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D K Spady, … , S D Turley, J M Dietschy

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Research Article

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Receptor-independent Low Density Lipoprotein Transport in the Rat In Vivo

Quantitation, Characterization, and Metabolic Consequences

David K. Spady, Stephen D. Turley, and John M. Dietschy
Department of Internal Medicine, University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

Abstract

Receptor-independent low density lipoprotein (LDL) transport plays a critical role in the regulation of plasma cholesterol levels; hence, these studies were done to characterize this process in the tissues of the rat. High rates of receptor-independent clearance were found in the spleen, but other organs, like liver, gastrointestinal tract, and endocrine glands manifested lower clearance rates that varied from 3 to 9 μl/h per g, while the rates in nervous tissue, muscle, and adipose tissue were <1 μl/h per g. Receptor-dependent uptake was much higher in liver (85 μl/h per g) and adrenal gland (219 μl/h per g), but was also low in most other tissues. At normal plasma LDL concentrations, 67% of the receptor-dependent transport in the whole animal was accounted for by LDL uptake in the liver. In contrast, the receptor-independent uptake found in the whole animal took place in many organs, including skeletal muscle (20%), liver (16%), small bowel (15%), skin (10%), and spleen (7%). Furthermore, in liver, the rate of cholesterol synthesis could be varied 11-fold, yet the rate of receptor-independent LDL clearance remained constant at ~8 μl/h per g. When the circulating levels of LDL were systematically increased, receptor-independent LDL clearance also remained constant, so that hepatic LDL-cholesterol uptake by this mechanism increased linearly, from 1 to 20 μg/h per g, as the plasma LDL-cholesterol level was increased from 10 to 250 mg/dl. Finally, when equal amounts of LDL-cholesterol were delivered into the liver by either the receptor-dependent or receptor-independent mechanism, there was significant suppression of cholesterol synthesis and an increase in cholesteryl esters. Thus, in any situation in which receptor-dependent LDL degradation is lost, cholesterol balance in the whole animal and across individual organs is maintained by receptor-independent mechanisms, although when the new steady state is achieved, circulating levels of LDL must necessarily be very much increased.

Introduction

It is now recognized that low density lipoprotein (LDL)[1] is removed from the plasma by at least two distinct mechanisms that are generally described as receptor dependent and receptor independent. Despite marked variations in cholesterol turnover in different animal species (1, 2), the receptor-independent component plays an important and quantitatively similar role in the degradation of LDL in all animal species that have been examined, including the rat (25–43%) (3, 4), hamster (29%) (4), rabbit (24–38%) (5, 6), and guinea pig (21–22%) (7), as well as in man (20–33%) (8, 9). Furthermore, in animals or human subjects that lack LDL receptor activity, either because of an inherited defect in receptor synthesis (10, 11) or because of dietary suppression of receptor activity (12), this receptor-independent pathway must account for essentially all LDL turnover.

A limited number of studies are available, which suggests that nearly all organs in the body are capable of at least some degree of receptor-independent LDL uptake (6, 13, 14). Unfortunately, these studies have often yielded conflicting data with respect to the quantitative importance of this process because of the manner in which the measurements were made, or because of the use of heterologous LDL preparations or the use of in vitro cell systems. Thus, while the cell biology and physiology of receptor-dependent LDL turnover are understood in considerable detail (15, 16), there is relatively little quantitative data on the nature of receptor-independent LDL transport. Because of the potential importance of this process, the current studies were undertaken using a primed-continuous infusion technique to (*a*) quantitate the importance of receptor-independent LDL transport in the whole animal and in individual tissues of the rat; (*b*) define the kinetics of this uptake process; (*c*) examine possible mechanisms of regulation of receptor-independent transport; and (*d*) determine if cholesterol taken up by this pathway exerts the same metabolic effects as LDL taken up by the receptor-dependent pathway.

Methods

Animal preparation. Female Sprague Dawley-derived rats (CD [SD]BR, Charles River Breeding Laboratories, Inc., Wilmington, MA), purchased in the weight range of 125–150 g, were housed in an isolated room with alternating 12-h periods of light (1500–0300 h) and darkness (0300–1500 h), and were allowed free access to water and rodent diet (Wayne Laboratory Animal Diets, Allied Mills, Inc., Chicago, IL) for at least 2 wk before being used. In one experiment, groups of animals were fed either 3% (wt/wt) cholestyramine or 0.5% (wt/wt) cholesterol for 7 d before being used.

Lipoprotein preparations. Plasma was obtained from normal human subjects fasted overnight and from rats (groups of 25–300, depending upon the experiment) that had been maintained on the low-cholesterol rodent diet. Both rat LDL (rLDL) and human LDL (hLDL) were harvested in the density range of 1.020–1.055 g/ml, and purified as previously described (3, 14). On polyacrylamide gels, both LDL preparations contained essentially only apolipoprotein B_{100}. The rLDL and hLDL, as well as rat serum albumin (rSA) (Miles Laboratory, Inc., Elkhart, IN), were then labeled with either ^{125}I (17) or [^{14}C]sucrose (18). In some cases, a portion of the radiolabeled rLDL and hLDL was reductively methylated

Address correspondence to Dr. Spady, Gastroenterology Division.
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1. *Abbreviations used in this paper:* hLDL, human low density lipoprotein; methyl-hLDL, human LDL that has been reductively methylated; methyl-rLDL, rat LDL that has been reductively methylated; rLDL, rat LDL; rSA, rat serum albumin.

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(methyl-rLDL and methyl-hLDL) (19), which, by direct analysis, modified 77–88% of the free amino groups (20). All labeled lipoproteins were used within 12–24 h of preparation, and were passed through 0.45-μm Millipore filters just before being administered to the experimental animals.

Determination of whole-animal clearance rates. Whole-animal clearance (turnover) rates of the various lipoprotein preparations were measured using a constant infusion technique as previously described (3, 4). Each animal was fitted with a femoral vein catheter and administered a primed-continuous infusion of the labeled lipoprotein for 14 h. The rate of infusion of the labeled lipoprotein (dpm per hour) divided by the steady state plasma concentration of the same labeled molecule (dpm per microliter) gave the plasma clearance rate, which was expressed as the microliter of plasma cleared of a particular lipoprotein per hour. This value was then normalized to 100 g of body weight; hence, the whole-animal plasma lipoprotein clearance rates give the microliter of plasma cleared per hour per 100 g body weight (microliter per hour per 100 g).

Determination of lipoprotein tissue spaces, specific tissue spaces, and clearance rates. The rats were fitted with femoral vein catheters and placed in individual restraining cages. At the beginning of the studies, each animal was given a priming dose of a labeled lipoprotein or albumin, followed by a continuous infusion of the same protein fraction. In some studies, the infusion contained only trace amounts of the lipoprotein, but in others, mass quantities of unlabeled lipoprotein were also added to the infusates, so as to acutely elevate and maintain the circulating levels of LDL. Using the measured whole-animal clearance rates of each of these preparations, the amount of radioactivity (or lipoprotein mass) infused per hour was adjusted, relative to the dose given as a bolus, in order to maintain the specific activity of the radiolabeled protein in the plasma at a constant level throughout the period of infusion. Typically, 12–24 such animals were prepared, and then groups of 5–6 rats were killed at 10 min, 2 h, 4 h, and 6 h, and 15 different tissues were quickly removed. Aliquots of these organs, along with plasma samples, were solubilized and assayed for their ^{14}C content as previously described (21). The amount of LDL, methyl-LDL, or rSA present in a given tissue was then expressed in terms of the volume of plasma that would contain the same amount of the radiolabeled protein. Thus, the tissue space attained by each radiolabeled protein at each time point was calculated by dividing the radioactivity in 1 g of tissue (dpm per gram) by the steady state concentration of radioactivity in the plasma (dpm per microliter), and so has the units of microliters of plasma per gram of tissue (microliters per gram).

As illustrated diagrammatically in Fig. 1, these values for tissue spaces were used to calculate two sets of data. In many tissues of animals killed at 10 min, the tissue space of LDL was much higher than the tissue space achieved by rSA (Fig. 1 *A*). The difference in the two tissue spaces has been designated as the specific tissue space for that LDL preparation in that particular organ, and has the units of microliters per gram. Such specific tissue spaces have been shown to be saturable and regulable, and are essentially reduced to zero by reductive methylation of the lipoprotein (3, 21) and so were used in the present study as a measure of specific LDL binding to LDL transport sites.

In addition, rates of LDL transport were measured directly. As also shown in Fig. 1 *A*, under circumstances where the specific activity of the [^{14}C]sucrose-LDL preparation in the plasma was kept constant, the tissue space of LDL in many organs increased linearly with respect to time, as the lipoprotein was taken up by that tissue (14). The slope of the best-fit linear regression curve fitted to such data gives the rate of LDL clearance by that organ, expressed as the microliter of plasma cleared of its LDL content per hour per gram of tissue (microliter per hour per gram) (14). When multiplied by the concentration of LDL-cholesterol in the plasma (microgram per microliter), such data can also be expressed as the microgram of LDL-cholesterol taken up per hour by 1 g of that tissue (microgram per hour per gram). Finally, when such clearance rates are multiplied by whole-organ weights (normalized to a 100-g rat), the rates of clearance of LDL by the whole organ are obtained (microliter per hour per organ).

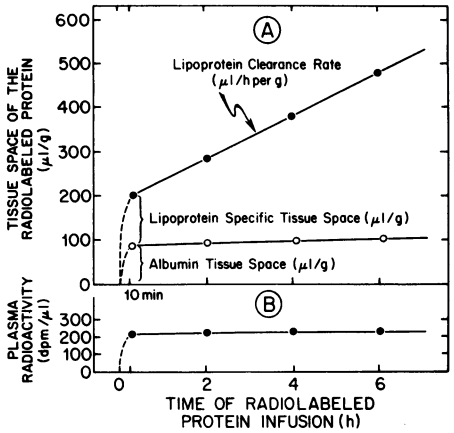

Figure 1. Diagrammatic representation of the protocol used in measuring the specific tissue space and clearance rate of labeled lipoproteins by the various tissues of the rat. As shown in *B*, a bolus of radiolabeled protein was administered intravenously at time zero, and was followed by a constant infusion of the same radiolabeled preparation for varying periods up to 6 h. Using the measured whole animal plasma clearance rate of each lipoprotein preparation, the amount of radioactivity infused per hour was adjusted relative to the dose given as a bolus in order to maintain the specific activity of the radiolabeled protein in the plasma at a constant level throughout the period of infusion. As shown in *A*, groups of animals were then killed at 10 min, 2 h, 4 h, and 6 h, and the plasma and 15 tissues were assayed for radioactive content. The radioactivity present in 1 g of each organ (dpm/g) was divided by the concentration of radiolabeled protein in the plasma (dpm/μl) to give the space of distribution achieved by each radiolabeled compound at each time point. The slope of the linear regression curves fitted to these values gives the rate of tissue uptake of the various test molecules expressed as the microliter of plasma cleared of its content of LDL or methyl-LDL each hour by 1 g of tissue. In *A*, typical curves are shown for a tissuelike liver in animals infused with either rLDL (●) or rSA (○).

Inherent in these calculations is the assumption that once taken up, the ^{14}C label is retained over a fairly long period of time (14, 18). A preliminary study, shown in Table I, was done to be certain that this

Table I. Loss of ^{14}C from Various Tissues of the Rat

	^{14}C loss after administration of	
	rLDL	Methyl-hLDL
	% per 6 h	*% per 6 h*
Liver	6.0±0.8	3.8±0.5
Ovary	3.5±0.6	2.5±0.5
Adrenal gland	2.0±0.3	3.0±0.3
Kidney	<1.0	<1.0
Spleen	3.0±0.3	3.3±0.4
Small bowel	16.0±3	11.0±2
Lung	6.0±0.8	5.5±0.7

Two groups of 25 rats each were administered intravenously a constant dose of either [^{14}C]sucrose-rLDL or [^{14}C]sucrose-methyl-hLDL. Groups of five animals were then killed every 24 h for 5 d (beginning 24 h after the dose of rLDL and 48 h after the methyl-hLDL dose), and the content of ^{14}C in seven organs was determined. The rate of disappearance of the ^{14}C in each organ over the 5-d period was determined and is presented in this table as the percentage of ^{14}C lost per 6 h. Mean values±1 SEM for data obtained in 25 animals are shown.

was, in fact, the case for LDL that was taken up into various tissues by either predominantly receptor-mediated (rLDL) or receptor-independent (methyl-hLDL) mechanisms. As shown in Table I, regardless of the mechanism of uptake, only ~2–6% of the ^{14}C was lost from the tissues over a 6-h interval. The single exception was the intestine which, because of its high cell turnover, lost significantly greater amounts of the radioactive label.

Determination of rates of cholesterol synthesis. In two studies, rates of cholesterol synthesis were measured in the livers of animals that had also been infused with [^{14}C]sucrose-LDL for measurement of lipoprotein clearance rates. At the completion of the infusions, aliquots of liver were taken and incubated in vitro with [^{3}H]water (1, 22, 23), and the rates of sterol synthesis were expressed as the nanomole of this precursor incorporated into digitonin precipitable sterols per hour per gram of tissue (nanomole per hour per gram).

Calculations. In most cases, data are presented as mean values±1 SEM. Clearance rates were determined by using the least-squares method to fit linear regression curves to the data describing the change in tissue space as a function of time (see Fig. 1), and are presented as mean values±1 SE.

Results

These studies were designed to provide quantitative data on the characteristics of receptor-independent LDL transport, and to compare the characteristics of this transport process with those of the receptor-dependent system. Two sets of preliminary studies were necessary, however, in order to establish that, in general, [^{14}C]sucrose-labeled LDL preparations accurately reflected the biological behavior of this class of lipoproteins, and, in particular, that [^{14}C]sucrose-methyl-hLDL was transported only by receptor-independent mechanisms.

Validation of the use of [^{14}C]sucrose-labeled LDL. Previously published studies have shown that [^{14}C]sucrose-labeled LDL generally reflects the behavior of LDL labeled with other isotopes such as ^{125}I (14, 24). However, because of the quantitative nature of the present studies, additional detailed comparisons were felt to be necessary. Initial experiments were performed to compare the specific binding characteristics in vivo of LDL preparations labeled with either ^{125}I or [^{14}C]sucrose. As shown in Fig. 2, the tissue spaces achieved by ^{125}I-rLDL and [^{14}C]sucrose-rLDL (Fig. 2 *A*) varied from just over 800 μl/g in the adrenal gland to much lower values in other organs. Most importantly, however, the values obtained in any organ were essentially identical when measured using LDL that was labeled with either isotope. This was true in the endocrine glands, spleen and small bowel, and in 10 other tissues not shown in Fig. 2. Only in the liver was the tissue space achieved with [^{14}C]sucrose-rLDL slightly less than that achieved with ^{125}I-rLDL. The tissue spaces measured with ^{125}I-rSA and [^{14}C]sucrose-rSA were, however, the same in all 15 organs (Fig. 1 *B*). Hence, when these data were used to calculate specific tissue spaces for rLDL, the ^{125}I- and [^{14}C]sucrose-rLDL preparations gave essentially identical results, as shown in Fig. 3 (Fig. 3 *A*), which suggested that both preparations bound equally well to the LDL receptors present in varying quantities in the different tissues. Furthermore, the specific tissue spaces achieved in the rat with hLDL were identical when measured with either ^{125}I-hLDL or [^{14}C]sucrose-hLDL, although the rat LDL receptors were capable of binding far less hLDL than rLDL, as is also evident in Fig. 3 *B*.

A comparison was also made of the amount of specific LDL binding that was evident using ^{125}I- and [^{14}C]sucrose-labeled LDL in rats treated with drugs known to alter rates of LDL turnover

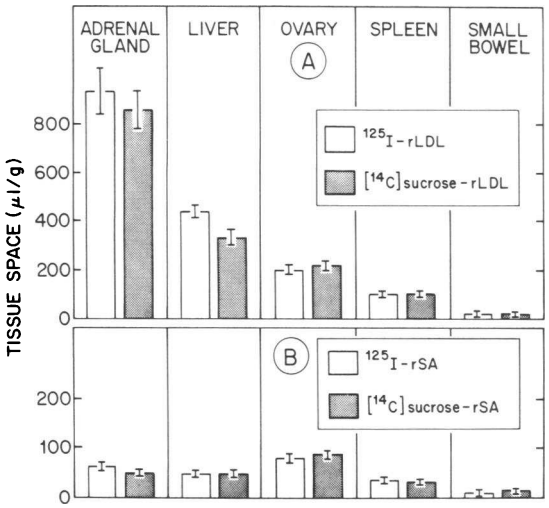

Figure 2. Comparison of the tissue spaces achieved by rLDL and rSA in the liver and various extrahepatic tissues when labeled with either [^{14}C]sucrose or ^{125}I. Groups of rats were killed 10 min after the intravenous administration of either [^{14}C]sucrose-rLDL, ^{125}I-rLDL, [^{14}C]sucrose-rSA, or ^{125}I-rSA, and the space achieved by each protein in 15 tissues, only five of which are shown, was determined. Each value represents the mean±1 SEM of data obtained in six animals.

in certain organs. As shown in Fig. 4, *A* and *B*, for example, treatment with dexamethasone selectively decreased specific LDL binding in the adrenal gland, and this reduction was quantitatively identical when measured with rLDL labeled with either isotope. Similarly, administration of ethinyl estradiol to the animals increased the binding of both ^{125}I-rLDL and [^{14}C]sucrose-rLDL to the endocrine glands and liver, but not to the spleen (Fig. 4 *C*).

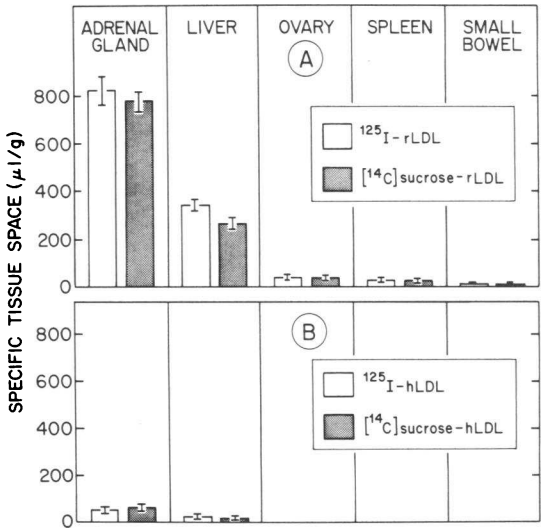

Figure 3. Comparison of the specific tissue spaces achieved by rLDL and hLDL in the liver and various extrahepatic tissues when labeled with either [^{14}C]sucrose or ^{125}I. Groups of rats were killed 10 min after the intravenous administration of either [^{14}C]sucrose-rLDL, ^{125}I-rLDL, [^{14}C]sucrose-hLDL, or ^{125}I-hLDL, and the specific tissue space achieved by each protein in 15 tissues, only five of which are shown, was determined. Each value represents the mean±1 SEM of data obtained in six animals.

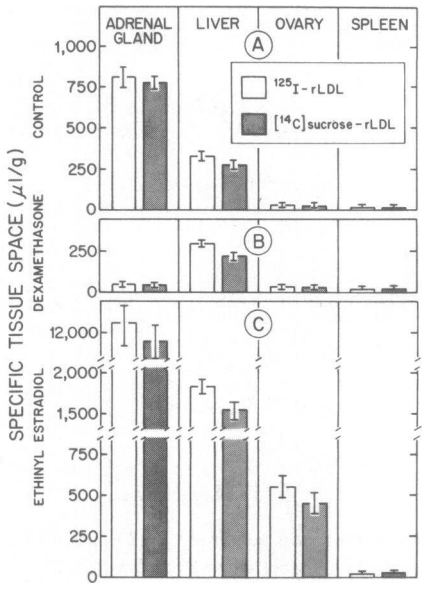

Figure 4. Comparison of the specific tissue spaces achieved by rLDL labeled with either [^{14}C]sucrose or ^{125}I in the liver and various extrahepatic tissues of rats treated with dexamethasone or ethinyl estradiol. Groups of rats were administered either 1 mg of decadron-LA or 1 mg of 17α-ethinyl estradiol in propylene glycol subcutaneously each day for 5 d, while control animals were given only buffer solution. They were then killed 10 min after the intravenous administration of either [^{14}C]sucrose-rLDL or ^{125}I-rLDL, and the specific tissue spaces were determined. Each value represents the mean±1 SEM of data obtained in six animals.

Finally, a comparison was also made of whole-animal clearance rates of both rLDL and hLDL when labeled with either ^{125}I- or [^{14}C]sucrose. As is evident in Table II, in studies run in parallel, the turnover of [^{14}C]sucrose-rLDL in the whole animal was slightly slower (659 μl/h per 100 g) than the turnover of ^{125}I-rLDL (731 μl/h per 100 g). The clearance rates of hLDL were much lower than those of rLDL, but were essentially identical when measured with either ^{125}I-hLDL (241 μl/h per 100 g) or [^{14}C]sucrose-hLDL (247 μl/h per 100 g).

Table II. Whole-Animal Clearance Rates in the Rat of LDL of Rat and Human Origin Labeled with either ^{125}I or [^{14}C]Sucrose

Lipoprotein fraction infused	Whole-animal clearance rate μl/h per 100 g
^{125}I-rLDL (5)	731±28
[^{14}C]Sucrose-rLDL (5)	659±17
^{125}I-hLDL (6)	241±9
[^{14}C]Sucrose-hLDL (6)	247±7

Rats were fitted with femoral vein catheters and infused for 14 h with a solution containing trace quantities of either rLDL or hLDL labeled with ^{125}I or [^{14}C]sucrose. At the end of this time, when the concentration of ^{125}I or [^{14}C]sucrose in the plasma had achieved constant values, the clearance rates were determined. These are given as the microliter of plasma cleared of a particular lipoprotein per hour per 100 g of body weight. Each value is the mean±1 SEM of data obtained in the number of animals shown in parentheses.

Thus, from these various studies, it was concluded that LDL of either rat or human origin labeled with [^{14}C]sucrose behaved nearly identically to the respective LDL preparation labeled with ^{125}I. This was true with respect to the distribution of specific LDL binding sites in the different tissues of the rat and the effect of drugs on these binding sites, as well as to the rates of degradation of rLDL and hLDL in the live animal. The only exception to this was the slightly lower level of binding of the [^{14}C]sucrose-rLDL to receptors in the liver (Fig. 3), and the slightly lower whole-animal clearance rate of this same LDL preparation. Since the liver is the most important organ in determining LDL turnover, the lower clearance rate of the [^{14}C]sucrose-rLDL presumably reflects the slightly lower level of hepatic LDL binding of this ^{14}C-labeled molecule.

Use of methyl-hLDL for measuring receptor-independent transport. The next study was undertaken to select the most suitable molecule for measuring rates of receptor-independent LDL transport. Previous work using rat fibroblasts (25) and the intact animal (3, 19, 26) has shown that both heterologous LDL and methyl-rLDL interact poorly with the LDL receptor in the rat. Thus, quantitative data were next obtained in vivo on the ability of four different [^{14}C]sucrose-labeled LDL preparations to specifically bind to the liver and to be cleared from the plasma space. As summarized in Table III, rLDL manifested a high degree of tissue binding and was rapidly cleared from the plasma at a rate of 695 μl/h per 100 g. Reductive methylation of the rLDL markedly reduced, but did not abolish, the tissue binding and whole-animal clearance. Human LDL also bound poorly to the liver, and was cleared from the plasma at a rate of 240 μl/h per 100 g. However, methylated hLDL manifested no specific tissue binding, i.e., the tissue spaces attained by methyl-hLDL and rSA were essentially equal, and was cleared from the plasma at the minimal rate of 186 μl/h per 100 g. Note that in rabbits lacking LDL receptors, i.e., the WHHL rabbit, the rate of homologous LDL and methyl-hLDL transport into tissues was essentially equal (unreported observation from this laboratory). Thus, taken together, all of these observations indicate that methyl-hLDL is taken up into tissues by mechanisms that do not depend upon the LDL receptor, and, therefore, this molecule is ideally suited for exploring the kinetics and metabolic consequences of receptor-independent LDL transport.

Rates of receptor-independent LDL transport in control rats.

Table III. Comparison of Specific Hepatic Binding and Whole-Animal Clearance Rates of LDL of Rat and Human Origin

Lipoprotein fraction infused	Specific hepatic tissue space μl/g	Whole-animal clearance rate μl/h per 100 g
rLDL	265±20	695±24
Methyl-rLDL	22±4	253±11
hLDL	12±4	240±15
Methyl-hLDL	0	186±10

Identical groups of rats were used for the measurement of specific LDL tissue spaces in the liver, and whole-animal clearance rates. These measurements were made the same day using [^{14}C]sucrose-labeled rLDL, methyl-rLDL, hLDL, and methyl-hLDL. Each value is the mean±1 SEM of data obtained in six animals.

Having chosen methyl-hLDL as the probe for receptor-independent LDL transport, the next group of studies was undertaken to measure the rates of both total LDL uptake (rLDL) and receptor-independent LDL uptake (methyl-hLDL) in the various tissues of control rats fed a low-cholesterol diet. As shown in Table IV, the highest rates of rLDL uptake in this species (Table IV, column 1) were found in the adrenal gland (222 μl/h per g), ovary (117 μl/h per g), liver (93 μl/h per g) and spleen (81 μl/h per g), while the lowest rates occurred in fat (1.3 μl/h per g), skin (0.9 μl/h per g), skeletal muscle (0.8 μl/h per g), and brain (<0.1 μl/h per g). The receptor-independent component of this uptake process (Table IV, column 2) was highest in the spleen (56 μl/h per g), but in the majority of other tissues equaled <10 μl/h per g.

From the difference between total and receptor-independent LDL uptake, rates of receptor-dependent LDL transport could be calculated from these values and are expressed as a percentage of total LDL uptake in Table IV, column 3. The receptor-dependent component accounted for the majority of the high rates of LDL uptake observed in organs like the endocrine glands and liver, while rates of rLDL and methyl-hLDL were essentially identical in tissues like heart, stomach, fat, skin, skeletal muscle, and brain, which indicated that the very low rates of LDL transport observed in these tissues could be accounted for entirely by receptor-independent mechanisms. Between these two extremes were tissues like spleen, and, particularly, the gastrointestinal tract, where both receptor-dependent and receptor-independent mechanisms contributed significantly to LDL uptake.

Table IV. Clearance Rates of rLDL and Methyl-hLDL Per Gram of Each Tissue in the Rat

	(1) rLDL clearance	(2) Methyl-hLDL clearance	(3) Receptor-dependent LDL uptake
	μl/h/g	μl/h/g	%
Adrenal gland	222.0±13	2.8±0.7	98.7
Ovary	117.0±7	6.1±0.7	94.8
Liver	93.0±4	7.8±0.5	91.6
Spleen	81.0±4	56.0±2.0	30.9
Kidney	28.0±2	4.8±0.5	82.9
Ileum	21.0±1	9.0±0.6	57.1
Jejunum	16.0±1	7.0±0.4	56.3
Lung	7.9±1	1.5±0.7	81.0
Colon	4.3±0.2	2.8±0.5	34.8
Heart	3.7±0.2	4.3±0.2	~0
Stomach	2.3±0.3	2.2±0.5	~0
Fat	1.3±0.1	1.2±0.1	~0
Skin	0.9±0.1	1.0±0.1	~0
Skeletal muscle	0.8±0.1	0.8±0.1	~0
Brain	<0.1	<0.1	~0
Urine	12.0±2	11.0±2.0	—
Bile	62.0±6	9.3±1.0	—

Using animals maintained on low-cholesterol control diet, groups of 12 rats were infused with tracer amounts of [^{14}C]sucrose-labeled rLDL or methyl-hLDL. Four animals from each group were killed 2, 4, and 6 h after beginning the infusions, and the spaces of distribution of the lipoprotein preparations were measured. The data from each organ in the two groups of 12 animals was used to calculate the clearance rates of the two preparations of LDL, and these rates are expressed as the microliter of plasma cleared of its LDL content per hour by 1.0 g of tissue. Column 1 shows the rate of total LDL uptake (receptor dependent and receptor independent), while column 2 gives the rate of receptor-independent uptake. In a parallel experiment, a group of rats was also prepared with indwelling catheters in the bladder and common bile duct, and then they were infused with the two LDL preparations. The rates of clearance of the degradation products of the [^{14}C]sucrose into urine and bile were thus determined and are also shown in this table.

Rates of clearance of rSA were also measured in these same organs, and in most cases were essentially equal to the rates of clearance observed with methyl-hLDL. In organs like kidney (16 μl/h per g) and lung (15 μl/h per g), however, the clearance of rSA was significantly higher than that of methyl-hLDL, while in small intestine (4 μl/h per g) it was lower.

At the same time that these studies were undertaken, whole-animal clearance rates were measured with the two LDL preparations. Since total rLDL clearance in the animals equaled 659 μl/h per 100 g, and receptor-independent clearance was 180 μl/h per 100 g, receptor-dependent degradation must have equaled 479 μl/h per 100 g. Taking into consideration the rates of LDL transport in each tissue (Table IV) and the weights of individual organs, it was next possible to determine the importance of each major organ to receptor-dependent and receptor-independent LDL degradation in the whole animal. As summarized in Table V, the liver and small bowel accounted for 53 and 9.7%, respectively, of total LDL turnover in these rats, while degradation in each of the other organs was quantitatively small (Table V, column 3). The receptor-independent component of LDL clearance was distributed in a number of tissues, since, of the 180 μl of plasma cleared of LDL in the whole animal by this mechanism, 20, 16, 15, and 10% took place in skeletal muscle, liver, small intestine, and skin, respectively (Table V, column 5). In contrast, of the 479 μl of plasma cleared of its LDL content by the receptor-dependent mechanism, fully 67% took place in the liver alone. Except for the small intestine (7%), receptor-dependent LDL transport was quantitatively unimportant in the other organs. As an aside, it should also be noted that the sum of the clearance rates found in all of the organs listed in Table V equaled 88% of the clearance rates measured simultaneously in whole animals, and, furthermore, the degradation and loss of the [^{14}C]sucrose label in urine and bile equaled only 5.8% of the whole-animal clearance rate during the period of observation (Table V, column 3).

Kinetic characteristics of receptor-independent LDL transport. While these data provided information on the relative importance of receptor-independent LDL transport in the liver and other organs, they applied only to the rat with normal circulating LDL-cholesterol levels. In the next experiment, the hepatic clearance rates were measured, under circumstances where the plasma concentration of methyl-hLDL was abruptly increased to a new value, and then maintained at that value using the primed-continuous infusion technique. As shown in Fig. 5 *A*, under these conditions, receptor-independent LDL clearance by the liver remained constant at 7–9 μl/h per g, as the plasma LDL-cholesterol level was raised from ~10 mg/dl to nearly 250 mg/dl. Thus, as shown in Fig. 5 *B*, the delivery of cholesterol into the liver by this mechanism was increased from ~1 μg/h per g to nearly 20 μg/h per g. In animals with normal plasma LDL-cholesterol concentrations, ~9 μg of LDL-cholesterol was taken up per hour per gram of liver (Fig. 5 *B*) and this rate is achieved because of the high, receptor-mediated clearance rate (Fig. 5 *A*) present in the normal liver. It should be noted, however,

Table V. Clearance Rates of rLDL and Methyl-hLDL by Each Whole Organ of the Rat

	(1) Organ weight per 100 g animal (g/100 g)	(2) rLDL clearance per organ	(3) Fraction of whole-animal rLDL clearance	(4) Methyl-hLDL clearance per organ	(5) Fraction of whole-animal methyl-hLDL clearance
		μl/h per organ	*%*	*μl/h per organ*	*%*
Liver	3.78	352.0	53.0	29.0	16.0
Skeletal muscle	45.50	36.0	5.5	36.0	20.0
Ileum	1.57	33.0	5.0	14.0	7.8
Jejunum	1.91	31.0	4.7	13.0	7.2
Kidney	0.76	21.0	3.2	3.6	2.0
Spleen	0.21	17.0	2.6	12.0	6.7
Skin	18.00	16.0	2.4	18.0	10.0
Fat	9.08	12.0	1.8	11.0	6.1
Adrenal gland	0.03	6.7	1.0	0.1	<0.1
Lung	0.66	5.2	0.8	1.0	0.5
Colon	1.22	5.2	0.8	3.4	1.9
Ovary	0.04	4.7	0.7	0.2	0.1
Heart	0.35	1.3	0.2	1.5	0.8
Stomach	0.54	1.2	0.2	1.2	0.7
Brain	0.55	<0.1	<0.1	<0.1	<0.1
Bile	0.44	27.0	4.1	4.1	2.3
Urine	0.95	11.0	1.7	10.5	5.8
Total		580	87.7	159	87.9

The values in columns 2 and 4 equal the product of the clearance rate for rLDL and methyl-hLDL shown in Table IV (μl/h per g) and the mean whole organ weight (per 100 g animal, column 1), and are expressed as the microliter of plasma cleared of its LDL content per hour by each whole organ, per 100 g body weight. In columns 3 and 5 the clearance of rLDL and methyl-hLDL by each whole organ is expressed as a percentage of the corresponding whole-animal clearance rate. The whole-animal clearance rates were measured in separate groups of animals the same day, and equaled 659 μl/h per 100 g body weight and 180 μl/h per 100 g body weight for rLDL and methyl-hLDL, respectively.

that in the absence of LDL receptors, just as much, or more, LDL cholesterol could be taken up by the liver using the receptor-independent pathway, provided that the plasma LDL-cholesterol concentration was elevated to sufficiently high levels.

Regulation of receptor-independent LDL transport. While alterations in cellular cholesterol content are known to effect receptor-dependent LDL transport (4, 10, 14), little information is available on the effect of such manipulations on receptor-independent transport. Hence, rates of hepatic cholesterol synthesis were varied by either cholestyramine or cholesterol feeding, and receptor-independent LDL clearance was measured. As summarized in Table VI, the clearance of methyl-hLDL was constant at ~7–9 μl/h per g under circumstances where the rate of hepatic sterol synthesis was varied from 89 to 1,007 nmol/h per g. Thus, hepatic receptor-independent LDL clearance remained constant not only under circumstances where the plasma LDL concentration was varied, but also where the rates of hepatic sterol synthesis were varied.

Regulatory effects of receptor-independent LDL-cholesterol uptake. A final group of studies was done to determine if LDL-cholesterol taken up by receptor-independent LDL transport was equally effective in regulating cellular cholesterol synthesis as that taken up by the receptor-dependent process. Using the primed-continuous infusion technique, varying amounts of LDL-cholesterol, carried in either rLDL or methyl-hLDL, were delivered to the liver over a 14-h period. As shown in Fig. 6 *A*, after 14 h of infusion, the cumulative hepatic clearance of rLDL equaled ~1,260 μl/g, while that of methyl-hLDL averaged 120 μl/g. Thus, even over this prolonged period of infusion, the hourly clearance rates of rLDL (92% receptor dependent) and methyl-hLDL (100% receptor independent) remained essentially linear with respect to time, and equaled ~90 μl/h per g and 9 μl/h per g, respectively. From these cumulative clearance data, the mass of LDL-cholesterol taken up by the liver over the 14-h infusion period was calculated and is shown in Fig. 6 *B*.

From these data, it was apparent that ~350 μg of LDL-cholesterol could be delivered to each gram of liver by elevating

Table VI. Rates of Receptor-independent LDL Clearance by the Livers of Rats with Various Rates of Hepatic Cholesterol Synthesis

Experimental group	Hepatic cholesterol synthesis	Hepatic receptor-independent LDL clearance
	nmol/h/g	*μl/h/g*
Control (5)	435±29	9.5±1.8
Cholestyramine (4)	1,007±86	8.5±1.9
Cholesterol (4)	89±28	7.2±1.1

Groups of rats that had been fed diets containing either 3.0% cholestyramine or 0.5% cholesterol for 7 d, as well as control animals, were infused with trace quantities of methyl-hLDL for determination of hepatic clearance rates. Aliquots of the livers were also taken for determination of rates of hepatic cholesterol synthesis in vitro. Each value is the mean±1 SEM of data obtained in the number of animals shown in parentheses.

and maintaining the plasma rLDL-cholesterol concentration to ~25 mg/dl, and the methyl-hLDL-cholesterol concentration to ~360 mg/dl over the 14-h infusion period. Such infusions were carried out in an additional 23 animals, and the results are summarized in Table VII. In this experiment, as shown in Table VII, column 1, essentially equal amounts of LDL-cholesterol were taken up by the liver, as determined directly by the uptake of the [^{14}C]sucrose marker, and this resulted in a similar degree of suppression of the rate of hepatic cholesterol synthesis (Table VII, column 2). Furthermore, the content of cholesteryl esters increased 66 and 140% in the animals infused with rLDL and methyl-hLDL, respectively. Thus, when nearly equal amounts of sterol were taken up by either the receptor-dependent or receptor-independent pathway, there was significant suppression of *de novo* cholesterol synthesis and a rise in the level of hepatic cholesteryl esters.

Discussion

Receptor-independent LDL degradation accounts for the turnover of ~20–40% of the circulating LDL pool in normal animals and man (3–9), and for essentially 100% of the pool in the rabbit and human lacking LDL receptors (5, 10). As shown in the present studies, nearly every tissue in the body manifests at least some level of receptor-independent LDL transport, which, like receptor-dependent LDL uptake, contributes cholesterol to the intracellular pools and leads to feedback regulation of the rate of cholesterol synthesis. Thus, most organs can potentially acquire sterol from at least three different sources including *de novo* cholesterol synthesis within the cell and the receptor-dependent and receptor-independent uptake of LDL-cholesterol. While the interaction of these three processes are remarkably effective in maintaining sterol balance across individual organs under even pathological conditions, this balance is often achieved at the expense of potentially harmful shifts in the concentration of LDL in the circulating plasma.

Table VII. Inhibition of Hepatic Cholesterol Synthesis by LDL-Cholesterol Taken Up by Either Receptor-dependent or Receptor-independent Mechanisms

Experimental group	(1) Hepatic LDL-cholesterol uptake	(2) Hepatic cholesterol synthesis
	μg/g	*nmol/h/g*
Control (12)		356±35
rLDL infused (5)	353±22	124±21
Methyl-hLDL infused (6)	381±22	154±16

As described in the legend to Fig. 6, these animals were infused with either rLDL (a bolus of 2.5 mg followed by 0.5 mg of LDL-cholesterol per hour) or methyl-hLDL (a bolus of 40 mg followed by 1.7 mg of LDL-cholesterol per hour) for 14 h. At the end of this time, the animals were killed and the content of ^{14}C in each liver was determined. From this value, and the specific activity of the [^{14}C]sucrose-LDL preparation infused (dpm per microgram of LDL cholesterol), the amount of cholesterol taken up by the liver from the two LDL preparations was calculated (column 1). Aliquots of these same livers were also assayed for rates of cholesterol synthesis in vitro (column 2). Mean values±1 SEM for the number of animals shown in parentheses are given.

Because of the importance of the interaction of these three processes, the present studies were undertaken to provide detailed, quantitative data on the characteristics of receptor-independent LDL transport in the whole animal and in individual organs of the rat. There are three technical features of these studies that should be emphasized. First, homologous, purified LDL (rLDL) was used to measure rates of total LDL clearance in the whole animal and in individual organs. It is now recognized that serious quantitative errors in assessing the importance of receptor-independent LDL transport in vivo and in isolated cells have resulted from measuring total LDL clearance with heterologous lipoprotein preparations, which interact poorly with the LDL receptor. Second, at least in the rat, reductive methylation alone does not totally abolish interaction of rLDL with the LDL receptor (Table III). However, reductive methylation of heterologous LDL (methyl-hLDL) does eliminate all specific binding and reduces whole-animal turnover to a minimal value. Thus, methyl-hLDL was used to quantitate receptor-independent LDL transport in these studies. Third, uptake rates were measured using LDL preparations labeled with [^{14}C]sucrose, which is retained by the tissues for fairly long periods of time after uptake of the LDL molecule (13, 18). Such [^{14}C]sucrose-labeled LDL behaves nearly identically with ^{125}I-labeled lipoprotein (Table II, and Figs. 2–4). By using such labeled preparations with the primed-continuous infusion technique, it was possible to measure absolute rates of tissue clearance under circumstances where the rate of tissue uptake was linear with respect to time and independent of plasma levels of radioactivity, and at sufficiently short intervals to minimize differences in rates of tissue degradation and loss of the ^{14}C-label (Table I). Note that, using this technique, the sum of the rates of clearance of the various LDL preparations by all major organs in the body essentially equalled the rate of whole-animal turnover (Table V and reference 14).

From the data derived using this technique, a number of important conclusions can be drawn with respect to both receptor-independent and receptor-dependent LDL transport in the various organs of the rat. As in the hamster (14), the rates of total LDL clearance by the various organs varied over a 200-fold range when expressed per gram of tissue. The highest rates were found in the endocrine glands, liver, spleen, kidney, and small intestine, while very low rates were encountered in adipose tissue, skin, and skeletal muscle (Table IV). The receptor-independent component of this uptake process was high in spleen, but much lower in most other organs. As a consequence of these differences it is apparent that in nearly all of those organs that manifested rapid LDL uptake, the high rates of clearance were achieved through the presence of large amounts of LDL receptor activity. Thus, receptor-dependent LDL uptake accounted for most of the total LDL uptake observed in the adrenal gland (99%), ovary (95%), liver (92%), kidney (83%), and lung (81%). The two exceptions were the small intestine (56%) and spleen (31%), both of which had large components of receptor-independent uptake. In all other tissues in the body, rates of LDL uptake were very low, and no receptor-dependent component could be identified (Table IV). Note that previous reports which suggested that receptor-independent transport was quantitatively much more important in organs like liver (13, 27) were probably the result of using heterologous LDL preparations that grossly underestimated the receptor-dependent component.

More importantly, these data can also be viewed in terms of the role of each of the whole organs in receptor-dependent and receptor-independent LDL degradation. In the normal rat,

for example, receptor-dependent LDL clearance in the whole animal equalled ~479 μl/h per 100 g body weight, and most of this activity was found in the liver (67%) and small intestine (8%). Furthermore, the value in this latter tissue may have been significantly underestimated because of more rapid loss of the ^{14}C-label from the intestinal epithelial cells (Table 1). Receptor-dependent LDL uptake in the remaining organs was quantitatively of little importance. In contrast, receptor-independent LDL clearance in the whole animal equaled 180 μl/h per 100 g body weight, and this activity was distributed in a number of organs including skeletal muscle (20%), liver (16%), small intestine (15%), skin (10%), spleen (7%), and adipose tissue (6%). Thus, the observation that the liver is the major organ for LDL removal from the plasma in the normal rat (53%), hamster (73%) (14), and, probably, man (11), is due to the fact that the liver contains most of the receptor-dependent transport activity found in the whole body.

However, these quantitative relations apply only to the experimental animal and to man when the circulating LDL-cholesterol concentration is normal. Since receptor-mediated LDL clearance involves interaction with a finite number of binding sites on the cell surface (15), receptor-mediated transport in organs like the liver manifests saturation kinetics (12, 14). As demonstrated in these studies, the rate of receptor-independent LDL uptake is a linear function of plasma LDL concentration, so that the rate of clearance by this mechanism is constant over a very large range of plasma lipoprotein concentrations (Figs. 5 and 6). Thus, when the plasma LDL-cholesterol concentration is raised, e.g., by increasing LDL production, it necessarily follows that the receptor-independent component of transport becomes quantitatively more important in every organ, as well as in the whole animal. The same would be true if receptor activity were reduced, either for genetic reasons (10) or because of suppression by dietary components (12). In fact, in the complete absence of LDL receptor activity, receptor-independent LDL transport presumably accounts for all LDL uptake by the various tissues of the body. Under these circumstances, there is a major shift in the quantitative importance of the different organs for the degradation of LDL. Rather than the liver accounting for 53 and 73%, respectively, of whole-animal LDL degradation, as is true in the normal rat and hamster (14), tissues like skeletal muscle, the gastrointestinal tract, spleen, and adipose tissue assume a far more important role in degrading LDL when receptor activity is suppressed or absent.

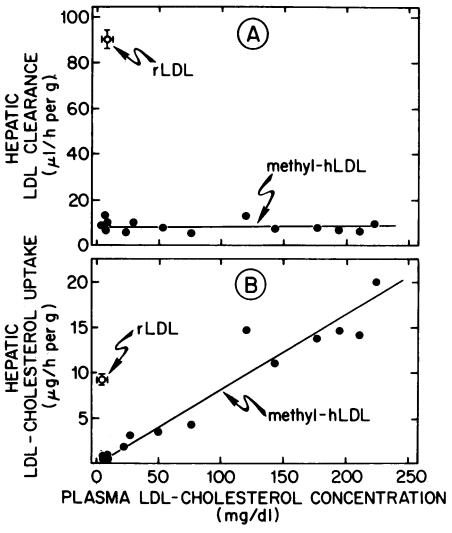

Figure 5. Relationship between rates of receptor-independent LDL transport by the liver and plasma LDL-cholesterol concentrations. Groups of animals received a primed-continuous infusion of varying mass amounts of [^{14}C]sucrose-methyl-hLDL for 6 h. Rates of methyl-hLDL clearance (*A*) and methyl-hLDL-cholesterol uptake by the liver were then determined in each animal and are plotted as a function of the steady state plasma LDL-cholesterol concentration in the same rat. Also shown are the rates of rLDL clearance and rLDL-cholesterol uptake by the liver of animals infused with trace amounts of rLDL. These latter values represent the means±1 SEM of data obtained in six animals.

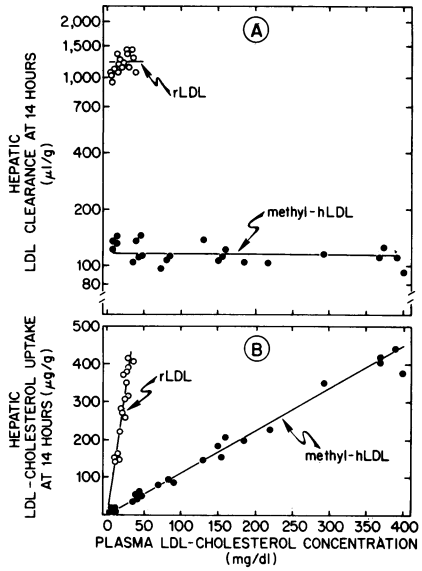

Figure 6. Relationship between rates of rLDL and methyl-hLDL transport in the liver and the plasma LDL-cholesterol concentrations. Groups of animals received a primed-continuous infusion of varying amounts of [^{14}C]sucrose-labeled rLDL or methyl-hLDL for 14 h. Rates of LDL clearance (*A*) and LDL-cholesterol uptake (*B*) of the two preparations by the liver were then calculated for each animal and are plotted as a function of the steady state plasma LDL-cholesterol concentration. Each point represents the result obtained in an individual animal.

A question of critical importance, however, is whether LDL-cholesterol taken up into the liver and other tissues by the receptor-independent pathway enters into the same intracellular pools as sterol transported via the LDL receptor, and whether it is capable of being a substrate for the esterification reaction and of acting as a regulator of the rate of intracellular cholesterol synthesis. Although earlier studies suggested that cholesterol taken up by receptor-independent means might not exert metabolic regulation (reviewed in reference 28), several lines of evidence suggest that this conclusion is not warranted, at least in vivo. In liver, for example, as much LDL-cholesterol could be delivered to the hepatocytes by the receptor-independent pathway as by the receptor-dependent system, although much higher plasma LDL levels were required (Fig. 6). Under these circumstances, there was comparable suppression of cholesterol synthesis and elevation in the levels of cholesteryl esters (Table VII). In other studies, the infusion of large amounts of hLDL, which in the rat is taken up predominately by the receptor-independent pathway, clearly inhibited cholesterol synthesis and raised the

level of cholesterol esters in the liver (29), and suppressed the rates of sterol synthesis in most extrahepatic organs (30). Furthermore, in the rabbit lacking LDL receptor activity, the rates of cholesterol synthesis in nearly all of the organs in the body essentially equalled the rates found in the normal animal (31). Thus, LDL-cholesterol taken up by the receptor-independent pathway can regulate intracellular metabolism.

Finally, the receptor-independent LDL uptake quantitated in the various organs in this study presumably reflected the presence of this transport process in the parenchymal cells of each of these tissues rather than uptake in a second set of cells, such as the vascular endothelium or tissue macrophages or fibroblasts. In the intestine and liver, at least, an essentially identical pattern of receptor-independent and receptor-dependent LDL uptake was found after isolation and partial purification of the respective parenchymal cells (6, 32; and D. K. Spady and J. M. Dietschy, unpublished observations), as was found in the whole organs (Table IV). The kinetics of this process, its lack of regulability and the fact that the clearance rates of albumin and methyl-hLDL were similar in most tissues, suggests that receptor-independent LDL transport may be mediated by a mechanism like fluid endocytosis. The occasional differences seen in the uptake rates of the different probe molecules could easily be explained by differences in the sieving effects of the endothelial diffusion barriers, or by charge or nonsolvent water effects within the endocytotic vesicles. Nevertheless, it is clear that the molecular mechanisms responsible for receptor-independent LDL transport require further work.

Thus, these studies demonstrate that nearly every organ in the body is capable of taking up LDL-cholesterol by receptor-independent mechanisms. In the normal animal and man, however, most LDL-cholesterol is removed from the plasma by receptor-mediated transport mechanisms that are located primarily in the liver and intestine, so that the cholesterol can be readily excreted from the body. If the LDL receptors are absent or lost with aging or the intake of dietary cholesterol and saturated fatty acids, receptor-independent LDL transport becomes quantitatively much more important, and is capable of maintaining sterol balance across the individual tissues as well as the whole animal. However, in order to achieve the rates of LDL transport via the receptor-independent mechanism that are necessary to maintain this balance, the plasma LDL-cholesterol levels must necessarily rise.

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