Nuclear Imaging Analysis of Human Low-Density Lipoprotein Biodistribution in Rabbits and Monkeys

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We have evaluated the biodistribution of human low-density lipoprotein (LDL) radiolabeled with ^{99m}Tc or with ^{123}I-tyramine cellobiose in rabbits and in rhesus monkeys. Biodistribution was assessed after intravenous injection of radiolabeled LDL by quantitative analysis of scintigrams, counting of excreta, and counting of tissues at necropsy. Both rabbits and monkeys showed lower renal uptake (^{123}I:^{99m}Tc ~1:3, as regional percent injected activity corrected for physical decay) and excretion (1:2 to 1:4), but higher hepatic (1.5:1 to 2:1) and cardiac (1.7:1 to 4:1) uptake of ^{123}I than of ^{99m}Tc. Adrenals were visualized in normolipemic animals with ^{123}I-tyramine cellobiose-LDL but not with ^{99m}Tc-LDL. Hyperlipemic animals showed increased cardiac (up to six-fold) and decreased hepatic activity (by 50%–60%) of both radionuclides. We conclude that ^{123}I-tyramine cellobiose-LDL is better suited than ^{99m}Tc-LDL for dynamic studies of LDL metabolism in vivo.

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Over the past decade a few research groups have explored methods to prepare radiolabeled low-density lipoprotein (LDL) suitable for nuclear imaging studies. Such agents would be useful for a broad range of experimental and clinical applications. In addition to measuring plasma turnover of radiolabeled LDL, which has traditionally employed ^{125}I-LDL or ^{131}I-LDL (*1,2*), one could dynamically monitor LDL uptake by a particular tissue, simultaneously evaluate biodistribution of LDL among several tissues or organs, and quantify the effects of diet, drugs, and disease states upon these parameters.

Although Lees, Lees, and Strauss (*3*) were able to demonstrate uptake of conventionally labeled ^{125}I-LDL in atherosclerotic human carotid arteries, they advocated the use of radionuclides with better imaging characteristics, such as ^{99m}Tc or ^{123}I. They subsequently developed a method for preparing ^{99m}Tc-LDL (*4*), used it for biodistribution analysis in rabbits (*4*) and in humans (*5*), and later described focal uptake of LDL by carotid and iliac arteries in some patients with atherosclerosis (*6,7*).

Vallabhajosula et al. (*8*) claimed to show comparable tissue distribution of ^{99m}Tc-LDL and ^{131}I-tyramine cellobiose-LDL in rabbits and in monkeys, but discounted major differences in plasma retention of the two agents as an artifact of their tyramine cellobiose (TyC) labeling procedure. They maintained that ^{99m}Tc-LDL, having plasma retention properties in their study similar to conventionally prepared ^{131}I-LDL, behaves more like native LDL than does ^{131}I-TyC-LDL. In contrast, Moerlein et al. (*9*) concluded that both ^{131}I-TyC-LDL and ^{123}I-TyC-LDL are more reliable than their directly-labeled counterparts (^{131}I-LDL and ^{123}I-LDL) as tracers of native LDL metabolism.

Vallabhajosula et al. subsequently showed enhanced uptake of ^{99m}Tc-LDL in spleen and bone marrow of patients with myeloproliferative disorders and hypocholesterolemia (*10*), and Ginsberg, Goldsmith, and Vallabhajosula recently described the uptake of ^{99m}Tc-LDL by xanthomata in hypercholesterolemic patients (*11*).

Within the past three years, ^{111}In-LDL has been proposed as an alternative to ^{99m}Tc-LDL or radioiodinated LDL for nuclear imaging studies (*12–17*), and ^{68}Ga-LDL has shown promise as an agent suitable for PET analysis of lipoprotein metabolism (*17,18*).

We have been evaluating human LDL labeled with selected radionuclides as potentially useful nuclear imaging agents. In this report, we compare the biodistribution of ^{123}I-TyC-LDL with that of ^{99m}Tc-LDL in rabbits and in monkeys.

MATERIALS AND METHODS

Isolation and Derivatization of Human LDL

For these animal studies, we elected to use human LDL rather than autologous rabbit or monkey LDL. Human LDL is available in large quantity and at minimal cost, and apolipoprotein B is virtually its sole protein component. Rabbit and monkey LDL are more difficult to obtain, and often they either contain signif-

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icant levels of non-B apolipoproteins or are contaminated by other lipoprotein classes such as intermediate density lipoproteins (*19*). All human LDL used for these studies was obtained from the same young healthy male donor, and a fresh preparation of LDL was used for each experiment.

LDL was isolated by the method of Pittman et al. (*20*), except that we included a broader density range of particles. Blood (about 60 ml) was collected from a fasting human subject by venipuncture and anticoagulated with neutralized disodium EDTA (5 m*M* final concentration), and plasma was separated from the cells by low-speed centrifugation. The plasma was then subjected to ultracentrifugal flotation (minimum of 3.2×10^6 g-hr) at d = 1.006 g/ml to remove particles of very low-density lipoprotein (VLDL). The infranatant fraction from this spin was adjusted with NaCl to a buoyant density of 1.063 g/ml and recentrifuged as for VLDL. LDL was collected as the topmost 2–3 ml from the second spin. This sample was dialyzed exhaustively at 4°C against either 0.1 *M* $NaHCO_3$, pH 8.0 (prior to labeling with ^{99m}Tc) or 10 m*M* sodium phosphate, 0.15 *M* NaCl, and 1 m*M* EDTA, pH 7.4 (prior to labeling with ^{123}I-tyramine cellobiose). The dialyzed samples were sterilized by passage through a 0.22-μm filter and stored under sterile conditions in aliquots at 4°C. The final product of LDL has been found free of pyrogens by the *Limulus* test. Addition of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) and the antioxidant butylated hydroxytoluene (BHT) to plasma was attempted initially but discontinued due to their interference with the tyramine cellobiose conjugation reaction.

Protein concentrations in the isolated and dialyzed LDL samples were estimated by modifications of the method of Lowry et al. (*21*). The apolipoprotein composition of the LDL was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on tandem gradient gels (*22*).

LDL was labeled with ^{99m}Tc by dithionite reduction of pertechnetate in the presence of lipoprotein according to Lees et al. (*4*) with minor modifications suggested by them. Technetium-99m as the TcO_4^- ion was obtained from a commercial generator (New England Nuclear, N. Billerica, MA) used in the section of clinical nuclear medicine. We typically reacted 3–5 mg of LDL by protein with 10–30 mCi of ^{99m}Tc. Following gel filtration of the reaction mixtures (see below), the mean and standard deviation for radiochemical yield were 40% ± 13% for six separate preparations, resulting in estimated specific activities of 2.3 ± 0.7 mCi ^{99m}Tc per mg LDL protein.

For ^{123}I labeling of LDL we adapted protocols developed by Pittman and his colleagues for labeling LDL with ^{125}I or ^{131}I (*20*). In this procedure, tyramine cellobiose is first radioiodinated and then conjugated with LDL apolipoproteins by reductive amidation in the presence of cyanuric chloride. We chose this approach in order to avoid the low tissue retention of ^{123}I and quantitation problems resulting from deiodination of iodotyrosine following cellular uptake of directly radioiodinated LDL (*9,23*). The details of our protocol, which differ considerably from the procedure published earlier by Moerlein et al. (*24*), are as follows:

Two conical glass reaction vials (0.3 ml Reacti-Vial, Pierce Chemical Co.) were acid washed, rinsed thoroughly with water followed by HPLC-grade acetone (Aldrich), and dried under a stream of nitrogen gas. One vial was coated with 20 μl of a 1-mg/ml (2.3 m*M*) solution of Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril; Sigma Chemical Co., St. Louis, MO) in HPLC-grade dichloromethane (Aldrich). After excess solvent was evaporated under nitrogen, the vial was rinsed several times with water and shaken dry. To this vial was then added 5 μl of 10 m*M* tyramine cellobiose (TyC; generously provided by Dr. Ray Pittman) in 0.1 *M* sodium phosphate, pH 7.4, followed by an additional 15 μl of 1.0 *M* sodium phosphate, pH 7.4. About 5 mCi of ^{123}I in 20–160 μl of 0.1 N NaOH (obtained as NaI from Nordion/Atomic Energy of Canada, Ltd.) was first buffered in the shipping vial by adding one-tenth volume of 1.0 *M* sodium phosphate, pH 7.0, and then adjusted to contain 10 nmole of carrier NaI (from a 0.1-*M* stock in 10 m*M* sodium phosphate, 150 m*M* NaCl, pH 7.4) per mCi of ^{123}I. This radioactive mixture was added to the reaction vial. The vial was tightly capped, gently vortexed, and incubated at room temperature for 30 min.

Meanwhile, (i) to the second Reacti-Vial was added 5 μl each of 0.1 *M* NaI and 0.2 *M* $NaHSO_3$; (ii) in a separate glass tube was prepared a fresh solution of cyanuric chloride (purchased from Sigma, then recrystallized from toluene and stored desiccated in the dark at 4°C), 0.46 mg/ml (2.5 m*M*) in acetone; and (iii) approximately 1 ml of LDL (3–5 mg by protein) in a 15-ml conical plastic tube (Falcon) was adjusted to pH 9.5-10 by adding 0.3-0.5 ml of 0.3 *M* sodium borate, pH 10.0.

To quench the iodination reaction, the contents of the first vial were transferred to the second vial and briefly vortexed. Immediately thereafter 20 μl of cyanuric chloride solution was added to the second vial, and the vial was again vortexed. The vial contents were then quickly transferred to the LDL solution; the mixture was gently vortexed, and then incubated for 2 hr at room temperature with occasional mixing. Care was taken to complete both transfers and all three vortexing steps within a total period of 40 sec.

In six trial labelings of human LDL with ^{123}I-TyC, we obtained a mean radiochemical yield and standard deviation of 59% ± 7%. However, when we first attempted to label LDL with ^{123}I-TyC by Pittman's original protocol (i.e., without adding 1.0 *M* sodium phosphate to the first reaction vial and without adding sodium phosphate or carrier NaI to the shipping vial) we achieved radiochemical yields of only 21% ± 15% (n = 5). After amending the protocol to provide stronger buffer and carrier, we obtained radiochemical yields of 45% ± 11% (n = 4), with resultant estimated specific activities of 0.31 ± 0.11 mCi ^{123}I per mg LDL protein following gel filtration.

In order to separate LDL-associated radioactivity from unreacted radionuclide, each LDL derivatization mixture was subjected to gel filtration on 1.0 × 50 cm columns (Econo-Columns, Bio-Rad) containing Sephadex G-25 or Sephadex G-50 equilibrated in sterile and pyrogen-free buffer isotonic for injection. Radiolabeled LDL eluting at the void volume, accounting for >90% of the protein applied to the column, was collected in a total volume of 3–5 ml.

Samples of radiolabeled LDL after gel filtration but prior to injection were analyzed by SDS-PAGE followed by autoradiography (*22*) or by direct counting of gel slices. Technetium-99m-LDL included radioactivity migrating in the molecular weight range of apolipoprotein B, but an estimated one-third to one-half of the total radioactivity, presumably ^{99m}Tc aggregates, did not enter the separating gel. About 75% of the ^{123}I associated with LDL comigrated with apolipoprotein B (data not shown).

Experimental Animals

Protocols for animal housing, handling and anesthesia, and for administration and disposal of radioactivity were each approved by the responsible institutional committee.

Six New Zealand White rabbits weighing 3.2–4.9 kg were maintained on ad libitum rabbit chow and drinking water. Each rabbit was injected intravenously with an estimated 2–2.5 mg (0.4 to 0.8 mg/kg) by protein of either ^{99m}Tc-LDL (1-9 mCi) or ^{123}I-TyC-LDL (0.2-0.9 mCi) and imaged immediately after injection and again between 18 and 24 hr postinjection. After the final images were obtained, the animals were euthanized. At necropsy whole organs were removed, weighed, and assayed for activity by comparison with appropriate aliquots of the injected solutions using similar detector geometry.

We were given temporary access to five hypercholesterolemic rabbits weighing 3.4–4.8 kg (Watanabe hereditary hyperlipemic rabbits heterozygous for functional LDL receptors). Each Watanabe rabbit received an injection of an estimated 2 to 2.5 mg (0.4 to 0.7 mg/kg) by protein of ^{99m}Tc-LDL (4-9 mCi) followed two days later by injection of an equivalent mass of ^{123}I-TyC-LDL (0.2-0.6 mCi). Background images were taken just prior to each injection, and in vivo organ uptake for each preparation was evaluated by conjugate imaging of the intact animal at 18–26 hr following injection.

We also were permitted temporary use of five male rhesus monkeys kept as plasma donors. Two monkeys (weighing 13.8 and 10.7 kg) had been maintained on Purina Monkey Chow for 61 mo and 132 mo, respectively. One monkey (13.7 kg) had been fed a diet containing 25% w/w peanut oil and 2% w/w cholesterol for 42 mo, and two monkeys (7.5 and 9.9 kg) had been maintained on 25% w/w coconut oil and 2% w/w cholesterol for 74 and 75 mo, respectively. Each monkey received intravenously an estimated 2.5–5-mg (0.2–0.7 mg/kg) by protein of ^{99m}Tc-LDL (4-10 mCi) and was imaged 22–26 hr later for conjugate counting. Immediately thereafter each monkey received an equivalent mass of ^{123}I-TyC-LDL (0.1-2.0 mCi) and was imaged again the following day. Although we were not able to evaluate organ activity in these animals by direct counting, the aortas and coronary arteries of the hyperlipemic monkeys revealed extensive atherosclerosis at necropsy within 1 mo of our imaging studies.

Imaging Procedure and Analysis

Imaging was performed with a Pho Gamma IV system using appropriate collimation and on-line computer acquisition. Colbat-57 point sources were used as fiducial markers at anatomic landmarks. Established conjugate counting and data reduction techniques (*25–27*) were used to estimate the percent of injected activity localized in various regions. Statistical calculations were performed with the program Minitab (Minitab, Inc.) using Student's t-test for paired or unpaired samples as appropriate.

RESULTS

Biodistribution of LDL in Normolipemic Rabbits

Table 1 shows biodistribution data for human ^{99m}Tc-LDL and for human ^{123}I-TyC-LDL in normolipemic rabbits, together with related data published earlier by Lees et al. (*4*) and with data for ^{125}I-TyC-LDL kindly provided by Dr. Ray Pittman (personal communication). Our data generally agree with theirs. We found higher values for

TABLE 1
Biodistribution in Normal Rabbits for ^{99m}Tc and ^{123}I Injected as Radiolabeled Human LDL

Organ	^{99m}Tc	^{123}I	*p*	Lees' ^{99m}Tc	Pittman's ^{125}I
Lungs	0.46 ± 0.22	1.9 ± 1.3		0.7 ± 0.2	0.9
Heart	0.14 ± 0.07	0.25 ± 0.02			
Liver	21 ± 11	34 ± 15		21.1 ± 1.3	58.4
Gallbladder*	0.12 ± 0.03	0.75 ± 0.40			
Spleen	0.44 ± 0.32	0.53 ± 0.37		0.4 ± 0.1	0.66
Stomach	2.9 ± 2.0	2.5 ± 1.2			
Pancreas*†	0.06 ± 0.02	0.01		0.02 ± 0.00	
SD‡	1.4 ± 0.7	2.0 ± 0.8		1.3 ± 0.2	
ULI/cecum‡	11 ± 5	12 ± 5		2.3 ± 0.3	2.2
LLI*‡	0.83 ± 0.67	0.93 ± 0.93			
Omentum*	0.11 ± 0.07	0.27 ± 0.15			
Kidneys	8.5 ± 1.5	2.5 ± 0.1	0.019	3.8 ± 0.6	1.4
Adrenals*	0.17 ± 0.06	0.87 ± 0.46		0.2 ± 0.1	0.55
Gonads	0.07 ± 0.05	0.20 ± 0.08	0.098	0.1 ± 0.0	
Bladder‡	1.1 ± 0.7	9.4 ± 9.8			
Mixed excreta	38 ± 17	8.3 ± 1.9	0.096	27.2 ± 1.1¶	15.8¶
Blood§	0.04 ± 0.02	0.16 ± 0.03	0.009	0.03 ± 0.01	
Carcass	12 ± 3	25 ± 1	0.020		
Totals	99 ± 8	103 ± 9			

Tabulated values express percent administered activity corrected for physical decay (%IA) recovered in the indicated organ at 18–24 hr postinjection, as determined by counting excised organs at necropsy: * n = 2 for respective ^{99m}Tc values; † n = 1 for the ^{123}I pancreas value. All other values in the ^{99m}Tc and ^{123}I columns represent the mean ± 1 s.d. for three rabbits.

‡ Including contents.

§ Activity per gram blood.

¶ Urine values only.

SI = small intestine; ULI = upper large intestine; LLI = lower large intestine.

p values are given only for organs in which ^{99m}Tc and ^{123}I activity differed significantly at $\alpha = 0.10$. (Lees' ^{99m}Tc values from rabbits injected with human ^{99m}Tc LDL are reprinted by permission of Ref. [*4*]; Pittman's ^{125}I values from rabbits injected with human ^{125}I-TyC-LDL were obtained by personal communication.)

renal, pancreatic, and large intestinal uptake of ^{99m}Tc-LDL than Lees and his colleagues observed. We also noticed higher values for renal and large intestinal uptake, but lower values for hepatic uptake and for excretion of ^{123}I-TyC-LDL than Pittman observed for ^{125}I-TyC-LDL. Factors that could account for these differences include the broader density range of lipoprotein particles we used and technical details of the quantifying methods. We conclude that our preparations of human ^{99m}Tc-LDL satisfactorily duplicate those of Lees and colleagues, and that our human ^{123}I-TyC-LDL is a reasonable analogue for Pittman's ^{125}I-TyC-LDL.

Our data show that the in vivo metabolism of ^{123}I-TyC-LDL differs in several respects from that of ^{99m}Tc-LDL. The mean percent injected activity corrected for physical decay (%IA) was higher for ^{123}I than for ^{99m}Tc in 14 of the 18 organs listed in Table 1. Based on unpaired t-tests, there were significant differences at $\alpha = 0.10$ between ^{123}I-TyC-LDL and ^{99m}Tc-LDL for mean %IA in kidneys (^{123}I:^{99m}Tc ~1:3), gonads (^{123}I:^{99m}Tc ~3:1), mixed excreta (^{123}I:^{99m}Tc ~1:4), blood (^{123}I:^{99m}Tc ~4:1), and the eviscerated carcasses (^{123}I:^{99m}Tc ~2:1). Assuming a mean fractional catabolic rate (FCR) of 0.041 h^{-1} for human ^{123}I-TyC-LDL in the bloodstream of normolipemic rabbits (based on our unpublished data for ^{131}I-TyC-LDL), an FCR of about 0.107 h^{-1} can be estimated for ^{99m}Tc-LDL in normal rabbits. Finally, the mean adrenal %IA was much higher in normal rabbits injected with ^{123}I-TyC-LDL (^{123}I:^{99m}Tc ~5:1), but high individual variability in adrenal ^{123}I activity made this difference not statistically significant.

In preparation for biodistribution analyses in hyperlipemic rabbits and monkeys, for which necropsy data would not be available, we also used the group of normal rabbits to compare the regional percent injected activities obtained by conjugate counting of liver and heart with those obtained by counting of individual organs at necropsy. The organ counts were within 10% of and were highly correlated with the imaging estimates ($r^2 = 0.989$).

Effects of Hyperlipemia Upon Biodistribution of Radiolabeled LDL in Animals

Some potentially important differences emerged between ^{123}I-TyC-LDL and ^{99m}Tc-LDL when compared in the same animal, as well as between hyperlipemic and normolipemic animals of both species with respect to particular radionuclide uptake. The pertinent data are presented in Figure 1, Figure 2, and Table 2, and may be summarized as follows:

1. Renal activity of ^{99m}Tc was readily visualized on scintigrams of both animal species, while renal uptake of ^{123}I was much less conspicuous.
2. Adrenals were visualized in normolipemic rabbits and monkeys with ^{123}I-TyC-LDL, but not with ^{99m}Tc-LDL. Adrenals were not detected in hyperlipemic animals with either agent.

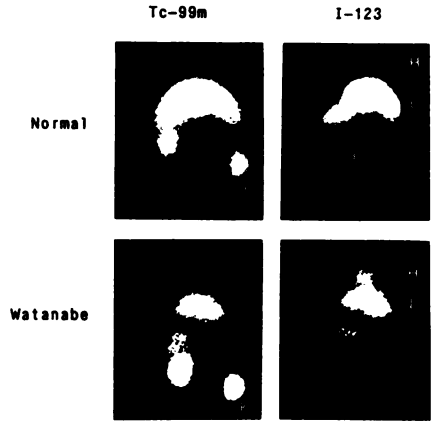

FIGURE 1. Nuclear images of rabbits injected with radiolabeled LDL. Normal rabbits (upper panels) and heterozygous Watanabe hereditary hyperlipemic rabbits (lower panels) were injected intravenously with radiolabeled human LDL either as ^{99m}Tc-LDL (left panels) or as ^{123}I-TyC-LDL (right panels). Displayed are heart and upper abdominal gamma camera images obtained at 18–24 hr following injection. The ovoid structures indicated by *K* in both left panels represent activity in the left kidney. *H* indicates the level of the heart; *L* the level of the liver; and arrows at *a* indicate activity in the region of the adrenals.

3. For each group of animals the mean %IA for ^{99m}Tc excreted was between about 1.6 and 5 times that for ^{123}I. This difference achieved greater statistical significance when all rabbits or all monkeys were considered as a group.
4. For all groups the mean cardiac %IA for ^{123}I was between 1.8 and 3.8 times and the mean hepatic activity between 1.6 and 2 times that of the corresponding organ %IA for ^{99m}Tc. These differences were highly significant for hearts of Watanabe rabbits and for the livers of both groups of monkeys.
5. Mean cardiac %IA of both radionuclides was in-

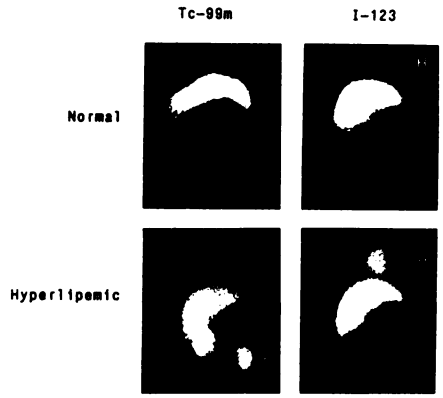

FIGURE 2. Nuclear images of monkeys injected with radiolabeled LDL. Chow-fed rhesus monkeys (upper panels) and monkeys maintained on high-fat, high-cholesterol atherogenic diets (lower panels) were injected intravenously with radiolabeled human LDL either as ^{99m}Tc-LDL (left panels) or as ^{123}I-TyC-LDL (right panels). Shown are heart and upper abdominal gamma camera images obtained 22–26 hr postinjection. Structures are the same as for Figure 1.

TABLE 2
Recovery in Animals of ^{99m}Tc and ^{123}I Introduced as Radiolabeled LDL

Organ	n	^{99m}Tc	^{123}I	p
Normolipemic rabbits				
Heart	3	0.14 ± 0.07*	0.25 ± 0.02†	
Liver	3	21 ± 11	34 ± 15	
Excreta	3	38 ± 17	8.3 ± 1.9	0.10
Hyperlipemic (Watanabe) rabbits				
Heart	5	0.42 ± 0.20*	1.6 ± 0.7†	0.001
Liver	5	7.9 ± 3.3	13 ± 7	
Excreta	3	50 ± 18	10 ± 1	0.07
All rabbits				
Excreta	6	41 ± 14	10 ± 2	<0.001
Normolipemic monkeys				
Heart	2	0.64 ± 0.52	2.2 ± 1.6	
Liver	2	31 ± 10	61 ± 9	0.01
Excreta	2	20 ± 4	12 ± 7	
Hyperlipemic monkeys				
Heart	3	1.4 ± 0.4	2.4 ± 2.5	
Liver	3	16 ± 5	30 ± 5	0.001
Excreta	3	27 ± 1	13 ± 7	0.09
All monkeys				
Excreta	5	24 ± 4	13 ± 6	0.02

Data are expressed as the mean ± 1 s.d. for percent of administered activity corrected for physical decay (as determined by conjugate counting or by counting excised organs at necropsy) recovered in the indicated organ at 18–26 hr postinjection of radiolabeled LDL. In the rightmost column, all other comparisons for ^{99m}Tc versus ^{123}I gave $p \geq 0.10$.

*$p = 0.035$ for normolipemic versus hyperlipemic rabbits, ^{99m}Tc activity in heart.

†$p = 0.010$ for normolipemic versus hyperlipemic rabbits, ^{123}I activity in heart. All other normolipemic versus hyperlipemic comparisons for ^{99m}Tc-LDL or ^{123}I-LDL gave $p \geq 0.10$.

creased to between 1.1- and 6.4-fold in the hyperlipemic state. This increase was statistically significant in rabbits but not in monkeys.

6. The mean hepatic %IA for both radionuclides in hyperlipemic monkeys was about half that in normolipemic monkeys. In rabbits, hyperlipemia was associated with reductions in hepatic activity of both radionuclides to about 38% of the normolipemic levels. However, these differences did not achieve statistical significance.

DISCUSSION

We have demonstrated that the biodistribution of ^{123}I injected as ^{123}I-TyC-LDL differs substantially from that of ^{99m}Tc injected as ^{99m}Tc-LDL in rabbits and in rhesus monkeys. Iodine-123-TyC-LDL resulted in lower renal uptake, lower levels of excretion, and higher levels of hepatic and cardiac uptake than did ^{99m}Tc-LDL. Adrenals, steroidogenic organs expected to have high uptake rates for LDL, preferentially accumulated ^{123}I. To the best of our knowledge, ours is only the second study (after ref. *9*) to evaluate the metabolism of ^{123}I-TyC-LDL in vivo, and the first to compare its metabolism directly with that of ^{99m}Tc-LDL.

Our biodistribution data with human ^{99m}Tc-LDL in normal rabbits essentially confirm those reported by Lees et al. (*4*), and our ^{123}I-TyC-LDL data with normal rabbits generally fit Pittman's ^{125}I-TyC-LDL data. For normal rabbits, we estimated a mean FCR for ^{99m}Tc-LDL that is 2.6 times that for ^{123}I-TyC-LDL. Although detailed results of human biodistribution studies using these agents will be presented elsewhere (Hay R et al., manuscript submitted), here it is worth nothing that such catabolic discrimination by rabbits is echoed in humans. The mean FCR value we have calculated in normolipemic human subjects for ^{99m}Tc-LDL [0.044 h^{-1}, which agrees with the result of Lees et al. (*5*)] is 2.8 times that for ^{123}I-TyC-LDL [0.016 h^{-1}, comparable to the mean value reported for conventionally prepared ^{125}I-LDL (*2*)]. Since we have used only one individual as the source of human LDL for all animal studies, and since our human subjects received autologous LDL, it is unlikely that the metabolic disparity we have observed between ^{99m}Tc-LDL and ^{123}I-TyC-LDL in three mammalian species results from differences among LDL preparations prior to radiolabeling.

Our data regarding effects of a hyperlipemic state on LDL biodistribution are consistent with those of Williams et al. (*28*), who observed a two-fold difference in ^{99m}Tc-LDL uptake between livers of homozygous Watanabe rabbits and New Zealand White rabbits, as well as with those of Moerlein et al. (*9*), who measured about twice as much cardiac uptake and about half as much hepatic uptake of ^{123}I-TyC-LDL in rabbits made hypercholesterolemic by diet compared to normocholesterolemic controls. Although Vallabhajosula et al. (*8*) observed only a 20% decrease in hepatic uptake, they documented a 74% decrease in adrenal uptake of ^{99m}Tc-LDL in rabbits fed a hypercholesterolemic diet compared to normals. Unlike their experience or that of Isaacsohn et al. (*29*), we were not able to image adrenals with ^{99m}Tc-LDL even in normolipemic subjects. Nevertheless, our scintigrams in Figures 1 and 2 do suggest suppression of adrenal LDL uptake of ^{123}I-TyC-LDL in association with hyperlipemia.

The data we have obtained with rhesus monkeys complement the finding of Fox et al. (*30*) that hepatic LDL receptor mRNA levels are reduced to about half the control values in baboons fed a cholesterol- and saturated fat-containing diet. Moreover, Portman and Alexander (*31*) have reported values for hepatic uptake and for combined excretion of ^{125}I following injection of ^{125}I-TyC-LDL into hyperlipemic squirrel monkeys that are comparable to our respective values in hyperlipemic rhesus monkeys.

Such findings support the view that hepatic and adrenal uptake of LDL can be altered in similar fashion by dietary and genetic hyperlipemias and indicate that ^{123}I-TyC-LDL should be useful for monitoring such alterations in vivo.

Of previously published studies, the one nearest in design and scope to ours is that of Vallabhajosula et al. (*8*),

who examined the metabolism of ^{99m}Tc-LDL, directly iodinated ^{131}I- or ^{125}I-native LDL, and ^{131}I-TyC-LDL in rabbits and in cynomolgus monkeys. They found comparable FCR values in rabbits for ^{99m}Tc-LDL and for radioiodinated native LDL, but a considerably lower FCR for ^{131}I-TyC-LDL. Regardless of their dissimilar plasma retention, the authors concluded that ^{99m}Tc-LDL and ^{131}I-TyC-LDL behave alike in terms of both overall tissue distribution and hepatic acquisition. They inferred that the metabolism of ^{99m}Tc-LDL adequately reflects that of native LDL, and they implied and subsequently reaffirmed (*11*) that ^{99m}Tc-LDL and radioiodine-TyC-LDL can be considered equivalent in terms of their use as residualizing labels for in vivo studies. Our own data and those published by other investigators (*9,20,31*) do not support these assertions.

Our findings do confirm some results of Vallabhajosula and colleagues. The data we have obtained for ^{99m}Tc distribution following injection of ^{99m}Tc-LDL correspond to theirs for seven organs. We both have observed an FCR of ~1.0 per day for ^{131}I-TyC-LDL in rabbits, and their reported FCR ratio of 2.97 for iodine-TyC-LDL:^{99m}Tc-LDL is close to the ratio of 2.6 we have calculated for normal rabbits. In addition, their published scintigrams of monkeys injected with radiolabeled human LDL suggest higher renal uptake of ^{99m}Tc-LDL than of ^{131}I-TyC-LDL by up to 8 hr postinjection, although the authors did not comment on this feature in the text of their report (*8*).

We also have some discordant observations. Our ^{99m}Tc-LDL results in rabbits differ in terms of mean %IA recovered in spleen (0.44 versus 1.46), large intestine (11.8 versus 1.22), kidneys (8.5 versus 3.0), and possibly excreta [38 (mixed) versus ~17 (urine only)]. Our ratios for ^{99m}Tc:^{123}I-TyC mean %IA are the inverse of theirs for ^{99m}Tc-:^{131}I-TyC mean %IA in rabbit liver, kidneys, and adrenals at 24 hr postinjection. Technical differences between our protocols may account for some of these discrepancies. Vallabhajosula et al. used rabbit LDL for their rabbit experiments, while we and Lees et al. (*4*) have both evaluated human LDL in rabbits; they injected ^{99m}Tc-LDL in the presence of carrier human serum albumin, whereas we and Lees et al. have used carrier-free ^{99m}Tc-LDL.

These considerations notwithstanding, we must respectfully disagree with the interpretation Vallabhajosula et al. have given to their findings. First, although we have not directly compared radioiodinated native LDL and ^{123}I-TyC-LDL in the present study, we question the generality of their claim that derivatization of LDL with radioiodine-TyC markedly retards (by two-thirds in their study) the catabolism of LDL. Pittman et al. (*20*) detected no differences in plasma retention between radioiodine-TyC-labeled rat apoA-I, human LDL, or rabbit albumin, and their respective directly radioiodinated counterparts. Portman and Alexander (*31*) observed a mean FCR for ^{125}I-TyC-LDL in squirrel monkeys that was only 26% lower than that of biosynthetically labeled LDL, and only 21%–32% lower than other preparations of radioiodinated LDL. If the claim of Vallabhajosula and colleagues concerning the altered behavior of rabbit ^{131}I-TyC-LDL is valid, perhaps radioiodine-TyC inordinately perturbs the structure and metabolism of rabbit LDL. On the other hand, we note that Vallabhajosula and colleagues have reportedly stored their ^{131}I-TyC-LDL preparations for up to two days and their radioiodinated native LDL preparations for up to two weeks before injection, whereas we have injected both ^{99m}Tc-LDL and ^{123}I-TyC-LDL immediately after radiolabeling.

Second, we suggest that the postinjection interval of 8 hr used by Vallabhajosula et al. for monitoring monkeys is probably inadequate for detecting biodistribution differences between ^{99m}Tc-LDL and ^{131}I-TyC-LDL. Consequently, they may have overstated the degree to which residualizing analogues of LDL are actually "trapped" in the liver in vivo. They claim to have observed no decrease in hepatic activity of either ^{99m}Tc or of ^{131}I-TyC in monkeys over 8 hr postinjection. Yet their rabbit data point to roughly a two-fold difference in hepatic activity between ^{99m}Tc and ^{131}I-TyC at 24 hr postinjection, and our data from rhesus monkeys confirm significant differences in hepatic activity between ^{99m}Tc and ^{123}I-TyC (albeit in inverse ratio to their rabbit data) by 22–26 hr postinjection. While Vallabhajosula et al. apparently did not measure excreted activity for either ^{99m}Tc or radioiodine in their monkey studies, our data from rabbits and monkeys reveal significant differences in combined excretion between ^{99m}Tc and ^{123}I-TyC by one day postinjection. If the initial rates of hepatic uptake of ^{99m}Tc-LDL and of ^{131}I-TyC-LDL from the bloodstream are both rapid, but the excretion rates of the radionuclides from liver into bile or blood are slow yet unequal, extended monitoring would be required for any differences in retained hepatic activity to surface. Indeed, Moerlein et al. (*9*) observed initially rapid uptake of ^{123}I-TyC-LDL in livers of rabbits, followed by a slow but monotonic decline in hepatic activity, and Pittman et al. (*20*) reported significant excretion (~25% of injected activity) of ^{125}I into the gut and stool by 24 hr after rapid hepatic clearance of ^{125}I-TyC-asialofetuin. Rather than considering sustained hepatic activity as representing truly "trapped" radionuclide—activity that enters the organ but does not leave by biologic turnover—we think it more likely reflects a near steady-state between hepatic acquisition of LDL and hepatic excretion of radiolabeled LDL catabolites.

The ideal tracer for evaluating LDL metabolism in vivo would have a physical half-life short enough to give a small radiation absorbed dose to the subject, so that sequential studies could be performed in a given individual, but long enough and of the proper energy for tissues of interest to be visualized by external scintillation cameras. It should contain radionuclide stably bound to LDL for at least the lifetime of the lipoprotein in the circulation, and it ought to display biologic half-life, biodistribution properties, and interactions with tissue receptors resembling those of na-

tive LDL. Our findings argue that ^{123}I-TyC-LDL better satisfies these criteria and that it is more suitable for dynamic studies of LDL metabolism than ^{99m}Tc-LDL.

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REFERENCES

1. Vega GL, Grundy SM. Estimation of turnover of low density lipoproteins by simplified methods. *Atherosclerosis* 1989;76:139–148.
2. Kesaniemi YA, Grundy SM. Significance of low density lipoprotein production in the regulation of plasma cholesterol level in man. *J Clin Invest* 1982;70:13–22.
3. Lees RS, Lees AM, Strauss HW. External imaging of human atherosclerosis. *J Nucl Med* 1983;24:154–156.
4. Lees RS, Garabedian HD, Lees AM, et al. Technetium-99m low density lipoprotein: preparation and biodistribution. *J Nucl Med* 1985;26:1056–1062.
5. Lees RS, Lees AM, Strauss HW, et al. The distribution and metabolism of Tc-99m labeled low density lipoprotein in human subjects [Abstract]. *J Nucl Med* 1985;26:P35.
6. Lees AM, Lees RS, Schoen FJ, et al. Imaging human atherosclerosis with ^{99m}Tc-labeled low density lipoproteins. *Arteriosclerosis* 1988;8:461–470.
7. Lees RS, Lees AM, Fischman AJ, Strauss HW. External imaging of active atherosclerosis with ^{99m}Tc-LDL. In: Glagov S, Newman WP III, Schaffer SA, eds. *Pathobiology of the human atherosclerotic plaque.* New York: Springer-Verlag; 1990:841–851.
8. Vallabhajosula S, Paidi M, Badimon JJ, et al. Radiotracers for low density lipoprotein biodistribution studies in vivo: technetium-99m-low density lipoprotein versus radioiodinated low density lipoprotein preparations. *J Nucl Med* 1988;29:1237–1245.
9. Moerlein SM, Dalal KB, Ebbe SN, Yano Y, Budinger TF. Residualizing and non-residualizing analogues of low-density lipoprotein as iodine-123 radiopharmaceuticals for imaging LDL catabolism. *Nucl Med Biol* 1988;15:141–149.
10. Vallabhajosula S, Gilbert HS, Goldsmith SJ, Paidi M, Hanna MM, Ginsberg HN. Low-density lipoprotein (LDL) distribution shown by technetium-99m-LDL imaging in patients with myeloproliferative diseases. *Ann Intern Med* 1989;110:208–213.
11. Ginsberg HN, Goldsmith SJ, Vallabhajosula S. Noninvasive imaging of ^{99m}Tc-labeled low density lipoprotein uptake by tendon xanthomas in hypercholesterolemic patients. *Arteriosclerosis* 1990;10:256–262.
12. Rosen JM, Butler SP, Ginsberg HN, et al. Development of In-111-labeled LDL for imaging atherosclerotic lesions [Abstract]. *J Nucl Med* 1989;30:787.
13. Nicolas JM, Leclef B, Jardez H, Keyeux A, Melin JA, Trouet A. Imaging atherosclerotic lesions with In-111-labelled low density lipoproteins [Abstract]. *J Nucl Med* 1989;30:788.
14. Gross MD, Skinner RWS, Schwendner SW, DeForge LE, Counsell RE, Newton RS. Indium-111-DTPA-low density lipoprotein (LDL) imaging of LDL-receptor activity [Abstract]. *J Nucl Med* 1989;30:768.
15. Hay RV, Ryan JW, Williams KA, et al. Indium-111-[SCN-Bz-DTPA]-low density lipoprotein: a new agent for extended nuclear imaging and detection of atheromata [Abstract]. *Cardiovascular Science & Technology: Basic and Applied I* 1989;203–204.
16. Rosen JM, Butler SP, Meinken GE, et al. Indium-111-labeled LDL: a potential agent for imaging atherosclerotic disease and lipoprotein biodistribution. *J Nucl Med* 1990;31:343–350.
17. Moerlein SM, Daugherty A, Welch MJ. Assessment of in-vivo lipoprotein metabolism with Ga-68 and In-111-labeled low-density lipoprotein [Abstract]. *J Nucl Med* 1990;31:745.
18. Moerlein SM, Daugherty A, Welch MJ. Ga-68-DTPA-LDL: a potential radiopharmaceutical for in-vivo imaging of low-density lipoprotein receptor activity with PET [Abstract]. *J Nucl Med* 1988;29:848–849.
19. Chapman MJ. Animal lipoproteins: chemistry, structure, and comparative aspects. *J Lipid Res* 1980;21:789–853.
20. Pittman RC, Carew TE, Glass CK, Green SR, Taylor CA Jr, Attie AD. A radioiodinated, intracellularly trapped ligand for determining the sites of plasma protein degradation in vivo. *Biochem J* 1983;212:791–800.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275.
22. Hay R, Fleming R, O'Connell W, Kirschner J, Oppliger W. Apolipoproteins of the orotic acid fatty liver: implications for the biogenesis of plasma lipoproteins. *J Lipid Res* 1988;29:981–995.
23. Chisolm GM III, Sila CA, Hmiel SP. Measurements of the degradation products of radioiodinated proteins. *Anal Biochem* 1981;111:212–219.
24. Moerlein SM, Dalal KB, Yano Y. Synthesis of ^{123}I- and ^{131}I-labelled derivatives of low-density liproprotein for radiopharmaceutical use. *J Lab Comp Radiopharm* 1987;24:1325–1339.
25. Tsui BMW, Chen C-T, Yasillo NJ, et al. A whole-body scanning system for collection of quantitative in vivo biodistribution data in humans. In: Watson EE, Schlafke-Stelson AT, Coffey JL, Cloutier RJ, eds. *Proceedings of the third international symposium on radiopharmaceutical dosimetry.* Rockville, MD: The Bureau of Radiological Health; 1981:138–156.
26. Ryan JW, Harper PV, Stark VJ, Peterson EL, Lathrop KA. Radiation absorbed dose estimate for rubidium-82 determined from in vivo measurements in human subjects. In: Schlafke-Stelson AT, Watson EE, eds. *Fourth international radiopharmaceutical dosimetry symposium.* Oak Ridge, TN: Oak Ridge Associated University Publishers; 1986:346–358.
27. Lathrop KA, Bartlett RD, Chen C, et al. Quantitative clinical uptake measurements using conjugate counting. In: Schlafke-Stelson AT, Watson EE, eds. *Fourth international radiopharmaceutical dosimetry symposium.* Oak Ridge, TN: Oak Ridge Associated University Publishers; 1986:135–147.
28. Williams KJ, Vallabhajosula S, Rahman IU, et al. Low density lipoprotein receptor-independent hepatic uptake of a synthetic, cholesterol-scavenging lipoprotein: implications for the treatment of receptor-deficient atherosclerosis. *Proc Natl Acad Sci USA* 1988;85:242–246.
29. Isaacsohn JL, Lees AM, Lees RS, Strauss HW, Barlai-Kovach M, Moore TJ. Adrenal imaging with technetium-99m-labelled low density lipoproteins. *Metabolism* 1986;35:364–366.
30. Fox JC, McGill HC Jr, Carey KD, Getz GS. In vivo regulation of hepatic LDL receptor mRNA in the baboon. *J Biol Chem* 1987;262:7014–7020.
31. Portman OW, Alexander M. Metabolism of [^{125}I]tyramine cellobiose-labeled low density lipoproteins in squirrel monkeys. *Atherosclerosis* 1985;56:283–299.
32. Hay R, Fleming R, Stark V, Lathrop K, Harper P. Biodistribution of low density lipoprotein in normal and hyperlipemic animals [Abstract]. *Cor Notes* 1988;3:5–6.