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Impaired receptor-mediated catabolism of low density lipoprotein in the WHHL rabbit, an animal model of familial hypercholesterolemia

(protein turnover/lipoprotein receptors/modified lipoproteins)

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ABSTRACT The homozygous WHHL (Watanabe heritable hyperlipidemic) rabbit displays either no or only minimal low density lipoprotein (LDL) receptor activity on cultured fibroblasts and liver membranes and has therefore been proposed as an animal model for human familial hypercholesterolemia. To assess the impact of this mutation on LDL metabolism *in vivo*, we performed lipoprotein turnover studies in normal and WHHL rabbits using both native rabbit LDL and chemically modified LDL (i.e., methyl-LDL) that does not bind to LDL receptors. The total fractional catabolic rate (FCR) for LDL in the normal rabbit was 3.5-fold greater than in the WHHL rabbit. Sixty-seven percent of the total FCR for LDL in the normal rabbit was due to LDL receptor-mediated clearance and 33% was attributable to receptor-independent processes; in the WHHL rabbit, essentially all of the LDL was catabolized via receptor-independent processes. Despite a 17.5-fold elevated plasma pool size of LDL apoprotein (apo-LDL) in WHHL as compared to normal rabbits, the receptor-independent FCR—as judged by the turnover of methyl-LDL—was similar in the two strains. Thus, the receptor-independent catabolic processes are not influenced by the mutation affecting the LDL receptor. The WHHL rabbits also exhibited a 5.6-fold increase in the absolute rate of apo-LDL synthesis and catabolism. In absolute terms, the WHHL rabbit cleared 19-fold more apo-LDL via receptor-independent processes than did the normal rabbit and cleared virtually none by the receptor-dependent pathway. These results indicate that the homozygous WHHL rabbit shares a number of metabolic features in common with human familial hypercholesterolemia and should serve as a useful model for the study of altered lipoprotein metabolism associated with receptor abnormalities. We also noted that the *in vivo* metabolic behavior of human and rabbit LDL in the normal rabbit differed such that the mean total FCR for human LDL was only 64% of the mean total FCR for rabbit LDL, whereas human and rabbit methyl-LDL were cleared at identical rates. Thus, if human LDL and methyl-LDL had been used in these studies, the magnitude of both the total and receptor-dependent FCR would have been underestimated.

Familial hypercholesterolemia (FH) is a human disease characterized clinically by accelerated atherosclerosis, elevated plasma levels of low density lipoprotein (LDL), xanthoma formation in tendons and skin, and inheritance as an autosomal dominant trait with a gene-dosage effect (1, 2). The defect is biochemically defined by the absence or near-absence of LDL receptors on cells obtained from patients with the homozygous form of the disease and half the normal number of LDL receptors on the cells of heterozygotes (1, 2). One major result of diminished or absent LDL receptor activity is an impaired rate of LDL catabolism *in vivo*; on average, heterozygotes catabolize LDL at a fractional rate that is two-thirds of normal, whereas homozygotes catabolize LDL at a rate only one-third of normal (3). These results led to the proposal that normal subjects have at least two processes for clearance of LDL from plasma: an LDL receptor-dependent process and one or more LDL receptor-independent processes (4). In FH homozygotes, the receptor-dependent mechanisms are absent. Because these subjects clear LDL at one-third the normal rate, it was suggested that the receptor-independent processes may account for one-third of the clearance of LDL in normal subjects (4).

To estimate the relative contributions of LDL receptor-dependent and receptor-independent processes to the overall catabolism of LDL, Shepherd and co-workers (5–7) introduced a double-label turnover technique in which the metabolism of native LDL is compared with that of chemically modified LDL. The latter include methyl-LDL and cyclohexandione-LDL which do not bind to the LDL receptor (5–11). In these studies, native and chemically modified LDL—each radiolabeled with a different isotope of iodine—are simultaneously injected into animals or humans and the fractional catabolic rate (FCR) for each is determined. The FCR for native LDL is presumed to reflect both receptor-dependent and receptor-independent processes, whereas the FCR for chemically modified LDL presumably reflects only receptor-independent catabolic processes. Subtraction of the FCR for chemically modified LDL from the FCR for native LDL gives an estimate of the FCR attributable to receptor-mediated catabolism of LDL. Such studies in normal humans indicate that 20–50% of the total FCR for LDL is attributable to the LDL receptor (5, 8). In FH heterozygotes with about half the normal number of LDL receptors, the portion of the FCR for LDL attributable to the LDL receptor drops to about half of the normal value (5, 6), and in FH homozygotes essentially no LDL is cleared via the LDL receptor (8). Similar studies in normal rats, monkeys, and rabbits indicate that the LDL receptor mechanism accounts for ≈50% of the total FCR for LDL in these species (7, 9–11).

Validation of the above model for LDL catabolism *in vivo* requires the use of suitable animal models with a genetic defect in the LDL receptor. Indeed, such a model—the Watanabe heritable hyperlipidemic (WHHL) rabbit—has recently been described. Homozygous WHHL rabbits exhibit the following manifestations: spontaneous hyperlipidemia on a low fat diet; atherosclerosis; tendon xanthomas; a markedly decreased num-

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Abbreviations: apo, apoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate; FH, familial hypercholesterolemia; WHHL, Watanabe heritable hyperlipidemic; NZW, New Zealand White.

ber of LDL receptors in several tissues, including cultured skin fibroblasts, liver, and adrenal gland; and impaired LDL catabolism *in vivo* (12–17). Therefore, the WHHL rabbit manifests clinical, metabolic, and biochemical features that are strikingly similar to those observed in human FH.

To examine the metabolic behavior of LDL in this unique animal model, we compared the turnover of homologous LDL and methyl-LDL in both normal and homozygous WHHL rabbits. The results indicate that about two-thirds of the LDL catabolized in normal rabbits is cleared via the LDL receptor pathway, whereas essentially none is cleared by this mechanism in the WHHL rabbits. In addition, WHHL rabbits exhibit marked overproduction of LDL. We also noted that the receptor-independent FCR for LDL was similar in the normal and WHHL rabbits, indicating that this process (or processes) was not affected by the mutation in the gene for the LDL receptor.

METHODS

Rabbits. New Zealand White (NZW) rabbits (referred to as "normal rabbits") were purchased from Sunny Acres Rabbitry (Tyler, TX). Homozygous WHHL rabbits were raised in Dallas from a mating pair of homozygous WHHL rabbits obtained from Watanabe (12) and Japanese White rabbits were obtained from Chubu Kagaku Shizai (Takamatsu, Japan). All animals were fed Purina Lab Rabbit Chow and all were more than 4 months of age at the time of study.

Lipoproteins. Normal rabbit LDL was prepared from the plasma of fasted (7–9 days) rabbits by ultracentrifugation between salt densities of 1.030 and 1.050 g/ml (16). The LDL migrated as a single band on agarose gel electrophoresis and was shown to contain apoprotein (apo)-B and only trace quantities of apo-E on $NaDodSO_4$/polyacrylamide gel electrophoresis (18). LDL was isolated in a similar fashion from the plasma of WHHL rabbits and from the plasma of normal human donors. Iodination of LDL with either ^{125}I or ^{131}I was performed by the iodine monochloride method (19). Lipid labeling averaged 7.4% for ^{125}I-labeled LDL (^{125}I-LDL) and 7.5% for ^{131}I-labeled LDL (^{131}I-LDL) preparations. Reductive methylation of LDL was performed with formaldehyde plus sodium borohydride by using a 60-min reaction sequence as described by Weisgraber *et al.* (20). Typically, >90% of the free amino groups on LDL were modified by this reaction as judged by the trinitrobenzenesulfonic acid reaction (21). Preparations of radioiodinated methyl-LDL showed no LDL receptor binding activity when using the liver membrane binding assay (16).

Lipoprotein Turnover Studies. Animals were studied in groups of four (two normal and two WHHL) as designated by the letters in Tables 1 and 2. Each animal was anesthesized with xylazine and ketamine, and a catheter (Tygon Microbore no. S-54-HL) was surgically inserted and secured in the internal jugular vein and exteriorized at the base of the neck posteriorly where it was secured to the skin with a wire suture (22). The catheter was flushed daily with citrate/phosphate/dextrose anticoagulant solution to keep it patent. The animals were allowed to recover from surgery for 48–72 hr before they were studied.

Lipoprotein turnover studies were initiated by the simultaneous injection of radiolabeled LDL and radiolabeled methyl-LDL into the marginal ear vein of each rabbit. The amount of radioactivity injected per study ranged from 27 to 163 μCi for ^{125}I and from 19 to 78 μCi for ^{131}I (1 Ci = 3.7×10^{10} becquerels). Blood samples drawn 2–3 min later were used to calculate the plasma volume by the isotope dilution method. Serial 2-ml blood samples were then obtained for the next 96 hr. All morning samples were taken from animals fasted for 12 hr.

Measurement of Plasma Lipoproteins. Lipoprotein quantification was performed by standard techniques including ultracentrifugation at salt density = 1.019 g/ml and heparin/manganese precipitation (23). Precipitation of very low density lipoprotein (VLDL) and LDL by the heparin/manganese method was complete as judged by agarose gel electrophoresis of the supernatant which showed only a faint HDL band. The plasma apo-LDL pool was obtained by multiplying the plasma LDL-cholesterol concentration first by the plasma volume and then by the protein/cholesterol ratio for normal rabbit LDL (mean ± SD = 0.76 ± 0.06; range = 0.71–0.84). The mean protein/cholesterol ratio for normal rabbit LDL was nearly identical to that obtained when three WHHL-derived LDL preparations were assayed (0.76 ± 0.05). Apoprotein content was measured by a modification of the method of Lowry *et al.* by using bovine serum albumin as a standard (24). Plasma cholesterol and triglyceride assays were performed by using commercially available enzyme methods as described (3, 19).

Gel Filtration Chromatography of Lipoproteins. To determine whether or not LDL and methyl-LDL were metabolically converted to other lipoproteins during the lipoprotein turnover, plasma samples (0.75–2 ml) obtained from normal and WHHL rabbits at 0 time, 48 hr, and 96 hr were subjected to gel filtration by using Bio-Gel A-5m (Bio-Rad) packed in 1.5 × 90 cm glass columns (25). In each case >95% of the total plasma radioactivity eluted in a peak that corresponded with the elution position of authentic LDL and methyl-LDL, suggesting that virtually all the plasma radioactivity remained in LDL or methyl-LDL throughout the course of these studies.

Calculations. The kinetic parameters for LDL and methyl-LDL turnover were calculated by using methods originally described by Matthews (26), assuming a simple two-compartment model (3, 19, 27). Curve fitting was performed by using the SAAM-25 computer program (28) to derive the two exponential components of the 96-hr plasma die-away curves. These methods yielded results for the following metabolic parameters for LDL: the absolute rate of catabolism, the percent of the apo-LDL pool in the intravascular space, and the FCR (defined as the fraction of the intravascular pool of LDL catabolized per day). The absolute rate of catabolism for apo-LDL was calculated by using the following formula: ACR = (FCR) × (PV) × (apo-LDL concentration), in which ACR = absolute catabolic rate and PV = plasma volume. This absolute or total rate of catabolism was then corrected for body weight and is expressed as milligrams of apo-LDL or methyl-apo-LDL catabolized per kilogram of body weight per day.

Statistical analyses were performed by using the Kruskal–Wallis analysis of variance followed by the nonparametric multiple-comparisons procedure or by the paired-sample *t* test (29).

RESULTS

In confirmation of the data of Watanabe and co-workers (12, 13), we observed that the plasma lipid and lipoprotein levels in the WHHL rabbits differed markedly from those in the normal rabbits. The total plasma cholesterol was nearly 8-fold higher and the plasma triglyceride level was ≈3.9-fold higher in WHHL as compared with normal rabbits (Table 1). The cholesterol content of the VLDL + intermediate density lipoprotein (IDL) fraction (density <1.019) was 21 ± 6 mg/dl in the normal and 206 ± 35 mg/dl in the WHHL rabbits. The LDL-cholesterol (density = 1.019–1.063) was 17 ± 3 mg/dl in normal and 298 ± 26 mg/dl in WHHL rabbits (Table 1). High density lipoprotein (HDL)-cholesterol levels were lower in the WHHL rabbits as compared to the NZW strain (18 ± 4 and 28 ± 2 mg/dl, respectively); this difference approached but did not reach statistical significance ($P = 0.10$) (Table 1).

To determine the effects of the WHHL mutation on LDL metabolism *in vivo*, we measured the simultaneous turnover of radioiodinated LDL and radioiodinated methyl-LDL in both normal and WHHL rabbits (Table 2 and Fig. 1). To eliminate

Table 1. Plasma lipid and lipoprotein levels in normal and WHHL rabbits during LDL turnover studies

Rabbit	Weight, kg	Sex	Plasma cholesterol, mg/dl				Plasma triglyceride, mg/dl
			Total	VLDL + IDL*	LDL*	HDL	
Normal							
A-1	1.73	F	97	38	18	31	123
A-2	2.13	F	64	15	21	25	96
B-1	2.26	F	73	35	7	30	199
B-2	2.22	F	52	23	6	21	137
C-1	3.12	F	65	6	24	32	51
C-2	2.55	F	62	6	25	29	41
Mean ± SEM	2.34 ± 0.19		69 ± 6	21 ± 6	17 ± 3	28 ± 2	108 ± 24
WHHL†							
A-3	1.80	F	529	235	239	13	412
A-4	2.27	F	631	202	379	14	383
B-3	1.85	F	578	231	296	29	370
B-4	1.78	F	623	286	326	27	547
C-3	2.54	F	347	78	248	7	369
Mean ± SEM	2.05 ± 0.15		542 ± 52	206 ± 35	298 ± 26	18 ± 4	416 ± 34
P value‡	NS		0.005	<0.005	<0.005	0.100	0.005

Values for plasma lipids and lipoproteins in each animal are the mean of two measurements made during the turnover study.

* Lipoprotein quantification was performed after ultracentrifugation at density = 1.019; thus, the cholesterol content of the density <1.019-fraction is derived from both VLDL and IDL.

† The intravenous catheter in animal C-4 became inoperative before sufficient data for the turnover study were collected.

‡ *P* values refer to statistical comparisons between the normal and WHHL groups for each of the parameters listed; NS = not significant.

any systematic error induced by an isotope effect, we reversed the ^{131}I and ^{125}I on the native and methyl-LDL preparations in sequential studies. No apparent metabolic differences were observed between ^{125}I- and ^{131}I-labeled lipoproteins. After the intravenous injection of the native and methyl-LDL, the plasma die-away curve for each lipoprotein was curvilinear with the terminal linear exponential becoming apparent after 12 hr (Fig. 1). The mean FCR for native LDL (total FCR in Table 2) in the normal rabbits was 1.65 ± 0.09 pools per day but the FCR for methyl-LDL was only 0.54 ± 0.10 pools per day. These results confirm the previous observations by Mahley and coworkers that methylation retards the clearance of LDL from the body—presumably by blocking the interaction of the lipoprotein with the LDL receptor (11, 20). If one assumes that the FCR for native LDL reflects both receptor-dependent and receptor-independent clearance (total clearance), whereas the FCR for methyl-LDL reflects only receptor-independent clearance, then the difference between the two gives an estimate of receptor-mediated clearance. Thus, in the normal rabbit, receptor-mediated clearance of LDL was 1.11 ± 0.16 pools per day (Table 2), indicating that 67% of the FCR for LDL can be attributed to the LDL-receptor pathway, whereas 33% is accounted for by receptor-independent mechanisms.

As reported by Tanzawa *et al.* (13), the clearance of native

Table 2. Kinetic parameters for LDL and methyl-LDL turnover in normal and WHHL rabbits

Rabbit	Total plasma apo-LDL pool, mg	FCR,* pools per day			Rate of catabolism of apo-LDL, mg/kg/day		
		Total (a)	Receptor-independent (b)	Receptor-mediated (a − b)	Total (c)	Receptor-independent (d)	Receptor-mediated (c − d)
Normal							
A-1	15	1.54	0.27	1.27	8.7	1.5	7.2
A-2	18	2.00	0.55	1.45	13.1	3.6	9.5
B-1	6	1.80	0.44	1.36	3.5	0.8	2.7
B-2	5	1.44	0.95	0.49	3.2	2.1	1.1
C-1	18	1.40	0.64	0.76	6.0	2.7	3.3
C-2	19	1.69	0.37	1.32	8.6	1.9	6.7
Mean ± SEM	14 ± 3	1.65 ± 0.09	0.54 ± 0.10	1.11 ± 0.16	7.2 ± 1.5	2.1 ± 0.4	5.1 ± 1.3
WHHL†							
A-3	201	0.36	0.28	0.08	30.0	23.3	6.7
A-4	318	0.38	0.50	(−0.12)	44.1	58.1	(−14)
B-3	249	0.49	0.37	0.12	44.9	34.1	10.8
B-4	274	0.51	0.46	0.05	45.5	41.0	4.5
C-3	184	0.62	0.76	(−0.14)	38.6	47.3	(−8.7)
Mean ± SEM	245 ± 24	0.47 ± 0.05	0.47 ± 0.08	0.00 ± 0.05	40.6 ± 1.9	40.8 ± 5.9	(−0.14) ± 4.8
P values‡	0.005	0.005	NS	0.005	0.005	0.005	NS

* FCR of apo-LDL or methyl-apo-LDL from the intravascular space.

† The intravenous catheter in animal C-4 became inoperative before sufficient data for the turnover study were collected.

‡ *P* values refer to statistical comparisons between the normal and WHHL groups for each of the parameters listed; NS = not significant.

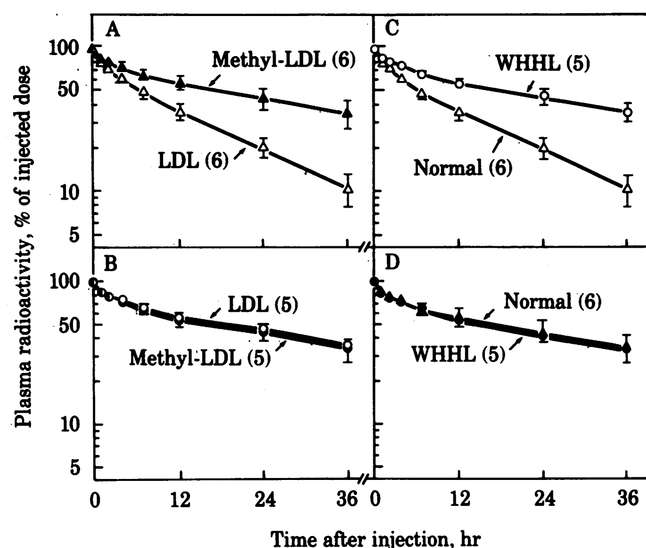

FIG. 1. Plasma die-away curves after the intravenous injection of radiolabeled LDL and methyl-LDL in normal (*A*) and WHHL (*B*) rabbits. The plasma die-away curves for native LDL in normal and WHHL rabbits are compared in *C*, and the plasma die-away curves for methyl-LDL are compared in these same two strains in *D*. Each point on a curve represents the mean ± 1 SD for studies in six normal and five WHHL rabbits (see Table 2); *n* values are given in parentheses. The SD bars were omitted when they were smaller than the symbols used for the data points.

LDL in the WHHL strain was markedly decreased; we observed a mean FCR of only 0.47 ± 0.05 pools per day (Table 2). The FCR for methyl-LDL (0.47 ± 0.08 pools per day) was identical to that for native LDL, indicating that there was no receptor-mediated clearance of LDL. The FCR for methyl-LDL in the WHHL rabbit was not statistically different from the FCR for methyl-LDL in the normal rabbit. Thus, despite a 17.5-fold difference in the plasma apo-LDL pool size between the WHHL and normal rabbits, the receptor-independent FCR in each was similar.

To rule out the unlikely possibility that the decreased FCR for LDL was also present in the Japanese White strain of rabbit in which the WHHL mutation arose, we performed LDL and methyl-LDL turnovers in two Japanese White rabbits. The FCRs for LDL (2.34 and 2.34 pools per day) and for methyl-LDL (0.37 and 0.53 pools per day) (experiment 1, Table 3) were within the range of values for these lipoproteins observed in the normal NZW rabbits (Table 2), indicating that the decreased FCR for LDL in the WHHL rabbit was due to the mutation and not to some underlying metabolic characteristic of the original Japanese White strain.

To test whether or not the LDL in the WHHL rabbit was capable of normal interaction with the LDL receptor *in vivo*, we compared the metabolic behavior of LDL isolated from WHHL rabbits with that of LDL isolated from normal rabbits. The FCRs for both WHHL-LDL and normal-LDL were identical in the two normal rabbits (1.55 and 1.21 pools per day in each study) (experiment 2, Table 3). The FCRs for WHHL-LDL and normal-LDL in the two WHHL rabbits (0.41 versus 0.53 and 0.43 versus 0.54 pools per day, respectively) were also quite similar (experiment 2, Table 3). In each case, WHHL-LDL was metabolically indistinguishable from normal-LDL and the FCR for WHHL-LDL was appropriate for the rabbit strain in which it was being tested.

The rates of apo-LDL catabolism in the normal rabbit were 7.2, 5.1, and 2.1 mg/kg per day via the total, receptor-dependent, and receptor-independent pathways, respectively (Table 2). In the WHHL rabbit, the rates of apo-LDL catabolism via these same pathways were 40.6, −0.14, and 40.8 mg/kg per day, respectively. These results indicate that the normal rabbit clears 71% of its apo-LDL via the LDL receptor and 29% via receptor-independent processes, whereas the WHHL rabbit clears essentially no apo-LDL via the LDL receptor, thereby leaving virtually all of the apo-LDL to be cleared by receptor-independent processes. Furthermore, the total flux of apo-LDL in the WHHL rabbit was much greater than normal. Thus, total catabolism and receptor-independent catabolism in the WHHL rabbit were 5.6-fold and 19-fold greater than normal, respectively. However, receptor-dependent clearance was greater in the normal than in the WHHL rabbit (Table 2). If one assumes that metabolic steady state conditions were approximated during these turnover studies, then apo-LDL synthesis was also increased 5.6-fold above normal in the WHHL rabbit.

When the intravascular–extravascular distribution of LDL was calculated, similar amounts of the lipoprotein were contained in the intravascular space in both the normal and WHHL rabbits (59.0 ± 1.9 and 61.1 ± 1.6%, respectively). A similar proportion of the methyl-LDL was also found within the intravascular space (60.4 ± 5.2 and 57.6 ± 1.9% in normal and WHHL rabbits, respectively).

The turnover of human LDL and normal rabbit LDL was compared in four normal rabbits (experiment 3, Table 3). The mean FCR for human LDL was 1.03 pools per day and the mean FCR for rabbit LDL was 1.61 pools per day. In every case, human LDL was cleared from the plasma at a slower rate than was rabbit LDL. However, when human methyl-LDL and rabbit methyl-LDL were simultaneously injected into each of two normal rabbits, they were cleared from the plasma at essentially identical rates (0.53 and 0.60 pools per day for human methyl-LDL and 0.53 and 0.55 pools per day for rabbit methyl-LDL) (experiment 4, Table 3). In these experiments, the receptor-dependent FCR for rabbit LDL was 1.07 pools per day (1.61–0.54). However, for human LDL, the receptor-dependent FCR was 0.47 pools per day (1.03–0.56). Thus, human LDL was cleared only 44% as well as was rabbit LDL by the LDL receptor in the rabbit.

Table 3. Comparative turnover studies in normal NZW, normal Japanese White, and WHHL rabbits

	Source of donor lipoprotein			FCR, pools per day	
	LDL	Methyl-LDL	Recipient rabbit	LDL	Methyl-LDL
Exp. 1	NZW	NZW	Japanese White	2.34	0.37
			Japanese White	2.34	0.53
Exp. 2*	NZW/WHHL		NZW	1.55/1.55	
			NZW	1.21/1.26	
			WHHL	0.53/0.41	
			WHHL	0.54/0.43	
Exp. 3*	Human/NZW		NZW	1.43/2.20	
			NZW	0.91/1.56	
			NZW	1.02/1.64	
			NZW	0.74/1.03	
Exp. 4*		Human/NZW	NZW		0.53/0.53
			NZW		0.60/0.55

* FCRs are listed in the same order as are the sources of donor LDL or donor methyl-LDL.

DISCUSSION

A major conclusion of this study is that ≈70% of the FCR in the normal rabbit is attributable to the LDL receptor and 30% is

cleared by one or more receptor-independent processes. This conclusion emerges from two separate pieces of evidence. (*i*) An estimate of the receptor-dependent contribution to the FCR based on methyl-LDL turnover in the normal rabbit is 67%. (*ii*) The total FCR in the WHHL rabbit—a mutant strain that is deficient in LDL receptors—is only 28% of the total FCR in normal rabbits, suggesting that ≈73% of the normal total FCR is attributable to LDL-receptor clearance.

The FCR attributable to receptor-independent clearance did not significantly differ between the normal and WHHL rabbit despite a 17.5-fold difference in the plasma apo-LDL pool size. This finding supports the use of methyl-LDL as a valid probe for estimating receptor-independent catabolism of LDL and further indicates that this catabolic process (or group of processes) is neither readily saturable nor influenced by the mutation affecting the LDL receptor.

Two lines of evidence indicate that the reduced FCR in the WHHL rabbit results from the LDL receptor defect that is demonstrable *in vitro*. (*i*) The FCR for normal LDL in the Japanese White strain from which the WHHL mutation arose was normal, indicating that the reduced FCR in the WHHL rabbit was not actually a metabolic characteristic of the Japanese White strain. (*ii*) The metabolic behavior of LDL isolated from WHHL rabbits was identical to that of LDL isolated from NZW rabbits, indicating that the reduced FCR in WHHL rabbits was not due to a structural defect in LDL that rendered it incapable of interaction with the LDL receptor.

We also noted that the *in vivo* metabolic behavior of human and rabbit LDL differed such that the mean total FCR for human LDL was only 64% of the mean total FCR for rabbit LDL. However, the *in vivo* metabolic behavior of human and rabbit methyl-LDL was essentially identical. Thus, if human LDL and methyl-LDL had been used in these studies, the magnitude of the total FCR and of the receptor-dependent FCR would have been significantly underestimated.

The reciprocal of the total FCR for LDL equals the mean residence time, an estimate of the total time interval spent by an LDL particle in the plasma compartment (30). Thus, the mean residence time for LDL in the normal rabbit is 0.61 days, whereas it is 2.13 days in the WHHL rabbit. In other words, because of the mutation affecting the LDL receptor, an LDL particle resides in the plasma compartment of a WHHL rabbit 3.5 times longer than it does in the plasma compartment of a normal rabbit.

Despite the low total FCR for apo-LDL in the WHHL rabbit, the total rate of apo-LDL catabolism in this strain standardized for body weight is increased 5.6-fold above normal. This sharp increase in the total rate of apo-LDL catabolism occurs at the expense of a 17.5-fold elevation in the plasma apo-LDL pool. Thus, the WHHL rabbit clears 19-fold more apo-LDL than normal via the receptor-independent process (or processes) but clears essentially none via the LDL receptor—a finding in keeping with the minimal LDL receptor activity found on cultured cells and isolated membranes from this mutant strain (15–17).

The abnormalities of LDL metabolism associated with deficient LDL receptor function in the homozygous WHHL rabbit are qualitatively identical to those observed in patients with homozygous FH. The mutation in both cases results in (*i*) a reduced FCR for apo-LDL, (*ii*) enhanced rates of apo-LDL synthesis and catabolism, and (*iii*) elevated plasma apo-LDL concentrations. In both cases, spontaneous atherosclerosis also results but the mechanistic relationship of the arterial damage to receptor-independent clearance of apo-LDL remains unknown. The answers to this and other important questions regarding the physiological role of the LDL receptor and the pathophysiological consequences of its absence should be more easily obtained from studies in this animal model for FH.

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