Selective uptake of HDL cholesteryl esters is active in transgenic mice expressing human apolipoprotein A-I

John C. Khoo,[1,*] Ray C. Pittman,[*] and Edward M. Rubin[†]

Department of Medicine,[*] 0682, University of California, San Diego, La Jolla, CA 92039-0682, and Division of Research Medicine and Radiation Biophysics,[†] Division of Cell and Molecular Biology, Lawrence Berkeley Laboratory, University of California, Berkeley, Berkeley, CA 94720

Abstract The direct non-endocytotic uptake of cholesteryl esters (CE) from high density lipoprotein (HDL) plays a major role in HDL CE metabolism in rats and rabbits. In vitro evidence indicates it may also play such a role in humans. However, a study in mice (tracing the CE and apoA-I moieties of HDL) concluded that, while selective uptake played a role in normal animals, it did not in transgenic mice which express predominantly human apoA-I (Chajek-Shaul et al., 1991. *Proc. Natl. Acad. Sci. USA.* **88:** 6731–6735); thus human apoA-I was apparently unable to support selective uptake. These conclusions rested on plasma decay data that represent a composite of all tissue and which may obscure tissue-specific factors. Thus we reexamined the matter by measuring the rates of uptake of HDL components by individual tissues using intracellularly trapped tracers. Plasma decay data were much as reported in the referenced study. Nonetheless the fractional rate of uptake of HDL CE was greater than that of apoA-I in adrenal gland and liver, indicating selective uptake. Kidney took up apoA-I tracer at a greater fractional rate than CE tracer, apparently by filtration and reabsorption of free apoA-I, and this uptake was at a greater fractional rate in the transgenic mice than in normal mice. Thus, the lack of evidence for selective uptake in the plasma decay data of the transgenic mice was explained by a higher rate of renal uptake of apoA-I and not by a diminished rate of selective uptake in other tissues. This result was the same whether examined using tracers incorporated into normal or transgenic mouse HDL. The result was also confirmed using synthetic HDL that carried [^{14}C]sucrose octaoleate as an independent marker of HDL particle uptake. Thus, human apoA-I supports selective uptake with no substantial evidence that it is less efficacious than mouse apoA-I.—**Khoo, J. C., R. C. Pittman, and E. M. Rubin.** Selective uptake of HDL cholesteryl esters is active in transgenic mice expressing human apolipoprotein A-I. *J. Lipid Res.* 1995. **36:** 593–600.

Supplementary key words reverse cholesterol transport

The direct non-endocytotic uptake of cholesteryl esters (CE) from high density lipoprotein (HDL), which we term "selective uptake," is an established pathway that has been described in vivo in several species (1–3), in perfused organs (4, 5) and in vitro in a number of cell types (6–9). Human cell lines, including hepatoma cells, exhibit selective uptake from human HDL (8–11). Recently, Rinninger et al. (12) reported the pathway in primary cultures of human hepatocytes. As human cells, including primary hepatocytes, exhibit the pathway, one might expect the pathway to play a significant role in normal human HDL CE metabolism as it does in rats (2) and in rabbits (3).

Nevertheless, a study of mice transfected with the gene for human apolipoprotein A-I (apoA-I) reached a contrary conclusion (13). The plasma level of apoA-I in these mice was about twice that of control mice, and was predominantly the product of the human apoA-I gene (13, 14). This study found in control mice that the plasma fractional catabolic rate (FCR) for HDL CE was greater than the FCR for apoA-I, which the authors interpreted to represent selective uptake. Their results were different for the transgenic mice. In the transgenic mice, the FCR for CE was not greater than the FCR for apoA-I, largely due to a lower FCR for CE. From this the authors concluded that selective uptake did not take place in the transgenic animals. As they did not find evidence that the ostensible lack of selective uptake was secondary to the higher plasma HDL levels in the transgenic animals, they further concluded that human apoA-I apparently was unable to support the selective uptake pathway. If correct, this seemingly would preclude an important role for selective uptake of HDL CE in humans.

However, the conclusions of that paper rested upon plasma decay data, the only data available for the apoA-I moiety in that study (13). This was not adequate to support their conclusions. Only more limited inferences

Abbreviations: HDL, high density lipoprotein; apoA-I, apolipoprotein A-I; CE, cholesteryl ester; [^{3}H]CEt, [^{3}H]cholesteryl-oleyl ether; ^{125}I-labeled NMTC-apoA-I, apoA-I derivatized with the radioiodinated N-methyltyramine-cellobiose ligand; FCR, fractional catabolic rate; CETP, cholesteryl ester transfer protein.

[1]To whom correspondence should be addressed.

about selective uptake can be reached from plasma decay data alone, even in animals (such as rats) where plasma CE transfer activity is absent and the rate of apoA-I clearance indicates the maximum rate of HDL particle clearance (1, 2). If all pertinent facets of HDL metabolism in mice are like those in rats (the best understood model), it would seem reasonable to interpret a plasma FCR for HDL CE in excess of that for HDL apoA-I in mice as indicating a minimum value for selective uptake. On the other hand, a fractional rate of HDL CE clearance equal to or less than the fractional rate of apoA-I clearance would not necessarily indicate that selective uptake is absent. This is largely because the necessary use of the apoA-I plasma clearance rate to indicate HDL particle clearance substantially overestimates the actual rate of that particle clearance (1, 2). This more rapid clearance of apoA-I than of HDL particles is largely due to the renal filtration and reabsorption of free apoA-I dissociated from HDL (2, 15, 16), which accounts for over 20% of apoA-I clearance in rats (2). Such renal uptake of free apoA-I might play a prominent role in mice as well as rats. Furthermore, it would not be surprising if the direct renal uptake of apoA-I played an even greater role in transgenic mice than in normal mice due to their higher production rates and higher plasma levels of apoA-I. Thus, the data collected in the study of Chajek-Shaul et al. (13) could not, in principle, support a conclusion one way or the other as to whether selective uptake plays a role in the transgenic animals. To more directly address the question it was necessary to measure the rates of uptake of the CE and apoA-I moieties of HDL in each tissue individually.

Consequently we reexamined selective uptake of HDL CE in transgenic mice expressing predominantly human apoA-I by measuring rates of uptake of HDL CE and apoA-I tracers by individual tissues in vivo. This approach abolishes the uncertainties resulting from the renal uptake of free apoA-I and mitigates other tissue-specific sources of ambiguity. In these experiments we used a strain of transgenic mice, designated "A2" (17) that is similar to the strain used by Chajek-Shaul et al. (13). The two strains were developed independently but in parallel. Both strains exhibit a total plasma apoA-I level about twice that of normal mice, and this is about 95% human apoA-I (13, 14, 17, 18). HDL CE levels are similar in the two transgenic strains, about twice that of normal mice (13, 14, 17, 18). The HDL patterns of both transgenic strains are complex, exhibiting HDL_3 and HDL_1 fractions as well as the HDL_2 fraction that predominates in normal mice (13, 14, 17, 18).

To determine the rate of uptake of HDL components by individual organs, we used intracellularly trapped tracers that remain at their site of uptake and thereby serve as cumulative markers of tracee uptake (19, 20). As in previous studies (1, 6), [^{3}H]cholesteryl-oleyl ether ([^{3}H]CEt) traced cholesteryl esters and apoA-I derivatized with ^{125}I-labeled N-methyltyramine-cellobiose (^{125}I-labeled NMTC) ligand traced apoA-I. Whereas mice lack plasma CE transfer activity (21), the [^{3}H]CEt tracer remains with the particles originally labeled so that its only known routes of catabolism are by uptake of the labeled particles or by selective uptake independent of particle uptake. If uptake of apoA-I tracer is assumed to occur only by uptake of HDL particles except in kidney (an assumption we test here), then an excess of CE uptake over apoA-I uptake in tissues represents selective uptake (except in kidney, of course). The results of these experiments presented below lead to the conclusion that selective uptake plays a prominent role in HDL CE metabolism both in normal mice and in the transgenic mice.

METHODS

Animals

The transgenic mouse line studied here was derived from the C57BL/6 strain by transfection with the human apoA-I gene (17). Characteristics of the A2 strain used in the present study have been described previously (17, 18). Some experiments in this report used male mice and others female, as dictated by the availability of animals. Normal C57BL/6 mice were obtained from Jackson Laboratories. All experiments used adult mice of 20–25 g.

Lipoprotein preparations

Mouse HDL was prepared in the density range 1.09–1.21 g/ml from the plasma of either normal C57BL/6 mice or transgenic mice using sequential preparative ultracentrifugation by standard techniques (22). Isolated lipoprotein fractions were dialyzed against phosphate-buffered saline containing 0.01% EDTA and 0.02% NaN_3. HDL particles containing apoE were removed from the HDL preparation by heparin-Sepharose affinity chromatography, as previously described (6, 23).

Preparations of labeled HDL

Three types of labeled HDL particles were used; HDL from normal mice was labeled with CE and mouse apoA-I tracers (described below); HDL from transgenic mice was labeled with CE and human apoA-I tracers; synthetic HDL was labeled with CE and human apoA-I tracers, as well as a sucrose polyester tracer of the HDL particle per se as described below. All tracers were designed to accumulate at their sites of cellular uptake, and so mark the rates of uptake of their tracees (19, 24).

Human apoA-I and mouse apoA-I were isolated and labeled as previously described (6, 15). Briefly, HDL was delipidated using ethanol–diethyl ether, and apoA-I was separated by gel exclusion chromatography on Sephacryl S-200 (Pharmacia, Piscataway, NJ). Residual apoE was removed by heparin-Sepharose affinity chromatography.

Purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (25). ApoA-I was labeled by covalent attachment of the intracellularly trapped ^{125}I-NMTC ligand (19, 20).

[^{3}H]cholesteryl-oleyl ether ([^{3}H]CEt), was prepared (26) and incorporated into HDL (d 1.09–1.21 g/ml) from a donor liposomal preparation (27) using partially purified human plasma cholesteryl ester transfer protein (CETP) (28), as previously described (6). The donor liposomes were then separated from the labeled HDL by ultracentrifugation at a salt density of d 1.06 g/ml. ^{125}I-labeled NMTC-apoA-I was then associated with the ^{3}H-labeled HDL by exchange (37°C, 24 h). Unbound apolipoprotein was removed by centrifugation at a salt density of 1.21 g/ml. The supernatant fraction containing doubly labeled HDL was dialyzed against phosphate-buffered saline at pH 7.4 containing EDTA (0.3 mM), and then sterile-filtered (0.45 μm) before use.

Preparation of labeled synthetic HDL

In some studies tracers were incorporated into synthetic HDL in order to accommodate [^{14}C]sucrose octaoleate, a definitive marker of the particle per se that could not be incorporated by transfer techniques (29, 30). [^{14}C]sucrose octaoleate was prepared as previously described (29). [^{14}C]sucrose octaoleate, [^{3}H]CEt, and ^{125}I-labeled NMTC-human apoA-I were then included during the preparation of the synthetic HDL by a cosonication method (29). Human apoA-I was used in the synthetic preparation. These particles have been shown to reproduce closely the physical and metabolic properties of biological HDL (29, 30).

Tracer studies in vivo

Experiments to determine the plasma FCR of HDL tracers and their rates of uptake by various tissues were carried out as previously described (2, 19). Food was removed at 16:00 h the night before turnover studies, which were initiated at 10:00 h by tail vein injection of labeled HDL (about 40 μg protein). Animals were fasted throughout the 24-h study period but had free access to water.

Periodic blood samples (20 μl) were withdrawn from the retro-orbital sinuses during the 24 h after tracer injection; samples were generally taken at 0.08, 0.5, 2, 6, 12, 20, and 24 h. Plasma samples were directly radioassayed for ^{125}I and subsequently radioassayed for ^{3}H after lipid extraction, as previously described (6).

The animals were anesthetized with ether and exsanguinated 24 h after tracer injection. The vasculature was perfused using at least 50 ml of phosphate-buffered saline. Whole organs, samples of large or diffuse tissues, gut contents, feces, and urine were collected, weighed, homogenized, and radioassayed. In the case of adipose tissue, muscle, and skin, literature values were used to estimate total tissue weight, as previously described (15, 19). As in previous studies, tracers in the feces and gut lumen were attributed to primary uptake by liver (2, 19); this represented a correction of about 10% in the case of [^{3}H]cholesteryl-oleyl ether tracer and about 40% in the case of ^{125}I-labeled NMTC-apoA-I tracer. Tissue content of ^{3}H was assayed by liquid scintillation spectrometry after lipid extraction (6).

Computer analysis using an iterative curve peeling program was used to fit a least-squares biexponential curve to each set of plasma decay data and to calculate fractional catabolic rates (FCR) according to Matthews (31).

Uptake of HDL components by individual tissues was calculated as previously described (2). Selective uptake was calculated as the rate of CE uptake minus that due to HDL particle uptake. In some cases this was the fractional rate of uptake of [^{3}H]CEt less the fractional rate of uptake of ^{125}I-labeled NMTC-apoA-I, while in other cases it was the fractional rate of uptake of [^{3}H]CEt less the fractional rate of uptake of [^{14}C]sucrose octaoleate. These are shown as the FCR attributable to the whole organ in each animal.

Statistical analysis

Values shown are mean ± standard deviation. Statistical significance was determined using the Student's *t*-test; where appropriate, comparison was for paired data (e.g., plasma decay of doubly labeled HDL particles). A difference was considered significant at $P < 0.05$.

RESULTS

The plasma decay kinetics of HDL CE and apoA-I tracers were determined in normal and transgenic male mice (**Table 1**). In normal mice the plasma FCR for [^{3}H]CEt was greater than the FCR for ^{125}I-labeled NMTC-apoA-I ($P < 0.05$ for unpaired data). As outlined above, this difference may be taken as a minimum value for whole-body selective uptake. In the transgenic mice the FCR for the CE moiety of HDL was not greater than that for apoA-I, and, in fact, it was somewhat smaller.

TABLE 1. Plasma fractional catabolic rates

	[^{3}H]CEt	^{125}I-labeled NMTC-ApoA-I
	h^{-1}	
Normal male mice	0.083 ± 0.010	0.060 ± 0.011
Transgenic male mice	0.072 ± 0.021	0.084 ± 0.084

The HDL_2 fraction isolated from the plasma of transgenic mice was labeled with [^{3}H]CEt and ^{125}I-labeled NMTC-apoA-I. Normal and transgenic mice (n = 4 for each set) were injected with the reisolated tracer HDL_2 via a tail vein, and plasma decay kinetics were followed for 24 h as described in the Methods section.

Thus, there was no evidence for selective uptake in the transgenic animals. The data shown are from experiments in which HDL_2 from transgenic mice (carrying predominantly human apoA-I) was labeled and injected into both normal and transgenic animals; similar results were obtained when HDL_2 from normal mice was labeled and used in both transgenic and normal animals. Thus, plasma decay kinetics largely reproduced the results of Chajek-Shaul et al. (13), providing evidence for selective uptake in normal mice but not in transgenic mice.

The next step was to examine the rates of uptake of the two tracers by individual tissues. To do this, experiments were carried out in normal and transgenic male mice using tracers incorporated into HDL endogenous to each strain. Thus, normal mice received normal mouse HDL labeled with [^{3}H]CEt and ^{125}I-labeled NMTC-mouse apoA-I by gentle transfer/exchange methods, while transgenic mice received HDL from transgenic animals labeled with [^{3}H]CEt and ^{125}I-labeled NMTC-human apoA-I. Plasma decay kinetics were followed for 24 h, when organs were removed to measure the accumulated tracers. Whereas the tracers were almost completely removed from plasma by 24 h (>95%), a theoretically correct measure of tracee uptake by individual organs could be calculated (20, 32, 33).

The data from male mice injected with the doubly labeled HDL tracers are shown in **Table 2.** The plasma FCR for apoA-I was about the same in normal and transgenic animals, whereas the FCR for CE was somewhat lower in the transgenic animals. Thus, again plasma decay kinetics gave evidence for selective uptake in normal mice but not in transgenic mice. However, the tissue data told a different story. These data indicated selective uptake in organs of both mouse strains. The tissue results generally resembled those previously reported in rats (2). Thus, liver was the main site of uptake of the CE tracer, accounting for about 54% of total HDL CE clearance in normal mice and 46% in transgenic mice. Apparent selective uptake, calculated as the difference between the fractional rates of [^{3}H]CEt and ^{125}I-labeled NMTC-apoA-I uptake, accounted for 35% of total [^{3}H]CEt uptake in the liver of normal mice and 40% in transgenic mice. Although the fractional rate of apparent selective uptake was 1.3-times as high in the normal animals (Table 2), the HDL CE mass flux to the liver was 1.5-times greater in the transgenic animals. ([Mass flux] = [liver FCR for selective uptake] × [HDL CE plasma pool size].) Thus, selective uptake was apparent in the livers of transgenic animals, where it mediated a greater mass flux of CE and made a greater fractional contribution to total hepatic HDL CE uptake than in normal animals.

Results for HDL tracer uptake by other tissues were

TABLE 2. Fractional rates of uptake of tracers from homologous HDL by specific organs of normal and transgenic mice

Organ Strain	Fractional Rate of Tracer Uptake		Apparent Selective Uptake ^{3}H − ^{125}I
	[^{3}H]CEt	^{125}I-labeled NMTC-ApoA-I	
	FCR per organ ($10^4 h^{-1}$)		
Plasma			
Normal	*922* ± 78	836 ± 16	86 ± 84
Transgenic	717 ± 209	835 ± 108	−130 ± 69
Liver			
Normal	500 ± 79	327 ± 61	173 ± 66
Transgenic	328 ± 104	193 ± 44	130 ± 69
Adrenal glands			
Normal	3.67 ± 0.33	0.88 ± 0.28	2.79 ± 0.22
Transgenic	4.21 ± 1.19	0.80 ± 0.03	3.44 ± 1.39
Testes			
Normal	7.17 ± 1.51	6.78 ± 1.84	0.38 ± 0.71
Transgenic	6.32 ± 1.86	6.62 ± 0.50	−0.31 ± 1.02
Lungs			
Normal	6.46 ± 1.61	3.49 ± 1.49	2.97 ± 1.02
Transgenic	6.85 ± 2.07	2.86 ± 0.23	4.00 ± 1.48
Spleen			
Normal	20.5 ± 4.5	12.7 ± 3.8	7.8 ± 1.8
Transgenic	7.77 ± 2.38	4.22 ± 0.99	3.55 ± 1.47
Kidneys			
Normal	8.37 ± 1.49	117 ± 34	−109 ± 33
Transgenic	10.2 ± 3.1	145 ± 31	−134 ± 30

Normal and transgenic male mice (n = 4 for each set) were administered homologous HDL labeled in the CE and apoA-I moieties. Thus, normal mice received normal mouse HDL labeled with [^{3}H]CEt and ^{125}I-labeled NMTC-mouse apoA-I, while transgenic mice received HDL from transgenic animals labeled with [^{3}H]CEt and ^{125}I-labeled NMTC-human apoA-I. Plasma decay kinetics were followed for 24 h, after which the vasculature was perfused and organs were removed (or aliquoted) for radioassay.

also reminiscent of previous results in rats (2). For example, selective uptake played a dominant role in CE uptake by adrenal gland, but no detectable role in HDL CE uptake by testes (Table 2).

The fractional rate for kidney uptake of ^{125}I-labeled NMTC-apoA-I was in great excess of the rate for [^{3}H]CEt uptake. Whereas mice lack signicant plasma CETP activity, the rate of [^{3}H]CEt uptake is the upper limit for the rate of HDL particle uptake. This leads to the interpretation that renal ^{125}I-labeled NMTC-apoA-I uptake was predominantly independent of HDL particle uptake, presumably representing its filtration and tubular reabsorption as in rats (2, 15, 16). This renal uptake of ^{125}I-labeled NMTC-apoA-I in excess of [^{3}H]CEt was at a greater fractional rate in the transgenic mice than in the normal mice. This explains, at least in part, the apparent contradiction of CE selective uptake in animals in which plasma clearance of ^{125}I-labeled NMTC-apoA-I is at a greater fraction rate than [^{3}H]CEt.

The above interpretation of the data in Table 2 depends on the assumption that ^{125}I-labeled NMTC-apoA-I adequately traces uptake of HDL particles, except in kidney. Indeed, this assumption has been supported in the case of rats (29, 30). However, we could not be sure that ^{125}I-labeled NMTC-apoA-I would play a similar role in normal mice; even if it did, it might not do so in the transgenic mice with their higher rates of production and higher plasma levels of human apoA-I.

To explore these possibilities, we used a marker of the HDL particle and its lipid core per se, [^{14}C]sucrose octaoleate (29). This is a high molecular weight, nonpolar, nonhydrolyzable lipid that is not subject to monomolecular transfers such as selective uptake or CETP-mediated transfer. It is also retained at its site of cellular uptake (29). Consequently, the [^{14}C]sucrose octaoleate tracer is irrevocably tied to the HDL particle in which it is introduced, marking its eventual site of cellular uptake. We have previously used this marker to validate the use of ^{125}I-labeled NMTC-apoA-I as a tracer of HDL particle uptake in most organs of the rat (29, 30). To apply this approach in mice, [^{14}C]sucrose octaoleate was incorporated into synthetic HDL particles (along with [^{3}H]CEt and ^{125}I-labeled NMTC-human apoA-I) by methods previously established (29). The resulting triply labeled particles were then injected into both normal and transgenic female mice. As in the study of Table 2, plasma decays were followed, and organs were sampled for tracer content 24 h after injection.

As shown in **Table 3,** the sites of [^{3}H]CEt uptake, and the apparent contribution of selective uptake to that uptake, were much the same as shown above for tracers in biological HDL. More than 60% of total [^{3}H]CEt clear-

TABLE 3. Fractional rates of uptake of tracers from synthetic HDL by specific organs of normal and transgenic mice

Organ Strain	Fractional Rate of Tracer Uptake			Apparent Selective Uptake	
	[^{3}H]CEt	^{125}I-labeled NMTC-ApoA-I	[^{14}C]Sucrose Octaoleate	^{3}H – ^{125}I	^{3}H – ^{14}C
	FCR per organ (10^{4} h^{-1})				
Plasma					
Normal	1046 ± 138	800 ± 83	632 ± 135	246 ± 86	414 ± 53
Transgenic	986 ± 196	910 ± 117	473 ± 83	76 ± 221	513 ± 207
Liver					
Normal	622 ± 103	316 ± 47	290 ± 78	305 ± 62	332 ± 33
Transgenic	597 ± 144	332 ± 34	191 ± 31	264 ± 135	406 ± 145
Adrenal gland					
Normal	18.2 ± 3.2	2.1 ± 0.5	5.0 ± 1.4	16.1 ± 2.7	13.2 ± 2.2
Transgenic	14.9 ± 2.8	1.6 ± 0.3	2.5 ± 0.8	13.3 ± 2.6	12.4 ± 0.6
Ovaries					
Normal	2.3 ± 0.5	1.7 ± 0.1	1.8 ± 0.5	0.6 ± 0.4	0.5 ± 0.3
Transgenic	3.0 ± 0.8	2.0 ± 0.2	1.4 ± 0.5	1.0 ± 0.8	1.6 ± 0.7
Lungs					
Normal	7.6 ± 2.4	3.0 ± 1.0	3.4 ± 1.1	4.6 ± 1.6	4.2 ± 1.3
Transgenic	6.9 ± 1.7	3.0 ± 0.4	2.0 ± 0.5	4.0 ± 1.6	4.9 ± 1.6
Spleen					
Normal	15.5 ± 6.2	15.0 ± 3.2	17.7 ± 7.4	0.6 ± 3.6	−2.1 ± 2.3
Transgenic	13.1 ± 3.7	10.5 ± 1.4	8.8 ± 1.1	2.7 ± 3.3	4.3 ± 3.6
Kidneys					
Normal	7.3 ± 1.0	70.3 ± 8.7	5.6 ± 0.8	−64.0 ± 8.0	1.7 ± 1.0
Transgenic	7.0 ± 2.1	123 ± 10	3.21 ± 0.5	−116 ± 11	3.8 ± 1.8

Triply labeled synthetic HDL was prepared using human apoA-I and injected into five normal female mice and five female transgenic mice. Plasma decays were followed for 24 h, after which the vasculature was perfused and organs were removed (or aliquoted) for radioassay.

ance from plasma was due to uptake by the liver, with most of that uptake attributable to selective uptake (using either ^{125}I-labeled NMTC-apoA-I or [^{14}C]sucrose octaoleate as a measure of HDL particle uptake). The rates of uptake of the ^{125}I-labeled NMTC-apoA-I and [^{14}C]sucrose octaoleate tracers agreed reasonably well in all organs of normal mice (except kidney, of course). Agreement between the particle markers was also reasonably close in most organs of the transgenic animals, except in the case of liver where the uptake of ^{125}I-labeled NMTC-apoA-I tracer was at a clearly greater fractional rate than the uptake of the [^{14}C]sucrose octaoleate tracer. The reason for this is uncertain. It does suggest a pathway for hepatic uptake of apoA-I in mice that is independent of HDL particle uptake, and that makes a greater contribution to hepatic apoA-I uptake in transgenic animals than in normal animals. It also suggests that selective uptake may have been underestimated in the experiments of Table 2 where ^{125}I-labeled NMTC-apoA-I served as the marker of HDL particle uptake in transgenic animals.

Using [^{14}C]sucrose octaoleate uptake as the measure of HDL particle uptake, the data of Table 3 indicate that selective uptake made an even greater contribution to hepatic uptake in transgenic animals than it did in normal mice (68% of total hepatic [^{3}H]CEt uptake in transgenic animals, and 53% of that uptake in normal animals). Regardless of whether [^{14}C]sucrose octaoleate or ^{125}I-labeled NMTC-apoA-I is taken as the measure of HDL particle uptake, selective uptake made a major contribution to HDL CE uptake by the liver in the transgenic animals.

Also shown in Table 3 is that uptake of ^{125}I-labeled NMTC-apoA-I by kidney was at a much greater fractional rate than the uptake of either [^{3}H]CEt (1.8 times) or [^{14}C]sucrose octaoleate (2.2 times), verifying that ^{125}I-labeled NMTC-apoA-I uptake by kidney was predominantly independent of HDL particle uptake. The fractional rate of this renal ^{125}I-labeled apoA-I uptake was substantially greater in the transgenic animals than in the normal animals, as was also seen in Table 2. Thus, again the higher plasma FCR for ^{125}I-labeled NMTC-apoA-I than of [^{3}H]CEt in the transgenic animals was at least largely explained by a greater fractional rate of renal uptake of apoA-I independent of HDL particle uptake.

DISCUSSION

The above results demonstrate that selective uptake of HDL CE is active in normal mice, as it has previously been shown to be in rats and rabbits (1–3). The results also show that selective uptake is active in transgenic mice where human apoA-I is the predominant form of that apolipoprotein produced and circulating. As in rats, liver plays the dominant role in mice both in total HDL CE uptake and in selective uptake. There is no substantial evidence here that selective uptake is even quantitatively less in the liver of transgenic mice. In fact, selective uptake made a greater contribution to total hepatic HDL CE uptake in the transgenic than in normal animals, and selective uptake mediated a greater mass flux of HDL CE in the transgenic animals. Thus there is no evidence at all that human apoA-I is any less able to support selective uptake than mouse apoA-I. This conclusion is in agreement with previous in vitro studies reporting selective uptake in the presence of only human apoA-I (8–12), which did not note a diminished capacity for that process.

Some problems of quantitation alluded to in the Results section should be further addressed. When the uptakes of ^{125}I-labeled NMTC-apoA-I and [^{14}C]sucrose octaoleate were compared (Table 3), the two tracers were taken up at similar rates by all organs of normal mice except kidney, indicating that ^{125}I-labeled NMTC-apoA-I reasonably marks uptake of HDL particles in normal mice (except kidney). However, ^{125}I-labeled NMTC-apoA-I was taken up in excess of [^{14}C]sucrose octaoleate by the liver of transgenic mice. This result suggests that the assumptions used in calculating selective uptake may not have been fully justified, at least in the case of hepatic uptake in the transgenic animals. There are several possible explanations for this. First, it is possible that the difference between the uptakes of ^{125}I-labeled NMTC-apoA-I and [^{14}C]sucrose octaoleate may have been the consequence of HDL metabolic heterogeneity that is more evident in the transgenic mice; the ^{125}I-labeled NMTC-apoA-I tracer would mark all subfractions while the [^{14}C]sucrose octaoleate tracer would mark only the injected tracer particles. Certainly there is greater HDL physical heterogeneity in the transgenic mice, which exhibit HDL_3 and HDL_1 fractions as well as the HDL_2 fraction that dominates in normal mice (17, 18). Another possible explanation is that the synthetic particles may have been metabolically different from the endogenous HDL; again, the immobile [^{14}C]sucrose octaoleate tracer would reflect only the fate of the synthetic particles, while the mobile ^{125}I-labeled NMTC-apoA-I tracer would largely reflect the fate of endogenous particles. It is also possible that apoA-I is cleared from lipoprotein fractions other than HDL to a greater extent in the transgenic mice. However, regardless of whether [^{14}C]sucrose octaoleate or ^{125}I-labeled NMTC-apoA-I more accurately reflects the actual rate of HDL particle uptake by the liver of the transgenic animals, selective uptake is apparent in that organ; only its rate is in some question.

The above problems of quantitation apply only to the experiments with labeled endogenous HDL particles, and were precluded in the experiments shown in Table 3 which used tracers incorporated into synthetic HDL particles. Upon injection of these particles, both the CE tracer and particle tracer ([^{3}H]CEt and [^{14}C]sucrose octaoleate) remained in the particles originally labeled, so that

selective uptake and particle uptake were necessarily measured from the same set of particles. Clearly [^{3}H]CEt was taken up without parallel uptake of [^{14}C]sucrose octaoleate, without the possibility of problems arising from metabolic heterogeneity or tracer/tracee differences. As the synthetic HDL particles were prepared using human apoA-I and injected into mice carrying predominantly (>95%) human apoA-I (17), there is little question of mouse apoA-I involvement. Thus, these data serve as an important confirmation that selective uptake is not only present, but is not compromised in the transgenic mice. Clearly human apoA-I can support the process in vivo, as previously shown in vitro (8–12).

From this and preceding studies there is much reason the think that selective uptake operates in humans as it does in rats, rabbits, and now mice. That is not to say that selective uptake is necessarily a major contributor to total clearance of HDL CE or to reverse cholesterol transport in humans as it is in rats and mice. That role usually is thought to be played in humans by the transfer of HDL CE to more buoyant lipoproteins in a process mediated by plasma CETP activity (34, 35). However, even a quantitatively minor role in normal individuals might have major implications to disease states. For example, it is a credible hypothesis that the CETP-mediated transfer of CE from HDL to more buoyant lipoproteins is a pro-atherogenic pathway (36). By the same token, selective uptake does not enrich the atherogenic lipoproteins in CE and thus should be anti-atherogenic in this context. Consequently, there might be reason to amplify this pathway at the expense of the competing CETP-mediated pathway as a prophylactic or therapeutic measure. In fact, it might be that selective uptake plays a protective role in normal individuals that we do not know about because we have not looked. We hope that this paper will once again make the point that this pathway should be studied in the human context where it may play a central role in mediating the apparent protective effect of HDL in reducing arteriosclerotic risk.

We thank Simone Green and Mercedes Silvestre for their excellent assistance in these studies. This work was supported by funds provided by the Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco-Related Disease Program of the University of California Grant No. IRT399. E.M.R. is an American Heart Association Established Investigatorship.

Manuscript received 24 June 1994 and in revised form 15 September 1994.

REFERENCES

1. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesteryl ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesteryl to liver, adrenal and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
2. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1984. Uptake of HDL-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* **260:** 744–750.
3. Goldberg, D. I., W. F. Beltz, and R. C. Pittman. 1990. Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. *J. Clin. Invest.* **87:** 331–346.
4. Arbeeny, C. M., V. A. Rifici, and H. A. Eder. 1987. The uptake of the apoprotein and cholesteryl ester of high-density lipoproteins by the perfused rat liver. *Biochim. Biophys. Acta.* **917:** 9–17.
5. Reaven, E., J. Boyles, M. Spicher, and S. Azhar. 1988. Evidence for surface entrapment of cholesterol-rich lipoproteins in luteinized ovary. *Arteriosclerosis.* **8:** 298–309.
6. Pittman, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor, Jr. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262:** 2443–2450.
7. Bachorik, P. S., D. G. Virgil, and P. O. Kwiterovich, Jr. 1987. Effect of apolipoprotein E-free high density lipoproteins on cholesterol metabolism in cultured pig hepatocytes. *J. Biol. Chem.* **262:** 13636–13645.
8. Rinninger, F., and R. C. Pittman. 1988. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters by human fibroblasts and HepG2 cells. *J. Lipid Res.* **29:** 1179–1194.
9. Wishart, R., and M. Mackinnon. 1990. Uptake and metabolism of high-density lipoproteins by cultured rabbit hepatocytes. *Biochim. Biophys. Acta.* **1044:** 375–381.
10. Despres, J. P., B. S. Fong, J. Jimenez, P. Julien, and A. Angel. 1988. Selective uptake of HDL cholesterol ester by human fat cells. *Am. J. Physiol.* **254:** E667–675.
11. Rinninger, F., and R. C. Pittman. 1989. Mechanism of the cholesteryl ester transfer protein-mediated uptake of high density lipoprotein cholesteryl esters by HepG2 cells. *J. Biol. Chem.* **264:** 6111–6118.
12. Rinninger, F., M. Brundert, S. Jackle, P. R. Galle, C. Busch, J. R. Izbicki, X. Rogiers, D. Herne-Bruns, B. Kremer, C. E. Broelsch, and H. Greten. 1994. Selective uptake of high density lipoprotein-associated cholesteryl esters by human hepatocytes in primary culture. *Hepatology.* **19:** 1100–1114.
13. Chajek-Shaul, T., T. Hayek, A. Walsh, and J. L. Breslow. 1991. Expression of the human apolipoprotein A-I gene in transgenic mice alters high density lipoprotein (HDL) particle size distribution and diminishes selective uptake of HDL cholesteryl esters. *Proc. Natl. Acad. Sci. USA.* **88:** 6731–6735.
14. Walsh, A., Y. Ito, and J. L. Breslow. 1989. High levels of human apolipoprotein A-I in transgenic mice result in increased plasma levels of small high density lipoprotein (HDL) particles comparable to human HDL$_3$. *J. Biol. Chem.* **264:** 6488–6494.
15. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **258:** 7161–7167.
16. Saku, K., G. S. Reddy, B. A. Hynd, and L. Kashyap. 1984. Renal handling of high density lipoproteins by isolated perfused kidneys. *Metabolism.* **33:** 432–438.
17. Rubin, E. M., B. Y. Ishida, S. M. Clift, and R. M. Krauss. 1991. Expression of human apolipoprotein A-I in transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new high density lipoprotein size subclasses. *Proc. Natl. Acad. Sci. USA.* **88:** 434–438.

18. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. *Nature.* **353:** 265–267.
19. Pittman, R. C., and C. A. Taylor, Jr. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–628.
20. Pittman, R. C., T. E. Carew, C. K. Glass, S. R. Green, C. A. Taylor, Jr., and A. D. Attie. 1983. A radioiodinated, intracellularly trapped ligand for determining the sites of plasma protein degradation in vivo. *Biochem. J.* **212:** 791–800.
21. Jiao, S., T. G. Cole, R. T. Kitchens, B. Pfleger, and G. Schonfeld. 1990. Genetic heterogeneity of lipoproteins in inbred strains of mice: analysis by gel-permeation chromatography. *Metabolism* **39:** 155–160.
22. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
23. Weisgraber, K. H., and R. W. Mahley. 1978. Apoprotein (E-A-II) complex of human plasma lipoproteins. I. Characterization of this mixed disulfide and its identification in a high density lipoprotein subfraction. *J. Biol. Chem.* **253:** 6281–6288.
24. Pittman, R. C., and D. Steinberg. 1984. Sites and mechanisms of uptake and degradation of high density and low density lipoproteins. *J. Lipid Res.* **25:** 1577–1585.
25. Weber, K., and M. Osborne. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
26. Pool, G. L., M. E. French, R. A. Edwards, L. Huang, and R. H. Lumb. 1982. Use of radiolabeled hexadecyl cholesteryl ether as a liposome marker. *Lipids.* **17:** 448–452.
27. Hough, J. L., and D. B. Zilversmit. 1984. Comparison of various methods for in vitro cholesteryl ester labeling of lipoproteins from hypercholesterolemic rabbits. *Biochim. Biophys. Acta.* **792:** 338–347.
28. Morton, R. E., and D. B. Zilversmit. 1981. A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256:** 11992–11995.
29. Pittman, R. C., C. K. Glass, D. Atkinson, and D. M. Small. 1987. Synthetic high density lipoprotein particles: application to studies of the apoprotein specificity for selective uptake of cholesterol esters. *J. Biol. Chem.* **262:** 2435–2442.
30. Richard, B. M., M. A. Pfeuffer, and R. C. Pittman. 1992. Transport of HDL cholesterol esters to the liver is not diminished by probucol treatment in rats. *Arterioscler. Thromb.* **12:** 862–869.
31. Matthews, C. M. E. 1957. The theory of tracer experiments with ^{131}I-labeled plasma proteins. *Phys. Med. Biol.* **2:** 36–53.
32. Pittman, R. C., A. D. Attie, T. E. Carew, and D. Steinberg. 1982. Tissue sites of catabolism of rat and human low density lipoproteins in rats. *Biochim. Biophys. Acta.* **710:** 7–14.
33. Carew, T. E., R. C. Pittman, and D. Steinberg. 1982. Tissue sites of degradation of native and reductively methylated [^{14}C]sucrose-labeled low density lipoprotein in rats: contribution of receptor-dependent and receptor-independent pathways. *J. Biol. Chem.* **257:** 8001–8008.
34. Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and W. Patsch. 1987. High density lipoprotein$_2$. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. *J. Clin. Invest.* **80:** 341–347.
35. Schwartz, C. C., L. A. Zech, J. M. VandenBroek, and P. S. Cooper. 1989. Reverse cholesterol transport measured in vivo in man: the central roles of HDL. *In* High Density Lipoproteins and Atherosclerosis II. N. E. Miller, editor. Elsevier Science Publisher B. V., Amsterdam. 321–329.
36. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27:** 361–367.