Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters by human fibroblasts and Hep G2 hepatoma cells

Franz Rinninger and Ray C. Pittman

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

Abstract We have previously shown that the liver and steroidogenic tissues of rats in vivo and a wider range of cells in vitro, including human cells, selectively take up high density lipoprotein (HDL) cholesteryl esters without parallel uptake of HDL particles. This process is regulated in tissues of rats and in cultured rat cells according to their cholesterol status. In the present study, we examined regulation of HDL selective uptake in cultured human fibroblasts and Hep G2 hepatoma cells. The cholesterol content of these cells was modified by a 20-hr incubation with either low density lipoprotein (LDL) or free cholesterol. Uptake of HDL components was examined in a subsequent 4–6-hr assay using intracellularly trapped tracers: ^{125}I-labeled N-methyl-tyramine-cellobiose-apoA-I (^{125}I-NMTC-apoA-I) to trace apoA-I, and [^{3}H]cholesteryl oleyl ether to trace cholesteryl esters. In the case of fibroblasts, pretreatment with either LDL or free cholesterol resulted in decreased selective uptake (total [^{3}H]cholesteryl ether uptake minus that due to particle uptake as measured by ^{125}I-NMTC-apoA-I). In contrast, HDL particle uptake increased with either form of cholesterol loading. The amount of HDL that was reversibly cell-associated (bound) was increased by prior exposure to free cholesterol, but was decreased by prior exposure to LDL. In the case of Hep G2 cells, exposure to free cholesterol only slightly increased HDL particle uptake; selective uptake decreased after both forms of cholesterol loading, and reversibly bound HDL increased after exposure to free cholesterol, but either did not change or decreased after exposure to LDL. It was excluded that either LDL carried over into the HDL uptake assay or that products secreted by the cultured cells influenced these results. Thus, selective uptake by cells of both hepatic and extrahepatic origin was down-regulated by cholesterol loading, under which conditions HDL particle uptake increased. Total HDL binding was not directly correlated with either the rate of selective uptake or the rate of HDL particle uptake or the cholesterol status of the cells, suggesting more than one type of HDL binding site. —**Rinninger, F., and R. C. Pittman.** Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters by human fibroblsts and Hep G2 hepatoma cells. *J. Lipid Res.* 1988. **29:** 1179–1194.

Supplementary key words cholesterol homeostasis • reverse cholesterol transport • cholesteryl ester metabolism • apoA-I

It is generally accepted that HDL (high density lipoproteins) play an important role in the transport of cholesterol from extrahepatic tissues to the liver for reutilization or excretion into bile (reverse cholesterol transport) (1). Such a role for HDL has been reinforced by studies in cultured cells showing that HDL can accept free cholesterol from cells in a process regulated by their cholesterol status (2–5). Less attention has been paid to HDL as a vehicle for delivery of cholesterol to extrahepatic tissues, with the notable exception of rat steroidogenic tissues where a clear role has been outlined (6, 7).

Previous studies in rats have shown that the liver and steroidogenic tissues take up cholesteryl esters from HDL at a greater fractional rate than apolipoprotein A-I (apoA-I), i.e., cholesteryl esters are taken up without parallel uptake of HDL-associated apoA-I (selective uptake) (8, 9). Liver accounts for most of this cholesteryl ester uptake in excess of apoA-I uptake (9), a result supported by Arbeeny, Rifici, and Eder (10) who showed a high rate of selective uptake by perfused rat livers. These results were paralleled by high rates of selective uptake by primary cultures of rat hepatocytes (9, 11) and adrenal cells (9, 12, 13), but also by rat fibroblasts (14), a result that would not have been predicted from the in vivo studies (9). In addition, cells of other species, including human fibroblasts and human-derived Hep G2 hepatoma cells, also selectively take up HDL cholesteryl esters (15). Clearly, HDL can deliver cholesteryl esters to a variety of cell types in vitro as well as remove free cholesterol from them (2, 3). Apparent selective uptake has also been observed in perfused

Abbreviations: HDL, high density lipoprotein (d 1.063–1.21 g/ml); HDL_2, high density lipoprotein$_2$ (d 1.063–1.125 g/ml); HDL_3, high density lipoprotein$_3$ (d 1.125–1.21 g/ml); LDL, low density lipoprotein (d 1.019–1.063 g/ml); apoA-I, apolipoprotein A-I; apoE, apolipoprotein E; apoB, apolipoprotein B; NMTC, N-methyl-tyramine-cellobiose; TC, tyramine-cellobiose; ^{125}I-NMTC-apoA-I, apoA-I radiolabeled by covalent attachment of the NMTC ligand; ^{125}I-TC-LDL, LDL labeled by covalent attachment of the radioiodinated TC ligand; ^{125}I-LDL, directly radioiodinated LDL; [^{3}H]CEt, [^{3}H]cholesteryl oleyl ether; [^{14}C]SOO, [^{14}C]sucrose octaoleate; PBS, phosphate-buffered saline; BSA, bovine serum albumin; DME, Dulbecco's modified Eagle medium; CETP, cholesteryl ester transfer protein.

rabbit livers (16), suggesting that selective uptake in vivo may not be restricted to rats. Thus the process may occur in animals with high levels of LDL and plasma cholesteryl ester transfer activity and not just in animals, like rats, with very little LDL (17) and negligible plasma cholesteryl ester transfer activity (18).

In a previous study in rats, we showed that selective uptake of HDL-associated cholesteryl esters is regulated according to the cholesterol status of cells both in vitro and in vivo (14). In rat fibroblasts, cholesterol loading decreased selective uptake of HDL cholesteryl esters, whereas regulation was not observed in primary cultures of rat hepatocytes. In rats made hypocholesterolemic by either of two drug treatments, selective uptake of HDL cholesteryl esters was induced in tissues that did not exhibit the pathway in normocholesterolemic animals (skin, muscle, and adipose tissue). In normal rats, enough cholesterol is apparently available to down-regulate selective uptake in all tissues except liver and steroidogenic tissues (14).

In the present investigation we show regulation of selective uptake in human cells. To do this we have studied cells loaded with cholesterol, using LDL as well as free cholesterol. In rat fibroblasts we had used only free cholesterol because of the low levels of LDL receptor activity. Selective uptake by human fibroblasts was regulated according to their cholesterol content as it was in rat fibroblasts (14). Selective uptake by human Hep G2 hepatoma cells was similarly regulated. We also examined the possibility that products potentially secreted by Hep G2 cells (e.g., lipoproteins (19, 20), apolipoproteins (21, 22), cholesteryl ester transfer protein (CETP) (23, 24), lecithin:cholesterol acyltransferase (25)) may have contributed to, or interfered with, our measure of selective uptake or its regulation, and concluded that they did not under our experimental conditions. The relationship of selective uptake to HDL binding (26–28) measured by two approaches was also examined. The change in binding on increasing cell cholesterol levels depended, even qualitatively, on the mode of introduction of cholesterol into the cells, and total HDL binding could not be clearly associated with either increased cell cholesterol levels or with selective uptake.

MATERIALS AND METHODS

Lipoprotein and apolipoprotein preparation

Human HDL (d 1.063–1.21 g/ml), HDL_3 (d 1.125–1.21 g/ml), and LDL (low density lipoprotein, d 1.019–1.063 g/ml) were isolated ultracentrifugally from pooled plasma of healthy donors using conventional techniques (29). The various HDL preparations originated from a relatively constant pool of five or six donors. Analysis of some of these preparations showed levels of free cholesterol and ester cholesterol of 132 and 235 μg cholesterol/mg protein for HDL (n = 4), and 52 and 126 μg cholesterol/mg protein for HDL_3 (n = 3), respectively. Heparin-Sepharose (Pharmacia) affinity chromatography was used to remove any HDL particles containing apoE (30). No apoE was detected by SDS-PAGE (31) after this procedure. Lipoprotein-deficient serum (LDS) was prepared as the d > 1.25 g/ml fraction of human serum. Lipoproteins and LDS were exhaustively dialyzed against phosphate-buffered saline at pH 7.4 (PBS) containing EDTA (1 mM).

As previously described (9), apoA-I was prepared from HDL after delipidation with ethanol diethyl ether (32). The delipidated apoproteins were solubilized in 0.05 M sodium phosphate buffer (pH 7.4) containing 4 M guanidine HCl, 1 M NaCl, and 0.02% sodium azide. The apoproteins were then chromatographed on Sephacryl S-200 (Pharmacia). Fractions containing apoA-I were pooled, dialyzed against water containing 0.01% EDTA (pH 7.4), and lyophilized. Purity of apoA-I was assessed by SDS-PAGE (31).

Preparation of tracers

Purified human apoA-I was labeled with ^{125}I-NMTC-apoA-I (^{125}I-labeled N-methyl-tyramine-cellobiose apoA-I) as described elsewhere (33). [^{3}H]Cholesteryl oleyl ether was prepared from [1,2-^{3}H]cholesterol (Amersham) as previously described (15). [^{14}C]Sucrose octaoleate was prepared from [U-^{14}C]sucrose (Amersham) and purified as described in detail elsewhere (15). ^{125}I-labeled HDL_3 and ^{125}I-labeled LDL were prepared by direct iodination using Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril, Pierce) as oxidant (34). In some cases, LDL was labeled with the radioiodinated tyramine-cellobiose ligand (^{125}I-TC-LDL) (33). Specific activity in all cases was 150–600 cpm/ng apoprotein.

Preparation of doubly labeled human HDL

Human HDL and HDL_3 were labeled with [^{3}H]cholesteryl oleyl ether by exchange from donor liposomal particles using partially purified human plasma CETP as previously described (15). Donor particles were removed from the labeled HDL by flotation at d 1.063 g/ml. ^{125}I-NMTC-apoA-I was associated with the [^{3}H]cholesteryl ether-labeled HDL by exchange (15). The HDL protein/apoA-I mass ratio was at least 10. The mixture was incubated for 24 hr at 37°C. The resulting doubly labeled HDL was then separated from unbound apoA-I by flotation at d 1.21 g/ml and exhaustively dialyzed against PBS containing 1 mM EDTA. Throughout the preparative procedure and during storage, the labeled HDL was kept in 1.5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) to inhibit lecithin:cholesterol acyltransferase activity (35). Before use of the HDL, this inhibitor was removed by dialysis and the HDL was sterile-filtered (45 μm, Millipore). In some cases the doubly labeled HDL (d 1.063–

1.21 g/ml) was separated into HDL_2 and HDL_3 subfractions by flotation of HDL_2 at d 1.125 g/ml, with subsequent flotation of HDL_3 at d 1.21 g/ml. The final specific activities of doubly labeled HDL were 8–34 cpm/ng protein for ^{125}I and 5–35 dpm/ng protein for ^{3}H.

Synthetic HDL particles

Synthetic HDL particles were prepared as described in detail elsewhere (36). Briefly, cholesteryl oleate, egg phosphatidylcholine, and lipid core markers ([^{3}H]cholesteryl oleyl ether, [^{14}C]sucrose octaoleate) in near trace mass were sonicated for 30 min at 49–52°C to form microemulsion particles. The temperature was lowered to 39–42°C, and apolipoprotein A-I (including ^{125}I-NMTC-apoA-I) in 2.5 M urea was added gradually over 5–7 min during continued sonication. Particles in the HDL size range were separated from unassociated material by gel filtration chromatography on 8% agarose (Bio-Rad). Appropriate density subfractions were then prepared by equilibrium density gradient ultracentrifugation (37). Fractions in the HDL density range were pooled, dialyzed against PBS, and sterile-filtered (0.45 μm).

Human fibroblasts

Human skin fibroblasts were grown as described elsewhere (38). Cells were used between the 6th and 10th passage. Cells were plated in 60-mm plastic culture dishes (Lux) at a density of 100,000/plate. The culture medium (Dulbecco's Modified Eagle Medium, DME, Gibco) containing fetal calf serum (10% v/v, Gibco) and gentamycin (50 mg/ml) was changed every 2 or 3 days. Near confluency, usually 5–6 days after plating, the cells were washed with PBS (2×) and 2.5 ml of fresh DME containing human lipoprotein-deficient serum (2.5 mg protein/ml) was added. After 24 hr, the cells were washed again (PBS, 2×) and fresh DME was added for a 20-hr incubation to modify cell cholesterol content. This medium contained BSA (5 mg/ml, Sigma) and other additions as indicated. These were LDL or unesterified cholesterol (tissue culture grade, Sigma) in a final concentration of 1% or less ethanol, or appropriate parallel concentrations of ethanol alone. Ethanolic solutions of cholesterol were freshly prepared for each experiment. The LDL was dialyzed against DME before addition to the fibroblasts when addition volume was significant.

Hep G2 cells

Human Hep G2 hepatoma cells were cultured as described (39). Either 150,000 cells (producing plates of low cell density) or 500,000 cells (producing plates of high cell density) were plated in 60-mm plastic culture dishes (Lux). Medium (DME containing 10% v/v fetal calf serum and gentamycin (50 mg/ml)) was changed every 2 or 3 days. Usually 4–6 days after plating, the cells were washed (PBS, 2×) and incubated for 20 hr with fresh DME containing additions to modify cell cholesterol content as described for fibroblasts. This DME also contained BSA (5 mg/ml, Sigma), gentamycin, and the additions indicated in the figures and tables.

Uptake of doubly labeled HDL

The standard assay to determine the uptake of labeled HDL or synthetic HDL by cells was performed essentially as described previously (15). After the 20-hr incubation of fibroblasts or Hep G2 cells in the presence or absence of additions to modify the cell cholesterol content, the cells were washed (PBS, 4×). Fresh DME (1.5 ml/dish) containing BSA (5 mg/ml, Sigma) and the indicated labeled HDL or synthetic HDL preparation was then added for the HDL uptake assay. After incubation of the cells at 37°C for the indicated time periods, the medium was removed and the cells washed with PBS (4×). Then, DME with BSA (5 mg/ml) and unlabeled HDL (100 μg protein/ml) was added for a 2-hr "chase" period at 37°C. After this chase period, the medium (designated "chase medium") was aspirated and saved, and the cells were washed (PBS 1×). The cells were then released from the plates with 1.0 ml of trypsin/EDTA solution (0.5 g/l trypsin, 0.2 g/l EDTA, Irvine Scientific). Trypsin activity was quenched by the addition of 1.0 ml PBS containing albumin (50 mg/ml). The cell suspension was transferred to tubes with a PBS rinse of the dishes. The cells were then pelleted by centrifugation (2000 *g*, 10 min), and the supernatant ("trypsin medium") was aspirated and saved. The cell pellet was washed with PBS (10 ml). The cell pellet was dissolved in 0.1 N NaOH, sonicated, and aliquots were taken for protein determination (40), direct ^{125}I radioassay, and ^{3}H radioassay after lipid extraction according to the method of Dole (41). Cellular uptake of HDL tracers is shown as apparent particle uptake as indicated by the cell content of each tracer, as we have done previously. This apparent uptake is expressed in terms of HDL protein.

The chase and the trypsin media were centrifuged (2000 *g*, 10 min) immediately after harvest to remove possibly contaminating cells. Aliquots of each supernatant were analyzed for ^{125}I by direct radioassay. Radioactivity in these two fractions was summed and designated "reversibly cell-associated HDL." This value, too, is expressed as apparent association of HDL particles, expressed in terms of HDL protein.

In fibroblasts, the chase period removed approximately 25% more ^{125}I-NMTC-apoA-I tracer from the cells than did trypsin treatment alone. Apparent uptake of apoA-I from HDL_3 was 51.6 ± 0.6 ng HDL_3 protein/mg cell protein/6 hr after the chase and subsequent trypsin treatment versus 64.6 ± 8.9 after treatment with trypsin only.

Direct assay of ^{125}I-labeled HDL_3 binding to fibroblasts

Binding of ^{125}I-labeled human HDL_3 to human fibroblasts was assayed according to the method described by Oram (42). All of these assays used directly radioiodinated HDL_3 prepared as outlined above.

In some cases binding was assayed at 4°C (42). After overnight cholesterol loading of the cells with LDL, the cells were washed (PBS containing BSA 2 mg/ml, 2×) and fresh DME containing BSA (5 mg/ml, Sigma) was added. Cells were then incubated for 1 hr at 37°C. The cells were washed twice with PBS containing BSA (2 mg/ml) and then chilled in that medium to 4°C (15 min). The PBS was aspirated and fresh cold DME was added. This DME contained BSA (5 mg/ml) and ^{125}I-labeled HDL_3 (5 μg protein/ml), buffered with HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 20 mM/l) instead of $NaHCO_3$ (42). To determine "nonspecific" binding for each incubation condition, an excess of unlabeled HDL_3 (200 μg HDL_3 protein/ml) was added to parallel sets of plates. All plates were then incubated for 2 hr at 4°C. The medium was removed and the cells were washed 3× at 4°C with PBS containing BSA (2 mg/ml). After incubation of the cells in the final wash medium for 5 min at 4°C, the medium was aspirated, fresh PBS was added, and the cells were incubated at 4°C for 5 min. Finally, the cells were rapidly washed 2× at 4°C with PBS. The cells were then dissolved in 0.1 N NaOH and sonicated, and aliquots were taken for protein determination (40) and direct ^{125}I radioassay.

Binding assays were also carried out at 37°C as described (42). After overnight incubation to modify cell cholesterol levels, the medium was aspirated and the cell monolayers were washed 3× with PBS containing BSA (2 mg/ml). Fresh DME containing BSA (5 mg/ml) and ^{125}I-labeled HDL_3 (5 or 20 μg HDL_3 protein/ml) was then added. Some plates also received an excess of unlabeled HDL_3 (concentrations indicated in the legends). The fibroblasts were then incubated for 1 hr at 37°C, after which the plates were chilled to 4°C (10 min). The cells were washed 3× at 4°C with PBS containing BSA (2 mg/ml) and incubated in the last wash medium for 5 min at 4°C. The cells were washed again in the same medium and incubated again at 4°C for 5 min. Finally the cells were washed rapidly 2× with PBS. The final cell monolayer was then analyzed as described above for the 4°C assay.

Assay of LDL uptake

LDL uptake by human fibroblasts and Hep G2 cells was assessed using ^{125}I-TC-LDL (LDL derivatized with the radioiodinated tyramine-cellobiose ligand) (33). After the 20-hr loading incubation described above, the cells were washed 4× with PBS. The cells were then incubated for 30 min at 37°C in DME containing BSA (5 mg/ml) to allow internalization or dissociation of residual cell-associated lipoprotein. The cells were washed 2× with PBS, and ^{125}I-TC-LDL at the indicated concentrations was then added in 1.5 ml DME containing BSA (5 mg/ml). Cells were incubated for the indicated times at 37°C. The medium was removed and the cells were washed with PBS. Cells were harvested after trypsin–EDTA treatment and assayed as described above. The cell pellet was dissolved in NaOH (0.1 N) and sonicated, and aliquots were taken for protein determination (40) and direct radioassay of LDL uptake.

Cholesterol mass determination

Cell cholesterol mass was determined on separate dishes incubated in parallel with dishes used for labeled lipoprotein uptake. Cells were washed 6× with PBS, and then harvested as described for the doubly labeled HDL uptake assay except that no chase period was carried out. The cell pellet was sonicated in water, and aliquots were taken for protein determination (40) and lipid extraction according to the method described by Folch, Lees, and Sloane Stanley (43). Unesterified and total cholesterol were measured using a fluorometric assay (Huang, Kuan, and Guilbault (44) as modified by Daniels et al. (4)).

Cholesteryl ester transfer protein preparation and assay

Partially purified human plasma CETP was prepared according to the method of Morton and Zilversmit (45). The CETP was dialyzed against DME before addition to cells.

CETP activity was assayed in terms of the rate of transfer of [^{3}H]cholesteryl ether from HDL to LDL. The usual assay contained 20 μg synthetic HDL protein as [^{3}H]cholesteryl ether donor, 36 μg LDL protein as [^{3}H]cholesteryl ether acceptor, and the source of transfer activity (or mock source) in a total volume of 0.6 ml. All components were in DME containing 5 mg/ml·BSA. Samples were incubated at 37°C and aliquots (50 μl) were removed at various time periods up to 24 hr. Transfer to LDL was quantified after precipitation of LDL with heparin–manganese (46). Transfer rates were calculated according to Morton (47).

RESULTS

Studies with human fibroblasts

To modify cell cholesterol levels, human fibroblasts were first depleted of cholesterol by incubation with lipoprotein-deficient serum before cell cholesterol content was increased by incubation with either LDL, which is taken up by a well-defined receptor-mediated mechanism

(48), or with free cholesterol (introduced in ethanol) which is taken up independently of receptors (49). LDL loading increased the cholesteryl ester content of the cells predominantly, whereas exposure to free cholesterol increased both the cholesteryl ester and unesterified cholesterol levels (**Table 1**). After the loading incubation, the uptake of doubly labeled HDL was examined in a 6-hr incubation in the absence of LDL or cholesterol, after which excess unlabeled HDL was added for a "chase" incubation. The cells were then trypsin-treated. Tracer appearing in the chase medium and the medium from the trypsin treatment were summed to represent reversibly cell-associated or bound HDL.

In the first series of experiments we used doubly labeled HDL_3 to study uptake of particle components. To compare the uptake of ^{125}I-NMTC-apoA-I and [^{3}H]cholesteryl oleyl ether tracers on the same basis, uptake is shown as the HDL particle uptake (expressed in terms of HDL protein) that would be necessary to account for the observed tracer uptake (15). Expressed in this way, uptake of HDL particles is represented by equal uptakes of the two tracers.

As shown in Table 1, increasing cell cholesterol content by exposure to either lipoprotein or nonlipoprotein cholesterol led to an increase in ^{125}I-NMTC-apoA-I uptake from HDL_3. Selective uptake (determined as the difference between total [^{3}H]cholesteryl ether uptake and ^{125}I-NMTC-apoA-I uptake) decreased after either type of cholesterol loading. Total [^{3}H]cholesteryl ether uptake (selective uptake plus particle uptake) changed relatively little (not statistically significant). Reversibly cell-associated ^{125}I-NMTC-apoA-I, a measure of HDL binding, decreased after loading with LDL but increased after loading with free cholesterol, a matter addressed in greater detail below.

Fig. 1 shows the consequences of cholesterol loading with LDL as a function of LDL concentration. Down-regulation of HDL selective uptake (Fig. 1A) and up-regulation of HDL particle uptake (Fig. 1B) were seen with as little as 20 μg LDL protein/ml, and these regulatory consequences were about in parallel with the increase in cell cholesterol (Fig. 1E) and the down-regulation of LDL uptake (Fig. 1D). Reversibly cell-associated apoA-I (Fig. 1C) also decreased about in parallel with the other measured parameters.

The time course of uptake of the two HDL_3 tracers was examined (**Fig. 2**). In cholesterol-deficient fibroblasts, as well as in cells loaded with LDL or free cholesterol, the uptake of ^{125}I-NMTC-apoA-I was linear while the uptake of [^{3}H]cholesteryl ether usually was not. This nonlinearity may be due to changes in the HDL_3 particles during incubation, as shown by experiments in which a more nearly linear time course resulted from hourly changes of the medium containing labeled HDL_3. Selective uptake was down-regulated by either type of cholesterol loading throughout the time course.

The concentration dependency of uptake of doubly labeled HDL_3 is shown in **Fig. 3.** Following either type of cholesterol loading, selective uptake (the difference between ^{3}H uptake and ^{125}I uptake) decreased, while particle uptake (represented by ^{125}I uptake) increased at all HDL_3 concentrations. Eadie-Scatchard transformation of the data for selective uptake disclosed complex kinetics in the case of cells loaded with free cholesterol. In contrast, selective uptake by LDL-loaded cells and control cells produced more linear Eadie-Scatchard plots (v/[S] vs. v). The kinetic constants for selective uptake by LDL-loaded cells in the experiment shown in Fig. 3 were: apparent V_{max} = 2.2 μg HDL_3 protein/mg cell protein per 6 hr and apparent K_m = 80 μg HDL_3 protein/ml (r = 0.9949).

TABLE 1. Effects of increasing the cell cholesterol content of human fibroblasts on uptake of doubly labeled HDL_3

	LDL Loading		Free Cholesterol Loading	
	Control	LDL-Loaded	Control	FC-Loaded
Uptake of ^{125}I-NMTC-apoA-I (ng HDL_3 protein/mg cell protein)	54 ± 3.4	229 ± 28[a]	78 ± 7.0	390 ± 32[a]
Selective uptake of [^{3}H]cholesteryl ether (ng HDL_3 protein/mg cell protein)	546 ± 31	408 ± 21[a]	658 ± 49	299 ± 47[a]
Reversibly cell-associated ^{125}I-NMTC-apoA-I (ng HDL_3 protein/mg cell protein)	811 ± 170	662 ± 175[b]	662 ± 175	2083 ± 321[a]
Cell total cholesterol (μg/mg cell protein)	18.1 ± 1.6	50 ± 3.2[a]	16.9 ± 2.6	72 ± 4.3[a]
Cell free cholesterol (μg/mg cell protein)	15.9 ± 2.8	25 ± 2.9[b]	12.5 ± 1.2	42 ± 5.7[a]

Cells were exposed to LDL (200 μg protein/ml) or free cholesterol (100 μg/ml) as described under Materials and Methods. Doubly labeled HDL_3 (20 μg protein/ml) was then added for a 6-hr uptake period. Mean ± SEM are shown for 14 experiments in the case of LDL loading, and 6 experiments in the case of free cholesterol loading. Experiments used three independent preparations of labeled HDL_3 in the case of LDL loading and two in the case of free cholesterol loading.

[a] $P < 0.01$ for paired data.

[b] $P < 0.05$ for paired data.

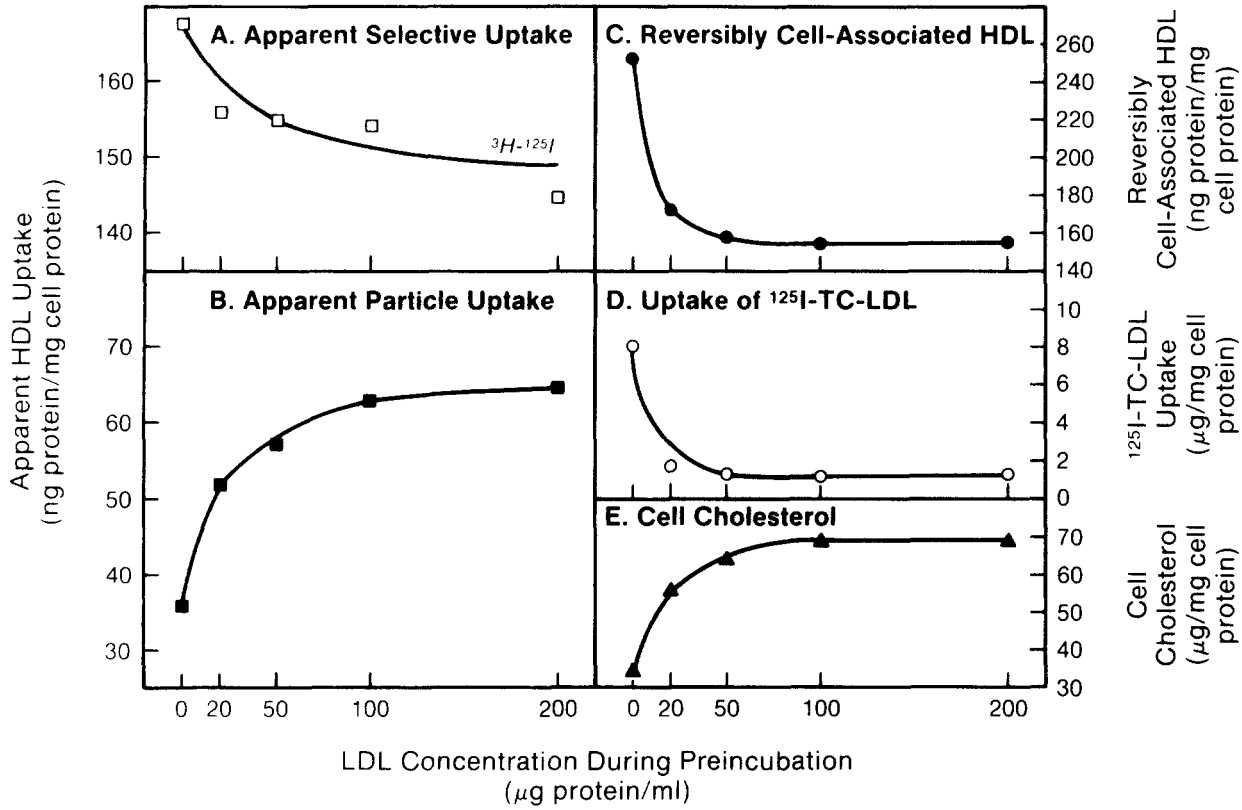

Fig. 1. Effect of prior incubation with various concentrations of LDL on the uptake of doubly labeled HDL and LDL by fibroblasts. Fibroblasts were treated with the indicated concentrations of LDL (20 hr) before assay for uptake of doubly labeled HDL (20 μg protein/ml) or uptake of ^{125}I-TC-LDL (50 μg protein/ml), as described in Materials and Methods. The uptake period was 6 hr. Apparent HDL selective uptake (A), apparent HDL particle uptake (B), reversibly cell-associated HDL (C), and LDL uptake (D) were determined as described in Materials and Methods. Total cell cholesterol (E) was determined in parallel plates before the uptake assays. Values in A, B, and C represent means of three or four replicates, values in D represent means of two or three replicates and values in E represent means of two cholesterol determinations. A similar experiment yielded similar results.

This may be compared to less linear transformed data for cells not exposed to LDL: apparent V_{max} = 3.0 and apparent K_m = 62 (r = 0.8743). The clear complexity of the Eadie-Scatchard plots of cells loaded with free cholesterol (r = 0.5423 for this plot) compared to those loaded with LDL is not explained, but suggests that more processes may significantly affect selective uptake in cells loaded with free cholesterol than in cells loaded with LDL.

As shown above (Table 1), loading the fibroblasts with nonlipoprotein cholesterol (100 μg/ml) in every case increased the amount of HDL_3 reversibly cell-associated, in agreement with the earlier studies (26, 28, 50). In contrast, and contrary to the results of the earlier studies (26), loading the cells with LDL cholesterol in every case decreased the amount of HDL_3 reversibly cell-associated, although in some experiments the decrease was small (Table 1, Fig. 1). Thus, while prior exposure to either LDL or free cholesterol had similar consequences on increasing cell cholesterol content, decreasing HDL_3 selective uptake and increasing HDL_3 particle uptake, the consequences were dissimilar with respect to reversibly cell-associated HDL_3.

The apparent disagreement between these results and earlier studies of HDL binding to fibroblasts (26) was further explored. The differences could have been due to a number of factors, such as the carry-over of competing LDL from the loading incubation into the HDL uptake incubation, the use of different binding assays, different methods for lipoprotein labeling, or differences in the cell lines studied.

To examine the possibility that in spite of extensive washing some LDL was carried over into the HDL uptake assay, in some experiments we used a post-loading (preuptake) incubation to allow dissociation or internalization of remaining surface-bound LDL before adding labeled HDL. After the loading period (20 hr), the fibroblasts were washed and then incubated for 1 or 1.5 hr in serum-free medium before adding labeled HDL. Results were not distinguishable from incubations without the post-loading incubation. Further evidence was that the decrease in reversibly cell-associated HDL was seen after

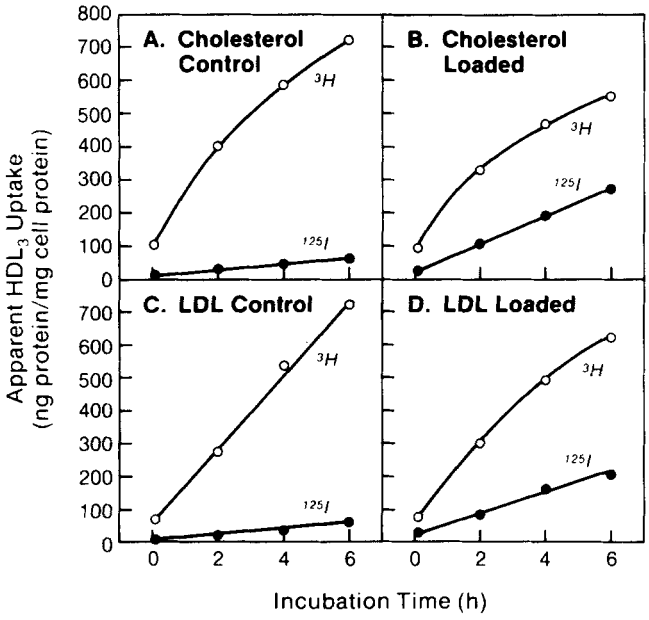

Fig. 2. Time course of uptake of HDL_3 by fibroblasts. Fibroblasts were exposed to either LDL, cholesterol, or appropriate control media for 20 hr before assay of HDL_3 uptake as described in Materials and Methods. During the 20-hr loading period, the medium in A contained 1% ethanol (v/v), and that in B contained cholesterol (100 μg/ml) in 1% ethanol (v/v); the medium in C contained no additions, and that in D contained LDL (200 μg protein/ml). During the uptake assay, doubly labeled HDL_3 was present at a concentration of 20 μg protein/ml for the indicated times. Data shown represent apparent holo-HDL uptake as indicated by [^{3}H]cholesteryl ether uptake (○), and by ^{125}I-NMTC-apoA-I uptake (●). Values are means of two or three determinations. Two similar experiments yielded similar results.

loading with as little as 20 μg LDL protein/ml, maximal inhibition was sometimes achieved with as little as 50 μg LDL protein/ml, and routinely no further inhibition of apparent binding was seen between 100 and 800 μg LDL protein/ml. To examine further the possibility of LDL carry-over, we incubated cells with ^{125}I -labeled LDL (200 μg protein/ml) for 20 hr and the amount of cell-associated, noninternalized, protein-bound ^{125}I was determined (trypsin-releasable, trichloroacetic acid-precipitable ^{125}I). This represented 1.62 ± 0.1 μg LDL protein/mg cell protein, or about 0.2 μg LDL protein/ml if free in the medium. The parallel experiments, the presence of LDL during the HDL_3 uptake assay (20 μg HDL protein/ml) did not significantly affect the amount of reversibly cell-associated HDL_3 at LDL concentrations up to 10 μg LDL protein/ml. We conclude that not enough LDL was carried over into the HDL uptake assay to explain the decreased apparent HDL binding.

As an alternative explanation for the decrease in HDL binding after exposure to LDL where an increase had been reported previously (26), we considered the possibility that qualitatively different results might be obtained at different HDL concentrations. This possibility was ruled out by data of the sort shown in **Fig. 4.** Reversibly cell-associated HDL_3 decreased after LDL loading at every HDL_3 concentration, but increased at every concentration after loading with free cholesterol.

To see whether the decrease in apparent HDL binding after LDL treatment might depend on the method used to assess binding, we also carried out studies in which binding was assayed by the methods described in studies reporting increased HDL_3 binding after LDL uptake (26, 42) (**Table 2**). To do this, cells were loaded with LDL or free cholesterol as described. Directly radioiodinated HDL_3 was then used to determine saturable binding after a 1-hr incubation at 37°C, or after a 2-hr incubation at 4°C (42). After LDL loading, ^{125}I-labeled HDL_3 binding determined at either 4°C or 37°C decreased, whereas binding increased after loading with free cholesterol. Both results are in agreement with binding measured in terms of reversibly cell-associated ^{125}I-NMTC-apoA-I tracer. Thus, no matter which binding assay was used, pretreating cells with LDL led to decreased HDL_3 binding.

We also investigated selective uptake from HDL isolated in a broader density range (d 1.063–1.21 g/ml). Fibroblasts selectively took up [^{3}H]cholesteryl ether from this HDL, although the rate of this uptake (either in terms

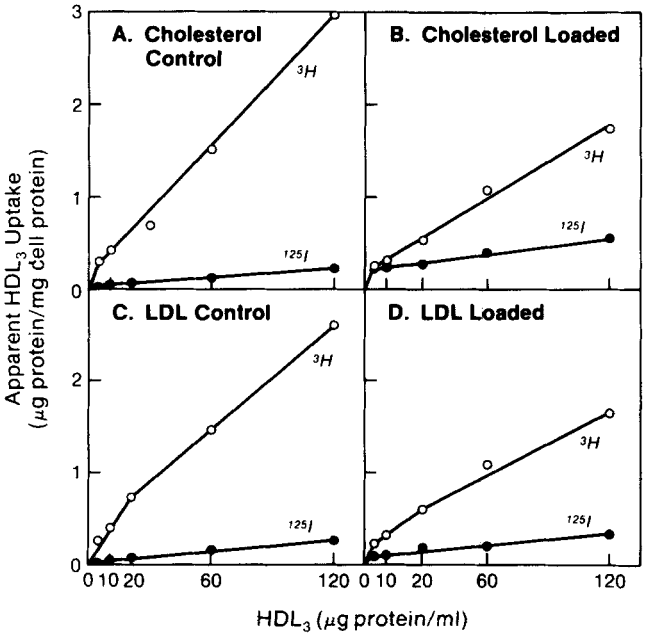

Fig. 3. Uptake of HDL_3 by fibroblasts as a function of HDL_3 concentration. Fibroblasts were exposed to either LDL, cholesterol, or appropriate control media for 20 hr before assay of HDL_3 uptake as described in Materials and Methods. During the 20-hr loading period, the medium in A contained 1% ethanol (v/v), and that in B contained cholesterol (100 μg/ml) in 1% ethanol (v/v); the medium in C contained no additions, and that in D contained LDL (200 μg protein/ml). During the HDL_3 uptake assay, cells were incubated with the indicated concentrations of doubly labeled HDL_3 for 6 hr. Apparent holo-HDL uptake as indicated by [^{3}H]cholesteryl ether uptake (○), and as indicated by ^{125}I-NMTC-apoA-I uptake (●), is shown. Values are means of duplicate or triplicate determinations.

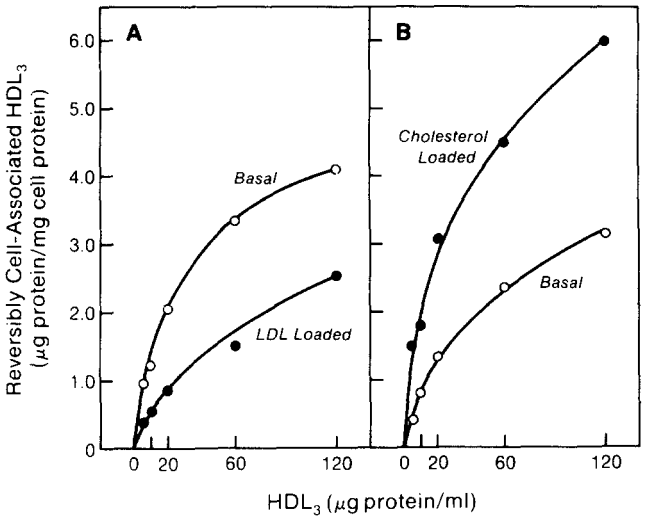

Fig. 4. Reversible cell-association of HDL_3 with fibroblasts as a function of HDL_3 concentration. Fibroblasts were exposed to either LDL or cholesterol or appropriate control conditions for 20 hr before addition of HDL_3 labeled with ^{125}I-NMTC-apoA-I. Reversibly cell-associated ^{125}I was determined as outlined under Materials and Methods. In A, cells were incubated either with no additions or with LDL (200 μg protein/ml). In B, cells were incubated either with 1% ethanol (v/v) added or with cholesterol (100 μg/ml) added in 1% ethanol (v/v). Incubation with doubly labeled HDL_3 was for 6 hr. Values are means of duplicate or triplicate incubations.

of apparent particle uptake or cholesteryl ester mass uptake) was lower than in the case of HDL_3 (data not shown). On loading the cells with LDL or cholesterol, selective uptake again decreased and apparent particle uptake increased. In agreement with the data for HDL_3, the amount of HDL reversibly cell-associated decreased after exposure to LDL, but increased after exposure to free cholesterol. Results were qualitatively similar over a range of LDL concentrations during the loading incubation from 20 to 800 μg protein/ml, as they were for levels of free cholesterol of either 50 or 100 μg/ml.

Selective uptake of HDL-cholesteryl ester by Hep G2 hepatoma cells

To modify the cell cholesterol content of Hep G2 cells, the cells were incubated with either LDL (usually 200 μg protein/ml, representing about 430 μg cholesterol/ml) or free cholesterol (usually 100 μg/ml), as described above for fibroblasts. As with fibroblasts, LDL increased predominantly cellular cholesteryl ester content, whereas free cholesterol increased both cellular free and ester cholesterol content (**Table 3**). These increases were less pronounced than in the case of human fibroblasts under similar conditions.

After the loading incubation, the uptake of doubly labeled HDL_3 and its reversible association with the cells were determined. As shown in Table 3, loading the cells with either LDL or free cholesterol increased slightly, but significantly, the uptake of HDL_3-associated ^{125}I-NMTC-apoA-I. This was in contrast to the several-fold increase in the case of fibroblasts. As was true for fibroblasts, selective uptake of [^{3}H]cholesteryl ether in every case decreased after cholesterol loading. Total uptake of cholesteryl ethers (selective uptake plus particle uptake) decreased after LDL loading (from 173 ± 5.3 to 109 ± 6.9, mean ± SEM, $P < 0.001$), and decreased much less but still significantly after loading with free cholesterol

TABLE 2. Saturable binding of ^{125}I-labeled HDL_3 to fibroblasts

Exp. No.	^{125}I-Labeled HDL_3	Temp.	Time	Saturable ^{125}I-Labeled HDL_3 Binding: LDL in Preincubation (μg LDL protein/ml) 0	100	200	Cholesterol in Preincubation (μg/ml) 0	50	100
	μg protein/ml		*hr*	*ng HDL_3 protein/mg cell protein*					
I	5	4°C	2	39.1 ± 7.3 (21.1)	3.8 (36.8)	15.1 ± 3.7 (40.6)			
II	5	37°C	1	162 ± 41 (20.8)		95 ± 31 (30.6)	90 ± 24 (23.8)		175 ± 10 (40.3)
II	20	37°C	1	384 (20.8)		231 (30.6)			
III	5	37°C	1	351 ± 81 (26.4)	230 ± 14 (33.0)		221 ± 52 (32.1)	262 ± 16 (51.0)	328 ± 11 (100.2)
III	20	37°C	1	1172 (26.4)	457 (33.0)		359 (32.1)	745 (51.0)	601 (100.2)

Human fibroblasts were loaded with LDL or cholesterol and then binding of ^{125}I-labeled HDL_3 was measured at the indicated concentration, temperature, and time conditions as described in detail in Materials and Methods. Nonspecific binding, i.e., binding of ^{125}I-labeled HDL_3 in the presence of an excess unlabeled HDL_3 (200 μg HDL_3 protein/ml in experiments I and II, 300 μg/ml in experiment III), was determined for each loading condition and subtracted from total binding of ^{125}I-labeled HDL_3. Parallel sets of dishes were used to determine cell cholesterol mass. Values represent means of triplicate (mean ± SD) or duplicate (mean) incubations. Cell unesterified cholesterol levels for each experiment are shown in parentheses (μg cholesterol/mg cell protein).

TABLE 3. Effect of increasing cell cholesterol on uptake of doubly labeled HDL_3 by dense cultures of Hep G2 cells

	LDL Loading		Free Cholesterol Loading	
	Control	LDL-Loaded	Control	FC-Loaded
Uptake of ^{125}I-NMTC-apoA-I (ng HDL_3 protein/mg cell protein)	52 ± 3.8	58 ± 4.2[a]	63 ± 1.3	67 ± 1.7[a]
Selective uptake of [^{3}H]cholesteryl ether (ng HDL_3 protein/mg cell protein)	121 ± 3.3	59 ± 4.1[a]	117 ± 6.5	97 ± 9.4[a]
Reversibly cell-associated ^{125}I-NMTC-apoA-I (ng HDL_3 protein/mg cell protein)	59 ± 6.9	54 ± 5.9[b]	55 ± 3.7	82 ± 6.3[a]
Cell total cholesterol (μg/mg cell protein)	23.5 ± 1.0	37.3 ± 0.8[a]	22.5 ± 0.8	46.5 ± 2.8[a]
Cell free cholesterol (μg/mg cell protein)	18.8 ± 0.2	22.7 ± 0.5[b]	18.1 ± 1.1	33.7 ± 2.9[a]

Cells, grown to high cell density as outlined under Materials and Methods, were exposed to LDL (200 μg protein/ml), or free cholesterol (100 μg/ml) or appropriate mock conditions for 20 hr before washing and adding doubly labeled HDL_3 (20 μg protein/ml) for a 4-hr incubation period. Mean ± SEM are shown for ten experiments in the case of LDL loading, and six in the case of loading with free cholesterol. In both cases three preparations of labeled HDL_3 were used.

[a] $P < 0.01$ for t test of paired data.

[b] Not significant.

(from 180 ± 7.5 to 164 ± 10.9, $P < 0.02$). A qualitatively similar pattern of HDL tracer uptake was observed over a wide range of LDL concentrations during the loading incubation (20–300 μg LDL protein/ml) and at either 50 or 100 μg free cholesterol/ml.

As was true with fibroblasts, the effects of the two types of cholesterol loading on the amount of HDL_3 that was reversibly cell-associated were dissimilar (Table 3). Treatment with free cholesterol resulted in an increase in reversibly cell-associated HDL_3, as recently reported (51), while there was not a significant change after exposure to LDL.

The data shown in Table 3 were gathered using Hep G2 cells at high density on the culture plates (1.17 ± 0.42 (SD) mg cell protein/60-mm plate). Cells were dense but did not pile up. (These cells do not become confluent before piling up.) Results were qualitatively similar but quantitatively quite different when cells were plated at lower density and examined at the same time after plating as the denser cultures. Cell density at termination of the experiments was 0.216 ± 0.01 mg cell protein/60-mm plate. The uptake of ^{125}I-NMTC-apoA-I was not different from that observed with the dense cultures, and the small increase in ^{125}I-NMTC-apoA-I uptake seen after LDL loading did not reach significance in this modest series of experiments. In contrast, selective uptake of [^{3}H]cholesteryl ether was much higher in the cells at lower density, and down-regulation was more dramatic after cholesterol loading. The amount of reversibly cell-associated ^{125}I-NMTC-apoA-I was also much greater than in the denser cultures, and there was a significant decrease in this parameter after LDL loading (511 ± 29 vs. 333 ± 29 ng protein/mg cell protein, $P < 0.001$) that had not been observed in the denser cultures. No such differences between cells of high and low density were observed in the case of fibroblasts.

The time course of uptake of HDL_3 by Hep G2 cells was examined (**Fig. 5**). As with fibroblasts, uptake of the ^{125}I-NMTC-apoA-I moiety was linear, whereas uptake of [^{3}H]cholesteryl ether was somewhat nonlinear. This was true both for the cells that had been cholesterol-loaded by exposure to LDL and for those that had not. Again, hourly changes of the medium containing fresh labeled HDL produced a linear rate of [^{3}H]cholesteryl ether uptake (data not shown). Down-regulation of selective uptake was evident after only 10 min of incubation with doubly labeled HDL_3.

Broad density range HDL (d 1.063–1.21 g/ml) was also studied in Hep G2 cells at high cell density. Regulation of HDL metabolism by pretreatment of the cells with LDL was qualitatively similar to the results with HDL_3 (data

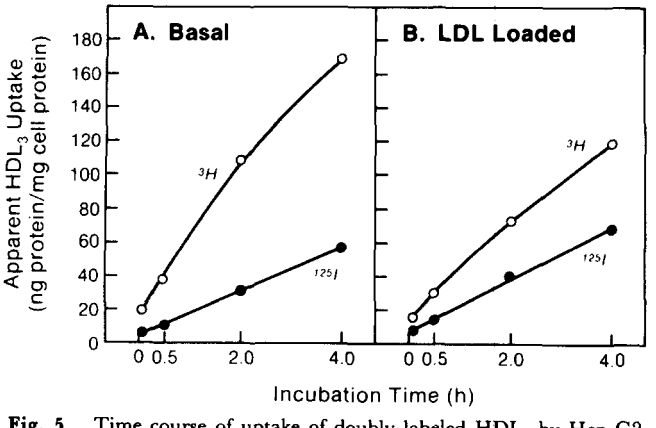

Fig. 5. Time course of uptake of doubly labeled HDL_3 by Hep G2 cells. Hep G2 cells at high density were incubated for 20 hr with 200 μg LDL protein/ml (LDL-loaded) or without LDL (basal) before assay of HDL_3 uptake. HDL_3 concentration was 20 μg protein/ml. Apparent holo-HDL uptake as indicated by [^{3}H]cholesteryl ether (○), and as indicated by ^{125}I-NMTC-apoA-I (●) is shown for each time point. Values are means of duplicate or triplicate determinations. Another similar experiment yielded similar results.

not shown). Selective uptake was down-regulated, but there was not a significant increase in ^{125}I-NMTC-apoA-I uptake. However, reversibly cell-associated ^{125}I-NMTC-apoA-I was decreased by LDL pretreatment, as had been observed in the case of HDL_3 uptake by sparse cultures. In a limited series of experiments using Hep G2 cells pretreated with free cholesterol, the increase in cell cholesterol was again accompanied by an increase in reversibly cell-associated HDL, as had been the case for Hep G2 cells exposed to HDL_3 and for fibroblasts exposed to either HDL_3 or HDL.

As was the case with fibroblasts, we considered the possibility that LDL carried over from the preincubation period influenced the results above, and similar approaches were used to examine the question. A post-loading, pre-uptake incubation of 1 hr again altered none of the parameters of HDL uptake. In a related experiment shown in **Table 4,** medium containing the doubly labeled HDL_3 was changed hourly during the 4-hr uptake period giving ample time for any residual LDL to be either internalized or dissociated and removed. ^{125}I-NMTC-apoA-I uptake was not changed by this maneuver; [^{3}H]CEt uptake and selective uptake were increased, but only in parallel with cells that had not been exposed to LDL. In other experiments, Hep G2 cells were exposed to LDL (200 μg protein/ml) for only 30 min or 60 min at 37°C, times thought adequate to allow near steady-state binding without significant alteration in cell cholesterol content. No significant effect of this brief exposure was observed on any of the parameters of HDL_3 uptake. As a final test, the amount of LDL bound to Hep G2 cells was determined after 20 hr incubation with ^{125}I-labeled LDL (200 μg protein/ml), as described earlier for fibroblasts. This was equivalent to about 0.2 μg LDL protein/ml incubation medium; LDL added to the HDL_3 uptake assay at concentrations up to 10 μg LDL protein/ml did not change any of the parameters of HDL_3 uptake, including the amount of HDL_3 reversibly cell-associated.

It was suggested by Granot, Tabas, and Tall (52) that human cholesteryl ester transfer protein (CETP), which mediates the exchange of cholesteryl esters and triglycerides between lipoprotein particles in plasma (53), can also mediate the direct uptake of cholesteryl esters from HDL to various cells in vitro, including Hep G2 cells and human fibroblasts. In addition, it has been shown that Hep G2 cells secrete CETP that appears to be identical to plasma CETP (23, 24). The possibility was considered that CETP might contribute to the selective uptake observed in Hep G2 cells. To test this, one approach was to see whether added CETP enhanced the selective transfer of HDL cholesteryl esters into cells. We incubated Hep G2 cells and human fibroblasts in the presence of partially purified CETP and labeled HDL or synthetic HDL. The concentration of CETP used was double that which we used in our usual procedure for incorporating [^{3}H]cholesteryl ether into HDL (or HDL_3) using 10–20 mg HDL protein (15). This amount of CETP was directly assayed for transfer activity in parallel with medium that had been exposed for 5 hr to Hep G2 cells, and was shown to have four times the activity of the conditioned medium. Addition of this amount of CETP did not increase the uptake of HDL-associated [^{3}H]cholesteryl ether in either Hep G2 cells or fibroblasts (data not shown).

It was also possible that lipoproteins secreted by Hep G2 cells (19, 20), acting in conjunction with secreted CETP (23), might contribute to our measure of selective uptake. CETP might mediate the transfer of [^{3}H]cholesteryl ether tracer to apoB- or apoE-containing lipoproteins secreted by the cells which were then rapidly taken up into the cells again. One approach to examine this possibility was to block uptake of lipoproteins by apoB,E receptors. To do this, heparin (54) was added to the medium during incubation of Hep G2 cells with doubly labeled HDL_3 (**Table 5**). Heparin inhibited uptake of LDL by about 90%. It should be noted that heparin also substantially inhibits uptake of HDL enriched in apoE, and

TABLE 4. Effect of frequent medium changes on uptake of HDL_3 tracers by untreated and LDL-loaded Hep G2 hepatoma cells

Additions during Loading Period	Total Cell Cholesterol	Medium Changes	Apparent HDL_3 Uptake		Selective Uptake
			^{125}I-Labeled ApoA-I	[^{3}H]CEt	^{3}H − ^{125}I
	μg/mg cell protein		*ng HDL_3* protein/mg cell protein per 4 hr		
None	21.8	None	56.4	242	186
None	21.8	Four mock changes	56.0 ± 6.0	249 ± 14	193 ± 18
None	21.8	Four changes	53.9 ± 2.1	296 ± 15	242 ± 13
LDL (200 μg protein/ml)	31.4	Four mock changes	54.9 ± 2.6	191 ± 8	136 ± 6
LDL (200 μg protein/ml)	31.4	Four changes	60.2 ± 4.8	265 ± 24	205 ± 21

Hep G2 cells at low cell density were exposed to LDL or to mock loading conditions for 20 hr, as described under Materials and Methods, before assay of HDL_3 tracer uptake during a 4-hr incubation. The 4-hr uptake was either conducted as usual (no medium changes), or the medium containing labeled HDL_3 was replaced hourly (four changes), or plates were handled exactly as those receiving medium changes but without the changes (four mock changes). Data are means of triplicate (± SD) or duplicate plates.

TABLE 5. Effect of heparin on the uptake of HDL_3 and LDL by Hep G2 cells

Additions during Loading Period	Total Cell Cholesterol	Labeled Lipoprotein	Apparent Lipoprotein Uptake in the Absence of Heparin		Apparent Lipoprotein Uptake in the Presence of Heparin	
			[^{3}H]CEt	^{125}I-Labeled Apoprotein	[^{3}H]CEt	^{125}I-Labeled Apoprotein
	μg/mg cell protein		*ng protein/mg cell protein*			
None	24.4	HDL_3	195 ± 10	61.5 ± 2.9	179 ± 4	24.9 ± 2.1
None	24.4	LDL		1403 ± 20		80.4 ± 9.8
LDL (200 μg protein/ml)	36.5	HDL_3	137 ± 4.7	66.7 ± 2.7	137 ± 7	32.2 ± 1.2
LDL (200 μg protein/ml)	36.5	LDL		370 ± 7		27.4 ± 1.6

Hep G2 cells at high density were exposed to LDL (200 μg protein/ml) or appropriate mock conditions before a 4-hr assay for uptake of doubly labeled HDL_3 or ^{125}I-TC-LDL, as described under Materials and Methods. Heparin (10 mg/ml) was added during the uptake assay where indicated. Cell cholesterol levels were determined in parallel sets of plates prior to the HDL_3 uptake assay. Values shown are the mean ± SD in cases of three or more replicate plates, or simply the mean in the case of duplicate plates.

blocks the reuptake of secreted buoyant lipoproteins to which [^{3}H]CEt transfers on addition of purified CETP (Rinninger, F., and R. C. Pittman, unpublished results). However, heparin had little effect on uptake of [^{3}H]cholesteryl ether in the experiments of Table 5. Uptake of ^{125}I-NMTC-apoA-I was more inhibited (about 50%, as previously reported (15)). In those instances where heparin slightly decreased [^{3}H]cholesteryl ether uptake, the effect could be entirely attributed to decreased HDL_3 particle uptake, and selective uptake was not at all decreased. This argues against involvement of apoB,E lipoprotein receptors in apparent selective uptake. It does, however, suggest that apoB,E receptors play a role in uptake of ^{125}I-NMTC-apoA-I and thus of HDL particles by these cells. Evidence to be presented elsewhere suggests that the HDL particle uptake blocked by heparin is mediated by apoE secreted by the Hep G2 cells and acquired by HDL (Rinninger, F., and R. C. Pittman, unpublished results). In similar studies in human fibroblasts (not shown), no such decrease in ^{125}I-NMTC-apoA-I uptake was seen in the presence of heparin. The failure of heparin to inhibit selective uptake also argues against the involvement of cell-bound lipoprotein lipase in the uptake of HDL-associated [^{3}H]cholesteryl ether, a process reported to be involved in the uptake of chylomicron lipids and inhibited by heparin (55).

Secretion of apolipoprotein A-I by Hep G2 cells has been reported (19, 22). To examine the possibility that secretion of this or any other factor by the Hep G2 cells altered apoprotein specific activity and thus distorted our measurement of HDL selective uptake, we made use of synthetic HDL particles which allowed measurement of selective uptake without reference to a coat apoprotein. These synthetic particles composed only of human apoA-I, cholesteryl oleate, egg yolk phosphatidylcholine, and tracers, resemble native HDL particles in size, density, and metabolism (15, 36). ApoA-I was traced by ^{125}I-NMTC-apoA-I and cholesteryl oleate by [^{3}H]cholesteryl oleyl ether. The particles also contained a very large non-polar, nontransferable tracer, [^{14}C]sucrose octaoleate which is excluded from selective uptake in rats and mouse Y1-BS1 adrenal cells and which serves as a marker of the particle core per se (15, 36). Several density classes of synthetic HDL were examined in this way (**Table 6**). Particle uptake by Hep G2 cells was similar in all density fractions whether measured in terms of ^{125}I-NMTC-apoA-I or [^{14}C]sucrose octaoleate, and selective uptake was consequently similar. The same result was obtained in parallel studies with human fibroblasts, also shown in Table 6. Besides ruling out dilution of apoA-I specific activity as significantly contributing to selective uptake, these results also indicate that apoA-I is a sufficient apolipoprotein for the selective uptake by Hep G2 cells and human fibroblasts, a result previously demonstrated in mouse Y1-BS1 adrenocortical tumor cells (36).

Table 6 makes another unrelated point. We have previously shown in mouse Y1-BS1 adrenocortical tumor cells that cholesteryl esters are selectively taken up from all HDL density subfractions, but at a progressively greater fractional rate from particles of progressively higher density (36). Table 6 shows a similar result for uptake by human fibroblasts and Hep G2 cells. [^{3}H]Cholesteryl ether was taken up by both cell types at a greater rate from denser synthetic particles. Apparent uptake of HDL particles by Hep G2 cells, represented by uptake of either ^{125}I-NMTC-apoA-I or [^{14}C]sucrose octaoleate, was somewhat higher for more buoyant HDL particles than for the denser particles. Such preferential uptake of more buoyant fractions is even more pronounced in the case of Y1-BS1 mouse adrenocortical tumor cells (36). In contrast to both the Y1-BS1 cells and Hep G2 cells, human fibroblasts preferentially took up denser synthetic HDL particles.

Table 4, discussed above in the context of a possible carry-over of LDL into the HDL uptake assay, also addresses in a more general way possible effects of products secreted by Hep G2 cells. In the case of any interfering secretory product, the effect should depend on

TABLE 6. Uptake of synthetic HDL by Hep G2 hepatoma cells and human fibroblasts

Synthetic HDL Fraction	Apparent Particle Uptake			Selective Uptake	
	^{125}I-Labeled ApoA-I	[^{3}H]CEt	[^{14}C]SOO	^{3}H – ^{125}I	^{3}H – ^{14}C
g/ml	*ng apoA-I/mg cell protein*				
Hep G2 cells					
1.068	64.8	266.0	49.5	201	217
1.088	57.2	338.2	49.0	281	289
1.104	52.3	395.9	43.2	344	353
1.142	52.7	524.9	40.7	472	484
Human fibroblasts					
1.068	19.7	263.0	13.6	243	249
1.088	26.5	335.8	16.7	309	319
1.104	36.4	382.4	18.0	346	364
1.142	40.9	513.4	22.7	473	491

Cells were incubated for 20 hr in DME with 5 mg/ml albumin before addition of triply labeled synthetic HDL of the indicated modal density at a concentration of 20 μg protein/ml. Hep G2 cells at low density were incubated for 4 hr with the labeled HDL, and fibroblasts for 6 hr. All density fractions were from a single preparation of synthetic HDL separated by density gradient ultracentrifugation. The indicated densities are the modal densities of each subfraction which spanned a density range of no more than 0.04 g/ml. Tracers included [^{14}C]sucrose octaoleate ([^{14}C]SOO) as well as [^{3}H]cholesteryl oleyl ether and ^{125}I-NMTC-apoA-I. Values are means of duplicate plates. Duplicates varied <5% in the case of Hep G2 cells, and <10% in the case of fibroblasts.

the quantity of interfering substances accumulating in the medium and thus on the time of incubation. In the experiments of Table 4, tracer uptake during a 4-hr incubation was compared to parallel incubations during which the medium containing labeled HDL_3 was changed every 1 hr, or carried through mock medium changes. There was no significant change in uptake of ^{125}I-NMTC-apoA-I under any conditions, and no decrease in [^{3}H]cholesteryl ether uptake due to frequent medium changes. The modest increase in [^{3}H]cholesteryl ether uptake due to the frequent medium changes may relate to the nonlinearity of the time course of [^{3}H]cholesteryl ether uptake pointed out earlier. Thus there was no evidence for any type of interfering secretory product accumulation in the incubation medium.

DISCUSSION

Selective uptake of HDL cholesteryl esters by fibroblasts was down-regulated by prior cholesterol loading using either LDL or free cholesterol, while apparent particle uptake (measured in terms of apoA-I uptake) increased several-fold. These results for human fibroblasts are parallel to results for rat fibroblasts (14).

However, the results for Hep G2 human hepatoma cells were different from both human and rat fibroblasts in that the large increases in particle uptake seen in cholesterol-loaded fibroblasts were not seen in cholesterol-loaded Hep G2 cells (**Table 7**). Furthermore, the results for Hep G2 cells were also different from results for primary rat hepatocytes (Table 7). Cholesterol loading of Hep G2 cells resulted in clear down-regulation of selective uptake and a marginal increase in ^{125}I-NMTC-apoA-I uptake; regulation of neither of these parameters could be demonstrated in rat hepatocytes even though significant increases in cell cholesterol were achieved (14). It is not known whether this difference between Hep G2 cells and rat hepatocytes represents a true species-specific difference, a difference between cells in primary and continuous culture, or a difference between normal and tumor cells.

In the case of Hep G2 cells, total [^{3}H]cholesteryl ether uptake decreased on cholesterol loading in spite of a small increase in HDL particle uptake. In the case of cholesterol-loaded fibroblasts, total [^{3}H]cholesteryl ether uptake was *not* significantly changed because an increase in particle uptake after cholesterol loading almost compensated for the decrease in selective uptake. This does not neces-

TABLE 7. Summary of the effects of cholesterol loading on HDL metabolism by human and rat cells

	Human		Rat	
	Hep G2[a]	Fibroblasts	Hepatocytes	Fibroblasts
Selective uptake				
FC loading	↓[b]	↓[b]	→[c]	↓[c]
LDL loading	↓	↓		
Particle uptake				
FC loading	→(↑)[b]	↑[b]	→[c]	↑[c]
LDL loading	→(↑)[b]	↑[b]		
Binding				
FC loading	↑[b]	↑[b]	→[d]	↑[d]
LDL loading	→(↓)[b]	↓[b]		

[a]Arrows not in parentheses are for cells at high density, and arrows in parentheses are for cells at low density.
[b]Data from the present study.
[c]Data from Reference 14.
[d]Unpublished results (Rinninger, F., and R. C. Pittman).

sarily mean that down-regulation of selective uptake in fibroblasts is obligatorily accompanied by a compensating increase in HDL particle uptake. Earlier studies (15) have indicated that selective uptake and particle uptake involve different mechanisms, and the relative expression of these pathways may vary under other conditions. Many factors that might differentially modulate the two pathways were not considered here.

This study has focused on cellular uptake of HDL components, but this uptake did not result in a net delivery of cholesterol to the cells. Many studies have shown that exposure to HDL results in a net efflux of free cholesterol from various cultured cells in a process regulated according to the cell cholesterol status (2–5). In the present study the cholesterol content of cholesterol-loaded cells did decrease during the 4- or 6-hr exposure to HDL; total cell cholesterol of Hep G2 cells previously exposed to 200 μg LDL protein/ml fell a mean of 13% while the cholesterol content of similarly treated fibroblasts fell 20%. The cholesterol content of basal cells did not significantly change on exposure to HDL. However, HDL can deliver cholesteryl esters in a net fashion to some cells under some conditions. For example, ACTH-stimulated mouse adrenocortical tumor cells take up HDL cholesteryl ester predominantly by selective utpake without evidence for appreciable efflux of free cholesterol from the cells (15). There is also evidence that cultured adrenal cells (56) and hepatocytes (57) can, under some conditions, take up HDL free cholesterol in a net fashion without particle uptake. It seems, then, that the net change in cellular cholesterol due to exposure to HDL is the resultant of at least three pathways, all of which may vary as a function of cell cholesterol status: the flux of free cholesterol; selective uptake of HDL cholesteryl esters; or uptake of HDL particles. These pathways need not be regulated in strict parallel, and net cholesterol flux may be modulated by any of them. In the present studies, the resultant was a net mass efflux after cholesterol loading, but no significant net change in basal cells.

The increase in HDL particle uptake resulting from cholesterol loading of fibroblasts was unexpected. The maintenance of cholesterol homeostasis would seemingly best be served by decreased HDL particle uptake by such cholesterol-rich cells. However, HDL particle uptake remained small relative to total binding, in agreement with the work of others (26, 50). The mechanism of this increased uptake per binding is not known.

We have not previously addressed the relationship between HDL binding and selective uptake directly. Saturable binding of HDL to several cell types has been described (26–28, 50), and several HDL binding proteins have been isolated and postulated to represent HDL receptor proteins (50). Saturable HDL binding to cells (26, 28) and the cellular level of an HDL binding protein (50) have been shown to increase on cholesterol loading. This binding may not directly lead to increased acceptance of free cholesterol by HDL (58), but may be involved in mobilization of cholesterol to the plasma membrane (59). We found an increase in HDL binding after exposing fibroblasts and Hep G2 cells to free cholesterol, as have others (26, 28, 51). In contrast, we observed a significant *decrease* in binding to fibroblasts after exposure to LDL. This occurred even after uptake of an amount of LDL sufficient to down-regulate both LDL uptake and selective uptake, and to up-regulate HDL particle uptake. These binding data, determined in terms of reversibly cell-associated tracer, were corroborated using the same methodology employed in previous studies that showed *increased* HDL_3 binding by fibroblasts after uptake of LDL (26, 42) and increased HDL_3 binding to aortic endothelial cells after uptake of acetylated LDL (60). It is possible that the increased binding observed in the earlier studies occurs only above a threshold of cell free cholesterol that is greater than that necessary for regulation of selective uptake and particle uptake, and that we did not reach that threshold. However, the fractional increases in cell free cholesterol observed here overlapped those of the previous study (26). It is also possible that the HDL preparations used in the two studies differed in some pertinent way or that the fibroblast cell lines differed in the two studies. Whatever the reason, it is clear that the increased HDL binding observed by others is not necessary for the mobilization of cell cholesterol to HDL in the medium observed here.

The provision of LDL cholesterol to fibroblasts produced a decrease in both selective uptake and in HDL binding. This suggests that much of the HDL binding measured under the conditions of cholesterol demand, as in the "basal" cells under our experimental conditions, could be related to selective uptake. Such a notion is supported by studies in rat adrenal cells in which both HDL binding (61) and selective uptake (36) were increased by ACTH treatment. In concordance with this, both HDL binding and selective uptake were greater in rapidly growing sparse cultures of Hep G2 cells than in dense cultures. However, no method now exists for directly distinguishing between the several possible types of HDL binding.

Whole-body selective uptake of HDL cholesteryl esters and of HDL particles has been quantified only in the rat (9, 14, 36), an animal pertinently different from humans at least in its use of HDL as the predominant carrier of plasma cholesterol and its lack of plasma CETP activity (18). In rats, we showed that selective uptake must play a role in reverse cholesterol transport due to the predominance of liver in whole-body selective uptake, and the large contribution of selective uptake to total hepatic uptake of HDL cholesteryl esters. It might be asked whether the present data are consistent with a role for

selective uptake in reverse cholesterol transport in humans as well. Certainly rat and human fibroblasts are at least qualitatively similar in their regulation of the pathways that determine net cholesterol flux. However, the easy regulation of selective uptake and particle uptake by Hep G2 cells is in contrast to the inability to demonstrate such regulation in primary rat hepatocytes or in rat livers in vivo (14). This appears to mitigate against a role for human liver as an unregulated (or insensitively regulated) HDL cholesteryl ester sink, as appears to be the case in rats. Of course, hepatoma-derived Hep G2 cells may not share the regulatory properties of normal hepatocytes. Nonetheless, the differences between cultured human and rat hepatic cells suggest that if selective uptake contributes to reverse cholesterol transport in humans as it does in rats, it may be regulated differently.

The expert assistance of F. Casanada, S. R. Green, Lorna Joy, Jennifer Pattison, and M. Silvestre in these studies is gratefully acknowledged. This work was supported by Research Grant HL-14197 from the National Heart, Lung, and Blood Institute. F. R. was supported by a Research Grant from the Deutsche Forschungsgemeinschaft, Bonn, F.R.G.

Manuscript received 7 January 1988, in revised form 29 March 1988, and in re-revised form 20 April 1988.

REFERENCES

1. Miller, N. E. 1984. Current concepts of the role of HDL in reverse cholesterol transport. *In* Clinical and Metabolic Aspects of High-density Lipoproteins. N. E. Miller and G. J. Miller, editors. Elsevier Science Publishers B.V., Amsterdam. 187–216.
2. Oram, J. F., J. J. Albers, M. C. Cheung, and E. L. Bierman. 1981. The effects of subfractions of high density lipoprotein on cholesterol efflux from cultured fibroblasts. *J. Biol. Chem.* **256:** 8348–8356.
3. Oram, J. F. 1983. Effects of high density lipoprotein subfractions on cholesterol homeostasis in human fibroblasts and arterial smooth muscle cells. *Arteriosclerosis.* **3:** 420–432.
4. Daniels, R. J., L. S. Guertler, T. S. Parker, and D. Steinberg. 1980. Studies on the rate of efflux of cholesterol from cultured human skin fibroblasts. *J. Biol. Chem.* **256:** 4978–4983.
5. Daerr, W. H., S. H. Gianturco, J. R. Patsch, L. C. Smith, and A. M. Gotto, Jr. 1980. Stimulation and suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in normal human fibroblasts by high density lipoprotein subclasses. *Biochim. Biophys. Acta.* **619:** 287–301.
6. Gwynne, J. T., and J. F. Strauss III. 1982. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. *Endocrine Rev.* **3:** 299–329.
7. Gwynne, J. T., B. Hess, T. Hughes, S. Holland, R. Rountree, D. Mahaffee, O. Irsula, and J. Reynolds. 1985. The "HDL pathway" in the rat adrenal cortex. *In* Lipoprotein and Cholesterol Metabolism in Steroidogenic Tissues. J. F. Strauss III and K. M. J. Menon, editors. George F. Stickley Company, Philadelphia. 111–124.
8. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesteryl ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesteryl ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
9. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1985. Uptake of high-density lipoprotein-associated apoprotein A-I and cholesteryl esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* **260:** 744–750.
10. Arbeeny, C. M., V. A. Rifici, and H. A. Eder. 1987. The uptake of the apoprotein and cholesteryl ester of high-density lipoproteins by the perfused rat liver. *Biochim. Biophys. Acta.* **917:** 9–17.
11. Leitersdorf, E., O. Stein, S. Eisenberg, and Y. Stein. 1984. Uptake of rat plasma HDL subfractions labeled with [^{3}H]cholesteryl linoleyl ether or with ^{125}I by cultured rat hepatocytes and adrenal cells. *Biochim. Biophys. Acta.* **796:** 72–82.
12. Gwynne, J. T., and B. Hess. 1980. The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis. *J. Biol. Chem.* **255:** 10875–10883.
13. Leitersdorf, E., A. Israeli, O. Stein, S. Eisenberg, and Y. Stein. 1986. The role of apolipoproteins of HDL in the selective uptake of cholesteryl linoleyl ether by cultured rat and bovine adrenal cells. *Biochim. Biophys. Acta.* **878:** 320–329.
14. Rinninger, F., and R. C. Pittman. 1987. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters. *J. Lipid Res.* **28:** 1313–1325.
15. Pittman, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor, Jr. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesteryl esters. *J. Biol. Chem.* **262:** 2443–2450.
16. Mackinnon, M., J. Savage, R. Wishart, and P. Barter. 1986. Metabolism of high density lipoproteins by the perfused rabbit liver. *J. Biol. Chem.* **261:** 2548–2552.
17. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21:** 789–853.
18. Barter, P. J., and J. I. Lally. 1978. The activity of an esterified cholesterol transferring factor in human and rat serum. *Biochim. Biophys. Acta.* **531:** 233–236.
19. Dashti, N., and G. Wolfbauer. 1987. Secretion of lipids, apolipoproteins, and lipoproteins by human hepatoma cell line, HepG2: effects of oleic acid and insulin. *J. Lipid Res.* **28:** 423–436.
20. Ellsworth, J. L., S. K. Erickson, and A. D. Cooper. 1986. Very low and low density lipoprotein synthesis and secretion by the human hepatoma cell line Hep-G2: effects of free fatty acid. *J. Lipid Res.* **27:** 858–874.
21. Dashti, N., P. Alaupovic, C. Knight-Gibson, and E. Koren. 1987. Identification and partial characterization of discrete apolipoprotein B containing lipoprotein particles produced by human hepatoma cell line. *Biochemistry.* **26:** 4837–4846.
22. Zannis, V. I., J. L. Breslow, T. R. SanGiacomo, D. P. Aden, and B. B. Knowles. 1981. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry.* **20:** 7089–7096.
23. Faust, R. A., and J. J. Albers. 1987. Synthesis and secretion of plasma cholesteryl ester transfer protein by human hepatocarcinoma cell line, HepG2. *Arteriosclerosis.* **7:** 267–275.
24. Drayna, D., A. Stephens Jarnagin, J. McLean, W. Henzel, W. Kohr, C. Fielding, and R. Lawn. 1987. Cloning and

sequencing of human cholesteryl ester transfer protein cDNA. *Nature.* **327:** 632–634.
25. Chen, C-H, T. H. Forte, B. E. Cahoon, R. N. Thrift, and J. J. Albers. 1986. Synthesis and secretion of lecithin-cholesterol acyltransferase by the human hepatoma cell line HepG2. *Biochim. Biophys. Acta.* **877:** 433–439.
26. Oram, J. F., E. A. Brinton, and E. L. Bierman. 1983. Regulation of high density lipoprotein receptor activity in cultured human skin fibroblasts and human arterial smooth muscle cells. *J. Clin. Invest.* **72:** 1611–1621.
27. Tauber, J-P., D. Goldminz, and D. Gospodarowicz. 1981. Up-regulation in vascular endothelial cells of binding sites of high density lipoprotein induced by 25-hydroxycholesterol. *Eur. J. Biochem.* **119:** 327–339.
28. Tabas, I., and A. R. Tall. 1984. Mechanism of the association of HDL_3 with endothelial cells, smooth muscle cells, and fibroblasts. *J. Biol. Chem.* **259:** 13897–13905.
29. 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.
30. Weisgraber, K. H., and R. W. Mahley. 1980. Subfractionation of human high density lipoproteins by heparin-Sepharose affinity chromatography. *J. Lipid Res.* **21:** 316–325.
31. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
32. Scanu, A. 1966. Forms of human serum high density lipoprotein protein. *J. Lipid Res.* **7:** 295–306.
33. Pittman, R. C., and C. A. Taylor, Jr. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–627.
34. Markwell, M. A. K., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3,6-diphenylglycoluril. *Biochemistry.* **17:** 4807–4817.
35. Stokke, K. T., and K. R. Norum. 1971. Determination of lecithin:cholesterol acyltransfer in human blood plasma. *Scand. J. Clin. Lab. Invest.* **27:** 21–27.
36. Pittman, R. C., C. K. Glass, D. Atkinson, and D. M. Small. 1987. Synthetic high density lipoprotein particles. Application to studies of the apoprotein specificity for selective uptake of cholesteryl esters. *J. Biol. Chem.* **262:** 2435–2442.
37. Foreman, J. R., J. B. Karlin, C. Edelstein, D. J. Juhn, A. H. Rubenstein, and A. M. Scanu. 1977. Fractionation of human serum lipoproteins by single-spin gradient ultracentrifugation: quantification of apolipoproteins B and A-I and lipid components. *J. Lipid Res.* **18:** 759–767.
38. Pittman, R. C., S. R. Green, A. D. Attie, and D. Steinberg. 1979. Radiolabeled sucrose covalently linked to protein. A device for quantifying degradation of plasma proteins catabolized by lysosomal mechanisms. *J. Biol. Chem.* **254:** 6876–6879.
39. Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science.* **209:** 497–499.
40. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1957. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
41. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35:** 150–154.
42. Oram, J. F. 1986. Receptor-mediated transport of cholesterol between cultured cells and high-density lipoproteins. *Methods Enzymol.* **129:** 645–659.
43. Folch, J., M. Lees, and G. H. Sloane Stanley. 1954. A simple method for preparation of total pure lipide extracts from brain. *Fed. Proc.* **13:** 209.
44. Huang, H-s., J-c. W. Kuan, and G. G. Guilbault. 1975. Fluorometric enzymatic determination of total cholesterol in serum. *Clin. Chem.* **21:** 1605–1608.
45. Morton, R. E., and D. B. Zilversmit. 1981. A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256:** 11992–11995.
46. Bachorik, P. S., and J. J. Albers. 1986. Precipitation methods for quantification of lipoproteins. *Methods Enzymol.* **129:** s78–100.
47. Morton, R. E. 1985. Binding of plasma-derived lipid transfer protein to lipoprotein substrates. The role of binding in the lipid transfer process. *J. Biol. Chem.* **260:** 12593–12599.
48. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–42.
49. Lange, Y., and B. V. Ramos. 1983. Analysis of the distribution of cholesterol in the intact cell. *J. Biol. Chem.* **258:** 15130–15134.
50. Oram, J. F., C. J. Johnson, and T. A. Brown. 1987. Interaction of high density lipoprotein with its receptor on cultured fibroblasts and macrophages. *J. Biol. Chem.* **262:** 2405–2410.
51. Hoeg, J. M., S. J. Demosky, Jr., S. B. Edge, R. E. Gregg, J. C. Osborne, Jr., and H. B. Brewer, Jr. 1984. Characterization of a human hepatic receptor for high density lipoproteins. *Arteriosclerosis.* **5:** 228–237.
52. Granot, E., I. Tabas, and A. R. Tall. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured HepG2 cells. *J. Biol. Chem.* **262:** 3482–3487.
53. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27:** 361–367.
54. Goldstein, J. L., S. K. Basu, G. Y. Brunschede, and M. S. Brown. 1976. Release of low density lipoprotein from its cell surface receptor by sulfated glycosaminoglycans. *Cell.* **7:** 85–95.
55. Stein, O., G. Halperin, E. Leitersdorf, T. Olivecrona, and Y. Stein. 1984. Lipoprotein lipase-mediated uptake of non-degradable ether analogues of phosphatidylcholine and cholesteryl ester by cultured cells. *Biochim. Biophys. Acta.* **795:** 47–59.
56. Gwynne, J. T., and D. D. Mahaffee. 1987. Esterification of cholesterol in high density lipoprotein decreases its ability to support ACTH-stimulated steroidogenesis by rat adrenocortical cells. *J. Biol. Chem.* **262:** 16349–16356.
57. Phillips, M. C., W. J. Johnson, and G. H. Rothblat. 1987. Mechanisms and consequences of cellular cholesterol exchange and transfer. *Biochim. Biophys. Acta.* **906:** 223–276.
58. Karlin, J. B., W. J. Johnson, C. R. Benedict, G. K. Chacko, M. C. Phillips, and G. H. Rothblat. 1987. Cholesterol flux between cells and high density lipoprotein. *J. Biol. Chem.* **262:** 12557–12564.
59. Slotte, J. P., J. F. Oram, and E. L. Bierman. 1987. Binding of high density lipoproteins to cell receptors promotes

translocation of cholesterol from intracellular membranes to the cell surface. *J. Biol. Chem.* **262:** 12904–12907.

60. Brinton, E. A., R. D. Kenagy, J. F. Oram, and E. L. Bierman. 1985. Regulation of high density lipoprotein binding activity of aortic endothelial cells by treatment with acetylated low density lipoprotein. *Arteriosclerosis.* **5:** 329–335.
61. Gwynne, J. T., and B. Hess. 1978. Binding and degradation of human ^{125}I-HDL by rat adrenocortical cells. *Metabolism.* **27:** 1593–1600.