± 0.3	0.9 ± 0.3	0.7 ± 0.2	0.9 ± 0.2
Fat	1.7 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Heart	1.1 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	1.0 ± 0.3
Colon	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	0.9 ± 0.2
Stomach	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
Skeletal muscle	< 0.5	< 0.5	< 0.5	< 0.5
Skin	< 0.5	< 0.5	< 0.5	< 0.5
Brain	< 0.5	< 0.5	< 0.5	< 0.5

Rates of LDL clearance were determined using the primed-continuous infusion of [^{14}C]sucrose-labeled LDL as described in Fig. 1. Each value equals the slope of the relationship between tissue space and time of infusion for data obtained in 12 NZ control animals and 6 WHHL rabbits.

WHHL rabbits between the rates of homologous LDL transport and methyl-hLDL uptake in virtually any organ. Thus, as expected, there was no evidence of receptor-dependent LDL transport in these animals, and all LDL removal from the plasma could be accounted for by transport via the receptor-independent process. Second, the rates of LDL uptake observed in the WHHL animals were virtually identical to the rates of methyl-hLDL clearance observed in the tissues of the NZ control animals. Thus, even though the WHHL rabbit lacked LDL receptor activity, the receptor-independent transport process appeared to be intact and operating at rates identical to those found in the normal animal.

In order to quantitate the contribution of each organ to the whole-animal turnover of homologous LDL and methyl-hLDL, the clearance rates shown in Table 2 were multiplied by the corresponding organ weights measured in these same animals to obtain the rates at which each whole organ removed LDL from the plasma. These values are summarized in **Table 3.** The data in the four columns show the rates of clearance of both homologous LDL and methyl-hLDL by each major tissue of the NZ control and WHHL rabbits. In addition, values for the clearance of LDL in the whole animal were independently measured in the four different experimental groups so that the rate of LDL clearance in each organ could be related directly to the total rate of LDL degradation found in a parallel group of intact animals. In the NZ control animals, homologous LDL and methyl-hLDL were cleared by the whole-animal at rates of 3,880 and 1,040 μl/hr per kg of body weight, respectively. In such animals, the clearance of homologous and methyl-hLDL by the whole liver equaled 2,686 and 321 μl/hr per organ, respectively, so that this tissue accounted for the degradation of nearly 70% of the circulating plasma LDL and contained 83% of the receptor-dependent LDL transport activity detected in the whole animal. In contrast, the liver accounted for only about 30% of the LDL transport and degradation that occurred via the receptor-independent pathway. Thus, in the WHHL rabbit, which lacks receptor-dependent LDL transport, the importance of the liver as a major site for LDL degradation decreases from the control value of 70% to only about 30%. Therefore, nearly 70% of the LDL-cholesterol in the receptor-deficient animals must have been taken up and degraded by the extrahepatic tissues. It is also evident from the data in Table 3 that no particular extrahepatic tissue was primarily responsible for this clearance. Rather, nearly all of the extrahepatic organs contributed to this receptor-independent LDL degradation. Finally, it should also be noted that in these studies the sum of the rates of receptor-independent uptake in the individual tissues was somewhat less than the clearance rates determined in the whole animal. This discrepancy very likely was due to the fact that it was difficult to quantitate the very low rates of receptor-independent LDL transport in large tissues like muscle and skin and that some tissues such as bone marrow, omentum, and pancreas, etc. were not sampled.

DISCUSSION

In the steady-state the concentration of LDL-cholesterol in the plasma is determined by the complex interaction of

TABLE 3. Clearance rates of homologous LDL and methyl-hLDL by each whole organ in NZ control and WHHL rabbits

Organ	NZ Control Rabbits		WHHL Rabbits	
	Homologous LDL	Methyl-hLDL	Homologous LDL	Methyl-hLDL
	μl/hr per organ per kg			
Adrenal gland	98	2.0	0.96	0.77
Spleen	256	58	72	36
Liver	2,686	321	352	320
Ovary	1.3	0.40	0.48	0.38
Lung	62	4.5	4.0	12
Kidney	78	23	27	20
Ileum	50	33	43	29
Jejunum	20	9.9	8.4	11
Fat	151	36	27	36
Heart	3.5	2.9	2.5	3.1
Colon	33	30	40	32
Stomach	5.5	6.6	6.0	5.0
Skeletal muscle	< 272	< 272	< 272	< 272
Skin	< 59	< 59	< 59	< 59
Brain	< 1.9	< 1.9	< 1.9	< 1.9
Whole animal	3,880	1,040	1,080	1,010

These values equal the product of the clearance rates (μl/hr per g) shown in table 2 and the corresponding whole organ weights (g per kg body weight) that were determined at the time each animal was killed (skeletal muscle and fat were assumed to weigh 544 g and 89 g per kg body weight, respectively). Total plasma LDL clearance was determined in separate groups of animals as described in materials and methods.

a number of different processes. On the one hand, the rate of LDL production determines the rate at which this lipoprotein fraction is introduced into the plasma space, and this process, in turn, can be altered by changing either the rate of VLDL secretion by the liver or the proportion of this lipoprotein fraction that is converted to LDL. On the other hand, the rate of removal of LDL from the plasma depends upon the still more complex interaction of at least two processes, receptor-dependent and receptor-independent LDL transport. Each of these processes, in turn, may be affected differently by various environmental, physiological, or pharmacological factors. Any approach to understanding the regulation of plasma LDL-cholesterol levels is thus critically dependent upon quantitative data describing each of the processes that either contributes to or removes LDL from the circulation. While such data are now available in the rat and hamster (6, 12), the rabbit offers a unique opportunity to characterize several of these processes in detail under circumstances where receptor-dependent LDL transport is normal (the NZ control rabbit) or where this activity is genetically essentially absent (the WHHL rabbit).

The first set of observations obtained in these studies dealt with the characteristics of receptor-independent LDL transport in this species. In the whole animal, receptor-independent LDL degradation, measured with methyl-hLDL, accounted for approximately 27% of total LDL turnover (Table 1) and the absolute rate of receptor-independent LDL clearance was essentially identical in both the NZ control and WHHL rabbits at approximately 1,080 μl/hr per kg body weight. This percentage figure is very similar to other reported values in this species based upon measurements of fractional catabolic rates (FCR) in the normal and receptor-deficient animal (2, 3). In normal humans with an average plasma LDL-cholesterol concentration of about 76 mg/dl, 39% of LDL turnover is by the receptor-independent pathway (25). When corrected to a plasma LDL-cholesterol concentration of about 20 mg/dl (as found in the normal rabbit), this figure in humans would equal approximately 29% (26). Furthermore, the absolute clearance rate of LDL by the receptor-independent mechanism in humans can be calculated to equal approximately 280 μl/hr per kg body weight in control subjects and in patients with homozygous familial hypercholesterolemia (25). Thus, at comparable plasma LDL-cholesterol concentrations, receptor-independent transport accounts for 27–29% of LDL turnover in normal humans and rabbits. Furthermore, the absolute clearance rate of LDL by this mechanism is the same in the normal and receptor-deficient human and rabbit, suggesting that in the whole animal this degradative pathway is not altered by the genetic mechanisms that influence the receptor-dependent pathway.

In the rabbit, this receptor-independent LDL transport activity is widely distributed in a number of organs (Table 2) and, in each of these organs, the absolute rate of LDL clearance per g of tissue is essentially the same in the NZ control and WHHL animals. The fact that in the whole animal, as well as in the individual organs, the rate of clearance of LDL is constant even though the plasma LDL-cholesterol concentration is nearly 20-fold higher in the receptor-deficient animals, indicates that LDL uptake

by this process is a linear function of the plasma LDL concentration. Such kinetic characteristics are identical to those that have been reported in the hamster and rat where direct measurements have demonstrated that receptor-independent LDL-cholesterol uptake is a linear function of the plasma LDL level so that the clearance rate is constant (6). When whole organ weight is taken into consideration (Table 3), approximately 30% of the receptor-independent LDL transport detected in the whole animal is attributable to uptake by the liver in both the NZ control and WHHL animals. Other organs such as skeletal muscle, spleen, and intestine are also important in this regard and nearly all of the remaining tissues manifest at least a small amount of LDL-cholesterol uptake. Thus, as has been described in the rat and hamster (6, 7, 12), receptor-independent LDL uptake activity is distributed in nearly every organ in the body. Since uptake by this process is a linear function of the plasma LDL-cholesterol concentration and, further, is unaffected by loss of LDL receptor activity, this process accounts for the absolute clearance of approximately 1,080 and 280 μl of plasma of its LDL content per hr per kg body weight, respectively, in the rabbit and human, in both the normal and receptor-deficient individuals.

The second set of observations obtained in these studies deals with the quantitative nature of the receptor-dependent LDL transport process found in the organs of the normal rabbit. As has been observed in the rat and hamster (6, 7), the high rates of LDL uptake in tissues like the liver and endocrine glands are due to the presence of significant amounts of receptor-dependent LDL transport in these organs (Table 2). When whole organ weight is again taken into consideration, the liver of the normal rabbit accounts for the uptake and degradation of nearly 70% of the LDL that is turned-over in the whole animal. This compares with the figures of 73% and 53% found for hepatic LDL degradation in the hamster and rat, respectively (6, 7). However, since receptor-dependent LDL clearance in the liver equals 2,365 μl/hr and in the whole animal is only 2,840 μl/hr, it can be further calculated that, in the rabbit, the liver contains 83% of all receptor-dependent LDL transport that can be detected in the whole animal (Table 3). Again, this figure compares favorably with similar values calculated for the hamster (90%) and rat (67%) (5–7). Unlike the receptor-independent degradation process, however, this receptor-dependent uptake is saturable so that its quantitative importance to overall LDL turnover decreases as the plasma LDL-cholesterol level is increased (12, 26). Furthermore, receptor-dependent LDL transport is subject to genetic and metabolic regulation (10, 11, 27). Thus, in contrast to the situation seen with receptor-independent LDL degradation, the absolute clearance rate of LDL by way of the receptor-dependent process can vary considerably depending upon the plasma LDL-cholesterol concentration and the metabolic and genetic state of the animal.

From these characteristics of the kinetics of LDL degradation and the quantitative data on both receptor-dependent and receptor-independent LDL transport, it is possible to predict how cholesterol balance across individual tissues and the plasma LDL-cholesterol concentration will change given alterations in either receptor number or production rate. As illustrated in panel A of **Fig. 2** for example, the NZ control rabbit has a mean LDL-cholesterol concentration of about 20 mg/dl and an LDL clearance rate of 3,880 μl/hr per kg. From these two values it follows that 776 μg of LDL-cholesterol must be degraded each hr per kg of body weight (the plasma LDL-cholesterol concentration times the LDL clearance rate), which, in the steady-state, must also equal the LDL-cholesterol production rate. Since most of the LDL receptor activity is located in the liver, 69% of this degradation takes place in this organ (Table 3) while the remaining 31% of the cleared LDL is removed by the other organs of the body.

If all receptor activity were suddenly lost but the production rate were kept constant (Fig. 2, panel B), then the plasma LDL-cholesterol level would have to increase to 72 mg/dl (the metabolic production rate divided by the clearance rate). Thus, by allowing the plasma cholesterol level to increase modestly to 72 mg/dl, the receptor-independent transport system would be able to fully clear from the plasma 776 μg of LDL-cholesterol/hr per kg. However, since most of the receptor activity that was lost was originally located in the liver, it follows that the other organs of the body must assume a far greater role in this degradative process. Hence, in this situation, only 30% of the LDL is degraded in the liver while the remainder is taken up by various extrahepatic organs in proportion to their respective rates of receptor-independent LDL transport (Table 3). Thus, total loss of LDL receptor activity alone leads to only a modest increase in the plasma LDL cholesterol concentration but a major shift in the anatomical sites for LDL degradation.

However, in the WHHL animal there is not only loss of LDL receptor activity but, in addition, there is a 5.6-fold increase in the metabolic production rate of LDL (3). Thus, under conditions where the LDL-cholesterol production rate equals 4,380 μg/hr per kg and the clearance rate is 1,080 μl/hr per kg, the plasma LDL-cholesterol concentration must necessarily increase to 405 mg/dl in order for the receptor-independent LDL transport system to degrade this amount of LDL cholesterol in the steady-state. It is noteworthy that, in the WHHL animals in which these measurements were made, the experimentally determined LDL-cholesterol level averaged about 408 mg/dl. Furthermore, since the amount of LDL-cholesterol taken up by each organ is a linear function of the plasma LDL-cholesterol concentration, it follows that the absolute

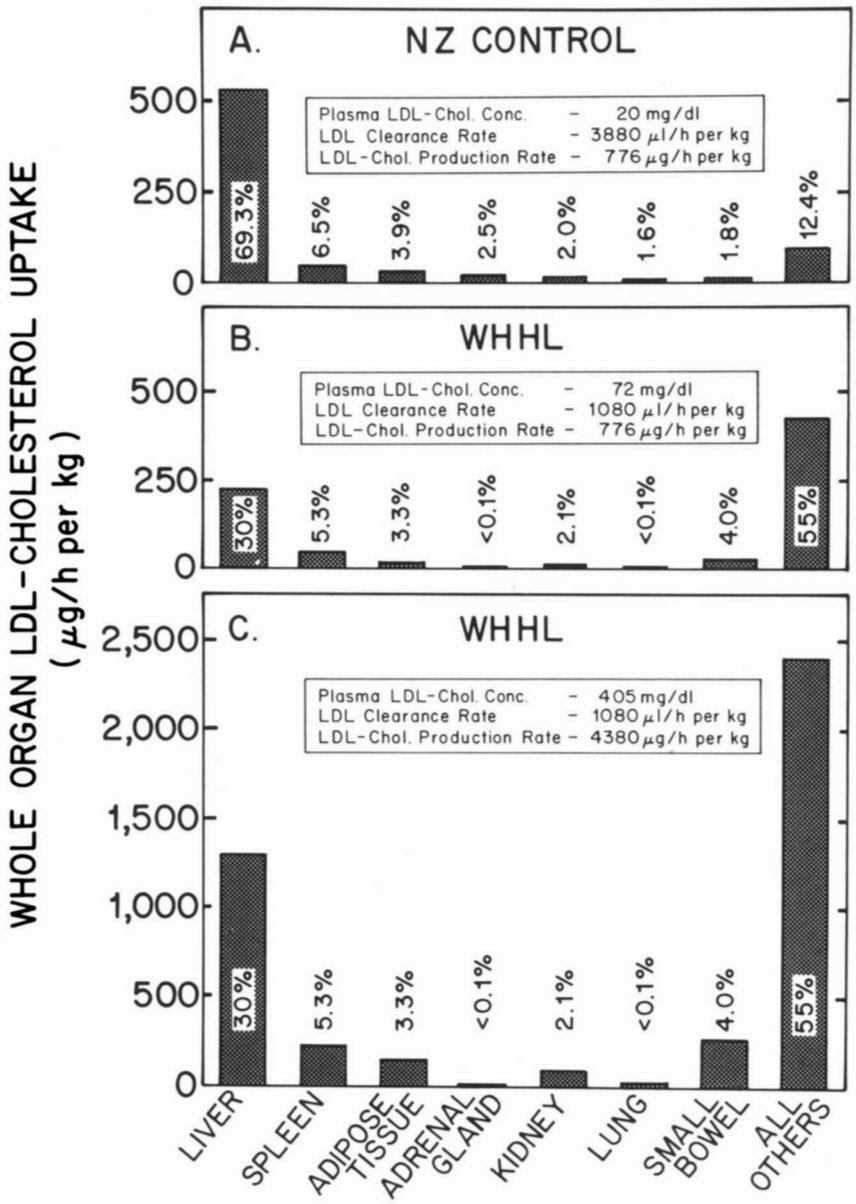

Fig. 2. Distribution of LDL-cholesterol uptake in the various organs of the NZ control and WHHL rabbit under conditions where the amount of receptor-dependent LDL transport and the rate of LDL-cholesterol production were varied. Panel A shows the situation in the NZ control rabbit. Panel B illustrates the situation that would occur if all receptor-dependent LDL transport was eliminated but the metabolic production rate of LDL was kept constant at the normal value. Panel C shows the results where all receptor-dependent LDL transport was absent and where the metabolic production rate of LDL-cholesterol was increased 5.6-fold. In all three panels the absolute rate of LDL-cholesterol uptake in each tissue is shown, as well as the percentage of total LDL degradation that is accounted for by such uptake in that same organ.

amount of LDL-cholesterol taken up by each tissue must also increase by 5.6-fold (Fig. 2, panel C) even though the relative importance of each organ with respect to total LDL degradation remains unchanged.

The quantitative effect of these two events, i.e., loss of receptor activity and increased LDL production, varies in the different organs depending upon the relative importance of receptor-dependent and receptor-independent LDL transport in a particular tissue. In the liver, for example, the amount of LDL-cholesterol actually taken up increases from 537 µg/hr per kg in the NZ control rabbit (Fig 2, panel A) to 1,300 µg/hr per kg in the WHHL animals (Fig. 2, panel C). This is due to the fact that receptor-independent LDL transport in the liver is relatively high (11 µl/hr per g) compared to total uptake (92 µl/hr per g). This enhanced cholesterol uptake is reflected

in a significantly lower rate of cholesterol synthesis in the liver of the WHHL rabbits (310 nmol/hr per g) compared to the normal animals (538 nmol/hr per g) (28). In contrast, receptor-independent uptake in adrenal gland is low (22 μl/hr per g) compared to total transport (1,090 μl/hr per g) so that the absolute amount of LDL-cholesterol taken up by this organ is actually lower in the WHHL rabbits (8 μg/hr per kg) than in the NZ control animals (20 μg/hr per kg). Hence, in contrast to the liver, the rate of cholesterol synthesis is actually increased several-fold in the adrenal gland of the WHHL rabbits (28). Thus, the net effect of loss of LDL receptor activity and LDL-cholesterol overproduction results in a rise in the plasma LDL-cholesterol concentration, a marked change in the relative importance of the various organs in LDL degradation, and a variable effect on the absolute amount of LDL-cholesterol taken up in any particular tissue. Despite the complexity of these alterations, however, the absolute magnitude of these changes can be predicted from the rate constants measured in these studies.

This work was supported by U.S. Public Health Service Research Grants HL-09610, AM-19329, and HL-15949 and by grants from the Moss Heart Fund and the American Heart Association, Texas Affiliate Grant G155. Dr. Spady is also a recipient of the Clinical Investigator Award AM-01221 from the U.S. Public Health Service.

Manuscript received 4 March 1986 and in revised form 25 July 1986.

REFERENCES

1. Havel, R. J., J. L. Goldstein, and M. S. Brown. 1980. Lipoproteins and lipid transport. *In* Metabolic Control and Disease. P. K. Bondy and L. E. Rosenberg, editors. W. B. Saunders Company, Philadelphia. 393–494.
2. Pittman, R. C., T. E. Carew, A. D. Attie, J. L. Witztum, Y. Watanabe, and D. Steinberg. 1982. Receptor-dependent and receptor-independent degradation of low density lipoprotein in normal rabbits and in receptor-deficient mutant rabbits. *J. Biol. Chem.* **257:** 7994–8000.
3. Bilheimer, D. W., Y. Watanabe, and T. Kita. 1982. Impaired receptor-mediated catabolism of low density lipoprotein in the WHHL rabbit, an animal model of familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **79:** 3305–3309.
4. Kesaniemi, Y. A., J. L. Witztum, and U. P. Steinbrecher. 1983. Receptor-mediated catabolism of low density lipoprotein in man. Quantitation using glucosylated low density lipoprotein. *J. Clin. Invest.* **71:** 950–959.
5. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.
6. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Receptor-independent low density lipoprotein transport in the rat in vivo. Quantitation, characterization, and metabolic consequences. *J. Clin. Invest.* **76:** 1113–1122.
7. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
8. Stange, E. F., and J. M. Dietschy. 1983. Cholesterol synthesis and low density lipoprotein uptake are regulated independently in rat small intestinal epithelium. *Proc. Natl. Acad. Sci. USA.* **80:** 5739–5743.
9. Huettinger, M., W. J. Schneider, Y. K. Ho, J. L. Goldstein, and M. S. Brown. 1984. Use of monoclonal anti-receptor antibodies to probe the expression of the low density lipoprotein receptor in tissues of normal and Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* **74:** 1017–1026.
10. Spady, D. K., and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. USA.* **82:** 4526–4530.
11. Spady, D. K., E. F. Stange, L. E. Bilhartz, and J. M. Dietschy. 1986. Bile acids regulate hepatic low density lipoprotein receptor activity in the hamster by altering cholesterol flux across the liver. *Proc. Natl. Acad. Sci. USA.* **83:** 1916–1920.
12. Spady, D. K., J. B. Meddings, and J. M. Dietschy. 1986. Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. *J. Clin. Invest.* **77:** 1474–1481.
13. Kovanen, P. T., D. W. Bilheimer, J. L. Goldstein, J. J. Jaramillo, and M. S. Brown. 1981. Regulatory role for hepatic low density lipoprotein receptors in vivo in the dog. *Proc. Natl. Acad. Sci. USA.* **78:** 1194–1198.
14. Andersen, J. M., S. D. Turley, and J. M. Dietschy. 1979. Low and high density lipoproteins and chylomicrons as regulators of rate of cholesterol synthesis in rat liver in vivo. *Proc. Natl. Acad. Sci. USA.* **76:** 165–169.
15. Andersen, J. M., and J. M. Dietschy. 1977. Regulation of sterol synthesis in 15 tissues of rat. II. Role of rat and human high and low density plasma lipoproteins and of rat chylomicron remnants. *J. Biol. Chem.* **252:** 3652–3659.
16. Tanzawa, K., Y. Shimada, M. Kuroda, Y. Tsujita, M. Arai, and H. Watanabe. 1980. WHHL-rabbit: a low density lipoprotein receptor-deficient animal model for familial hypercholesterolemia. *FEBS Lett.* **118:** 81–84.
17. Shimada, Y., K. Tanzawa, M. Kuroda, Y. Tsujita, M. Arai, and Y. Watanabe. 1981. Biochemical characterization of skin fibroblasts derived from WHHL-rabbit, a notable animal model for familial hypercholesterolemia. *Eur. J. Biochem.* **118:** 557–564.
18. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
19. Pittman, R. C., S. R. Green, A. D. Attie, and D. Steinberg. 1979. Radiolabeled sucrose covalently linked to protein. A device for quantifying degradation of plasma proteins catabolized by lysosomal mechanisms. *J. Biol. Chem.* **254:** 6876–6879.
20. Mahley, R. W., K. H. Weisgraber, G. W. Melchior, T. L. Innerarity, and K. S. Holcombe. 1980. Inhibition of receptor-mediated clearance of lysine and arginine-modified lipoproteins from the plasma of rats and monkeys. *Proc. Natl. Acad. Sci. USA.* **77:** 225–229.
21. Koelz, H. R., B. C. Sherrill, S. D. Turley, and J. M. Dietschy. 1982. Correlation of low and high density lipoprotein binding in vivo with rates of lipoprotein degradation in the rat. A comparison of lipoproteins of rat and human origin. *J. Biol. Chem.* **257:** 8061–8072.
22. Munford, R. S., J. M. Andersen, and J. M. Dietschy. 1981. Sites of tissue binding and uptake in vivo of bacterial lipopolysaccharide-high density lipoprotein complexes.

Studies in the rat and squirrel monkey. *J. Clin. Invest.* **68:** 1503–1513.

23. Tonks, D. B. 1967. The estimation of cholesterol in serum: a classification and critical review of methods. *Clin. Biochem.* **1:** 12–29.
24. Innerarity, T. L., R. E. Pitas, and R. W. Mahley. 1980. Disparities in the interaction of rat and human lipoproteins with cultured rat fibroblasts and smooth muscle cells. *J. Biol. Chem.* **255:** 11163–11172.
25. Bilheimer, D. W., N. J. Stone, and S. M. Grundy. 1979. Metabolic studies in familial hypercholesterolemia. Evidence for a gene-dosage effect in vivo. *J. Clin. Invest.* **64:** 524–533.
26. Meddings, J. B., and J. M. Dietschy. 1986. Regulation of plasma low density lipoprotein-cholesterol levels: interpretation of LDL turnover data in man. *Circulation.* **74:** 805–814.
27. Brown, M. S., and J. L. Goldstein. 1979. Receptor-mediated endocytosis: insights from the lipoprotein receptor system. *Proc. Natl. Acad. Sci. USA.* **76:** 3330–3337.
28. Dietschy, J. M., T. Kita, K. E. Suckling, J. L. Goldstein, and M. S. Brown. 1983. Cholesterol synthesis in vivo and in vitro in the WHHL rabbit, an animal with defective low density lipoprotein receptors. *J. Lipid Res.* **24:** 469–480.