Lipoprotein [a] is cleared from the plasma primarily by the liver in a process mediated by apolipoprotein [a]

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Abstract **The cellular and molecular mechanisms responsible for lipoprotein [a] (Lp[a]) catabolism are unknown. We examined the plasma clearance of Lp[a] and LDL in mice using lipoproteins isolated from human plasma coupled to radiolabeled tyramine cellobiose. Lipoproteins were injected into wild-type, LDL receptor-deficient (*Ldlr*$^{-/-}$), and apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice. The fractional catabolic rate of LDL was greatly slowed in *Ldlr*$^{-/-}$ mice and greatly accelerated in *Apoe*$^{-/-}$ mice compared with wild-type mice. In contrast, the plasma clearance of Lp[a] in *Ldlr*$^{-/-}$ mice was similar to that in wild-type mice and was only slightly accelerated in *Apoe*$^{-/-}$ mice. Hepatic uptake of Lp[a] in wild-type mice was 34.6% of the injected dose over a 24 h period. The kidney accounted for only a small fraction of tissue uptake (1.3%). To test whether apolipoprotein [a] (apo[a]) mediates the clearance of Lp[a] from plasma, we coinjected excess apo[a] with labeled Lp[a]. Apo[a] acted as a potent inhibitor of Lp[a] plasma clearance. Asialofetuin, a ligand of the asialoglycoprotein receptor, did not inhibit Lp[a] clearance. In summary, the liver is the major organ accounting for the clearance of Lp[a] in mice, with the LDL receptor and apolipoprotein E having no major roles. Our studies indicate that apo[a] is the primary ligand that mediates Lp[a] uptake and plasma clearance.**—Cain, W. J., J. S. Millar, A. S. Himebauch, U. J. F. Tietge, C. Maugeais, D. Usher, and D. J. Rader. **Lipoprotein [a] is cleared from the plasma primarily by the liver in a process mediated by apolipoprotein [a].** ***J. Lipid Res.*** **2005.** 46: **2681–2691.**

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Lipoprotein [a] (Lp[a]) is a LDL-like lipoprotein that has been associated with increased risk of coronary heart disease, stroke, and restenosis (1). Lp[a], which closely resembles LDL in lipid composition, contains a single apolipoprotein B-100 (apoB-100) molecule and an additional apolipoprotein, called apolipoprotein [a] (apo[a]), which is connected via a disulfide linkage to apoB-100 (1). Apo[a] is a polymorphic glycoprotein that contains repeating domains of varying length that are homologous to kringle IV of plasminogen. More than 30 different isoforms of apo[a] have been described in humans, ranging in size from <300 kDa to >800 kDa (2).

Plasma levels of Lp[a] vary greatly between individuals, from <1 mg/dl to >100 mg/dl (2). The source of most of this variability in plasma concentrations is variability in the production rate of Lp[a] (3, 4), which, in turn, is controlled largely by the apo[a] gene locus (5). Apo[a] is synthesized by hepatocytes and rapidly associates with LDL after secretion to form Lp[a] in the sinusoids of the liver (2, 6). Although the steps involved in Lp[a] production are known, the major mechanisms involved in Lp[a] clearance from plasma are not currently known. It has been suggested that the kidney might play a major role in Lp[a] clearance, after several clinical studies reported increased plasma Lp[a] levels in patients with renal failure (7, 8). In addition, Kronenberg et al. (9) used measurements of renovascular arteriovenous differences in Lp[a] plasma concentrations in patients without renal insufficiency to demonstrate that the human kidney may play an active role in Lp[a] catabolism.

Several receptors that mediate the binding and uptake of lipoproteins containing apoB-100 have been proposed as receptors for Lp[a] catabolism. These include the LDL receptor (LDLR) (10–15), megalin/gp330 (16), the LDL receptor-related protein (LRP) (17), and the VLDL receptor (18). The latter two receptors can mediate binding to lipoproteins through apoE. According to the secretion-capture model, apoE that is secreted by the liver rapidly

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binds to remnants associated with heparan sulfate proteoglycans. These captured lipoproteins are then cleared by receptor-mediated endocytosis (19). ApoE has been proposed to play a similar role in Lp[a] catabolism (20).

The current studies were designed to test potential pathways responsible for Lp[a] uptake in mice through the use of a radioiodinated, intracellularly trapped ligand, tyramine cellobiose (TC), which was covalently attached to human Lp[a] and injected into mouse models in the C57BL/6 genetic background. Our results demonstrate that in this model, the liver is the major organ responsible for the plasma clearance of Lp[a] and that the kidney plays a very minor role under normal conditions. In addition, we examined the role of the LDLR, apoE, and the asialoglycoprotein receptor (ASGPR) in the clearance of Lp[a] in mice. These studies show that the LDLR, apoE, and the ASGPR do not have a major role in Lp[a] catabolism in mice. Lastly, we demonstrated that the administration of excess apo[a] can effectively block the plasma clearance of Lp[a], suggesting that it is the apo[a] moiety that mediates the plasma clearance of Lp[a].

METHODS

Animals

Wild-type C57BL/6, LDL receptor-deficient ($Ldlr^{-/-}$), and apoE-deficient ($Apoe^{-/-}$) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were maintained on a chow diet under a 12 h light/dark cycle.

Isolation of lipoproteins and apo[a]

Lp[a] was isolated from single-donor plasma as described previously (3, 4, 21). Briefly, plasma was immediately brought to 0.01% NaN_3, 0.01% Na_2EDTA, and 1 mM benzamidine and adjusted to a density of 1.21 g/ml, and total lipoproteins were isolated by ultracentrifugation. The total lipoprotein fraction was dialyzed against phosphate buffer (0.1 M sodium phosphate, 0.01% NaN_3, 0.01% Na_2EDTA, and 1 mM benzamidine, pH 7.4) and then applied to a lysine-Sepharose column (Amersham Pharmacia) equilibrated with phosphate buffer. The column was then washed with phosphate buffer, and the unbound fraction, which contained LDL, was collected. The bound fraction, which is composed mostly of Lp[a], was eluted with phosphate buffer containing 100 mM ε-aminocaproic acid (ACA). This fraction was brought to 7.5% (w/w) CsCl and centrifuged in a Beckman 60Ti rotor at 50,000 rpm and 15°C for 27 h. The self-forming density gradient was pumped from the centrifuge tubes. Those fractions containing purified Lp[a] were pooled and dialyzed against PBS (0.1 M NaCl, 0.01 M sodium phosphate, 0.01% NaN_3, 0.01% Na_2EDTA, and 1 mM benzamidine, pH 7.4). The unbound fraction from the lysine-Sepharose column was dialyzed against histidine buffer (0.2 M NaCl, 0.025 M histidine, and 0.01% Na_2EDTA, pH 6.0) and applied to a column containing PBE94 (Bio-Rad) equilibrated with histidine buffer. The column was washed with histidine buffer, and the unbound fraction, which contained LDL, was collected and dialyzed against PBS. This fraction was concentrated using an Amicon stirred pressure cell, and the density was then adjusted to 1.35 g/ml with NaBr. Five milliliters of this solution was placed into 25 ml Beckman 60Ti centrifuge tubes and overlaid with a 0–25% NaBr gradient. The tubes were centrifuged for 90 min at 15°C and 50,000 rpm. The density gradient was pumped from the centrifuge tubes, and those fractions containing purified LDL were pooled and dialyzed against PBS.

Apo[a] was prepared by a modification of the procedure described by Edelstein et al. (22). Lp[a] was brought to a final concentration of 100 mM ACA and 2 mM dithioerythritol and incubated at room temperature for 1 h. The reaction mixture was dialyzed against PBS for ~1 h and adjusted to a density of 1.3 g/ml with NaBr. Approximately 5 ml of the density-adjusted reaction mix was placed into a Beckman 60Ti centrifuge tube and overlaid with a 10–25% NaBr gradient to a volume of 25 ml. This was centrifuged at 50,000 rpm and 15°C for 90 min to separate apo[a] from the LDL released by reduction and intact Lp[a]. The density gradient was pumped from the centrifuge tubes, and those fractions containing purified apo[a] were pooled, dialyzed against PBS, and concentrated using Vivaspin concentrators (Vivascience).

The BCA protein assay (Pierce) was used to quantify all lipoprotein and apolipoprotein preparations. When Lp[a] was coinjected with excess apo[a], we estimated the molar excess of apo[a] as follows. We determined that the apparent molecular weight of apo[a] used in this study was the same as that of apoB-100 and made the simplifying assumption that an equimolar amount of Lp[a] would have twice the amount of protein as apo[a] when measured by the BCA assay. The fold molar excess was then calculated as (micrograms of apo[a] injected)/(micrograms of Lp[a] injected × 0.5).

Radioactive labeling of proteins

Lipoproteins, apo[a], and asialofetuin (from fetal calf serum; Sigma) were directly iodinated with either $Na^{131}I$ or $Na^{125}I$ (New England Nuclear) using a modification of the iodine monochloride procedure (4); alternatively, TC was iodinated with either $Na^{131}I$ or $Na^{125}I$ and then chemically coupled to LDL or Lp[a] using the procedure described by Pittman and Taylor (23). Typically, 0.1 μmol of TC was iodinated using Iodobeads (Pierce) and then chemically coupled to ~5 mg of protein with 0.1 μmol of cyanuric chloride. The purity of the radiolabeled preparations was verified using SDS-PAGE and 1% agarose native gels (Ciba Corning).

Metabolic studies

Radioiodinated lipoproteins in saline were injected via a tail vein. Blood was drawn from the orbital sinus into heparin-coated tubes, and plasma was separated by low-speed centrifugation. Radioactivity in 10 μl aliquots was quantified in a γ counter. The fractional catabolic rates (FCRs) were calculated using SAAM II (SAAM Institute, Seattle, WA).

Tissue uptake of proteins labeled with TC was examined in mice housed in metabolic cages (four mice per cage). One day after injection of radiotracer, the mice were anesthetized and bled from the inferior vena cava immediately followed by perfusion with saline. Unless stated otherwise, organs were removed whole, weighed, and counted. Tissue samples were taken from visceral adipose tissue, muscle from the hind leg, and skin from the abdomen. These samples were weighed and counted. The accumulations of radiotracer in skin, muscle, and adipose tissue were calculated by assuming that these tissues accounted for 16.5, 38.4, and 6.4% (w/w), respectively, of the mass of a mouse (24). Feces and urine were collected and counted. The injected dose was calculated using the plasma counts at 5 min as follows: (counts per minute per milliliter of plasma at 5 min) × (mouse weight in grams) × (0.035 ml of plasma per gram of mouse body weight). The counts remaining in the plasma at 24 h were calculated as follows: (counts per minute per milliliter of plasma at 24 h) × (mouse weight in grams) × (0.035 ml of plasma per gram of mouse body weight). The tissue distribution of radiotracer is expressed as a percentage of the injected dose.

Statistical analysis

All statistical analyses were performed using the unpaired two-tailed Student's *t*-test.

RESULTS

Analysis of radiolabeled Lp[a] and LDL

Lipoproteins were either radiolabeled directly by the iodine monochloride method (4) or, alternatively, radiolabeled TC was chemically cross-linked to lipoproteins by the method of Pittman and Taylor (23). For each study, lipoprotein preparations were analyzed by SDS-PAGE under reducing and nonreducing conditions. A representative gel is shown in **Fig. 1A**. The Lp[a] used in this study had a single apo[a] isoform with the same apparent molecular weight as apoB-100, and this was verified by immunoblotting (data not shown). Nondenaturing agarose gel electrophoresis was also performed (Fig. 1B), and for all preparations, a single band with preβ mobility was observed. Lipoproteins labeled by either of the two methods showed essentially identical migration patterns when examined using both electrophoretic methods. The ^{125}I-TC-Lp[a] contained ~10% of the label on apo[a] and the remainder on apoB-100 (data not shown).

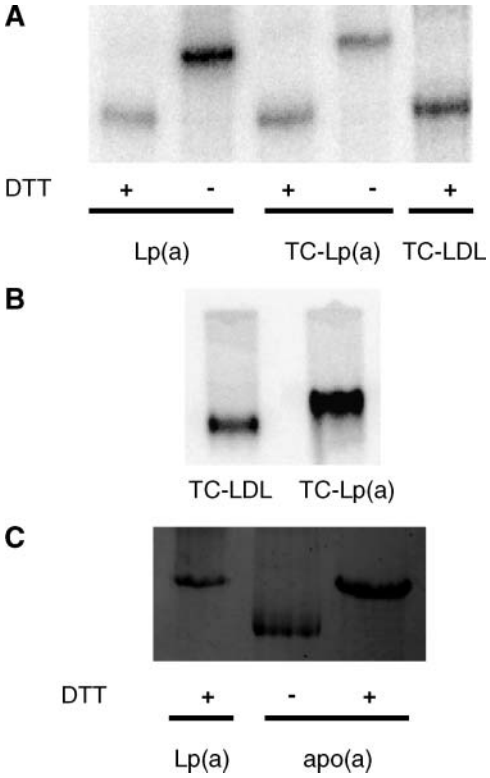

Fig. 1. Analysis of radiolabeled lipoproteins. The lipoprotein [a] (Lp[a]) and LDL used for this study were isolated from a donor who possessed a single apolipoprotein [a] (apo[a]) isoform with the same apparent molecular weight as apolipoprotein B-100. A: Analysis by SDS-PAGE. The panel shows an autoradiograph of ^{125}I-Lp[a], reduced (lane 1) and not reduced (lane 2); ^{125}I-tyramine cellobiose (TC)-Lp[a], reduced (lane 3) and not reduced (lane 4); ^{131}I-TC-LDL, reduced (lane 5). B: Autoradiograph of ^{125}I-TC-Lp[a] and ^{131}I-TC-LDL separated by nondenaturing agarose gel electrophoresis. C: Analysis by SDS-PAGE. The panel shows Coomassie staining of Lp[a], reduced (lane 1); and apo[a], not reduced (lane 2) and reduced (lane 3).

Tissue uptake of Lp[a] and LDL in wild-type mice

To assess which organs or tissues were responsible for Lp[a] uptake and catabolism, male C57BL/6 mice were injected simultaneously with both ^{125}I-TC-Lp[a] and ^{131}I-Lp[a]. For comparison, another set of male mice was injected with ^{125}I-TC-LDL and ^{131}I-LDL. The clearance of LDL was significantly faster than the clearance of Lp[a] for both ^{125}I-TC-labeled lipoproteins ($P = 0.02$) and ^{131}I-labeled lipoproteins ($P = 0.0002$) (**Fig. 2**). Furthermore, the plasma clearance of ^{125}I-TC-labeled lipoproteins was similar to that of the ^{131}I-labeled forms. The plasma clearance of ^{125}I-TC-LDL (3.0 ± 0.2 d^{-1}) and ^{131}I-LDL (3.2 ± 0.3 d^{-1}) were not significantly different ($P = 0.3$), whereas the clearance of ^{125}I-TC-Lp[a] (2.0 ± 0.2 d^{-1}) was slightly slower than that of ^{131}I-Lp[a] (2.5 ± 0.3 d^{-1}) ($P = 0.03$).

After 24 h, the animals were euthanized and tissues counted. The results are shown in **Fig. 3**, and values are expressed as percentages of the injected dose retained by each tissue. The tissue distribution of TC-Lp[a] and TC-LDL were quite similar. The liver was the major organ that accumulated the TC-labeled lipoproteins, 21.3 ± 3.1% of the injected Lp[a] and 20.5 ± 2.5% of the injected LDL. The kidney contained only 1.3 ± 0.3% of the Lp[a] and <1% of the LDL. Intestinal tissue contained 2.1 ± 0.3% and 1.9 ± 0.5% of the Lp[a] and LDL, respectively. Combined values for the stomach, spleen, adrenals, testes, heart, lungs, and brain contained <2% of either lipoprotein. We used tissue samples to estimate the amount of

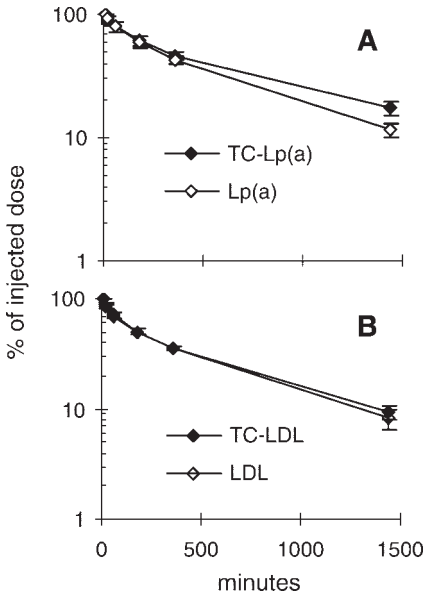

Fig. 2. Plasma clearance of human Lp[a] and LDL in wild-type mice. A: Four male mice were injected with both ^{125}I-TC-labeled Lp[a] (TC-Lp[a]) and ^{131}I-Lp[a] (Lp[a]). B: Four male mice were injected with both ^{125}I-TC-labeled LDL (TC-LDL) and ^{131}I-LDL (LDL). Error bars indicate SD.

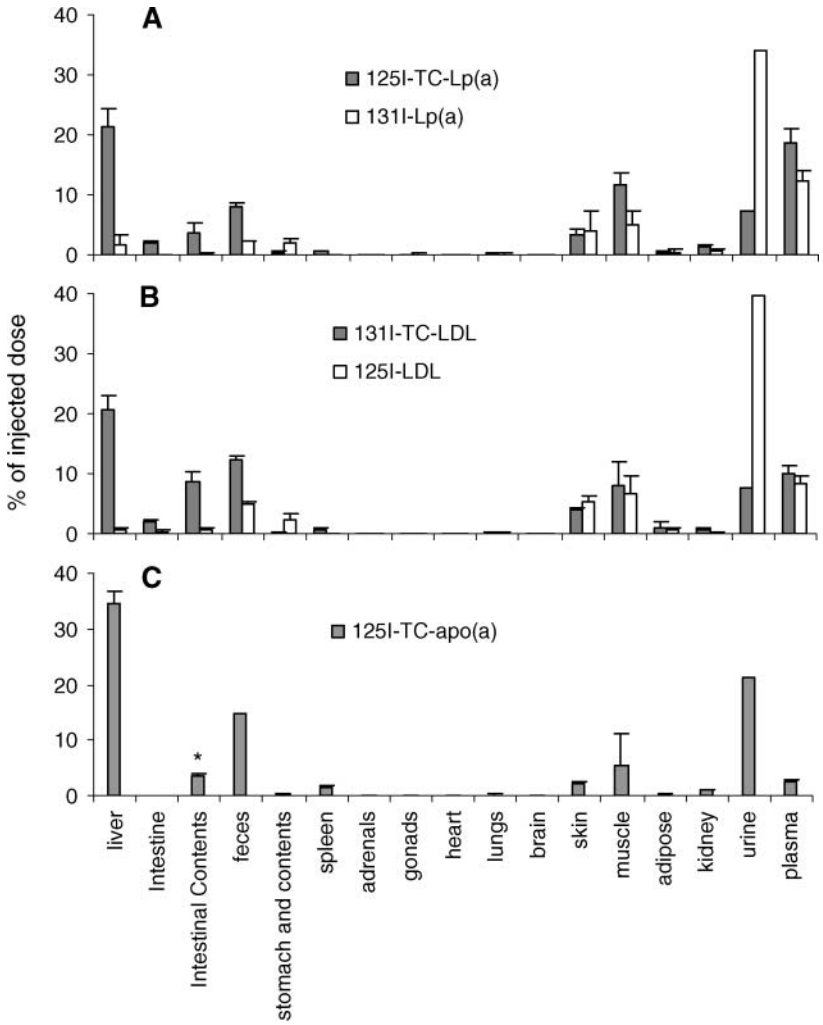

Fig. 3. Tissue distribution of radiolabeled human Lp[a] and LDL in wild-type mice. Groups of four mice each were housed in metabolic cages. Twenty-four hours after injection of radiotracer, the animals were euthanized and their tissues were counted. The results are expressed as percentages of the injected dose. A: Four male mice were injected with both ^{125}I-TC-Lp[a] and ^{131}I-Lp[a]. B: Four male mice were injected with both ^{125}I-TC-LDL and ^{131}I-LDL. C: Four male mice were injected with ^{125}I-TC-apo[a]. Error bars indicate SD.

TC-labeled lipoproteins that accumulated in the skin, muscle, and adipose tissue (see Methods). Both the muscle and skin retained a considerable amount of lipoprotein. The muscle contained 11.7 ± 2.0% of the Lp[a] and 7.9 ± 4.4% of the LDL, whereas the skin retained 3.2 ± 1.1% of the Lp[a] and 4.0 ± 0.3% of the LDL. Adipose tissue retained only a small amount of label.

As expected, the tissue distribution of ^{131}I-LDL and ^{131}I-Lp[a] that lacked the trapped TC ligand was considerably different from that of the TC-labeled lipoproteins (Fig. 3). The liver retained relatively little LDL and Lp[a] when these were not coupled to TC. Most of the labeled lipoprotein retained by tissues was found in the muscle and skin. The largest amount of radiolabel was recovered in the urine and accounted for 43% and 39% of the injected dose for LDL and Lp[a], respectively. This is an underestimation because there were some urine losses during some of the mouse bleeds. Indeed, in a similar study, when blood samples were not taken over the course of the urine collection, the radiolabel recovered in the urine accounted for almost all of the counts not recovered in the carcass when lipoproteins were labeled directly with iodine (data not shown).

To determine whether the ^{125}I-TC-Lp[a] injected into mice was still intact in the plasma after 24 h, plasma samples were adjusted to 7.5% (w/w) CsCl and ultracentrifuged to separate Lp[a] from LDL. Under these conditions, LDL floats near the top of the self-forming density gradient, whereas Lp[a] is found toward to the bottom. **Figure 4** clearly shows that almost all of the radiolabel in the plasma after 24 h was found as intact Lp[a]. Free apo[a], which would be found in the bottom of the tube, was not detected.

Metabolism of Lp[a] and LDL in *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice

Wild-type, *Ldlr*$^{-/-}$, and *Apoe*$^{-/-}$ male mice were injected with both ^{125}I-TC-Lp[a] and ^{131}I-TC-LDL. The plasma clearance of LDL was considerably slower in *Ldlr*$^{-/-}$ mice than

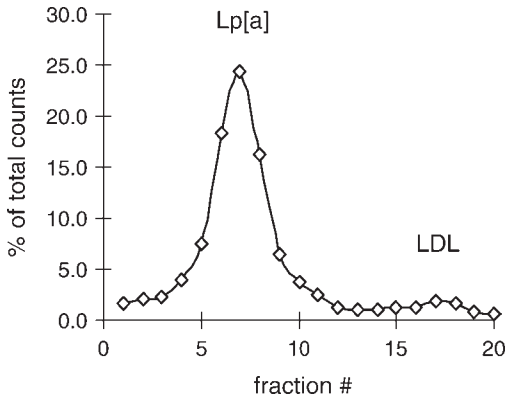

Fig. 4. Distribution of ^{125}I-TC between Lp[a] and LDL at 24 h after injection of ^{125}I-TC-Lp[a]. Ten microliters of plasma, obtained form a mouse at 24 h after injection with ^{125}I-TC-Lp[a], was added to a 7.5% CsCl solution, and the LDL was separated from Lp[a] by density gradient ultracentrifugation.

in wild-type controls (1.47 vs. 2.96 d^{-1}; $P = 0.0002$), whereas the clearance was accelerated considerably in *Apoe*$^{-/-}$ mice (4.69 vs. 2.96 d^{-1}; $P < 0.0001$) (**Fig. 5A**). In contrast, the plasma clearance rate of Lp[a] was not affected significantly by the absence of the LDLR and was modestly increased by 15% in the *Apoe*$^{-/-}$ mouse (Fig. 5B) compared with the wild-type mouse (2.95 vs. 2.43 d^{-1}; $P = 0.01$).

The distribution of the trapped TC ligand among the tissues of the three mouse strains is shown in **Table 1**. As shown previously for wild-type mice, the uptake of radiolabel by different tissues was very similar for TC-Lp[a] and TC-LDL when injected into wild-type mice, with the liver accounting for 23.2% and 21.4% of the injected dose for TC-Lp[a] and TC-LDL, respectively. LDLR deficiency resulted in a markedly lower accumulation of TC-LDL in the liver (8.0%) but only a small decrease in the accumulation of TC-Lp[a] in the liver (19.5%). *Apoe*$^{-/-}$ mice had a marked increase in the accumulation of TC-LDL in the liver (41.8%) but only a small increase in the accumulation of TC-Lp[a] in the liver (27.7%).

Hepatic uptake of Lp[a] and LDL

Pittman and Taylor (23) showed in rat and in rabbit that trapped radiotracer from TC-labeled plasma protein did not redistribute between tissues after initial uptake, within 24 h after injection. However, they did detect significant leakage of radiolabel from the liver, which appeared in the gut contents and the feces. Because the liver is a major site for Lp[a] and LDL uptake in mice, we believe that radiotracer appearing in the gut contents and feces is probably hepatic in origin. Therefore, a better estimate for the hepatic uptake and catabolism of LDL and Lp[a] in mice would be obtained by adding the amounts recovered in the liver, gut contents, and feces. The net hepatic contribution to plasma clearance was calculated and expressed as a percentage of the injected dose (**Fig. 6**). In these studies, the intestinal tissue and contents were not separated. We estimated the net hepatic clearance as the sum of the label found in the liver, intestine with contents,

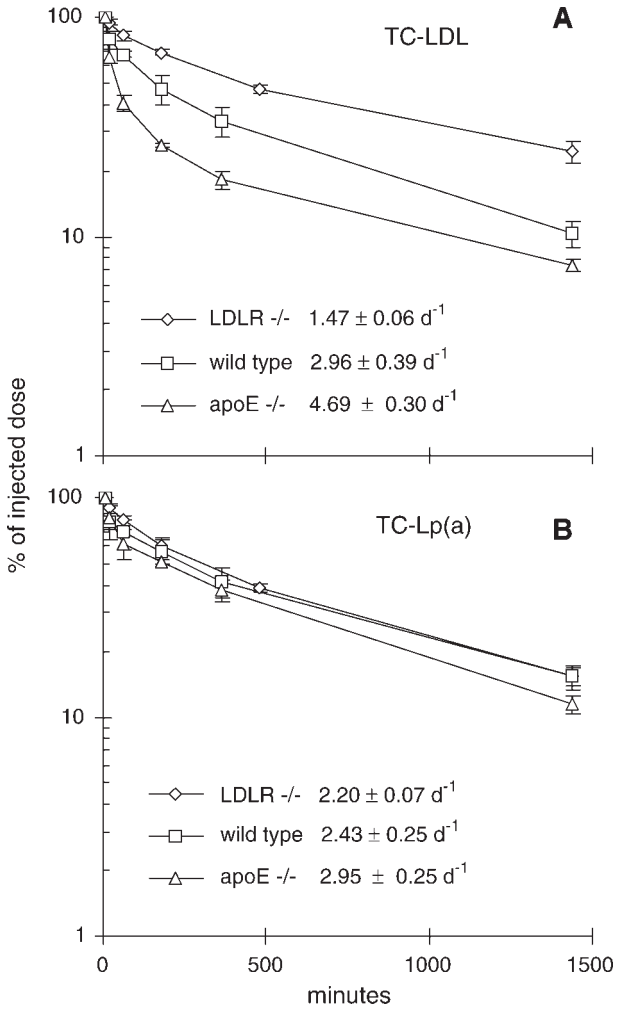

Fig. 5. Plasma clearance of TC-Lp[a] and TC-LDL. Mice were injected with both ^{125}I-TC-Lp[a] and ^{131}I-TC-LDL. A: Clearance of ^{131}I-TC-LDL. B: Clearance of ^{125}I-TC-Lp[a]. Wild-type mice, n = 7; LDL receptor-deficient (*Ldlr*$^{-/-}$) mice, n = 4; apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice, n = 4. Error bars indicate SD.

and feces. Because intestinal tissue contained only ~2% of the injected dose for both TC-LDL and TC-Lp[a] in wild-type mice, the error caused by including the intestinal tissue in the net hepatic uptake should be relatively small. In wild-type mice, the net hepatic uptake of TC-labeled LDL after 24 h was 41.7 ± 2.3% of the injected dose. Hepatic uptake of TC-LDL was decreased by >2-fold in *Ldlr*$^{-/-}$ mice to 18.7 ± 0.7% ($P < 0.0001$), whereas hepatic uptake in *Apoe*$^{-/-}$ mice was increased nearly 2-fold to 70.25 ± 4.0% ($P < 0.0001$). In contrast, hepatic uptake of TC-Lp[a] showed a small decrease in *Ldlr*$^{-/-}$ mice to 27.9 ± 1.3% compared with 34.6 ± 3.1% in wild-type mice ($P = 0.003$), and it increased in *Apoe*$^{-/-}$ mice to 39.33 ± 2.1% ($P = 0.04$).

Plasma clearance and tissue uptake of apo[a]

Apo[a] that was purified from Lp[a] had the same apparent molecular weight as apoB-100 when analyzed by SDS-PAGE under reducing conditions and, as expected, migrated considerably faster under nonreducing conditions

TABLE 1. Tissue distribution of ^{125}I-TC-Lp[a] and ^{131}I-TC-LDL

Organ	TC-LDL			TC-Lp[a]		
	Apoe$^{-/-}$	Wild Type	*Ldlr*$^{-/-}$	*Apoe*$^{-/-}$	Wild Type	*Ldlr*$^{-/-}$
Liver	41.76 ± 0.68[a]	21.39 ± 3.11	8.00 ± 0.56[a]	27.74 ± 1.40[b]	23.17 ± 2.34	19.53 ± 1.72[b]
Intestine and contents	6.10 ± 0.58[b]	9.26 ± 1.23	5.76 ± 1.12[b]	4.78 ± 0.58[b]	6.42 ± 1.09	4.08 ± 1.06[b]
Feces	23.85	13.60	6.29	9.48	7.21	6.2
Stomach and contents	0.36 ± 0.06	0.34 ± 0.17	0.81 ± 0.30[b]	0.40 ± 0.04	0.40 ± 0.22	0.88 ± 0.35[b]
Adrenals	0.29 ± 0.12[b]	0.13 ± 0.05	0.04 ± 0.01[b]	0.07 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
Spleen	0.98 ± 0.05	1.48 ± 0.62	0.81 ± 0.04	0.93 ± 0.03	0.91 ± 0.27	0.43 ± 0.03[b]
Gonads	0.09 ± 0.00[b]	0.13 ± 0.02	0.11 ± 0.05	0.08 ± 0.01	0.10 ± 0.02	0.07 ± 0.03
Heart	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.17 ± 0.05	0.13 ± 0.03	0.09 ± 0.02
Lungs	0.19 ± 0.03	0.23 ± 0.07	0.27 ± 0.09	0.35 ± 0.10	0.34 ± 0.13	0.20 ± 0.04
Brain	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.01[b]	0.02 ± 0.00	0.03 ± 0.01	0.01 ± 0.01[b]
Skin	3.13 ± 0.11	4.66 ± 3.40	5.56 ± 3.08	5.97 ± 1.47	5.75 ± 3.17	3.67 ± 1.96
Muscle	2.91 ± 0.35	8.61 ± 6.18	6.77 ± 1.78	4.58 ± 0.63	11.39 ± 8.10	5.54 ± 1.53
Adipose	0.47 ± 0.20	1.39 ± 2.68	2.72 ± 4.12	0.71 ± 0.33	2.08 ± 4.20	2.48 ± 4.11
Kidney	1.40 ± 0.08	1.10 ± 0.43	0.61 ± 0.14	0.94 ± 0.05	1.06 ± 0.16	0.82 ± 0.09[b]
Urine	17.16	6.62	2.59	11.69	7.16	3.97
Plasma	7.47 ± 0.23[b]	11.02 ± 1.48	26.27 ± 3.00[a]	11.90 ± 1.13[b]	16.33 ± 1.61	16.32 ± 2.04

Apoe$^{-/-}$, apolipoprotein E-deficient; *Ldlr*$^{-/-}$, LDL receptor-deficient; Lp[a], lipoprotein [a]; TC, tyramine cellobiose. Mice were injected with both ^{125}I-TC-Lp[a] and ^{131}I-TC-LDL. Wild-type (n = 7), *Ldlr*$^{-/-}$ (n = 4), and *Apoe*$^{-/-}$ (n = 3) mice were housed in separate metabolic cages. Animals were euthanized after 24 h, and the organs and tissue samples were collected. Plasma samples were also taken, and the results are shown in Fig. 4. The results are expressed as percentages of the injected dose. Data for feces and urine are from pooled samples. *t*-tests were used to compare each tissue in the knockout lines with wild-type tissues.

[a] $P < 0.0005$.

[b] $P < 0.05$.

(Fig. 1C). The plasma clearance of ^{125}I-TC-apo[a] was monitored in four male mice, and after 24 h, tissues were harvested and counted. Plasma clearance of apo[a] was faster than that of Lp[a], with a FCR of 7.38 ± 0.53 d^{-1}. The tissue distribution of ^{125}I-TC-apo[a] was similar to that for Lp[a] (Fig. 3C). The liver was the predominant site of apo[a] catabolism and retained 34.7 ± 1.9% of the injected dose, whereas the kidney retained only 0.9 ± 0.2%. The hepatic contribution to plasma clearance (liver + intestinal contents + feces) was 52.7%.

Apo[a] mediates the plasma clearance of Lp[a]

Because we found that Lp[a] was not cleared to any significant extent by pathways involving the LDLR or apoE, we hypothesized that Lp[a] clearance could be mediated by the apo[a] moiety of the lipoprotein. To test this hypothesis, we measured the clearance of Lp[a] in the presence of a large (~30-fold) molar excess of apo[a] (**Fig. 7A**). Lp[a] clearance was measured in mice injected with 72 μg of ^{125}I-TC-Lp[a] protein and 1.1 mg of unlabeled apo[a] protein and compared with Lp[a] clearance in mice injected with label only. The plasma clearance of Lp[a] was greatly reduced by apo[a]. In mice coinjected with label and apo[a], 99.1 ± 6.2% of the labeled Lp[a] remained in the plasma after 30 min, whereas in mice injected with label only, 76.8 ± 1.2% of the ^{125}I-TC-Lp[a] remained in the plasma ($P = 0.007$). By 1 h, this effect was still apparent, with 93.5 ± 5.6% of the label remaining in the plasma of mice coinjected with apo[a] compared with 65.7 ± 3.3% in mice receiving label only ($P = 0.003$).

To assess the contribution of the liver in the plasma clearance of Lp[a] during the first hour after injection, mice were injected with ^{125}I-TC-Lp[a] and at 1 h the animals were euthanized and perfused. After 1 h, 39.6 ± 3.5% of the injected dose was cleared from the plasma and 10.5% of the injected dose was recovered in the liver (**Fig. 8**). The spleen and kidney contained 0.6 ± 0.3% and 0.7 ± 0.1% of the injected dose, respectively.

Apo[a] is a highly glycosylated protein rich in sialic acid, and it has been suggested by Hrzenjak et al. (25) that the ASGPR found in the liver may play an important role in the plasma clearance of Lp[a]. We examined the clearance of ^{125}I-TC-Lp[a] and ^{125}I-TC-asialofetuin, an ASGPR ligand, in both the presence and absence of excess unlabeled asialofetuin (Fig. 7B). Mice were injected with 60 μg each of labeled Lp[a] and asialofetuin, either with or without 7.5 mg of asialofetuin. The plasma clearance of asialofetuin is very rapid, so that the initial plasma sample

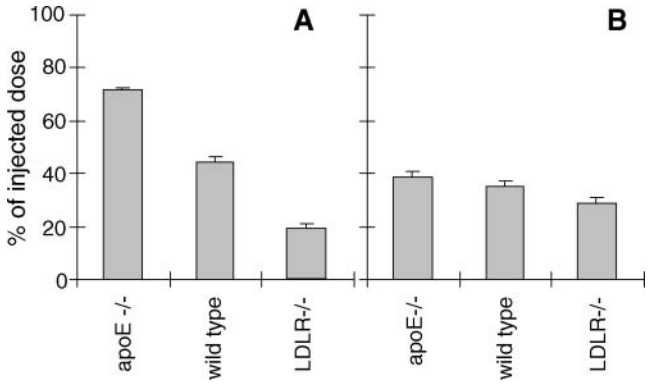

Fig. 6. Hepatic clearance of TC-LDL (A) and TC-Lp[a] (B). Hepatic clearance was estimated by adding the tissue counts found in the liver, intestine with contents, and feces at 24 h after the injection of ^{125}I-TC-Lp[a] and ^{131}I-TC-LDL. The results are expressed as percentages of the injected dose. Wild-type mice, n = 7; *Ldlr*$^{-/-}$ mice, n = 4; *Apoe*$^{-/-}$ mice, n = 3. Error bars indicate SD.

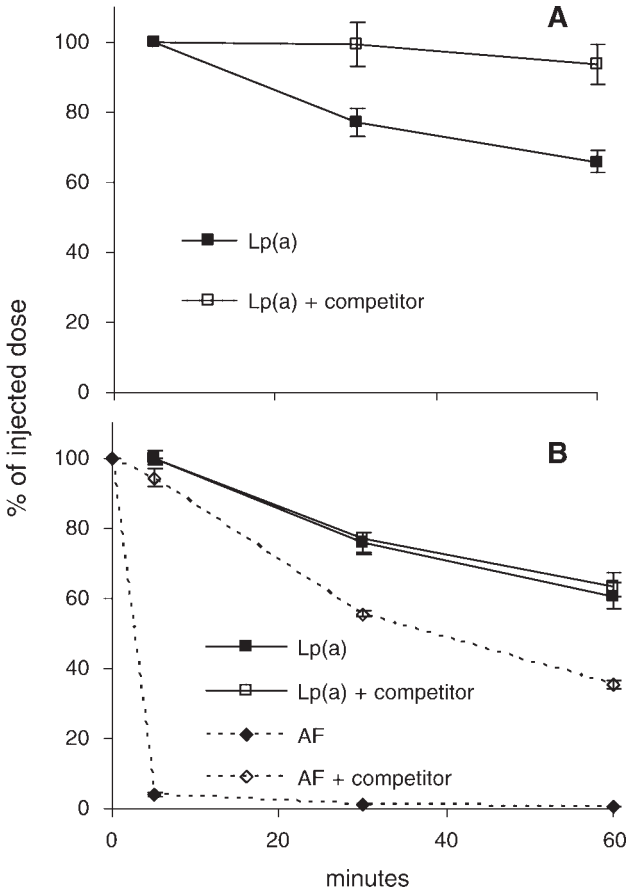

Fig. 7. Effect of apo[a] and asialofetuin on the plasma clearance of Lp[a]. A: Effect of apo[a]. Mice were injected with ^{125}I-TC-Lp[a] either without or with 1.1 mg of apo[a] (+ competitor) (n = 4 for each group). B: Effect of asialofetuin (AF). Mice were injected with ^{125}I-TC-Lp[a] and ^{131}I-TC-asiaolfetuin either without or with 7.5 mg of asialofetuin (+ competitor) (n = 4 for each group). Error bars indicate SD.

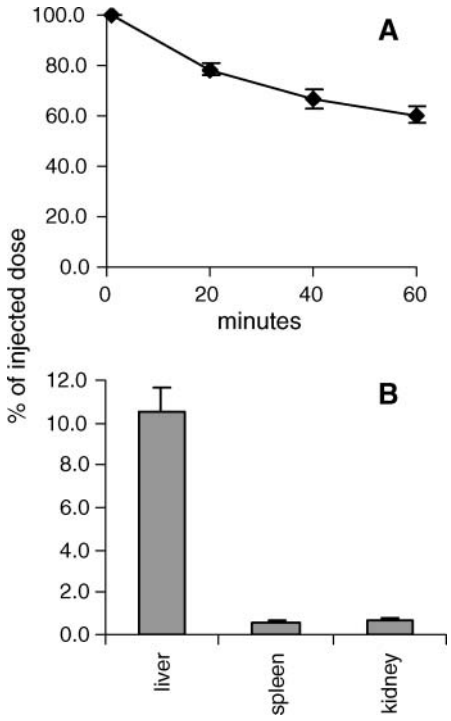

Fig. 8. Plasma clearance (A) and tissue distribution (B) of ^{125}I-TC-Lp[a] at 1 h after injection (n = 4). Results are expressed as percentages of the injected dose. Error bars indicate SD.

taken at 5 min occurs after most of the label has been cleared. To obtain meaningful data for the clearance of asialofetuin, we found it necessary to estimate the actual injected dose at time zero based upon the volume injected rather than use the 5 min time point. At 5 min, only 3.9 ± 0.4% of the label remained in the plasma. In contrast, in those mice also injected with excess unlabeled asialofetuin, 95.3 ± 2.8% of the injected label remained in the plasma after 5 min. The inhibitory effect is still clearly seen after 1 h. The plasma clearance of Lp[a] was essentially unaffected by the presence of asialofetuin over the 1 h study period.

DISCUSSION

Mice do not possess Lp[a], which has been described only in certain primates and hedgehogs (26). Despite this limitation, studies using transgenic mice producing human Lp[a] have already contributed much to our understanding of Lp[a] synthesis (27–30) and its role in atherosclerosis (31–33). Furthermore, we believe that mice may also offer an attractive model for the study of Lp[a] catabolism, because they are able to clear the lipoprotein in a way that appears to be similar to that in humans. In this study, we examined the plasma clearance and tissue uptake of both human LDL and Lp[a] in mice. As we demonstrated previously in humans (3, 4, 21), in wild-type mice the clearance of LDL is faster than that of Lp[a], and the relative FCR of LDL to Lp[a] is similar to that in humans. In this study in mice, FCR values for LDL were ~20% higher than the values for Lp[a], whereas in humans, FCR values were ~40% higher for LDL than for Lp[a]. The plasma clearance of Lp[a] has been examined in human subjects with homozygous familial hypercholesterolemia who lack LDLRs (21). In the present study, the influence of LDLR deficiency in mice on Lp[a] turnover is remarkably similar to the results seen in humans (**Table 2**). In both species, LDLR deficiency reduces the LDL FCR to ~50% of normal, while only reducing the Lp[a] FCR by ~10% (a difference that in both the mouse and human studies was not statistically significant). Gabelli et al. (34) examined the plasma clearance of LDL in a human subject who was apoE-deficient and reported an FCR that was 100% greater than normal. Similarly, in *Apoe*$^{-/-}$ mice, the LDL FCR was increased by 58%. Thus, the plasma clearance of both human LDL and Lp[a] in mice resembles what occurs in humans, suggesting that the pathways of catabolism in these species are similar.

We used radioiodinated TC to examine the tissue uptake of both human LDL and Lp[a] in mice. For both lipoproteins, the liver was by far the major organ responsible for clearance, whereas the kidney retained only ~1%

TABLE 2. Comparison of FCR values for human Lp[a] and LDL in normal and *Ldlr*$^{-/-}$ individuals

Sample	FCR		Percentage Change
	Ldlr$^{-/-}$	Normal	
	pools/day		
LDL			
Human	0.19	1.47	−53
Mouse	0.40	2.96	−50
Lp[a]			
Human	0.25	2.20	−13
Mouse	0.29	2.43	−9

FCR, fractional catabolic rate. The data for mice are from this study; the data for humans are from Rader et al. (21) and Gabelli et al. (34).

of the injected dose. Furthermore, the spleen retained only ~1% of the injected label, indicating that the labeled proteins were not recognized as foreign or denatured. The hepatic uptake, as measured by the sum of the label found in the liver, intestines, and feces, accounted for 34.6 ± 3.1% of the injected dose. A better indicator of the liver's role in Lp[a] catabolism is obtained when uptake is expressed as a percentage of the cleared dose (injected dose minus the plasma counts at 24 h). When expressed in this manner, hepatic uptake of Lp[a] accounts for 42.3 ± 4.9% of the cleared dose. In rat, Ye et al. (35) found similar results when they examined the tissue uptake of Lp[a] that was labeled with [^{3}H]cholesteryl linoleyl ether, a nonhydrolyzable lipid. After 24 h, the liver contained 21.4% of the injected dose, compared with 23.2% (Table 1) in the mice used in this study. They also found <1% of the injected dose retained by the kidney.

The kidney has been hypothesized to play a major role in Lp[a] metabolism. Patients with impaired renal function have increased plasma Lp[a] levels (7, 8, 36, 37). In addition, Kronenberg et al. (9) found arteriovenous differences in Lp[a] plasma concentrations, indicating that Lp[a] was removed from the renal circulation in these patients. Several studies have identified large apo[a] fragments in human urine (7, 38, 39), and evidence suggests that these fragments are formed extrarenally and excreted by the kidney (38, 40, 41). In our study, ~7% of the injected radiolabel from ^{125}I-TC-Lp[a] was found in the urine after 24 h (Table 1), and only ~10% of the radiolabel was TCA-precipitable (data not shown). We did not detect any fragmentation of Lp[a] in the plasma. Mooser et al. (38) injected human Lp[a] into mice and detected only trace amounts in the urine by ELISA, and they detected no apo[a] fragments in either the urine or plasma by immunoblot analysis. Thus, in mice with normal renal function, the kidney does not play a major role in Lp[a] catabolism.

It has been proposed that Lp[a] is a ligand for the LDLR and that the liver plays a prominent role in plasma clearance of Lp[a] through the LDLR pathway. Although some in vitro studies support this proposal (11–13), others do not (42–44). In addition, several studies have reported that individuals with familial hypercholesterolemia, in which there is a genetic absence of functional LDLRs, have increased plasma Lp[a] levels, suggesting a role for the LDLR and the liver in the plasma clearance of Lp[a] (10, 14, 15). However, kinetic studies in humans injected with ^{125}I-labeled Lp[a] have shown that Lp[a] plasma clearance is essentially the same in individuals lacking the LDLR compared with normal and heterozygous familial hypercholesterolemia family members (21), and only a minor role for the LDLR was suggested. Furthermore, Lp[a] isolated from patients with familial defective apoB-100 did not contain a higher proportion of defective apoB-100 than normal apoB-100, supporting the concept that the role of the LDLR in Lp[a] clearance is minimal (45).

Transgenic mice and rabbits have been used to further explore the potential role of the LDLR in Lp[a] clearance. Mice overexpressing the human LDLR were found to have accelerated clearance of human Lp[a], and the authors concluded that the LDLR has the potential to play a major role in the plasma clearance of Lp[a] in humans (46). Transgenic rabbits have been created that express human apo[a], and they form circulating Lp[a] that is composed of human apo[a] linked via a disulfide bound to rabbit LDL (47). When these rabbits were cross-bred with WHHL rabbits, progeny that produce Lp[a] but have defective LDLR function showed markedly increased plasma Lp[a] levels compared with apo[a] transgenic animals with normal receptor function (48). Presumably, this increase in Lp[a] was caused by a reduced plasma clearance rate, although no in vivo studies of Lp[a] turnover were performed in this model.

The tissue distributions in wild-type mice of TC-labeled LDL and Lp[a] were very similar. For both lipoproteins, the liver was the primary site of uptake and catabolism. When expressed as a percentage of the cleared dose, hepatic uptake accounted for 42.3% and 47.8% of the plasma clearance of Lp[a] and LDL, respectively, in wild-type mice. This suggests the possible role of the LDLR in Lp[a] clearance in mice. Indeed, in *Ldlr*$^{-/-}$ mice, the FCR for Lp[a] was ~9% less and hepatic uptake was reduced by ~20% during 24 h. On the other hand, for LDL, the FCR was reduced by ~50% and the hepatic uptake was less than half that for wild-type mice. Thus, it is possible that in wild-type mice the LDLR may make a minor contribution to the hepatic uptake of Lp[a] but that its role is much more limited than it is in the uptake of LDL.

ApoE can associate with apoB-containing lipoproteins and facilitate receptor-mediated endocytosis by several receptors in the LDLR family (19). In addition, van Barlingen et al. (20) showed that enrichment of Lp[a] with apoE resulted in increased lipoprotein lipase-enhanced binding to heparan sulfate proteoglygans, concluding that the secretion-capture process of apoE was a possible catabolic route for Lp[a]. We examined the possible role of apoE in the catabolism of Lp[a]. In mice deficient in apoE, the plasma clearance of Lp[a] was slightly accelerated compared with that in wild-type mice (Fig. 5B), and there was a small increase in hepatic uptake (Fig. 6A). This suggests that Lp[a] catabolism is not primarily facilitated through the acquisition of apoE. In these mice, plasma clearance and hepatic uptake of LDL were greatly

accelerated (Figs. 5A, 6B). Woollett et al. (49) made similar observations when they examined the catabolism of both mouse and human LDL in *Apoe*$^{-/-}$ mice. Their studies revealed that the increased catabolism of LDL was not attributable to an increase in hepatic LDLRs but rather to the lack of apoE-containing lipoproteins to compete for binding to the receptor.

Other members of the LDLR family have also been suggested to participate in Lp[a] catabolism. It has been shown that the VLDL receptor mediates the uptake and catabolism of Lp[a] in fibroblasts expressing the VLDL receptor and that this uptake is mediated by apo[a] (18). Furthermore, the plasma clearance of Lp[a] was delayed in VLDL receptor knockout mice. However, the VLDL receptor is found primarily in the heart, muscle, and adipose tissue (50) and is expressed only at very low levels in the liver. Hence, the VLDL receptor may play an important role in the apo[a]-mediated clearance of Lp[a] in certain nonhepatic tissues. Another candidate receptor, megalin/gp330, can mediate the uptake and degradation of Lp[a] in in vitro studies (16), but unlike the VLDL receptor, the uptake is primarily mediated by apoB-100. However, megalin/gp330 is found only in certain resorptive epithelial cells, such as the proximal tubules of the kidney, and is not found in the liver (51). Hence, it is unlikely that the VLDL receptor or megalin/gp330 participates significantly in the hepatic uptake of Lp[a]. The LRP is highly expressed by the liver, and one in vitro study suggested that it may act as a receptor for high molecular weight isoforms of Lp[a] (17), but other in vitro studies do not support this notion (44, 52). It is well known that the LRP can mediate the hepatic catabolism of chylomicron and VLDL remnants containing apoE (24). However, because apoE deficiency in mice did not reduce the hepatic uptake of ^{125}I-TC-Lp[a], it is doubtful that the LRP plays a similar role in the catabolism of Lp[a].

We demonstrate here for the first time that the plasma clearance of Lp[a] by the liver is greatly reduced when coinjected with a large molar excess of apo[a]. These results strongly suggest that the apo[a] moiety of Lp[a] is the major ligand that mediates plasma clearance by the liver. In separate studies, labeled apo[a] has a similar pattern of tissue uptake to Lp[a], with the liver being the predominant site, consistent with Lp[a] and apo[a] being ligands for a common receptor(s). Even if Lp[a] and apo[a] largely share the same receptor(s), they are still different molecules and might be expected to have different binding kinetics. In addition, the presence of the large LDL moiety found in Lp[a] could affect its access to putative receptors to which apo[a] also binds. This could explain why apo[a] is cleared more than twice as fast as Lp[a], and why uptake by the liver is enhanced compared with that of Lp[a].

Apo[a] is a heavily sialylated protein (53), and if desialylated it is a possible ligand for the ASGPR, which is highly expressed in hepatocytes (54). Asialofetuin is a well-characterized ligand of the ASGPR that is rapidly cleared from the plasma by the liver (55). We found that asialofetuin at concentrations that greatly reduced its own plasma clearance rate had no effect on Lp[a] clearance. Hrzenjak et al. (25) showed that asialo Lp[a] is rapidly cleared from the plasma of mice and hedgehogs. In addition, they showed that in ASGPR-deficient mice the plasma clearance of native Lp[a] is delayed. However, in the absence of the ASGPR, Lp[a] clearance was only slowed by a small percentage, leading the authors to speculate that other mechanisms are involved in Lp[a] clearance. Indeed, the plasma clearance of Lp[a] expressed as a percentage of the injected dose was not significantly slower in ASGPR-deficient mice until 4 h after injection. We also saw a small but nonsignificant slowing of Lp[a] clearance in mice injected with asialofetuin, which was only apparent after 4 h (data not shown).

Keesler et al. (56) identified an apo[a] receptor that mediates the binding and internalization of apo[a] and Lp[a] in cholesterol-loaded macrophages. They showed that the region around kringle IV_{6-7} of apo[a] was important in receptor binding. Recently, Devlin et al. (57) suggested the existence of one or more receptors in the liver that bind to Lp[a] through the kringle IV_{5-8} region of apo[a] and that also mediate the plasma clearance of remnant lipoproteins.

In this study, we used Lp[a] possessing a single isoform of apo[a] with the same apparent molecular weight as apoB-100. It is possible that the catabolism of other isoforms may be somewhat different. However, the FCR is independent of isoform size in humans (3), and presumably the sites of tissue uptake are independent of isoform size as well. We believe that this is probably also true for mice, although that issue was not addressed in this study.

In conclusion, we demonstrate that the liver is the primary organ responsible for the uptake and catabolism of Lp[a] in mice, whereas the kidneys are responsible for only a small fraction of the tissue uptake. Neither the LDLR nor apoE plays an important role in the hepatic uptake of Lp[a]. We show that a large molar excess of apo[a] dramatically reduces the plasma clearance of Lp[a], which suggests that the apo[a] moiety plays a major role in mediating the plasma clearance and tissue uptake of Lp[a]. The nature of the major receptor(s) that mediate the plasma clearance and tissue uptake of Lp[a] remains to be determined.

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REFERENCES

1. Scanu, A. M. 2001. The role of lipoprotein(a) in the pathogenesis of atherosclerotic cardiovascular disease and its utility as predictor of coronary heart disease events. *Curr. Cardiol. Rep.* **3:** 385–390.

2. Hobbs, H. H., and A. L. White. 1999. Lipoprotein(a): intrigues and insights. *Curr. Opin. Lipidol.* **10:** 225–236.
3. Rader, D. J., W. Cain, K. Ikewaki, G. Talley, L. A. Zech, D. Usher, and H. B. Brewer, Jr. 1994. The inverse association of plasma lipoprotein(a) concentrations with apolipoprotein(a) isoform size is not due to differences in Lp(a) catabolism but to differences in production rate. *J. Clin. Invest.* **93:** 2758–2763.
4. Rader, D. J., W. Cain, L. A. Zech, D. Usher, and H. B. Brewer, Jr. 1993. Variation in lipoprotein(a) concentrations among individuals with the same apolipoprotein (a) isoform is determined by the rate of lipoprotein(a) production. *J. Clin. Invest.* **91:** 443–447.
5. Boerwinkle, E., C. C. Leffert, J. Lin, C. Lackner, G. Chiesa, and H. H. Hobbs. 1992. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. *J. Clin. Invest.* **90:** 52–60.
6. Koschinsky, M. L., and S. M. Marcovina. 2004. Structure-function relationships in apolipoprotein(a): insights into lipoprotein(a) assembly and pathogenicity. *Curr. Opin. Lipidol.* **15:** 167–174.
7. Kostner, K. M., M. Clodi, G. Bodlaj, B. Watschinger, W. Horl, K. Derfler, and K. Huber. 1998. Decreased urinary apolipoprotein (a) excretion in patients with impaired renal function. *Eur. J. Clin. Invest.* **28:** 447–452.
8. Sechi, L. A., L. Zingaro, S. De Carli, G. Sechi, C. Catena, E. Falleti, E. Dell'Anna, and E. Bartoli. 1998. Increased serum lipoprotein(a) levels in patients with early renal failure. *Ann. Intern. Med.* **129:** 457–461.
9. Kronenberg, F., E. Trenkwalder, A. Lingenhel, G. Friedrich, K. Lhotta, M. Schober, N. Moes, P. Konig, G. Utermann, and H. Dieplinger. 1997. Renovascular arteriovenous differences in Lp[a] plasma concentrations suggest removal of Lp[a] from the renal circulation. *J. Lipid Res.* **38:** 1755–1763.
10. Wiklund, O., B. Angelin, S. O. Olofsson, M. Eriksson, G. Fager, L. Berglund, and G. Bondjers. 1990. Apolipoprotein(a) and ischaemic heart disease in familial hypercholesterolaemia. *Lancet.* **335:** 1360–1363.
11. Floren, C. H., J. J. Albers, and E. L. Bierman. 1981. Uptake of Lp (a) lipoprotein by cultured fibroblasts. *Biochem. Biophys. Res. Commun.* **102:** 636–639.
12. Krempler, F., G. M. Kostner, A. Roscher, F. Haslauer, K. Bolzano, and F. Sandhofer. 1983. Studies on the role of specific cell surface receptors in the removal of lipoprotein (a) in man. *J. Clin. Invest.* **71:** 1431–1441.
13. Havekes, L., B. J. Vermeer, T. Brugman, and J. Emeis. 1981. Binding of Lp(a) to the low density lipoprotein receptor of human fibroblasts. *FEBS Lett.* **132:** 169–173.
14. Mbewu, A. D., D. Bhatnagar, P. N. Durrington, L. Hunt, M. Ishola, S. Arrol, M. Mackness, P. Lockley, and J. P. Miller. 1991. Serum lipoprotein(a) in patients heterozygous for familial hypercholesterolemia, their relatives, and unrelated control populations. *Arterioscler. Thromb.* **11:** 940–946.
15. Utermann, G., F. Hoppichler, H. Dieplinger, M. Seed, G. Thompson, and E. Boerwinkle. 1989. Defects in the low density lipoprotein receptor gene affect lipoprotein (a) levels: multiplicative interaction of two gene loci associated with premature atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **86:** 4171–4174.
16. Niemeier, A., T. Willnow, H. Dieplinger, C. Jacobsen, N. Meyer, J. Hilpert, and U. Beisiegel. 1999. Identification of megalin/gp330 as a receptor for lipoprotein(a) in vitro. *Arterioscler. Thromb. Vasc. Biol.* **19:** 552–561.
17. Marz, W., A. Beckmann, H. Scharnagl, R. Siekmeier, U. Mondorf, I. Held, W. Schneider, K. T. Preissner, L. K. Curtiss, W. Gross, et al. 1993. Heterogeneous lipoprotein (a) size isoforms differ by their interaction with the low density lipoprotein receptor and the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor. *FEBS Lett.* **325:** 271–275.
18. Argraves, K. M., K. F. Kozarsky, J. T. Fallon, P. C. Harpel, and D. K. Strickland. 1997. The atherogenic lipoprotein Lp(a) is internalized and degraded in a process mediated by the VLDL receptor. *J. Clin. Invest.* **100:** 2170–2181.
19. Mahley, R. W., and Z. S. Ji. 1999. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* **40:** 1–16.
20. van Barlingen, H. H., H. A. Kleinveld, D. W. Erkelens, and T. W. de Bruin. 1997. Lipoprotein lipase-enhanced binding of lipoprotein(a) [Lp(a)] to heparan sulfate is improved by apolipoprotein E (apoE) saturation: secretion-capture process of apoE is a possible route for the catabolism of Lp(a). *Metabolism.* **46:** 650–655.
21. Rader, D. J., W. A. Mann, W. Cain, H. G. Kraft, D. Usher, L. A. Zech, J. M. Hoeg, J. Davignon, P. Lupien, M. Grossman, et al. 1995. The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans. *J. Clin. Invest.* **95:** 1403–1408.
22. Edelstein, C., M. Mandala, D. Pfaffinger, and A. M. Scanu. 1995. Determinants of lipoprotein(a) assembly: a study of wild-type and mutant apolipoprotein(a) phenotypes isolated from human and rhesus monkey lipoprotein(a) under mild reductive conditions. *Biochemistry.* **34:** 16483–16492.
23. Pittman, R. C., and C. A. Taylor, Jr. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–628.
24. Brown, R. P., M. D. Delp, S. L. Lindstedt, L. R. Rhomberg, and R. P. Beliles. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health.* **13:** 407–484.
25. Hrzenjak, A., S. Frank, X. Wo, Y. Zhou, T. Van Berkel, and G. M. Kostner. 2003. Galactose-specific asialoglycoprotein receptor is involved in lipoprotein (a) catabolism. *Biochem. J.* **376:** 765–771.
26. Lawn, R. M., N. W. Boonmark, K. Schwartz, G. E. Lindahl, D. P. Wade, C. D. Byrne, K. J. Fong, K. Meer, and L. Patthy. 1995. The recurring evolution of lipoprotein(a). Insights from cloning of hedgehog apolipoprotein(a). *J. Biol. Chem.* **270:** 24004–24009.
27. Callow, M. J., L. J. Stoltzfus, R. M. Lawn, and E. M. Rubin. 1994. Expression of human apolipoprotein B and assembly of lipoprotein(a) in transgenic mice. *Proc. Natl. Acad. Sci. USA.* **91:** 2130–2134.
28. Cheesman, E. J., R. J. Sharp, C. H. Zlot, C. Y. Liu, S. Taylor, S. M. Marcovina, S. G. Young, and S. P. McCormick. 2000. An analysis of the interaction between mouse apolipoprotein B100 and apolipoprotein(a). *J. Biol. Chem.* **275:** 28195–28200.
29. Chiesa, G., H. H. Hobbs, M. L. Koschinsky, R. M. Lawn, S. D. Maika, and R. E. Hammer. 1992. Reconstitution of lipoprotein(a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein(a). *J. Biol. Chem.* **267:** 24369–24374.
30. Linton, M. F., R. V. Farese, Jr., G. Chiesa, D. S. Grass, P. Chin, R. E. Hammer, H. H. Hobbs, and S. G. Young. 1993. Transgenic mice expressing high plasma concentrations of human apolipoprotein B100 and lipoprotein(a). *J. Clin. Invest.* **92:** 3029–3037.
31. Callow, M. J., J. Verstuyft, R. Tangirala, W. Palinski, and E. M. Rubin. 1995. Atherogenesis in transgenic mice with human apolipoprotein B and lipoprotein (a). *J. Clin. Invest.* **96:** 1639–1646.
32. Lawn, R. M., D. P. Wade, R. E. Hammer, G. Chiesa, J. G. Verstuyft, and E. M. Rubin. 1992. Atherogenesis in transgenic mice expressing human apolipoprotein(a). *Nature.* **360:** 670–672.
33. Sanan, D. A., D. L. Newland, R. Tao, S. Marcovina, J. Wang, V. Mooser, R. E. Hammer, and H. H. Hobbs. 1998. Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). *Proc. Natl. Acad. Sci. USA.* **95:** 4544–4549.
34. Gabelli, C., R. E. Gregg, L. A. Zech, E. Manzato, and H. B. Brewer, Jr. 1986. Abnormal low density lipoprotein metabolism in apolipoprotein E deficiency. *J. Lipid Res.* **27:** 326–333.
35. Ye, S. Q., J. Keeling, O. Stein, Y. Stein, and W. J. McConathy. 1988. Tissue distribution of [^{3}H]cholesteryl linoleyl ether-labeled human Lp(a) in different rat organs. *Biochim. Biophys. Acta.* **963:** 534–540.
36. Kronenberg, F., K. Lhotta, P. Konig, R. Margreiter, H. Dieplinger, and G. Utermann. 2003. Apolipoprotein(a) isoform-specific changes of lipoprotein(a) after kidney transplantation. *Eur. J. Hum. Genet.* **11:** 693–699.
37. Kronenberg, F., A. Lingenhel, K. Lhotta, B. Rantner, M. F. Kronenberg, P. Konig, J. Thiery, M. Koch, A. von Eckardstein, and H. Dieplinger. 2004. The apolipoprotein(a) size polymorphism is associated with nephrotic syndrome. *Kidney Int.* **65:** 606–612.
38. Mooser, V., M. C. Seabra, M. Abedin, K. T. Landschulz, S. Marcovina, and H. H. Hobbs. 1996. Apolipoprotein(a) kringle 4-containing fragments in human urine. Relationship to plasma levels of lipoprotein(a). *J. Clin. Invest.* **97:** 858–864.
39. Oida, K., H. Takai, H. Maeda, S. Takahashi, A. Shimada, J. Suzuki, T. Tamai, T. Nakai, and S. Miyabo. 1992. Apolipoprotein(a) is present in urine and its excretion is decreased in patients with renal failure. *Clin. Chem.* **38:** 2244–2248.
40. Doucet, C., V. Mooser, S. Gonbert, F. Raymond, J. Chapman, C. Jacobs, and J. Thillet. 2000. Lipoprotein(a) in the nephrotic syn-

drome: molecular analysis of lipoprotein(a) and apolipoprotein(a) fragments in plasma and urine. *J. Am. Soc. Nephrol.* **11:** 507–513.
41. Mooser, V., S. M. Marcovina, A. L. White, and H. H. Hobbs. 1996. Kringle-containing fragments of apolipoprotein(a) circulate in human plasma and are excreted into the urine. *J. Clin. Invest.* **98:** 2414–2424.
42. Armstrong, V. W., B. Harrach, H. Robenek, M. Helmhold, A. K. Walli, and D. Seidel. 1990. Heterogeneity of human lipoprotein Lp[a]: cytochemical and biochemical studies on the interaction of two Lp[a] species with the LDL receptor. *J. Lipid Res.* **31:** 429–441.
43. Maartmann-Moe, K., and K. Berg. 1981. Lp(a) lipoprotein enters cultured fibroblasts independently of the plasma membrane low density lipoprotein receptor. *Clin. Genet.* **20:** 352–362.
44. Reblin, T., A. Niemeier, N. Meyer, T. E. Willnow, F. Kronenberg, H. Dieplinger, H. Greten, and U. Beisiegel. 1997. Cellular uptake of lipoprotein[a] by mouse embryonic fibroblasts via the LDL receptor and the LDL receptor-related protein. *J. Lipid Res.* **38:** 2103–2110.
45. Perombelon, Y. F., J. J. Gallagher, N. B. Myant, A. K. Soutar, and B. L. Knight. 1992. Lipoprotein(a) in subjects with familial defective apolipoprotein B100. *Atherosclerosis.* **92:** 203–212.
46. Hofmann, S. L., D. L. Eaton, M. S. Brown, W. J. McConathy, J. L. Goldstein, and R. E. Hammer. 1990. Overexpression of human low density lipoprotein receptors leads to accelerated catabolism of Lp(a) lipoprotein in transgenic mice. *J. Clin. Invest.* **85:** 1542–1547.
47. Ramharack, R., T. M. Bocan, M. J. Imperiale, and M. A. Spahr. 1999. Recombinant adenovirus vector mediated expression of lipoprotein (a) [Lp(a)] in rabbit plasma. *Biochim. Biophys. Acta.* **1438:** 322–328.
48. Fan, J., M. Challah, H. Shimoyamada, M. Shiomi, S. Marcovina, and T. Watanabe. 2000. Defects of the LDL receptor in WHHL transgenic rabbits lead to a marked accumulation of plasma lipoprotein [a]. *J. Lipid Res.* **41:** 1004–1012.
49. Woollett, L. A., Y. Osono, J. Herz, and J. M. Dietschy. 1995. Apolipoprotein E competitively inhibits receptor-dependent low density lipoprotein uptake by the liver but has no effect on cholesterol absorption or synthesis in the mouse. *Proc. Natl. Acad. Sci. USA.* **92:** 12500–12504.
50. Tiebel, O., K. Oka, K. Robinson, M. Sullivan, J. Martinez, M. Nakamuta, K. Ishimura-Oka, and L. Chan. 1999. Mouse very low-density lipoprotein receptor (VLDLR): gene structure, tissue-specific expression and dietary and developmental regulation. *Atherosclerosis.* **145:** 239–251.
51. Zheng, G., D. R. Bachinsky, I. Stamenkovic, D. K. Strickland, D. Brown, G. Andres, and R. T. McCluskey. 1994. Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/alpa 2MR, and the receptor-associated protein (RAP). *J. Histochem. Cytochem.* **42:** 531–542.
52. Bottalico, L. A., G. A. Keesler, G. M. Fless, and I. Tabas. 1993. Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein(a) and apoprotein(a) internalization and degradation. *J. Biol. Chem.* **268:** 8569–8573.
53. Fless, G. M., M. E. ZumMallen, and A. M. Scanu. 1986. Physicochemical properties of apolipoprotein(a) and lipoprotein(a) derived from the dissociation of human plasma lipoprotein (a). *J. Biol. Chem.* **261:** 8712–8718.
54. Spiess, M. 1990. The asialoglycoprotein receptor: a model for endocytic transport receptors. *Biochemistry.* **29:** 10009–10018.
55. Wu, J., P. Liu, J. L. Zhu, S. Maddukuri, and M. A. Zern. 1998. Increased liver uptake of liposomes and improved targeting efficacy by labeling with asialofetuin in rodents. *Hepatology.* **27:** 772–778.
56. Keesler, G. A., B. R. Gabel, C. M. Devlin, M. L. Koschinsky, and I. Tabas. 1996. The binding activity of the macrophage lipoprotein(a)/apolipoprotein(a) receptor is induced by cholesterol via a post-translational mechanism and recognizes distinct kringle domains on apolipoprotein(a). *J. Biol. Chem.* **271:** 32096–32104.
57. Devlin, C. M., S. J. Lee, G. Kuriakose, C. Spencer, L. Becker, I. Grosskopf, C. Ko, L. S. Huang, M. L. Koschinsky, A. D. Cooper, et al. 2005. An apolipoprotein(a) peptide delays chylomicron remnant clearance and increases plasma remnant lipoproteins and atherosclerosis in vivo. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1704–1710.