Characterization of High Density Lipoprotein Binding to Human Adipocyte Plasma Membranes

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Abstract

Freshly isolated human adipocytes showed specific uptake of 125I-labeled human high density lipoprotein (HDL2 and HDL3), a portion of which could be released by subsequent incubation with excess unlabeled ligand. To study the mechanism of HDL binding, sucrose gradient-purified adipocyte plasma membranes were incubated with radiiodinated lipoprotein particles under equilibrium conditions in the absence (total binding) or presence (nonspecific binding) of 100-fold excess unlabeled ligand. Specific binding of HDL2 and HDL3, calculated by subtracting nonspecific from total binding, was Ca++ independent, unaffected by EDTA, and not abolished by pronase treatment of the membranes. Modification of HDL3 by reductive methylation or cyclohexanedione treatment also failed to affect its binding to adipocyte plasma membranes. High salt concentration (200 mM NaCl) inhibited specific binding of HDL2 and HDL3 but had no effect on LDL binding. A significant portion of 125I-HDL2 or 125I-HDL3 binding was consistently inhibited by adding excess unlabeled LDL, but this inhibition was incomplete as compared with a similar molar excess of unlabeled HDL2 or HDL3.

The role of apoproteins (apo) in HDL binding to adipocyte membranes was examined by comparing binding of HDL2 and HDL3 isolated from normal, abetalipoproteinemic (abeta) and apo E-deficient (apo E0) plasma. Specific binding was observed with all normal and mutant HDL particles. Furthermore, a significant portion (61–78%) of abeta-HDL2, apo E0-HDL2, and apo E0-HDL3 binding was inhibited by adding 100-fold excess of unlabeled low density lipoproteins (LDL). The cross-competition of LDL and HDL binding was confirmed by the ability of normal, abeta, and apo E0-HDL2 to completely inhibit 125I-LDL binding. These data suggest that HDL binding is independent of apo E and that the responsible apoprotein(s) of HDL compete with LDL-apo B for binding to the same or closely related site in the adipocyte plasma membrane. Normal and apo E0-HDL3 binding was also completely inhibited by normal HDL2, which suggested that HDL2 and HDL3 probably bind to the same site. Scatchard analysis of normal HDL2, normal HDL3, and apo E0-HDL3 binding data best fitted a one-component binding profile with similar equilibrium dissociation constants (40–96 nM). HDL3 binding was found to be effectively inhibited by anti-human apo AI or anti-human apo AI, but not by anti-human apo B antisera. This binding was also unaffected by monoclonal anti-human apo B or E antibodies known to inhibit binding of apo B or E containing lipoprotein to the LDL receptor of cultured fibroblasts.

These findings, taken together, suggest that human fat cells possess HDL binding sites with apo AI and/or apo AI specificity. The significant but partial inhibition of HDL2 and HDL3 binding by LDL along with the complete inhibition of LDL binding by HDL2 and HDL3 tends to exclude a single binding site that interacts with both lipoproteins and favors the interpretation that LDL and HDL particles bind to multiple recognition sites or to different conformations of the same lipoprotein binding domain on the human fat cell.

Introduction

Plasma high density lipoproteins (HDL) are critically important in the metabolism of cellular cholesterol, as these particles are believed to act as efflux acceptors of cholesterol from peripheral tissues for excretion by the liver (1, 2) and also deliver cholesterol to steroidogenic tissues for hormone synthesis (3–8). Variations in plasma HDL concentrations have clinical implications, in that low concentrations of HDL are associated with enhanced risk for premature atherosclerosis, whereas high concentrations indicate some protection against the future development of coronary artery disease (9, 10).

The catabolic mechanisms and sites of HDL particle interaction in man remain to be defined. Several reports have shown specific high-affinity binding sites for HDL in steroidogenic tissues of rodents (11–15), especially the adrenal and gonads. Specific binding of human HDL to hepatocytes (16–18), intestinal mucosal cells (19), as well as cultured human skin fibroblast systems (20) have also been described. While these studies have identified a variety of parenchymal and mesenchymal cells that are capable of interacting with HDL particles, the quantitative importance of each of these tissues in vivo may not be reliably predicted from this information. Additionally, in vitro characteristics of HDL binding to cells in culture may bear little relationship to in vivo circumstances.

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where variations in permeability of vascular endothelium and physiological regulation of receptor activities dictate the balance and distribution of cholesterol from tissue to tissue.

Attempts to quantitate the relative importance of different tissues in the irreversible catabolism of HDL have been made using cumulative markers (21). In the rat, the principal tissue sites of degradation of HDL as monitored by labeled apoprotein (apo) A1 are kidney, liver, skin, small intestine, and adipose tissue, in descending order of quantitative significance (21). The sites of HDL catabolism in man are not known, and in vivo assessment using existing methods is not feasible. Alternatively, analysis of tissue biopsy specimens can provide some insight into lipoprotein receptor status and may approximate in vivo characteristics. Several studies have been carried out using human tissue biopsy specimens showing the presence of low density lipoprotein (LDL) and HDL receptors in liver (22) and adipose cells (23).

Human adipose tissue is an important cholesterol storage organ, but does not synthesize significant amounts of cholesterol de novo (24, 25). The flux of cholesterol in this tissue is thus mediated by interaction with lipoproteins. In previous studies from this laboratory, high-affinity specific binding of LDL was shown in freshly isolated human adipocytes (23) and purified plasma membranes (26). Specificity was not absolute, in that human HDL2 and HDL3 both competed effectively with the LDL binding site (23, 26). This observation implied the presence of binding sites for HDL on adipocytes, and the fact that adipose tissue in man is a large organ (24) indicated that adipose tissue could be an important site of HDL interactions in man despite its quantitative insignificance in the rat (21).

In the present study, we examined in detail the interactions of purified adipocyte plasma membranes from freshly isolated adipocytes with a variety of HDL particles isolated from normal subjects and from patients with genetic apoprotein deficiencies. These studies suggest that interactions of human adipocytes with HDL involve specific binding sites for apo AI and/or apo AI-I, and are independent of apo E.

**Methods**

**Lipoprotein isolation, idation, and chemical modification.** Human lipoproteins (LDL d 1.024–1.045; HDL2 d 1.075–1.125; HDL3 d 1.125–1.210) were purified from human plasma obtained from the Canadian Red Cross by sequential ultracentrifugation as previously described (27). Apo E-free HDL2 and HDL3 (apo E2) were isolated from plasma of a patient genetically deficient in apo E (28). Lipoproteins free of apo B were isolated from plasma of a patient with abetalipoproteinemia (29).

All lipoproteins used in the present study were washed once by ultracentrifugation and dialyzed against 0.15 M NaCl and 1 mM EDTA, pH 8.6. Lipoproteins were analyzed by agarose gel electrophoresis (Beckman Paragon electrophoresis system) and by SDS polyacrylamide gel electrophoresis (30). Lipoproteins were iodinated by the iodine monochloride method of McFarlane with minor modifications (31).

Reductive methylation of lipoprotein was carried out at 0°C using sodium borohydride and aqueous formaldehyde in accordance to Weisgraber et al. (32). Cyclohexanedione treatment was performed by incubating lipoprotein (0.5 mg/ml final concentration) in 0.2 M borate buffer, pH 8.1, and 0.15 M cyclohexanedione at 35°C for 2 h as previously described (33).

**Isolation of adipocytes and adipocyte plasma membranes.** 3–15 g of human peritoneal fat were obtained from patients (aged 37–76) undergoing elective cardiovascular surgery. Human adipocytes were freshly isolated by a modified procedure of Rodbell (34) as previously described (23). Fat tissue was incubated with collagenase (1.3 mg/ml) in Krebs-Ringer bicarbonate buffer (KRB) containing 5% bovine serum albumin (BSA) for 1 h at 37°C with constant shaking. Adipocytes were separated from undigested tissue by filtering through a fine nylon mesh and washed thrice with 3 vol of KRB containing 2% BSA. Approximately 5 × 10^6 adipocytes were obtained from 1 g of fat tissue.

Plasma membranes were prepared from the washed, isolated adipocytes by the procedure of McKeel and Jarrett (35). Cells were mixed with 8 ml of 10 mM Tris-HCl buffer containing 0.25 M sucrose and 1 mM EDTA, pH 7.4 (medium I), and broken by 10 up-and-down strokes in a Dounce homogenizer. The crude homogenate was chilled on ice and the congealed fat removed before centrifugation at 16,000 g for 15 min. The pellet was resuspended in medium I and centrifuged at 1000 g for 10 min. The resultant supernatant was used to prepare plasma membranes and was subjected to centrifugation at 16,000 g for 20 min. The pellet, resuspended in 1 ml of medium I, was layered onto 11-ml linear sucrose gradient (32–52% sucrose in 1 mM EDTA, 10 mM Tris-HCl, pH 7.4), and centrifuged at 36,000 rpm for 50 min in a Beckman SW 40 rotor. The plasma membranes banded near the top of the gradient and were collected, diluted with buffer containing 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, and centrifuged at 22,800 rpm for 30 min in a Beckman SW 40 rotor. The plasma membrane pellet (referred to as adipocyte membranes) was resuspended in 0.5–1 ml of 10 mM Tris-HCl, pH 7.4 and stored at −20°C for later use. The above isolation procedure yielded on the average 105-106 μg membrane protein/10^6 cells.

The purity of the plasma membrane fraction was examined by enzyme marker assays. 5' nucleotidase (36), succinic dehydrogenase (37), acid phosphatase (38), and NADH dehydrogenase (39) were measured as previously described. The purified adipocyte membranes were found to be 3–5-fold higher in 5' nucleotidase activity compared with cell homogenate, and contained negligible mitochondrial, lysosomal, and microsomal contamination. It was also observed that lipoprotein binding activities of the purified plasma membranes remained unchanged throughout a storage period of 3 mo at −20°C.

**Lipoprotein uptake and degradation by adipocytes.** Freshly isolated adipocytes were incubated with shaking at 37°C in 1-oz plastic bottles in 2 ml KRB buffer containing 5% BSA (KRBa), 5 mM glucose, 125I-labeled lipoprotein, and 2.5 μg/ml lima bean trypsin inhibitor. Previous studies (23) have indicated that inclusion of trypsin inhibitor in the assay reduces nonspecific degradation of lipoprotein. After 4 h of incubation, the medium and cells were separated and the cells were washed four times with 3 ml KRBa. The cells were then denatured with 5 ml 10% TCA and TCA at 80°C for 10 min. The TCA precipitate and supernatant were delipidated and the organic extracts were pooled and used for determination of total cell lipid weight. The TCA-insoluble lipid-extracted residues were dried, dissolved in 1 ml of NaOH, and their radioactivity determined. The latter was taken to represent cellular uptake of 125I-labeled lipoprotein.

To measure lipoprotein degradation the medium was precipitated with 10% TCA. The amount of 125I-radioactivity in the TCA-soluble fraction was determined after chloroform extraction and represented cell-mediated lipoprotein degradation. Noncell-mediated degradation was measured in flasks containing medium but not cells and the values were subtracted from those with cells.

**Lipoprotein binding assay.** Binding of 125I-labeled lipoproteins to purified adipocyte plasma membranes was assayed by a modified procedure of Basu et al. (40). Standard binding assays were carried out in 75 μl buffer containing 100 mM NaCl, 0.5 mM CaCl2, 50 mM Tris-HCl, pH 7.5, 2 mg/ml BSA, 10 μg purified plasma membrane protein, and varying amounts of 125I-labeled lipoprotein. After 1 h of incubation at 0°C, the membranes were resolubilized by centrifugation in a Beckman airfuge (100,000 g) or in an Eppendorf centrifuge (15,600 g) for 15 min. Both centrifugation procedures pelletled all the membranes that were washed once by centrifugation with 200 μl KRB buffer containing 2% BSA, pH 7.5. The bottom of the centrifuge tube containing the pellet was then cut off with a razor blade and the amount of membrane-associated radioactivity was determined in a
gamma counter. Nonspecific binding was measured by adding 100-fold excess unlabeled ligand. Parallel assays were also set up as no membrane controls with the corresponding amounts of $^{125}$I-labeled ± unlabeled ligand, and these values were subtracted from the membrane containing assays.

**Apoprotein antibodies.** Anti-human apo AI, anti-human apo AII, and anti-human apo B antisera were purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN. Monoclonal antibodies directed against apo E (1D7) and apo B (3A10) were kindly provided by Dr. R. Milne and Dr. Y. Marcel of the Clinical Research Institute of Montreal, Quebec H2W 1R7, Canada.

**Binding data analysis.** Binding data were analyzed by the method of Scatchard (41) and were fitted by an iterative nonlinear least square procedure (42) on a MEDAC S-100 Z80 microcomputer (kindly provided by Dr. D. Isenman, Department of Biochemistry, University of Toronto).

## Results

The apoprotein compositions of the various human HDL$_2$ (d 1.075–1.25) and HDL$_3$ (d 1.12–1.210) used in the present study were analyzed by SDS-polyacrylamide gel electrophoresis and the results are shown in Fig. 1. Apo E was present in HDL$_2$ isolated from plasma of normal individuals but was clearly missing in the HDL$_2$ isolated from the apo E-deficient (apo E$_0$) patient. The apoproteins of HDL$_3$ from both the normal and apo E$_0$ individuals showed similar polyacrylamide gel patterns, indicating the absence of apo E in our normal HDL$_3$ ultracentrifugal preparations.

The interaction of normal $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ by adipocytes isolated from human fat tissue was assayed after 4 h of incubation with 10 μg/ml $^{125}$I-labeled HDL at 37°C. Table I shows that $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ radioactivity was found to be associated with the cells (uptake). About 90% of this cellular uptake of $^{125}$I-labeled HDL was specifically inhibited by the addition of 25-fold excess unlabeled HDL in the incubation medium. Results in Table I also show that in three out of the four experiments, TCA-soluble degradation products (small peptides) of $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ could be detected in the incubation medium, but this degradation did not appear to be inhibited when excess unlabeled HDL was added (experiments 2 and 3).

A portion of the $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ uptake by adipocytes was observed to be reversibly dissociated. In these experiments, cells were reisolated and washed with KRBA after 4 h of incubation with $^{125}$I-labeled HDL and were then resuspended in fresh KRBA. After incubation for 30–120 min at 37°C with constant shaking, the amount of $^{125}$I-radioactivity that remained associated with the cells was measured. Results in Fig. 2 show that the cell-associated $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ radioactivity decreased with time. This decrease appeared to level off between 1 and 2 h after addition of unlabeled ligand and 59–61% of the $^{125}$I-HDL$_2$ radioactivity remained associated with the cells. The dissociated radioactivity (measured in the incubation medium) was all TCA-precipitable and could represent HDL particles that were reversibly bound to the surface of the adipocytes.

The binding characteristics of HDL particles to human adipocytes were more specifically examined by employing plasma membranes purified from the freshly isolated adipocytes. Fig. 3 shows a time course of $^{125}$I-HDL$_2$ binding to adipocyte plasma membranes at 0°C. Total and nonspecific binding, measured respectively in the absence and presence of 100-fold excess unlabeled HDL$_2$, reached equilibrium after 60 min of incubation. Similar results were obtained for $^{125}$I-HDL$_3$ binding (data not shown). All subsequent experiments on HDL$_2$ and HDL$_3$ binding were thus carried out at 0°C for 1 h.

Human adipocyte membranes were previously shown to specifically bind LDL, and this binding was completely inhibited

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**Table I. Uptake and Degradation of $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ by Freshly Isolated Human Adipocytes**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cell</th>
<th>$^{125}$I-HDL</th>
<th>Uptake</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>+ Cold</td>
</tr>
<tr>
<td>1</td>
<td>F 70 yr</td>
<td>HDL$_2$</td>
<td>0.45±0.07</td>
<td>0.04±0.002</td>
</tr>
<tr>
<td>2</td>
<td>M 66 yr</td>
<td>HDL$_2$</td>
<td>0.84±0.20</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>3</td>
<td>M 46 yr</td>
<td>HDL$_3$</td>
<td>2.29±0.23</td>
<td>0.21±0.04</td>
</tr>
<tr>
<td>4</td>
<td>M 53 yr</td>
<td>HDL$_3$</td>
<td>1.23±0.12</td>
<td>0.17±0.004</td>
</tr>
</tbody>
</table>

Adipocytes were obtained by collagenase digestion of peritoneal fat obtained from four different individuals. The freshly isolated adipocytes were incubated with 10 μg/ml $^{125}$I-HDL$_2$ or $^{125}$I-HDL$_3$ for 4 h at 37°C with constant shaking. Parallel flasks were set up containing excess unlabeled HDL$_2$ or HDL$_3$ (250 μg/ml). The amounts of TCA-precipitable radioactivity associated with the cells (uptake) and TCA-soluble radioactivity in the incubation medium (degradation) were determined as described in Methods. Each value represents the mean±1 SD of triplicate assays.

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**Figure 1.** Apoproteins of normal and apo E$_2$ HDL$_2$ and HDL$_3$ separated on SDS-polyacrylamide (10%) gel electrophoresis. 100 μg of each lipoprotein was delipidated in ethanol-diethyl ether (3:1 vol/vol) and reduced with 10 mM dithiothreitol before electrophoresis. Apo E was present in normal but not in apo E$_2$ HDL$_2$. The similar electrophoretic pattern of normal and apo E$_2$ HDL$_3$ indicates that normal HDL$_3$ contains no apo E.

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containing 250 µg/ml of the corresponding unlabeled ligand. After further incubation for 30-120 min at 37°C, aliquots of the cells were reisolated and the amount of TCA-precipitable radioactivity that remained associated with the cells was determined. Each point represents the mean±1 SD of triplicate assays.

The specific binding of HDL2 and HDL3 to adipocyte plasma membranes was unaffected when EDTA was added to the binding buffer at a final concentration of 10 mM or when calcium was omitted (Table II). The effect of pronase treatment on HDL binding was also examined. In three separate experiments, preincubation of adipocyte membranes with pronase (30 µg/ml) failed to abolish 125I-HDL2 specific binding (2.68±1.24 µg/mg for control vs. 3.23±1.19 µg/mg for pronase-treated membranes). Similar characteristics have been observed for LDL binding to adipocyte membranes (26). High salt, however, affected LDL and HDL binding differently. Table III shows that HDL2 and HDL3 specific binding was reduced by HDL2 and HDL3 (26). The effect of LDL on HDL2 and HDL3 binding was therefore examined. Fig. 4, A and B show that excess unlabeled LDL inhibited 125I-HDL2 and 125I-HDL3 binding, but not to the same extent as as unlabeled HDL2 or HDL3 at similar molar excess. The results show that ~75% of HDL2 (Fig. 4 A) and 65% of HDL3 (Fig. 4 B) binding was inhibited by 25-fold molar excess of unlabeled LDL as compared with 85-95% inhibition obtained in the presence of 25-fold molar excess of the homologous ligand. Inhibition of 125I-HDL2 and 125I-HDL3 binding appeared to level off between 50- and 100-fold molar excess of unlabeled lipoproteins. At 100-fold molar excess of LDL, 85% of HDL2 (Fig. 4 A) and 80% of HDL3 (Fig. 4 B) binding was inhibited. Thus, LDL inhibited HDL2 and HDL3 binding significantly but incompletely.

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Table III. Effect of NaCl on HDL2, HDL3, and LDL Specific Binding

<table>
<thead>
<tr>
<th>NaCl concentration (mM)</th>
<th>Specific binding (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>HDL2 (n = 2) 4.82±0.92*</td>
</tr>
<tr>
<td>200</td>
<td>HDL3 (n = 7) 2.66±0.90§</td>
</tr>
<tr>
<td></td>
<td>LDL (n = 4) 1.88±1.82§</td>
</tr>
</tbody>
</table>

Adipocyte plasma membranes were purified from human peritoneal fat as described in Methods. Binding assays were carried out using 10 μg (protein) of purified membranes and 40 μg/ml 125I-labeled lipoprotein in binding buffer containing 50 mM Tris-HCl (pH 7.5), 0.5 mM CaCl2, 2 mg/ml BSA, and 100 mM (standard) or 200 mM NaCl. Specific binding was determined as the difference in binding between that in the presence and that in the absence of 100-fold excess unlabeled ligand. * Number of different membrane preparations. § Average±range. Mean±SD.

Table IV. Effect of Various Lipoproteins on Normal HDL3, Apo E-deficient HDL3, and Normal LDL Binding to Human Adipocyte Plasma Membranes

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Membrane</th>
<th>125I-Lipoprotein</th>
<th>Control</th>
<th>HDL2</th>
<th>HDL3</th>
<th>E0-HDL2</th>
<th>E0-HDL3</th>
<th>Abeta-HDL3</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M 50 yr</td>
<td>HDL3</td>
<td>2.50</td>
<td>0.23</td>
<td>0.23</td>
<td>ND</td>
<td>0.18</td>
<td>ND</td>
<td>1.04</td>
</tr>
<tr>
<td>2</td>
<td>M 53 yr</td>
<td>E0-HDL3</td>
<td>2.42</td>
<td>0.21</td>
<td>0.17</td>
<td>ND</td>
<td>0.15</td>
<td>0.21</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>M 50 yr</td>
<td>LDL</td>
<td>1.46</td>
<td>0.10</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>0</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Adipocyte plasma membranes were obtained from peritoneal fat tissues as described in Methods. 10 μg (protein) adipocyte membranes were incubated with 40 μg/ml 125I-labeled lipoprotein (control). Parallel assays contained, in addition, 100-fold protein excess of the indicated unlabeled lipoprotein. Each value represents the average of duplicate assays. Normal and apo E0 HDL3 bound specifically to adipocyte membranes. Both bindings were partially inhibited by unlabeled LDL but were completely inhibited by HDL2 and by each other. These results suggest that HDL2 and HDL3 probably bind to the same site and that apo E is not required for binding. The various unlabeled HDL particles also completely inhibited 125I-LDL binding.

Adipocyte plasma membranes were purified from human peritoneal fat as described in Methods. Binding assays were carried out using 10 μg (protein) of purified membranes and 40 μg/ml 125I-labeled lipoprotein in binding buffer containing 50 mM Tris-HCl (pH 7.5), 0.5 mM CaCl2, 2 mg/ml BSA, and 100 mM (standard) or 200 mM NaCl. Specific binding was determined as the difference in binding between that in the presence and that in the absence of 100-fold excess unlabeled ligand. * Number of different membrane preparations. § Average±range. Mean±SD.

The possibility that apo AI and/or apo AII are primarily involved in the binding of HDL2 and HDL3 to adipocyte membranes was tested by using monoclonal and polyclonal antibodies to specific apoproteins. Fig. 5 shows that HDL3 binding was inhibited by anti-human apo AI and anti-human apo AII, but not by anti-human apo B antibodies. Furthermore, two monoclonal antibodies directed against apo E (1D7) and apo B (3A10) that have been shown to inhibit apo E-HDL and LDL binding, respectively, to human fibroblasts (43, 44), also failed to have any effect on HDL3 binding to adipocyte membrane. Similar inhibition by anti-apo AI and anti-apo AII was observed for HDL2 binding (data not shown).

The affinity of HDL2 and HDL3 binding to human adipocyte membranes was examined by dose-response binding assays. Fig. 6 A shows that total binding of 125I-HDL2 was ligand concentration dependent and was saturable at ~40 μg/ ml 125I-HDL2. Nonspecific binding, measured in the presence of 100-fold excess unlabeled HDL2, also appeared to be curvilinear and saturable and represented <10% of total binding. Specific binding was obtained by subtracting nonspecific binding from total. The addition of 100-fold protein (25-fold molar) excess of unlabeled LDL reduced total 125I-HDL2 binding by ~50%. The Scatchard plot for 125I-HDL2 specific binding (Fig. 6 B) best fitted a one-component system. Dose-response binding assays were also carried out with normal HDL3 (Fig. 7 A and B) and with apo E0-HDL3, with similar results.

The equilibrium dissociation constant (Kd) and maximum binding capacity (Bmax) obtained for normal HDL2, normal HDL3, and apo E0-HDL3 by Scatchard analysis are listed in Table VI. With the exception of exp. 2, the binding affinities and capacities for various HDL particles were remarkably similar.

Discussion

The present study extends our previous work on LDL interaction with adipocytes (23, 26) and demonstrates that human

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fat cells interact with a variety of normal and mutant HDL particles in a specific manner. These findings are consistent with recent studies showing in vivo catabolism of HDL apoprotein in fat tissue (21), and emphasize the possibility that fat tissue in man, because of its mass, may be quantitatively more important compared with lower animals. The observed degradation of HDL by the freshly isolated human adipocyte system was, however, variable. While HDL degradation was apparently cell dependent, its relation to cellular internalization and processing remains to be elucidated. Our studies on HDL interaction with freshly isolated human adipocytes demonstrated that a significant portion of the HDL taken up by the fat cells was reversibly dissociated. While the latter probably represents surface-bound HDL particles, the undissociable portion probably represents internalized or otherwise sequestered particles. Furthermore, because cellular processing (presumably internalization and degradation) of HDL could occur simultaneously, the accurate definition of binding in intact cells is difficult. Thus, in order to characterize binding of HDL to adipocytes independent of cellular processing, we resorted to the use of purified plasma membranes.

The results of the membrane studies demonstrated that $^{125}$I-HDL$_2$ and $^{125}$I-HDL$_3$ were bound to purified adipocyte membranes in a specific and saturable manner. This binding was insensitive to EDTA, independent of calcium (Table II), and not inhibited by pronase, thus resembling HDL binding in a number of ex vivo tissue studies (13, 45) including human liver membranes (46). In addition, HDL$_2$ and HDL$_3$ inhibited each other’s binding to human adipocyte membranes, showed similar $K_d$ (Table VI), and were inhibited by high salt (Table III). These similar binding characteristics suggest that HDL$_2$ and HDL$_3$ particles bind to the same site in human adipocyte membranes.

The nonspecific binding of $^{125}$I-labeled HDL to adipocyte plasma membranes accounts for only 5–10% of total HDL binding and appeared to have a saturable profile (Figs. 6 and 7). This is consistent with the curvilinear nonspecific LDL binding previously observed (26). A saturable nonspecific binding curve may be due in part to the constant ratio of unlabeled to $^{125}$I-labeled ligand (100:1) in the assay for all ligand concentrations used. This explanation is inadequate, however, as total binding was also saturable. The highly vesiculated nature of the adipose cell surface as frequently observed in electron microscopic studies (47) could have

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**Table V. Specific Binding of Different HDL Particles to Human Adipocyte Plasma Membranes**

<table>
<thead>
<tr>
<th>Specific binding</th>
<th></th>
<th>% Inhibited by LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg HDL/mg membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal HDL$_2$ (n = 11)</td>
<td>3.50±1.21*</td>
<td>61.5±10.6</td>
</tr>
<tr>
<td>Normal HDL$_3$ (n = 4)</td>
<td>1.95±0.72</td>
<td>57.6±8.8</td>
</tr>
<tr>
<td>Abeta HDL$_2$ (n = 3)</td>
<td>2.76±1.05</td>
<td>78.0±5.2</td>
</tr>
<tr>
<td>Apo E$_2$ HDL$_2$ (n = 3)</td>
<td>3.83±1.16</td>
<td>74.0±1.7</td>
</tr>
<tr>
<td>Apo E$_2$ HDL$_3$ (n = 7)</td>
<td>2.78±1.21</td>
<td>61.3±8.3</td>
</tr>
</tbody>
</table>

Specific binding of the various normal and mutant HDL particles was measured at 40 µg/ml $^{125}$I-labeled ligand and 10 µg (protein) purified adipocyte plasma membranes as described in Methods. The portion of HDL binding inhibited by LDL was assayed in the presence of 100-fold protein excess unlabeled LDL. Human adipocyte plasma membranes displayed similar specific binding of normal, abeta, or apo E$_2$ HDL particles and all were partially inhibited by LDL.

* Mean±SD.
concentrations of 
subtracting nonspecific (o) excess unlabeled 
7.

Membrane HDL 3

Table VI. K_d and B_{max} of HDL Particles to Various Human Adipocyte Plasma Membrane Preparations

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Membrane</th>
<th>HDL</th>
<th>K_d*</th>
<th>B_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M 67 yr</td>
<td>Normal HDL3</td>
<td>40</td>
<td>3.05</td>
</tr>
<tr>
<td>2</td>
<td>M 62 yr</td>
<td>Normal HDL3</td>
<td>96</td>
<td>7.68</td>
</tr>
<tr>
<td>3</td>
<td>M 65 yr</td>
<td>Normal HDL3</td>
<td>56</td>
<td>2.76</td>
</tr>
<tr>
<td>4</td>
<td>M 61 yr</td>
<td>Normal HDL3</td>
<td>69</td>
<td>2.15</td>
</tr>
<tr>
<td>5</td>
<td>F 59 yr</td>
<td>Apo E_HDL3</td>
<td>48</td>
<td>1.56</td>
</tr>
<tr>
<td>6</td>
<td>M 44 yr</td>
<td>Apo E_HDL3</td>
<td>51</td>
<td>3.47</td>
</tr>
<tr>
<td>7</td>
<td>M 64 yr</td>
<td>Apo E_HDL3</td>
<td>67</td>
<td>3.27</td>
</tr>
</tbody>
</table>

Binding assays and methods of calculation for all the experiments were similar to that described in the legend to Fig. 6.

* K_d for experiments 1 and 4 correspond to values shown in Figs. 6 and 7, respectively.

Figure 7. ^125^I-HDL3 binding to human adipocyte plasma membranes. Plasma membranes were purified from adipocytes that were isolated from peritoneal fat obtained from a 61-yr-old male. (A) Purified plasma membranes (9.6 μg protein) were incubated with varying concentrations of ^125^I-HDL3 in the absence (e) or presence of 100-fold excess unlabeled HDL3 (o) or in the presence of 100-fold protein excess unlabeled LDL (o). Specific binding (w) was calculated by subtracting nonspecific (o) from total binding (e) and was saturable. Excess unlabeled LDL partially inhibited ^125^I-HDL3 specific binding. Each point represents the average of duplicate assays. (B) Scatchard plot of ^125^I-HDL3 specific binding. Scatchard analysis was carried out as described in legend to Fig. 6. The molecular weight of 1.75 × 10^6 (55% protein) was used in the calculation.

We conclude that HDL binding to human fat cells is probably related to apo A apoproteins. Indeed, studies on the effects of anti-apo A1 and anti-apo AII antibodies on HDL binding suggest that both apo A1 and apo AII, the major apoprotein components of HDL particles, may be involved in the binding of the lipoprotein particles to human adipocyte membranes (Fig. 5). While this is consistent with other cell types known to interact with HDL in a specific manner (14, 18, 49), a role for other minor apoproteins (e.g., apo C's) and lipoprotein ligands in the binding process has not been excluded.

The cross-competition between LDL and HDL binding to adipocytes and purified membranes is substantial, and may be interpreted in a number of ways. The simplest interpretation is that LDL and HDL particles both bind to the same site. This could explain why HDL2 and HDL3 completely inhibit LDL binding, but fails to adequately explain the partial inhibition of HDL2 and HDL3 binding by LDL or the differential effect of high NaCl concentration on LDL and HDL specific binding. Another interpretation is that human adipocytes contain multiple lipoprotein binding sites consisting of one class that recognizes both LDL and HDL particles and another class that binds HDL particles only. Alternatively, the multiple sites may be constituted of distinct but juxtaposed LDL and HDL binding sites. The cross-competition of LDL and HDL binding may be accounted for in this case by steric hindrance or negative co-operativity. A third interpretation is that LDL and HDL bind to different conformations of the same binding domain. These different conformations may exhibit preferential interactions with LDL or HDL and may be differently affected by high NaCl concentration. This model may explain the relaxed binding specificity of human adipocytes for various lipoprotein particles and the latter's ability to modulate each other's binding.

We conclude from our present study that human adipose tissue, functioning through its lipoprotein binding sites or domain, is probably an important site of interaction for both low and high density lipoproteins in vivo. The lower than normal level of plasma HDL-cholesterol (50–53) and the increased rate of LDL turnover (54) observed in obesity might in fact be explained in part by an increase in lipoprotein metabolism by the expanded adipose mass in these individuals.

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References


