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Interaction of Plasma Lipoprotein Subfractions With Differentiating 3T3-L1 and Human Mammary Preadipocytes in Culture

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Abstract Differentiating 3T3-L1 preadipocytes (murine fatty fibroblasts) and human preadipocytes interact with human lipoprotein subfractions (HDL_2 and $LDL_{II/III}$) at all stages of the differentiation program, displaying saturable binding behavior. Both cell types interact similarly with $LDL_{II/III}$ as differentiation proceeds, showing increased binding affinities and capacities and maximal rates of uptake in the mature cells, as compared with the preadipocyte stage. These changes coincide with the intracellular appearance of lipid droplets. However, with regard to HDL_2, a markedly different pattern of interaction is evident in both cell types. For 3T3-L1 cells, lowered binding and uptake affinities and capacities are apparent in the fully differentiated state for HDL_2, as compared with $LDL_{II/III}$. Human preadipocytes displayed two distinct affinity binding sites for HDL_2 during the early stages of differentiation (days 2 and 3), as compared with a single affinity site for $LDL_{II/III}$ at all stages. However, in the fully differentiated human cells, only a single affinity site, indistinguishable from the high-affinity site present on day 2, is evident, and probably represents the only binding site of physiological significance in these cells. All the cellular developments appear to be largely unaffected by exposure of both preadipocyte types to added lipoproteins (HDL + LDL) in the medium during the early stages of the conversion process. J. Cell. Biochem. 74:181–193, 1999.

Key words: HDL_2; $LDL_{II/III}$; binding; internalization; uptake; cell culture; 3T3-L1; mammary; preadipocytes

Aberrations in plasma lipoprotein levels have been observed in many inheritable disorders of lipoprotein metabolism, e.g., familial hypertriglyceridemia and familial hypo-alphalipoproteinemia. Similarly, increased adiposity has been observed in association with some of these disorders. Obesity is itself characterized by altered levels of plasma lipoproteins [Angel et al., 1986; Mahaney et al., 1995]. Low-density lipoprotein (LDL) is the main carrier of cholesterol in the bloodstream [Gigli et al., 1992] and, together with high-density lipoprotein (HDL), plays an important role in cholesterol metabolism in adipocytes [Fong et al., 1984], as they fulfill the cholesterol requirements of adipocytes, which possess a limited capacity for cholesterol biosynthesis [Bernini et al., 1991].

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The first event in cell-mediated lipoprotein metabolism is binding of the lipoprotein particles to appropriate receptors. In human adipocytes, LDL binding and internalization readily occur through the LDL (B/E) receptor [Angel et al., 1979]. This lipoprotein receptor recognizes apoB on LDL, and apoE on chlyomicrons, VLDL and HDL enriched with apoE [Fielding, 1992]. The LDL binding reaction does, however, lack absolute specificity in that very low-density lipoprotein (VLDL) and HDL can inhibit these processes in adipocytes in vitro [Mahley et al., 1981]. In addition to the LDL receptor, adipocytes also possess VLDL [Sakai et al., 1994] and LDL-receptor-related protein (LRP)[Descamps et al., 1993] receptors. Although both receptors are members of the LDL receptor family, lipoprotein interactions with these receptors in the present study were considered minimal, as the LRP receptor does not bind apoB [Lestavel and Fruchart, 1994], while the VLDL receptor binds LDL only with low affinity [Sakai et al., 1994].

HDL binding proteins have been identified on adipocytes [Barbaras et al., 1987; Shen and

Angel, 1993]. However, apart from the identification of the binding of HDL via apoA-I to these sites [Morrison et al., 1992], these sites remain poorly characterized. Indeed, it could be speculated as to whether these binding sites are associated with HDL uptake or cholesterol efflux.

The present study did not aim to distinguish between the range of lipoprotein binding sites on adipocytes but viewed the lipoprotein recognition system as a whole.

With the knowledge that lipoproteins are important in supplying cholesterol to tissues of the body, and that their levels are altered in obesity, closer scrutiny of lipoprotein metabolism by differentiating preadipocytes may yield important information as to the role of lipoprotein subfractions during various stages of the differentiation process. Several well-established cell lines, including the 3T3-L1 cell line, are available and have been extensively used as models for studying the integrated in vivo mechanisms of adipogenesis [Pairault and Lasnier, 1987].

Various lipoproteins and their subfractions have been analyzed in terms of their binding properties in various cell types, including human adipocytes [Angel et al., 1986; Angel and Fong, 1983]. These studies, however, used freshly isolated mature adipocytes and adipocyte membranes. Little, if any, information is available concerning binding of lipoprotein subfractions to cultured adipose cells at various stages of preadipocyte differentiation. In addition, previous studies of lipoproteins with various cell systems used single lipoprotein fractions. The aim of the present study was to directly compare subfractions from two prominent lipoprotein classes, for differences and similarities in their metabolism under identical conditions, and at various stages of preadipocyte differentiation. Cell surface binding (affinity and capacity), as well as the internalization of these fractions by the same differentiating preadipocytes were investigated simultaneously. Studies published to date have addressed binding and uptake phenomena independently. The two lipoprotein subfractions chosen for the present study ($LDL_{II/III}$ and HDL_2), were found recently in our laboratory to yield significantly enhanced GPDH activity development in human preadipocytes [Stanton et al., 1998]. LDL fractions II and III, with densities 1.037−1.041 and 1.041–1.047 $g \cdot ml^{-1}$, respectively, were pooled to increase their yields, thus allowing a greater number of experiments to be performed. Of all HDL subfractions tested, only HDL_2 significantly increased the differentiation of human preadipocytes [Stanton et al., 1998], and therefore was the only HDL subfraction used in the present study.

MATERIALS AND METHODS

Chemicals

All standard chemicals obtained from commercial sources were of analytical reagent grade and were used without further purification. The $Na^{125}I$ (2 mCi)(carrier free) in 0.1 N NaOH was supplied by Amersham Radiochemical Centre (Buckingham, UK). Boehringer Mannheim (Mannheim, Germany) supplied the following: D-biotin, bovine serum albumin (BSA), fraction V, defatted according to the method of Ramachandran et al. [1972]; transferrin and bovine insulin (tissue culture reagents); and trypsin (2.5% v/v)(cell culture reagent). Dulbecco's modified Eagle's medium (DMEM) with L-glutamine (powder), fetal bovine serum (FBS), Ham's F12 medium (Ham's F12 or F12)(powder), and penicillin/streptomycin (1% w/v) were purchased from Highveld Biological (Johannesburg, RSA). Filter count was obtained from Packard (Meriden, CT) and Sephadex G-25M (Pharmacia PD-10; bed volume 9.1 ml) from Pharmacia Fine Chemicals (Brussels, Belgium). Collagenase Type II from *Clostridium histolyticum*, dexamethasone, hematoxylin, heparin (cell culture reagent), 3-isobutyl-1-methylxanthine (IBMX), Oil red O, and triiodothyronine (T3)(cell culture reagent) were obtained from Sigma Chemical Company (St Louis, MO). The human serum used in the isolation of human preadipocytes was obtained from the Blood Transfusion Service, Provincial Hospital, Port Elizabeth, RSA, as was the human plasma used in the isolation of lipoproteins.

Maintenance and Culture of 3T3-L1 Preadipocyte Cultures

Stock cultures of 3T3-L1 preadipocytes were grown in a humidified atmosphere containing 5% CO_2 at 37°C. The cells were maintained at low density in a stock culture medium (growth medium) which consisted of DMEM:F12 (1:1) supplemented with 10% (v/v) FBS and 0.01% (w/v) penicillin/streptomycin. Subconfluent cultures were detached from the culture matrix

using trypsin/EDTA in phosphate-buffered saline (PBS), containing no calcium or magnesium. They were suspended in DMEM: F12 (1:1) containing 10% FBS and plated into 96-well plates at a density of 3.2×10^4 cells·cm^{-2} and incubated for 24 h in the above growth medium. Where cells were used for binding assays on day 1, they were plated into 96-well plates at 6.4×10^4 cells·cm^{-2} as described above. After a 3-h incubation under culture conditions (37°C; 5% CO_2), FBS was replaced with 10% lipoprotein-deficient serum (LPDS). For cells that were not assayed for lipoprotein binding, growth medium was replaced with a supplemented medium 24 h after plating into the multi-well plates; this day was referred to as day 1. The supplemented medium consisted of DMEM: F12 (1 : 1) supplemented with 5% (v/v) FBS, 5 µg insulin·ml^{-1}, 10^{-8} M dexamethasone and 0.1 mM IBMX. Cells were refed 72 h later (day 4) with supplemented medium, similar to that described above but lacking dexamethasone and IBMX. After a further 48-h incubation (day 6), the cells were refed with fresh medium (same as on day 4) and assayed 24 h thereafter (day 7). Day 7 was assumed to represent the late stage of differentiation, as was apparent from visual observation and glycerol 3-phosphate dehydrogenase (GPDH) activity. When cells were to be assayed for lipoprotein binding, the medium containing 5% FBS was replaced 15–20 h before assaying with medium appropriate for that stage of the differentiation program and which was supplemented with 5% LPDS instead of FBS. The effect of lipoproteins, added to the differentiation medium, on the binding and uptake of HDL_2 and $LDL_{II/III}$, was investigated in cultures which were fed daily for the initial 3 days of their differentiation program with a combination of 20 µg HDL and 20 µg LDL protein·ml^{-1} (complete fractions). Cells were not exposed to lipoproteins after day 4 when the medium was replenished with one from which IBMX and dexamethasone was omitted.

Isolation and Culture of Human Adipocyte Precursors

Human mammary preadipocytes were isolated and cultured as described previously [Stanton et al., 1998]. When the effects of lipoproteins were investigated, cultures were fed daily for the initial 4 days of their differentiation program with a combination of 20 µg HDL and 20 µg LDL protein·ml^{-1} (complete fractions). They were not exposed to lipoproteins after day 5 when the medium was replenished with one from which IBMX was omitted. All cultures were kept at 37°C in a humidified atmosphere containing 5% CO_2. The indicated times of assay refer to the number of days after the cells were plated into the 96-well plates and fed the defined medium, i.e., the day they were trypsinized, plated into multi-well plates and fed the defined medium was assumed to be day 1.

Determination of Preadipocyte and Adipocyte Numbers

Cell numbers were determined as described previously [Stanton et al., 1998], either by trypsinization or after staining of the cells with hematoxylin.

Isolation of Lipoprotein Fractions and Subfractions

HDL and LDL were isolated from human male plasma as previously described [Stanton et al., 1998]. Lipoprotein fractions were further subfractionated and isolated according to the methods of Jaakkola et al. [1989] and Strachan et al. [1988], for LDL and HDL, respectively. The subfractions were pooled according to the following densities: $LDL_{II/III}$ = 1.037–1.047 g·ml^{-1} and HDL_2 = 1.08–<1.13 g·ml^{-1}. The pooled fractions were dialyzed against physiological saline with 1 mM EDTA (pH 7.4), filtered through a 0.2-µm syringe filter and stored at 4°C.

Preparation of Lipoprotein-Deficient Serum

LPDS was prepared as described by Goldstein et al. [1983] from FBS using ultracentrifugation techniques. The LPDS was sterilized by passage through a 0.2-µm syringe filter and stored in aliquots at −80°C until required for use.

Lipoprotein Radioiodination

The procedure for the preparation of ^{125}I-labeled lipoproteins for the present study was described by Goldstein et al. [1983]. It is also the procedure of choice for tissue culture studies and is based on Bilheimer's modification [Bilheimer et al., 1972] of the iodine monochloride method of MacFarlane [1958]. Labeling was performed using 1 mCi Na^{125}I per 5 mg

protein of the lipoprotein subfraction. The specific radioactivities of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ were within the range of 180–300 cpm.ng^{-1} protein and 200–500 cpm·ng^{-1} protein, respectively. More than 95% of the radioactivity in ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ preparations were precipitable by 10% trichloroacetic acid, and less than 5% was extractable with chloroform: methanol (2:1, v/v).

Binding and Uptake of ^{125}I-LDL$_{II/III}$ and ^{125}I-HDL$_2$ by 3T3-L1 and Human Preadipocytes

Binding and uptake of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ by differentiated and undifferentiated cells were determined as described by Goldstein et al. [1983] with minor modifications. The assays were performed at 37°C, which allowed the measurement of both binding and uptake by the same cells [Tauber et al., 1981]. Briefly, cell monolayers were washed twice with KRB-5% (w/v) defatted BSA-5 mM glucose (pH 7.5) and then incubated at 37°C for 1 h in the same buffer containing various amounts of labeled lipoprotein (3–30 µg lipoprotein protein·ml^{-1}). Nonspecific binding and uptake was determined from cells that had also been incubated in the presence of a 100-fold excess of unlabeled lipoprotein. The differences between total and nonspecific binding was considered to be specific binding (heparin-releasable). After incubation, the cells were kept at 2–4°C for 15–20 min before 2 rapid washes with buffer B (which contained 50 mM Tris-HCl, 150 mM NaCl and 2 mg BSA·ml^{-1}; pH 7.4), followed by two additional washes of 7 min each, also with buffer B. The cells were then washed once with buffer C (which consisted of buffer B without BSA) and incubated for 1 h at 4°C in buffer D (10 mM HEPES, 50 mM NaCl; pH 7.4) which contained 2 mg sodium heparin·ml^{-1}. The supernatant was recovered and the amount of radioactivity determined in 2 ml Filter Count. This heparin-releasable fraction represented surface-bound ^{125}I-labeled lipoprotein. For uptake results, the same cells were further incubated with 0.1 N NaOH for 15 min at room temperature and the resulting solution counted in 2 ml Filter Count. This fraction (nonreleasable by heparin), was taken to represent cellular incorporation of ^{125}I-LDL$_{II/III}$ or HDL$_2$ and is referred to as internalized lipoprotein.

The binding assays were performed at various stages of the differentiation program of 3T3-L1 and human preadipocytes. The first day for the assay depended on the time of the initial exposure of the cells to the medium for inducing the differentiation program, which in the case of the human preadipocytes was only possible on day 2 after trypsinization, owing to the need for at least a 20-h incubation period without exposure to serum, which in itself is a source of lipoproteins. In order to allow attachment to the plate, cells were exposed to human serum after the trypsination, and although this time was brief (3 h), a 20-h period was allowed to expire before executing the first assays. For 3T3-L1 preadipocytes, day 1 was the first possible day for assay, owing to the need for at least a 20-h incubation period without exposure to FBS, which in itself is also a source of lipoproteins.

Protein Assays

Protein was quantified by the Folin-Lowry microtiter plate method [Fryer et al., 1986] using BSA as the standard.

Calculations and Data Analysis

All binding assays were carried out in triplicate within each experiment. Binding data was analyzed by the method of Scatchard [1974] using the slope of the graph ($-1/K_d$) as a measure of binding affinity, and the intercept with the horizontal axis as a measure of the binding capacity (B_{max}). K_d was expressed as µg lipoprotein protein·ml^{-1} and B_{max} as ng lipoprotein protein-bound·10^{-6} cells. Internalization data were analyzed by the method of Angel et al. [1979], using double reciprocal plots, where the inverse of the intercept with the vertical axis gives the maximal rate of specific uptake of ^{125}I-lipoprotein (V_m), expressed as ng lipoprotein protein·10^{-6} cells·h^{-1}. The inverse of the intercept with the horizontal axis represents the concentration of lipoprotein required to achieve one-half the maximal rate of specific uptake (K_m), expressed as µg lipoprotein protein·ml^{-1}. The results are reported as specific binding and uptake. Specific binding was calculated as the difference between the total and nonspecific binding. Similarly for uptake, specific uptake was calculated by subtracting nonspecific uptake from total uptake results. Nonspecific binding and uptake were determined from cells incubated in the presence of a 100-fold excess of unlabeled lipoprotein. Nonspecific uptake was considered to represent internalization of the label in the presence of

100-fold excess unlabeled lipoprotein, and which was heparin resistant.

Statistical analysis. Differences in binding and internalization between various treatments or between different stages of differentiation were analyzed using the two-sample Student's *t*-test.

RESULTS

Binding and Uptake of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ as a Function of ^{125}I-Lipoprotein Concentration

As indicated by dose-response curves, saturation binding by both differentiated 3T3-L1 preadipocytes (day 7) and human mammary adipocytes (day 11) was observed at a concentration of 18–25 µg lipoprotein protein·ml^{-1} for both HDL$_2$ and LDL$_{II/III}$ (Fig. 1). The specific uptake of ^{125}I-HDL$_2$ by human cells appeared to reach equilibrium at 15–20 µg lipoprotein protein· ml^{-1}, as did the uptake of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ by 3T3-L1 cells. However, the uptake of ^{125}I-LDL$_{II/III}$ by human cells continued to increase even at a level of 30 µg protein·ml^{-1} (Fig. 2).

The greater binding and uptake capacities observed in human cells compared with murine cells may be attributed to species-specific differences in lipoprotein metabolism. It may also originate from the use of human lipoproteins as probes for the 3T3-L1 cell line. Investigation of mouse lipoprotein metabolism using mouse-derived lipoproteins may yield valuable insight regarding these observed differences. However, because of the practical difficulty of obtaining sufficient quantities of mouse lipoprotein fractions, this aspect was not included in the present study.

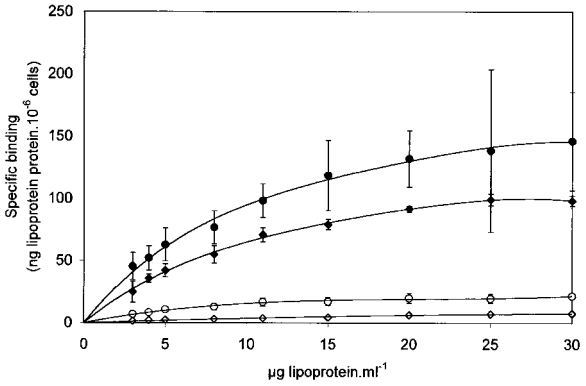

Fig. 1. Saturation binding of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ by differentiated 3T3-L1 (day 7) and human (day 11) cells. Saturation binding of ^{125}I-HDL$_2$ (◆◇) and ^{125}I-LDL$_{II/III}$ (●○) by 3T3-L1 (◇○) and human (◆●) cells were performed at 37°C in KRB as described under Materials and Methods. Each point signifies the mean specific binding ± SEM of three experiments. Error bars lying within the confines of the symbol used were not indicated on the graphs. Specific binding was calculated as the difference between the mean values of total and nonspecific binding, each measured in triplicate for each experiment (SEM within 7% and 9% of each mean value for 3T3-L1 and human cells, respectively). For each cell type, the experiment was performed three times, with human cells obtained from different donors. Nonspecific binding was determined from cells incubated in the presence of a 100-fold excess of unlabeled lipoprotein.

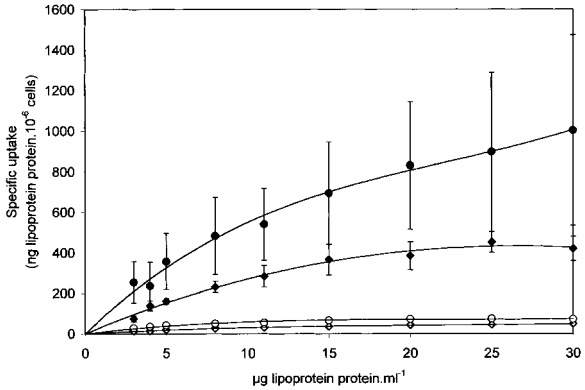

Fig. 2. Internalization of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ by differentiated 3T3-L1 (day 7) and human cells (day 11). Internalization of ^{125}I-HDL$_2$ (◆◇) and ^{125}I-LDL$_{II/III}$ (●○) by 3T3-L1 (◇○) and human (◆●) cells was performed at 37°C in KRB as described under Materials and Methods. Each point signifies the mean specific uptake ± SEM of three experiments. Error bars lying within the confines of the symbol used were not indicated on the graphs. Specific uptake was calculated as the difference between the mean values of total and nonspecific uptake, each measured in triplicate for each experiment (SEM within 7% and 9% of each mean value for 3T3-L1 and human cells, respectively). For each cell type, the experiment was performed three times, human cells being obtained from different donors. Nonspecific uptake was determined from cells incubated in the presence of a 100-fold excess of unlabeled lipoprotein.

Changes in Specific ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ Binding and Uptake During 3T3-L1 and Human Preadipocyte Differentiation

The days of the differentiation program chosen for assessing the binding and uptake of the lipoprotein subfractions were taken as representing the immature preadipocyte stage (days 1 and 2), mature adipocytes (days 7 and 11), as well as a time when the first lipid droplets became visible and GPDH activity began to increase rapidly (days 4 and 5) for 3T3-L1 and human cells respectively. The specific binding of ^{125}I-LDL$_{II/III}$ by 3T3-L1 cells increased during the differentiation program in what appeared to be a sigmoidal manner (Fig. 3). The specific binding of this lipoprotein fraction was very low in undifferentiated preadipocytes but increased

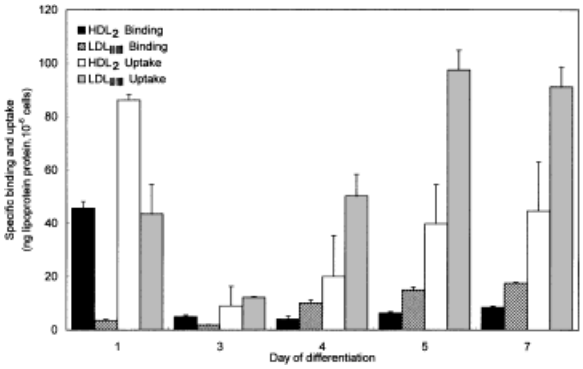

Fig. 3. Changes in specific binding and internalization of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ during the differentiation of 3T3-L1 preadipocytes. 3T3-L1 cells were maintained and differentiated (in the absence of exogenously added lipoproteins), as described under Materials and Methods. The cells were incubated in KRB medium at various stages of the differentiation program with 10 μg ^{125}I-HDL$_2$ protein·ml^{-1} or 4 μg ^{125}I-LDL$_{II/III}$ protein·ml^{-1}. The nonspecific values were determined by simultaneous incubation with a 100-fold excess of unlabeled lipoprotein. Data points represent the mean ± SEM of three independent experiments in each of which all determinations were made in triplicate.

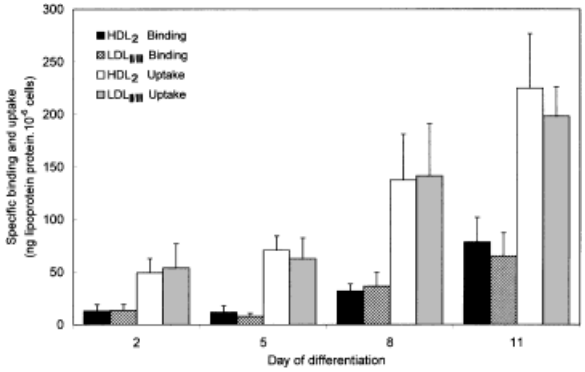

Fig. 4. Changes in the specific binding and uptake of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ during human preadipocyte differentiation. Human cells were maintained and differentiated (in the absence of exogenously added lipoproteins), as described under Materials and Methods. The cells were then incubated in KRB medium at various stages of the differentiation program with 10 μg ^{125}I-HDL$_2$ protein·ml^{-1} or 4 μg ^{125}I-LDL$_{II/III}$ protein·ml^{-1}. Values represent the mean specific value ± SEM of three experiments. Specific ^{125}I-lipoprotein binding and uptake was calculated for each experiment as the difference between the mean values of total and nonspecific binding and uptake, each measured in triplicate (SEM within 6% and 7% of each mean value, within a given experiment, for binding and uptake, respectively). Each experiment was peformed with cells from different donors. Nonspecific binding and uptake was determined from cells incubated in the presence of a 100-fold excess of unlabeled lipoprotein.

rapidly by day 4, which coincided with the microscopically visible appearance and accumulation of lipid droplets.

The binding of ^{125}I-HDL$_2$ by differentiating 3T3-L1 preadipocytes (Fig. 3) deviated markedly from the pattern observed with ^{125}I-LDL$_{II/III}$. Whereas ^{125}I-HDL$_2$ bound specifically was high in undifferentiated cells (day 1) it decreased drastically by day 3, amounting to only 10–20% of the specific binding on day 1. The specific ^{125}I-HDL$_2$ binding increased slightly as the cells differentiated further.

The specific uptake of ^{125}I-LDL$_{II/III}$ by differentiating 3T3-L1 and human preadipocytes increased during differentiation (Figs. 3 and 4, respectively), after the increased binding.^{125}I-HDL$_2$ specific uptake by 3T3-L1 cells followed the same pattern as its specific binding (Fig. 3). The uptake was high on day 1 but decreased dramatically to day 3 before showing an increase in internalization as time proceeded. It should be noted, however, that the amount of ^{125}I-HDL$_2$ which was internalized on day 7, although being more than that taken up on days 3–5, was still less than that on day 1. Thus, mature 3T3-L1 adipocytes appear to internalize less ^{125}I-HDL$_2$ than preadipocytes.

The specific binding of ^{125}I-LDL$_{II/III}$ by human cells also displayed a lag phase (Fig. 4) before starting to increase after the appearance of lipid droplets and GPDH activity in the terminal stages of differentiation. Specific binding of ^{125}I-LDL$_{II/III}$ was limited in human preadipocytes but increased considerably with maturation (Fig. 4). Likewise, specific binding of ^{125}I-HDL$_2$ by human cells demonstrated an increase after day 5 (Fig. 4).

Except for a relatively sharp increase between days 8 and 11, ^{125}I-HDL$_2$ uptake by human cells followed trends similar to those observed for ^{125}I-LDL$_{II/III}$ uptake during the differentiation program (Fig. 4). Thus, the specific binding and uptake of both lipoprotein subfractions by human cells displayed similar trends, although the actual amounts of ^{125}I-lipoprotein subfraction bound and internalized differed considerably between donors.

Determination of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ Binding and Uptake Parameters for Differentiating 3T3-L1 and Human Preadipocytes

^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ binding by 3T3-L1 cells on days 1 and 7 occurs via a single binding site, as was evident from the apparent linearity of the respective Scatchard plots (not shown). The binding site for ^{125}I-LDL$_{II/III}$ displayed a

slight but not statistically significant decrease in the K_d value of untreated cells as they differentiated, as compared with a significant decrease ($P < 0.001$) in lipoprotein treated cells, indicative of an increase in affinity (Table I). On the contrary, the affinity of the binding sites for ^{125}I-HDL_2 appears to have decreased slightly as the cells differentiated, but not to an extent that was statistically significant.

The binding capacity of the 3T3-L1 cells for ^{125}I-$LDL_{II/III}$ (Table I) demonstrated an increase with differentiation, while the capacity of the differentiated preadipocytes for ^{125}I-HDL_2 (day 7) displayed a slight decrease compared with day 1 preadipocytes. These changes were, however, not statistically significant. Furthermore, these parameters were unaffected in cells exposed to lipoproteins during their differentiation as compared with cells that did not receive lipoproteins during the early stages of the 3T3-L1 preadipocyte differentiation period.

Comparison of the affinities between ^{125}I-HDL_2 and ^{125}I-$LDL_{II/III}$ binding sites for their respective lipoprotein subfractions in undifferentiated 3T3-L1 preadipocytes indicates that these cells bound both of the lipoprotein subfractions equally well (Table I). As 3T3-L1 cells differentiated, however, the increase in the affinity of the ^{125}I-$LDL_{II/III}$ binding sites resulted in a statistically significant ($P < 0.05$) increase in affinity of differentiated 3T3-L1 preadipocytes to bind ^{125}I-$LDL_{II/III}$ compared with ^{125}I-HDL_2. Together with an apparently increased capacity of differentiated 3T3-L1 preadipocytes to bind ^{125}I-$LDL_{II/III}$, the differentiating cells appear to favor the latter subfraction with regard to cell surface binding development. Lipoprotein treatment did not significantly alter any of the binding parameters in differentiating 3T3-L1 preadipocytes for either of the lipoprotein subfractions.

The specific binding data of ^{125}I-$LDL_{II/III}$ were best fitted to a single affinity binding system by Scatchard analysis throughout the different stages of human preadipocyte differentiation. The influence of lipoproteins on these parameters was investigated in different cells that had been exposed to lipoprotein fractions during the initial stages of differentiation. The results suggested that the exposure of preadipocytes to lipoproteins during their differentiation did not significantly affect the binding parameters for ^{125}I-$LDL_{II/III}$ (Table II). When comparing the results from all stages of differ-

TABLE I. Summary of the Specific Binding Affinities (K_d) and Capacities (B_{max}), Lipoprotein Concentration Required for Half-maximal Internalization (K_m) and Maximal Rate of Specific Internalization (V_m) of ^{125}I-HDL_2 and ^{125}I-$LDL_{II/III}$[a]

Day of differentiation	K_d		B_{max}		K_m		V_m	
	^{125}I-HDL_2	^{125}I-$LDL_{II/III}$	^{125}I-HDL_2	^{125}I-$LDL_{II/III}$	^{125}I-HDL_2	^{125}I-$LDL_{II/III}$	^{125}I-HDL_2	^{125}I-$LDL_{II/III}$
1	5.3 ± 0.8	4.8 ± 0.6	23.9 ± 7.8	15.3 ± 2.2	5.6 ± 1.3	8.7 ± 0.6	16.1 ± 2.2	11.6 ± 4.2
7 (untreated)	7.0 ± 0.6	3.0 ± 0.3[c]	12.7 ± 1.8	29.2 ± 5.3	16.8 ± 3.4[b]	4.0 ± 1.1[b]	60.3 ± 4.5[e]	90.1 ± 7.9[e]
7 (treated)	7.8 ± 0.8	2.2 ± 0.5[d,e]	16.1 ± 1.0	28.7 ± 4.5	25.0 ± 4.5[b]	3.4 ± 0.8[b]	65.5 ± 1.3[e]	95.2 ± 12.6[e]

[a]Expressed as μg lipoprotein protein · ml^{-1}; ng lipoprotein protein · 10^{-6} cells; μg lipoprotein protein · ml^{-1}, and ng lipoprotein protein · 10^{-6} cells · h^{-1}, respectively, by differentiating 3T3-L1 preadipocytes. Values at all stages of differentiation are the means ±SEM of three independent experiments, each measured in triplicate. The binding parameters were calculated from Scatchard transformations, where the K_d equals −1/slope, and the intercept with the horizontal axis represents maximum binding (B_{max}). The internalization parameters were calculated from double reciprocal transformations, where V_m represents the inverse of the vertical axis intercept, and K_m the inverse of the intercept with the horizontal axis. Untreated cells received no lipoprotein exposure during their differentiation, while treated cells received a combination of 20 μg HDL and 20 μg LDL protein · ml^{-1} daily for the initial 3 days of the differentiation program. For each metabolic parameter, within each lipoprotein subfraction: [b]significantly different from cells on day 1 ($P < 0.05$); [c]significantly different from the binding affinity for ^{125}I-HDL_2 of untreated cells on day 7 ($P < 0.05$); [d]significantly different from the binding affinity for ^{125}I-HDL_2 of treated cells on day 7 ($P < 0.05$); [e]significantly different from cells on day 1 ($P < 0.001$).

TABLE II. Summary of the Specific Binding Affinities (K_d), Binding Capacities (B_{max}), Lipoprotein Concentration Required for Half-maximal Internalization (K_m), and Maximal Rate of Specific Internalization (V_m) of ^{125}I-LDL$_{II/III}$[a]

Day of differentiation	K_d	B_{max}	K_m	V_m
2	14.6 ± 2.0	19.7 ± 3.3	11.9 ± 1.7	64.8 ± 15.0
4 (untreated)	24.2 ± 4.4	22.6 ± 5.0	9.9 ± 2.6	71.4 ± 24.9
4 (treated)	41.2 ± 10.6[b]	18.2 ± 4.0	12.8 ± 4.5	42.7 ± 13.0
11 (untreated)	10.1 ± 3.5	129.5 ± 41.3[b]	12.1 ± 0.8	1196.1 ± 652.5[b]
11 (treated)	11.7 ± 3.4	129.9 ± 51.0[b]	9.1 ± 1.3	800.0 ± 200.0[c,d]

[a]Expressed as μg lipoprotein protein · ml^{-1}; ng lipoprotein · 10^{-6} cells; μg lipoprotein protein · ml^{-1} and ng lipoprotein protein · 10^{-6} cells · h^{-1}, respectively, by differentiating human mammary preadipocytes. Values for saturation binding and uptake parameters are the mean ±SEM, based on at least three independent experiments (different cell donors), each measured in triplicate at each lipoprotein concentration used. The binding parameters were calculated from Scatchard transformations, where the K_d equals −1/slope and the intercept with the horizontal axis represents maximal binding (B_{max}). The internalization parameters were calculated from double reciprocal transformations, where V_m represents the inverse of the vertical axis intercept, and K_M the inverse of the intercept with the horizontal axis. Untreated cells received no exogenously added lipoprotein during their differentiation, while treated cells received a combination of 20 μg HDL and 20 μg LDL protein · ml^{-1} daily for the initial 4 days of the differentiation program. Within each metabolic parameter: [b]significantly different from cells on day 2 ($P < 0.05$); [c]significantly different from cells on day 2 ($P < 0.001$); [d]significantly different from day 4 treated and untreated cells ($P < 0.05$).

entiation, certain trends were evident, even though statistical significance was difficult to achieve owing to the large inter-donor variation which became apparent. Different donors (batches of cells) had to be used in order to obtain sufficient numbers of cells to cover the various stages of differentiation and lipoprotein treatments included in the experiments described. Although not statistically significant, the mean K_d values for ^{125}I-LDL$_{II/III}$ binding decreased (Table II), with increasing time beyond day 4, indicating increased affinity of the receptors for LDL$_{II/III}$ as the preadipocytes differentiated. The capacity for ^{125}I-LDL$_{II/III}$ binding increased significantly during the differentiation period from day 2 to day 11 (Table II). The apparent increases in affinity and capacity of the binding sites were more pronounced after the stage of lipid droplet formation (i.e., from day 4 onward).

The specific binding of ^{125}I-HDL$_2$ by human preadipocytes on days 2 and 3 of the human preadipocyte differentiation program was characterized consistently by biphasic Scatchard plots exhibiting two distinct slopes and hence the presence of two nonidentical affinity binding sites. By day 4, however, Scatchard transformations of the specific binding data revealed a single affinity binding system (Table III). The high and low affinities of the binding sites in preadipocytes (days 2 and 3), as shown by their K_d values, are significantly different ($P < 0.05$) from each other, while on days 2 and 3, the low-affinity site also exhibits a significantly lower affinity ($P < 0.05$) than the single binding site present on day 11. The single affinity site present on day 4 (untreated) was significantly lower in affinity than the high-affinity sites present on day 2 ($P < 0.05$) and on day 11 for both treated and untreated cells ($P < 0.05$). The presence of lipoproteins during the initial stages of preadipocyte development did not appear to significantly affect the biphasic nature of the Scatchard plots on day 3. Neither did the presence of lipoproteins significantly affect any of the binding parameters in differentiating and undifferentiated human cells at comparable stages of differentiation. As differentiation proceeded, the low-affinity site for ^{125}I-HDL$_2$ binding appeared to diminish and ultimately vanished completely as differentiation proceeded towards day 11. The end result was a single binding site with an affinity not significantly different from that of the high-affinity site of day 2 preadipocytes. There was little difference in affinities on days 2 and 3. On day 4, the single binding affinity was significantly higher in lipoprotein treated cells as compared with the low-affinity site from the corresponding cells on day 3. Irrespective of lipoprotein exposure during differentiation, mature cells (day 11) displayed a single high-affinity binding site for ^{125}I-HDL$_2$. It is noteworthy that the affinity of ^{125}I-HDL$_2$ binding by the high-affinity site on day 2 is significantly greater ($P < 0.05$) than that of ^{125}I-LDL$_{II/III}$ on day 2 (Table II).

TABLE III. Summary of the Specific Binding Affinities (K_d), Binding Capacities (B_{max}), Lipoprotein Concentration Required for Half-maximal Internalization (K_m), and Maximal Rate of Specific Internalization (V_m) of ^{125}I-HDL$_2$[a]

Day of differentiation	K_d	B_{max}	K_m	V_m
2	2.7 ± 0.8; 27.0 ± 5.6[b,c]	16.3 ± 5.5; 63.5 ± 33.6	9.2 ± 1.0	90.0 ± 21.4
3 (untreated)	5.1 ± 0.9; 45.0 ± 9.7[b,c]	9.7 ± 3.3; 19.6 ± 5.5	12.3 ± 1.6	90.7 ± 23.4
3 (treated)	8.4 ± 1.5; 54.1 ± 11.3[b,c]	9.6 ± 2.6; 23.0 ± 6.0	14.4 ± 4.4	70.4 ± 13.7
4 (untreated)	32.9 ± 7.8[c,d]	45.0 ± 20.8	12.0 ± 2.6	124.8 ± 11.5
4 (treated)	11.2 ± 1.0[c]	18.2 ± 8.1	29.3 ± 14.9	70.8 ± 10.5[j]
11 (untreated)	4.9 ± 1.4	287.6 ± 144.0[e,f,g]	10.6 ± 1.3	1534.2 ± 902.9[i]
11 (treated)	5.0 ± 1.5	228.3 ± 98.3[e,f]	13.3 ± 0.8[h]	1274.9 ± 615.6[i]

[a]Expressed as μg lipoprotein protein · ml^{-1}; ng lipoprotein protein · 10^{-6} cells; μg lipoprotein protein · ml^{-1}, and ng lipoprotein protein · 10^{-6} cells · h^{-1}, respectively, for differentiating human mammary preadipocytes. Values for saturation binding and uptake parameters are the mean ±SEM, based on at least three independent experiments (different cell donors), each measured in triplicate at each lipoprotein concentration used. The binding parameters were calculated from Scatchard transformations, where the K_d equals −1/slope, and the intercept with the horizontal axis represents maximum binding (B_{max}). The internalization parameters were calculated from double reciprocal transformations, where V_m represents the inverse of the vertical axis intercept, and K_m the inverse of the intercept with the horizontal axis. Untreated cells received no lipoprotein exposure during their differentiation, while treated cells received a combination of 20 μg HDL and 20 μg LDL protein · ml^{-1} daily for the initial 4 days of the differentiation program. Within each metabolic parameter: [b]significantly different from high-affinity binding sites of cells on same day (days 2 and 3) and from single affinity sites on day 11 ($P < 0.05$); [c]significantly different from treated and untreated cells on day 11 ($P < 0.05$); [d]significantly different from the high affinity binding sites on day 2 ($P < 0.05$); [e]significantly different from the high affinity binding site capacity on day 2 ($P < 0.05$); [f]significantly different from the capacity of the high- and low-affinity binding sites of cells on day 3 ($P < 0.05$); [g]significantly different from the capacity of the low-affinity binding site on cells from day 2 ($P < 0.05$); [h]significantly different from affinity of uptake on day 2 ($P < 0.05$); [i]significantly different from the rate of uptake on day 2 ($P < 0.05$); [j]significantly different from the rate of uptake of untreated cells on day 4 ($P < 0.05$). On days 2 and 3, the data for the high-affinity sites appear first.

The binding capacity of ^{125}I-HDL$_2$ by differentiated preadipocytes (day 11) was significantly higher ($P < 0.05$) than the capacity of the high-affinity binding site of preadipocytes (day 2)(Table III). Although the ^{125}I-HDL$_2$ binding capacity of cells on day 11 also exceeded that of the low-affinity sites in the preadipocytes, this difference was only significant in untreated cells. Unlike the binding site affinities, the capacities of the high- and low-affinity binding sites, for both treated and untreated cells, did not significantly differ during the early stages of the differentiation program. Although general trends could be observed in the capacities, a lack of significance, attributable to the large inter-batch variation in human cells, was evident, thus indicating considerable donor dependence in the human data.

The maximum specific uptake rate (V_m) of ^{125}I-LDL$_{II/III}$ by mature 3T3-L1 preadipocytes appears to be significantly more effective ($P < 0.001$) than in undifferentiated 3T3-L1 preadipocytes (Table I). These observations are in agreement with the observed increase in the affinity and capacity of the binding sites for ^{125}I-LDL$_{II/III}$ discussed above (Table I). However, ^{125}I-HDL$_2$ also displayed an increased rate of uptake despite the concentration required for half maximal uptake (K_m) having increased significantly (Table I). Although the higher K_m values coincide with the observed lower affinities of the binding sites for ^{125}I-HDL$_2$ (Table I), the increased rate of uptake would seem to be contradictory. It is possible that recycling times for ^{125}I-HDL$_2$ are increased (if such a mechanism exists), or alternatively it may be speculated that HDL is taken up by non-receptor-mediated mechanisms in 3T3-L1 cells. However, these suggestions require further investigation. As with the binding parameters, the presence of complete HDL and LDL fractions during the initial stage of 3T3-L1 preadipocyte development did not affect the uptake parameters of lipoprotein subfractions by differentiated 3T3-L1 cells significantly.

The uptake of ^{125}I-HDL$_2$ and ^{125}I-LDL$_{II/III}$ by human cells was determined in the same cells used for lipoprotein subfraction binding. As with the binding affinities and capacities for both lipoprotein subfractions, which increased with differentiation, the maximal rate of specific uptake (V_m) of these subfractions also increased significantly ($P < 0.05$) between preadipocytes and fully differentiated human adipocytes

(Tables II and III). The rate of uptake remained relatively constant during the first phase of the differentiation process, and increased rapidly after lipid droplets had started to accumulate, i.e., after day 5. Whereas some differences in the rates of uptake of both subfractions during the initial stages of the differentiation program were evident for both $LDL_{II/III}$ and HDL_2 uptake, such differences were not significant. However, it would appear that the rate of HDL_2 uptake tended to exceed that of $LDL_{II/III}$, at all stages of human preadipocyte development (Tables II and III). Although the general trend indicated a lowered uptake rate of both lipoprotein subfractions in the treated as compared with untreated cells, exposure of the human cells to lipoproteins during the early stages of differentiation showed no significant effects on uptake rates, except on day 4 in the case of ^{125}I-HDL_2 uptake. Except for ^{125}I-HDL_2 uptake by lipoprotein-treated cells on day 11, the lipoprotein concentration (K_m) required for half-maximal uptake of both HDL_2 and $LDL_{II/III}$ also remained insignificantly changed during the different stages of the differentiation program, irrespective of whether the developing preadipocytes were exposed to lipoproteins during the early stages of differentiation (Tables II and III).

DISCUSSION

Previous studies in this laboratory [Stanton et al., 1997], as well as subsequent studies using lipoprotein subfractions [Stanton et al., 1998], demonstrated that lipoproteins enhance 3T3-L1 preadipocyte differentiation. In the present study, these cells have been shown to metabolize both HDL_2 and $LDL_{II/III}$ albeit to varying degrees, and depending on the stage of differentiation of the preadipocytes (Fig. 3; Table I). Steinberg et al. [1983] found differentiated 3T3-L1 preadipocytes to degrade significantly higher amounts of LDL than undifferentiated cells. This observation was suggested to result from a significant induction of LDL receptors which accompany 3T3-L1 preadipocyte differentiation. These workers also reported that the LDL receptor of 3T3-L1 cells is subject to down-regulation analogous to that reported for other cell types. The present study demonstrated that lipoproteins present during the initial 3 days of differentiation of 3T3-L1 preadipocytes did not significantly alter the ability of the cells to bind and metabolize lipoproteins with differentiation (Table I). Furthermore, as the untreated cells differentiated, their affinity and capacity for HDL_2 and $LDL_{II/III}$ binding was not significantly altered (Table I). The maximal rate of uptake, however, did increase significantly ($P < 0.001$) with differentiation (Table I). In view of the insignificant changes in the binding affinities for the lipoproteins, and even a slight decrease in affinity and capacity for HDL_2 binding, the observed significantly increased rate of HDL_2 uptake remains unexplained as to the mechanism involved. However, $LDL_{II/III}$ binding did appear to increase in capacity and affinity with 3T3-L1 cell differentiation, thus matching its observed increased affinity and rate of uptake.

Recent studies in our laboratory indicated that exposure of preadipocytes to both HDL_2 and $LDL_{II/III}$ during the early stages of differentiation appeared to enhance differentiation in terms of GPDH activity development and lipid accumulation [Stanton et al., 1998]. However, the changes in binding and uptake parameters, noted in the present study, appeared to be an inherent feature of the differentiation process itself and were largely unaffected in both cell types by the presence of lipoproteins during the early stages of the differentiation process (Tables I, II, and III).

Nonspecific binding of HDL_2 and $LDL_{II/III}$ varied considerably for both cell types, in particular depending on the stage of differentiation. Previously published data [Salter et al., 1987; Angel et al., 1979; Fong et al., 1984] concerning nonspecific binding by mature adipocytes and adipocyte membranes showed nonspecific binding amounting to 30% of total binding. Whereas similar values were found for fully differentiated preadipocytes in the present study, 3T3-L1 cells, which had not commenced with the differentiation program (day 1), displayed exceptionally high nonspecific binding characteristics (88% and 74% of the LDL and HDL fractions, respectively), dropping to much lower values by day 4 (66% and 64% for LDL and HDL, respectively). Likewise, a decrease in nonspecific HDL_2 and $LDL_{II/III}$ binding became evident as differentiation proceeded in the human preadipocytes. The high nonspecific binding of undifferentiated preadipocytes may relate to the structure of the extracellular matrix, which upon differentiation may undergo changes in various components. However, binding of lipoproteins via a receptor-independent pathway has been recog-

nized [Eisenberg, 1984] and may influence nonspecific binding values. In our hands, purified plasma membranes yielded similar high nonspecific binding values, indicating that it is cell mediated. Further investigation is required to explain these observations.

Both ^{125}I-HDL_2 and ^{125}I-$LDL_{II/III}$ were found to bind to 3T3-L1 preadipocytes and differentiated cells by a single affinity binding site, as was evident from linear Scatchard transforms. As was the case for 3T3-L1 differentiation (Fig. 3), preadipocytes isolated from the stromal vascular fraction of mammary adipose tissue were shown in the present study to specifically bind HDL_2 and $LDL_{II/III}$ at all stages of preadipocyte differentiation (Fig. 4). Binding of $LDL_{II/III}$ also yielded linear plots by Scatchard analysis at all stages of the differentiation program, suggesting the presence of a single type of LDL binding site, not unlike that reported by Fong et al. [1984] in human mediastinal and properitoneal adipocyte plasma membranes. The affinity and capacity of this site were largely unaffected by exposure to lipoproteins during the early stages of the differentiation program (Table II). $LDL_{II/III}$ binds avidly to a high-affinity receptor recognition site on the human adipocyte surface, displaying saturable binding (Fig. 1). The affinity for $LDL_{II/III}$ of the differentiated mammary adipocytes (K_d = 10.1 and 11.7 μg lipoprotein protein·ml^{-1} for untreated and treated cells, respectively), compares favorably with those exhibited by fibroblasts with K_d values of 2.6 and 3.2 μg protein·ml^{-1} for LDL fractions II and III, respectively [Jaakkola et al., 1989], and 11 μg LDL protein·ml^{-1} in the human carcinoma cell subclone HT29-18 [Mazière et al., 1992]. The K_d value in the present study of 3 and 2.2 μg protein·ml^{-1} for ^{125}I-$LDL_{II/III}$ with untreated and treated 3T3-L1 cells, respectively, on day 7 (Table I) also compares favorably with these reported values.

The functional significance of HDL interactions with adipocytes with regard to cholesterol flux remains to be established. HDL is believed to be primarily involved in "reverse" cholesterol transport, removing cholesterol from peripheral tissues for delivery to the liver [Tall, 1990]. However, depending on the species, the tissue and the particular HDL fraction involved, HDL may also serve to deliver cholesterol to peripheral cells, e.g., adrenals and gonads [Andersen and Dietschy, 1978]. In view of the results in the present study, one might infer that HDL_2 delivers its core of cholesterol to adipocytes for processing or storage, or both. Such a view would be consistent with clinical and epidemiological studies demonstrating an inverse relation between excess body weight and plasma HDL-cholesterol [Philips et al., 1981], and is further supported by studies in which plasma HDL-cholesterol levels increase with weight reduction [Wolf and Grundy, 1983]. The interaction of HDL_2 with developing human preadipocytes appears to occur through binding sites which are dependent on the stage of the differentiation program (Fig. 4). The physiological significance of the low-affinity site, present only on days 2 and 3, is questionable, owing to its relatively high K_d values (Table III), which implies a requirement for HDL_2 concentrations above the expected interstitial levels. By contrast, the low K_d value of the high-affinity site for ^{125}I-HDL_2 binding on day 2 favors physiological relevance. Its values fall within the physiological range reported for lipoprotein levels in the interstitial fluid [Angel et al., 1979].

The possible significance of the two distinct binding sites for HDL_2, which was consistently observed in undifferentiated human preadipocytes, but never in the 3T3-L1 preadipocytes in the present study, is unclear. Salter et al. [1987] described binding affinities for HDL_2 by omental and subcutaneous depots, which were similar to the affinity observed in the present study for HDL_2 binding to differentiated mammary preadipocytes, but confined to a single affinity binding site. The latter study, based on isolated mature cells that differentiated in vivo, therefore confirmed the results from the present study with respect to mature cultured adipose cells but did not consider cells in the preadipocyte stage of development.

As with the 3T3-L1 cells, ^{125}I-$LDL_{II/III}$ binding conformed to a single affinity site throughout the differentiation program of human preadipocytes (Table II), reaching an affinity in the mature state (day 11) which was comparable to that of the HDL_2 high-affinity site (Table III). The binding affinity for ^{125}I-$LDL_{II/III}$, while relatively high at the onset of differentiation (day 2), was diminished by day 4, before increasing to its final high value in the mature state by day 11 (Table II). The relatively high inherent inter-donor variation in the binding parameters of the human cells at different stages of their development, impeded statistically significant comparisons of some apparent differences.

High levels of inter-batch variation was also reported by Salter et al. [1987] for the binding of HDL_2 to omental and subcutaneous adipocytes and plasma membranes and by Fong et al. [1984].

The specific uptake of both HDL_2 and $LDL_{II/III}$ by human cells increased with differentiation (Fig. 4) and the maximal rates of uptake (V_m) of the two subfractions appeared to be comparable (Tables II and III). As with the binding parameters, the present study also demonstrated substantial inter-individual variation in HDL_2 and $LDL_{II/III}$ uptake by human cells (Tables II and III). This hampered the demonstration of statistically significant differences in some of the data, including the apparently negative effect of lipoprotein treatments on rates of uptake, which was consistently observed.

The present study further indicated that the uptake of both HDL_2 and $LDL_{II/III}$ by 3T3-L1 and human cells occurs via holoparticles, as is evident from the intracellular detection of labeled apoproteins. This finding indicates that at least a portion of the cholesterol ester transfer to the cell involves a specific apoprotein-mediated process. It would thus appear possible for adipose tissue to be involved in the regulation of plasma HDL levels. For example, in obesity, where adipose tissue mass is enlarged and HDL binding and internalization would be enhanced, HDL-cholesterol ester delivery would be increased and thereby augment plasma cholesterol ester turnover. It has been shown that adipocyte HDL binding is increased in obesity [Angel et al., 1986], and that HDL uptake correlates with fat cell size [Despres et al., 1987]. The increased uptake of HDL particles by enlarged fat cells in obesity would thus contribute to changes in the level of plasma HDL. This proposed mechanism may explain in part the established association between obesity and low plasma HDL-cholesterol levels.

In conclusion, the present study confirmed that human adipocytes possess a remarkable capacity to metabolize lipoprotein in vitro. This is dependent, however, on the stage of differentiation of the preadipocytes. A comparison of the results presented in the present study on cells matured in culture with results from the literature [e.g., Angel et al., 1979; Fong et al., 1984] suggests that human preadipocytes differentiated in culture may serve as a convenient model to further investigate lipoprotein interactions in vivo. Furthermore, despite much similarity, certain differences were also noted in the qualitative and quantitative behavior of 3T3-L1 preadipocytes with differentiation, as compared with human preadipocytes. Such differences would question the general validity of the former cell line as a model for the study of human preadipocyte-lipoprotein interactions.

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