Low Density Lipoprotein Receptor Activity in Homozygous Familial Hypercholesterolemia Fibroblasts*

Clay F. Semenovich, Richard E. Ostlund, Jr., Richard A. Levy, and Steven R. Osa

From the Metabolism Division, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri

(Received for publication, November 5, 1981)

We have identified specific low affinity low density lipoprotein (LDL) receptors in skin fibroblasts from two patients previously classified as having LDL receptor-negative homozygous familial hypercholesterolemia (FHC). $K_r$ and maximum capacity for cell-associated and degraded $^{125}$I-LDL were measured in homogenates of normal and FHC fibroblasts. The $K_r$ values of normal cells and the maximum specific capacity was reduced to 11% of normal. Thus, some FHC cells have reduced affinity as well as reduced capacity for LDL.

The FHC cell receptors share many but not all properties of the normal skin fibroblasts LDL receptor. Specific degradation of bound $^{125}$I-LDL occurred concomitantly with LDL binding and was greatly reduced by the addition of chloroquine, an inhibitor of lysosomal function. Preincubation of FHC cells with cholesterol or LDL resulted in significant suppression of receptor function. Modification of lysine residues of LDL abolished receptor activity in both normal and FHC cells. Treatment of FHC cells with compactin, a cholesterol synthesis inhibitor, resulted in significant increases in specific $^{125}$I-LDL binding and degradation compared to FHC cells without compactin treatment. Normal cells also showed increases in $^{125}$I-LDL binding and degradation with compactin treatment, but the mean percentage increase in specific $^{125}$I-LDL degradation was significantly greater in FHC cells (strain GM 2000, 160 $\pm$ 18%) than in normal cells (29 $\pm$ 8%).

Homozygous familial hypercholesterolemia is a well described clinical phenotype which is accompanied by a deficiency in the cell surface receptor for LDL, and accelerated atherosclerosis with premature death (1, 2). Despite elegant characterization of the clinical syndrome over the past 20 years by Khachadurian, Frederickson and Levy, and Brown and Goldstein, the exact nature of the genetic defects involved remains unknown. Goldstein and Brown (3) have postulated that the clinical phenotype can be accounted for by three disorders that are clinically similar but biochemically distinct. Thus, cultured fibroblasts from patients with homozygous FHC are classified as receptor-negative in which less than 2% of normal LDL receptor activity is detectable, receptor-defective in which 2-25% of normal LDL receptor activity is detectable, and “internalization-defective” in which LDL receptors are able to bind LDL particles normally but LDL is not taken up by the cell. This paper focuses on the receptor-negative form of homozygous FHC.

LDL receptor-negative fibroblasts clearly have a gross reduction in $^{125}$I-LDL binding activity, but it is unresolved whether or not some residual activity is present. Several lines of evidence suggest the existence of significant LDL receptor activity in “receptor-negative” cells. LDL receptor activity can be assessed either directly by the measurement of $^{125}$I-LDL bound to cultured fibroblasts or indirectly by a number of methods that measure the effects of LDL on cellular metabolic functions. Indirect methods include determinations of the effect of LDL on 3-hydroxy-3-methylglutaryl-CoA reductase activity, cholesteryl ester formation, and the release of amino acids by LDL degradation. Goldstein et al. (4) classify cells as “receptor-negative” if LDL fails to stimulate any incorporation of oleate into cholesteryl esters. However, when receptor-specific $^{125}$I-LDL binding was studied in five fibroblast strains that were later classified as LDL receptor-negative on the basis of oleate incorporation studies (4), these cells bound 11.2 $\pm$ 3.7% of the expected amount of LDL (5). Breslow et al. (6) found 12% of normal $^{125}$I-LDL specific binding in fibroblasts from a patient apparently classified as LDL receptor-negative. Using a dose-response curve, measurable levels of specific degradation of LDL (15% of normal) were demonstrated by Goldstein and Brown in fibroblasts from a typical homozygote with the receptor-negative form of FHC (4, 7). Haba et al. (31) found from 0-7.5% of the expected specific LDL internalization and degradation in receptor-negative fibroblasts. Fung et al. (10, 11) have repeatedly demonstrated LDL-mediated suppression of hydroxymethylglutaryl-CoA reductase activity and of acetate incorporation into sterols in receptor-negative fibroblasts. This is evidence for specific receptor activity since LDL entering fibroblasts by nonspecific pinocytosis evidently does not regulate sterol synthesis or esterification (7, 12, 15). Using fluorescent and immunofluorescent microscopy to study individual fibroblasts, Kruth and Vaughn (35) reported that some homozygous receptor-negative cells bind small amounts of LDL and accumulate intracellular cholesterol.

The present study was undertaken to characterize receptor activity present in fibroblasts from patients with the receptor-negative form of homozygous FHC. We have identified specific low affinity LDL receptor activity in these fibroblasts which
is distinct from nonspecific uptake of LDL by cells and binding of LDL by blank culture dishes.

**EXPERIMENTAL PROCEDURES**

**Materials**—The diphosphate salt of chloroquine, Hepes buffer, and chollophosphatidylcholine were purchased from Mallinckrodt Chemical Works. Dextran sulfate was purchased from Calbiochem-Behring. Compartment in the lactone form was obtained from Dr. A. Endo, Sankyo Co., Ltd., Tokyo, Japan. The lactone was converted to the sodium salt by heating in base; 0.385 mM chollophosphatidylcholine was subsequently adjusted to physiologic pH and stored at −20 °C (14). Newborn calf serum was purchased from Gibco, Inc., Grand Island, NY. 125I was purchased from Amersham. Radiation-stabilized plastic 35-mm diameter six-well cluster dishes were purchased from Costar Corp., Bedford, MA. LDL (density 1.019–1.063 g/ml) from normal, healthy donors was prepared by ultracentrifugation and iodinated as previously reported (7, 15). Unlabeled LDL prepared from an unrelated patient with homozygous FHC was used for displacement of specifically bound 125I-LDL. This FHC LDL was indistinguishable from LDL prepared from normal donors in displacing 125I-LDL in cultured fibroblasts. Lipoprotein-deficient human serum was prepared from human volunteers. 125I-LDL was added to each well to induce receptor activity. After 22 h, the medium was removed and cells and then were assayed for 125I-LDL binding in previous studies as previously described (8). Human 125I-thrombin (9) was a gift of Dr. Douglas Tollefsen and topical bovine thrombin was purified from Parke-Davis.

**Cells**—Skin fibroblast strains from two patients with the "receptor-negative" form of FHC (lines GM 2000 and 1915) were purchased from the Genetic and Mutant Cell Repository, Camden, NJ. Consistent results were obtained using different shipments of the same cell type over 3 years. Skin fibroblasts from normal controls were obtained by skin biopsies of the deltoid area. Cell plating density was 103 cells/35-mm well. The cells were grown for 4 days in growth media consisting of Eagle’s minimum essential medium containing 15–20% NBCS and antibiotics (15, 23). Cells were then washed twice with Puck’s saline G and internalized LDL was then determined by dissolving the cells in NaOH. Cell-associated 125I-LDL was determined either by dissolving the washed cells directly in 0.625 N NaOH for 20 min at room temperature without first eluting the surface LDL or as the sum of dextran/SO4-releasable + internalized LDL in experiments where there was too little surface-bound LDL to analyze.

**Calculations**—Statistical comparisons were performed by the Student’s t-test with mean ± standard error of the mean presented. Unless otherwise stated, specific 125I-LDL binding or degradation was computed as the difference of values determined in the absence (total) and the presence (nonspecific) of 20-fold or greater excess of unlabeled LDL. The estimated standard error of this difference was computed as SEM (specific) = √SEM (total) + SEM (nonspecific) where the number of samples for total and nonspecific binding was identical. Experiments were routinely performed in triplicate. 

**RESULTS**

**Characterization of LDL Receptor Activity in FHC Cells**—A dose-response curve for cell-associated 125I-LDL in LDL receptor-negative GM 2000 cells is presented in Fig. 1. Although the absolute level is low, appreciable cell-associated 125I-LDL, displaceable by unlabeled LDL is apparent and easily distinguishable from binding to culture dishes without cells (blank dishes). In subsequent data, receptor-specific cell-associated or degraded 125I-LDL was calculated by subtracting nonspecific values (seen in the presence of cells but with excess unlabeled LDL) from total values (seen in the presence of cells without excess unlabeled LDL). The estimated standard error of this difference is routinely presented in the figures and tables. Blank culture dishes reproducibly manifest a minor degree of specific binding which has been disregarded in subsequent calculations. No LDL specific degradation products were observed in blank culture dishes. Data of the experiment of Fig. 1 were recalculated as nanograms of
125I-LDL specifically cell-associated and degraded per mg of cell protein and are displayed in Fig. 2. Specific LDL degradation occurred and increased in parallel with cell-associated LDL, suggesting that the cell-associated material was transferred to lysosomes. This was further supported by the data of Table I in which addition of 60 μM chloroquine (lysosomal enzyme inhibitor) to the assay solution during a 5-h incubation in the presence of 50 μg/ml of 125I-LDL resulted in the nearly complete inhibition of LDL degradation in FHC cells accompanied by a significant increase in the amount of internalized LDL.

The time course of specific cell-associated and degraded LDL in receptor-negative GM 1915 cells is shown in Fig. 3. The FHC cells qualitatively resembled normal cells. Cell-associated 125I-LDL values rose progressively after addition of 125I-LDL and no degradation was observed until 45 min after which degradation rose linearly with time. Maximum cell associated 125I-LDL was observed at 5 h (data not shown).

**Table I**

| Assay conditions          | Dextran-SO₄-releasable binding | LDL internalized | LDL degraded |
|---------------------------|--------------------------------|------------------|-------------|
| 50 μg/ml 125I-LDL only    | 5.54 ± 1.58                    | 32.3 ± 4.7       | 135 ± 15.9  |
| 50 μg/ml 125I-LDL plus 60 | 4.62 ± 0.82                    | 98.5 ± 4.3       | 6.7 ± 13.7  |
| μM chloroquine            |                                | (p < 0.001)      | (p < 0.001) |

Little LDL binding and no significant degradation were observed in blank culture dishes.

Specificity of FHC cell receptor for lipoproteins is demonstrated in Table II. In both normal and FHC cells, unlabeled LDL reduced the specific degradation of 125I-LDL to a similar extent. However, methyl LDL and acetoacetyl LDL, lipoproteins modified at critical lysine residues involved in receptor binding (27), did not compete significantly for either normal or FHC receptors.

Since only a very small portion of the radioactivity added to the cultured cells became cell-associated, the nature of this material was further examined. The 125I-LDL tracer added contained 3.6% of material that remained in the organic phase after a Folch lipid extraction and wash (26) followed by evaporation of the organic solvent. Internalized 125I radioactivity dissolved in 0.5 ml of 0.625 N NaOH from an experiment identical with that described in Table II was neutralized with 0.5 ml of 1 N HCl and immediately vortexed with 1.0 ml of chloroform. The chloroform phase was removed, dried, counted, and compared to that remaining in the aqueous phase. In GM 2000 cells, 7.5% of the radioactivity was chloroform-soluble, whereas in normal cells 3.6% was chloroform-soluble. Hence, this material did not appear to be primarily lipid in either normal or FHC cells. Since apoprotein B, the protein constituent of LDL, is insoluble in aqueous solutions of TMU whereas other apoproteins are soluble in TMU (25), the solubility of bound radioactivity in this solvent was tested (Table III). In both normal and GM 2000 cells, over 87% of the bound counts were TMU-insoluble. Hence, the cell-associated radioiodinated material appears to be 125I-LDL apoprotein and not a contaminant.

Saturability of FHC cell receptor activity is demonstrated by the displacement curve shown in Fig. 4. In the presence of a constant amount of 125I-LDL (50 μg/ml) in the medium, the amount of cell-associated 125I-LDL tracer decreased from 104 ± 2.8 ng/mg of cell protein to 18.2 ± 1.1 ng/mg as the concentration of unlabeled LDL in the assay medium was increased from 0 to 2000 μg/ml. In the same experiment, the amount of degraded tracer decreased from 223 ± 15.4 ng/mg of cell protein to 51.9 ± 12.9 ng/mg. The displacement of 125I-LDL by large amounts of unlabeled LDL does not appear to be an artifact since similar amounts of unlabeled LDL did not displace specifically bound 125I-thrombin or 125I-mouse epidermal growth factor from normal cell receptors (Table IV).

LDL binding in FHC cells was calcium-sensitive (Table V). The effect of calcium on specific LDL binding was assessed by a modification of the technique used by Goldstein and Brown to characterize high affinity receptor activity (7). In the presence of 50 μg/ml of 125I-LDL, both specific dextran-SO₄-releasable surface-bound and internalized LDL were significantly increased in GM 2000, GM1915, and normal cells after the addition of 2 mM calcium to the assay medium.
LDL Receptors in Homozygous Hypercholesterolemia

**Calculation of Binding Parameters—Two methods were used to calculate the apparent $K_a$ and maximum capacity for specific cell-associated $^{125}$I-LDL and degraded LDL in GM 2000 cells (Table VI).** First, a Scatchard analysis was applied to the equilibrium dose-response data of Fig. 2 in which increasing quantities of $^{125}$I-LDL were added with and without excess unlabeled LDL (Fig. 5). It is clear that only low affinity binding (flat slope) is present in GM 2000 cells. The apparent $K_a$ (reciprocal of affinity) of these cells was 127 µg/ml compared to 16.5 µg/ml in the normal cell type. The mean $K_a$ for receptor-negative cells (103 µg/ml) was 4.6 times that of normals, while the mean maximum binding capacity (364 ng/
TABLE IV

Effects of unlabeled LDL on binding of thrombin and mouse epidermal growth factor to fibroblasts.

A. normal human fibroblasts were plated at 10^5/well in MEM + 15% NBCS. The medium was removed after 5 days, the cells were washed with saline G, and 25 mm Hesper-buffered MEM, pH 7.3, containing 0.1% bovine serum albumin was added for 5 min to decrease nonspecific binding. This medium was removed and 0.7 ml of the same medium containing 200-fold excess of unlabeled bovine thrombin or unlabeled LDL was added. The cells were incubated for 45 min at 37 °C in air and then washed as for LDL binding experiments. The cells were dissolved in 0.625 N NaOH and counted for total cell-associated binding.

| 125I-a-thrombin bound | fm/mg protein |
|-----------------------|--------------|
| A. 125I-a-thrombin    |              |
| +200 ßg/ml unlabeled bovine thrombin | 8.1 ± 1.8 |
| +100 ßg/ml LDL        | 325 ± 12.7   |
| +200 µg/ml LDL        | 337 ± 12.6   |

B. 125I-mEGF

| 125I-mEGF bound | pg/mg protein |
|-----------------|--------------|
| +500 ßg/ml mEGF | 85 ± 12.2    |
| +200 µg/ml mEGF | 2160 ± 237   |
| +200 µg/ml LDL  | 2430 ± 133   |

Table V

Calcium sensitivity of LDL binding

Cells were grown for 3 days in MEM + 15% NBCS and washed, and 1.0 ml of MEM + 10% LPDS was added to each well. After 2 days of receptor induction, the medium was removed. One group of cells was then washed with calcium and magnesium-free Puck's saline G and 1.5 ml of 50 mm TrisCl (pH 7.5), 0.1 m NaCl buffer containing 50 ßg/ml of 125I-LDL with or without 2 mg/ml of unlabeled LDL, was added to each well. A second group of cells was treated identically except CaCl2 was added to the assay medium yielding a final calcium concentration of 2 mm. The cluster dishes were sealed with parafilm, incubated in air at 37 °C for 3 h, and then processed for LDL binding.

| Cells | 2 mm Ca^2+ | Dextran SO4-releasable binding | 125I-LDL internalized |
|-------|------------|-------------------------------|-----------------------|
| GM 2000 | 6.84 ± 2.97 | 20.5 ± 2.67 | p < 0.01 |
| GM 1915 | 4.58 ± 1.56 | 30.2 ± 1.26 | p < 0.01 |
| Normal | 30.2 ± 2.87 | 82.3 ± 9.07 | p < 0.01 |
| Blank dishes | 27.7 ± 2.16 | 202 ± 31.6 | p < 0.01 |

* Not significant.

Fig. 5. Scatchard analysis of specific cell-associated 125I-LDL. The data of Fig. 2 for specific cell-associated 125I-LDL were plotted according to the method of Scatchard (18). The slope is the negative of the apparent affinity for LDL and the x-intercept is the maximum capacity. GM 2000 cells; normal cells.

Table VI

125I-LDL processing characteristics of normal and FHC fibroblasts

| Method of determination of Ks | Cells | 125I-LDL bound (ng/mg) | g/mL | ng/mg | g/mL |
|-------------------------------|-------|------------------------|------|-------|------|
| Scatchard analysis | GM 2000 | 127 (r = 0.99) | 310 | 116 (r = 0.87) | 777 |
| Displacement analysis | GM 2000 | 24.7 (r = 0.99) | 150 | 6900 ± 780 |

r is the correlation coefficient.

LDL Receptors in Homozygous Hypercholesterolemia

The degree of competition will be imperfect depending on the per
cent saturation of the LDL receptors, which is itself a function of binding affinity. If receptors are present in great excess, no competition will occur; if receptors are saturated with LDL, perfect competition takes place. The data from Fig. 4 (GM 2000 cells) and a normal cell type are plotted in Fig. 6 in such a way as to linearize the data, analogously to the Lineweaver-Burk method of enzyme analysis. In Fig. 6, the x-intercept = \(-K_m\). It can be seen that the apparent affinities of FHC cells (48.7 \(\mu\)g/ml) and a normal cell type (6.6 \(\mu\)g/ml) differ markedly. By this method, maximum capacity for cell-associated \(^{125}\)I-LDL of receptor-negative cells was 10.6% of normal (Table VI).

The above parameters were computed for cell-associated (surface-bound plus internalized) \(^{125}\)I-LDL and degraded LDL. Although it was possible to measure dextran-SO\(_4\)-releasable cell surface binding (Tables I and V), the level of binding was too low to determine reliable \(K_m\) and maximum binding values. More precise surface binding data could be obtained from the measurement of cell-associated \(^{125}\)I-LDL at 4 °C, a condition which prevents LDL internalization and degradation (1). Receptor-specific \(^{125}\)I-LDL binding at 4 °C in three normal cell strains performed as specified in the legend to Fig. 7 demonstrated half-maximum receptor saturation at 3.8 \(\pm\) 1.0 \(\mu\)g/ml and maximum capacity of 109 \(\pm\) 15 mg/mg of protein. When similar studies were done on GM 2000 cells it was not possible consistently to demonstrate specific \(^{125}\)I-LDL binding at concentrations of added \(^{125}\)I-LDL less than 10 \(\mu\)g/ml. However, at higher amounts of added \(^{125}\)I-LDL (Fig. 7), appreciable specific binding activity was observed and the binding capacity/mg of protein at 125 \(\mu\)g/ml of added \(^{125}\)I-LDL was 35.5 \(\pm\) 2.6% of a normal strain analyzed simultaneously. Half-maximum receptor occupancy occurred at 75 \(\mu\)g/ml of added \(^{125}\)I-LDL, consistent with reduced affinity of the GM 2000 receptor for LDL.

**Effect of Physiologic Manipulations on LDL Receptor Activity**—Up to this point, only data from cells grown in LPDS have been presented. To approximate more closely the physiologic extracellular environment, normal fibroblasts and GM 1915 FHC cells were preincubated for 3 days in MEM + 10% LPDS containing 80 \(\mu\)g/ml of LDL. The cells were then aspirated and replaced with MEM + 10% LPDS containing 80 \(\mu\)g/ml of \(^{125}\)I-LDL ± 1 mg/ml of unlabeled LDL and the cells were incubated 21 h at 37 °C. Mean \(\pm\) S.E. of triplicate dishes is presented.

| Cell type             | \(^{125}\)I-LDL specific degradation |
|-----------------------|-------------------------------------|
| Normal                | 5820 \(\pm\) 336                   |
| FHC heterozygote      | 3230 \(\pm\) 261                   |
| FHC homozygote (GM 195)| 1970 \(\pm\) 173                   |

**Fig. 7. Binding of \(^{125}\)I-LDL at 4 °C. Normal fibroblasts (a), GM 2000 (b), and dishes without cells ( ■) were prepared for \(^{125}\)I-LDL binding as described under "Experimental Procedures." The cells were cooled to 4 °C for 40 min and all subsequent operations were performed in a 4 °C cold room. \(^{125}\)I-LDL ± unlabeled LDL in MEM + 10% LPDS buffered with 25 mM Hepes to pH 7.3 was added and the dishes were incubated 2 h, followed by the usual washing procedure except that 2 extra 10-min washes with albumin-containing buffer were done before washing the cells with albumin-free buffer. The cells were dissolved in 0.625 N NaOH for 20 min and counted.**
cially induced by LPDS. The ability of LDL or cholesterol preincubation to down-regulate LDL receptors of normal and FHC cells grown in LPDS is presented in Table VIII. LDL receptor activity was determined in both cell types, but the FHC cells demonstrated more resistance to down-regulation than normal cells.

The effect of 48-h preincubation with compactin, an inhibitor of cholesterol biosynthesis, was also studied. GM 2000 cells were washed and preincubated for 72 h in MEM + 10% silicic acid-treated NBCS LPDS containing either LDL, cholesterol, or no addition. LDL receptor activity was determined by washing twice with saline G and incubating 5 h at 37 °C with MEM + 10% silicic acid-treated NBCS LPDS containing 100 pg/ml of tritiated LDL ± 1.0 mg/ml of unlabeled LDL. Results are the mean from 3-6 wells/condition ± S.E. All statistical comparisons are with untreated cells of the same type.

| Experiment 1 | 10% LPDS | 100 pg/ml | 5 h | ±7.5 pg/ml cholesterol | 5 h | 20-fold excess of LDL | Degraded |
|--------------|----------|-----------|-----|------------------------|-----|------------------------|----------|
| GM 2000      | 12.0 ± 0.83 | 164 ± 8.4 | 502 ± 17.3 |
| Normal       | 6.72 ± 0.76 | 122 ± 11.0 | 322 ± 26.4 |
| No addition  | 137 ± 0.73 | 1660 ± 60 | 4440 ± 251 |
| +50 μg/ml LDL| 20.5 ± 2.6 | 258 ± 12.8 | 589 ± 38.3 |
| GM 2000      | 5.73 ± 0.71 | 135 ± 4.8 | 255 ± 22.5 |
| No addition  | 3.34 ± 0.41 | 97.0 ± 4.8 | 90.4 ± 23.2 |
| +7.5 μg/ml cholesterol | 392 ± 27.2 | 2980 ± 219 | 7540 ± 537 |
| Normal       | 110 ± 5.3 | 1310 ± 50.1 | 3530 ± 170 |

* p < 0.001.  
** p < 0.02.

**DISCUSSION**

The data presented in this paper concur with the studies of Brown and Goldstein (1, 5, 7) in that no high affinity LDL receptor activity was found in skin fibroblasts from patients with the receptor-negative form of homozgyous FHC (Fig. 5). However, specific but low affinity LDL receptor activity was identified which is distinct from the nonspecific bulk phase pinocytosis of 125I-LDL measurable in FHC cells: in the presence of large amounts of unlabeled LDL (Fig. 4, Ref. 7) and from nonspecific binding of 125I-LDL by culture dishes without cells (Figs. 3, 4, and 7). The cell-associated 125I-labeled material appears to be apo-LDL since it is not extractable into chloroform and is insoluble in aqueous tetramethylurea (Table III). Such specific low affinity receptor activity is consistent with previous findings of small amounts of specific binding of 125I-LDL (5, 6), small amounts of specific degradation of 125I-LDL (7), and regulation of sterol synthesis by LDL (10, 11) in receptor-negative FHC fibroblasts.

The LDL receptors in FHC cells demonstrated by our experiments resemble the normal high affinity LDL receptor in several ways. 1) The binding of 125I-LDL is accompanied by appropriate degradation (Fig. 2). During a 5-h incubation at 37 °C with 100 μg/ml of 125I-LDL, the ratio of dextran-S04-releasable surface-bound LDL:internalized LDL:degraded LDL was 10.0:100:152 in GM 2000 cells and 10.0:100:182 in normal cells. The degradation of 125I-LDL was linear after a 45-min delay in both normal and receptor-negative cells (Fig. 3B), consistent with a requirement that LDL be transferred to lysosomes before degradation. Chloroquine, a lysosomal enzyme inhibitor, reduced 125I-LDL degradation to 5% of normal in GM 2000 cells. Thus, the usual pathway of LDL uptake is apparently present in FHC cells. 2) The time course for cell-associated 125I-LDL was similar in normal and FHC cells (Fig. 3A). 3) Modification of lysine residues reduced the capacity of LDL to interact with both FHC and normal receptors (Table II). 4) Binding to both cell types was calcium-sensitive (Table V). 5) FHC cells demonstrated down-regulation of LDL receptors in response to pretreatment with cholesterol and LDL (Table VIII) and increased receptor activity in response to compactin, a cholesterol biosynthesis inhibitor (Table IX).

The LDL receptors of FHC cells are also easily distinguished from those of normal cells. The maximum specific capacity for cell-associated 125I-LDL in FHC cells was 9.8-13% of normal cells assayed in the same manner (Table VI). But receptor affinity of LDL was also reduced to 14-28% of normal.
The $K_m$ for LDL in FHC cells (49-127 $\mu$g/ml) was 3.5-7.3 times that of normal cells and much nearer the estimated normal extracellular LDL apoprotein B concentration of 70 $\mu$g/ml. Thus, FHC cells should metabolize significant amounts of LDL when grown chronically under conditions in which large amounts of LDL are present in the medium. This was confirmed in Table VII in which FHC cells metabolized 34% of the normal amount of LDL when 80 $\mu$g/ml of LDL was present. Reduced affinity of $^{125}$I-LDL in GM 2000 cells was also noted in assays conducted at 4°C (Fig. 7), a condition preventing LDL internalization and degradation.

The data presented do not allow for a distinction between altered expression of the classic LDL receptor and a genetically separate class of low affinity LDL receptors that might be present in all cell types. It should be noted, however, that in another FHC mutation characterized by increased LDL receptor affinity but low capacity there is no evidence for a class of low affinity receptors even though they should have been more easily detected than in normal cells (17). Of interest is the recent report by Beisiegel et al. (38) that some LDL receptor-negative fibroblasts have much more binding of radiolabeled anti-LDL receptor antibody than of radiolabeled LDL, suggesting that the high affinity LDL receptor might be present but modified.

The regulation of receptors in GM 2000 cells was also altered. Preincubation of normal cells for 48 h with compactin in LPDS medium resulted in increased specific $^{125}$I-LDL degradation compared to cells grown in LPDS without compactin (Table IX). This confirms similar previous work with compactin in normal skin fibroblasts (29). Thus, LPDS medium apparently is not a maximum stimulus to LDL receptor accumulation. GM 2000 cells also demonstrated increased receptor activity after compactin preincubation. The mean percentage increase in specific LDL degradation in this cell type after compactin preincubation was significantly greater (160 ± 18%) than in normal cells from three individuals (29 ± 8%). Since the absolute increase in specific receptor activity after compactin addition was considerably greater in normal cells than in FHC cells, the greater percentage increase in specific LDL degradation in GM 2000 cells may be physiologically insignificant and merely represent an artifact of experimental conditions or data treatment. An alternative explanation is that the greater percentage increase in degradation in GM 2000 cells after compactin preincubation represents the unmasking of specific LDL receptors usually down-regulated by endogenous cellular production of cholesterol. Prolonged preincubation with compactin appears important in order to demonstrate increased receptors since Haba et al. (31) did not observe any changes in LDL receptor activity in either normal or FHC fibroblasts incubated with both $^{125}$I-LDL and compactin for only 6 h. Thus, our finding of specific low affinity LDL receptors and hyper-responsiveness to compactin in GM 2000 cells raises the possibility that the primary abnormality in FHC fibroblasts may not necessarily be a defect in the structural gene for the LDL receptor but rather a defect in the genes regulating LDL receptor expression or cholesterol metabolism. A mechanism for FHC implicating increased endogenous production of cholesterol has been proposed previously by Fogelman et al. (22).

Low affinity receptors may have been overlooked in previous experiments for several reasons. The curve describing $^{125}$I-LDL binding of fibroblasts is sigmoidal and very little binding is seen at $^{125}$I-LDL concentrations far below the $K_m$. Thus, when $^{125}$I-LDL at concentrations of 5-20 $\mu$g/ml is used, normal cells have appreciable receptor occupancy whereas receptor-negative cells show very little occupancy. $^{125}$I-LDL concentrations above 50 $\mu$g/ml should be employed for FHC cells and, for this reason, 1-2 mg/ml of unlabeled LDL in alternate wells is required to detect specific binding of the labeled material. We have often used large quantities of LDL prepared from the pheresis plasma of an FHC homozygote for this purpose. We also induced LDL receptors by growing cells in 7 mg/ml of LPDS for 72 h, a larger amount and longer time than customarily used.

The major classification scheme for FHC fibroblasts has utilized principally an indirect method for determination of LDL receptor function, e.g., radiolabeled oleate incorporation into cholesteryl esters after exposure of cells to LDL (2, 4). Receptor-negative cells revealed no oleate incorporation in response to LDL whereas receptor-defective cells had 5-20% of normal incorporation. These are measurements of the acute effect of metabolized LDL on cells, not the actual LDL processed by surface binding, internalization, and degradation. Since low levels of specific LDL receptor activity can be identified in at least some receptor-negative fibroblasts, we believe that the phenotype of homozygous FHC may be associated with a spectrum of qualitative and quantitative receptor abnormalities encompassing the functional categories receptor-negative and receptor-defective. Since it is agreed that almost all homozygous FHC fibroblasts are severely deficient in LDL receptor function, future studies should more clearly define the exact nature of the LDL binding abnormalities present in these cells.

Acknowledgments—We thank Stephen Block, Mark Mendelsohn, and Kay Zorn for excellent technical and secretarial assistance.

REFERENCES
1. Goldstein, J. L., and Brown, M. S. (1977) Annu. Rev. Biochem. 46, 867-890
2. Fredrickson, D. S., Goldstein, J. L., and Brown, M. S. (1978) in The Metabolic Basis of Inherited Disease (Stambury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., eds) pp. 617-633, McGraw-Hill, New York
3. Goldstein, J. L., and Brown, M. S. (1979) Annu. Rev. Genet. 13, 259-296
4. Goldstein, J. L., Dana, S. E., Brunschede, G. Y., and Brown, M. S. (1975) Proc Natl. Acad. Sci. U.S.A. 72, 1092-1096
5. Brown, M. S., and Goldstein, J. L. (1974) Proc Natl. Acad. Sci. U.S.A. 71, 788-792
6. Berlin, J. L., Spaulding, D. R., Lux, S. E., Levy, R. I., and Lees, R. S. (1975) N. Engl. J. Med. 293, 900-906
7. Goldstein, J. L., and Brown, M. S. (1974) J. Biol. Chem. 249, 5153-5162
8. Carpenter, G., and Cohen, S. (1976) J. Cell Biol. 71, 159-171
9. Tollefsen, D. M., Feagler, J. R., and Majerus, P. W. (1974) J. Biol. Chem. 249, 2046-2051
10. Hung, C. H., Khachadurian, A. K., Wang, C. H., and Durr, I. F. (1977) Biochim. Biophys. Acta 487, 445-457
11. Hung, C. H., Wang, C. H., and Khachadurian, A. K. (1978) Biochim. Biophys. Acta 528, 445-455
12. Goldstein, J. L., and Brown, M. S. (1976) Curr. Top. Cell Regul. 11, 147-181
13. Deleted in proof
14. Brown, M. S., Faust, J. R., Goldstein, J. L., Kaneko, I., and Endo, A. (1978) J. Biol. Chem. 253, 1121-1128
15. Ostlund, R. E., Jr., Pfleger, B., and Schönfeld, G. (1979) J. Clin. Invest. 63, 75-84
16. Puck, T. T., Cieciura, S. J., and Robinson, A. (1958) J. Exp. Med. 108, 949-956
17. Ostlund, R. E., Jr., Levy, R. A., Witztum, J. L., and Schönfeld, G. (1981) J. Clin. Invest., in press
18. Scharf, G. (1949) Annu. N.Y. Acad. Sci. 51, 660-672
19. Deleted in proof
20. Deleted in proof
21. Deleted in proof
22. Deleted in proof
23. Ostlund, R. E., Jr., Hajek, S. V., Levy, R. A., and Witztum, J. L.
LDL Receptors in Homozygous Hypercholesterolemia

(1981) Metabolism 30, 285–289

24. Deleted in proof

25. Kane, J. P. (1973) Anal. Biochem. 53, 350–364

26. Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957) J. Biol. Chem. 226, 497–509

27. Weisgraber, K. H., Innerarity, T. L., and Mahley, R. W. (1978) J. Biol. Chem. 253, 9053–9062

28. Deleted in proof

29. Filipovic, I., and Menzel, B. (1981) Biochem. J. 196, 625–628

30. Oram, J. F., Albers, J. J., and Bierman, E. L. (1980) J. Biol. Chem. 255, 475–485

31. Haba, T., Mabuchi, H., Yoshimura, A., Watanabe, A., Wakasugi, T., Tatami, R., Ueda, K., Ueda, R., Kametani, T., Koizumi, J., Miyamoto, S., Takeda, H., and Takeshita, H. (1981) J. Clin. Invest. 67, 1532–1540

32. Fieser, L. F. (1953) J. Am. Chem. Soc. 75, 5421–5422

33. Dana, S. E., Brown, M. S., and Goldstein, J. L. (1977) Biochem. Biophys. Res. Commun. 74, 1369–1376

34. Holstee, B. H. J. (1959) Nature (Lond.) 184, 1296–1298

35. Kruth, H. S., and Vaughn, M. (1980) J. Lipid Res. 21, 123–130

36. Beisiegel, U., Schneider, W. J., Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1981) J. Biol. Chem. 256, 11923–11931