Diversity in Expression of Heterozygous Familial Hypercholesterolemia

Characterization of a Unique Kindred

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Abstract

Clinical and biochemical characteristics of familial hypercholesterolemia (FH) heterozygotes possessing an abnormally high molecular weight low density lipoprotein receptor (HMWR) are reported. The disorder is transmitted as an autosomal dominant trait and is not distinguishable from classic heterozygous FH on clinical grounds. The average plasma low density lipoprotein (LDL) level is 360 mg/dl and tendon xanthomata and early coronary disease are present.

LDL receptor activity is higher than expected. In skin fibroblast cultures two types of functional LDL receptors are present, one with a normal apparent native molecular weight of 140,000, and the other of 176,000. When immobilized on nitrocellulose paper both receptors bind LDL. Maximum ^{125}I-LDL binding capacity of fibroblast monolayers is reduced only 20%, compared with 50% in typical heterozygous FH. Affinity for ^{125}I-LDL is increased and a 38% reduction in the Michaelis constant for LDL is observed. When autologous ^{125}I-LDL was injected intravenously, the fractional catabolic rate of LDL was 205% and the LDL apoprotein B production rate was 328% of that found in a typical heterozygous FH subject. Thus, both in vitro and in vivo testing indicated only a modest deficiency of LDL receptor activity.

Kindred members possessing the HMWR had an associated abnormality of cholesterol biosynthesis. Cholesterol balance studies in three individuals with the HMWR trait demonstrated elevated cholesterol biosynthesis of two to three times the mean of normal subjects.

These findings suggest that increased LDL production and increased cholesterol production may assume a significant role in the pathologic manifestations of heterozygous FH. Functional abnormalities in LDL receptor activity as measured in fibroblast culture may be relatively small.

Introduction

Research into the molecular basis of familial hypercholesterolemia (FH)[1] has revealed considerable heterogeneity in low density lipoprotein (LDL) receptor abnormalities. Mutations that alter receptor molecular weight and affinity for LDL and lower the capacity for LDL binding have been described in skin fibroblasts derived from FH homozygotes (1–4). The pathophysiologic significance of these differences is not known but may be important. For example, FH homozygotes whose skin fibroblasts possess residual LDL receptor activity appear to have an improved life expectancy when compared with clinically identical subjects in whom LDL receptor activity is not readily detectable by standard assay (1).

The skin fibroblasts of FH heterozygotes have also been shown to be heterogeneous (3–5). Immunoprecipitation and gel electrophoresis have revealed that cells from some of these patients express both a normal LDL receptor and an abnormal receptor, which may differ in molecular weight or fail to be properly processed to a mature form. However, little is known of the specific effects of these mutations on cholesterol and lipoprotein physiology, and it is possible that very diverse pathophysiologic effects might result. In this paper we characterize FH heterozygotes from a kindred with classic clinical FH in which an LDL receptor of increased molecular weight is present in addition to the normal receptor. This mutation appears to be associated with increased cholesterol biosynthesis and a relatively mild reduction in functional LDL receptor activity.

Methods

Subjects. This kindred (Fig. 1) has been the object of several previous reports (2, 4, 6–9). Plasma lipids and lipoproteins were determined by the Lipid Research Clinics method (10) and heterozygotes were diagnosed from LDL cholesterol levels which exceeded the age- and sex-specific 95th percentile values (11). LDL receptor-negative homozygote skin fibroblasts (lines GM1915 and GM2000) were purchased from the American Type Culture Collection, Rockville, MD. The cells of hypercholesterolemic subject 9 have been classified as heterozygous FH with one normal allele and one allele determining failure of maturation of the LDL receptor precursor (4).

In vitro assays. Deltoid area skin fibroblasts were explanted and cultured in Eagle's minimum essential medium containing 15% fetal bovine serum or newborn calf serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. Cells were assayed for ^{125}I-LDL surface binding, internalization, and degradation as previously described (6, 12). Cells were plated at 10^5/35-mm well in six-well plastic culture dishes. After 3–5 d the medium was removed, the cells were washed twice with Puck's saline G and 1 ml Eagle's minimal essential medium containing 10% lipoprotein-deficient human serum (LPDS) was added. After 3 d of LDL receptor induction the medium was aspirated and replaced with 0.7 ml of the same medium containing ^{125}I-LDL (2.5–100 μg/ml, 100 cpm/ng) with or without a 15-fold or greater excess of unlabeled LDL. The cells were incubated for 5 h at 37° and specific LDL receptor activity was calculated by subtracting values obtained in the presence of unlabeled LDL from those obtained in its absence. In certain experiments the cells were cooled to 4° for 40 min before assay for LDL binding. All experiments were performed in triplicate wells and the means±SEM are presented. The Michaelis constant (K_m) and maximum binding capacity were calculated from Scatchard-like plots as described previously (2, 5).

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1. *Abbreviations used in this paper:* apo, apoprotein; FH, familial hypercholesterolemia; HMWR, high molecular weight receptor mutation; LPDS, lipoprotein-deficient human serum.

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[^3H]*Oleate incorporation into cholesteryl esters.* This procedure was performed as described (2). Fibroblasts in logarithmic growth were treated for 24 h with 10% LPDS and then received either 200 μg/ml LDL protein or 5 μg/ml 25-hydroxycholesterol and were incubated at 37° for 7 h. During the last 3.5 h, 0.1 mM bovine albumin-[^{3}H]oleate complex was added to the medium. The cells were washed, scraped from the dish, and the cholesteryl esters were isolated by thin-layer chromatography and counted.

Cholesterol balance. The heterozygotes were studied as outpatients. All food was prepared by the metabolic kitchen of the Washington University Clinical Research Center. Homozygous children were studied in the hospital. The subjects were not taking drugs and consumed an isocaloric, low cholesterol, high saturated fat diet of mixed solid food and formula divided into three approximately equal feedings per day (8, 13). The polyunsaturated/saturated fat ratio was 0.2. Stools were collected throughout the 3-wk study and pooled into aliquots of several days for analysis. Fecal neutral and acidic steroids were isolated and their masses determined by quantitative gas-liquid chromatography (14, 15). β-sitosterol, 225 mg twice daily, was given to correct for possible intestinal losses of neutral steroids (16). Excretion of bile acids was corrected for variation in fecal flow by use of chromic oxide, 180 mg twice daily (17). Cholesterol balance was calculated by subtracting the small amount of dietary cholesterol intake from total fecal steroids.

LDL kinetics. Studies were performed on an outpatient basis while the subjects were not taking drugs and were on an isocaloric diet of 40% fat (polyunsaturated/saturated ratio 0.2), 45% carbohydrate, 15% protein, and 4.6 mg/kg cholesterol per d. The diet began 7 d before injection. 5 d before injection 300 ml plasma was obtained by pheresis. The subjects began taking saturated solution of KI 150 mg three times daily, which was continued throughout the study in order to block thyroid uptake of radiolabeled iodide. Using sterile technique, we prepared autologous LDL (density 1.019–1.063) by ultracentrifugation and washed it once at each density limit. The lipoproteins were iodinated with ^{125}ICl (18), passed through 0.22-μm filters, and tested for pyrogenicity with the limulus amebacyte lysate clot test (Pyrogent; Mallinckrodt, Inc., Science Products Div., St. Louis, MO). All materials contacting the lipoprotein were depyrogenated by heating at 240° for 45 min, by extensive washing in pyrogen-free water, or by passing through 10,000-D molecular filters. Each subject received 7.5–40 μCi ^{125}I-LDL of specific activity, 6–21 cpm/ng. Fasting blood samples were collected for 13 d. Each was immediately centrifuged at 2,500 rpm and the plasma separated. LDL was isolated by ultracentrifugation and apoprotein (apo) B prepared as described for counting and protein determination (19). A compartmental model was used to calculate LDL fractional catabolic rate (20).

Western blotting. Cells were grown as described for LDL binding studies in 10% lipoprotein-deficient human serum and then harvested and the LDL receptor extracted as previously described (21, 22). Briefly, cells were homogenized 100 strokes at 4° with 1.6% Triton X-100, 0.3 mM leupeptin, 5.0 M urea, and 1.5 mM phenylmethylsulfonyl fluoride. After extraction for 30 min the receptor-containing supernatant was separated by ultracentrifugation and the extract made 0.5% in sodium dodecyl sulfate (SDS), 10% in glycerol, and 0.1% in bromphenol blue. Polyacrylamide gel electrophoresis was done in 6% gels by the method of Laemmli (23) and the gel proteins were transferred to nitrocellulose paper at 4° in 20 mM tris-glycine, pH 8.6. The blot was blocked overnight with buffer containing 50 mg/ml albumin and then incubated with 20 μg/ml LDL for 1 h at room temperature. It was thoroughly washed and then incubated with 3.0 μg/ml radioiodinated affinity-purified anti-LDL IgG for 30 min at room temperature. After extensive washing the blot was dried and subjected to autoradiography. Control blots using nonimmune goat IgG or calcium-free binding buffer failed to result in visualization of LDL receptors.

Results

Clinical characteristics. Fig. 1 shows the pedigree of the subjects who have inherited FH with the high molecular weight receptor (HMWR) gene. The gene originated with subject 4 and the inheritance is consistent with an autosomal dominant trait. Subjects 14 and 15 are phenotypic FH homozygotes, having received a gene for the HMWR from their father and a gene for typical FH from their mother. Table I gives plasma lipoprotein concentrations in family members separated into hypercholesterolemic and normal groups using age- and sex-specific normal

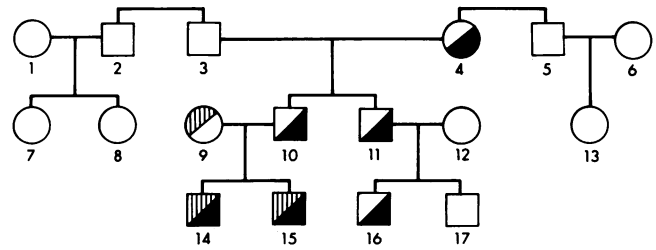

Figure 1. Pedigree of HMWR patients. Solid symbols indicate FH patients with the HMWR trait. Crosshatched symbols depict a gene for typical FH. Squares are males and circles are females. Plasma lipoproteins levels are found in Table I.

Table I. Plasma Lipoproteins

Subject	Age	Cholesterol LDL	Cholesterol HDL	Cholesterol VLDL	Triglycerides
	yr	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
Normocholesterolemic					
2	55	150	35	65	288
3	63	166	49	12	156
5	65	115	35	12	146
8	28	126	68	3	40
12	39	119	57	2	53
17	16	102	31	8	99
Mean±SEM		129±10	44±5	16±10	130±37
HMWR FH heterozygotes					
4	59*	496	28	21	192
10	33‡	362	35	20	128
11	28	279	37	11	76
16	14	303	44	7	69
Mean±SEM		360±49	36±3	15±3.4	116±29
Typical FH heterozygote					
9	33	258	65	4	59
Phenotypic FH homozygote with HMWR gene					
14	5§	940	25	5	115
15	2‖	913	22	5	63

Abbreviations used in this table: HDL, high density lipoprotein; VLDL, very low density lipoprotein.
Plasma lipoprotein levels are given for fasting specimens collected at least 6 wk after cessation of medication.
* Angina at age 39, myocardial infarction and death at age 59, and tendon xanthomata.
‡ Tendon xanthomata.
§ Tendon and cutaneous xanthomata.
‖ Cutaneous xanthomata.

values (11). FH heterozygotes possessing the HMWR FH gene were severely affected clinically. LDL cholesterol was 360 mg/dl, 2.8 times as much as in unaffected kindred members, and tendon xanthomata and early death from coronary heart disease were present. Fig. 2 shows Western blots of fibroblast extracts from kindred normals and kindred heterozygotes. In this technique LDL receptors are separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper where they are detected by binding of LDL to the receptor and subsequent exposure of the LDL-receptor complex to affinity-purified radioiodinated anti-LDL IgG. The LDL receptor of normal individuals has an apparent molecular weight of 140,000 (Fig. 2, lane *1*), similar to a reported estimate of 130,000 (21). This molecular weight is lower than the value of 160,000 obtained when LDL receptors were reduced before electrophoresis (21). Reduction destroys LDL binding capacity and increases the apparent molecular weight of the receptor. Each of the three heterozygous family members descended from subject 4 (Fig. 2, lanes *2–4*) has two LDL receptors, one of normal apparent molecular weight 140,000 and one of 176,000. Both receptors bind LDL about equally well on the blot. In contrast, normal family members (lanes *5–7*) and an unrelated normal (lane *1*) have only a 140,000-mol-wt receptor. Thus, the diagnosis of FH is established by both clinical and molecular criteria in the HMWR heterozygotes.

LDL binding to skin fibroblasts. Quantitative LDL binding parameters were determined from ^{125}I-LDL dose-response curves in skin fibroblasts from three HMWR heterozygotes and three normal members of the kindred using Scatchard plotting and linear regression on the data points (Table II, Fig. 3). To eliminate interassay variation, fibroblasts of similar passage number from family members and controls were thawed from liquid nitrogen, passaged, and analyzed together in triplicate at the same time.

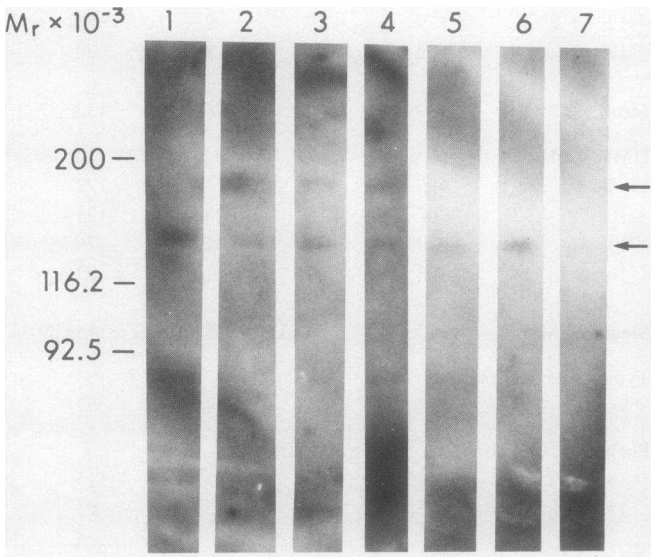

Figure 2. Ligand blotting of LDL receptors. Fibroblast extracts were prepared as described in Methods, electrophoresed in SDS-polyacrylamide gels, and transferred to nitrocellulose paper. The blots were then reacted with LDL followed by radioiodinated affinity-purified anti-LDL IgG, washed, and subjected to autoradiography. The lanes contained 25–50 μg protein. *Lane 1,* unrelated normal; *lanes 2–4,* HMWR FH heterozygotes 10, 11, 16; *lanes 5–7,* family normocholesterolemic subjects 3, 12, 17.

Table II. ^{125}I-LDL Binding Kinetics in Skin Fibroblasts

Subject	K_M	Maximum binding capacity
	μg/ml	*ng/mg*
Family normocholesterolemic		
3	26.3	1447
12	27.0	1335
17	26.5	1517
Mean±SEM	26.6±0.2	1433±53.0
HMWR heterozygotes		
10	13.9	1237
11	16.4	1161
16	19.3	1010
Mean±SEM	16.5±1.6*	1136±66.7‡
Typical FH heterozygote		
9	25.1	897

Fibroblasts were prepared and assayed as described in the legend to Fig. 3. Parameters of binding were computed from Scatchard-like plots for which correlation coefficients were >0.94.
* $P < 0.005$ compared with normocholesterolemic subjects.
‡ $P < 0.05$ compared with normocholesterolemic subjects.

Fig. 3 shows a Scatchard plot of the mean values obtained for the normals and HMWR heterozygotes. Only a slight decrease in maximum binding capacity for LDL (x intercept) was observed and an increase in affinity for LDL (increasing negative slope of the line) was seen in the HMWR heterozygote cells. Table II shows that the HMWR heterozygotes demonstrated a 38±6.0% reduction in K_m for LDL and a 21±4.7% decrease in ^{125}I-LDL binding capacity.

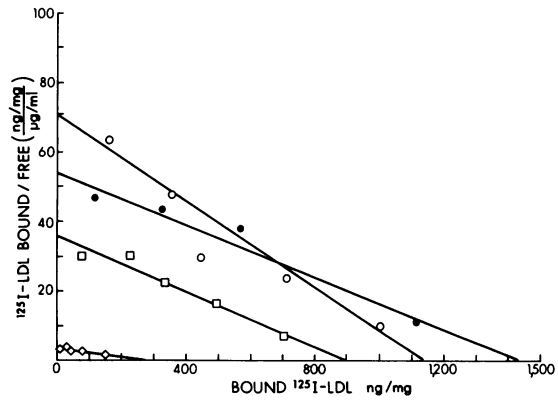

Figure 3. Scatchard plot of ^{125}I-LDL binding by skin fibroblasts. Cells were thawed from liquid nitrogen, passaged, and assayed together as described in Methods. After incubation for 72 h in Eagle's minimum essential medium plus 10% LPDS, medium containing ^{125}I-LDL (2.5, 7.5, 15, 30, and 100 μg/ml) plus or minus a 15-fold or greater excess of unlabeled LDL was added to triplicate wells and the cells were incubated at 37° for 5 h. The cells were washed and dissolved in 0.625 N NaOH for determination of the specific cell-associated ^{125}I-LDL. ●, family normocholesterolemic cells (Table I); ○, HMWR FH heterozygotes (see Table I); □, typical FH heterozygote 9; ◇, unrelated LDL receptor-negative homozygote GM1915. Lines were drawn through the data points by the method of least squares.

To be sure that these changes were consistent over time, LDL binding characteristics of skin fibroblasts from HMWR heterozygote 10 were compared with typical heterozygote 9 and with normal subjects in seven experiments conducted in triplicate over 2 yr. Receptor-specific surface binding, internalization, and degradation of ^{125}I-LDL were determined at saturating concentrations of LDL (60–200 μg/ml). The typical heterozygote 9 expressed 51±2% of normal LDL receptor activity, a value expected for a genotype consisting of one normal LDL receptor and one physiologically silent LDL receptor. In contrast, the HMWR heterozygote 10 expressed 81±3% of normal activity ($P < 0.001$ with respect to the typical heterozygote.)

Since LDL stimulates the esterification of cholesterol in fibroblasts that possess LDL receptors, the incorporation of [^{3}H]oleate into cholesteryl esters can be used as an indirect measure of LDL receptor activity (1). Table III (column *B*) shows that all cell types demonstrated similar amounts of cholesterol esterification in response to 25-hydroxycholesterol, the action of which does not require the presence of LDL receptors (1). However, considerable differences were seen when LDL was used to stimulate cholesterol esterification (column *A*) and these are most apparent when reading column *C*, in which the ratio of esterification observed in response to LDL and 25-hydroxycholesterol is presented. Cells from an unrelated LDL receptor-negative homozygote (GM2000) had a ratio that was 3.5% of normal, indicating severe impairment of the LDL receptor pathway. The typical FH heterozygote demonstrated a ratio that was 27% of normal, in the range previously reported for heterozygotes (1, 24). But the HMWR heterozygotes had esterification ratios that were 90% of normal. Thus, the HMWR heterozygotes can be distinguished from classic FH heterozygotes by this technique, since they exhibit 2.7–3.6 times as much cholesterol esterifying activity in response to LDL. It should be remembered that the esterification assay is conducted under non-steady state conditions and is not linear with respect to receptor activity, tending to give low values to cells with deficient LDL receptors (1).

Table III. Stimulation of Cholesterol Esterification in Skin Fibroblasts

Subject	*A* LDL added	*B* 25-OH-cholesterol added	*C* Ratio *A/B*
	pM/mg protein per h		
Family normocholesterolemic			
3	481±19	2254±205	0.213
12	729±50	2073±861	0.352
17	697±23	1759±60	0.396
Mean±SEM	636±78	2029±145	0.313
HMWR heterozygotes			
10	714±67	2276±53	0.314
11	406±55	1726±101	0.235
16	694±29	2186±63	0.317
Mean±SEM	604±100	2062±170	0.293
Typical FH heterozygote			
9	206±1	2367±139	0.087
LDL receptor negative homozygote (GM2000)			
12	20±6	1789±36	0.011

Fibroblasts were incubated with either LDL (200 μg/ml) or 25-hydroxycholesterol (5 μg/ml) and then with [^{3}H]oleate as described in Methods. The radioactivity incorporated into cholesterol esters is presented ±SEM for triplicate determinations.

Plasma LDL kinetics. It was possible to perform further clinical studies in subjects 9 and 10, a typical FH heterozygote and a HMWR heterozygote, respectively. LDL turnover was determined by injection of autologous ^{125}I-LDL and the measurement of radioactivity decay in plasma over the ensuing 14 d. The subjects consumed a diet containing moderate amounts of cholesterol and a polyunsaturated/saturated fatty acid ratio of 0.2 (Fig. 4). A distinct difference is seen between the HMWR heterozygote and the typical heterozygote. The HMWR heterozygote had a fractional catabolic rate of 0.375 pools/d while in the typical FH heterozygote the value was only 0.183 pools/d. The absolute LDL apo B production rate in the HMWR heterozygote was 36.1 mg/kg per d, whereas the classic FH heterozygote produced only 11.0 mg/kg per d. These calculations assume kinetic homogeneity of the LDL tracers used.

Cholesterol biosynthesis. Cholesterol synthesis was determined by cholesterol balance studies performed with the chromatographic technique described in Methods (Table IV). The patients consumed a diet low in cholesterol and polyunsaturated/saturated fat ratio and remained on the diet for at least 20 d. Stool specimens were analyzed from pools of several days' duration after equilibration of stool markers was complete. HMWR heterozygote 10 had cholesterol biosynthesis of 19.7 mg/kg per d, a value 4.4 SD above the mean of normal subjects. This is the highest rate of synthesis observed in an FH heterozygote and is 3.5 SD above the mean of six heterozygous FH patients. The increased sterol excretion was seen in both neutral steroids and acidic steroids. The children of this HMWR heterozygote have the clinical phenotype of homozygous hypercholesterolemia but are genetic compounds (Fig. 1). Cholesterol biosynthesis in both these children, who express only the high molecular weight receptor, was 22 mg/kg per d, whereas most FH homozygotes have normal rates of cholesterol biosynthesis (27). The mother

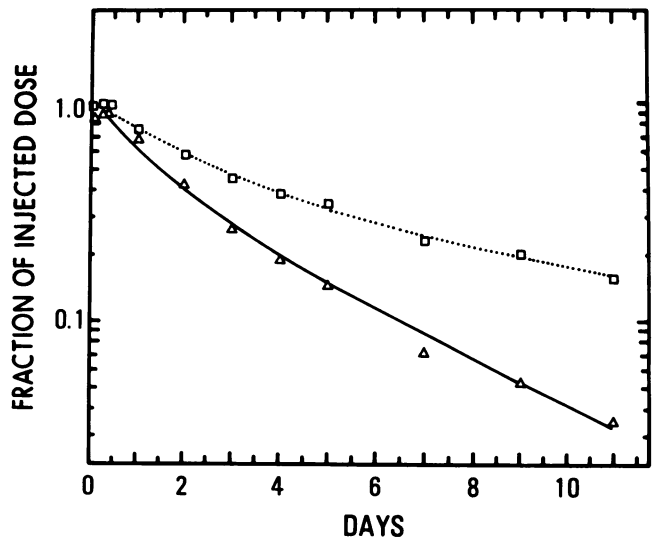

Figure 4. ^{125}I-LDL turnover. The specific activity of apo B was determined after intravenous injection of autologous ^{125}I-LDL in HMWR heterozygote 10 (△) and classic FH heterozygote 9 (□). The fraction of the injected dose present in plasma is plotted as a function of time.

Table IV. Cholesterol Balance Data

Subject	Weight	Dietary cholesterol	Fecal neutral steroids	Fecal acidic steroids	Total fecal steroids	Cholesterol balance (biosynthesis)
	kg		*mg/kg per d*	*mg/kg per d*	*mg/kg per d*	*mg/kg per d*
HMWR heterozygote 10	89.3	1.6	11.8	9.6	21.3	19.7*
Typical heterozygote 9	48.8	1.7	6.6	7.8	14.3	12.6
Phenotypic homozygote 14	45.3	1.4	8.5	14.6	23.1	21.7*
Phenotypic homozygote 15	19.3	3.2	13.6	12.0	25.6	22.4*
Normals (n = 4)		1.8±0.1	6.5±0.5	4.9±0.6	11.4±0.7	9.6±0.6
FH heterozygotes‡ (n = 6)		1.0±0.1	8.4±0.7	2.8±1.2	11.2±1.2	10.2±1.1
FH homozygotes§ (n = 14)						12.8±1.2

Data represent the mean of 2–5 specimens each pooled from 3–7 collection d. Data from patient series are ±SEM. * $P < 0.05$ with respect to normals, computed using tolerance factors with 95% population coverage (25). ‡ Subjects 1–6 from reference 26. § Subjects tabulated in reference 27.

of these children, typical FH heterozygote 9, had a normal cholesterol biosynthetic rate, 12.6 mg/kg per d.

Discussion

HMWR FH heterozygotes are not distinguishable from typical FH patients by means of clinical criteria. The mean LDL cholesterol is 360 mg/dl and tendon xanthomata and early coronary disease are present (Table I). Mating of one HMWR heterozygote with a typical FH heterozygote has produced two children with LDL cholesterol levels over 900 mg/dl and with cutaneous xanthomata in early childhood, findings typical of homozygous FH (Fig. 1, Table I).

The LDL receptor is not absent in the kindred, but is rather abnormal. We found previously that the two phenotypic homozygotes (subjects 14 and 15, who express only the high molecular weight receptor) have a single dose of a disorder which produces LDL receptors with reduced capacity but increased affinity for LDL (2). Then Tolleshaug et al., using an anti-LDL receptor antibody, demonstrated that subject 14 (designated FH homozygote 295 in reference 4) had inherited an LDL receptor of increased molecular weight from his father (4), our subject 10. The present work confirms that formulation and demonstrates that other hypercholesterolemic family members also possess the high molecular weight receptor. HMWR heterozygotes express two LDL receptors, a normal receptor with apparent molecular weight of 140,000 and a receptor with an increased apparent molecular weight of 176,000 (Fig. 2). In our blotting method LDL is bound to the receptor and detected by radioiodinated anti-LDL antibody rather than an anti-receptor antibody. Both normal and abnormal receptors are capable of binding LDL on the blot in vitro. LDL receptors are not reduced during electrophoresis and, consequently, the apparent molecular weights are less than those observed previously in reduced SDS gels (160,000 and 210,000, for normal and mutant receptors, respectively, [21, 4]).

Characterization of LDL binding in intact skin fibroblasts from three HMWR heterozygotes and three normocholesterolemic family members has established that an LDL receptor abnormality is present in the heterozygote state, but it is mild. While one might have expected that the LDL binding capacity would be 50% of normal, a reduction of only 20% was observed (Table II). A typical FH heterozygote was studied simultaneously and was found to have 50% of the expected maximum binding capacity. Nonhypercholesterolemic controls both from within the family (Table II) and unrelated to the family were used. An indirect indication of LDL receptor function is found in the capacity of endocytosed LDL to stimulate cholesterol esterification. Table III shows that LDL-induced cholesterol esterification was normal in cells of the HMWR heterozygotes, but reduced to 28% of normal in classic heterozygous FH. Fibroblasts from HMWR heterozygotes had a 38% reduction in K_m for LDL (thus showing increased affinity for LDL, Table II). These findings strongly suggest that the high molecular weight LDL receptor is expressed in cultured HMWR skin fibroblasts.

Consistent with this is the study of in vivo LDL apo B turnover in a HMWR heterozygote (Fig. 4). The fractional catabolic rate for LDL is 2.0 times that of a typical FH heterozygote. Assuming kinetic homogeneity of the LDL tracer, one can estimate that the LDL apo B production rate is 3.3 times that of the typical FH heterozygote. Taken together with the skin fibroblast data, this evidence suggests that LDL receptor function is only mildly reduced in HMWR heterozygotes.

Perhaps equally as important as the alteration of LDL receptor activity in this kindred is an associated increase of cholesterol biosynthesis. Usually cholesterol synthesis is normal in FH patients (28, 27, 8). However, three subjects in this kindred who have inherited the high molecular weight receptor have a cholesterol biosynthetic rate that is approximately twice that of normal (Table IV). Patient 10, a HMWR heterozygote, has a cholesterol biosynthetic rate of 19.7 mg/kg per d, significantly higher than the normal mean of 9.6 ($P < 0.05$). To our knowledge, increased cholesterol synthesis has not been reported previously in FH heterozygotes. Patients 14 and 15 are genetic compounds (phenotypic homozygotes) who have inherited both an HMWR gene and a typical FH gene (Fig. 1). Cholesterol synthetic rates are elevated at 21.7 and 22.4 mg/kg per d, respectively, in these subjects. The mother of the phenotypic homozygotes, subject 9, has a normal cholesterol biosynthetic rate and typical heterozygous FH. Thus, increased synthesis of cholesterol segregates with the HMWR trait in the family members we have been able to study.

It is not clear why, in the HMWR kindred, increases in cholesterol biosynthesis and LDL production rate are prominent, whereas the expected LDL receptor deficiency is mild. It is pos-

sible that only a single defect is present, a qualitative abnormality of the LDL receptor, and that this results in secondary increases in cholesterol and LDL synthesis. The liver would be expected to be a prominent site of these changes. Alternatively, the overproduction of cholesterol (and possibly of LDL) may be a separate defect which is linked to the mutant HMWR LDL receptor.

These studies emphasize the unusual pathophysiology with which clinically classic FH can present, and they encourage further research into the mechanisms of hypercholesterolemia in FH patients.

Acknowledgments

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