

Imaging of hepatic low density lipoprotein receptors by radionuclide scintiscanning *in vivo*

(¹²³I-labeled lipoproteins/Watanabe-heritable hyperlipidemic rabbits/regulation of hepatic lipoprotein receptors/familial hypercholesterolemia)

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ABSTRACT The low density lipoprotein (LDL) receptor mediates the cellular uptake of plasma lipoproteins that are derived from very low density lipoproteins (VLDL). Most of the functional LDL receptors in the body are located in the liver. Here, we describe a radionuclide scintiscanning technique that permits the measurement of LDL receptors in the livers of intact rabbits. ¹²³I-labeled VLDL were administered intravenously, and scintigraphic images of the liver and heart were obtained at intervals thereafter. In seven normal rabbits, radioactivity in the liver increased progressively between 1 and 20 min after injection, while radioactivity in the heart (reflecting that in plasma) decreased concomitantly. In Watanabe-heritable hyperlipidemic rabbits, which lack LDL receptors on a genetic basis, there was little uptake of ¹²³I-labeled VLDL into the liver and little decrease in cardiac radioactivity during this interval. These findings demonstrate that the LDL receptor is necessary for the hepatic uptake of VLDL-derived lipoproteins in the rabbit. Two conditions that diminish hepatic LDL receptor activity, cholesterol-feeding and prolonged fasting, also reduced the uptake of ¹²³I-labeled VLDL in the liver as measured by scintiscanning. The data suggest that radionuclide scintiscanning can be used as a noninvasive method to quantify the number of LDL receptors expressed in the liver *in vivo*.

Low density lipoprotein (LDL) receptors mediate the catabolism of two atherogenic plasma lipoproteins, intermediate density lipoproteins (IDL) and LDL (reviewed in ref. 1). Projecting outward from the surfaces of cells in the liver and other organs, these receptors bind plasma IDL and LDL and mediate their cellular uptake and delivery to lysosomes, where the lipoproteins are degraded. IDL and LDL are both derived from very low density lipoproteins (VLDL), which are triacylglycerol-rich lipoproteins that are secreted by the liver. In extrahepatic tissues, the triacylglycerols of VLDL are hydrolyzed by lipoprotein lipase and the VLDL are converted to cholesterol-rich IDL. Most of the IDL are removed rapidly from plasma by binding to LDL receptors in the liver. Some of the IDL particles escape hepatic uptake, undergo further lipolysis in the circulation, and become LDL. Eventually, most of the LDL particles are also cleared from plasma by binding to LDL receptors in liver and in other tissues. The liver takes up IDL much more rapidly than LDL because IDL contains a protein, apoprotein E, that binds to LDL receptors with higher affinity than the apoprotein B of LDL.

When the number of LDL receptors is diminished, either as a result of defects in the gene encoding the receptor or by environmental factors that suppress production of receptors,

IDL is not cleared normally by the liver and is converted to LDL in increased amounts (1). Catabolism of LDL is also slowed. Because of the combined overproduction and undercatabolism, the level of LDL in plasma rises and atherosclerosis is accelerated. For this reason, it is important to develop methods to assess the number of LDL receptors operating *in vivo*.

In experimental animals (2-5) and in man (6-8), the total number of LDL receptors in the body has been estimated by measuring the rate of disappearance of ¹²⁵I-labeled LDL from the circulation. In animals, the number of receptors in various tissues can be estimated by removal of organs after intravenous administration of radiolabeled lipoproteins and measurement of the radioactive lipoprotein that has been taken up by those organs. Such studies have revealed that the vast bulk of LDL receptors in animals are in the liver (4, 9, 10) and that these hepatic receptors are subject to regulation (2, 5, 10-13). In rabbits, LDL receptor activity is suppressed by high cholesterol diets (11), by diets composed of wheat starch and casein (12), and by prolonged fasting (13). Conversely, LDL receptor activity is stimulated by agents that lower the cholesterol content of liver, such as bile acid-binding resins and inhibitors of cholesterol synthesis (2, 8).

A method for the noninvasive measurement of LDL receptor activity in liver would be desirable, initially for experimental studies in animals, and eventually for clinical studies in humans. The possibility of such measurement emerges from recent advances in scintiscanning and computer techniques and in the availability of low-energy isotopes (14). Theoretically, these methods should allow quantitative estimates *in vivo* of the receptor-mediated uptake of radiolabeled lipoproteins in the liver.

To this end, we administered ¹²³I-labeled VLDL intravenously to rabbits and measured the uptake of the lipoprotein in livers of these animals by radionuclide scintiscanning. We have chosen VLDL as a ligand because this lipoprotein is converted in the bloodstream to IDL, which enters the liver rapidly, due to its content of apoprotein E (15, 16). In earlier studies, we showed that, in normal rabbits, 32% of intravenously administered ¹²⁵I-labeled VLDL radioactivity was found in the liver 30 min after injection (15). Evidence that this uptake occurred by a receptor-mediated mechanism was confirmed by comparative studies in Watanabe-heritable hyperlipidemic (WHHL) rabbits, which are homozygous for a genetic defect in the LDL receptor (1, 15, 17). When ¹²⁵I-labeled VLDL was injected intravenously into WHHL rabbits, the uptake of the lipoprotein into the liver was <10% of that observed in normal rabbits, owing to the lack of functional LDL receptors (1, 15). In this report, we show that

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Abbreviations: IDL, intermediate density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; WHHL rabbits, Watanabe-heritable hyperlipidemic rabbits.

radionuclide scintiscanning with ^{123}I -labeled VLDL as a tracer can be used as a noninvasive method to quantify receptor-mediated uptake of lipoproteins in the liver.

METHODS

Materials. We obtained carrier-free iodine-123 from Crocker Nuclear Laboratory (University of California, Davis); Rompun (xylazine) and Vetalar (ketamine hydrochloride) from Pioneer Veterinary Supply (Houston, TX); and short catheters (Quick-Cath) from Travenol Laboratories (Deerfield, IL). All other materials were obtained as previously described (15).

Rabbits. New Zealand White rabbits were purchased from Hickory Hill Rabbitry (Flint, TX). Homozygous WHHL rabbits were raised in Dallas (15). Animals were 3–36 months old (1.9–4.7 kg) at the time of study (Table 1). As indicated in Table 1, animals were fed either Purina rabbit chow (normal diet) or a diet containing 0.2% cholesterol and 10% corn oil (0.2% cholesterol diet) (11) or were fasted for 3 weeks. The fasted animals had access to tap water.

^{123}I -Labeled VLDL. VLDL was prepared from plasma of WHHL rabbits fed a standard diet without fasting (15). One milliliter of this preparation containing 2.5 mg of protein was radiolabeled with 5 mCi (0.185 GBq) of ^{123}I by the iodine monochloride method (18), after which bovine serum albumin was added to a final concentration of 40 mg/ml. Free iodine was removed by chromatography on a Sephadex G-25 column (PD-10, Pharmacia) equilibrated in 150 mM NaCl/0.24 mM EDTA, pH 7.4, followed by dialysis (2×2 hr) against 6 liters of the same buffer at 4°C . Of the total radioactivity in ^{123}I -labeled VLDL, <15% was extractable

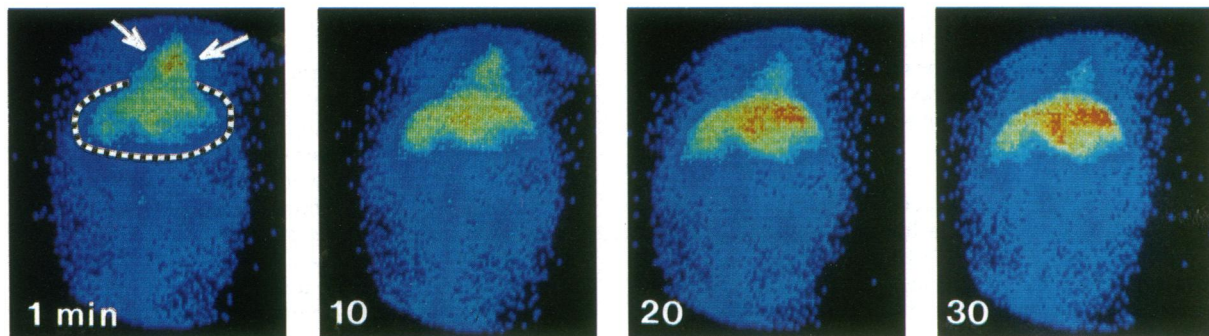
into chloroform/methanol (2:1, vol/vol) and >97% was precipitable by 10% (wt/vol) trichloroacetic acid.

Injection of ^{123}I -Labeled VLDL into Animals. Six hours after the iodination reaction, $47\text{--}279 \times 10^6$ cpm (≈ 0.3 ml) of ^{123}I -labeled VLDL was injected via a short catheter into the marginal ear vein of animals lightly anesthetized with Rompun (3 mg/kg of body weight) and Vetalar (25 mg/kg of body weight) to prevent movement during scanning.

Nuclear Imaging and Analysis. Images were obtained using a standard field-of-view gamma camera (DynaMo, Picker International) equipped with a low-energy, general-purpose, parallel-hole collimator. Energy discrimination was provided by a 20% window centered on the 159 keV photopeak. Pixel dimensions were 2.2×2.2 mm. System resolution was measured to be 2.5 mm full-width at half maximum amplitude on the collimator face. Animals were positioned with the anterior torso on the collimator face and taped to the head of the camera. Following equilibration for 1 min after intravenous injection of ^{123}I -labeled VLDL, an initial image was obtained by acquiring counts for 4 min. Then, further images were obtained over periods of 4 min each, starting at 10, 20, and 30 min after injection. Images containing $\approx 100,000$ counts each were stored on a dedicated nuclear medicine computer system (Technicare Model 450) at a digital resolution of 128×128 . Images were analyzed by manually assigning areas of interest over the heart and liver, and time vs. activity curves were generated for each animal. The areas used for the calculations were maintained constant throughout a study.

Other Assays. Protein was determined by the method of Lowry *et al.* (19). Cholesterol levels were measured as described (6).

A Normal rabbit



B WHHL rabbit

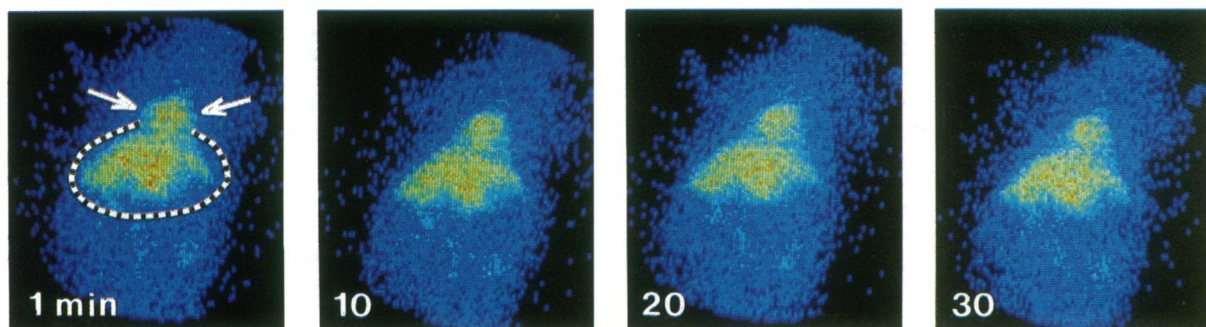


FIG. 1. Scintigraphic images of the liver and heart of a normal rabbit (A) and a WHHL rabbit (B) after intravenous injection of ^{123}I -labeled VLDL. The normal rabbit (animal 3 in Table 1) and the WHHL rabbit (animal 11 in Table 1) each received 1.1×10^8 cpm of ^{123}I -labeled VLDL intravenously. Scintigrams were obtained at 1, 10, 20, and 30 min after injection as described in *Methods*. The heart is indicated by the arrows and the liver is indicated by the dotted lines. Red indicates a higher concentration of radioactivity than green.

RESULTS

Fig. 1 shows scintigraphic images of radioactivity in the heart and liver of a normal and of a WHHL rabbit at different times after intravenous administration of ¹²³I-labeled VLDL. In the normal animal, the amount of radioactivity in the heart was highest at the initial time point (1 min after injection) and declined progressively thereafter, as evidenced by the decreasing intensity of the heart image (Fig. 1A). The liver, on the other hand, showed progressive accumulation of radioactivity from 1 min to 30 min after injection, as indicated by the transition from a green to a red color. In the WHHL rabbit, these changes did not occur (Fig. 1B). The amount of radioactivity in the heart declined only slightly and the amount of radioactivity in the liver did not increase during the course of the experiment. We interpret these findings to indicate that, at 1 min after injection, the radioactivity in both heart and liver largely reflects extracellular ¹²³I-labeled VLDL. The progressive increase of radioactivity in the liver and the progressive decrease in the heart after 1 min reflect the receptor-mediated uptake of labeled lipoprotein into liver cells, with consequent reduction in the blood level.

Table 1 summarizes the quantitative results of scintiscanning measurements of ¹²³I-labeled VLDL in four groups of rabbits. The radioactivity detected over the liver at the initial time point (1 min after injection) varied within each group. This was due in part to differences in the specific radioactivity of the injected ¹²³I-labeled VLDL and in part to differences in the anatomy of individual animals, which affects the efficiency of scintiscanning. Thus, to quantify the scintiscanning data, we calculated the radioactivity at the 20-min time point and expressed it as a fraction of the radioactivity at the 1-min time point. When the data were thus normal-

ized, consistent results were obtained [(b)/(a) ratios in Table 1].

Fig. 2 graphically illustrates the ¹²³I radioactivity in liver and heart at 10, 20, and 30 min after injection of labeled VLDL, expressed as a fraction of the counts that were present in each organ at 1 min. In all seven normal rabbits, the radioactivity in the liver increased progressively over the initial 20 min, with a corresponding decrease in the radioactivity in the heart (Fig. 2A). At the 20-min time point, which was chosen for comparative quantitative analysis, the normal animals had a 1.31-fold mean increase in the amount of ¹²³I in the liver compared with that at the 1-min time point (Table 1). In the heart, the counts had declined to a mean of 65% of the initial value. There was little difference in these parameters between male and female animals and between animals that were 3 and 36 months old (Table 1).

In four WHHL rabbits, the plasma cholesterol level averaged 604 mg/dl, which is 9-fold the mean value of 67 mg/dl in the seven normal rabbits (Table 1). In the WHHL animals, there was no increase in ¹²³I in the liver (91% of the initial value) and only a slight decrease in cardiac radioactivity (87% of the initial value) between 1 and 20 min after the administration of ¹²³I-labeled VLDL (Table 1 and Fig. 2B).

Several treatments have been shown to markedly reduce LDL receptors in livers of normal rabbits, as shown by direct assays of the binding of ¹²⁵I-labeled VLDL and LDL to liver membranes (11–13). One of these treatments is cholesterol feeding (11). Accordingly, we placed two normal rabbits on a 0.2% cholesterol diet for 4 weeks. At the end of this time, the plasma cholesterol level had risen to an average value of 771 mg/dl. When these animals were administered ¹²³I-labeled VLDL intravenously, there was no progressive increase in liver radioactivity and only a slight decrease in

Table 1. Quantitative ¹²³I scintiscanning of liver and heart of rabbits injected intravenously with ¹²³I-labeled VLDL

Animal	Sex	Age, mo	Weight, kg	Plasma cholesterol, mg/dl	¹²³ I-labeled VLDL injected, cpm × 10 ⁻⁶	¹²³ I in organs, counts × 10 ⁻³					
						1 min (a)		20 min (b)		(b)/(a) ratio	
						Liver	Heart	Liver	Heart	Liver	Heart
<i>Group A (normal rabbits fed normal diet)</i>											
1	M	3	2.3	63	47	8.5	3.6	10.5	2.3	1.24	0.64
2	M	3	2.5	83	228	38.0	15.7	47.0	12.0	1.24	0.76
3	M	3	2.5	50	110	44.2	6.6	64.0	3.7	1.45	0.57
4	F	3	2.3	65	180	30.2	14.2	38.7	9.7	1.28	0.68
5	F	3	2.3	41	180	42.0	12.8	48.3	8.5	1.15	0.66
6	M	36	4.7	82	228	57.7	16.9	78.5	11.5	1.36	0.68
7	M	36	4.5	82	228	45.8	9.8	66.8	5.8	1.44	0.59
Mean ± SD										1.31 ± 0.11	0.65 ± 0.07
<i>Group B (WHHL rabbits fed normal diet)</i>											
8	M	3	2.1	701	180	24.7	13.9	22.5	11.7	0.91	0.84
9	F	3	2.1	513	47	4.3	3.6	4.1	2.9	0.97	0.78
10	F	3	2.4	662	87	6.4	2.6	5.0	2.4	0.78	0.92
11	M	5	2.7	540	110	16.8	4.2	16.3	4.1	0.97	0.94
Mean ± SD										0.91 ± 0.09	0.87 ± 0.07
<i>Group C (normal rabbits fed 0.2% cholesterol diet)</i>											
12	F	3	2.5	769	47	5.0	2.0	4.8	1.7	0.96	0.85
13	F	3	2.5	773	47	6.9	3.7	6.7	2.9	0.97	0.78
Mean										0.97	0.82
<i>Group D (normal rabbits, fasted 3 wk)</i>											
14	M	3	2.8 → 1.9	375	47	11.4	4.0	10.2	3.0	0.89	0.75
15	M	3	2.6 → 2.0	345	279	31.2	22.8	29.5	18.6	0.95	0.82
Mean										0.92	0.79

Animals were fasted or fed as indicated, and received the indicated amount of ¹²³I-labeled VLDL intravenously. The number of counts detected over a constant area of the liver and the heart of each animal was determined at 1 min (a) and 20 min (b) after injection, as described in Methods. In group D, the weights of the animals before and after fasting are both given.

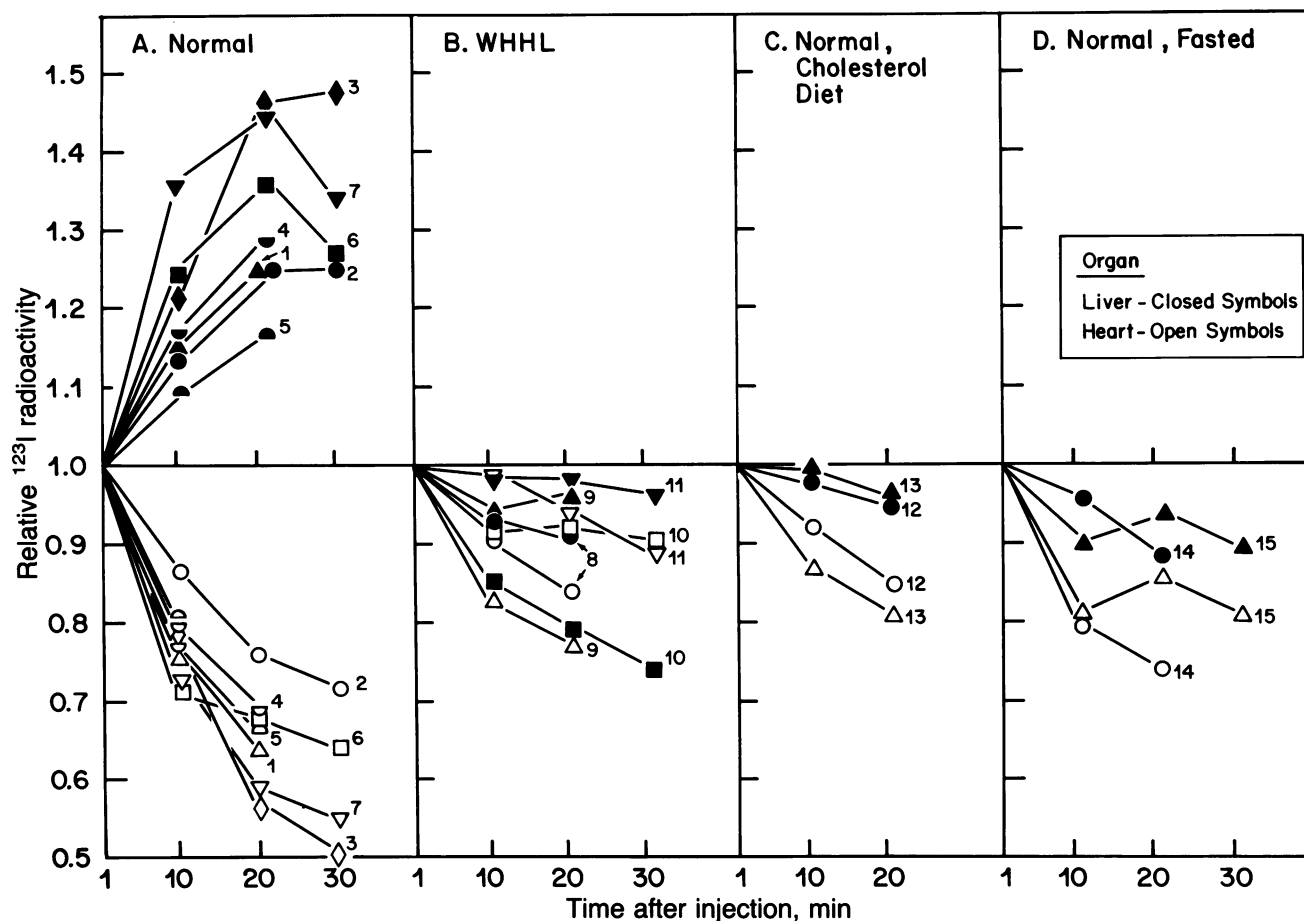


FIG. 2. Quantitative scintiscanning of the liver and heart in rabbits at various times after injection of ^{123}I -labeled VLDL. The data are expressed as the ratio of the radioactivity in the liver (closed symbols) or heart (open symbols) at the indicated time relative to that in the same organ at 1 min after injection. These data were obtained in the experiments described in Table 1; each number in the figure refers to the corresponding animal in Table 1, and A–D correspond to groups A–D in the table. All animals were scanned at 1, 10, and 20 min; animals 2, 3, 6, 7, 10, 11, and 15 were also scanned at 30 min.

cardiac radioactivity between 1 and 20 min after injection (Table 1 and Fig. 2C).

Another treatment known to reduce hepatic LDL receptor levels in rabbits is prolonged fasting (13). We subjected two normal rabbits to a total fast for 3 weeks and then administered ^{123}I -labeled VLDL. In these animals, the plasma cholesterol levels had risen to an average value of 360 mg/dl. Again, there was no progressive increase in radioactivity in the liver and only a slight decrease in radioactivity in the heart over 20 min (Table 1 and Fig. 2D).

DISCUSSION

In these experiments, we have applied scintiscanning techniques to the measurement of the uptake of VLDL-derived lipoproteins by rabbit liver. Entry of ^{123}I -labeled VLDL radioactivity into the liver of normal rabbits was rapid. This uptake required the LDL receptor, since it did not occur in WHHL rabbits and was diminished by cholesterol feeding and by prolonged fasting, both of which are known to diminish hepatic LDL receptor activity (10–13).

In the case of the WHHL rabbit, the failure to take up the ^{123}I -labeled VLDL is attributable exclusively to a lack of LDL receptors, since a primary genetic deficiency of LDL receptors has been demonstrated directly in these animals (reviewed in ref. 1). In the cholesterol-fed and the fasted rabbits, the situation is somewhat more complicated. A diminished production of LDL receptors has been demonstrated by *in vitro* membrane-binding assays, and this deficiency initiates the buildup of IDL and LDL in plasma (11–13). As the

plasma levels of these lipoproteins rise, the remaining LDL receptors become saturated, and the excess lipoproteins compete with each other for the few receptors that are functional (11). Thus, when a radiolabeled ligand is injected into the cholesterol-fed or fasted animals, the diminished uptake is attributable to two factors: (i) a reduced number of receptors and (ii) saturation of the remaining receptors with endogenous ligands that compete with the radiolabeled ligand. The observed reduced uptake of the radioactive ligand implies that the receptors are the limiting factor in lipoprotein clearance, but these measurements alone cannot tell how much of the limitation is caused by a reduced number of receptors and how much is caused by saturation of the remaining receptors.

We do not know whether this scintiscanning technique can be adapted to humans and whether it would be discriminating enough to detect the 50% deficiency of LDL receptors in heterozygous familial hypercholesterolemia or to reveal the subtle changes in hepatic LDL receptors that occur in response to diet and drug therapy. Humans, in general, seem to express fewer LDL receptors than do lower animal species such as rabbits (20). The clearance rate for ^{125}I -labeled VLDL is much slower in humans (21) than in rabbits (15), and the percentage conversion of VLDL to LDL is higher in humans. The clearance rate of LDL in humans (6) is also markedly less than that in rabbits (3). A rapid rate of hepatic uptake is necessary for the scintiscanning method to work because the ^{123}I -labeled-VLDL-derived radioactivity is released from the liver within 60 min after the lipoprotein is

taken up and digested in lysosomes. In rabbits, rapid degradation does not present a problem for the assay, since the uptake is rapid and the steady-state level of radioactivity in the liver is relatively high. However, in humans the rate of uptake of ^{123}I -labeled VLDL may be so slow that a sufficiently high intracellular level of ^{123}I within the liver would not be attained.

In this regard, it may be possible to use VLDL covalently attached to ^{123}I -labeled tyramine-cellobiose as a ligand (22). When ^{125}I -labeled tyramine-cellobiose is covalently attached to LDL and the lipoprotein is taken up by the liver or other organs, the radioactive tyramine-cellobiose remains trapped in lysosomes and accumulates progressively (22). Since the labeled tyramine-cellobiose is not excreted from the cells, its use should increase the sensitivity of the scintiscanning technique quite markedly.

Another possible approach for measurement of hepatic LDL receptors in humans involves the use of radionuclide-labeled monoclonal antibodies directed against the LDL receptor. Some of these antibodies bind to the receptor at a site that is different from the LDL binding site; their binding is not inhibited competitively by LDL or other ligands for the LDL receptor. After binding to the LDL receptor, these antibodies are carried into the cell and delivered to lysosomes, as is LDL (23). When such a monoclonal antibody is labeled with ^{125}I and injected into rabbits, most of the radioactivity is taken up rapidly in the liver in a process that depends on the antibody binding to the LDL receptor (24).

If administration of radiolabeled mouse monoclonal antibodies to humans can be shown to be a safe procedure, then it should be feasible to use ^{123}I -labeled monoclonal antibodies against the LDL receptor as probes for hepatic scintiscanning. Such antibodies would overcome the difficulties in interpretation that might result from saturation of the receptors by endogenous lipoproteins, and their rapid uptake would also deliver sufficient radioactivity to the liver to produce a highly sensitive and quantitative assay for LDL receptors in intact humans.

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