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Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: Selective delivery of cholesterol ester to liver, adrenal, and gonad

(cholesterol ether/tyramine cellobiose/lipoprotein turnover)

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ABSTRACT **The metabolic fate of homologous high density lipoprotein (HDL) was studied in the rat, tracing the apoprotein A-I (apo A-I) and cholesterol ester moieties simultaneously. The apo A-I was labeled with covalently linked ^{125}I-labeled tyramine cellobiose, which accumulates in the cells degrading the apoprotein; [^{3}H]cholesterol ethers, which cannot be hydrolyzed or mobilized after uptake, were incorporated into the lipid core of reconstituted HDL to reflect the fate of the cholesterol esters. Several lines of evidence, including direct comparison with biologically labeled HDL, are presented to support the validity of this approach. The liver was the major organ of cholesterol ether uptake, accounting for 65% of the total; the adrenal gland and ovary were the most active organs per gram (wet) of weight. Uptake of cholesterol ether was 7-fold greater than that of apo A-I in adrenal, 4-fold greater in the ovary, and >2-fold greater in the liver. The remaining tissues took up apo A-I and cholesterol ethers at more nearly equal rates. Transfer of HDL-associated cholesterol ethers and ^{125}I-labeled apo A-I to other lipoprotein fractions was not observed; thus, the results reflect direct uptake from HDL itself. Whereas uptake of low density lipoprotein appears to involve endocytosis of intact particles, uptake of HDL in at least some rat tissues involves additional, more complex, transfer mechanisms.**

The increasingly impressive evidence that risk of coronary heart disease correlates inversely with plasma high density lipoprotein (HDL) levels (1) has stimulated a great deal of research on HDL metabolism. This protective effect may be related to HDL-mediated transport of cholesterol from extrahepatic tissues to the liver, as postulated by Glomset (2), but evidence for such "reverse cholesterol transport" *in vivo* is largely indirect and almost no quantitative data are available. HDL can also, at least in the rat, deliver cholesterol to steroidogenic tissues (3, 4). This delivery may be receptor mediated although the targeting of HDL for such uptake is poorly understood.

We have used nondegradable markers to determine simultaneously the uptake of HDL-associated apoprotein A-I (apo A-I) and cholesterol esters by various tissues *in vivo*. Apo A-I was traced by covalently attaching to it ^{125}I-labeled tyramine-cellobiose (^{125}I-TC). The TC moiety, which can be radioiodinated to high specific activity, remains trapped in tissues after uptake and degradation of the labeled protein because it cannot be degraded by mammalian cells and does not readily cross cell membranes (5). The fate of cholesterol esters was determined by using [^{3}H]cholesteryl linoleyl ether as a marker, as described (6). Since cholesterol ethers cannot be hydrolyzed by mammalian cells, they remain trapped in tissues after uptake in the same manner as the nondegradable protein marker. Cholesterol ethers were introduced into HDL by a modification of the procedure for reconstituting low density lipoprotein (LDL) (7).

The rat was chosen for these studies in part to evaluate further the special role of HDL in this animal for delivering cholesterol to the adrenal gland and gonads and in part because the rat has little or no cholesterol ester exchange protein in its plasma (8). In the rabbit—and probably in other species with plasma cholesterol ester-exchange protein—cholesterol ethers, like cholesterol esters, exchange rapidly among lipoprotein fractions (9). This makes it very difficult to be certain which lipoprotein fraction is responsible for observed tissue uptake. In the present studies in the rat, HDL-associated cholesterol ethers did not redistribute to other lipoproteins after injection. Thus, accumulation of cholesterol ethers by tissues resulted from delivery by HDL itself.

MATERIALS AND METHODS

Lipoproteins. HDL was isolated in the density interval 1.09–1.21 g/ml from serum of large female Sprague–Dawley rats (retired breeders) by using sequential ultracentrifugation according to standard techniques (10). Apo A-I was purified and labeled with ^{125}I-TC as described (11). ^{125}I-TC-labeled apo A-I (^{125}I-TC-apo A-I) was reassociated with HDL by incubation for 1 hr at 37°C in phosphate-buffered saline (pH 7.4) (P_i/NaCl) at a ratio of 1 mol of ^{125}I-TC-apo A-I per 20 mol of HDL. Unbound apo A-I was removed by flotation of the HDL at a salt density of 1.21 g/ml.

To biologically label rat HDL in the cholesterol ester moiety, rats were fasted overnight and then fed [1α,2α-^{3}H]cholesterol (Amersham) in 0.5 ml of corn oil delivered through a gastric tube. Serum was recovered 22 hr later and HDL was isolated by sequential ultracentrifugation. At this time, 80% of the ^{3}H label was associated with cholesterol esters and 20%, with free cholesterol. To reduce the fraction of ^{3}H in free cholesterol, this preparation was injected into a donor animal and, 1 hr later, blood was drawn and HDL was reisolated. This procedure decreased the radioactivity in free cholesterol to <5% of total.

Incorporation of [^{3}H]Cholesterol Ethers into HDL. Radiolabeled cholesteryl linoleyl ether was prepared from [1α,2α-^{3}H]-cholesterol and linoleyl methanesulfonate (12) as described (13).

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Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; apo A-I, apoprotein A-I; TC, tyramine cellobiose; ^{125}I-TC, ^{125}I-labeled TC; FCR, fractional catabolic rate; P_i/NaCl, phosphate-buffered saline (pH 7.4).

Rat HDL was brought to a concentration of 2 mg/ml in P_i/NaCl, and aliquots (2 mg of HDL protein) were lyophilized onto 25 mg of purified potato starch (Sigma) in siliconized 15-ml extraction tubes. The HDL was partially delipidated by extraction with 5 ml of heptane at −20°C for 1 hr. The starch was then pelleted by low-speed centrifugation and the heptane phase was removed. The samples were extracted two more times with heptane at −20°C for 30 min. Exogenous lipids consisting of 2 mg of unlabeled cholesteryl linoleate, 0.5–5 × 10^8 dpm of [^{3}H]cholesteryl linoleyl ether (<1 μg total), and 0.1 mg of unlabeled free cholesterol were added to each sample in 200 μl of heptane and incubated at room temperature for 1 hr. The heptane was then evaporated under nitrogen until the starch appeared powder dry. HDL was solubilized from the starch by addition of P_i/NaCl/0.02% sodium azide and incubated overnight at 37°C with gentle agitation. The starch was then sedimented at 10,000 × *g* for 20 min and the resulting supernate was filtered through an 0.8-μm filter (Unipore). The resulting HDL containing [^{3}H]cholesteryl linoleyl ether was turbid. Equilibrium density gradient ultracentrifugation resolved the product into two peaks of radioactivity: the first, recovered in a density range similar to that of native HDL, was associated with HDL apoproteins; the second, which accounted for 30–40% of recovered radioactivity, had a density of <1.02 g/ml and was unassociated with protein. This light fraction accounted for all of the turbidity observed in the starting material. We routinely reisolated the reconstituted HDL in the density interval 1.07–1.21 g/ml by sequential ultracentrifugation to remove this light component. The resulting preparation is referred to below as "reconstituted" HDL. The infranate of the reconstituted HDL reisolated at 1.07 g/ml was dialyzed against P_i/NaCl and reassociated with ^{125}I-TC-apo A-I as described above.

***In Vivo* Studies.** Sprague–Dawley female rats (175–225 gm) were fasted overnight prior to and throughout the course of turnover studies but were allowed free access to water. On the morning of the study, animals were anesthetized and silastic catheters were placed in the left jugular vein. After a 3-hr recovery period, radiolabeled samples were injected and plasma decay data were obtained by drawing periodic blood samples of 100–125 μl. Plasma decay data were fitted to a biexponential function and fractional catabolic rates (FCR) were calculated using a simple kinetic model (14). Tissues were collected 24 hr after injection and homogenized as described (11). In some experiments, hepatocytes and nonparenchymal cells were isolated after collagenase perfusion of the liver (15).

Radioassay Procedures. Samples containing ^{125}I or ^{3}H were radioassayed using standard techniques. To assay ^{3}H in tissue homogenates containing both ^{125}I and ^{3}H, samples were brought to a volume of 1 ml with water and extracted using the method of Dole (16). This resulted in nearly quantitative recovery of cholesterol ethers in the nonpolar phase (93.5 ± 2.7%) with virtually no contamination by ^{125}I-TC-labeled protein or its degradation products (<0.5% of total ^{125}I).

RESULTS

Characterization of doubly labeled reconstituted HDL reisolated in the density interval 1.07–1.21 g/ml is shown in Fig. 1. ^{125}I-TC-apo A-I and ^{3}H-labeled cholesteryl linoleyl ether migrated together with α mobility on agarose gel electrophoresis (Fig. 1*A*). There was no evidence for a class of particles containing cholesterol ethers but no apo A-I. As shown in Fig. 1*B*, the density distribution of [^{3}H]cholesteryl linoleyl ether in reconstituted HDL paralleled that of cholesterol esters in control HDL. However, the protein distribution of reconstituted HDL

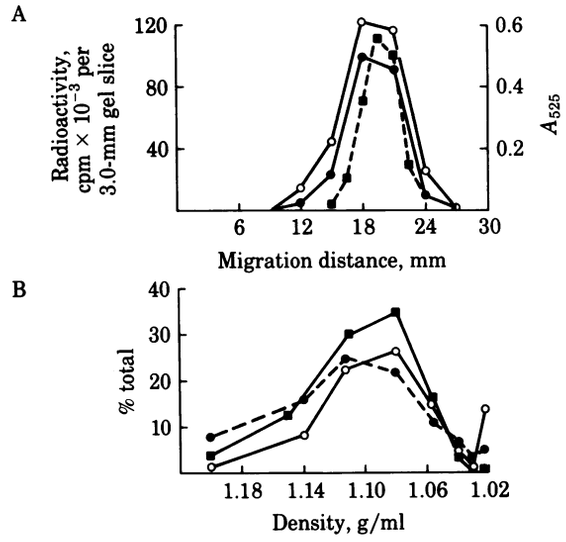

FIG. 1. (*A*) Agarose gel electrophoresis of doubly labeled reconstituted HDL. Doubly labeled reconstituted HDL and native HDL were electrophoresed in parallel lanes. The reconstituted HDL lane was cut into 3-mm slices. Each slice was homogenized in 1 ml of water and assayed for ^{125}I (●) and ^{3}H (○). The native HDL lane was stained with Oil Red 0 and scanned at 525 nm (■). (*B*) Density gradient ultracentrifugation of doubly labeled reconstituted HDL. Reconstituted HDL was adjusted to 2.0 M NaCl and centrifuged for 48 hr in the gradient system of Foreman *et al.* (17). Fractions (1.2 ml) were collected and assayed for density, A_{280} (●), and ^{3}H content (○). Native HDL was run in a parallel gradient and fractions were assayed for density, A_{280} (data not shown), and cholesterol ester mass (■). The distribution of A_{280} values coincided with that of cholesterol ester mass in this case.

peaked at a slightly higher density than did [^{3}H]cholesterol ether. The protein distribution of native HDL was identical to that of its cholesterol ester component (data not shown).

The apoprotein compositions of control and reconstituted HDL were similar but not identical (Table 1). Apo A-I accounted for 24% of total HDL mass for reconstituted HDL compared with 30% for control HDL. Reconstitution caused some enrichment in C apo proteins and a decrease in apoprotein A-IV content. Lipid analysis of reconstituted HDL and native HDL is also shown in Table 1. The free cholesterol/protein and phospholipid/protein ratios were not significantly different for the two preparations, but the cholesterol ester/

Table 1. Composition of native and reconstituted HDL

Component	% of total HDL mass: Native HDL	Reconstituted HDL
Apoproteins		
A-I	30 ± 3.0	24 ± 1.2
Cs	6.8 ± 0.5	13 ± 0.7
A-IV	4.1 ± 0.3	2.4 ± 0.5
E	1.4 ± 0.4	1.0 ± 0.5
Other HDL-associated proteins	2.3 ± 1.0	7.0 ± 1.5
Lipids		
Phospholipid	24 ± 6.1	29 ± 3.5
Free cholesterol	4.5 ± 0.9	3.5 ± 0.2
Cholesterol ester	27 ± 1.4	20 ± 2.1
Triglyceride	<2.0	<1.0

Results represent mean ± SEM. The apoprotein distribution was determined after NaDodSO$_4$/polyacrylamide gel electrophoresis using densitometric scanning of Coomassie blue-stained gels.

protein ratio of reconstituted HDL was 25–35% less than that of control HDL. Electron microscopy of negatively stained preparations of native and reconstituted HDL showed that most of the reconstituted HDL particles were spherical, with diameters not significantly different from that of control HDL (data not shown). There was, in addition, a population of discoidal particles, identified on the basis of their formation of rouleaux, that accounted for 15–20% of the total particle number. This subclass, presumably incompletely reconstituted, may account for the slight difference in the density distribution of protein and lipid components of reconstituted HDL mentioned above and for its overall lower cholesterol ester/protein ratio.

In studies to be reported elsewhere, the effect of reconstitution on HDL–cell interaction was tested in cultured rat hepatocytes. Uptake of TC-labeled native HDL was identical to that of TC-labeled reconstituted HDL. Unlabeled native HDL competed equally for uptake of both labeled preparations.

The plasma decay kinetics of reconstituted HDL were compared with those of cholesterol esters in a native particle. Reconstituted doubly labeled HDL was biologically screened by passage through a donor animal for 1 hr. Serum from the donor was then injected into recipient animals. Plasma decay curves for ^{125}I-TC-apo A-I and [^{3}H]cholesteryl linoleyl ether and a representative plasma decay curve for rat HDL biosynthetically labeled with cholesterol esters are shown in Fig. 2. The FCR of ^{125}I-TC-apo A-I in the reconstituted HDL was 0.11 ± 0.01 hr^{-1}. The FCR for the [^{3}H]cholesterol ether component was 0.14 ± 0.01 hr^{-1} (n = 4), compared with the FCR for biologically labeled HDL cholesterol esters of 0.14 ± 0.02 hr^{-1} (n = 7).

Tissues were assayed for radioactivity 24 hr after injection of the biologically screened reconstituted HDL. At that time, >90% of the initial serum ^{125}I and ^{3}H had left the circulation. The fraction of total recovered extravascular radioactivity found in selected tissues is shown in Table 2. The liver was the predominant organ of cholesterol ether uptake and was also the major organ of apo A-I uptake. Isolation of hepatocytes and nonhepatocytes showed that 93–96% of both trapped labels was associated with hepatocytes. The kidney exhibited a high rate of apo A-I uptake, as in previous studies (11), but took up <1% of the injected dose of cholesterol ethers. Recoveries of ^{125}I and ^{3}H at 24 hr ranged from 78% to 90% of the injected dose. Redistribution or leakage of the two labels was not examined directly in these experiments but has previously been shown to be insignificant for up to 48 hr in the case of ^{125}I-TC-apo A-I (11) and for up to 6 days after uptake of [^{3}H]cholesterol ethers (6).

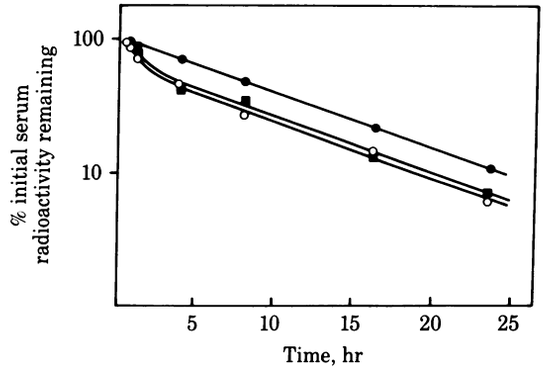

FIG. 2. Representative plasma decay curve of biologically screened doubly labeled reconstituted HDL. ●, ^{125}I-TC-apo A-I; ○, [^{3}H]cholesteryl linoleyl ether. Also shown is a representative plasma decay curve of biosynthetically labeled HDL cholesterol esters (■).

Table 2. Tissue uptake of ^{125}I-TC-apo A-I and [^{3}H]cholesteryl linoleyl ether from reconstituted HDL

Organ	% of total body uptake	
	^{125}I-TC-apo A-I	[^{3}H]Cholesteryl ether
Liver	37 ± 2.9	64 ± 2.3
Kidney	18 ± 2.6	0.5 ± 0.1
Adrenal	0.6 ± 0.1	2.8 ± 0.3
Ovary	0.9 ± 0.3	2.9 ± 0.6
Other	43 ± 7.9	30 ± 5.9

To assess the relative activities of the various tissues in uptake per unit weight of tissue, the data were calculated in terms of an organ FCR that represents the fraction of the plasma pool cleared per hour per gram of tissue (Table 3). Calculated this way, the adrenal glands and ovaries were the most active organs in uptake of [^{3}H]cholesteryl linoleyl ether per gram of tissue. The liver exhibited the next highest rate of uptake. The kidney was the most active organ in uptake of ^{125}I-TC-apo A-I, followed by adrenal, ovary, and liver. These tissues were substantially more active in uptake of ^{125}I-TC-apo A-I and [^{3}H]cholesterol ethers than any of the remaining tissues.

A particularly striking finding was the differential rate of uptake of apo A-I and cholesterol ethers in adrenal, ovary, and liver. Cholesterol ether/apo A-I uptake ratios for these and several other tissues are also shown in Table 3. The ratio was highest in adrenal (7.0) and definitely >1 in ovary (4.6) and liver (2.2). The kidney also exhibited a striking degree of dissociation but in the opposite direction. Apo A-I uptake in kidney occurred some 30 times faster than that of the ether. Most of the remaining tissues exhibited ether/apo A-I uptake ratios that were not significantly different from 1.

Several possibilities were considered that might account for the differential rates of uptake observed in liver, adrenal, and ovary. One possible explanation is selective uptake of an ether-rich particle. However, a minor contaminant of ether-rich particles in the injected preparation could not account for the findings in liver. Tissue content of radioactivity was measured after >90% of the two labels had decayed from the plasma and the liver had taken up more than half of the injected dose. The large fraction of ether-rich particles needed to explain these findings could not have been overlooked. Nevertheless, to directly assess this possibility, doubly labeled reconstituted HDL was separated into heavy and light density subfractions by preparative equilibrium density gradient ultracentrifugation and the tissue uptake of the two fractions was compared. The heavy HDL fraction (1.10–1.21 g/ml) was relatively rich in ^{125}I-TC-apo A-I while the light fraction (1.065–1.10 g/ml) was relatively

Table 3. Comparative organ FCRs for HDL-associated [^{3}H]cholesterol linoleyl ether and ^{125}I-TC-apo A-I in selected tissues

Tissue	Organ FCR, $hr^{-1} \cdot g^{-1} \times 10^{3}$		
	Cholesterol ether	apo A-I	Organ FCR ratio*
Adrenal	60 ± 8.5	9.1 ± 2.8	7.0 ± 2.3*
Ovary	39 ± 11	9.8 ± 5.0	4.6 ± 2.3*
Liver	15 ± 1.1	6.5 ± 0.4	2.3 ± 0.2*
Kidney	0.4 ± 0.1	12 ± 3.7	0.03 ± 0.01*
Lung	1.3 ± 0.1	0.8 ± 0.1	1.7 ± 0.4*
Other	0.1–5.7	0.1–6.0	0.9–1.4

Results represent mean ± SEM.

* Cholesterol ether organ FCR/apo A-I organ FCR: FCR calculated as plasma FCR × fraction of total body uptake in organ ÷ organ wet weight (g).

rich in [^{3}H]cholesteryl linoleyl ethers. Despite the differences in the relative amounts of ^{125}I-TC-apo A-I and [^{3}H]cholesteryl linoleyl ether in the two fractions, there was no evidence of metabolic heterogeneity. As shown in Table 4, uptake of ether label was at least 2-fold greater than apo A-I uptake in liver, adrenal, and ovary; there were no apparent differences between dense and light HDL subfractions.

To address the possibility that these differential rates of uptake were the consequence of an artifact of reconstitution, HDL that had been biologically labeled in the cholesterol ester moiety was reassociated with ^{125}I-TC-apo A-I and injected into experimental animals. One hour after injection, hepatocytes and adrenal cells were isolated as described (15, 18) and their contents of ^{3}H and ^{125}I were determined. The 1-hr time period was chosen to minimize hydrolysis of cholesterol esters and loss of radiolabeled cholesterol from tissues. Radioactivity was determined in isolated cells to avoid contamination by the large fraction of the injected dose still in the plasma compartment or extravascular space 1 hr after injection. Results of these experiments are shown in Table 4. The ratio of the uptake of cholesterol esters and apo A-I by hepatocytes was in good agreement with those obtained using reconstituted HDL. The ratio in adrenal, however, was significantly less than that observed using reconstituted HDL. Since the adrenal uses lipoprotein cholesterol for steroid hormone production (19), it was possible that radiolabeled cholesterol esters might have been lost at a significant rate due to conversion to steroids and secretion during the course of the experiment. This possibility was examined by administration of 25 mg of aminoglutethamide to each of two rats 1 hr before injection of the labeled HDL. Aminoglutethamide is known to block side-chain cleavage of cholesterol, a step required in the production of corticosteroids (20). Prior treatment with this agent doubled the cholesterol ester/apo A-I ratio subsequently found in adrenal cells after injection of the doubly labeled HDL. Thus, even though blockade of cholesterol label leakage was probably incomplete, these experiments show the preferential uptake of esters compared with apo A-I from "native" HDL (i.e., HDL not subjected to the reconstitution procedure).

Another possible explanation for the disproportionately greater uptake of cholesterol ether and esters is their transfer to lower density lipoprotein fractions without parallel transfer of apo A-I followed by more rapid tissue uptake of these lighter lipoproteins. Since rat plasma contains little or no cholesterol ester exchange activity, this possibility seemed unlikely but it was checked. A rat was injected with doubly labeled reconstituted HDL. Serum was obtained 1 hr later and chromatographed on 6% agarose. As shown in Fig. 3, both the apo A-I and cholesterol ether labels eluted exclusively in the HDL range; there was no evidence of transfer of cholesterol ethers to lighter lipoproteins.

Table 4. Relative uptake of HDL-associated [^{3}H]cholesterol ethers or esters and ^{125}I-TC-apo A-I from various HDL preparations

Preparation	Liver	Adrenal	Ovary
Reconstituted HDL*			
Light subfraction (1.065–1.10 g/ml)	2.5 (2.7, 2.3)	5.6 (5.1, 6.0)	2.7 (3.1, 2.3)
Heavy subfraction (1.10–1.21 g/ml)	2.2 (2.1, 2.3)	5.7 (4.6, 6.7)	2.7 (2.4, 2.9)
Biologically labeled HDL†	2.2 (2.3, 2.1)	1.4 (1.4, 1.5)	ND
Biologically labeled HDL (rats treated with aminoglutethamide)†	1.8 (2.0, 1.6)	2.9 (2.9, 2.9)	ND

Results represent apparent uptake of HDL as determined by ^{3}H ÷ apparent uptake of HDL as determined by ^{125}I. Values given are means of results from two experiments and values in parentheses are for individual experiments. ND, not determined.

* Tissue content of radioactivity was determined 24 hr after injection.

† Content of ^{3}H and ^{125}I was determined in isolated hepatocytes and adrenal cells 1 hr after injection.

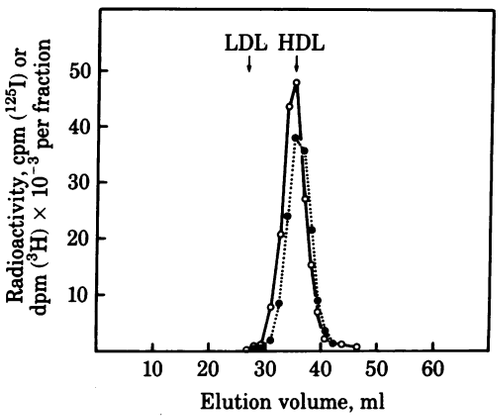

FIG. 3. Gel filtration (6% agarose) of serum obtained 1 hr after injection of doubly labeled reconstituted HDL. Arrows denote the elution volumes of human LDL and native rat HDL determined in independent calibration runs. ●, ^{125}I-TC-apo A-I; ○, [^{3}H]cholesteryl linoleyl ether.

DISCUSSION

Several lines of evidence support the conclusion that the metabolism of the nondegradable markers on reconstituted HDL reflects the metabolism of apo A-I and cholesterol esters of native HDL. (*i*) The physical properties (electrophoretic mobility and hydrated density), apoprotein composition, and lipid composition of the reconstituted HDL closely resemble those of native HDL except for a somewhat lower cholesterol ester/protein ratio. (*ii*) Unlabeled native HDL competed equally with reconstituted ^{125}I-TC-labeled HDL and with HDL directly conjugated with ^{125}I-TC for uptake and degradation in a cultured rat hepatocyte system. (*iii*) The *in vivo* plasma decay rate for cholesterol *esters* in biosynthetically labeled HDL was identical to that of cholesterol *ethers* in HDL reconstituted *in vitro* (and previously biologically screened in a donor animal). We have shown previously that the *in vivo* plasma decay rate for TC-labeled apo A-I is not different from that of conventionally iodinated apo A-I (11).

The present results are in good agreement with those previously reported for the sites of degradation of apo A-I on HDL that had not been reconstituted (11). In those studies, the overall fraction of apo A-I degraded by kidney (37%) was significantly greater than that observed in the present studies. This difference is likely to be due to the combination of ultracentrifugal flotation and biological screening carried out on reconstituted HDL in the present studies. Although the kidneys accounted for 18% of total apo A-I uptake, they took up almost no cholesterol ether (<1%). This supports our previous proposal that the renal degradation represents primarily tubular degradation of filtered free apo A-I in equilibrium with apo A-I on HDL (11). Estimating the filtration fraction for apo A-I from published data for other macromolecules (21), we calculate that as little as 1–2% unassociated apo A-I could account for the observed results. This selective renal degradation of a significant fraction of plasma apo A-I without concomitant uptake of the cholesterol ether would tend to make the FCR of apo A-I greater than that of cholesterol ether. However, the dispro-

portionate uptake of cholesterol ether in liver, adrenal, and ovary overrides this, so that the observed FCR for the ether (0.14 hr^{-1}) is greater than that for A-I (0.11 hr^{-1}).

The liver was the major site of HDL cholesterol ester uptake, accounting for 65% of the total. Based on a HDL cholesterol ester pool size of 4.2 mg for a 200-g rat and a plasma FCR of 0.14 hr^{-1}, we estimate delivery of 9.2 mg of cholesterol ester back to the liver daily. The data are compatible with a significant role of HDL in reverse cholesterol transport, even in the rat.

The large dissociation of cholesterol ether uptake from that of apo A-I in liver, adrenal, and ovary was striking. Uptake of the cholesterol ether was 2- to 7-fold greater than that of apo A-I in these tissues. Could the disproportionate cholesterol ether uptake be artifactual? The method for reconstitution did not lead to complete incorporation of the labeled cholesterol ether into HDL. Thus, the possibility that the observed dissociation might reflect heterogeneity in the preparation had to be considered. Reisolation of HDL by sequential ultracentrifugation removed all detectable unassociated cholesterol ether as shown by electrophoresis and density gradient centrifugation of the repurified reconstituted HDL. Further fractionation of the reconstituted HDL into heavy and light density subfractions and comparison of the two in recipient animals did not provide evidence for metabolic heterogeneity. Finally, differential rates of hepatic and adrenal uptake of apo A-I and cholesterol *esters* were observed for preparations of HDL containing biosynthetically labeled cholesterol esters.

It is unlikely that the differential uptake of cholesterol ethers and apo A-I is due to transfer of the cholesterol ethers to LDL or very low density lipoprotein, which are then taken up without apo A-I. The rat has very low levels of transfer factor activity when assayed *in vitro* (8), and we failed to detect any evidence for transfer of cholesterol ethers from HDL to lighter lipoproteins *in vivo*. Furthermore, our preliminary studies in primary cultures of rat hepatocytes and adrenal cells carried out in the absence of other lipoprotein fractions also show selective uptake of cholesterol ethers from reconstituted HDL (unpublished data). Preferential uptake of HDL cholesterol by isolated rat adrenal cells was previously postulated (3) but from studies without direct measurement of cholesterol uptake and without differentiation between free and esterified cholesterol uptake.

The rat may not be a representative animal with respect to HDL metabolism. For example, the rat adrenal may be unique in its ability to use cholesterol from HDL as a major source of steroid precursor whereas the adrenal in some other species seems to use predominantly LDL as a source of sterol (19). This may be the consequence of receptors specific for apo A-I or the HDL uptake may be mediated, at least in part, by the presence of receptors for other apoproteins. For example, the high proportion of apoprotein E on rat HDL would permit uptake through the LDL receptor, which recognizes apoproteins B and E (22).

The present studies do not establish the mechanisms responsible for selective uptake of cholesterol esters. At least two categories of mechanism may be considered. In the first case, uptake of cholesterol esters could occur without uptake of the remainder of the HDL particle—e.g., through binding of HDL to the cell surface with consequent selective transfer of cholesterol esters into the cell. In the second case, uptake of the HDL particle could occur without parallel degradation of apo A-I, either through loss of apo A-I before or during binding of HDL to the cell surface or by uptake of the entire HDL particle with subsequent release of apo A-I from the cell. Differential rates of uptake of the apoprotein and cholesterol ester moieties of chylomicrons have been observed in the perfused rat heart (23) and in rat heart mesenchymal cells in culture (24, 25). The selective uptake of cholesterol esters from chylomicrons appears to be facilitated by lipoprotein lipase (25), but the mechanisms responsible are not known. In the present studies, substantial dissociation in the rates of uptake of HDL-associated cholesterol esters and apo A-I occurred only in three tissues, each of them highly active in uptake of apo A-I, and each of them having a specialized role in cholesterol metabolism.

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