Selective uptake of cholesteryl esters from low density lipoproteins in vitro and in vivo

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Abstract Evidence for the direct uptake ("selective uptake") of cholesteryl esters (CE) from low density lipoproteins (LDL) by perfused luteinized rat ovaries (Azhar, S., A. Cooper, L. Tsai, W. Maffe, and E. Reaven. 1988. *J. Lipid Res.* **29:** 869–882) led to this examination of LDL selective uptake in cultured cells and in rats using LDL doubly labeled with intracellularly trapped tracers of the CE and apoB moieties. Studies in vitro demonstrated LDL selective uptake by human fibroblasts at a low rate relative to LDL particle uptake; the fractional rate of this selective uptake increased with decreasing LDL particle size. Mouse Y1-BS1 adrenal cortical tumor cells also selectively took up LDL CE; on ACTH treatment, LDL selective uptake increased in parallel with high density lipoproteins (HDL) selective uptake, and accounted for the majority of LDL CE uptake. Metabolism of doubly labeled LDL was examined in rats. Adrenal gland and liver selectively took up CE from rat LDL, as did lung and adipose tissue. Selective uptake from human LDL was at a lower fractional rate than from rat LDL, and could not be demonstrated in as many organs. Although selective uptake from LDL by ovaries of adult rats was not significant, ovaries of immature rats consistently exhibited LDL selective uptake; on treatment of these rats with hormones to produce superovulated, luteinized ovaries, LDL selective uptake increased in the ovaries and nowhere else. Selective uptake was also apparent in liver, where it accounted for 27% of total hepatic uptake of rat LDL CE. These studies indicate a significant contribution of selective uptake to LDL CE metabolism in rats, suggesting the possibility of a role in other animals as well. —**Green, S. R., and R. C. Pittman.** Selective uptake of cholesteryl esters from low density lipoproteins in vitro and in vivo. *J. Lipid Res.* 1991. **32:** 667–678.

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The liver, adrenal gland, and ovary of normal rats selectively take up HDL cholesteryl esters (CE) without parallel uptake of HDL particles (1). A wider range of cell types from several species exhibit selective uptake in vitro (2–5). The pathway is down-regulated in cultured cells by provision of lipoprotein cholesterol, and up-regulated in rats by lowering lipoprotein cholesterol levels (3, 6).

Selective uptake is a nonendocytotic process that does not require metabolic energy (7). HDL-associated cholesteryl esters are reversibly inserted into a plasma membrane pool, from which they are internalized by a process that appears essentially irreversible (8). The size of the membrane pool and the rate of selective uptake are regulated in parallel, evidently according to the membrane content of free cholesterol (8). There is no specific apolipoprotein required for this process (9), and there is no evidence for a specific protein receptor (7, 9).

Previous studies have indicated that the rate of selective uptake of HDL cholesteryl esters depends on particle size; CE are taken up from small HDL at a greater fractional rate in vitro than from large HDL (7), and there is little selective uptake from large particles (9). This relationship holds regardless of the apolipoprotein composition of synthetic particles in the HDL density range. Thus, it has seemed unlikely that the even larger LDL particles would be good donors for selective uptake. We explored the possiblity of LDL selective uptake directly in cultured human fibroblasts; there was a modestly greater uptake of CE tracer than of apoB tracer, which might have been explained by a more rapid endocytotic uptake of the larger LDL particles (9). Our interest in the possibility of selective uptake from LDL was rekindled by the work of Azhar and coworkers (10) who concluded that LDL cholesteryl esters may be taken up by perfused luteinized rat ovary at a rate many times that explained by LDL particle uptake.

To further explore the possibility of selective uptake from LDL in vitro, we again studied human fibroblasts, but this time using LDL of narrow size distribution in order to minimize metabolic heterogeneity. To reduce the background of LDL particle uptake and so unmask any low rate of selective uptake, cells deficient in LDL receptors were studied. Toward the same end, heparin was used

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; ^{125}I-TC-LDL, low density lipoprotein derivatized with the radioiodinated tyramine-cellobiose ligand; ^{125}I-NMTC-apoA-I, apoA-I derivatized with the radioiodinated N-methyl-tyramine-cellobiose ligand; FCR, fractional catabolic rate; CE, cholesteryl esters; PAGE, polyacrylamide gel electrophoresis; CEt, cholesteryl ether; PBS, phosphate-buffered saline; 4-APP, 4-aminopyrazolo-pyrimidine.

to inhibit receptor-mediated uptake of LDL particles (11) without inhibiting selective uptake of LDL CE (3, 12). Cultures of Y1-BS1 mouse adrenocortical tumor cells, which are very active in selective uptake of HDL cholesteryl esters, were also examined for evidence of LDL selective uptake.

Of course, the in vitro studies would not necessarily reflect what happens in vitro. This led us to examine the possibility of LDL selective uptake in rats using intracellularly trapped tracers (13). The in vivo experiments also offered methodological advantages over studies in cultured cells or organ perfusions. One advantage is that the usual in vivo use of intracellularly trapped tracers involves examination of tissues after the vasculature has been virtually cleared of tracer (13), so that the fate of the entire labeled LDL preparation is determined. In studies of cultured cells or perfused organs, only a very small fraction of the medium tracer is usually taken up so that there is the possibilty of mistaking the uptake of a minor subpopulation of atypical particles for authentic selective uptake from the general population of particles. Another advantage of examining tissues only after clearance of tracer from the circulation is mitigation of the possibility that results would be confounded by tracer bound to cells but not internalized, a complication encountered in the ovarian perfusion studies reporting apparent selective uptake from LDL (10). Of course, the overriding advantage of in vivo tracer experiments is that they describe what actually does occur in the intact animal, not what could possibly occur.

METHODS AND MATERIALS

Lipoprotein preparation and labeling

Human LDL was separated in the density range 1.02–1.057 g/ml by sequential equilibrium ultracentrifugation using NaBr to adjust densities (14). The preparations were washed at both the low and high ends of the density range. Rat LDL was prepared in the same manner. The rat LDL was then applied to an affinity column to remove particles containing apoE. The affinity column carried a mouse monoclonal antibody against rat apoE, which was purified from ascites fluid and then covalently linked to agarose. Analysis by PAGE showed apoB as the only discernible apolipoprotein component in the product LDL even on overloaded gels.

LDL was labeled with the radioiodinated tyramine-cellobiose ligand (^{125}I-TC-LDL) as previously described (15). Autoradiography of the labeled product after separation of apolipoproteins on SDS-PAGE gels disclosed a single discernible band in the region of apoB in the cases of both human and rat LDL. There was no discernible peak of ^{125}I at the apoE band in either case. In the case of rat LDL, from which particles with apoE had been removed by antibody affinity chromatography, direct radioassay of gel segments indicated less than 3% of total ^{125}I was in the segment containing apoE.

The cholesteryl ester moiety of LDL was traced by [^{3}H]cholesteryl-oleyl ether ([^{3}H]CEt). This was incorporated into LDL from donor synthetic HDL particles (9) by the action of partially purified human cholesteryl ester transfer protein (16), as previously described (7). The use of synthetic HDL as donor particles allowed labeling to the target specific activity of 30–60 dpm ^{3}H/ng LDL protein.

Rat and human HDL were prepared in the density range 1.063–1.21 g/ml. Particles containing apoE were removed from rat HDL by adsorption on a heparin affinity column (17). HDL was labeled as previously described (7). In brief, this involved transfer of [^{3}H]cholesteryl-oleyl ether from a donor liposomal preparation (18) using partially purified cholesteryl ester transfer protein (16), and subsequent exchange of homologous apoA-I labeled with the N-methyl-tyramine-cellobiose ligand (^{125}I-NMTC-apoA-I) (13) into the ^{3}H-labeled HDL.

LDL subfractionation

For in vitro studies, doubly labeled LDL was subfractionated by density gradient ultracentrifugation to obtain fractions of narrow density range, or by gel filtration chromatography to obtain fractions of narrow size range. Mixed salt-sucrose density gradients (19) were prepared in the density range 1.01–1.09 g/ml, and ultracentrifuged in SW41 rotors for 48 h at 38,000 rpm. The gradients were fractionated from the top using a "sipper" fractionator, and narrow density bands were chosen as outlined in the Results section. To separate particles by size, LDL was applied to a column of 4% agarose beads and eluted in phosphate-buffered saline (pH 7.4) containing 10^{-3} M EDTA. Fractions were collected and pooled as designated under Results.

In the case of either type of subfractionation, particle size and size distribution were examined by a dynamic laser light-scattering technique using a NICOMP Model 370 Submicron Particle Sizer (Pacific Scientific).

In vitro experiments

Uptake of LDL and HDL tracers by cultured cells was measured essentially as previously described for HDL (7). Human fibroblasts, either normal or LDL receptor-deficient, were plated in 60-mm culture dishes (Lux) at a density of 100,000/plate. The culture medium (1.5 ml of DME containing 10% v/v fetal calf serum and 50 μg/ml gentamycin was changed every 2–3 days. Near confluency, usually 5–6 days after plating, the cells were washed two times with PBS, and fresh DME containing 5 mg/ml bovine serum albumin was added for a 20-h incubation in the absence of plasma lipoproteins before addition of labeled lipoprotein and determination of tracer uptake rates.

The mouse Y1-BS1 adrenocortical tumor cell line, a stable subclone of the Y1 line (20), was also studied. These cells were grown on 60-mm plates grown in 1.5 ml of RPMI medium containing 10% horse serum and 2.5% fetal calf serum. Near confluency, the cells were incubated for 16 h in RPMI medium containing 5 mg/ml bovine serum albumin before addition of lipoprotein tracers. In the indicated cases, ACTH (10^{-7} M) was included during the 16-h preincubation.

In all cases (human fibroblasts or Y1-BS1 cells, LDL or HDL uptake) the serum-free incubation medium was changed to the same fresh medium but containing doubly labeled human HDL or human LDL at a lipoprotein concentration of 20 μg protein/ml. Where indicated, the medium for Y1-BS1 cells also contained 10^{-7} M ACTH. Cells were incubated for 4 h, during which time selective uptake was approximately linear in all cases. Cells were then washed four times with PBS. Fresh incubation medium containing unlabeled lipoprotein (100 μg protein/ml) in place of the labeled lipoprotein was added for a chase incubation of 2 h. The chase medium was removed and the cells were washed with PBS before release of the cells from the plates using a 1.0 ml of trypsin/EDTA solution (0.5 g/l trypsin, 0.2 g/l EDTA). Trypsin activity was quenched by addition 1.0 ml PBS containing 50 mg/ml bovine serum albumin. The cells were pelleted, washed with 10 ml PBS, and dissolved in 0.1 N NaOH with sonication. Aliquots were taken for protein assay (21) and for assay of tracers. The tracer contents of the chase medium and the trypsin medium were also assayed to determine amounts of reversibly cell-associated tracers.

Selective uptake was calculated as the excess of [^{3}H]cholesteryl-oleyl ether tracer over ^{125}I-TC-LDL tracer (or ^{125}I-NMTC-apoA-I tracer in the case of HDL) remaining in the cells after removal of the reversibly cell-associated tracers. This selective uptake of HDL CE is shown as apparent HDL particle uptake, expressed in terms of lipoprotein protein.

In vivo experiments

Studies were conducted generally as described previously (1). Female Sprague-Dawley rats, 180–250 g, were used except in the study of luteinized rats, in which case 105–132-g juvenile rats were used. The animals were fasted overnight before injection of tracer lipoproteins, and throughout the 24-h duration of the study. Tracer lipoproteins (normally 1–2 μCi of each tracer) were injected via a tail vein. In no case did the injected tracer represent more than 100 μg protein. Blood samples (usually 0.2 ml) were then periodically withdrawn by tail vein. Usually nine samples were taken over 24 h. At the termination of the experiment, rats were anesthetized, exsanguinated, and the vasculature was thoroughly perfused with PBS containing EDTA. Organs were then removed for analysis. Large or diffuse organs were sampled, and literature values for organ weight/body weight were used to calculate total organ weight. All organs or aliquots of organs were homogenized for direct assay of ^{125}I, and assay of ^{3}H- or ^{14}C-labeled lipid after extraction (22).

In some experiments the uptake of LDL tracers by liver and steroidogenic tissues was examined 4 h after tracer injection rather than the usual 24 h. These experiments were carried out as just described, except that to minimize the amount of LDL tracers bound to cells but not internalized, unlabeled rat plasma (10%) and human LDL (50 μg protein/ml) were added to the perfusion buffer (120 ml) at the termination of the experiment.

In some cases, rats were treated with 4-aminopyrazolopyrimidine (4-APP). The drug was given intraperitoneally at a dose of 2 mg/kg body weight 24 h prior to the study (23, 24).

In other cases, immature rats were treated with hormones to produce superovulation and luteinization of the ovaries (25, 26). Rats (24 days old) were injected subcutaneously with 50 I.U. of pregnant mare's serum gonadotropin (PMSG). Three days later they were injected subcutaneously with 25 I.U. of human chorionic gonadotropin (hCG). Six days after that the animals were studied.

RESULTS

To compare the rates of uptake of the cholesteryl ester and apoB moieties of LDL by cultured cells, LDL was doubly labeled with [^{3}H]cholesteryl-oleyl ether and ^{125}I-TC-apoB. These intracellularly trapped tracers allowed the uptake of the two LDL moieties to be determined solely by the cell content of accumulated tracers, obviating potential problems such as high blank values for degradation products in the medium and differing recoveries of cells and medium. To minimize potential problems of metabolic heterogeneity based on particle size, the labeled LDL was subfractionated by gel filtration, and a narrow range about the modal particle size was selected. To insure that only internalized tracers were measured at termination of the uptake period, bound but not internalized LDL tracers were chased from the cells by a 2-h incubation in the presence of excess unlabeled LDL, as described in the Methods section.

Table 1 shows results for tracer uptake from three such LDL preparations by human fibroblasts. Normal fibroblasts took up the CE tracer at a fractional rate about 1.3-times that of the apoB tracer (Table 1), a result similar to that previously obtained using the entire LDL fraction (9). The presence of heparin in the medium inhibited uptake of LDL particles, increasing the CE/apoB uptake ratio to about 2. Apparent selective uptake, calculated as the excess of ^{3}H uptake over ^{125}I uptake where both are

TABLE 1. Selective uptake of LDL cholesteryl esters by human fibroblasts

Cells	Apparent LDL Particle Uptake		Selective Uptake ($^3H - ^{125}I$)	Ratio $^3H/^{125}I$
	^{125}I-Labeled LDL	[^3H]CEt		
	ng LDL protein/mg cell protein			
Normal fibroblasts				
No additions	2226 ± 209	3002 ± 381	776 ± 313	1.35 ± 0.15
Heparin, 5 mg/ml	536 ± 193	1060 ± 230	524 ± 58	2.07 ± 0.36
FH fibroblasts				
No additions	164 ± 46	746 ± 236	582 ± 210	4.65 ± 1.15
Heparin	54 ± 10	674 ± 333	626 ± 158	12.38 ± 1.86

Uptake experiments were carried out as described in detail in the Methods section. Briefly, fibroblasts were incubated 20 h in the absence of lipoproteins before addition of doubly labeled LDL (20 μg protein/ml) for a 4-h uptake incubation. The cells were then washed, incubated for 4 h with unlabeled LDL (100 μg protein/ml), and harvested for assay. Where indicated, heparin was added during the 4-h uptake and the 2-h chase incubations.

expressed as apparent LDL particle uptake, was not significantly changed by exposure to heparin. The uptake of LDL particles by fibroblasts with defective internalization of LDL receptors was even lower than the uptake by normal cells in the presence of heparin. However, the rate of apparent selective uptake was not significantly different from normal cells, and consequently the ratio of CE uptake to apoB uptake increased to about 4. Addition of heparin to the internalization-defective cells decreased LDL particle uptake even further, and increased the CE/apoB uptake ratio to 12. Again, apparent selective uptake was not changed by heparin and was not significantly different from selective uptake in the normal cells.

Cholesteryl esters are selectively taken up from small HDL particles at a greater fractional rate than from large HDL particles by human fibroblasts and by rat mouse Y1-BS1 adrenal tumor cells (7, 9). The possibility was examined that a similar size dependency applied to selective uptake from LDL particles by human fibroblasts. To do this, doubly labeled LDL was separated into three fractions by density gradient ultracentrifugation; each fraction exhibited a single peak on gel filtration, and sizing by laser light-scattering confirmed the preparation of fractions of differing mean particle diameter. No apoE could be detected on SDS-PAGE in any of the preparations. As shown in **Table 2**, a small but significant selective uptake was detected with the set of larger particles, and somewhat more selective uptake was measured with the set of somewhat smaller particles. In the case of the smallest particles, there was a clearly higher rate of selective uptake, and this selective uptake was at a higher ratio of ^3H uptake to ^{125}I uptake than observed in the usual broad density range preparations of LDL ($^3H/^{125}I$ ratio of 1.15–1.4 in six preparations).

Y1-BS1 cells and primary cultures of rat adrenal cells take up HDL cholesteryl esters predominantly by selective uptake, and ACTH stimulates that process (7). If selective uptake from LDL represents the same process, then one might expect Y1-BS1 cells to also exhibit LDL selective, and that this uptake would be increased by ACTH. This proved to be the case (**Table 3**). Y1-BS1 cells took up LDL cholesteryl esters mostly by selective uptake, and this uptake increased 3-fold on ACTH treatment; uptake of LDL particles increased more than 3-fold so that the ratio of cholesteryl ester uptake to apoB uptake actually decreased due to hormone treatment. By comparison,

TABLE 2. Selective uptake of cholesteryl esters from LDL subfractions—human fibroblasts

Lipoprotein Fraction	Particle Diameter	Apparent Lipoprotein Particle Uptake		Selective Uptake ($^3H - ^{125}I$)	Ratio $^3H/^{125}I$
		^{125}I-Labeled Apolipoprotein	[^3H]CEt		
	nm	*ng protein/mg cell protein*			
LDL fraction					
I	20.6	8200 ± 345	9331 ± 286	1131 ± 59	1.14 ± 0.01
II	19.9	9948 ± 18	11791 ± 685	1844 ± 703	1.19 ± 0.07
III	18.2	4385 ± 100	9527 ± 102	5142 ± 14	2.17 ± 0.03
HDL		253 ± 11	5100 ± 968	4848 ± 957	20.1 ± 2.9

The uptake of doubly labeled HDL was carried out as described in detail in the Methods section and as more briefly described in Table 1. Doubly labeled LDL of varying size was prepared as described briefly in the text and in greater detail under Methods.

TABLE 3. Selective uptake of LDL cholesteryl esters by mouse Y1-BS1 adrenocortical tumor cells

Labeled Lipoprotein, Cell Treatment	Apparent Particle Uptake		Selective Uptake ($^3H - ^{125}I$)	Ratio $^3H/^{125}I$
	^{125}I-Labeled Apolipoprotein	[^3H]CEt		
	ng lipoprotein protein/mg cell protein			
Human LDL				
Basal cells	416 ± 19	1548 ± 71	1135 ± 63	3.69 ± 0.18
ACTH-treated cells	1597 ± 207	4556 ± 166	2959 ± 41	2.37 ± 0.44
Rat HDL				
Basal cells	357 ± 70	6393 ± 1403	6036 ± 1333	17.8 ± 0.5
ACTH-treated cells	452 ± 54	15982 ± 1460	15530 ± 1413	35.4 ± 2.3

Uptake of doubly labeled LDL and doubly labeled HDL by Y1-BS1 mouse adrenocortical tumor cells was measured as described in detail in the Methods section, and generally as described for fibroblasts in Table 1. However, Y1-BS1 cells were incubated 16 h (rather than 20) in the absence of serum before the lipoprotein uptake period. In the case of ACTH-treated cells, ACTH was added at 10^{-7} M both during the 16-h preincubation period and during the 4-h uptake incubation. Uptake of the two tracers is expressed as the apparent uptake of LDL particles so that the two can be compared on a common basis.

adrenal cell uptake of cholesteryl esters from rat HDL was at a much higher fractional rate than from LDL. Exposure to ACTH increased this selective uptake but, in contrast to results for LDL, this was accompanied by little effect on HDL particle uptake. On ACTH exposure, the fractional increase in LDL selective uptake was similar to the increase in HDL selective uptake (about 2.6-fold). Thus, selective uptake from LDL resembles selective uptake of HDL, in that adrenal uptake is regulated by ACTH about in parallel with HDL selective uptake, and adrenal cells are more dependent on this process than are human fibroblasts. Interestingly, the calculated rates of cholesteryl ester mass selectively taken up were similar for human LDL and rat HDL under the conditions of Table 3 (4–5 μg cholesteryl ester/mg cell protein during 4 h incubation of ACTH-stimulated cells).

Evidence indicates that HDL selective uptake is mediated by a plasma membrane pool of cholesteryl esters that can be assessed in rat adrenal cells or mouse Y1-BS1 adrenal cells in terms of reversibly cell-associated cholesteryl ester tracer in excess of reversibly cell-associated apoA-I tracer (8); this pool is usually measured by the release of tracers during a post-uptake incubation in the presence of unlabeled HDL. A parallel procedure was used in the present experiments examining selective uptake from LDL, as described in the Methods section. However, we were unable to discern a membrane pool of reversibly cell-associated cholesteryl ester tracer in excess of that accounted for by reversibly cell-associated LDL particles in Y1-BS1 cells. In fact, the ratio of ^3H to ^{125}I in this pool was in most cases less than unity, suggesting that small LDL particles relatively poor in ^3H preferentially bound to the cells, or that bound particles were significantly depleted of cholesteryl esters before release. Either case would have precluded observation of a relatively small reversibly cell-associated pool even if it had been present.

Evidence of selective uptake of LDL cholesteryl esters by cultured human fibroblasts and rat adrenal cells did not necessarily indicate that the pathway plays a role in vivo; we have observed high rates of selective uptake by cultured rat cells that were not exhibited by their counterparts in intact animals, evidently because the pathway is up-regulated under our usual culture conditions but down-regulated in vivo (6). In addition, it was possible that, in spite of our precautions, the selective uptake evident in cultured cells actually represented the uptake of an undetected, minor subset of particles. Therefore, metabolism of doubly labeled LDL was studied in intact rats where the fate of all particles in the labeled LDL preparation was determined.

First, the effect of luteinization on LDL cholesteryl ester metabolism in rate was examined. **Table 4** shows the plasma fractional catabolic rate (FCR) for each tracer, and that portion of the plasma FCR attributable to tissues of particular interest; tissue uptake data, therefore, represent the fraction of the plasma pool taken up by that tissue per hour. Of all organs examined, only adrenal gland and ovary consistently exhibited selective uptake in these juvenile rats. Significant differences ($P < 0.05$) between treated and mock-treated animals were seen in the ovary and in no other tissue. The fractional rate of uptake of both LDL tracers increased on luteinization. LDL selective uptake increased 5.4-fold on hormone treatment ($P < 0.05$), while LDL particle uptake increased 2.4-fold; in the mock-treated animals selective uptake accounted for about 40% of total HDL cholesteryl ester uptake, while in treated animals it accounted for about 60%. Thus, a significant amount of LDL selective uptake was detected in the ovaries of these small rats, and luteinization increased that uptake.

The increase in ovarian selective uptake on luteinization represented predominantly an increase in ovarian mass rather than specific activity. Selective uptake per

TABLE 4. Effect of luteinization on LDL uptake by selected organs

Organ, Isotope	Fractional Catabolic Rate: Control Rats (n = 4)	Fractional Catabolic Rate: Luteinized Rats (n = 5)	
	$10^3 \times h^{-1}$		
Plasma, ^{125}I	109 ± 18	126 ± 22	NS
Plasma, ^{3}H	120 ± 27	129 ± 33	NS
Liver, ^{125}I	53.7 ± 13.4	66.5 ± 13.5	NS
Liver, ^{3}H	59.2 ± 14.9	61.3 ± 20.3	NS
Adrenal, ^{125}I	0.315 ± 0.207	0.412 ± 0.183	NS
Adrenal, ^{3}H	0.602 ± 0.362	0.773 ± 0.418	NS
Adrenal, selective uptake	0.287 ± 0.177	0.361 ± 0.137	NS
Ovary, ^{125}I	0.286 ± 0.061	0.693 ± 0.267	$P < 0.05$
Ovary, ^{3}H	0.510 ± 0.112	1.91 ± 1.05	$P < 0.05$
Ovary, selective uptake	0.223 ± 0.071	1.21 ± 0.78	$P < 0.05$

Uptake of tracers from labeled human LDL was determined in immature female rats treated with hormones to produced luteinized ovaries and in parallel mock-treated rats, as described in detail under Methods. Plasma decay kinetics were followed for 24 h, after which tissues were harvested for assay. Tissue uptakes are expressed as the fraction of the plasma pool cleared by that organ per hour (plasma FCR × fraction of total catabolized tracer recovered in that organ).

gram of tissue increased by an average of only 92% on luteinization, while uptake of the apoB tracer actually decreased by 14%. However, there was a 2.8-fold increase in ovarian mass on hormone treatment (from 63 ± 4 to 178 ± 37 mg/rat) so that total ovarian selective uptake increased 5.4-fold and total apoB uptake increased 2.4-fold. The contribution of individual cell types to the increased total ovarian uptake was not examined, and it is not known to what degree the specific activity of any cell type(s) might have increased.

In this study of luteinized rats, selective uptake accounted for 40–60% of total ovarian LDL CE uptake. This was a considerably smaller contribution of selective uptake than reported by Azhar and coworkers (10) in their studies of perfused ovaries, where the apparent uptake of cholesteryl esters was about 12 times the apparent uptake of apoB. Those workers noted an ovarian pool of bound but not internalized labeled LDL particles that was large compared to internalized tracer. It was possible that a large pool of bound but not internalized LDL tracer, if present in the ovary at termination of our in vivo experiments, would lead to a substantial underestimation of the role of selective uptake. Similarly, the extracellular pool could have led to an overestimation of the role of selective uptake in the ovarian perfusion studies, particularly if bound LDL tracer was not representative of perfusate LDL tracer. The possibility of such a pool playing an important role in our in vivo experiments might be dismissed on conceptual grounds alone because it would require an incredibly large and slowly equilibrating pool. Nevertheless, the possibility was examined experimentally by measuring the relative amounts of apparently intact and degraded apoB tracer in tissues 24 h after tracer injection. In three animals, 79.7 ± 1.5% of the apoB tracer that had accumulated in liver was retained on a 10% agarose column; 75.3 ± 6.5% of adrenal and 72.2 ± 2.3% of ovarian tracer was retained on such a column. Thus the minimum fraction of tracer in the liver that was degraded was not substantially more than the minimum fraction of tracer in ovary and adrenal that was degraded, and there was no evidence for a major pool of intact apoB tracer in steroidogenic tissues. Interestingly, only 38.3 ± 0.6% of apoB tracer in liver was precipitated by 10% trichloroacetic acid in the same experiments, while 67.5 ± 5.5% of adrenal and 59.3 ± 9.1% of ovarian tracer was precipitated, in agreement both with our earlier findings that a large fraction of the catabolic products from liver (defined by sizing) was not precipitable (27), and with the findings of Azhar and coworkers (10) that sizing and precipitation agreed much more closely in steroidogenic tissues.

To further explore the role of LDL selective uptake in vivo, the effect of 4-APP treatment on the metabolism of LDL tracers was explored. This treatment lowers rat plasma cholesterol levels by about 90% (92.8 ± 4.8% in the present study), and up-regulates selective uptake from HDL in several organs (6). Various labeled LDL preparations were used in these studies: human LDL, human LDL that was reductively methylated to diminish LDL receptor recognition (28, 29), and rat LDL.

Table 5 shows the plasma fractional catabolic rates for the various LDL preparations in treated rats and in their mock-treated controls. Results for HDL from a previous study (6) are included for reference. One feature of these results is that the plasma FCR for the cholesteryl ester moiety of LDL was greater than that of the apoB moiety

TABLE 5. Plasma fractional catabolic rates for doubly labeled lipoproteins in rats

Lipoprotein, Treatment	Fractional Catabolic Rate	
	^{125}I-Labeled Apolipoprotein	[^{3}H]CEt
	h^{-1}	
Rat HDL[a]		
Control (n = 9)	0.158 ± 0.051	0.162 ± 0.017
4-APP (n = 4)	0.459 ± 0.077	0.436 ± 0.103
Human LDL		
Control (n = 5)	0.100 ± 0.021	0.111 ± 0.024[b]
4-APP (n = 5)	0.133 ± 0.036	0.151 ± 0.033[b]
Methylated human LDL		
Control (n = 4)	0.066 ± 0.015	0.080 ± 0.025
4-APP (n = 5)	0.084 ± 0.015	0.091 ± 0.019
Rat LDL		
Control (n = 4)	0.159 ± 0.014	0.193 ± 0.013[b]
4-APP (n = 4)	0.178 ± 0.023	0.272 ± 0.011[b]

Adult female rats were injected with the indicated doubly labeled lipoproteins, and the plasma decay kinetics were followed for 24 h for determination of plasma FCRs.

[a]From reference 6.

[b]$P < 0.05$ for the difference in ^{3}H and ^{125}I uptake using a two-tailed t-test for paired data.

in the case of both human and rat LDL, and in both control and drug-treated rats (Table 5). Since there is no known mechanism for transfer of cholesteryl esters between lipoproteins in rats, the direct interpretation is that selective uptake of cholesteryl esters was sufficient to affect plasma turnover rates of rat LDL.

Another feature of the results in Table 5 is that the FCR of every tracer increased on 4-APP treatment, reaching statistical significance in the case of the cholesteryl ester tracer of both human and rat LDL. However, in no case did 4-APP treatment produce as dramatic an increase in the plasma FCR of either LDL tracer as it did in the case of both HDL tracers. Reductive methylation of human LDL reduced the plasma FCR of both ^{3}H and ^{125}I tracers in untreated rats; although the mean FCR values for both tracers increased on 4-APP treatment, neither increase reached statistical significance.

Table 6 shows the effect of 4-APP treatment on the tissue uptakes of tracers from human LDL. Unlike the luteinization study of Table 4, selective uptake by steroidogenic tissues was marginal in both control and treated groups of the 4-APP study of Table 6, and generally did not reach statistical significance. These rats were larger than the juvenile animals used for the luteinization study where there was clear selective uptake by adrenal gland and ovary. Treatment of these larger rats with 4-APP resulted in increased fractional rates of LDL particle uptake by adrenal gland and ovary with no evidence for increased selective uptake, in contrast to the substantial increase in the fractional rate of selective uptake from HDL by these organs previously reported in a comparable study (6). Also, in contrast to the results with juvenile rats, hepatic selective uptake was apparent in the adult rats of Table 6. However, the fractional rate of this uptake did not greatly increase on 4-APP treatment, in

TABLE 6. Fractional rates of uptake of LDL-associated apoB (^{125}I) and cholesteryl esters (^{3}H) by various rat tissues—human LDL

Organ	Fractional Catabolic Rate			
	Control Rats (n = 5)		4-APP Rats (n = 5)	
	^{125}I	^{3}H	^{125}I	^{3}H
	$10^{3} \times h^{-1}$			
Liver-assoc.	53.2 ± 10.2	70.9 ± 18.7[a]	88.5 ± 59.9	106.9 ± 25.9
Adrenal	0.50 ± 0.34	0.61 ± 0.14	2.68 ± 2.60	3.01 ± 1.44
Ovary	0.38 ± 0.13	0.62 ± 0.19[a]	0.71 ± 0.36	0.98 ± 0.42
Spleen	11.0 ± 3.4	10.7 ± 6.1	10.0 ± 2.0	10.3 ± 4.1
Lymph nodes	2.73 ± 1.18	2.42 ± 1.07	2.84 ± 1.56	2.59 ± 0.63
Small int.	5.78 ± 2.58	6.01 ± 2.20	8.02 ± 3.59	7.95 ± 0.92
Lung	0.59 ± 0.11	0.86 ± 0.24[a]	0.55 ± 0.14	0.94 ± 0.38
Pancreas	0.36 ± 0.16	0.33 ± 0.12	0.56 ± 4113	0.50 ± 0.28
Thymus	0.13 ± 0.06	0.12 ± 0.03	0.09 ± 0.04	0.11 ± 0.05
Large int.	1.96 ± 0.89	0.86 ± 0.27[a]	1.73 ± 0.45	1.17 ± 0.24[a]
Kidney	3.23 ± 0.95	0.43 ± 0.15[a]	3.38 ± 0.74	0.39 ± 0.11[a]
Heart	0.14 ± 0.01	0.16 ± 0.04	0.12 ± 0.03	0.60 ± 0.99
Skin	8.22 ± 4.33	5.66 ± 6.38	5.24 ± 1.82	5.53 ± 2.80
Stomach	0.44 ± 0.21	0.39 ± 0.20	0.91 ± 0.43	1.02 ± 0.44
Adipose	1.77 ± 0.84	1.71 ± 0.81	1.53 ± 0.85	1.65 ± 0.57
Muscle	6.42 ± 3.05	7.64 ± 8.29	4.88 ± 1.86	9.17 ± 6.43

Uptake of tracers from labeled human LDL was determined in adult female rats treated with 4-aminopyrazolopyrimidine and in parallel mock-treated rats, as described in detail under Methods. Plasma decay kinetics were followed for 24 h, after which tissues were harvested for assay. Tissue uptakes are expressed as the fraction of the plasma pool cleared by that organ per hour.

[a]$P < 0.05$ for the difference in ^{3}H and ^{125}I uptake using a two-tailed t-test for paired data.

contrast to a large increase in HDL selective uptake previously shown (6).

Some general features of the data in Table 6 should be noted that also apply to the other in vivo experiments described here assessing tracer uptakes by individual tissues. In all cases, kidney and large intestine contained significantly more ^{125}I than ^{3}H. This result in kidney presumably represents the uptake and secondary trapping of some of the ^{125}I-labeled catabolic products leaking from other organs. Most of that leaking ^{125}I is excreted into urine. In the study of Table 5, 4.8 ± 2.2% of injected ^{125}I and 0.06 ± 0.04% of ^{3}H were recovered in urine. The sum of the ^{125}I recovered in urine and the excess ^{125}I in kidney, representing 7–8% of recovered ^{125}I, is in the range of the estimated rates of leakage of the ^{125}I-tyramine-cellobiose from cells in vivo (15). It might be noted that this relatively low rate of renal uptake of ^{125}I from LDL (about 3.3% of catabolized tracer) is in contrast to the high rate of renal uptake of apoA-I tracer from HDL (about 24% of total clearance (1)). The excess of ^{125}I over ^{3}H in large intestine presumably is a consequence of the previously observed high rate of biliary excretion of the ^{125}I-TC tracer into the gut lumen (1, 2, 15); there may be incomplete separation of intestinal contents or secondary sequestration after uptake by the wall. In reporting data here, tracer recovered in the gut contents and feces has been added to that recovered in liver to calculate total hepatic uptake (a large correction for liver ^{125}I). Similarly, ^{125}I recovered in urine has been distributed between tissues on the assumption that all tissues leak the label at the same fractional rate (a small correction for all tissues).

The inability to discern selective uptake by steroidogenic tissues in the larger rats used for the 4-APP experiments suggested that high rates of LDL particle uptake might have concealed relatively modest rates of selective uptake. This possibility was explored by tracing the uptake of human LDL that had been reductively methylated to block uptake of LDL particles via apoB/E receptors. In adrenal glands of untreated rats (n = 4), the fractional rate of uptake of ^{3}H from methylated LDL was 0.52 ± 0.28 h^{-1}, which was significantly greater ($P < 0.05$) than that of ^{125}I (0.17 ± 0.07 h^{-1}); in treated rats (n = 4), adrenal uptake of ^{3}H (1.59 ± 0.79 h^{-1}) was also greater than that of ^{125}I (0.87 ± .63 h^{-1}). In ovaries of untreated rats (n = 4), the fractional rate of uptake of ^{3}H was 0.38 ± 0.20 h^{-1}, which was significantly greater ($P < 0.05$) than that of ^{125}I (0.14 ± 0.20 h^{-1}); in treated rats (n = 4), ovarian uptake of ^{3}H (0.60 ± 0.17 h^{-1}) was also greater than that of ^{125}I (0.26 ± 0.07 h^{-1}). Thus, methylation of human LDL reduced particle uptake in steroidogenic tissues by 63–68%, allowing selective uptake to be observed.

Table 7 shows the fractional rates of uptake of rat LDL tracers in untreated rats and rats treated with 4-APP, which were significantly different from those obtained using human LDL as tracer. The liver of control rats took up both tracers of rat LDL at about twice the rate of the human LDL tracers. In fact, the difference in plasma FCR of the cholesteryl ester moiety of rat and human LDL, seen earlier in Table 5, was explained largely by the greater rate of hepatic selective uptake of the rat cholesterol ester tracer. In the mock-treated control rats of Table 7, liver accounted for about 75% of total cholesteryl ester uptake from rat LDL, but only about 55% of apoB uptake. The difference in fractional rates of uptake of the two tracers indicated that in the control rats about 37% of total hepatic uptake of rat LDL cholesteryl esters was due to selective uptake. Another difference between rat and human LDL was the greater rate of uptake of rat LDL tracers by steroidogenic tissues, due both to a greater rate of LDL particle uptake and, in the case of adrenal gland, to a greater rate of selective uptake. Thus, selective uptake played a role in uptake of rat LDL cholesteryl esters, and 4-APP treatment increased both LDL particle uptake and selective uptake. The effects of 4-APP on uptake of rat LDL tracers were qualitatively consistent with the effects of 4-APP on uptake of human LDL tracers, even though there were significant quantitative dissimilarities.

Some other features of the data in Table 7 might be noted. Selective uptake was evident in organs other than liver and steroidogenic tissues; these were lung, adipose tissue, pancreas, heart, and (less convincingly) stomach. These organs made relatively little contribution to whole-body selective uptake. It might be noted that selective uptake by lung was also apparent in the case of human LDL (Table 6), although it did not quite reach statistical significance in the 4-APP-treated rats; selective uptake by lung has been reported but not emphasized in the case of HDL (16).

Interpretation of the in vivo experiments above has assumed metabolic homogeneity of the LDL pool. However, metabolic heterogeneity, reported for clearance of LDL particles in other animals (30–32) and evident for LDL selective uptake in the experiments with cultured cells shown above, could confound these interpretations. To explore this possibility, subfractions of doubly labeled LDL were prepared by density gradient ultracentrifugation and examined in rats. Fractions were taken near the modal density (1.038 < d < 1.049 g/ml) and at higher density (1.052 < d < 1.058 g/ml). Laser particle sizing indicated that we had prepared particles of 25.6 nm and 23.1 nm modal diameter (the distribution of the 25.6 nm particles included a significant tail of particles resembling the 23.1 nm particles). The two sets of particles were injected into a total of seven rats and plasma decay kinetics were followed. The plasma fractional catabolic rate of ^{3}H was greater than that of ^{125}I in the case of both buoyant particles (0.085 ± 0.005 h^{-1} for ^{3}H, 0.066 ± 0.004 h^{-1} for ^{125}I, $P < 0.05$) and dense particles (0.095 ± 0.011 h^{-1}

TABLE 7. Fractional rates of uptake of LDL-associated apoB (^{125}I) and cholesteryl esters (^{3}H) by various rat tissues—rat LDL

Organ	Fractional Catabolic Rate			
	Control Rats (n = 4)		4-APP Rats (n = 4)	
	^{125}I	^{3}H	^{125}I	^{3}H
	$10^{3} \times h^{-1}$			
Liver-assoc.	107 ± 12	147 ± 9[a]	115 ± 17	203 ± 11[a]
Adrenal	2.81 ± 0.59	4.42 ± 0.58[a]	9.75 ± 3.72	13.4 ± 6.1[a]
Ovary	2.26 ± 0.66	1.76 ± 0.28	3.22 ± 0.76	3.66 ± 0.33
Spleen	4.97 ± 1.05	5.00 ± 0.94	5.44 ± 1.30	6.04 ± 1.11
Lymph nodes	0.75 ± 0.12	0.67 ± 0.08	1.06 ± 0.62	1.07 ± 0.45
Small int.	7.11 ± 2.30	7.04 ± 2.06	5.90 ± 2.61	7.76 ± 1.25
Lung	0.65 ± 0.07	1.21 ± 0.12[a]	0.88 ± 0.25	1.73 ± 0.29[a]
Pancreas	0.13 ± 0.05	0.18 ± 0.06[a]	0.19 ± 0.11	0.32 ± 0.11[a]
Thymus	0.09 ± 0.02	0.11 ± 0.01[a]	0.13 ± 0.03	0.23 ± 0.04[a]
Large int.	4.13 ± 1.61	1.80 ± 0.68[a]	2.83 ± 0.90	1.95 ± 0.65[a]
Kidney	7.41 ± 0.83	0.55 ± 0.09[a]	12.1 ± 1.71	0.86 ± 0.11[a]
Heart	0.17 ± 0.01	0.23 ± 0.03[a]	0.16 ± 0.08	0.25 ± 0.07[a]
Skin	14.5 ± 3.2	14.2 ± 4.4	14.4 ± 5.12	21.5 ± 7.33
Stomach	0.65 ± 0.17	0.51 ± 0.09	0.63 ± 0.14	0.74 ± 1.22[a]
Adipose	1.68 ± 0.61	2.17 ± 0.89[a]	1.42 ± 0.39	3.37 ± 1.57
Muscle	4.12 ± 0.44	6.03 ± 1.24	3.04 ± 1.33	5.74 ± 2.14[a]

Uptake of tracers from labeled rat LDL was determined in adult female rats treated with 4-aminopyrazolo-pyrimidine and in parallel mock-treated rats, as described in detail under Methods. Plasma decay kinetics were followed for 24 h, after which tissues were harvested for assay. Tissue uptakes are expressed as the fraction of the plasma pool cleared by that organ per hour.

[a] $P < 0.05$ for the difference in ^{3}H and ^{125}I uptake using a two-tailed t-test for paired data.

for ^{3}H, 0.077 ± 0.008 h^{-1} for ^{125}I, $P < 0.05$). The liver as well as the steroidogenic tissues of all animals exhibited selective uptake. Hepatic selective uptake was $5.70 \pm 1.66 \times 10^{-3}$ h^{-1} in the case of the buoyant particles, and $7.10 \pm 3.05 \times 10^{-3}$ h^{-1} in the case of the dense particles. Adrenal selective uptake of buoyant particles was $0.97 \pm 0.25 \times 10^{-4}$ h^{-1} and selective uptake of dense particles was $1.80 \pm 0.47 \times 10^{-4}$ h^{-1}. Thus, narrow ranges of particle density (and presumably narrow ranges of particle size) exhibited selective uptake, countering any suspicion that the selective uptake evident in vivo might instead be the consequence of metabolic heterogeneity.

Another experimental approach addressed the possibility that apparent selective uptake from LDL was somehow a consequence of metabolic changes in the tracer LDL particles occurring during the 24-h study period. Observation of selective uptake at short times after tracer injection would mitigate against this possibility. Thus, doubly labeled human LDL was injected into five rats (female, 200–220 g, as in other studies) and the uptake of LDL tracers by liver (including that excreted into the gut lumen) was determined 4 h later. Such short-term studies yield a legitimate measure of the relative rates of uptake of the two LDL tracers by those tissues rapidly exposed to the injected tracers, e.g., liver. Precise measurement of cellular uptake of tracers requires that any extracellular tracer be removed before assay of the tissue; this was accomplished by thorough perfusion of the liver with medium containing plasma lipoproteins before radioassay. These experiments disclosed a higher fraction of injected ^{3}H than of ^{125}I in liver; in five animals, the liver contained 20.2 ± 3.1% of injected ^{3}H and 11.0 ± 2.1% of injected ^{125}I after 4 h. At this time the fraction of injected ^{125}I remaining in plasma was 0.74 ± 0.11% times the fraction of injected ^{3}H remaining, so that the liver had been exposed to a somewhat lower time-integrated fraction of the injected dose of ^{125}I than of ^{3}H. It can be calculated from these data that [^{3}H]cholesteryl ether was taken up by liver at a rate that was, at a minimum, 1.4-times that of ^{125}I-labeled apoB (and more probably 1.6-times), demonstrating hepatic selective uptake 4 h after tracer injection.

DISCUSSION

The data here indicate that cholesteryl esters are selectively taken up from LDL in vitro by cells of mouse and human origin; a previous study using similar methodology showed LDL selective uptake by rat adrenal cells (33). The constancy of apparent selective uptake by human fibroblasts over a 40-fold range of LDL particle uptake and under blockade of LDL receptor binding indicates that the difference in rates of uptake of cholesteryl ester and apoB tracers was not somehow a consequence of holoparticle uptake, and that the process did not somehow de-

pend on the irreversible endocytotic uptake of LDL via apoB/E receptors. In these regards, LDL selective uptake resembles HDL selective uptake.

Although selective uptake of LDL cholesteryl esters by human fibroblasts was evident, it did not make as large a contribution to the total uptake of LDL cholesteryl esters as it does to the uptake of HDL cholesteryl esters (3). A similar result was found for Y1-BS1 cells. These results were, in part, due to a greater fractional rate of uptake of LDL particles than of HDL particles by both cell types. However, there was also a lower fractional rate of LDL selective uptake than of HDL selective uptake. This lower rate of LDL selective uptake did not necessarily indicate a lower rate of cholesteryl ester mass uptake.

Selective uptake of LDL cholesteryl esters by adrenal gland in vivo displayed characteristics parallel to those of HDL selective uptake: the rate of both processes was higher in adrenal gland than in other tissues; both processes were similarly regulated by drugs. Similarly, the relative rate of LDL selective uptake by various cultured cell types was parallel to HDL selective uptake (1, 6): both processes played a more prominent role in Y1-BS1 cells than in human fibroblasts; neither process depended on recognition by apoB/E receptors. LDL selective uptake also resembled HDL selective uptake (9) in that it was up-regulated in Y1-BS1 cells by ACTH treatment. Thus, it seems likely that HDL and LDL selective uptake represent the same pathway, and there is no substantial evidence suggesting otherwise.

Selective uptake of cholesteryl esters from human LDL played a role in vivo in several organs of the rat, and was observed in steroidogenic tissues and in liver, at least under some conditions. Results with rat LDL were somewhat different. Selective uptake from rat LDL was usually apparent in adrenal gland, but inconsistently reached significance in ovary except in the case of luteinized ovaries. Selective uptake from rat LDL was more consistent in liver, accounting for 35–40% of hepatic LDL cholesteryl ester uptake. Thus, it appears that LDL selective uptake in rats must contribute to reverse cholesterol transport, to the extent that LDL cholesteryl esters arise from cholesterol taken from extrahepatic cells. If such a pathway plays a role in animals with high LDL levels, such as humans, LDL conceivably could make a more substantial contribution to reverse cholesterol transport in these cases. This contribution might be especially important in people with familial hypercholesterolemia where hepatic LDL receptors are absent or deficient. It is more difficult to speculate about a potential role for LDL selective uptake in cases where LDL receptors are down-regulated due to hypercholesterolemia, since selective uptake may also be down-regulated (3, 6).

The use of rat LDL as a tracer raises concern that HDL_1 containing apoE may be isolated with the LDL and confound interpretation of the results. The problem has led some workers, including us (34), to trace endogenous LDL using buoyant subfractions evidently free of apoE. This approach, while solving one problem, creates another due to the metabolic heterogeneity of LDL. In the present study we took a different approach by studying the entire rat LDL fraction ($1.02 < d < 1.06$ g/ml) from which we removed apoE (and presumably the particles carrying apoE) by binding to an anti-apoE antibody affinity column. The resulting preparation was then studied in vivo, and tissue uptakes were measured only after virtually all tracer was cleared. The evident selective uptake of LDL cholesteryl esters by liver represented 10–20% of the injected cholesteryl ester tracer, which was considerably greater than uptake possibly due to residual apoE contaminating the rat LDL preparation.

Heterogeneity in the metabolism of LDL apoB has been reported in several species including humans (30–32), and in the above studies in terms of selective uptake by cultured cells. The possibility that such metabolic heterogeneity influenced the major conclusions of the present in vivo studies was examined both by tracing narrow subfractions of human LDL and by tracing the fate of the broad range of human LDL at short times after injection. In both of these circumstances selective uptake by liver and steroidogenic tissues was evident, reinforcing the conclusion that selective uptake from LDL plays a role in rats.

While the difference in FCR for the two density fractions of LDL examined here were not impressive, it is apparent that metabolic heterogeneity certainly must be taken into account in such in vivo studies. For example, in the present study the plasma FCR for the apoB moiety of rat LDL was about 1.5-times that of human apoB (Table 5), while we have previously reported similar FCRs for rat and human LDL (34). This difference may be explained by the fact that in the present study we isolated rat LDL in the traditional 1.02–1.06 g/ml density range and removed particles containing apoE using an anti-apoE antibody affinity column, while in the previous study we used a buoyant subfraction of LDL to minimize contamination by HDL_1 particles (34). The use of other strategies to exclude HDL_1 particles has produced even wider differences between plasma FCRs for human and rat LDL apoB (35). Even when the entire LDL fraction is studied, it is unlikely to fully account for metabolic heterogeneity since the particles most rapidly cleared will be underrepresented.

In the situations reported here, the fractional rate of LDL selective uptake was low compared to HDL selective uptake, and particle uptake made a greater contribution to the total uptake of LDL cholesteryl esters than is the case for HDL cholesteryl esters. We have emphasized evidence that particle size contributed to these differences. However, the rates of selective uptake from LDL by adrenal gland and particularly by liver are greater than

one would predict simply on the basis of relationship of HLD selective uptake and particle size (7, 9). Evidently other forces are at play in determining the rate of selective uptake. The data here suggest that LDL particle size may be a more important factor in adrenal gland than in liver.

The present results do not clearly indicate the probability of a role for LDL selective uptake in species other than rat. In a recent kinetic modeling study in rabbits (36) we were unable to detect selective uptake from LDL, even though there was a substantial rate of selective uptake from HDL. Humans, like rabbits and unlike rats, have high levels of LDL, larger LDL particles which are poorer substrates for selective uptake, and high plasma cholesteryl ester transfer activity. Consequently it seems improbable the selective uptake plays as large a role in humans as it does in rats in uptake of LDL cholesteryl esters. However, some contribution in humans is by no means ruled out, particularly under circumstances such as LDL receptor deficiency.

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