Receptor-mediated low density lipoprotein catabolism in man

James Shepherd, Susan Bicker, A. Ross Lorimer, and Christopher J. Packard

University Departments of Biochemistry and Medical Cardiology,[1] Royal Infirmary, Glasgow G4 OSF, United Kingdom

Abstract Binding of human low density lipoproteins (LDL) to their specific receptor on cultured cells can be inhibited by treatment with 1,2-cyclohexanedione which blocks a number of functionally significant arginyl residues on the apolipoprotein. We have used this observation to examine the role of the receptor pathway in LDL catabolism in man. The plasma clearance rates of ^{125}I-LDL and ^{131}I-cyclohexanedione-treated LDL were measured in four normal and four heterozygous familial hypercholesterolemic subjects. Chemical modification of the lipoprotein significantly reduced its fractional clearance rate and permitted calculation of receptor-mediated and receptor-independent catabolism in both groups. The normal subjects cleared 11% of their plasma LDL pool (corresponding to 3.0 mg/kg per day) by a receptor route and 22% (6.5 mg/kg per day) by a receptor-independent path. In the familial hypercholesterolemic subjects, 3% and 16% of the plasma apoLDL pool was cleared daily by these pathways, respectively. Because the mean apoLDL pool size in the group was increased 3-fold over normal, this gave absolute clearance rates for the apoprotein of 2.5 mg/kg per day via the receptors and 12.8 mg/kg per day by the nonreceptor pathway. We conclude that the specific LDL receptor mechanism operates in vivo and probably accounts for 33% and 16% of overall LDL catabolism in normal and heterozygous familial hypercholesterolemic subjects, respectively.—**Shepherd, J., S. Bicker, A. R. Lorimer, and C. J. Packard.** Receptor-mediated low density lipoprotein catabolism in man. *J. Lipid Res.* 1979. **20:** 999–1006.

Supplementary key words familial hypercholesterolemia 1,2-cyclohexanedione

Low density lipoprotein (LDL), the major transport vehicle for cholesterol in human plasma (1), has come under detailed scrutiny in recent years because of its putative involvement in the pathogenesis of atherosclerosis (2, 3). It has emerged that a number of cultured human cell species including fibroblasts (4), arterial smooth muscle cells (5, 6), lymphoid cells (7), and endothelial cells (8, 9) possess a common, specific LDL degradation pathway. This consists of an autoregulated, ordered sequence of events in which LDL is first bound to a high affinity receptor on the cell surface and is then internalized by endocytosis and delivered to lysosomes where the lipoprotein-associated cholesteryl esters are hydrolyzed, releasing free cholesterol for use by the cells (10).

Receptor recognition in the cells appears to be dependent (11) on a limited number of functionally significant arginyl residues on the LDL apoprotein (apoLDL). Although this process constitutes the main LDL degradation route in cell cultures exposed to low levels of the lipoprotein, introduction of physiological amounts of LDL to the medium results in suppression of receptor activity (7). Consequently, the importance of this pathway for LDL catabolism in vivo is not yet apparent. We have examined the problem by measuring the plasma clearance rate of LDL in man after chemical modification of the arginyl residues on apoLDL. The significant reduction in LDL clearance which we observed following this treatment indicates that the specific receptor binding mechanism has an important role in LDL degradation in vivo.

METHODS

Nine subjects whose clinical parameters are recorded in **Table 1,** gave informed consent to the study. Five were normolipemic and the remainder suffered from familial hypercholesterolemia (FH) according to the criteria of Goldstein and Brown (12). That is, each had a plasma cholesterol level greater than 350 mg/dl, demonstrated peripheral cholesterol deposition in the

Abbreviations: LDL, low density lipoprotein, $1.006 < d < 1.063$ kg/l; apoLDL, the apoprotein component of LDL; LDL_2, low density lipoprotein subfraction 2, $1.030 < d < 1.050$ kg/l; VLDL, very low density lipoprotein, $d < 1.006$ kg/l; HDL, high density lipoprotein, $1.063 < d < 1.21$ kg/l; FH, familial hypercholesterolemia; CHD, 1,2-cyclohexanedione; ^{131}I-CHD/LDL, low density lipoprotein labeled with ^{131}I-labeled CHD.

[1] Dr. Lorimer.

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TABLE 1. Clinical parameters of normal and heterozygous FH subjects

Subjects (Sex)	Age	Body Weight (n = 15)	Plasma Cholesterol (n = 6)	Plasma Triglyceride	VLDL	Cholesterol in LDL (n = 6)	HDL
	yr	*kg*	*mg/dl*	*mg/dl*		*mg/dl*	
Normal							
N1 (M)	21	56.4 ± 0.1	183 ± 15	106	22	104 ± 12	58
N2 (M)	22	70.0 ± 0.2	236 ± 16	190	35	146 ± 4	54
N3 (M)	24	62.8 ± 0.2	190 ± 14	63	16	108 ± 12	66
N4 (M)	22	55.0 ± 0.1	149 ± 8	62	7	89 ± 8	54
N5 (F)	23	52.7 ± 0.1	147 ± 3	93	13	64 ± 7	71
Heterozygous FH							
F1 (F)	46	55.9 ± 0.2	355 ± 15	128	23	276 ± 16	50
F2 (F)	38	57.7 ± 0.2	391 ± 22	168	31	321 ± 34	39
F3 (F)	28	63.1 ± 0.9	538 ± 25	124	27	445 ± 36	66
F4 (F)	50	49.4 ± 0.2	368 ± 17	186	35	277 ± 23	36

form of xanthelasmata or tendinous xanthomata, and had at least one first degree relative with the same condition. None of the volunteers exhibited clinical, biochemical, or hematologic evidence of other disease affecting the cardiovascular, renal, hepatic, or endocrine systems. They ate a normal diet and were investigated as outpatients, an arrangement which minimized perturbation of their lifestyle and which has been shown to provide steady-state conditions suitable for turnover studies (13). LDL metabolism was examined in eight of the subjects while, in the remaining normal volunteer (N5, Table 1), the kinetic parameters of high density lipoprotein (HDL) turnover were measured.

For 3 days before and throughout the investigation each subject received 120 mg of KI four times daily to prevent thyroidal sequestration of radioiodide. No other medications (including the contraceptive pill) were given. The subjects were weighed, while fasting, at 8:00 AM daily. Blood was also withdrawn at this time twice weekly for plasma cholesterol (14) and triglyceride (14), and three times a week for quantification of β-lipoproteins (14).

Preparation and chemical modification of labeled LDL

LDL_2 ($1.030 < d < 1.050$ kg/l) was isolated from the plasma of each subject by rate zonal ultracentrifugation (15), dialyzed against 0.15 M NaCl–0.01% Na_2-EDTA–0.01 M Tris, pH 7.0, and divided into two aliquots which were labeled separately (16) with ^{125}ICl or ^{131}ICl (The Radiochemical Centre, Amersham, England). The labeled lipoproteins were subsequently freed of unbound radioiodide and glycine buffer by gel filtration through a 1.5 × 70 cm column of G-10 Sephadex (Pharmacia, Uppsala, Sweden) using 0.15 M NaCl–0.01% Na_2EDTA (pH 7.0) in the elution process. Less than 5% of the lipoprotein-bound radioactivity was extractable with chloroform–methanol 2:1 (v:v).

The ^{131}I-labeled LDL_2 was treated with 1,2-cyclohexanedione (CHD) by a modification (11) of the method of Patthy and Smith (17) to block the charge on the arginyl residues of its protein moiety. Unreacted cyclohexanedione was removed by gel filtration through a 1.5 × 70 cm column of G-10 Sephadex using 0.15 M NaCl–0.01% disodium EDTA, pH 7.0, for elution. The ^{131}I-CHD/LDL was then exhaustively dialyzed against five changes of the same buffer at 4°C. Cyclohexanedione treatment increased substantially the anodic mobility of the lipoprotein (**Fig. 1**). Both normal and Type II LDL gave a homogeneous product with no residual material of native mobility. Treatment modified 55% of the arginyl residues in apoLDL as determined by amino acid analysis after acid hydrolysis in the presence of mercaptoacetic acid (17).

HDL labeling and modification

HDL ($1.063 < d < 1.21$ kg/l) was isolated by ultracentrifugal flotation (18) from the plasma of subject N5 (Table 1) and dialyzed against 0.15 M NaCl–0.01% disodium EDTA–0.01 M Tris, pH 7.0. Two aliquots of this material (each containing 5.0 mg of protein) were then labeled (19), respectively, with ^{125}I or ^{131}I and unbound radioiodide was removed by gel filtration (G-10 Sephadex) and subsequent dialysis at 4°C against 0.15 M NaCl–0.01% disodium EDTA, pH 7.0. The ^{131}I-HDL tracer was treated with cyclohexanedione (11, 17) followed by gel filtration and exhaustive dialysis as described above for LDL. Again, this treatment increased the anodic mobility of the labeled lipoprotein and modified 29% of its arginyl residues.

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Turnover study protocol

Fifteen microcuries each of autologous ^{125}I-LDL and ^{131}I-CHD/LDL (approximately 0.5 mg of protein) were mixed together and sterilized by membrane filtration (0.22-μm filters; Millipore Corp., Bedford, MA) prior to reinjection into the bloodstream of the donor. Each preparation was demonstrably free of bacteria and pyrogens. Following injection, blood samples were collected at 10 min and thereafter at daily intervals for 14 days. Twenty-four-hour urine collections were also made over this period. Sample handling and kinetic analysis by the mathematical procedures of Matthews (20) and Berson and Yalow (21) were performed as described elsewhere (13). The apoLDL concentration in the plasma of each subject was measured by the method of Langer, Strober, and Levy (22).

In one study (Subject N5, Table 1), the metabolism of autologous ^{125}I-HDL and ^{131}I-CHD/HDL was investigated as outlined above.

Stability of cyclohexanedione-modified LDL

Quantitation of receptor-mediated and receptor-independent LDL degradation required that the modified tracer be stable under physiological conditions. Two indirect assessments of its stability were made. In the first, the effects of incubation in vitro were determined using agarose gel electrophoresis. Trace amounts (approximately 2.0 μCi; less than 10 μg of LDL protein) of ^{125}I-LDL and ^{131}I-CHD/LDL were mixed with 2.0 ml of normal fasting plasma, sterilized by filtration, and incubated at 37°C for 19 hr. An aliquot was then subjected to agarose gel electrophoresis (Corning ACI, Palo Alto, CA) together with a control sample which had been maintained throughout at 4°C. The resulting eletrophoretograms were cut into 1.0-mm strips and counted

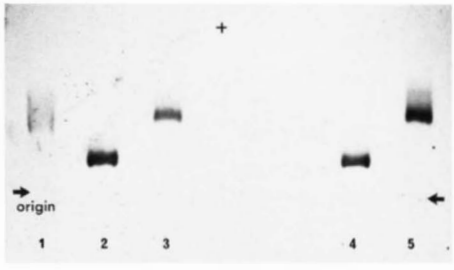

Fig. 1. Effect of cyclohexanedione on the electrophoretic mobility of normal and FH LDL in agarose. Samples of 1.0 μl were applied to preprepared agarose plates (Corning ACI, Palo Alto, CA) and electrophoresis was performed for 45 min. Lane 1, normal VLDL; lane 2, normal LDL; lane 3, CHD-treated normal LDL; lane 4, FH LDL; lane 5, CHD-treated FH LDL.

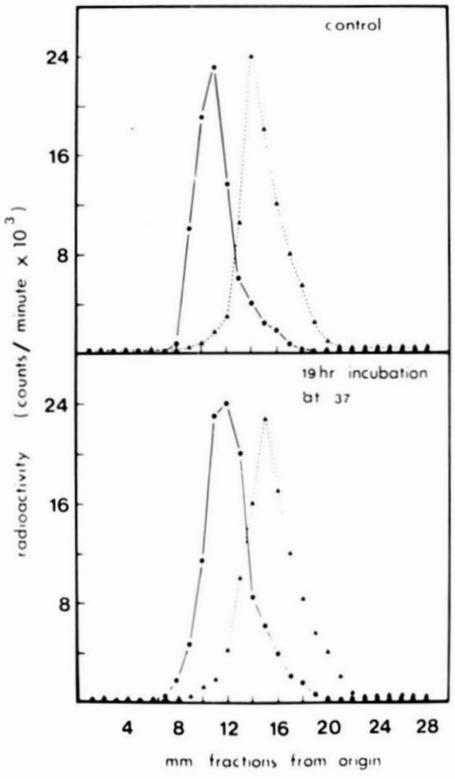

Fig. 2. In vitro stability of CHD-modified LDL. Trace amounts (approximately 2.0 μCi; 10 μg of protein) of ^{125}I-LDL and ^{131}I-CHD/LDL were mixed with 2.0 ml of fasting normal plasma, sterilized, and incubated for 19 hr at 4°C (control) and 37°C (test). Aliquots (1 μl) of each sample were then electrophoresed as described in Fig. 1. The electrophoretograms were cut into 1.0-mm strips and counted. —●— ^{125}I radioactivity; ···▲··· ^{131}I radioactivity.

(**Fig. 2**). Incubation did not alter the electrophoretic mobility of the CHD-treated LDL, suggesting that significant in vitro hydrolysis had not occurred.

A second stratagem was used to assess the in vivo stability of the modified LDL. We surmised that charge modification of the lipoprotein by CHD would interfere with its precipitation by heparin/Mn^{2+} (23) at suboptimal concentrations of Mn^{2+}. This was tested by adding trace amounts of ^{125}I-LDL and ^{131}I-CHD/LDL to normal fasting plasma (cholesterol 236 mg/dl; triglyceride 89 mg/dl) and treating 2.0-ml aliquots of the mixture with increasing amounts of Mn^{2+} at a final concentration of heparin of 1.29 mg/ml (Grade I, Sigma Chemical Co. Ltd, London, UK). The resulting precipitate was separated by centrifugation (23) and the supernatant was counted. At low Mn^{2+} concentrations, the CHD-modified LDL was consistently more resistant to precipitation than its untreated counterpart and optimal separation (whose magnitude varied inversely with the plasma cholesterol level) of the treated and untreated lipoprotein

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TABLE 2. Kinetic parameters of LDL turnover in normal and FH heterozygotes: effects of cyclohexanedione treatment on LDL metabolism

Subjects	Plasma Volume	Plasma apoLDL Concentration	%IV[a]		$T_{1/2}$	
			Control	CHD	Control	CHD
	ml	*mg/dl*			*days*	
N_1	2240	77	73	84	3.4	4.2
N_2	2810	92	78	72	3.6	4.5
N_3	2520	52	72	81	4.2	5.0
N_4	2190	65	63	69	4.3	5.1
Mean ± SD		72 ± 17	72 ± 6	77 ± 7	3.9 ± 0.4	4.7 ± 0.4
F_1	2260	173	77	75	6.0	7.1
F_2	2560	150	68	58	4.9	6.1
F_3	2490	295	79	72	6.3	7.9
F_4	1960	185	83	76	4.0	5.6
Mean ± SD		201 ± 64	77 ± 6	70 ± 8	5.3 ± 1.1	6.7 ± 1.0
Control *P* vs. FH		<0.02	NS	NS	NS	<0.01

[a] Percentage of labeled tracer in intravascular compartment.

was achieved at a final Mn^{2+} concentration of 0.033 M (**Fig. 3**). This concentration was used subsequently to measure the stability of the modified tracer in vivo. We argued that, if appreciable spontaneous regeneration of ^{131}I-LDL from ^{131}I-CHD/LDL did occur in vivo, the resistance of the CHD-treated lipoprotein to heparin/Mn^{2+} precipitation should diminish progressively with time. This did not occur. During an 11-day study on an FH heterozygote, the ^{131}I-CHD/LDL was consistently 25% (±2%; $n = 12$) more resistant to heparin/Mn^{2+} precipitation. We therefore concluded that the chemically modified LDL was stable under physiological conditions.

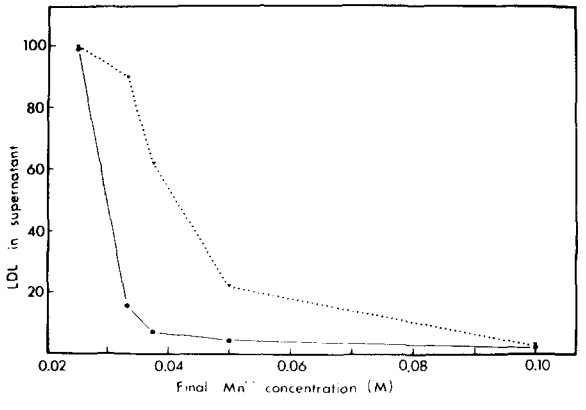

Fig. 3. Heparin/Mn^{2+} precipitation of ^{125}I-LDL and ^{131}I-CHD/LDL. Trace amounts of ^{125}I-LDL and ^{131}I-CHD/LDL were mixed with fasting normal plasma (cholesterol 235 mg/dl; triglyceride 89 mg/dl). Aliquots (2.0 ml) of the mixture were counted and then treated with increasing concentrations of Mn^{2+} at a final heparin level of 1.29 mg/ml (23). The resultant precipitate was separated by centrifugation and the supernatant ^{125}I and ^{131}I radioactivity was determined and expressed as a percentage of the original value. — ● — ^{125}I radioactivity; ···▼··· ^{131}I radioactivity.

RESULTS

The effects of cyclohexanedione modification on the normal and FH LDL used as tracers in this study were identical to those described by Mahley et al. (11) in respect to the percentage of arginine residues modified and the increase in electrophoretic mobility (Fig. 1). Moreover, after injection into the respective donor, both native and modified tracers retained the ultracentrifugal characteristics (15) of the subjects' endogenous LDL throughout the period of the study.

LDL metabolism in normal and FH subjects

It is now well established (13, 22, 24) that LDL accumulates in the plasma of FH patients largely as a result of defective plasma clearance. This phenomenon is evident in the present study (**Table 2**). When compared to the normal values, the plasma apoLDL levels in the FH group were increased 360% ($P < 0.02$) and the fractional clearance rate of the apoprotein reduced by 42% ($P < 0.02$). Moreover, its synthetic rate was significantly higher than normal in this group of unrelated FH subjects ($P < 0.05$), in agreement with our earlier findings on an FH family. This substantiates the contention that the abnormality in FH results in a combination of defective catabolism and oversynthesis of apoLDL.

Effects of cyclohexanedione modification on LDL metabolism in normal subjects

Treatment of LDL with cyclohexanedione reduced significantly (by 33%, $P < 0.05$) its rate of clearance from the plasma of the normal subjects (Table 2, **Fig. 4**). This occurred without change in the distribu-

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Fractional Clearance Rate				Absolute Catabolic Rate		
From Plasma Decay		U/P Ratio				
Control	CHD	Control	CHD	Native LDL	CHD-Treated LDL	Receptor-Mediated
pools/day				*mg/kg/day*		
0.353	0.212	0.39 ± 0.08	0.23 ± 0.03	10.9	6.5	4.4
0.300	0.245	0.26 ± 0.02	0.20 ± 0.01	11.0	9.0	2.0
0.323	0.196	0.37 ± 0.07	0.21 ± 0.05	6.7	4.1	2.6
0.357	0.237	0.35 ± 0.06	0.26 ± 0.03	9.3	6.2	3.1
0.333 ± 0.031	0.222 ± 0.020	0.34 ± 0.06	0.23 ± 0.03	9.5 ± 2.0	6.5 ± 2.0	3.0 ± 1.0
0.164	0.135	0.17 ± 0.02	0.12 ± 0.05	11.5	9.5	2.0
0.246	0.214	0.23 ± 0.04	0.20 ± 0.01	15.9	13.8	2.1
0.146	0.127	0.13 ± 0.01	0.11 ± 0.01	17.2	15.0	2.2
0.221	0.173	0.25 ± 0.03	0.16 ± 0.02	16.4	12.8	3.6
0.190 ± 0.040	0.162 ± 0.030	0.20 ± 0.06	0.15 ± 0.04	15.3 ± 2.5	12.8 ± 2.4	2.5 ± 0.8
<0.01	<0.02	<0.02	<0.02	<0.02	<0.05	NS

tion of the lipoprotein between intravascular and extravascular pools. On the assumption that cyclohexanedione-treated LDL measures receptor-independent catabolism, it was possible to calculate the fraction of the plasma LDL pool cleared each day via the receptor path. Knowledge of the LDL pool size then permitted quantitation of the absolute rate of catabolism achieved by each route. Table 2 shows that in the normal subjects 3.0 ± 1.0 and 6.5 ± 2.0 mg/kg per day of apoLDL were handled by the receptor and nonreceptor mechanisms, respectively.

In contrast, cyclohexanedione treatment of HDL did not change the metabolism of this fraction. Following injection of autologous ^{125}I-HDL and ^{131}I-CHD/HDL into the bloodstream of subject N5 (Table 1), the calculated fractional catabolic rates and half-lives of both preparations (FCR for ^{125}I-HDL = 0.172 pools/day; and for ^{131}I-CHD/HDL = 0.189 pools/day; $T_{1/2}$ for ^{125}I-HDL = 5.61 days; and for ^{131}I-CHD/HDL = 5.93 days) were within 9% and 5% of each other, respectively.

Effects of cyclohexanedione modification on LDL metabolism in FH subjects

Cyclohexanedione treatment of LDL consistently reduced its fractional clearance rate in the FH subjects (Table 2). However, the percentage reduction (16%) was significantly less than in the normals ($P < 0.05$). Again, this occurred without change in the compartmental distribution of the tracer. The group cleared a mean of 2.5 ± 0.8 and 12.8 ± 2.4 mg of apoLDL/kg per day by the receptor-dependent and independent pathways, respectively. Consequently, the increased turnover of apoLDL in the FH subjects was confined to the receptor-independent route. The flux through the receptor pathway was the same in both groups (Table 2).

DISCUSSION

The discovery of a specific, receptor-mediated catabolic pathway for LDL in cultured human cells (10) has advanced substantially our understanding of the processes that regulate LDL metabolism in man. Receptor recognition of the lipoprotein appears to be dependent on a limited number of functionally significant arginyl residues on its protein moiety (11). Chemical modification of 56% of these residues with

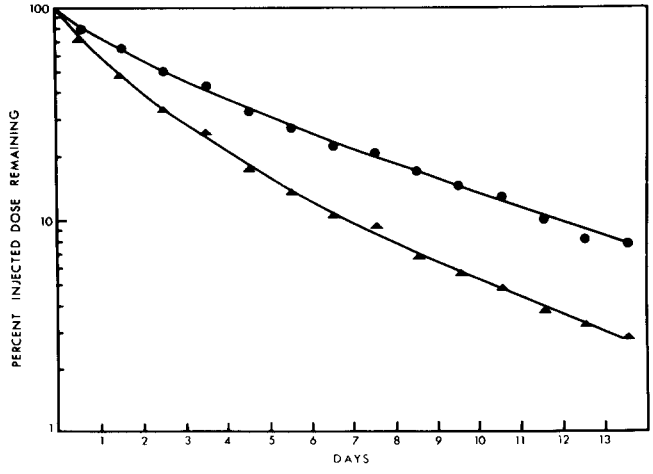

Fig. 4. Effect of cyclohexanedione treatment on plasma LDL clearance in subject N_1. ▲, ^{125}I-LDL decay curve; ●, ^{131}I-CHD/LDL decay curve.

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1,2-cyclohexanedione almost totally (>85%) abolished binding of the lipoprotein to the high-affinity receptor on human fibroblasts without affecting its other chemical or morphologic characteristics (11). We predicted from these results that, if the receptor mechanism operates in vivo, cyclohexanedione treatment of LDL should delay its clearance from the plasma to an extent that is dependent on the activity of the receptor pathway. By comparing the clearance rates of native and cyclohexanedione-treated LDL we hoped to determine the relative importance of this pathway, whose significance in vivo has not yet been established.

Our results show (Fig. 4, Table 2) that modification of the LDL arginyl residues to an extent that virtually eliminates receptor recognition in vitro (11) extends significantly the half-life of the lipoprotein in the plasma of both normal and heterozygous FH subjects. The observed prolongation of the half-life supports the view that we are measuring a physiological phenomenon, because nonspecific chemical damage to the lipoprotein would be expected to promote its clearance from, rather than prolong its stay in, the plasma (25). This is the first direct evidence that the interaction of LDL with a specific cell receptor is important for the metabolism of the lipoprotein in man.

Following their discovery of receptor-dependent LDL uptake into cultured cells, Goldstein and Brown (26) have made a quantitative prediction of the role of this pathway in regulating LDL catabolism in vivo. Central to their estimate is the finding that homozygous FH subjects, whose LDL receptor-mediated pathway is absent (10) continue to catabolize 0.15 of their circulating apoLDL pool per day (27). On the assumption that this pattern pertains in normal and heterozygous FH subjects also, Goldstein and Brown have predicted (26) from available kinetic data that 0.30 and 0.10 of the plasma apoLDL pool is cleared by the receptor route each day in normals and FH heterozygotes, respectively. Their prediction was based on the results of the study by Langer et al. (22) who measured LDL turnover in subjects consuming a diet low in cholesterol and with a high polyunsaturated/saturated fat ratio. Such a diet is known to increase the fractional catabolic rate of apoLDL (28, 29), presumably by stimulating receptor-dependent catabolism. Consequently, the predicted fractional LDL clearance rate via the receptor is high. Studies on subjects receiving a more normal diet (13, 30, 31) provide comparable total fractional clearance rates (±1 SD) of 0.34 ± 0.08 ($n = 22$) and 0.18 ± 0.04 ($n = 15$) in normals and FH heterozygotes, respectively. Assuming that 0.15 of the plasma apoLDL pool is cleared daily by the nonreceptor route, these data yield calculated fractional rates of receptor-mediated LDL catabolism of 0.19 and 0.03 of the plasma pool per day in the groups. A knowledge of their mean plasma apoLDL concentration (79 ±19 mg/dl in the normals; 211 ± 59 in the FH heterozygotes (13, 30, 31)) permits calculation of the absolute rate of apoLDL clearance via each pathway. These predicted values, culled from the literature, are compared in **Fig. 5** with our experimentally derived data.

In the FH group there is excellent agreement between the predicted and observed fractional and absolute apoLDL clearance rates for both receptor-dependent and receptor-independent pathways. The majority (84%) of apoLDL catabolism occurs by the latter route.

There was, however, a discrepancy between the predicted and measured rates in the normal subjects. Our estimate of fractional clearance of apoLDL via the receptor-independent path was higher than expected (0.22 vs. 0.15 of the plasma pool/day). Two technical problems may have contributed to this. *a*) Cyclohexanedione treatment may block incompletely

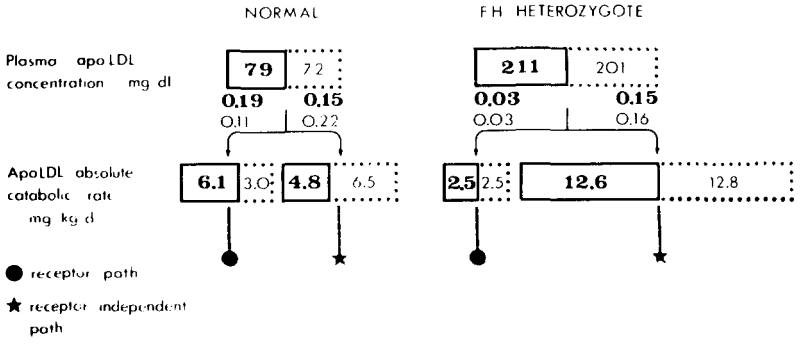

Fig. 5. Metabolic model for LDL catabolism. The experimentally observed data from the present study (▭) are compared with predicted mean values (▭) from the literature (13, 30, 31). Mean body weights used to calculate the latter were 67.8 kg ($n = 22$) for the normals and 62.7 kg ($n = 15$) for the FH heterozygotes.

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in vivo receptor recognition, particularly in normocholesterolemic subjects. We achieved a uniform change in the electrophoretic mobility of the tracer (Fig. 1) and the same degree of arginyl modification as reported by Mahley et al. (11). However, these authors found that, although such treatment inhibited more than 85% of receptor binding, it did not eliminate it. Consequently, some residual binding activity may account for the increment in nonreceptor metabolism over the predicted value of 0.15. *b*) Nonspecific in vivo hydrolysis of the ^{131}I-CHD/LDL may have occurred, resulting in an underestimate of receptor-dependent removal. Although we were unable to detect hydrolysis either in vitro or in vivo using the indirect methods described earlier, we cannot entirely preclude this possibility.

Despite the above reservations, two arguments support the proposed increased role of receptor-independent catabolism in normal subjects. First, according to our data (Fig. 5), both normal and FH subjects clear the same amount (cf the predicted values) of apoLDL by the receptor path. Goldstein and Brown (32, 33) suggest that this should be so, allowing a near normal amount of cholesterol to be delivered to the FH receptor-containing cells by this physiologically controllable route. Second, since the apoLDL pool size is apparently regulated by receptor activity, the ratio of receptor-mediated fractional catabolism in normal and FH subjects should approximate the reciprocal of their pool size ratio. In this study the experimentally observed fractional clearance ratio (0.27) rather than the predicted value (0.16) was closer to the ratio of the pool sizes (0.36) in the normal and FH groups.

Finally, the result of the turnover performed on subject N5 indicates that cyclohexanedione modification does not affect lipoprotein metabolism in a nonspecific way. Although 29% of the arginyl residues in the apoHDL had been blocked, its plasma half-life was unchanged.

This study shows for the first time that the LDL receptor, initially observed in cultured cells, has an important role in the in vivo regulation of LDL metabolism. Moreover, it confirms the general metabolic scheme proposed by Goldstein and Brown (26) but proposes different quantitative roles for the receptor-mediated and receptor-independent pathways in normal subjects.

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Informed consent was obtained from all subjects and the reported procedures were in accord with the ethical standards of the Human Studies Committee for the Royal Infirmary.

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