Metabolism of homologous and heterologous lipoproteins by cultured rat and human skin fibroblasts[1]

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Abstract Rat fibroblasts degraded human low density lipoprotein (LDL) very slowly, one-tenth to one-fortieth the rates observed in human fibroblasts. In rat cells, human LDL caused only very small increases in cell cholesterol content and acylCoA:cholesterol acyltransferase (ACAT) activity and caused only small decreases in β-hydroxy-β-methylglutaryl CoA (HMG CoA) reductase activity; in human cells, however, human LDL induced very large changes in all three of these parameters, as expected. The binding of human LDL to rat fibroblasts was not reduced by previous incubation with human LDL or with 25-hydroxycholesterol. Thus, in rat fibroblasts there appear to be few, if any, regulated high-affinity receptors that recognize human LDL. Rat LDL fractions (d 1.02–1.05 g/ml), in contrast, were degraded more rapidly than human LDL by rat fibroblasts, caused a significant increase in cell cholesterol content, an increase in ACAT activity, and a significant decrease in HMG CoA reductase activity. Moreover, the degradation of this rat LDL fraction by rat fibroblasts as a function of concentration was biphasic, i.e., there appeared to be a high-affinity component of degradation. Thus, it appears that rat fibroblasts do have a receptor for *homologous* lipoproteins. However, because both apoprotein B and apoprotein E are present in these rat lipoprotein fractions, the observed effects may relate to recognition of either or both of these apoproteins. The metabolism and metabolic effects of the conventionally defined high density lipoprotein (HDL) fraction of the rat by rat or human fibroblasts resembled those of human LDL in human fibroblasts. It is suggested that rat HDL may, because of its apo E content and higher concentration in rat plasma relative to that of LDL, play an important role in cholesterol homeostasis in vivo.—**Drevon, C. A., A. D. Attie, S. H. Pangburn, and D. Steinberg.** Metabolism of homologous and heterologous lipoproteins by cultured rat and human skin fibroblasts. *J. Lipid Res.* 1981. **22:** 37–46.

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The characteristics of the high-affinity receptor for low density lipoprotein (LDL) in human skin fibroblasts have been well established (1) and it appears that an analogous receptor system is operative in other human cell types (2, 3) and in cells of several other animal species (4, 5). Uptake of LDL by the high-affinity receptor system is associated with an increase in cell cholesterol content, an increase in acylCoA: cholesterol acyltransferase (ACAT) (6) activity and a decrease in β-hydroxy-β-methylglutaryl CoA (HMG CoA) reductase activity (7). The LDL receptor appears to recognize either apoprotein B or apoprotein E (8). Whether or not a given lipoprotein fraction will be bound and taken up rapidly by the high-affinity receptor mechanism appears to depend primarily on its apoprotein composition and to only a limited extent on its size, shape, or lipid composition (9, 10).

One of the purposes of the present study was to try to determine whether or not rat skin fibroblasts have an LDL receptor system analogous to that described in human skin fibroblasts. A second and related purpose was to compare the metabolism of homologous and heterologous lipoproteins, both LDL and HDL, in rat skin fibroblasts and human skin fibroblasts.

EXPERIMENTAL PROCEDURE

Materials

[9, 10-^{3}H]Oleic acid (sp act 8.26 Ci/mmol and [3-^{14}C]hydroxymethyl-glutaric acid (sp act 51.9 mCi/

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HMG CoA reductase, β-hydroxy-β-methylglutaryl coenzyme A reductase; ACAT, acylCoA:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; FCS, fetal calf serum; DME, Dulbecco's modified Eagle's medium; LDS, lipoprotein-deficient serum.

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mmol) were obtained from New England Nuclear Co., Boston, MA and carrier-free ^{125}I from Amersham-Searle Corp., Arlington Heights, IL. Cholesteryl ester hydrolase and cholesterol oxidase were obtained from Boehringer-Mannheim, West Germany. N,N-Dicyclohexylcarbodiimide, NADP+, glucose-6-phosphate dehydrogenase, oleic acid, Triton X-100, horseradish peroxidase, sodium cholate, and heparin were obtained from Sigma Chemicals, St. Louis, MO. Beta-sitosterol and 25-hydroxycholesterol were provided by Steroids, Wilton, NH and their purity was checked by gas–liquid chromatography using a Varian Aerograph, Series 2100, equipped with a 180 cm column, inner diameter 2 mm, packed with 3% SP-2250 or 80/100 mesh Supelcoport (Supelco) at 295°C with argon as a carrier gas (30 ml/min). Beta-sitosterol and 25-hydroxycholesterol were found to be ≧99.5% and 97.5% pure, respectively. [^{3}H]Oleic acid was mixed with unlabeled oleic acid and dissolved in ethanol. An equimolar amount of 1N KOH was added and the potassium soap was injected into a solution containing fatty acid-poor 5% bovine serum albumin dissolved in the standard tissue culture medium used (see below). The ratio between fatty acids and albumin in the final preparation was 1:1. Fetal calf serum (Irvine Scientific Sales Co., Fountain Valley, CA) was heat-inactivated at 56°C for 30 min before use. Penicillin G and streptomycin sulfate dissolved in saline were obtained from Grand Island Biologicals, Berkeley, CA. Kyro EOB was a gift from Dr. Fred H. Mattson.

Culture of cells

Human skin fibroblasts were grown in monolayer from a preputial biopsy of a normal infant. Metabolism of LDL and of HDL have been previously studied in this cell line (11, 12). Rat skin fibroblast cultures were initiated from subcutaneous tissue of three different rats, one neonatal and one adult Sprague-Dawley rat and one adult Buffalo rat. The cells from these three animals showed similar growth patterns and morphological features.

Cultures were maintained in a humidified incubator (95% air–5% CO_2) at 37°C in Dulbecco's modified Eagle's (DME) medium containing 24 mM $NaHCO_3$, 10% fetal calf serum (heat-inactivated), penicillin G (100 units/ml), and streptomycin (100 μg/ml). Cells were seeded at a density of about 1×10^5 cells in 60 × 15 mm polystyrene tissue culture dishes (Falcon) and used 3–4 days later. Growth curves showed that the doubling time for the rat cells was 19 hr and for the human cells 27 hr. The protein content of the dishes was usually 200–300 μg. In some studies rat cells were used at much lower cell density (70 μg/dish) with the same results obtained when the protein content was 300 μg/dish. The rat cells were more rounded, more granular, less uniform in shape, and tended to overlap more than the human cells. Karyotyping, generously performed in Dr. Kurt Benirschke's laboratory, demonstrated a normal number of chromosomes (42) and no abnormal chromosomes. The cells were used at a preconfluent stage unless otherwise indicated.

Isolation and labeling of lipoproteins

Human and rat lipoproteins were isolated from fresh plasma (containing EDTA, 1 mg/ml) by preparative ultracentrifugation (13) except that swinging-bucket rotors were used. The lipoproteins were washed once by reflotation and dialyzed exhaustively against phosphate-buffered saline containing 0.3 mM EDTA, pH 7.4. The purity of the lipoproteins was checked by agarose-electrophoresis using the method described by Noble (14) and apoprotein composition was determined by polyacrylamide gel electrophoresis (15).

Lipoproteins were iodinated by a modification (16) of the iodine monochloride method of MacFarlane (17) after which the lipoproteins were dialyzed against 0.02 M sodium phosphate, pH 7.4/0.9% NaCl/0.01% EDTA. The lipoproteins were passed through Millipore filters (0.45 μm) before use in tissue culture. More than 99% of the lipoprotein radioactivity was precipitated by 10% trichloroacetic acid (w/v). Human LDL and HDL were isolated in the density range 1.019–1.63 and 1.09–1.21 g/ml, respectively. Rat LDL was isolated in the narrow range 1.02–1.045 g/ml in an effort to limit the content of HDL_1, shown by Weisgraber, Mahley, and Assman (18) to be present in significant quantities in the d 1.02–1.063 g/ml fractions of normal rat plasma. This lipoprotein contains apoprotein E as the major protein and polyacrylamide gel electrophoresis of our 1.02–1.045 g/ml fraction showed a significant apo E band (about 10 to 15% by densitometric scanning). Rat HDL was isolated in the range d 1.09–1.21 g/ml. It contained apo A-1 as the major apoprotein, and apoproteins A-IV, E, and C; there was a minor band that did not penetrate the gel, presumably apo B, but this was estimated to account for no more than a few percent of the total protein.

Measurement of LDL binding

After 3 to 4 days in culture in DME medium containing 10% fetal calf serum, the medium was changed to DME containing human lipoprotein-deficient serum (LDS) (5 mg protein/ml) and the cells were incubated for 24 to 48 hr. The cells were chilled on ice and ^{125}I-labeled LDL at the indicated concentrations was added. Lipoprotein concentrations are expressed in terms of

lipoprotein protein throughout. The cells were shaken at about 30 oscillations per minute for 60 min. The culture dishes were then washed five times with a buffer containing 0.15M NaCl, 50 mM Tris, and 2 mg/ml bovine serum albumin, pH 7.4, as described by Goldstein et al. (19). The cells were washed once with 0.15 M NaCl–50 mM Tris (pH 7.4) and heparin was added to release LDL (50 mM NaCl/10 mM HEPES/0.4% heparin). The dishes containing the heparin solution were shaken as before for 60 min at 4°C. The medium (1 ml) was assayed for ^{125}I in an automatic gamma-counting system (Searle model 1197) to determine "heparin-releasable ^{125}I-labeled LDL". The rest of the medium was aspirated, the cells were washed once, 2 ml of 0.1 N KOH was added, and the dishes were incubated overnight at 37°C under water-saturated air–CO_2 19:1 (v/v). A sample was taken for determination of total cell protein and another for ^{125}I assay. The latter is taken to represent cell-associated ^{125}I-labeled LDL.

Measurement of lipoprotein degradation

After 3 to 4 days of culture, the medium was removed and replaced with 2 ml of fresh medium containing human LDS (5 mg protein/ml). After 24 hr, fresh medium containing the appropriate amount of ^{125}I-labeled lipoprotein was added to the cells. Most of the degradation experiments were carried out for 20–24 hr. After a 1- to 2-hr initial lag period, the degradation of lipoproteins was linear for up to 24 hr. At the end of the incubation, the medium was removed and precipitated with ice-cold trichloroacetic acid (10% final concentration, w/v). After centrifugation, the supernatant was filtered through an 0.45 μm Amicon filter to remove all precipitated material. Aliquots of the filtrates were cleared of free [^{125}I]-iodide by precipitation with silver nitrate using a method developed by Dr. David B. Weinstein.[3] To 0.5 ml of the trichloroacetic acid-soluble filtrate was added 0.25 ml of 5% $AgNO_3$ (w/v) dissolved in distilled water. After mixing on a vortex mixer and centrifugation (3000 *g* for 10 min), 200 μl of the supernatant, representing the noniodide, trichloroacetic acid-soluble radioactivity, was assayed in a gamma counter. This method was directly compared with the hydrogen peroxide-chloroform extraction procedure (20) and essentially identical results were obtained. Acid-soluble, iodide-free radioactivity from control dishes containing no cells was subtracted from the values for experimental samples. Data are expressed as ng or μg lipoprotein-protein degraded per mg of cell protein.

[3] Dr. David B. Weinstein. Personal communication.

Measurement of β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG CoA reductase)

[3-^{14}C]Hydroxymethylglutaric acid anhydride was prepared according to Goldfarb and Pitot (21) and [3-^{14}C]HMG CoA was formed by reacting the [3-^{14}C]hydroxymethylglutaric acid anhyride with reduced CoA on ice at pH 9.0 as described by Hilz et al. (22).

The medium from each flask was discarded, the cells were washed with phosphate-buffered saline, pH 7.4, and then scraped from the dishes with a rubber policeman. Cell extracts were prepared by adding 200 μl of a buffer containing 50 mM K_2HPO_4 (pH 7.2), 5 mM dithiothreitol, 1 mM EDTA, and 0.25% Kyro EOB to cell pellets containing 150–250 μg of protein (23). An amount of cell extract containing 50–120 μg of cell protein was used in each assay. Glucose-6-phosphate (final concentration, 30 mM) was preincubated with the solubilized enzyme for 10 min at 37°C in a volume of 150 μl. Then a reaction mixture containing glucose-6-phosphate (30 mM), glucose-6-phosphate dehydrogenase (3.5 units/ml), NADP+ (2.55 mM), and [3-^{14}C]HMG CoA (140 μM; sp act, 4.5 mCi/mmol) was added to start the reaction (final volume, 0.2 ml). The reaction was linear for more than 2 hr and routine assays were carried out for 60–120 min at 37°C in a shaking waterbath. Twenty μl of 12 N HCl was used to stop the reaction and 3 μmol (1.5×10^5 dpm) of [^3H]mevalonic acid was added as an internal standard. The samples were incubated for 30 min at 37°C or overnight at room temperature. Aliquots (100 μl) were applied to silica gel G (250 μm, Analtech) TLC plates that had been activated overnight (110°C). The developing system was dry acetone–benzene 1:1 (v/v) with anhydrous Na_2SO_4 present in the chamber. The mevalonic acid spots were located using I_2 vapor and the gel was scraped directly into vials containing 0.1 ml of IM acetic acid and 10 ml of scintillation fluid (PCS (Amersham)–toluene 1:2 (v/v)). Blank values obtained in incubations lacking the enzyme were subtracted in all cases. The activity of HMG CoA reductase is expressed as pmol of mevalonic aicd formed per mg of protein per min.

Measurement of cholesterol esterification

Acyl CoA:cholesterol acyltransferase (ACAT) activity was measured in intact cells by adding [^3H]oleic acid (bound to defatted bovine serum albumin) to the medium. The incorporation of precursor into cholesteryl ester was linear for at least 3 hr. A 60-min incubation period was used routinely. The final concentration of oleic acid was 50 μM and the molar ratio

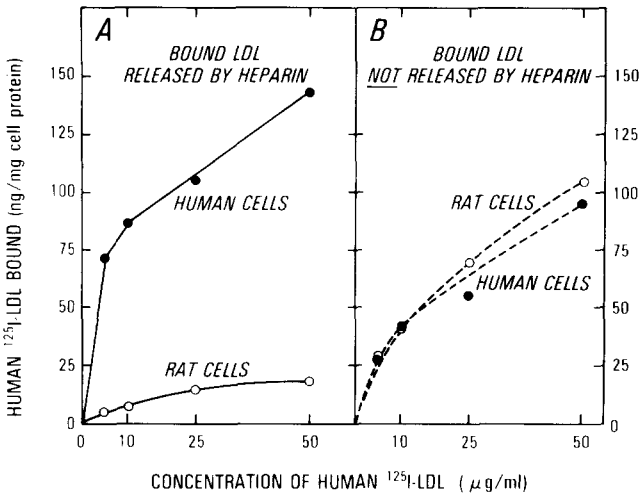

Fig. 1. Binding of human ^{125}I-labeled LDL to human skin fibroblasts (●) and to rat skin fibroblasts (○) at 4°C as a function of LDL concentration. Cells previously grown in a medium containing DME with 10% fetal calf serum were transferred to DME medium containing lipoprotein-deficient serum (5 mg protein/ml) 48 hr prior to study. The dishes were then chilled to 4°C and the medium was replaced with fresh medium containing the same concentration of lipoprotein-deficient serum plus the indicated concentrations of human ^{125}I-labeled LDL. After 60 min of incubation at 4°C on a metabolic shaker, the cells were washed six times as described under Methods and a buffer containing heparin (4 mg/ml), sodium chloride (50 mM), and HEPES buffer (10 mM) was added. The cells were then shaken at 4°C for 60 min and a sample of the medium was taken for radioassay (LDL released by heparin, Panel A). The cells were washed once, 2 ml of 0.1 N KOH was added to each dish and the dishes were incubated overnight at 37°C. One sample of KOH was taken for radioassay (bound LDL *not* released by heparin, Panel B) and another aliquot for protein determination. Data points represent mean values in three separate dishes.

of oleic acid to bovine serum albumin was 1:1. The specific activity of the added oleic acid was 25 dpm/pmol. At the end of the incubation, the dishes were placed on ice and the cells were washed three times with phosphate-buffered saline and scraped off with a rubber policeman. The cell pellet was suspended in 0.5 ml of distilled water and sonicated, a sample for protein analysis was taken, and the remaining cells were extracted with 20 volumes of chloroform–methanol 2:1 (v/v) as described by Folch, Lees, and Sloane Stanley (24). The chloroform phase was taken to dryness, the lipid residue was redissolved in 200 μl of chloroform–methanol 2:1 (v/v), and 100 μl was applied to silica gel G TLC plates and developed in hexane–diethylether–acetic acid 80:20:1, (v/v/v). The spots for cholesteryl ester were located using I_2 vapor and scraped into scintillation fluid. Using tritiated free cholesterol as a standard, 90% recovery of radioactivity was obtained overall. The activity of ACAT was expressed as pmol cholesteryl ester formed per mg of cell protein per hour. This represents a minimum estimate since it is based on the specific radioactivity of the [^{3}H]oleic acid in the medium. The remainder of the lipid extract was used for measurement of free and total cholesterol.

Chemical analysis

Protein was measured according to Lowry et al. (25) using bovine serum albumin as a standard. The sterol content of the cells was measured by the enzymatic method reported by Gamble et al. (26).

RESULTS

The binding of human ^{125}I-labeled LDL to the cell surface was measured at 4°C in human and in rat fibroblasts after 48 hr of preincubation of the cells in LDS-containing medium (**Fig. 1**). Plotted as a function of LDL concentration in the medium, the amount of bound LDL released from human cells by heparin showed a typical biphasic curve (Panel A), the break occurring at approximately 10 μg of LDL per ml. With increasing concentrations of LDL above 10 μg/ml there was a further increase in heparin-releasable LDL but the slope was much less than at low concentrations. The amount of heparin-releasable LDL bound to rat fibroblasts over the same concentration range of LDL was less than 10% of that bound to the human cells. For example, at an LDL concentration of 5 μg/ml the heparin-releasable LDL bound to rat cells was only 6% of that bound to human cells. The relationship between heparin-releasable LDL and LDL concentration was not strictly linear in the rat fibroblast system but the curvature was considerably less striking than that seen in the human fibroblast system. In both there was cell-associated ^{125}I-labeled LDL not released by heparin (Fig. 1, Panel B). In absolute terms, the amount of this bound LDL was comparable in the two cell lines but the proportion which was heparin-releasable was considerably greater in the case of the human cells.

These data suggested that the binding of human LDL to rat fibroblasts was largely nonspecific in contrast to the predominantly specific binding of LDL to human cells, at least at low concentrations of LDL. To explore this further, binding of ^{125}I-labeled LDL at a concentration of 5 μg per ml was measured in the absence and in the presence of 500 μg per ml of unlabeled LDL. In the case of human cells, the excess unlabeled LDL reduced heparin-releasable binding to less than 8% of that in the absence of unlabeled LDL. In contrast, in the case of the rat cells the binding in the presence of unlabeled LDL was about 50% of that observed in the absence of unlabeled LDL (data not shown).

Degradation of human ^{125}I-labeled LDL in human and in rat skin fibroblasts as a function of LDL con-

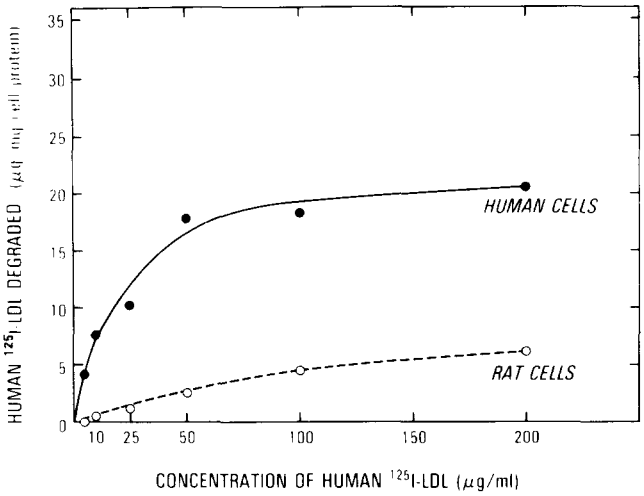

Fig. 2. Degradation of human ^{125}I-labeled LDL by human skin fibroblasts (●) and by rat skin fibroblasts (○) as a function of medium concentration of LDL. After 3 or 4 days in culture, cells were exposed to medium containing lipoprotein-deficient serum (5 mg protein/ml) for 24 hr. Then the cells were incubated in fresh LDS-containing medium with the indicated concentrations of human ^{125}I-labeled LDL for 20 hr and degradation was measured as described under Methods. Data points represent mean values for three separate dishes.

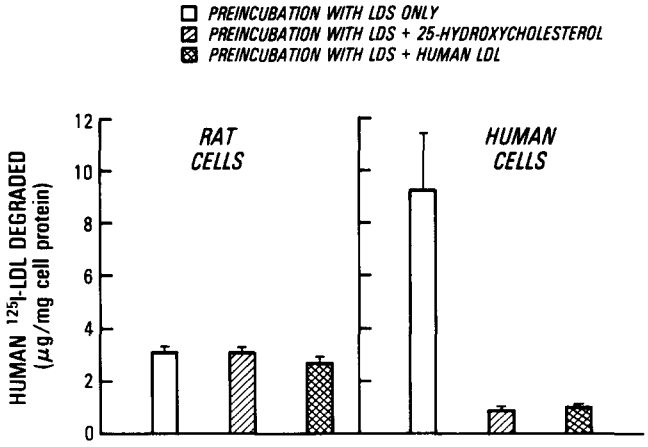

Fig. 3. Degradation of human ^{125}I-labeled LDL by rat skin fibroblasts (left panel) and by human skin fibroblasts (right panel) after preincubation in the presence of lipoprotein-deficient serum only (open bars), 25-hydroxycholesterol (10 μg/ml; hatched bars), or unlabeled human LDL (100 μg/ml; cross hatched bars). After 24 hr of incubation in medium containing lipoprotein-deficient serum (5 mg protein/ml), additions were made and incubation was then continued for an additional 24 hr at 37°C. Then human ^{125}I-labeled LDL (25 μg/ml) was added to all dishes and the amount of LDL degraded was determined after 7 hr of incubation at 37°C as described under Methods. Data represent mean values ± SD for three separate dishes.

centration in the medium is shown in **Fig. 2.** In human cells, there was at first a sharp increase in rate of degradation with increasing LDL concentration followed by a plateauing beginning between 25 and 50 μg/ml. In rat cells, degradation was much slower at all concentrations. At 5 μg/ml, degradation by rat cells was only 2 to 5% of that by human cells at the same LDL concentration.

It has been shown that the LDL receptor in human skin fibroblasts is under regulation by LDL itself and by cholesterol or more polar sterols, such as 25-hydroxycholesterol (27). This was confirmed in the present studies, as shown in **Fig. 3,** Panel B. The cells were first incubated 24 hr in medium containing LDS and then for an additional 24 hr with the additions indicated. Finally, degradation of ^{125}I-labeled LDL (25 μg/ml) over a 7-hr further incubation was measured. There was a 90% decrease in the rate of degradation in cells previously incubated either with human LDL or with 25-hydroxycholesterol (10 μg/ml). The results in rat cells were strikingly different, as shown in Fig. 3, Panel A. Previous incubation with human LDL decreased degradation rate by no more than 10 to 15%, while preincubation with 25-hydroxycholesterol had no detectable effect. These results together indicated that the binding and degradation of human LDL by rat fibroblasts was quite different from its binding and degradation by human fibroblasts. Moreover, the sites binding most of the human LDL on rat fibroblasts did not appear to be under regulation of the kind previously demonstrated for human fibroblast LDL receptors.

We then turned to a comparison of rat and human lipoproteins. As shown in **Fig. 4** (Panel A), the degradation of rat LDL by rat fibroblasts was about three times as fast as the degradation of human LDL. Moreover, degradation rate as a function of medium LDL

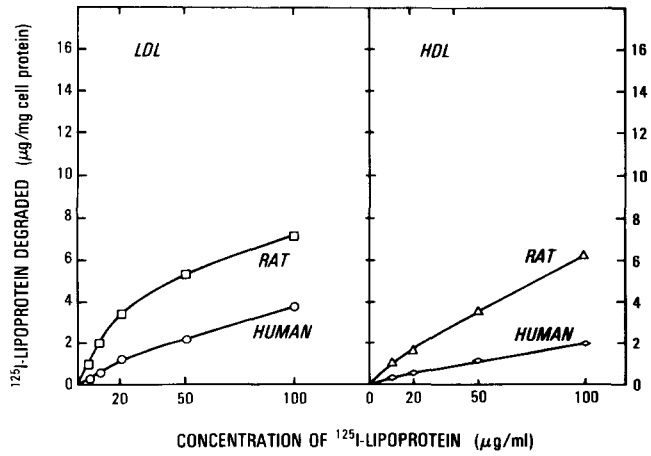

Fig. 4. Degradation of rat and of human lipoproteins by rat skin fibroblasts as a function of lipoprotein concentration. After 3 or 4 days in culture, cells were exposed to medium containing lipoprotein-deficient serum (5 mg protein/ml) for 24 hr. The cells were then washed and incubated for 20 hr at 37°C with the indicated concentrations of rat LDL (□), human LDL (○), rat HDL (△) or human HDL (◇). Degradation was determined as described under Methods. Data points represent mean values for results in three separate dishes.

TABLE 1. Degradation of human and rat ^{125}I-labeled lipoproteins by human skin fibroblasts[a]

^{125}I-labeled Lipoprotein Added	Lipoprotein Concentration	
	10 μg/ml	20 μg/ml
	Degradation (μg/mg cell protein)	
Human LDL	11.4 ± 0.8	15.3 ± 0.38
Rat LDL	6.0 ± 0.50	9.1 ± 0.51
Human HDL	0.14 ± 0.03	0.24 ± 0.02
Rat HDL	0.80 ± 0.12	1.09 ± 0.08

[a] After 3–4 days in culture in standard medium, the cells were incubated 24 hr in DME containing lipoprotein-deficient serum (5 mg/ml). Then the cells were incubated for 20 hr with ^{125}I-labeled lipoproteins at 10 or 20 μg protein/ml and degradation was measured in terms of acid-soluble noniodide ^{125}I accumulating in the medium. Data represent mean ± SEM of results in three separate dishes of cells.

concentration was decidedly curvilinear in the case of rat LDL but less so in the case of human LDL. Rat HDL was also degraded much more rapidly in rat fibroblasts than was human HDL (Fig. 4, Panel B).

A comparison of degradation rates for human and rat lipoproteins by human skin fibroblasts was also made (**Table 1**). Human LDL was degraded almost twice as rapidly as rat LDL at a medium concentration of 10 μg/ml; the difference was somewhat less at 20 μg/ml. Human HDL, however, was *not* degraded more rapidly by human cells. Quite the opposite, degradation of human HDL occurred at only about 25% the rate of degradation of rat HDL. This experiment was repeated with a second set of lipoprotein preparations and the results were essentially the same.

Regulation of HMG CoA reductase and ACAT activity by homologous and by heterologous lipoproteins

HMG CoA reductase and ACAT activity in human fibroblasts are, respectively, reduced and increased by previous incubation with human LDL. In view of the above findings it was of interest to examine responses of these two regulated enzymes to homologous and heterologous lipoproteins. Cells were incubated in DME medium containing LDS for 24 hr and then for an additional 20 hr either with 25-hydroxycholesterol, with rat or human LDL, or with rat or human HDL.

As shown in **Table 2,** 25-hydroxycholesterol depressed HMG CoA reductase activity by more than 90%, both in human and in rat cells. It stimulated ACAT activity 6-fold in human cells and 11-fold in rat cells. In human cells, human LDL (50–100 μg/ml) depressed HMG CoA reductase activity by more than 90% and stimulated ACAT activity 12-fold, in agreement with previous findings. In rat cells, however, human LDL at the same concentration had very little effect on either HMG CoA reductase (20% inhibition) or ACAT (15% stimulation). The effect of rat LDL in human cells was essentially the same as that of human LDL (over 90% depression of HMG CoA reductase and 7-fold stimulation of ACAT). The effects of rat LDL in rat cells, while qualitatively similar, were smaller (72% depression of HMG CoA reductase and 2.4-fold stimulation of ACAT).

The differences between the effects of human HDL and of rat HDL were marked (**Table 3**). Both in human and in rat cells, human HDL at a high concentration (600 μg/ml) slightly *increased* HMG CoA reductase activity (+36% and +67%, respectively). In contrast, rat HDL at the same high concentration *decreased* HMG CoA reductase markedly in both human and rat cells (−80% and −74%, respectively) and significantly *increased* ACAT activity in both cell lines (3.3-fold and 2.2-fold, respectively).

The concentration dependency of the inhibitory effect of rat HDL on HMG CoA reductase activity in rat cells is shown in **Fig. 5.** Exposure of the cells for

TABLE 2. Regulatory effects of homologous and heterologous LDL in human and in rat skin fibroblasts[a]

Additions to LDS Medium	Enzyme Activity Relative to Control (=100)			
	HMGCoA Reductase		ACAT	
	Human Cells	Rat Cells	Human Cells	Rat Cells
None	100	100	100	100
25-Hydroxycholesterol (10 μg/ml)	4.2 ± 0.8 (6)	7.6 ± 1.8 (6)	688 ± 179 (5)	1148 ± 142 (6)
Human LDL (50 μg/ml)	6.3 ± 1.1 (7)	80.0 ± 8.8 (7)	1127 ± 390 (5)	115 ± 20 (5)
Rat LDL (50 μg/ml)	5.9 ± 2.3 (2)	28.3 ± 6.3 (4)	732 ± 302 (2)	224 ± 103 (3)

[a] After 3–4 days in culture in standard medium, the cells were exposed to DME containing human LDS (5 mg protein/ml) for 24 hr. Then either 25-hydroxycholesterol, human LDL (50 μg/ml), or rat LDL (50 μg/ml) was added in fresh LDS. After 20 hr of incubation, labeled oleic acid was added and ACAT activity was measured as described under Materials and Methods. In separate dishes HMG CoA reductase was measured in cell-free extracts as described under Materials and Methods. Values in control cells incubated in DME containing only LDS were arbitrarily assigned a value of 100. Control values for HMG CoA reductase activity in human cells were 161 ± 43 (SEM; n = 7) and in rat cells 46.1 ± 14 (n = 6) pmol/mg/min; control values for ACAT activity in human cells were 253 ± 51 (n = 28) and in rat cells 149 ± 19 (n = 8) pmol/mg/hr. The data represent mean values ±SEM. Number of experiments is indicated in parentheses; triplicate dishes were analyzed in each experiment and results averaged.

TABLE 3. Regulatory effects of homologous and heterologous HDL in human and in rat skin fibroblasts[a]

Additions to LDS Medium	Enzyme Activity Relative to Control (=100)			
	HMGCoA Reductase		ACAT	
	Human Cells	Rat Cells	Human Cells	Rat Cells
None	100	100	100	100
25-Hydroxycholesterol (10 μg/ml)	4.2 ± 0.8 (6)	7.6 ± 1.8 (6)	668 ± 179 (5)	1148 ± 142 (6)
Human HDL (600 μg/ml)	136 ± 18.8 (4)	167 ± 14.1 (6)	63 ± 11.5 (4)	66 ± 11 (6)
Rat HDL (600 μg/ml)	19.8 ± 9.8 (4)	26.6 ± 3.7 (5)	332 ± 147 (4)	224 ± 43 (4)

[a] Methods and control values as described in footnote to Table 2.

20 hr to 100 μg/ml of rat HDL, a concentration well below the normal value for rat plasma HDL, suppressed reductase activity by almost 40% and in cells exposed to 600 μg/ml HDL, a concentration approximating normal rat plasma HDL levels, the inhibition was almost 80%.

Changes in cell cholesterol content following a 24 hr incubation with homologous or heterologous lipoproteins were determined (**Table 4**). Both human and rat LDL caused a marked increase in cholesterol content of human cells, consonant with the marked effect of both on reductase and ACAT activities. Human HDL had no effect on cholesterol content of human cells, in keeping with its lack of regulatory effect. In contrast, rat HDL increased cholesterol content of human cells by almost 60%, consonant with its significant regulatory effects in human cells. The changes in cholesterol content of rat fibroblasts were much smaller in all cases, again in keeping with the more modest changes seen in HMG CoA reductase and ACAT activities.

Mahley, Weisgraber, and Innerarity (8) and Weisgraber, Innerarity, and Mahley (28) have shown that the free amino groups of lysine residues play a critical role in the interaction of both LDL and HDL with the specific LDL receptor of human fibroblasts. The binding of HDL to the LDL receptor is attributable to its content of apoprotein E (29) and reductive methylation blocks this binding (8). Since rat HDL, as prepared in the present studies, had a significant content of apoprotein E, it seemed reasonable that its effects might relate to its apoprotein E moiety. As shown in **Fig. 6,** when rat HDL was reductively methylated by the methods of Mahley et al. (8), it no longer depressed HMG CoA reductase activity.

DISCUSSION

The curvilinear relationship between concentration of rat LDL and the rate of its degradation by rat fibroblasts, together with the significant regulation of HMG CoA reductase and of ACAT activity, suggested the presence in rat fibroblasts of a high affinity receptor analogous to that in human fibroblasts. However, the responses of rat fibroblasts were less marked with regard to each parameter studied. Compared at the same concentration of homologous LDL (50 μg/protein ml), degradation rate of LDL in the rat fibroblasts was about one-third that of human LDL in human fibroblasts; inhibition of reductase was about 70% instead of greater than 90%; and stimulation of ACAT was only 2-fold instead of greater than 10-fold. The smaller responses of reductase and ACAT were not due to some intrinsic limitations in the regulatory mechanisms because the responses to 25-hydroxycholesterol were similar in the two cell lines. The results are compatible with the interpretation

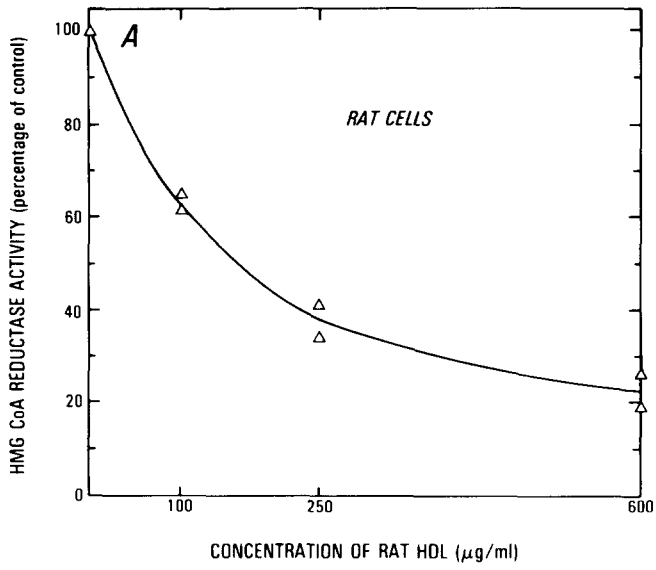

Fig. 5. Inhibition of HMG CoA reductase activity in rat skin fibroblasts after incubation with various concentrations of rat HDL. After 3 days of incubation in DME containing 10% fetal calf serum, the cells were washed and incubated with lipoprotein-deficient serum (5 mg protein/ml) for 24 hr. Rat HDL at the indicated concentrations was then added and the cells were incubated an additional 20 hr at 37°C. The cells were harvested and HMG CoA reductase activity was determined as described under Methods. The data points represent mean values of duplicate measurements taken from two separate experiments. HMG CoA reductase activity in control cells was 60.7 p mol/mg per min.

TABLE 4. Cell content of free cholesterol and esterified cholesterol in rat and human skin fibroblasts incubated with rat or human lipoproteins[a]

Lipoprotein Addition	Rat Cells		Human Cells	
	Free Cholesterol	Ester Cholesterol	Free Cholesterol	Ester Cholesterol
	(μg/mg protein)		*(μg/mg protein)*	
None	27.3 ± 1.8	2.9 ± 1.7	36.4 ± 2.3	0.9 ± 1.3
Rat LDL (50 μg/ml)	30.0 ± 2.5	6.4 ± 0.7	57.6 ± 3.6	12.9 ± 0.3
Human LDL (50 μg/ml)	29.8 ± 2.6	3.5 ± 1.5	58.9 ± 5.7	18.2 ± 1.3
Rat HDL (600 μg/ml)	30.6 ± 1.2	4.4 ± 0.6	47.3 ± 3.5	11.6 ± 1.3
Human HDL (600 μg/ml)	29.5 ± 2.7	3.0 ± 1.0	36.9 ± 3	1.2 ± 1.5

[a] The cells were cultured for 3 or 4 days (for rat and human cells, respectively) in medium containing DME and 10% FCS. Then the medium was changed to DME with human lipoprotein-deficient serum (5 mg protein/ml). After another 24 hr the cells were incubated with the indicated lipoproteins for 24 hr. The cells were washed six times with phosphate-buffered saline and scraped off the plate. Protein was determined and lipids were extracted with chloroform–methanol 2:1 and measured as described under Methods. Data represent the mean ± SEM for three to six individual dishes.

that the number of receptors on rat fibroblasts is smaller and that the rate of delivery of cholesterol by way of LDL is therefore limited. However, the results do indicate the presence of an active high-affinity lipoprotein receptor functionally similar to that previously described for other cell types (1–7). Weisgraber, Mahley, and Assman (18) have shown that electrophoresis of the d 1.02–1.063 g/ml fraction of rat plasma lipoproteins shows both a β-migrating band and an α-migrating band. The former ("LDL") contains primarily apoprotein B and the latter ("HDL_1") primarily apoprotein E. In the present studies, a d 1.02–1.045 g/ml fraction was used to reduce the amount of HDL_1 present but polyacrylamide gel electrophoresis showed that there was still a significant amount of apoprotein E present. Consequently the extent to which the observed results reflect affinity of the receptor for apo B, for apo E, or both cannot be assessed. Indirect in vivo evidence for a functioning high affinity LDL receptor in extrahepatic tissues of the rat has been presented by Anderson and Dietschy (30). They showed that rates of endogenous cholesterol synthesis in a number of tissues can be increased by drastically lowering plasma lipoprotein levels in the animal by treatment with 4-aminopyrazolo[3,4-d]pyrimidine. They showed further that intravenous infusion of LDL reduced rates of synthesis in some of the tissues examined toward control values.

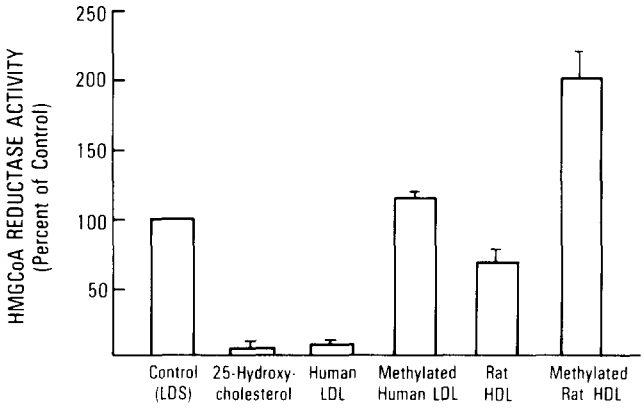

Fig. 6. Effects of reductive methylation of human LDL and of rat HDL on their ability to suppress HMG CoA reductase activity in human fibroblasts. Cell monolayers were incubated for 48 hr in DME containing lipoprotein-deficient serum (5 mg/ml). Then either 25-hydroxycholesterol (10 μg/ml), native or reductively methylated human LDL (50 μg/ml), or native or reductively methylated rat HDL (600 μg/ml) was added in fresh LDS-containing medium. After 15 hr, cells were harvested for the determination of HMG CoA reductase activity as described under Methods. Each bar represents the mean of triplicate incubations (error bar = 1 SD).

An interesting finding in the present studies was the effectiveness of rat HDL in suppressing HMG CoA reductase activity, stimulating ACAT activity and increasing cell cholesterol content in both human and rat cells. At a concentration comparable to that found in rat plasma (600 μg/ml), rat HDL had effects comparable to those of rat LDL also used at a concentration like that found in rat plasma (50 μg/ml). The responses of rat fibroblasts to human HDL were distinctly different. There was no inhibition of reductase nor stimulation of ACAT; in fact reductase was slightly enhanced and ACAT slightly inhibited. Rat HDL also inhibited reductase and stimulated ACAT in human fibroblasts.

HDL isolated from rat plasma by the methods used here contains a significant quantity of apo E, whereas HDL prepared from human plasma contains little or no apo E (31). Studies by Innerarity and Mahley (32) have established that the high-affinity receptor for LDL in human fibroblasts has a very high affinity for apo E, even higher than that for apo B. If the rat fibro-

blast also has a receptor that recognizes apo E, this would explain the present findings with rat HDL. It has been shown that reductive methylation of the epsilon lysine amino groups in apo B or in apo E reduces their ability to bind to the high affinity LDL receptor (8). In the present studies, reductive methylation of rat HDL almost completely abolished its ability to suppress HMGCoA reductase or stimulate ACAT activity. Thus, we suggest that the "anomalous" behavior of rat HDL vis-a-vis human HDL may reflect its apo E content. The possibility that other apoproteins are present whose binding also depends on lysine epsilon amino groups cannot be ruled out. The fact that rat HDL can deliver cholesterol to rat fibroblasts and thus regulate reductase and ACAT activities suggests that it may play a role in vivo analogous to that of LDL in man. The work of Gwynne et al. (33), of Anderson and Dietschy (30) and of Balasubramaniam et al. (34) indicates that at least in some specialized tissues (adrenal and ovary) HDL can indeed play such a role in vivo.

The present studies demonstrate important species differences in lipoproteins and lipoprotein receptors that could lead to incorrect generalizations when cells and lipoproteins are derived from different species. Binding and degradation of human LDL were almost an order of magnitude greater in human cells than in rat cells. The response of reductase and ACAT was also much greater in human cells. Finally, the uptake and degradation of human LDL in human cells was markedly reduced by prior incubation with human LDL or with 25-hydroxycholesterol but the uptake and degradation in rat cells was unaffected. These results imply either that the rat fibroblasts express very few high-affinity receptors for LDL or that these receptors have a configuration different from that of the receptor in human cells and therefore have a lower affinity for human LDL. Both may be true. The rate of degradation of human LDL by rat cells as a function of LDL concentration plateaued at a much lower level (Fig. 2), compatible with the presence of a limited number of receptors participating in the uptake process. Evidence for differences in the configuration of receptors in the two species of cells was also evident. Thus, human LDL was degraded more rapidly than rat LDL in human skin fibroblasts (Table 1); rat LDL was degraded more rapidly than human LDL in rat fibroblasts (Fig. 4). It should be noted that the absolute rates of degradation of LDL by rat fibroblasts were lower than the rates in human fibroblasts no matter which species of LDL is considered.

The present results show that human LDL is degraded much more slowly than rat LDL in rat fibroblasts and has little effect on endogenous cholesterol synthesis in rat fibroblasts. However, in vivo studies suggest that intravenously injected human LDL and rat LDL are metabolized at about the same rate and have similar sites of tissue degradation (35, 36). One explanation, of course, might be that the rat fibroblast under these culture conditions is not representative of the bulk of tissues catabolizing LDL in vivo. Another possibility is that human LDL, after its injection into the plasma, may acquire rat apoproteins by transfer from circulating rat lipoproteins. These homologous apoproteins (e.g., apoprotein E) could confer new specificity and determine a more rapid rate of catabolism for the heterologus LDL molecule. Similar considerations might apply to the apparent effectiveness of human LDL and HDL in regulation of cholesterogenesis by rat tissues in vivo (30, 34).

Note added in proof: Similar results have been obtained by T. L. Innerarity, R. E. Pitas, and R. W. Mahley. 1980. J. Biol. Chem. 255: 11163–11172.

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