A comparative study of surface binding of human low density and high density lipoproteins to human fibroblasts: regulation by sterols and susceptibility to proteolytic digestion

T. Koschinsky,[1] T. E. Carew, and D. Steinberg[2]

Division of Metabolic Disease, Department of Medicine, University of California, San Diego, La Jolla, CA 92093

Abstract Binding of ^{125}I-low density lipoprotein (LDL) and ^{125}I-high density lipoprotein (HDL) was determined in cultured human fibroblasts from a normal subject and two subjects with homozygous familial hypercholesterolemia (HFH). Binding was assayed at 0°C to minimize the internalization of labeled lipoproteins. The binding of LDL and of HDL were compared following interventions reported to affect LDL binding in normal fibroblasts. LDL binding to normal cells increased two- to threefold 24 hours after transfer from medium containing whole fetal calf serum to medium containing lipoprotein-deficient fetal calf serum. This increase was completely blocked in the presence of cycloheximide (200 μg/ml) or 7-ketocholesterol (2.5 μg/ml). This increased capacity of normal fibroblasts to bind LDL could be reduced 70–80% by a subsequent 18-hour incubation with cholesterol (50 μg/ml) or 7-ketocholesterol (2.5 μg/ml). In contrast, no significant change in HDL binding to normal fibroblasts was observed after any of these interventions. HFH cells failed to show any significant change in either LDL binding or HDL binding following these interventions. These results suggest that HDL binding sites on normal fibroblasts are for the most part distinct from LDL binding sites. They also support the conclusion that LDL binding sites on HFH cells are for the most part qualitatively different from those on normal cells.

Supplementary key words cholesterol · 7-ketocholesterol · pronase · homozygous familial hypercholesterolemia · lipoprotein receptors · cell culture

The potential importance of lipoprotein metabolism in peripheral cells has only recently been recognized. Animal studies in this laboratory showed that the apoprotein of low density lipoprotein (LDL) is probably degraded predominantly in the periphery rather than in the liver (1, 2). Goldstein and Brown (3) showed that fibroblasts cultured from the skin of patients with homozygous familial hypercholesterolemia (HFH) take up and degrade LDL at a much slower rate than do normal skin fibroblasts. In view of the low fractional catabolic rate of LDL protein in patients with familial hypercholesterolemia, a defect in peripheral metabolism thus may account for the high steady state levels of LDL in these patients (4–6). Brown and Goldstein (7) have carefully and comprehensively studied the nature of the interaction of LDL with normal and HFH fibroblasts. Their results support the following formulation of some of the essential steps in the process: *1*) LDL interacts with a high-affinity receptor on the cell surface; *2*) the LDL is internalized and carried to a primary lysosome; *3*) in the lysosome the LDL is degraded, the protein being converted to small peptides and amino acids and the cholesterol ester to free cholesterol; *4*) the liberated cholesterol, in a manner yet to be defined, suppresses the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase; *5*) the increased availability of exogenous cholesterol (either from LDL or from sterol added to the culture medium) reduces the number of high-affinity receptors on the cell surface; *6*) the rate of cholesterol esterification is increased; *7*) this overall mechanism provides a system for regulating the cell cholesterol content.

The metabolism of high density lipoprotein (HDL) has been less well studied. Whether or not peripheral metabolism of HDL is quantitatively important is still not known with certainty. Peripheral cells, including arterial smooth muscle and fibroblasts, can degrade HDL (8–11) although at a lower rate than LDL. Some peripheral degradation is suggested by the

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; HFH, homozygous familial hypercholesterolemia; FCS, fetal calf serum; LDS, lipoprotein-deficient serum; DME, Dulbecco's modification of Eagle's essential medium.

[1] Present address: Diabetes Forschungsinstitut, Universitaet Duesseldorf, Auf'm Hennekamp 65, D 4000 Duesseldorf 1, West Germany.

[2] To whom reprint requests and enquiries should be addressed.

failure of portacaval shunting to decrease HDL turnover (12). However, HDL certainly does not share the ability of LDL to suppress cholesterol synthesis in peripheral cells (13–16). HDL may be metabolized by different mechanisms and yet there is clearly some degree of interaction between LDL and HDL metabolism by cultured smooth muscle cells (8, 17, 18), endothelial cells (19, 20) and fibroblasts[3] (9). The present studies were undertaken to compare critically the properties of HDL and LDL binding sites on normal human fibroblasts. Interventions known to modify LDL binding in normal human fibroblasts (3, 21) were tested for their possible effects on HDL binding. Fibroblasts from patients with homozygous familial hypercholesterolemia (HFH) were studied to determine whether their binding of HDL differed from normal and also to determine whether LDL binding to them was affected by factors modifying LDL binding to normal cells.

METHODS

Materials

Pronase from *Streptomyces griseus* was purchased from Calbiochem, San Diego, CA. (B grade, 45,000 Kunitz units/g; Lot 502122); bovine trypsin (Lot 54C-8220, treated with diphenylcarbamyl chloride to inactivate chymotrypsin) and subtilopeptidase A from *B. subtilis* (lot 94C-0245) were purchased from Sigma, St. Louis, MO. 7-Ketocholesterol was obtained from Steraloids, Inc., Wilton, NH. Cholesterol (99% pure) was purchased from Applied Science Laboratories (State College, PA) and twice recrystallized from ethyl acetate. Aliquots of pure cholesterol in ethanol were stored at −20°C under nitrogen. Cycloheximide (Lot 41C-2610) was purchased from Sigma and sodium [^{125}I]iodide, carrier-free in 0.05 N NaOH, from Schwarz-Mann, Orangeburg, NY. Tissue culture supplies were obtained as previously described (14, 22).

Cells

Human fibroblast cultures were derived from a preputial biopsy of a normal infant (line BB) or from nongenital skin of two patients with the homozygous form of familial hypercholesterolemia—P.A. (HFH_1) and J.P. (HFH_2). The former line was started in this laboratory; the latter was obtained from Dr. Joseph L. Goldstein's laboratory and from the American Type Culture Collection. The clinical features and some aspects of LDL metabolism in vitro and in cell culture have been reported for patient P.A. (5, 22, 23) and for J.P. (24–27). Cells were maintained in Dulbecco's modification of Eagle's minimal essential medium (DME) (28) containing 10% (v/v) fetal calf serum (FCS). Medium was renewed every 2–3 days. Lipoprotein-deficient fetal calf serum (LDS) was prepared as described previously (15, 22). Its cholesterol content was less than 2.5 μg/ml.

Cells were studied between the 7th and 17th passages. Confluent monolayers of cells from stock flasks were harvested by incubation with 0.05% trypsin in a buffer containing 137 mM NaCl, 5.36 mM KCl, 5.55 mM dextrose, 6.9 mM $NaHCO_3$, and 0.54 mM disodium ethylenediaminetetraacetate (EDTA). Cells were seeded at a concentration of 1×10^5 cells per dish into 60 × 15 mm plastic dishes. The medium (final volume, 3 ml) contained FCS (10%), penicillin (50 U/ml), and streptomycin (50 μg/ml). On the 4th or 5th day, when the cells were still in logarithmic growth, the FCS medium was replaced by medium containing 5% LDS, yielding a final protein concentration of 2.5 mg/ml. Binding of lipoprotein was studied either immediately or after 18–66 hr of further incubation in LDS medium, with or without the addition of sterols or cycloheximide as indicated.

To study the effect of mild proteolytic digestion on lipoprotein binding, the cells were incubated 18–24 hr in 5% LDS medium, washed two times with 3 ml of phosphate buffered saline (PBS) (29) and then incubated at 37°C with the indicated concentrations of pronase, trypsin, or subtilopeptidase A. The cells were then washed again two times with 3 ml of PBS prior to the binding assays.

Preparation of lipoproteins

LDL (d 1.019–1.063) and HDL (d 1.09–1.21) from pooled human plasma were isolated by sequential density ultracentrifugation (30) and further purified by recentrifuging at the appropriate densities. The lipoprotein fractions were dialyzed against 0.15 M NaCl containing 0.01% EDTA and sterilized by passage through a Millipore filter (0.22 μm) (Millipore Corp., Bedford, MA). Purity of the final preparations was confirmed by agarose gel electrophoresis and by Ouchterlony double diffusion with specific antisera against human apolipoproteins B and A-I and against albumin.

Purified human LDL and HDL were iodinated in glycine–NaOH buffer, pH 10.0, by a modification of the MacFarlane iodine monochloride technique (31). Iodine monochloride was added in a molar

[3] Miller, N. E., D. B. Weinstein, T. E. Carew, T. Koschinsky, and D. Steinberg. Interaction between high density and low density lipoproteins during uptake and degradation by cultured human fibroblasts. *J. Clin. Invest.*, in press.

ratio of 5:1 to LDL protein (assumed mol wt 200,000) or to HDL protein (assumed mol wt 50,000) in the presence of carrier-free ^{125}I. The ^{125}I-labeled lipoproteins were dialyzed exhaustively against 0.15 M NaCl–0.01% EDTA. Prior to use in cell studies, the ^{125}I-LDL and ^{125}I-HDL were sterilized by passage through a Millipore filter (0.22 μm) and aliquots were taken for determination of protein (32) and radioactivity. The specific radioactivities ranged from 80 to 450 cpm/ng of lipoprotein protein.

Human LDL and HDL labeled in this way comigrated with unlabeled LDL or HDL of human plasma on agarose gel electrophoresis. Less than 1% of the total ^{125}I was soluble in 15% trichloroacetic acid; less than 3% of the total ^{125}I was in lipid (extractable with chloroform–methanol 2:1).

The integrities of the ^{125}I-LDL and ^{125}I-HDL were tested by diluting them up to 40-fold with unlabeled LDL or HDL, respectively, and measuring total cell-associated radioactivity after 3 hr at 37°C at a constant total lipoprotein concentration. The amount of radioactivity retained by the cells decreased in strict proportion to the extent of dilution, that is, the calculated cell-associated lipoprotein in ng/mg cell protein at each final specific activity was essentially the same.

Lipoprotein binding assay

In previous papers from this and other laboratories (22, 33), it has been shown that total cell-associated ^{125}I-LDL activity increases for several hours when cells are incubated at 37°C. The amount of ^{125}I-LDL released from the cells by brief trypsinization is very close to the total cell-associated ^{125}I-LDL when cells are incubated at 0°C, i.e., very little is internalized. At 37°C, the trypsin-releasable ^{125}I reaches a plateau value after incubation of human fibroblasts for about 60 min while total cell-associated ^{125}I-LDL continues to increase, as does the amount degraded. This suggests that trypsin-releasable ^{125}I represents ^{125}I-LDL bound to the cell surface, while that not released by trypsin represents LDL internalized by the cell. Similar observations have recently been made with respect to the metabolism of ^{125}I-HDL by arterial smooth muscle cells (8) and human fibroblasts (10).

Since the major interest in the present work was to focus attention on the LDL or HDL bound to the surface of the cell, all incubations with labeled lipoproteins were carried out at 0°C. When cells were incubated at this low temperature, 80–90% of ^{125}I-LDL and 85–95% of ^{125}I-HDL were released from the cells by brief trypsinization. The fraction not released by trypsin appeared to be independent of the concentration of labeled lipoprotein in the medium. Binding results in the present paper were calculated on the basis of total cell-associated radioactivity, since it is unclear whether the residual activity in the cells following trypsinization represents a small amount of internalization continuing even at 0°C or an incomplete release of surface-bound lipoprotein.

Cells to be studied at 0°C were placed on ice for 15 min and washed three times with 3 ml of ice-cold PBS. ^{125}I-LDL or ^{125}I-HDL was then added in ice-cold DME containing 5% LDS. During the following 2 hr incubation the dishes were held on ice in a 4°C cold room.

At the end of the incubation the medium was drawn off and an aliquot was directly assayed for total ^{125}I. The dishes were carefully washed five times with 3 ml of ice-cold PBS. The amount of ^{125}I in the final 3-ml wash was small relative to that still associated with the washed cells (less than 5%). Cells were released from the plate by incubating for 5 min at 37°C with 1 ml of the 0.05% trypsin solution described above. The plates were then placed back on ice and 1 ml of ice-cold medium containing 10% FCS was added to each dish to arrest the action of the trypsin. The cells were collected into a centrifuge tube and the dish was washed with an additional 1 ml of medium containing 10% FCS. The cells were pelleted by centrifugation at 3000 *g* for 5 min at 4°C and an aliquot of the supernatant fluid was assayed for ^{125}I radioactivity (trypsin-releasable radioactivity). The cells were washed by resuspending in 6 ml of ice-cold PBS, recentrifuged at 3000 *g* for 5 min, and assayed for ^{125}I. The cell pellet was then dissolved by overnight incubation in 1 N KOH and aliquots were removed for protein determination (32).

RESULTS

Effects of prolonged incubation in lipoprotein-deficient medium on LDL and HDL binding

Surface binding of LDL to normal human fibroblasts previously maintained in a medium containing 10% FCS increased markedly after 24 hr of incubation in lipoprotein-deficient medium. The increase was threefold, from 21 to 66 ng/mg cell protein with LDL at 5 μg/ml (**Table 1**). When, however, 7-ketocholesterol and cholesterol were present during the 24-hr incubation in LDS, LDL binding not only failed to increase but actually decreased, from 21 to 13.2 ng/mg cell protein. Cycloheximide also completely blocked the increase in LDL binding. These results on regulation of LDL binding sites by LDL and by sterols are in agreement with the findings of Brown and Goldstein with normal fibroblasts (21).

TABLE 1. Comparison of LDL and HDL with respect to effects of 24-hr incubation in lipoprotein-deficient medium on lipoprotein binding to normal and HFH fibroblasts[a]

	Incubation Prior to Measurement of Binding	Binding at 0° to Fibroblast Cell Lines		
		Normal	HFH_1	HFH_2
		ng/mg cell protein		
Binding of ^{125}I-LDL (5 μg/ml)	None (zero time)	21.0	7.2	7.2
	24 hr in 5% LDS alone	66.0	10.8	10.2
	24 hr in LDS plus 7-ketocholesterol (2.5 μg/ml) and cholesterol (12.5 μg/ml)	13.2	7.7	11.4[b]
	24 hr in LDS plus cycloheximide (210 μg/ml)	23.1		10.7
Binding of ^{125}I-HDL (5 μg/ml)	None (zero time)	5.7	5.7	6.4
	24 hr in LDS alone	5.1	5.8	6.5
	24 hr in LDS plus 7-ketocholesterol (2.5 μg/ml)	4.4	5.2	6.2

[a] All cell lines were grown in DME containing 10% fetal calf serum. On the first day of the study, LDL or HDL binding was measured in some plates immediately after repeatedly washing the preconfluent monolayer and cooling it to 0°C (see Methods). The medium in other plates was changed as indicated and incubation at 37°C was continued for an additional 24 hr after which 0°C binding was measured in the same way. Values shown represent the means of dulicate or triplicate determinations unless otherwise indicated. Replicate values differed by less than 15% from the indicated mean values.

[b] Single determination.

Binding of HDL, on the other hand, was unchanged after incubation in LDS either in the absence or in the presence of 7-ketocholesterol (Table 1).

Binding of LDL to HFH cells was less than that to normal cells—about one-third as great—and was changed little if at all after incubations in LDS, without or with sterols present. Binding of HDL to HFH cells was comparable in magnitude to its binding to normal cells and was unchanged on incubation with LDS or with LDS plus 7-ketocholesterol.

Effects of incubation with sterols on LDL and HDL binding

Cells were first incubated 18–24 hr in 5% LDS, which increased the number of LDL binding sites on normal fibroblasts as shown above. The cells were then incubated for an additional 24–48 hr either in LDS alone or in LDS to which sterols had been added. At the end of the second incubation the cells were cooled on ice and binding of LDL (**Table 2**) or of HDL (**Table 3**) was measured at 0°C. As shown in Table 2, incubation in the presence of sterols (cholesterol alone, 7-ketocholesterol alone, or a combination of the two) in every instance caused a marked decrease in LDL binding (49–80%, mean 64%). The absolute decrements, a function of the concentration of LDL used in the binding study, ranged from 11 to 53 ng/mg cell protein. The effect of incubation with sterols on LDL binding over a wide range of LDL concentrations is shown in **Fig. 1**.

The binding of LDL to HFH cells prior to incubation with sterol was less than that to normal cells, the binding ratios varying from as low as 1.7 to as high as 8.3 to 1. In contrast to the results with normal cells, incubation with sterols had only small and inconsistent effects on LDL binding to HFH cells (Table 2). The mean for all of the experiments shown was −4% for the HFH_1 line and −6% for the HFH_2 line.

As shown in Table 3, incubation of normal cells in the presence of sterols caused no change in HDL binding, a result in striking contrast to that for LDL (Table 2). The small and inconsistent changes (mean +5%) are probably within the limits of error of the experimental methods.

Prior to incubation with sterols, binding of HDL to the HFH cells was equal to or greater than that to the normal cells. Incubation with sterols caused no consistent change in HDL binding to HFH cells (Table 3).

Effects of incubation of fibroblasts with proteolytic enzymes on the binding of LDL and HDL

The sensitivity of binding sites to degradation by proteolysis was tested by first incubating the cells for 18 hr in LDS, washing, incubating with one of

TABLE 2. Effects of incubation with sterols on 0°C binding of LDL after prior incubation for 18–24 hr in LDS

Expt.	Labeled LDL Concentration	Incubation Conditions		Normal Cells		HFH_1 Cells		HFH_2 Cells	
		Time	Sterol Addition	LDL Bound	Sterol Effect	LDL Bound	Sterol Effect	LDL Bound	Sterol Effect
	μg/ml	*hr*		*ng/mg*	*%*	*ng/mg*	*%*	*ng/mg*	*%*
A	5	24	None	58		17		21	
		24	50 μg/ml cholesterol	23	−60	20	+18	23	+10
B	5	48	None	41		14		29	
		48	50 μg/ml cholesterol	21	−49	12	−14	18	−38
A	5	24	None	58		17		21	
			2.5 μg/ml, 7-ketocholesterol	14	−76	15	−12	21	0
E	5	24	None	20		9		10	
			2.5 μg/ml, 7-ketocholesterol	9	−55	9	0	9	−10
F	10	38	None	58		34		ND	
			2.5 μg/ml, 7-ketocholesterol + 12.5 μg/ml cholesterol	19	−67	37	+8	ND	
G	5	24	None	66		11		10	
			2.5 μg/ml, 7-ketocholesterol + 12.5 μg/ml cholesterol	13	−80	8	−27	11	+10

several proteolytic enzymes, and then measuring binding at 0°C. The time of exposure to and the concentration of the proteolytic enzymes were determined by trial and error to be such that the cells would not detach from the dish during the subsequent 2 hr incubation at 0°C used for the binding measurement or during the washing procedure.

A representative result with pronase digestion is shown in **Fig. 2**. Binding of LDL to normal cells was reduced by 82%; binding of LDL to the HFH lines was essentially unaltered. In other experiments the reduction in LDL binding to normal cells (measured at LDL concentrations from 1 to 20 μg/ml) ranged from 72 to 91% (mean, 83%; n = 8). In HFH cells the change in LDL binding ranged from −31 to +37% (mean, −1.6%; n = 7). Similar results were obtained using subtilopeptidase A (0.1 μg/ml for 10 min) and low concentration trypsin (1 μg/ml for 10

TABLE 3. Effects of incubation with sterols on 0°C binding of HDL after prior incubation for 16–24 hr in lipoprotein deficient medium

Expt.	Labeled HDL Concentration	Incubation Conditions		Normal Cells		HFH_1 Cells		HFH_2 Cells	
		Time	Sterol Addition	HDL Bound	Sterol Effect	HDL Bound	Sterol Effect	HDL Bound	Sterol Effect
	μg/ml	*hr*		*ng/mg*	*%*	*ng/mg*	*%*	*ng/mg*	*%*
A	5	24	None	11.0		18.0		25.3	
			50 μg/ml cholesterol	14.8	+34	17.5	−3	22.5	−11
A	5	24	None	11.0		18.0		25.3	
			2.5 μg/ml 7-ketocholesterol	9.9	−10	21.4	+20	24.8	−2
D	5	24	None	5.1		5.8		6.5	
			2.5 μg/ml 7-ketocholesterol	4.4	−14	5.2	−10	6.2	−5
F	10	16	None	30.3		60.1		ND	
			2.5 μg/ml 7-ketocholesterol + 12.5 μg/ml cholesterol	31.1	+3	47.5	−21	ND	
G	1	24	None	2.2		2.0		2.0	
			2.5 μg/ml 7-ketocholesterol + 12.5 μg/ml cholesterol	2.3	+5	2.5	+25	2.4	+20

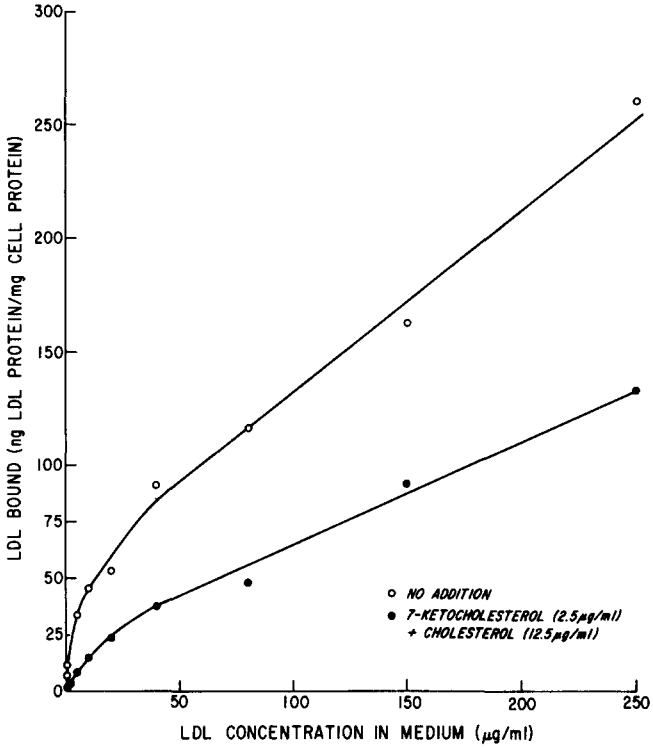

Fig. 1. Comparison of LDL surface binding to normal fibroblasts incubated either in the absence or presence of 7-ketocholesterol and cholesterol. The fibroblasts were incubated 19 hr in 3 ml of LDS-containing medium prior to addition of either 20 μl of ethanol alone (open circles) or 20 μl of ethanol containing 7.5 μg of 7-ketocholesterol and 37.5 μg of cholesterol (closed circles). After 24 hr cells were washed twice with 2 ml of ice-cold PBS. Then 2 ml of LDS-medium containing the indicated concentrations of ^{125}I-LDL (0.1–250 μg/ml) were added to duplicate dishes and incubated for 2 hr at 0°C. Cells were harvested and analyzed as described under Methods. The mean cellular protein per dish was 0.473 mg for control cells (○) and 0.486 mg for cells incubated with sterols (●).

min), i.e., LDL binding to normal cells was reduced by 30–45% while LDL binding to HFH cells was unaffected.

The binding of HDL, in contrast, was altered little if at all by pronase digestion in either normal cells or HFH cells (Fig. 2). In four separate experiments with normal fibroblasts, HDL binding was reduced on the average by only 11.3% (measured at HDL concentrations from 1 to 20 μg/ml).

DISCUSSION

The side-by-side comparisons of labeled HDL and labeled LDL reported here provide evidence for certain fundamental differences in the properties of the sites to which these two lipoproteins bind. LDL binding to normal fibroblasts was: *1*) increased by 24 hr incubation in the absence of lipoproteins, an increase blocked by cycloheximide; *2*) decreased by subsequent incubation with sterols in the medium; and *3*) reduced 70–90% by brief exposure of the cells to proteolytic enzymes. These results confirm the findings of Brown and Goldstein (21) with respect to the regulation of LDL binding to normal human fibroblasts by LDL itself and by sterols. The sensitivity of the binding sites to proteolytic digestion and the inhibition by cycloheximide of the increase in binding sites induced by incubation in LDS strongly suggest that a membrane protein (or proteins) is an essential element in the binding. New synthesis either of the membrane protein itself or of proteins necessary for its introduction into the membrane is probably essential to the induction of binding capacity produced by incubation in LDS.

In contrast, HDL binding to normal fibroblasts changed little if at all under the same conditions. Thus, HDL binding sites do not appear to be regulated by lipoproteins (or by sterols) as are LDL binding sites. Furthermore, the absence of any significant change in HDL binding after proteolytic digestion of the cell membrane implies: *1*) that membrane proteins play at most a small role in HDL binding or, if proteins are involved, that the proteins at the HDL binding sites are relatively resistant to pronase digestion; and *2*) that at most a small fraction of the bound HDL is bound to high-affinity LDL binding sites (which *are* destroyed by proteolytic enzymes).

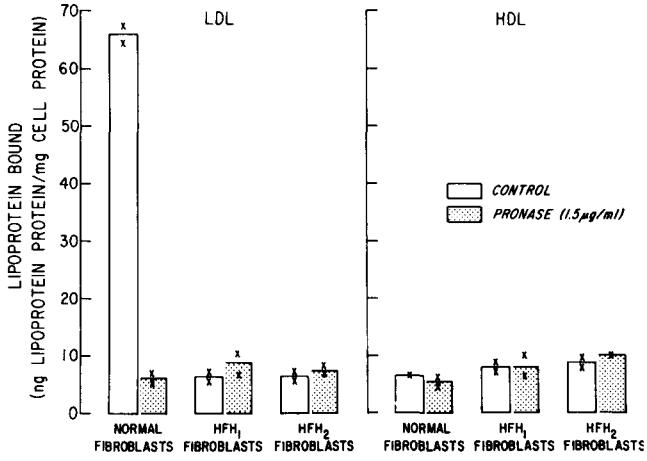

Fig. 2. Comparison of the effects of mild proteolytic digestion on surface binding of lipoproteins to normal and mutant fibroblasts. Fibroblasts were incubated 24 hr with 3 ml of LDS-containing medium. Then they were washed twice with 3 ml of PBS. Each dish received either 2 ml of DME alone (open bars) or, in addition, 3 μg of pronase in 10 μl of DME (closed bars). After 23 min at 37°C the cells were washed twice again with 3 ml of ice-cold PBS. Then 2 ml of fresh LDS-medium containing 5 μg of ^{125}I-LDL/ml or 5 μg of ^{125}I-HDL/ml were added to duplicate dishes and the cells were incubated for 2 hr at 0°C. Cells were harvested and analyzed as described under Methods. The mean cellular protein per dish was 0.396 mg for the normal cells, 0.198 mg for HFH_1 cells, and 0.212 mg for HFH_2 cells.

At first glance the latter conclusion seems at variance with the findings that HDL can reduce the binding, internalization, and degradation of LDL in normal human fibroblasts[3] (9). Actually, the findings are compatible since the apparent competition observed at high molar ratios of HDL to LDL in human fibroblasts could occur even if only a small fraction of the total bound HDL occupied LDL binding sites. Moreover, the interactions observed need not be the result of direct competition for the same binding sites (e.g., binding of HDL may perturb the cell membrane and reduce binding of LDL without necessarily occupying LDL binding sites directly). HDL has been previously shown to decrease LDL binding and uptake by swine arterial smooth muscle cells (18) and by human (32) and rabbit endothelial cells (19).

Brown and Goldstein (3) have shown that the binding of LDL to normal fibroblasts can be adequately described in terms of two classes of binding sites—of high and of low affinity, respectively. They found that treatment with pronase preferentially destroyed the high affinity binding and the present results with regard to LDL binding are in essential agreement. In contrast, HDL binding was not affected and the question arises as to whether there are any high-affinity binding sites for HDL analogous to those for LDL. Miller, Weinstein, and Steinberg (10) have shown that the relation between HDL binding and HDL concentration yields a curvilinear Scatchard plot, compatible with the presence of two or more classes of binding sites. Moreover, the total binding of HDL was only slightly less than that of LDL at equimolar concentrations of the two lipoproteins (up to 150 μg/ml of LDL and 25 μg/ml of HDL). Internalization and degradation of HDL, however, was much slower than that of LDL and, most relevant in the present context, these processes were approximately linearly related to the HDL bound. HDL uptake could be adequately accounted for by bulk endocytosis and adsorptive endocytosis occurring randomly wherever HDL was bound. In contrast, LDL uptake was much too large to be accounted for in this way. In this respect the cell handles LDL and HDL quite differently. Taken together the data suggest that, while there is a highly selective uptake process for LDL, there is no such analogous process for HDL or at least it accounts for only a relatively small fraction of HDL uptake.

Binding of LDL to fibroblasts from two patients with homozygous familial hypercholesterolemia (HFH) as studied here was not significantly increased by incubation in lipoprotein-deficient serum, was not decreased by incubation with sterols, and was not decreased by mild proteolytic digestion of the cells. These data show that the LDL binding sites on the HFH cells are qualitatively distinct from those on normal cells. Also, the total LDL bound to the normal cells at a low concentration of LDL in the medium (5 μg/ml) was always greater than that bound to HFH cells, the binding ratio varying from 2.7:1 to 9.3:1. In a previous paper from this laboratory, it was pointed out that the rate of internalization and degradation of LDL by normal fibroblasts can be as much as 20 to 50-fold greater than that by HFH fibroblasts, i.e., a difference out of proportion to the difference in binding (22). The reasons for this apparently disproportionate reduction in uptake remain to be elucidated. However, the *qualitative* differences in the LDL binding sites support the hypothesis that a highly specific cell membrane LDL receptor is either missing or radically altered in the HFH cells (3, 34).

HDL binding to normal cells and HFH cells was comparable at low HDL concentrations; at high HDL concentrations it was actually greater in the HFH cells. Thus, the clear deficiency in LDL binding to HFH cells was not paralleled by any decrease in HDL binding capacity, further evidence that the binding sites for these two lipoproteins are for the most part distinct.

The authors wish to thank Dr. Nicholas Myant for providing the skin biopsy from which the P.A. line of fibroblasts was started and Dr. Joseph L. Goldstein for the J.P. cell line. Ms. Susan Hayes' expert technical assistance is gratefully acknowledged. This project was supported by NIH Research Grants HL-14197 and HL-17471 awarded by the National Heart, Lung and Blood Institute and by Research Grant GM-17702 awarded by the National Institute of General Medical Sciences. Dr. Koschinsky was the recipient of research fellowship KO 570/1 from the Deutsche Forschungsgemeinschaft.

Manuscript received 4 November 1976 and accepted 17 February 1977.

REFERENCES

1. Sniderman, A. D., T. E. Carew, J. G. Chandler, and D. Steinberg. 1974. Paradoxical increase in rate of catabolism of low density lipoproteins after hepatectomy. *Science.* **183:** 526–528.
2. Steinberg, D., T. E. Carew, J. G. Chandler, and A. D. Sniderman. 1974. The role of the liver in metabolism of plasma lipoproteins. Alfred Benzon Symposium IV. *In* Regulation of Hepatic Metabolism. F. Lundquist and N. Tygstrup, editors. Munksgaard, Copenhagen. 144–156.
3. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured human fibroblasts. *J. Biol. Chem.* **249:** 5153–5162.
4. Langer, T., W. Strober, and R. I. Levy. 1972. The

metabolism of low density lipoprotein in familial Type II hyperlipoproteinemia. *J. Clin. Invest.* **51:** 1528–1536.
5. Simons, L. A., D. Reichl, N. B. Myant, and M. Mancini. 1975. The metabolism of the apoprotein of plasma low density lipoprotein in familial hyperbetalipoproteinemia in the homozygous form. *Atherosclerosis.* **21:** 283–298.
6. Bilheimer, D. W., J. L. Goldstein, S. M. Grundy, and M. S. Brown. 1975. Reduction in cholesterol and low density lipoprotein synthesis after portacaval shunt surgery in a patient with homozygous familial hypercholesterolemia. *J. Clin. Invest.* **56:** 1420–1430.
7. Brown, M. S., and J. L. Goldstein. 1976. Receptor-mediated control of cholesterol metabolism. *Science.* **191:** 150–154.
8. Carew, T. E., T. Koschinsky, and D. Steinberg. 1976. Binding, uptake, and degradation of ^{125}I-high and low density lipoprotein (HDL and LDL) by cultured swine aortic smooth muscle cells (SMC). *Clin. Res.* **24:** 154A.
9. Miller, N. E., D. B. Weinstein, and D. Steinberg. 1976. Metabolism of human lipoproteins, high density (HDL) and low density (LDL), by fibroblasts of normal and of homozygous familial hypercholesterolemia (FH) subjects. *Clin. Res.* **24:** 459A.
10. Miller, N. E., D. B. Weinstein, and D. Steinberg. 1976. Binding, internalization and degradation of high density lipoprotein by cultured human fibroblasts. *J. Lipid Res.* **18:** 438–450.
11. Stein, O., and Y. Stein. 1975. Comparative uptake of rat and human serum low density and high density lipoproteins by rat aortic smooth muscle cells in culture. *Circ. Res.* **36:** 436–443.
12. Carew, T. E., R. P. Saik, K. H. Johansen, C. A. Dennis, and D. Steinberg. 1976. Low density and high density lipoprotein turnover following portacaval shunt in swine. *J. Lipid Res.* **17:** 441–450.
13. Williams, C. D., and J. Avigan. 1972. In vitro effects of serum proteins and lipids on lipid synthesis in human skin fibroblasts and leukocytes grown in culture. *Biochim. Biophys. Acta.* **260:** 413–423.
14. Brown, M. S., S. E. Dana, and J. S. Goldstein. 1974. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human fibroblasts. *J. Biol. Chem.* **249:** 789–796.
15. Weinstein, D. B., T. E. Carew, and D. Steinberg. 1976. Uptake and degradation of low density lipoprotein by swine arterial smooth muscle cells with inhibition of cholesterol biosynthesis. *Biochim. Biophys. Acta.* **424:** 404–421.
16. Assman, G., B. G. Brown, and R. W. Mahley. 1975. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured swine aortic smooth muscle cells by plasma lipoproteins. *Biochemistry.* **14:** 3996–4002.
17. Koschinsky, T., T. E. Carew, and D. Steinberg. 1975. Effects of high density lipoprotein (HDL) on low density lipoprotein (LDL) metabolism by pig smooth muscle cells (SMC). *Circulation.* **51** (Suppl. II): 273.
18. Carew, T. E., T. Koschinsky, S. B. Hayes, and D. Steinberg. 1976. A mechanism by which high density lipoproteins may slow the atherogenic process. *Lancet.* **i:** 1315–1317.
19. Reckless, J. P. D., D. B. Weinstein, and D. Steinberg. 1976. Lipoprotein metabolism by rabbit arterial endothelial cells. *Circulation.* **54:** (Suppl. II): 56.
20. Stein, O., and Y. Stein. 1976. High density lipoproteins reduce the uptake of low density lipoproteins by human endothelial cells in culture. *Biochim. Biophys. Acta.* **431:** 363–368.
21. Brown, M. S., and J. L. Goldstein. 1975. Regulation of the activity of the low density lipoprotein receptor in human fibroblasts. *Cell.* **6:** 307–316.
22. Stein, O., D. B. Weinstein, Y. Stein, and D. Steinberg. 1976. Binding, internalization and degradation of low density lipoprotein by normal human fibroblasts and by fibroblasts from a case of homozygous familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **73:** 14–18.
23. Moutafis, C. D., and N. B. Myant. 1969. The metabolism of cholesterol in two hypercholesterolemic patients treated with cholestyramine. *Clin. Sci.* **37:** 443–454.
24. Goldstein, J. S., and M. S. Brown. 1973. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. *Proc. Natl. Acad. Sci. USA.* **70:** 2804–2808.
25. Starzl, T. E., M. P. Chase, C. W. Putnam, and J. J. Nora. 1974. Follow-up of patient with portacaval shunt for treatment of hyperlipidemia. *Lancet.* **ii:** 714–715.
26. Brown, M. S., and J. S. Goldstein. 1974. Suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. *J. Biol. Chem.* **249:** 7306–7314.
27. Goldstein, J. L., S. E. Dana, G. Y. Brunschede, and M. S. Brown. 1975. Genetic heterogeneity in familial hypercholesterolemia: evidence for two different mutations affecting functions of low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **72:** 1992–1996.
28. Dulbecco, R., and G. Freeman. 1959. Plaque production by the Polyoma virus. *Virology.* **8:** 396–397.
29. Dulbecco, R., and M. Vogt. 1960. Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med.* **99:** 167–182.
30. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
31. MacFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature (London).* **182:** 53.
32. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
33. Bierman, E. L., and J. J. Albers. 1975. Lipoprotein uptake by cultured human arterial smooth muscle cells. *Biochim. Biophys. Acta.* **388:** 198–202.
34. Anderson, R. G. W., J. L. Goldstein, and M. S. Brown. 1976. Localization of low density lipoprotein receptors on plasma membrane on normal human fibroblasts and their absence in cells from a familial hypercholesterolemia homozygote. *Proc. Natl. Acad. Sci. USA.* **73:** 2434–2438.