Determination of the tissue sites responsible for the catabolism of large high density lipoprotein in the African green monkey

Kevin W. Huggins,[1] Ellen R. Burleson, Janet K. Sawyer, Kathryn Kelly, Lawrence L. Rudel, and John S. Parks[2]

Department of Pathology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157

Abstract **In vivo multicompartmental modeling of the turnover of HDL subfractions has suggested that HDL containing four molecules of apoA-I per particle and no other apolipoproteins (large LpA-I) are terminal particles in plasma. We hypothesized that these terminal particles were the end product of HDL metabolism and, as such, would be cleared preferentially by the liver. Thus, the purpose of this study was to determine: *1*) the tissue sites of catabolism of large LpA-I in African green monkeys, and *2*) whether saturated versus n–6 polyunsaturated dietary fat affected tissue accumulation. Large LpA-I were isolated, without ultracentrifugation, by size exclusion and immunoaffinity chromatography and radiolabeled with either the residualizing compound, ^{125}I-labeled tyramine cellobiose (TC), or with ^{131}I. After injection into recipient animals, the plasma die-away of the radiolabels was followed for 12 or 24 h, after which the animals were killed and tissues were collected for determining radiolabel sites of catabolism. The plasma die-away of the ^{125}I-labeled TC-LpA-I and ^{131}I-labeled LpA-I doses was similar suggesting that the TC radiolabeling did not modify the metabolism of the large LpA-I dose. The liver, adrenal, kidney, and spleen had the greatest accumulation of large LpA-I degradation products on a per gram tissue basis. On a whole organ basis, the liver was the major site of large LpA-I degradation in both the 12-h (15.4 ± 0.3% of injected dose) and 24-h (9.1 ± 0.6% of injected dose) catabolic studies. The kidney, compared to the liver, had less uptake of large LpA-I radioactivity in either study (1.3 ± 0.4% and 1.2 ± 0.3% of injected dose). There was no apparent influence of dietary fat type on the tissue accumulation of large LpA-I. We conclude that the liver is the primary site of catabolism of large LpA-I in the African green monkey.**—Huggins, K. W., E. R. Burleson, J. K. Sawyer, K. Kelly, L. L. Rudel, and J. S. Parks. **Determination of the tissue sites responsible for the catabolism of large high density lipoprotein in the African green monkey.** ***J. Lipid Res.*** **2000.** 41: **384–394.**

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High density lipoproteins (HDL) are believed to play an integral role in cholesterol homeostasis through the process known as reverse cholesterol transport (1). This process results in the removal of excess free cholesterol from peripheral tissues by HDL, its subsequent esterification in plasma by lecithin:cholesterol acyltransferase, uptake by the liver, and secretion into bile or conversion into bile acids (2, 3). This hypothesized pathway for cholesterol removal may explain the inverse relationship between HDL cholesterol concentrations and development of coronary heart disease in humans (4) and coronary artery atherosclerosis in nonhuman primates (5, 6). Thus, understanding the metabolism of HDL is central to delineating the pathway of reverse cholesterol transport and the atheroprotective role of HDL.

Numerous analytical and preparative procedures have been used to separate HDL subfractions by size (7), density (8), and electrophoretic mobility (9). Recent investigations have focused on the use of immunoaffinity chromatography as a method for isolation of HDL based on apolipoprotein content (10). The advantage of this isolation procedure is that it does not subject the HDL to the effects of ultracentrifugation, which have been shown to cause modification of HDL subfraction distribution (11, 12). Two major classes of HDL have been the subject of study, those that contain apoA-I without apoA-II (LpA-I) and those that contain both apoA-I and apoA-II (LpA-I/A-II) (10). Studies have shown that LpA-I are more protective with regard to atherosclerosis development than LpA-I/A-II; LpA-I concentrations are inversely proportional to the prevalence of coronary heart disease in humans (13) and transgenic mice with elevated concentrations of LpA-I de-

Abbreviations: apo, apolipoprotein; CE, cholesteryl ester; FCR, fractional catabolic rate; HDL, high density lipoprotein; LpA-I, HDL containing apoA-I without apoA-II; SR-BI, scavenger receptor class B type I; TC, tyramine cellobiose; TCA, trichloroacetic acid.

[1] Current address: Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, OH 45267.

[2] To whom correspondence should be addressed.

velop less atherosclerosis compared to those with elevated concentrations of LpA-I/A-II (14). LpA-I is also more efficient at removing excess cholesterol from cells in culture (15, 16) and more efficient in the delivery of cholesteryl ester-derived sterol to bile in rats than LpA-I/A-II (17).

There is very little information concerning how HDL and its components are taken up by cells and metabolized. The current concept of HDL catabolism involves the selective uptake of individual constituents by different cell types. Early studies in rodents demonstrated that cholesteryl ester (CE) was selectively removed from HDL by the liver and steroidogenic tissues, whereas apoA-I was selectively filtered and catabolized by the kidney (18). Recently, a receptor in the liver (scavenger receptor BI, SR-BI) has been identified that mediates the selective uptake of CE, thus implicating it in the process of reverse cholesterol transport (19). HDL catabolism by the liver may also occur by pathways that involve hepatic lipase, apoE, heparan sulfate proteoglycans, and to a limited extent, LDL receptor-related protein (20). Hepatic lipase has been shown to increase HDL whole particle and selective CE uptake by a heparan sulfate proteoglycan-dependent pathway in McA-RH7777 cells, whereas apoE increased the uptake of whole HDL particles with little effect on selective uptake of CE (20).

There is experimental evidence that the size distribution of LpA-I may play a role in determining HDL metabolism. Rader et al. (21) showed that there was a precursor–product relationship between HDL_2 and HDL_3. Radiolabeled LpA-I or LpA-I/A-II particles were injected into subjects and the decay of radiolabel from HDL_2 and HDL_3 subfractions was monitored. For both LpA-I and LpA-I/A-II, the injected dose decayed from HDL_3 and appeared in HDL_2, and subsequently decayed from HDL_2. These data suggested that HDL_3 were converted to HDL_2 before being removed from plasma. Metabolic studies performed by Colvin et al. (22–24) demonstrated that small LpA-I containing two apoA-I molecules per particle were converted to medium (three apoA-I molecules) or large (four apoA-I molecules) LpA-I particles before being cleared from plasma. These results indicate that HDL plasma concentrations may be controlled by the flux of apoA-I into certain HDL subfractions and that HDL may be targeted for removal from plasma based on its size and/or apoA-I content. Currently, there is no information on the tissue sites of catabolism of HDL subfractions based on size and apolipoprotein content.

The purpose of the present study was to test the hypothesis that large LpA-I particles are the end products of HDL metabolism and are cleared from plasma preferentially by the liver. Large LpA-I were isolated by size exclusion and immunoaffinity chromatography and radiolabeled with ^{125}I-labeled tyramine cellobiose (TC), a residualizing compound that becomes trapped in cells once the LpA-I are metabolized. We found that the liver was the major site of apoA-I degradation for large LpA-I and that tissue degradation appears not to be influenced by the type of dietary fat.

EXPERIMENTAL PROCEDURES

Animals and diets

Seven adult male African green monkeys (*Cercopithecus aethiops*) were used for these studies. The animals were fed an atherogenic diet consisting of 35% of calories as either saturated (palm oil, n = 3) or polyunsaturated fat (safflower oil, n = 4) and 0.4 mg cholesterol/kcal. The average daily food consumption was 70 kcal/kg body weight. The animals had consumed the experimental diet for at least 24 weeks before the metabolic studies were performed. Average body weight (mean ± SEM) for the saturated fat group was 5.09 ± 0.42 kg and 4.23 ± 0.24 kg for the polyunsaturated fat group. All animals were individually housed in an enriched environment in the animal facility at the Wake Forest University School of Medicine, which is approved by the American Association for the Accreditation of Laboratory Animal Care and supervised by a veterinary staff. All procedures were approved by the Institutional Animal Care and Use Committee.

Isolation of LpA-I

Blood was obtained from the femoral vein of two donor animals from each diet group after injection of ketamine HCl (10 mg/kg), after an overnight fast. Plasma was immediately isolated by centrifugation at 2,000 *g* for 30 min at 4°C in the presence of the following preservatives and inhibitors: 0.01% EDTA, 0.01% NaN_3, 10 kallikrein inhibitor units of aprotinin/ml, and 0.08 mg PMSF/ml (final concentrations). Plasma (15 ml) was applied to an 8% agarose column (2.5 × 90 cm) and lipoproteins were eluted with 0.15 M NaCl, 0.01% EDTA, 0.01% NaN_3, pH 7.4 (column buffer) at 4°C. The distribution of the lipoproteins eluted from the column and collected in a fraction collector was determined by the measurement of total cholesterol in each of the column fractions using an enzymatic procedure (25). Large HDL fractions were pooled as the front portion of the HDL peak (22).

Large LpA-I were isolated from the large HDL region of the 8% agarose column eluate. The preparation of the anti-A-I and anti-A-II immunoaffinity columns used in this study has been previously described (22). The large HDL pool from the 8% agarose column was applied to the anti-A-II column and incubated overnight at 4°C. The unbound material was collected and the column was washed with 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4. The unbound material from the anti-A-II column was then applied to the anti-A-I column, the column was washed, and the LpA-I particles were eluted with 3 M NaSCN, pH 7.4, and immediately desalted over an 80 ml Sephadex G-25 medium-coarse column equilibrated with column buffer. The LpA-I particles were then dialyzed against 3 × 2 liters of column buffer over a 36-h period to ensure complete removal of NaSCN. LpA-I were then concentrated to at least 1 mg protein/ml using an Amicon nitrogen pressure cell.

Iodination of LpA-I

The isolated large LpA-I from each diet group were split into two aliquots (≈500–750 μg apoA-I per aliquot). One aliquot was coupled to ^{125}I-labeled TC as previously described (26). The TC used in these experiments was synthesized as described previously (27) and was a generous gift from Dr. Steve Adelman (Wyeth-Ayerst). Briefly, 0.01 μmol TC/mg HDL protein was incubated for 10 min with 5 mCi of ^{125}I (carrier-free) in a microreaction vessel coated with 10 μg Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril, Pierce Chemical Co.). The reaction was stopped by transferring the ^{125}I-labeled TC to a second (iodogen-free) reaction vessel containing 10 μl of 0.1 M sodium bisulfite and 5 μl of 0.1 M sodium iodide. LpA-I was iodinated by activating the ^{125}I-labeled TC with cyanuric chloride (1:1 molar ratio) and incubat-

ing at room temperature for 30 min. The ^{125}I-labeled TC-LpA-I was then passed over a desalting column (Bio-Rad) to remove any free iodine and dialyzed overnight in column buffer. After removal from dialysis, aliquots were taken for protein determination by the method of Lowry et al. (28) and for radioactivity quantification. Specific activities ranged from 150 to 750 cpm/ng protein.

The second aliquot of each large LpA-I preparation was iodinated with carrier-free ^{131}I according to the method of McFarlane (29), as modified by Bilheimer (30). Specific activities ranged from 350 to 1500 cpm/ng protein.

After iodination the radiolabeled LpA-I was subjected to a second size exclusion chromatography step using two Superdex 200HR FPLC columns (Pharmacia Biotech) connected in series. The particles were eluted at a flow rate of 0.5 ml/min with column buffer. Individual fractions were run on a 4–30% nondenaturing gradient gel for 1,400 V-h at 10°C. After electrophoresis the gels were fixed in 10% sulfosalicylic acid (1 h) and exposed to film. Fractions were pooled according to elution position to give homogeneous large LpA-I particles.

For the ^{125}I-labeled TC-large LpA-I and ^{131}I-labeled large LpA-I, trichloroacetic acid (TCA)-precipitable radioactivities (10% final concentration) were greater than 96% and radioactivity extractable in chloroform–methanol (vol/vol) (31) ranged from 1 to 2%. Crosslinking analysis with dimethyl suberimidate (Pierce Chemical Co.) showed that the large LpA-I contained four apoA-I molecules per particle as previously described (22).

In vivo study and necropsy

Before injection, the radiolabeled large LpA-I (^{125}I and ^{131}I) were combined and filter-sterilized by passage through a 0.45-μm filter (Millipore). Approximately 2.5×10^7 cpm of the ^{125}I-labeled TC-large LpA-I and ^{131}I-labeled large LpA-I were injected into the saphenous vein of recipient animals from the same diet group. The recipients had been fasted overnight and were anesthetized with ketamine HCl (10 mg/kg body weight). A solution containing 0.45% NaCl and 0.05% NaI was given ad libitum to the recipient animals 1 week before injection and throughout the study. Blood samples (2 ml) were taken from the femoral vein at 5 and 20 min and at 1, 2, 4, 8, 12, and 24 h after dose injection from four recipient animals to determine the plasma decay of radiolabeled LpA-I. Three recipient animals were studied for 12 h. Blood samples (1.5 ml) were collected into chilled tubes containing 0.1% EDTA and placed on ice. Plasma, obtained by low speed centrifugation of the blood samples, was subjected to TCA-precipitation (10% final concentration). Complete urine and fecal samples were collected throughout the study period using stainless-steel cage pans with a screen top to trap particulate matter (diet and feces). ^{125}I and ^{131}I radioactivity was determined from the resulting plasma precipitates and urine using a gamma counter (Beckman Gamma 4000, Beckman Instruments, Fullerton, CA) that was programmed for double isotope counting. Corrections were made for spillover of ^{131}I into the ^{125}I channel. Any radioactivity present in the feces was summed with the large intestinal contents.

The total of amount of count in the plasma time points was determined by multiplying the ^{125}I and ^{131}I count by the estimated plasma volume (3.5% of body weight). The % of injected dose was determined by taking the total counts in plasma at the various time points divided by the total counts injected times 100%. The plasma radioactivity data were fitted by a sum of two exponentials using a nonlinear least squares regression program. The FCR for the large LpA-I protein was calculated from the two exponentials (32).

Twelve or 24 h after dose injection, the recipient animals were exsanguinated and the vasculature was perfused through the left ventricle with 1.5 liters heparanized saline. The following tissues were removed and weighed: liver, kidneys, adrenals, spleen, heart, lung, small intestine, large intestine, stomach, pancreas, testes, and thyroids. Aliquots of the following tissues were taken from multiple sites: skin (abdominal, palmar, right thigh), skeletal muscle (bicep, sternocleidomastoid, soleus), lymph nodes (axillary, inguinal, omental), and adipose (axillary, omental, and perirenal). Bile was removed from the gall bladder using a 10-ml syringe. Multiple tissue samples ($n = 3–5$, ≈ 1 g each) were fixed in Karnovsky's solution (33) overnight, weighed, and then counted directly in a gamma counter.

In vitro tissue incubations

The in vitro tissue incubations were performed with liver and kidney samples obtained from the animals in the 12-h TC uptake study. After removal of the liver and kidney, approximately 2-g sections of each organ were removed and diced into small pieces with a razor blade. After the excess fluid had been removed, the tissue slices were transferred to a 125-ml disposable Erlenmeyer flask which contained 10 ml Dulbecco's modified Eagle's medium (Gibco) and 10% fetal bovine serum (Gibco). The tissue slices were incubated at 37°C (in duplicate) using an oscillation speed of 80 cycles per minute for 24 h. At 0, 1.5, 12, 18, and 24 h 0.5 ml of media was removed and placed in a 5-ml conical tube containing 0.5 ml 20% TCA. TCA-precipitation was allowed to proceed overnight. After low speed centrifugation, the supernatant was removed and the pellet was washed with 0.5 ml 10% TCA. The supernatant from the wash was combined with the first supernatant. The pellet and combined supernatant were counted for radioactivity (^{125}I and ^{131}I). The amount of radioactivity released from the tissues was calculated as the sum of the count appearing in the pellet and supernatant. Free iodide count was determined with 1 ml of the supernatant as previously described (34).

Data analysis

Results are given as mean ± standard error of the mean. Statistical analysis was done using paired and unpaired *t*-tests (Statview™ 4.5, Abacus Concepts, Inc.).

RESULTS

African green monkeys used in this study were fed a diet rich in saturated fat (palm oil) or n–6 polyunsaturated fat (safflower oil) for 24 weeks. Total plasma cholesterol (TPC), HDL cholesterol, and HDL apolipoprotein concentrations for the two groups of monkeys are shown in **Table 1**. There were no consistent differences between the two diet groups; however, there appeared to be a trend toward lower TPC (175 ± 25 vs. 245 ± 71 mg/dl) and HDL cholesterol (73 ± 6 vs. 80 ± 10 mg/dl) for the POLY-fed animals compared to those fed the SAT diet.

Large LpA-I isolated by gel filtration and immunoaffinity chromatography were used as radiolabeled tracers for the tissue uptake studies. The large LpA-I were radiolabeled with ^{125}I-labeled TC or with ^{131}I. **Figure 1A** shows an autoradiograph of a representative 4–30% non-denaturing gradient gel of the iodinated particles from each diet group used for injection. The gel demonstrates that the particles are homogeneous in size and migrate with a Stokes' diameter between 10.4 and 12.2 nm. It also shows that the addition of TC does not influence the migration of the LpA-I and that there is no apparent size difference

TABLE 1. Plasma concentrations of total cholesterol, HDL cholesterol, and HDL apolipoproteins

Diet Group, Animal #	TPC	HDL-C	ApoA-I	ApoA-II
		mg/d1		
SAT				
761	134	63	237	17
778	223	80	293	13
780	378	97	325	15
Mean ± SEM	245 ± 71	80 ± 10	285 ± 26	15 ± 1
POLY				
737	199	81	294	20
763	229	85	271	13
766	115	66	308	10
770	156	59	281	12
Mean ± SEM	175 ± 25	73 ± 6	288 ± 8	14 ± 2

Data are expressed as mg/dl. Total plasma cholesterol (TPC) and HDL cholesterol (HDL-C) are the mean values (n = 5) for each animal from 4–24 weeks on the experimental diet. HDL-C was determined by the heparin–manganese method (45). Apolipoprotein values (apoA-I and apoA-II), determined by ELISA (46), were measured from the plasma samples obtained during the metabolic studies (n = 6 or 7).

between the two diet groups. Separation of representative large LpA-I doses from a POLY donor on SDS polyacrylamide gradient gels demonstrated that nearly all (>93% of intensity) of the radiolabel was in the apoA-I region (Fig. 1B; lane 1, ^{125}I-labeled TC LpA-I and lane 2, ^{131}I-labeled LpA-I). ApoA-I was the only visible apolipoprotein on Coomassie-stained SDS polyacrylamide gradient gels as shown for representative large LpA-I preparations from SAT and POLY donors (lanes 3 and 4 of Fig. 1B, respectively). Crosslinking with dimethyl suberimidate showed that all large LpA-I had four apoA-I molecules per particle (data not shown). These results are consistent with the previous isolation of large LpA-I (22).

The large ^{125}I-labeled TC-LpA-I and large ^{131}I-labeled LpA-I doses were simultaneously injected into recipient animals and blood samples were taken over 12 and 24 h time periods before the animals were killed for determination of apoA-I tissue uptake. The individual plasma decay curves were averaged and are shown in **Fig. 2**. The dieaway curves for each isotope were highly reproducible and similar to each other in both the 12 and 24 h studies. The FCR values for the individual curves are shown in **Table 2**. By paired *t*-test analysis, there was not a statistically significant difference in plasma turnover between the ^{125}I-labeled TC-LpA-I and ^{131}I-labeled LpA-I doses, suggesting that the TC radiolabeling did not modify the metabolism of the large LpA-I dose. There was a trend towards faster FCR values for the POLY group compared to the SAT group, which was statistically significant ($P < 0.05$) for the ^{125}I-labeled TC-LpA-I dose but not the ^{131}I-labeled LpA-I dose due to increased variability in the ^{131}I-labeled LpA-I FCR values. However, the FCR values must be viewed with caution as the duration of the studies, 12 or 24 h, was short compared to the residence time of the radiolabeled tracers in plasma.

The amounts of radioactivity taken up per gram of organ or diffuse tissue 24 h after injection of radiolabeled large LpA-I are shown in **Fig. 3A**. The uptake data for a select number of tissues from the individual animals are shown in **Table 3**. There were no major differences in the sites of catabolism between the diet groups, therefore the animals were averaged together. The liver and spleen had the highest uptake of ^{125}I-labeled TC-apoA-I. Compared to the liver, the kidney and adrenal had 36% and 48% lower

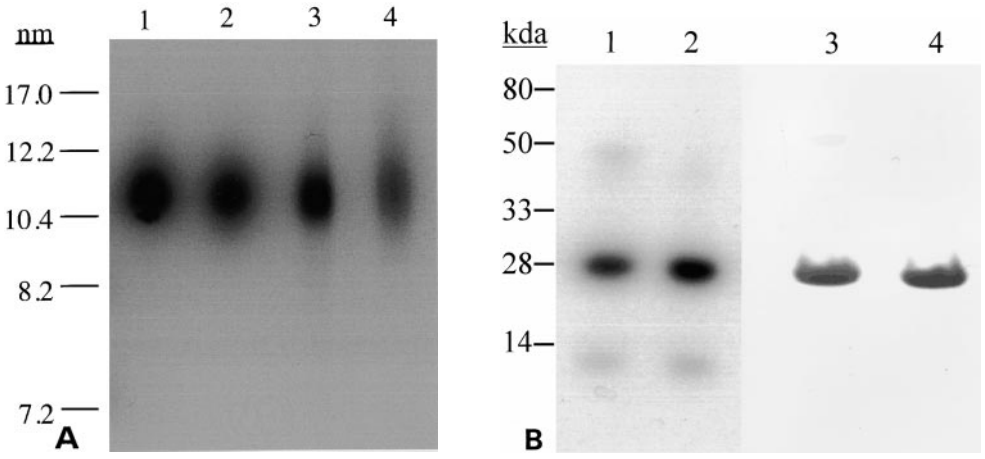

Fig. 1. (A) An autoradiograph of a 4–30% nondenaturing gradient gel of a representative set of large LpA-I purified by gel filtration and immunoaffinity chromatography and radioiodinated from animals fed a saturated (SAT) or polyunsaturated (POLY) diet. Gels were run as described in the Experimental Procedures section. Lane 1, SAT large ^{125}I-labeled TC-LpA-I; lane 2, SAT large ^{131}I-labeled LpA-I; lane 3, POLY large ^{125}I-labeled TC-LpA-I; lane 4, POLY large ^{131}I-labeled LpA-I. High molecular weight standard proteins are shown on the left side along with their hydrated Stokes' diameters: thyroglobulin (17.0 nm), ferritin (12.2 nm), catalase (10.4 nm), lactate dehydrogenase (8.2 nm), albumin (7.2 nm). (B) Autoradiograph of a SDS polyacrylamide gradient gel (4–30%) of POLY large ^{125}I-labeled TC-LpA-I (lane 1) and POLY large ^{131}I-labeled LpA-I (lane 2) and a Coomassie-stained gel of large LpA-I preparations from the SAT group (lane 3) and the POLY group (lane 4) prior to radiolabeling. SDS gels were run as previously described (47). Migration position of low molecular weight standard proteins is shown on the left side.

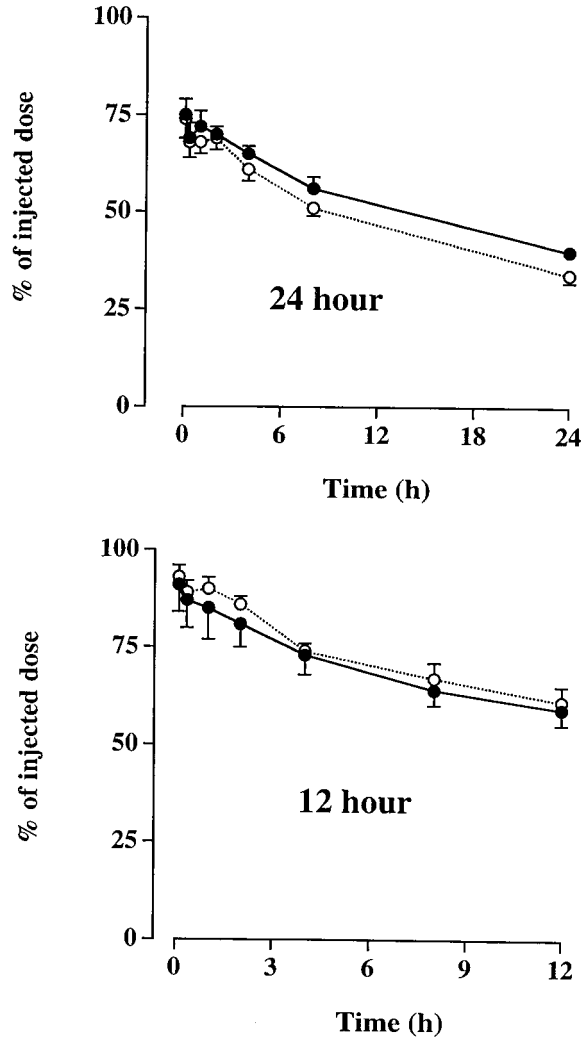

Fig. 2. Mean plasma decay curves for the 24-h (upper panel) and 12-h (lower panel) uptake studies of large ^{125}I-labeled TC-LpA-I (●) and large ^{131}I-labeled LpA-I (○) in African green monkeys. Details of the tracer preparations are given in the Experimental Procedures section. Tracers were simutaneously injected into the monkeys and the plasma decay curves represent plasma TCA-precipitable counts. Data represents the mean ± SEM of four (24-h study) and three (12-h study) animals.

^{125}I-labeled TC-apoA-I uptake. The ^{125}I radioactivity in bile and intestinal contents is thought to occur through leakage of the trapped ^{125}I-labeled TC from the liver and thus the amount of liver degradation of large LpA-I is likely underestimated in Fig. 3A (ref. 26 and Table 3). When an aliquot of bile from each of the animals was subjected to TCA-precipitation, the average amount of ^{125}I radioactivity that was TCA-soluble was 63 ± 7%. The average amount of free iodide present in the TCA-soluble ^{125}I count from four monkeys was 54 ± 1%. The amount of ^{131}I radiolabel, which represents undegraded large LpA-I, was low for nearly all tissues except adrenal, thyroid, and lymph nodes.

Figure 3B represents the percentages of injected radiolabeled large LpA-I recovered in the discrete tissues sampled as well as bile, intestinal contents, and urine after 24 h. Total recovery of injected dose ranged from 75 to 90% for

TABLE 2. Fractional catabolic rates of radiolabeled large LpA-I

Diet Group, Animal #	^{125}I-Labeled TC-LpA-I	^{131}I-Labeled LpA-I
	pools/h	
SAT		
761	0.031	0.038
778	0.035	0.055
780	0.028	0.023
Mean ± SEM	0.031 ± 0.002	0.039 ± 0.009
POLY		
737	0.036	0.026
763	0.044	0.050
766	0.048	0.052
770	0.037	0.051
Mean ± SEM	0.041 ± 0.003	0.045 ± 0.006

Data is expressed as pools/h. Values were calculated assuming a two-exponential die-away of large LpA-I protein and a metabolic steady state. Data are the individual animals consuming the SAT or POLY diet. Animals were killed 12 (#763, #766, #780) or 24 hours (#737, #761, #770, #778) after an injection of radiolabeled large LpA-I.

^{125}I and 60 to 80% for ^{131}I. The percentage of injected dose remaining in plasma at 24 h was 40 ± 1% for ^{125}I and 34 ± 2% for ^{131}I. On a per organ basis, the liver exceeded all other organs in the degradation of large LpA-I. The percentage of ^{125}I-labeled LpA-I dose measured in the liver at 24 h after injection was 9.1 ± 0.6%. The only other organ with an appreciable amount of ^{125}I-labeled LpA-I uptake was the kidney (1.2 ± 0.3%). There was also a significant amount of ^{125}I radioactivity in the urine (7.3 ± 0.6%). All of the ^{125}I radioactivity in the urine was TCA-soluble with 82 ± 0.6% of the soluble count attributable to free iodide. The data suggest that LpA-I degradation occurs primarily in the liver.

Due to the unexpected amount of ^{125}I-labeled TC appearing in the urine after 24 h, we conducted a 12-h uptake study to determine whether more of the ^{125}I-labeled TC radiolabel had remained trapped in the liver and less released into the urine. The uptake of radioactivity per gram tissue and the percentages of injected radiolabeled large LpA-I recovered after 12 h are shown in **Fig. 4A** and **B**. The data for individual animals are shown in Table 3. Total recovery of the injected dose ranged from 85 to 90% for ^{125}I and 75 to 85% for ^{131}I. The percentage of injected dose remaining in plasma after 12 h was 59 ± 4% for ^{125}I and 61 ± 4% for ^{131}I. Consistent with the 24-h uptake studies, the liver and spleen had the highest uptake of ^{125}I-labeled TC-apoA-I, followed by the adrenal and kidney, respectively. Approximately 93 ± 0.3% of the ^{125}I radioactivity in the bile was TCA-soluble with 22 ± 1% of the soluble count being free iodide. Significant amounts of ^{131}I radiolabel were only found in the adrenal and bile. When the data were expressed as the percentage of injected radiolabel recovered (Fig. 4B), the liver again exceeded all other organs in the degradation of large LpA-I. The kidney, spleen, and testis showed small amounts of large LpA-I accumulation after 12 h. Note that the urine contained approximately 5% of the injected ^{125}I-labeled TC radiolabel (all ^{125}I radioactivity in urine was TCA-soluble with 70 ± 2% being free iodide).

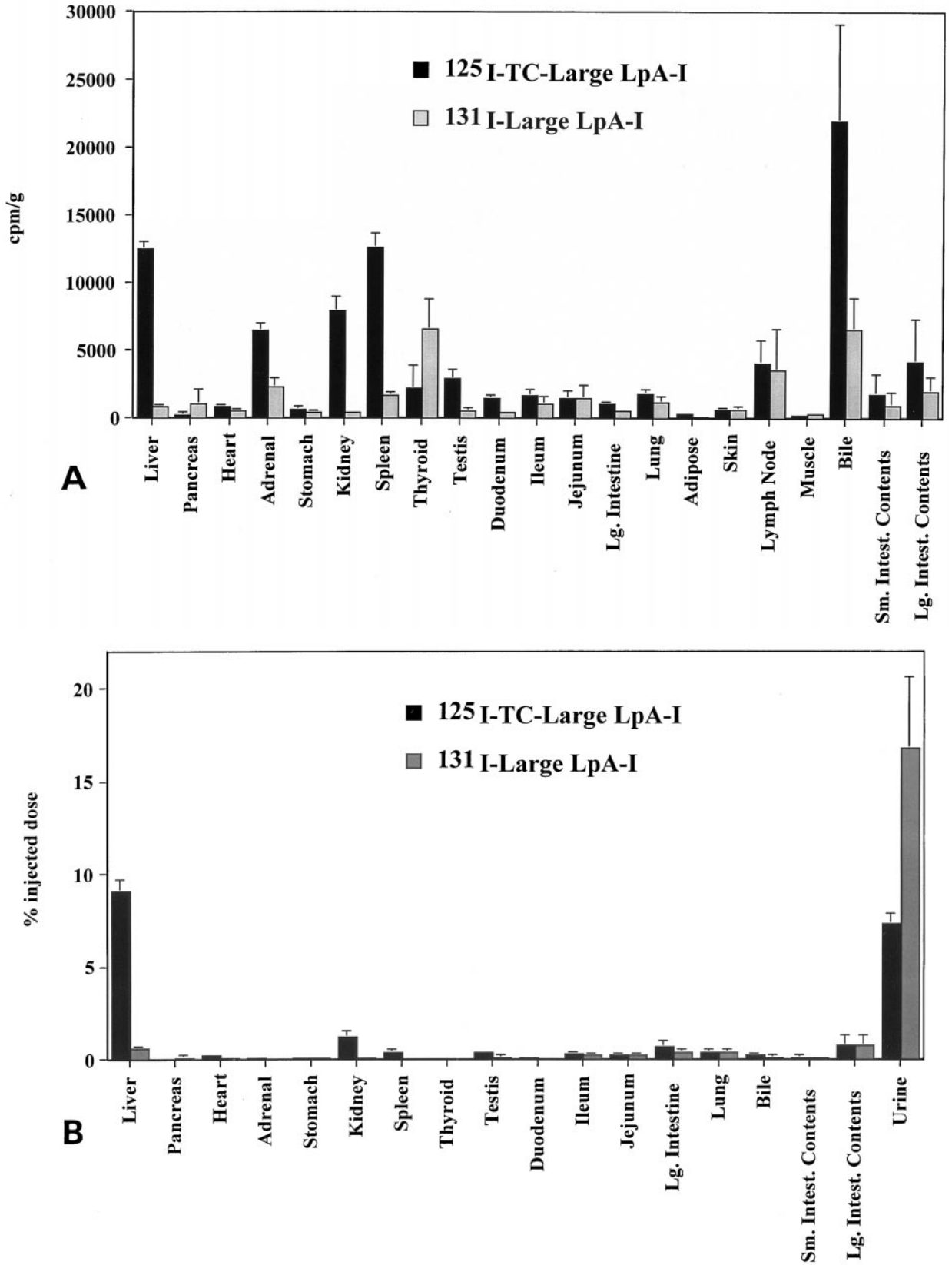

Fig. 3. Tissue sites of large LpA-I catabolism at 24 h after dose injection. (A) Radioactivity recovered in each tissue expressed as cpm/g. Data were adjusted to 5 μCi of injected dose to correct for differences in the amounts of tracer injected. (B) Radioactivity recovered in each tissue expressed as a percentage of injected dose. All data represent the mean ± SEM of four animals.

TABLE 3. Tissue sites of large LpA-I catabolism in African green monkeys

Tissue	12 Hours			24 Hours			
	#780 SAT	#763 POLY	#766 POLY	#761 SAT	#778 SAT	#737 POLY	#770 POLY
	% injected dose						
Liver-associated	17.3	16.3	17.1	8.2	10.0	11.4	10.9
Liver	15.9	14.8	15.4	8.0	9.6	10.6	10.4
Kidneys	2.1	0.9	0.9	2.0	1.4	2.0	2.2
Spleen	1.0	0.6	0.5	0.5	0.5	0.3	1.0
Adrenals	0.1	0.1	0.2	0.1	0.1	0.2	0.1
Testes	0.4	0.5	0.5	0.4	0.4	0.7	0.5

Data represent the radioactivity recovered in each organ expressed as percentage of injected dose. The liver-associated radioactivity is the radioactivity found in the intact liver plus the radioactivity found in the bile and intestinal contents.

Comparison of the tissue content of ^{125}I-labeled TC-apoA-I from the 12-h and 24-h studies is shown in **Fig. 5**. There was a significant decrease in the amount of ^{125}I-labeled TC-apoA-I in the liver between 12 and 24 h (15.4 $\pm$ 0.3% vs. 9.1 $\pm$ 0.6%, $P < 0.03$). There was also an increase in the amount of ^{125}I-labeled TC radiolabel in the urine at 24 h compared to the 12-h study (7.3 $\pm$ 0.6% vs. 4.8 $\pm$ 0.3%). There was no change in the amount of ^{125}I-labeled TC-apoA-I in the kidney between the two studies. No other tissues showed any major changes in the ^{125}I-labeled TC-apoA-I uptake between 12 and 24 h. These results suggest that the ^{125}I radiolabel appearing in the urine most likely leaked from the liver.

To verify that the kidney did not release the ^{125}I-labeled TC radiolabel at a faster rate than the liver, thus resulting in lower apparent apoA-I uptake in the kidney after 12 and 24 h, in vitro tissue incubations were performed. After the liver and kidney were removed during the 12-h uptake study, these organs were diced and incubated in media at 37°C for 24 h. At various timepoints, medium was removed and radioactivity was quantified. The results in **Fig. 6** show that the rate of release of ^{125}I radioactivity was essentially the same for both the liver and kidney. The data demonstrate that, at least in the liver and kidney, the in vitro release of ^{125}I-labeled TC label from cells occurs at a constant rate regardless of the type of tissue. When the media from the tissue incubations were subjected to TCA-precipitation, it was found that for the liver approximately 84 $\pm$ 5% of the ^{125}I radioactivity appearing in the media after 24 h was TCA-soluble (free iodide = 43 $\pm$ 4% of soluble count). When the media from the kidney incubations were analyzed by TCA-precipitation, it was found that 100% of the ^{125}I radioactivity was TCA-soluble in two of the three animals (#763 and #766) and, interestingly, all of the soluble count was free iodide. Animal #780 appeared to leak more ^{125}I-labeled TC-apoA-I because only 79% of the ^{125}I radioactivity was TCA-soluble (free iodide = 40% of soluble count). These results suggest that there is a certain amount of background leakage of the ^{125}I-labeled TC label and, as the liver is the major site of large HDL uptake and is the largest tissue of those sampled, the ^{125}I radiolabel appearing in the urine most likely originates from the liver as suggested in Fig. 5.

DISCUSSION

The purpose of this study was to test the hypothesis that large LpA-I particles, containing four molecules of apoA-I, are end products of HDL metabolism and are cleared from plasma preferentially by the liver. Results from recent studies have suggested that HDL subfractions containing two, three, and four apoA-I molecules per particle have distinct metabolic fates (22–24). Small LpA-I, containing two molecules of apoA-I, are converted in a unidirectional manner to either medium (three molecules of apoA-I) or large (four molecules of apoA-I) LpA-I, which are then catabolized from plasma. Furthermore, there was no evidence for the generation of pre-beta apoA-I during the catabolism of medium or large LpA-I. Kinetic evidence for such a metabolic pathway is evident when homogeneous-sized LpA-I particles, isolated by size-exclusion and immunoaffinity chromatography, are used as tracers. Ultracentrifugation has been reported to alter HDL subfraction distribution and lead to an under-representation of the large LpA-I particles (12). As the large LpA-I particles were cholesterol-enriched, relative to small LpA-I, and behaved kinetically as terminal particles, we reasoned that these particles represented the end product of reverse cholesterol transport and would be removed by the liver. Using the residualizing compound tyramine cellobiose, we found that the liver was the major site of apoA-I degradation, with at least 5- to 10-times more accumulation of large LpA-I than the kidney or spleen. To our knowledge this is the first study to quantify the tissue degradation of apoA-I originating from homogeneous-sized HDL subfractions of defined apoA-I content.

Previous studies have shown that the liver and kidney are the main sites of HDL apoA-I catabolism in rodents (18, 26) and nonhuman primates (35). However, our results showed that apoA-I degradation by the kidney was 10–20% that of the liver (Figs. 3 and 4 and Table 3). A potential explanation for the different results could be the method of HDL isolation. Previous studies have used ultracentrifugation to isolate HDL, which can result in the conversion of large HDL into smaller HDL particles with the generation of lipid-free apoA-I (12) (unpublished observations). Upon injection, the lipid-free apoA-I would become hypercatabolized by the kidney. There is experimental evidence to sup-

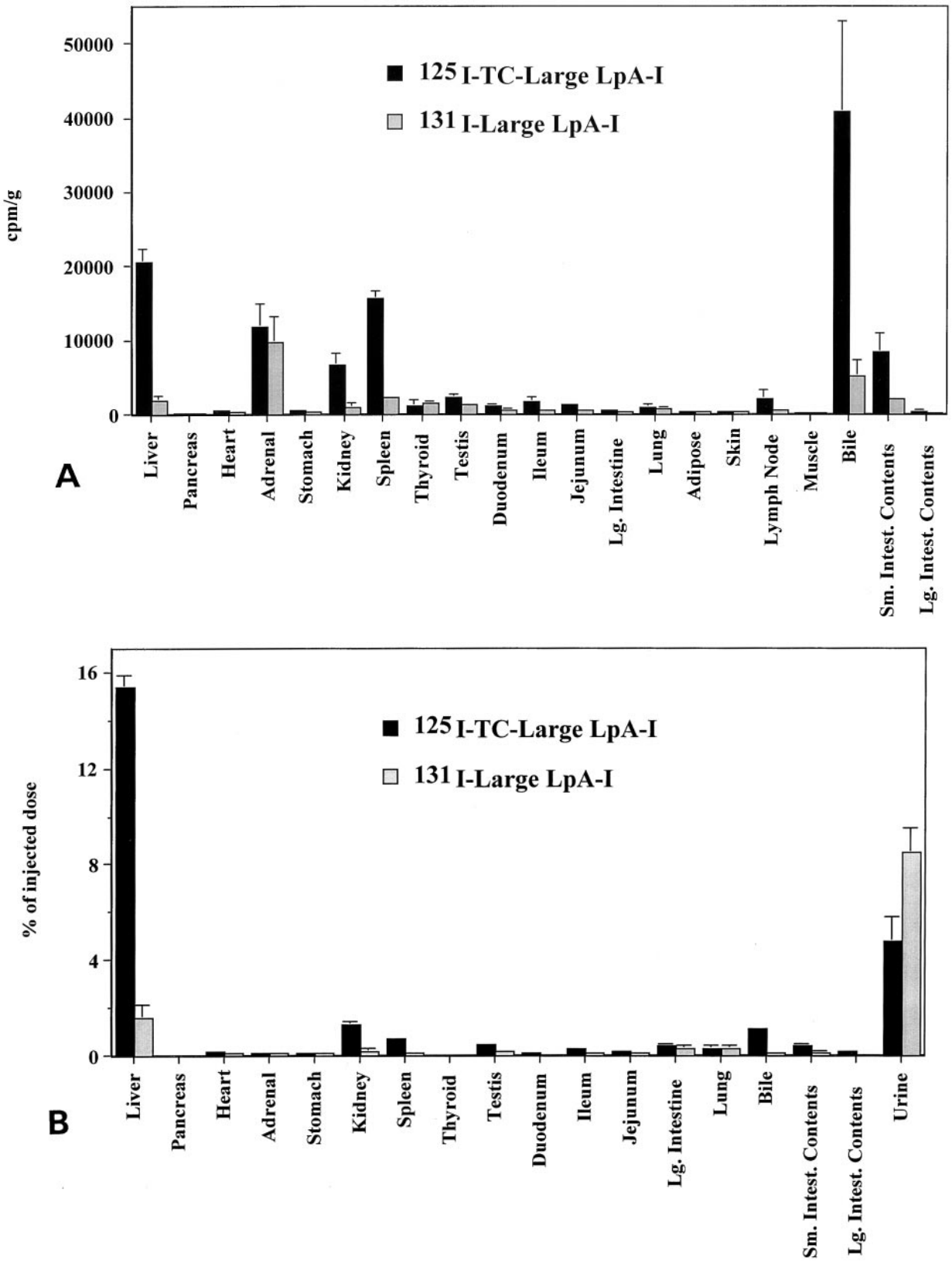

Fig. 4. Tissue sites of large LpA-I catabolism after 12 h. (A) Radioactivity recovered in each tissue expressed as cpm/g. Data were adjusted to 5 μCi to correct for differences in the amount of tracer injected. (B) Radioactivity recovered in each tissue expressed as a percentage of injected dose. All data represent the mean ± SEM of three animals.

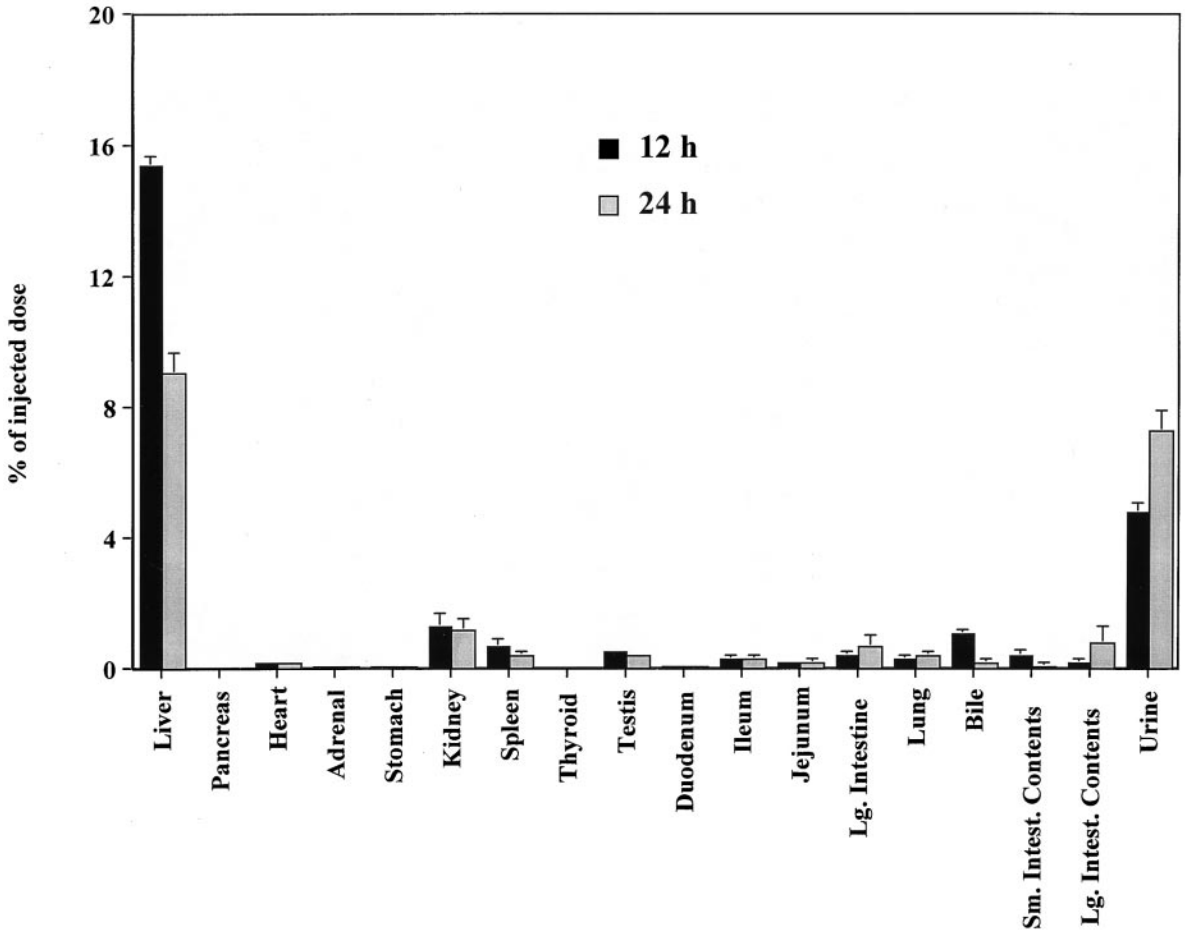

Fig. 5. Comparison of tissue sites of catabolism of large ^{125}I-labeled TC-LpA-I after 12 and 24 h. Data are expressed as the percentage of injected dose recovered in each tissue.

port this explanation. Goldberg et al. (35) showed that hypertriglyceridemic monkeys had an increased amount of apoA-I taken up by the kidney compared to control monkeys, which was attributed to the generation of increased amounts of lipid-free apoA-I upon hydrolysis of triglyceride-enriched HDL with hepatic lipase. In addition, reassociation of radiolabeled lipid-free apoA-I onto plasma HDL resulted in a faster FCR compared to radiolabeled apoA-I on native HDL (26, 35), presumably because the reassociated apoA-I is less stable on the HDL surface and ultimately dissociates from HDL and is catabolized by the kidney (26, 35). This concept is also supported by the data of Horowitz et al. (36), which demonstrated that men with low HDL cholesterol levels have a greater fraction of apoA-I that was isolated in the $d > 1.21$ g/ml fraction after ultracentrifugation. This led to a greater percentage of apoA-I that was taken up by the kidney in a rabbit kidney perfusion system compared to HDL from subjects with high HDL concentrations. These results taken together suggest that factors which increase the dissociation of apoA-I from the surface of HDL result in more uptake and catabolism by the kidney and less by the liver.

Large LpA-I catabolism by the liver is not likely mediated by SR-BI, which is the best-characterized receptor for HDL metabolism. SR-BI mediates selective CE uptake in tissues that are active in steroidogenesis (adrenal, testis, ovary) as well as the liver. However, we believe that the majority of tissue uptake of large LpA-I occurred as holoparticle uptake and, as such, did not involve SR-BI. Several lines of evidence support this conclusion. First, selective CE uptake by SR-BI at the liver surface would not directly result in the uptake and degradation of apoA-I, as observed in our data (Figs. 3 and 4). Second, if selective CE uptake were the major mechanism for the catabolism of large LpA-I, we would expect to see radiolabeled lipid-free apoA-I (i.e., pre-beta apoA-I) or the conversion of large to medium or small HDL as CE was removed from the particle core by SR-BI. Our kinetic studies have shown no evidence for the generation of pre-beta apoA-I or the conversion of large to smaller LpA-I, making this possibility unlikely (23). Third, if selective CE uptake by the liver were the primary catabolic pathway for the large LpA-I particles, we might also expect to see a large amount of kidney degradation of apoA-I. Thus, as SR-BI removed CE from LpA-I, lipid-free or lipid-poor apoA-I would be produced at the liver surface that would be filtered and degraded by the kidney. However, apoA-I degradation by the kidney was much less than degradation by the liver in our study

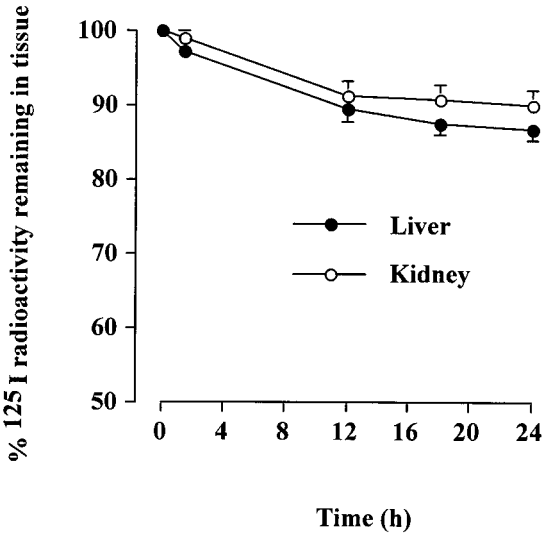

Fig. 6. Percentage of ^{125}I-labeled TC radioactivity remaining in liver and kidney tissue during in vitro incubation. Liver and kidney slices were incubated at 37°C for 24 h after the 12-h large LpA-I uptake study. At 0, 1.5, 12, 18, and 24 h, 0.5 ml of media was removed and subjected to TCA-precipitation. ^{125}I radioactivity in the pellet and supernatant were summed to determine the total amount of ^{125}I radioactivity released from each tissue. Data represent mean ± SEM of duplicate incubations from three animals.

(Table 3). Finally, we might anticipate an accumulation of ^{131}I-labeled LpA-I relative to ^{125}I-labeled TC LpA-I in the liver if SR-BI were the primary mode of large LpA-I catabolism. ^{131}I-labeled LpA-I radiolabel associated with tissues represents undegraded ligand, as internalization and degradation of LpA-I would result in the rapid release of ^{131}I into plasma, whereas the residualizing ^{125}I-labeled TC radiolabel would be trapped in the cell. We observed a relative enrichment of ^{131}I compared to ^{125}I radiolabel in several tissues (adrenals and testes) known to express SR-BI (Fig. 4A); however, this was not the case for the liver. Taken together these data suggest that large LpA-I are catabolized by hepatic holo-particle uptake.

The mechanism for the uptake and degradation of large LpA-I is presently unknown. Hepatic cells may recognize large HDL as the terminal particles in the HDL metabolic pathway and selectively target these particles for removal from the circulation by holo-particle internalization. These particles may acquire sufficient apoE in the circulation just prior to uptake by an LDL receptor or LDL receptor-related protein (LRP) pathway in the liver (20). Alternatively, large LpA-I may bind to apoE that is immobilized on the hepatocyte surface by hepatic lipase or heparan sulfate proteoglycans (HSPG). Subsequent uptake could be mediated by either a LDL or LRP-receptor-dependent pathway or by a HSPG-dependent pathway (20). Uptake by any of these pathways may be triggered when large LpA-I has acquired the optimal amount of CE enrichment during reverse cholesterol transport that occurs concomitant with the unidirectional conversion of small to large LpA-I (23). Another possibility might involve binding of hepatic lipase to large LpA-I, followed by uptake by hepatic bound HSPG, without the involvement of apoE or the LDL receptor or LRP pathway (20). It is also possible that an unidentified receptor may be involved in the hepatic uptake and catabolism of large LpA-I. The manner in which the hepatic cells would recognize large HDL for internalization may involve particle size, apoA-I content, and/or apoA-I conformation. Studies are currently underway to elucidate the pathways of large LpA-I uptake by the liver. We hypothesize that medium LpA-I particles are also terminal particles in HDL metabolism, based on kinetic data (23), and would have a similar metabolic fate as the large particles, but support for this hypothesis must await experimental confirmation.

It is well established that diets rich in polyunsaturated fats result in decreased plasma concentrations of HDL and apoA-I in nonhuman primates compared to diets rich in saturated fats (37, 38). The mechanism by which dietary fat influences these reductions is not known, but studies have suggested that clearance (39–42) and production (43, 44) of HDL are affected. The results reported here show a trend towards increased FCR (Table 2), decreased HDL concentrations (Table 1), and increased tissue uptake (Table 3) for large LpA-I from the POLY-fed animals compared to the animals fed the SAT diet. However, this study was not designed with enough statistical power to determine whether the FCR of LpA-I or the tissue uptake of LpA-I was influenced by dietary fat type. Furthermore, accurate FCR values are difficult to obtain for short duration turnover studies, which are necessary when investigating tissue retention of radiolabel. Because a limited number of non-human primates were available for these terminal studies of tissue uptake of LpA-I, only gross differences in tissue uptake as a function of dietary fat type could have been observed. In past studies we have generally observed a 20% lowering of plasma HDL cholesterol and apoA-I concentrations and a similar increase in FCR of HDL apoA-I in animals fed POLY versus SAT diets that was statistically significant with larger numbers of study animals (39). The differences in FCR, HDL concentrations, and LpA-I tissue uptake observed in this study were of similar magnitude.

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REFERENCES

1. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
2. Pieters, M. N., D. Schouten, and T. J. C. Van Berkel. 1994. In vitro and in vivo evidence for the role of HDL in reverse cholesterol transport. *Biochim. Biophys. Acta.* **1225:** 125–134.
3. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
4. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein:

the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
5. Rudel, L. L., M. G. Bond, and B. C. Bullock. 1985. LDL heterogeneity and atherosclerosis in nonhuman primates. *Ann. NY Acad. Sci. USA.* **454:** 248–253.
6. Quinet, E., A. Tall, R. Ramakrishnan, and L. Rudel. 1991. Plasma lipid transfer protein as a determinant of the atherogenicity of monkey plasma lipoproteins. *J. Clin. Invest.* **87:** 1559–1566.
7. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nichols. 1981. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **665:** 408–419.
8. Anderson, D. W., A. V. Nichols, S. S. Pan, and F. T. Lindgren. 1978. High density lipoprotein distribution. Resolution and determination of three major components in a normal population sample. *Atherosclerosis.* **29:** 161–179.
9. Noble, R. P., F. T. Hatch, J. A. Mazrimas, F. T. Lindgren, L. C. Jensen, and G. L. Adamson. 1969. Comparison of lipoprotein analysis by agarose gel and paper electrophoresis with analytical ultracentrifugation. *Lipids.* **4:** 55–59.
10. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
11. Kunitake, S. T., and J. P. Kane. 1982. Factors affecting the integrity of high density lipoproteins in the ultracentrifuge. *J. Lipid Res.* **23:** 936–940.
12. Cheung, M. C., and A. C. Wolf. 1988. Differential effect of ultracentrifugation on apolipoprotein A-I-containing lipoprotein subpopulations. *J. Lipid Res.* **29:** 15–25.
13. Puchois, P., A. Kandoussi, P. Fievet, J. L. Fourrier, M. Bertrand, and E. Koren. 1987. Apolipoprotein A-I containing lipoproteins in coronary artery disease. *Atherosclerosis.* **68:** 35–40.
14. Schultz, J. R., J. G. Verstuyft, E. L. Gong, A. V. Nichols, and E. M. Rubin. 1993. Protein composition determines the anti-atherogenic properties of HDL in transgenic mice. *Nature.* **365:** 762–764.
15. Fruchart, J. C., G. Ailhaud, and J. M. Bard. 1993. Heterogeneity of high density lipoprotein particles. *Circulation.* **87 Suppl.:** III22–III27.
16. Castro, G., L. P. Nihoul, C. Dengremont, C. De Geitère, B. Delfly, A. Tailleux, C. Fievet, N. Duverger, P. Denèfle, J. C. Fruchart, and E. M. Rubin. 1997. Cholesterol efflux, lecithin-cholesterol acyltransferase activity, and pre-β particle formation by serum from human apolipoprotein A-I and apolipoprotein A-I apolipoprotein A-II transgenic mice consistent with the latter being less effective for reverse cholesterol transport. *Biochemistry.* **36:** 2243–2249.
17. Pieters, M. N., G. R. Castro, D. Schouten, P. Duchateau, J.-C. Fruchart, and T. J. C. Van Berkel. 1993. Cholesterol esters selectively delivered in vivo by high-density-lipoprotein subclass LpA-I to rat liver are processed faster into bile acids than are LpA-I/A-II-derived cholesterol esters. *Biochem. J.* **292:** 819–823.
18. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1985. Uptake of high-density lipoprotein-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.
19. Acton, S., A. Rigotti, K. T. Landschulz, S. Z. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
20. Ji, Z. S., H. L. Dichek, R. D. Miranda, and R. W. Mahley. 1997. Heparan sulfate proteoglycans participate in hepatic lipase- and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. *J. Biol. Chem.* **272:** 31285–31292.
21. Rader, D. J., G. Castro, L. A. Zech, J. C. Fruchart, and H. B. Brewer, Jr. 1991. In vivo metabolism of apolipoprotein A-I on high density lipoprotein particles LpA-I and LpA-I, A-II. *J. Lipid Res.* **32:** 1849–1859.
22. Colvin, P., E. Moriguchi, H. Barrett, J. Parks, and L. Rudel. 1998. Production rate determines plasma concentration of large high density lipoprotein in non-human primates. *J. Lipid Res.* **39:** 2076–2085.
23. Colvin, P. L., E. Moriguchi, P. H. R. Barrett, J. S. Parks, and L. L. Rudel. 1999. Small HDL particles containing two apoA-I molecules are precursors in vivo to medium and large HDL particles containing three and four apoA-I molecules in nonhuman primates. *J. Lipid Res.* **40:** 1782–1792.
24. Colvin, P. L., and J. S. Parks. 1999. Metabolism of high density lipoprotein subfractions. *Curr. Opin. Lipidol.* **10:** 309–314.
25. Auerbach, B. J., J. S. Parks, and D. Applebaum-Bowden. 1990. A rapid and sensitive micro-assay for the enzymatic determination of plasma and lipoprotein cholesterol. *J. Lipid Res.* **31:** 738–742.
26. 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.
27. 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.
28. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
29. McFarlane, A. A. 1958. Efficient trace labelling of proteins with iodine. *Nature.* **182:** 53–57.
30. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
31. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
32. Yedgar, S., T. E. Carew, R. C. Pittman, W. F. Beltz, and D. Steinberg. 1983. Tissue sites of catabolism of albumin in rabbits. *Am. J. Phys.* **244:** E101–E107.
33. Carew, T. E., R. C. Pittman, E. R. Marchand, and D. Steinberg. 1984. Measurement in vivo of irreversible degradation of low density lipoprotein in the rabbit aorta. Predominance of intimal degradation. *Arteriosclerosis.* **4:** 214–224.
34. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem.* **249:** 5153–5162.
35. Goldberg, I. J., W. S. Blaner, T. M. Vanni, M. Moukides, and R. Ramakrishnan. 1990. Role of lipoprotein lipase in the regulation of high density lipoprotein apolipoprotein metabolism. Studies in normal and lipoprotein lipase-inhibited monkeys. *J. Clin. Invest.* **86:** 463–473.
36. Horowitz, B. S., I. J. Goldberg, J. Merab, T. M. Vanni, R. Ramakrishnan, and H. N. Ginsberg. 1993. Increased plasma and renal clearance of an exchangeable pool of apolipoprotein A-I in subjects with low levels of high density lipoprotein cholesterol. *J. Clin. Invest.* **91:** 1743–1752.
37. Rudel, L. L., J. S. Parks, and J. K. Sawyer. 1995. Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. *Arterioscler. Thromb. Vascular Biol.* **15:** 2101–2110.
38. Babiak, J., A. V. Nichols, E. L. Gong, C. A. McMahan, T. J. Kuehl, G. E. Mott, and H. C. McGill, Jr. 1985. Effects of dietary polyunsaturated and saturated fats on lipoproteins in the baboon. *Atherosclerosis.* **57:** 1–17.
39. Parks, J. S., and L. L. Rudel. 1982. Different kinetic fates of apolipoproteins A-I and A-II from lymph chylomicra of nonhuman primates. Effect of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* **23:** 410–421.
40. Chong, K. S., R. J. Nicolosi, R. F. Rodger, D. A. Arrigo, R. W. Yuan, J. J. MacKey, and P. N. Herbert. 1987. Effect of dietary fat saturation on plasma lipoproteins and high density lipoprotein metabolism of the rhesus monkey. *J. Clin. Invest.* **79:** 675–683.
41. Brousseau, M. E., E. J. Schaefer, A. F. Stucchi, J. Osada, D. B. Vespa, J. M. Ordovas, and R. J. Nicolosi. 1995. Diets enriched in unsaturated fatty acids enhance apolipoprotein A-I catabolism but do not affect either its production or hepatic mRNA abundance in cynomolgus monkeys. *Atherosclerosis.* **115:** 107–119.
42. Stucchi, A. F., L. K. Hennessy, D. B. Vespa, E. J. Weiner, J. Osada, J. M. Ordovas, and R. J. Nicolosi. 1991. Effect of corn and coconut oil-containing diets with and without cholesterol on high density lipoprotein apoprotein A-I metabolism and hepatic apoprotein A-I mRNA levels in cebus monkeys. *Arterioscler. Thromb.* **11:** 1719–1729.
43. Johnson, F. L., J. Babiak, and L. L. Rudel. 1986. High density lipoprotein accumulation in perfusates of isolated livers of African green monkeys. Effects of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* **27:** 537–548.
44. Sorci-Thomas, M., M. M. Prack, N. Dashti, F. Johnson, L. L. Rudel, and D. L. Williams. 1989. Differential effects of dietary fat on the tissue-specific expression of the apolipoprotein A-I gene: relationship to plasma concentration of high density lipoproteins. *J. Lipid Res.* **30:** 1397–1403.
45. Warnick, G. R., and J. J. Albers. 1978. A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J. Lipid Res.* **19:** 65–76.
46. Koritnik, D. L., and L. L. Rudel. 1983. Measurement of apolipoprotein A-I concentration in nonhuman primate serum by enzyme-linked immunosorbent assay (ELISA). *J. Lipid Res.* **24:** 1639–1645.
47. Auerbach, B. J., and J. S. Parks. 1989. Lipoprotein abnormalities associated with lipopolysaccharide-induced lecithin:cholesterol acyltransferase and lipase deficiency. *J. Biol. Chem.* **264:** 10264–10270.