

Characterization of High Density Lipoprotein Binding to Human Adipocyte Plasma Membranes

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Abstract

Freshly isolated human adipocytes showed specific uptake of ^{125}I -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 ^{125}I -HDL₂ or ^{125}I -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 ^{125}I -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 AII, 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 apo 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 AII 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)¹ 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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1. Abbreviations used in this paper: abeta, abetalipoproteinemic; apo, apoprotein; apo AI, apo AII, apo B, apo E, apoproteins AI, AII, B, and E; apo E₀, apo E-deficient; B_{max} , maximum binding capacity; K_d , equilibrium dissociation constant; KRB, Krebs-Ringer bicarbonate buffer; KRBA, KRB containing 5% BSA; medium I, 10 mM Tris-HCl buffer containing 0.25 M sucrose and 1 mM EDTA, pH 7.4.

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) AI 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 AII, and are independent of apo E.

Methods

Lipoprotein isolation, iodination, 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 E₀) 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 5×10^5 adipocytes were obtained from 1 g of fat tissue.

Plasma membranes were prepared from the washed, isolated adipocytes by the procedure of McKeel and Jarett (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 101.5 ± 9.6 - μ g 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, 125 I-labeled lipoprotein, and 2.5 μ g/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 heated 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 125 I-labeled lipoprotein.

To measure lipoprotein degradation the medium was precipitated with 10% TCA. The amount of 125 I-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 125 I-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 μ l buffer containing 100 mM NaCl, 0.5 mM CaCl₂, 50 mM Tris-HCl, pH 7.5, 2 mg/ml BSA, 10 μ g purified plasma membrane protein, and varying amounts of 125 I-labeled lipoprotein. After 1 h of incubation at 0°C, the membranes were reisolated by centrifugation in a Beckman airfuge (100,000 g) or in an Eppendorf centrifuge (15,600 g) for 15 min. Both centrifugation procedures pelleted all the membranes that were washed once by centrifugation with 200 μ l 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 \pm 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 HDL₂ (d 1.075–1.125) and HDL₃ (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 HDL₂ isolated from plasma of normal individuals but was clearly missing in the HDL₂ isolated from the apo E-deficient (apo E₀) patient. The apoproteins of HDL₃ from both the normal and apo E₀ individuals showed similar polyacrylamide gel patterns, indicating the absence of apo E in our normal HDL₃ ultracentrifugal preparations.

The interaction of normal ^{125}I -HDL₂ and ^{125}I -HDL₃ by adipocytes isolated from human fat tissue was assayed after 4 h of incubation with 10 $\mu\text{g}/\text{ml}$ ^{125}I -labeled HDL at 37°C. Table I shows that ^{125}I -HDL₂ and ^{125}I -HDL₃ 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 -HDL₂ and ^{125}I -HDL₃ 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 -HDL₂ and ^{125}I -HDL₃ uptake by adipocytes was observed to be reversibly dissociated. In these

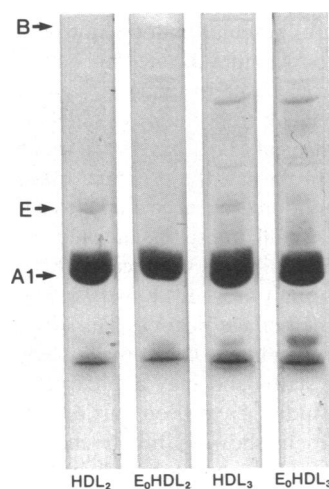


Figure 1. Apoproteins of normal and apo E₀ HDL₂ and HDL₃ separated on SDS-polyacrylamide (10%) gel electrophoresis. 100 μg of each lipoprotein was delipidated in ethanol-diethyl ether (3:1 vol/vol) and reduced with 10 mM dithiothreitol before electrophoresis. Apo E was present in normal but not in apo E₀-HDL₂. The similar electrophoretic pattern of normal and apo E₀-HDL₃ indicates that normal HDL₃ contains no apo E.

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 -HDL₂ and ^{125}I -HDL₃ 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 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 -HDL₂ binding to adipocyte plasma membranes at 0°C. Total and nonspecific binding, measured respectively in the absence and presence of 100-fold excess unlabeled HDL₂, reached equilibrium after 60 min of incubation. Similar results were obtained for ^{125}I -HDL₃ binding (data not shown). All subsequent experiments on HDL₂ and HDL₃ 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

Table I. Uptake and Degradation of ^{125}I -HDL₂ and ^{125}I -HDL₃ by Freshly Isolated Human Adipocytes

Experiment	Cell	^{125}I -HDL	μg HDL protein/g cell lipid/4 h			
			Uptake		Degradation	
			Total	+ Cold	Total	+ Cold
1	F 70 yr	HDL ₂	0.45 \pm 0.07	0.04 \pm 0.002	0	0
2	M 66 yr	HDL ₂	0.84 \pm 0.20	0.06 \pm 0.01	0.12 \pm 0.04	0.08 \pm 0.05
3	M 46 yr	HDL ₃	2.29 \pm 0.23	0.21 \pm 0.04	0.20 \pm 0.02	0.17 \pm 0.06
4	M 53 yr	HDL ₃	1.23 \pm 0.12	0.17 \pm 0.004	0.25 \pm 0.04	0.04 \pm 0.02

Adipocytes were obtained by collagenase digestion of periperitoneal fat obtained from four different individuals. The freshly isolated adipocytes were incubated with 10 $\mu\text{g}/\text{ml}$ ^{125}I -HDL₂ or ^{125}I -HDL₃ for 4 h at 37°C with constant shaking. Parallel flasks were set up containing excess unlabeled HDL₂ or HDL₃ (250 $\mu\text{g}/\text{ml}$). 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 \pm 1 SD of triplicate assays.

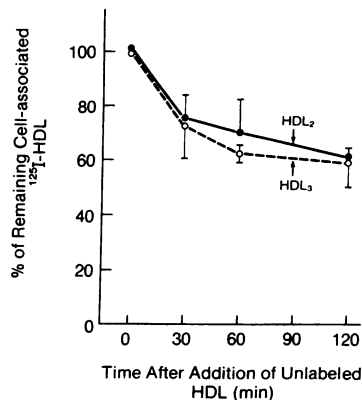


Figure 2. Release of cell-associated ^{125}I -HDL from human adipocytes by excess unlabeled HDL. Human adipocytes were freshly isolated from peritoneal fat tissues of two different individuals, a 53-yr-old male (\bullet) and a 61-yr-old male (\circ). The isolated adipocytes were incubated with $10\ \mu\text{g}/\text{ml}$ of ^{125}I -HDL₂ or ^{125}I -HDL₃ for 2 h at 37°C . The cells were reisolated, washed, and resuspended in fresh KRBA

containing $250\ \mu\text{g}/\text{ml}$ 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 \pm 1 SD of triplicate assays.

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 ^{125}I -HDL₂ and ^{125}I -HDL₃ binding, but not to the same extent as unlabeled HDL₂ or HDL₃ at similar molar excess. The results show that $\sim 75\%$ 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 ^{125}I -HDL₂ and ^{125}I -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.

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 ($30\ \mu\text{g}/\text{ml}$) failed to abolish ^{125}I -HDL₂ specific binding ($2.68 \pm 1.24\ \mu\text{g}/\text{mg}$ for control vs. $3.23 \pm 1.19\ \mu\text{g}/\text{mg}$ 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

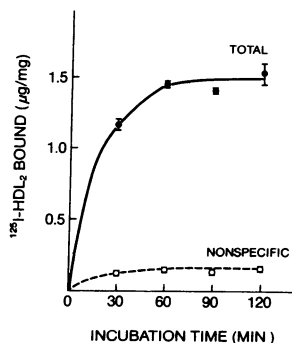


Figure 3. Time course of ^{125}I -HDL₂ binding to human adipocyte plasma membranes. Adipocytes were isolated from peritoneal fat of a 61-yr-old female by collagenase digestion for adipocyte plasma membrane purification. $10\ \mu\text{g}$ (protein) of purified plasma membranes were incubated at 0°C with $20\ \mu\text{g}/\text{ml}$ ^{125}I -HDL₂ in the absence (\bullet) or presence of 100-fold excess unlabeled HDL₂ (\circ) for 30–120 min. Each point represents the average and range of duplicate assays.

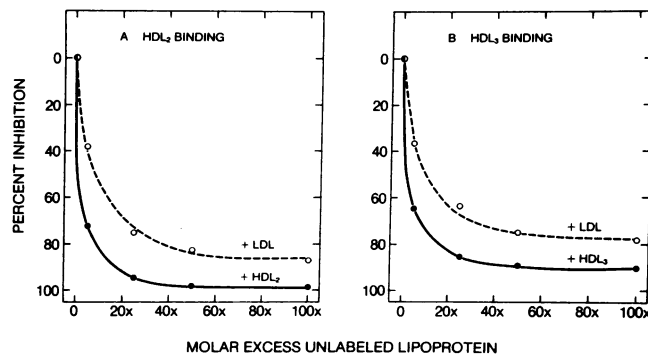


Figure 4. Effect of LDL on ^{125}I -HDL₂ (A) and ^{125}I -HDL₃ (B) binding to human adipocyte plasma membranes. Adipocyte plasma membranes were purified from peritoneal fat of two different male patients, aged 59 (A) and 63 (B), respectively. $10\ \mu\text{g}$ (protein) of the purified membranes was incubated with $2.5\ \mu\text{g}/\text{ml}$ ^{125}I -HDL₂ or ^{125}I -HDL₃ in the absence (\bullet) or presence (\circ) of varying amount of unlabeled lipoprotein for 1 h at 0°C . LDL did not inhibit ^{125}I -HDL₂ or ^{125}I -HDL₃ binding to the same extent as unlabeled HDL₂ or HDL₃ in equivalent molar excess.

$\sim 40\%$ in the presence of 200 mM NaCl as compared with 100 mM NaCl (standard condition used in our binding assay), while LDL specific binding was unaffected by high NaCl concentration as previously reported (26). Chemical modification of lysine (reductive methylation) or arginine (cyclohexanedione treatment) residues of HDL₃ had no effect on its binding to adipocyte membranes. In four experiments, specific binding of reductively methylated HDL₃ ($2.88 \pm 0.94\ \mu\text{g}/\text{mg}$, mean \pm SD) and cyclohexanedione-treated HDL₃ ($2.91 \pm 1.1\ \mu\text{g}/\text{mg}$) were not significantly different from native unmodified HDL₃ ($2.99 \pm 0.93\ \mu\text{g}/\text{mg}$).

The possible role of apo E in the recognition of lipoproteins by human adipocyte membranes was investigated using apo E-free HDL₂ and apo E-free HDL₃ fractions isolated from the plasma of a patient genetically deficient in apo E (apo E₀; Fig. 1). The results in Table IV show that $>90\%$ of normal ^{125}I -HDL₃ binding was inhibited by excess normal HDL₂ or HDL₃

Table II. Effect of EDTA and Calcium on HDL₂ and HDL₃ Specific Binding

	Specific binding (μg HDL protein/mg membrane protein)	
	HDL ₂	HDL ₃
Standard	$3.90 \pm 0.20^*$ (100%) \ddagger	2.14 ± 0.01 (100%)
+EDTA	3.85 ± 0.15 (99%)	2.33 ± 0.03 (109%)
Minus Ca ⁺⁺	4.35 ± 0.04 (112%)	2.31 ± 0.06 (108%)

Adipocyte plasma membranes were purified from peritoneal fat of a 59-yr-old male. Binding assays were carried out using $10\ \mu\text{g}$ (protein) of purified membranes and $40\ \mu\text{g}/\text{ml}$ ^{125}I -HDL₂ or ^{125}I -HDL₃ in standard binding buffer containing 50 mM Tris-HCl, 100 mM NaCl, 0.5 mM CaCl₂, and 2 mg/ml BSA, pH 7.5. In parallel assays, calcium was omitted or 10 mM EDTA (final concentration) was added. 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. Experiment was repeated with two other membrane preparations and similar results were obtained.

* Average \pm range of duplicate assays.

\ddagger As a percentage of standard condition binding.

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

	Specific binding ($\mu\text{g}/\text{mg}$)	
	NaCl concentration (mM)	
	100	200
HDL ₂ (n = 2)	4.82±0.92*	2.99±0.52 (62%)§
HDL ₃ (n = 7)	2.66±0.90‡	1.53±0.49 (57%)
LDL (n = 4)	1.88±1.82‡	1.86±1.55 (97%)

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 $\mu\text{g}/\text{ml}$ 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. n, number of different membrane preparations.

* Average±range.

‡ Mean±SD.

§ Binding as a percentage of 100 mM NaCl.

^{||} Significantly different from 100 mM NaCl, *P* < 0.01 by paired *t* test.

^{||} Not significantly different from 100 mM NaCl.

and that excess apo E₀-HDL₃ was as effective as normal HDL in inhibiting ¹²⁵I-HDL₃ binding. Only 58% of the ¹²⁵I-HDL₃ binding was inhibited by 100-fold (protein) excess unlabeled LDL. In exp. 2, ¹²⁵I-apo E₀-HDL₃ bound specifically to adipocyte plasma membranes, and this binding was also inhibited completely by normal HDL₂, normal HDL₃, and abeta HDL₃. Again excess LDL only partially inhibited apo E₀-HDL binding. These findings indicate that binding of HDL to fat cell membranes is independent of apo E. Table IV also shows the results of a cross-inhibition study on the effect of various normal and mutant HDL particles on LDL binding to adipocyte membranes. In exp. 3, ¹²⁵I-LDL binding was found to be completely inhibited by 100-fold excess unlabeled normal HDL₂, normal HDL₃, abeta HDL₃, apo E₀-HDL₂, and apo E₀-HDL₃.

The specific binding of various ¹²⁵I-labeled HDL₂ and

HDL₃ particles to a number of different human adipocyte plasma membrane preparations are listed in Table V. Binding assays were carried out using 40 $\mu\text{g}/\text{ml}$ ¹²⁵I-labeled ligand. Partial inhibition of normal, abeta, and apo E₀ HDL₂ or HDL₃ specific binding was observed in the presence of 100-fold protein excess unlabeled LDL in all cases.

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 $\mu\text{g}/\text{ml}$ 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 (*K_d*) and maximum binding capacity (*B_{max}*) 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

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

Experiment	Membrane	¹²⁵ I-Lipoprotein	Control	μg Lipoprotein bound/mg membrane protein					
				+ Excess unlabeled lipoproteins					
				HDL ₂	HDL ₃	E ₀ -HDL ₂	E ₀ -HDL ₃	Abeta-HDL ₃	LDL
1	M 50 yr	HDL ₃	2.50	0.23	0.23	ND	0.18	ND	1.04
2	M 53 yr	E ₀ -HDL ₃	2.42	0.21	0.17	ND	0.15	0.21	0.94
3	M 50 yr	LDL	1.46	0.10	0.07	0.06	0.05	0	0.15

Adipocyte plasma membranes were obtained from peritoneal fat tissues as described in Methods. 10 μg (protein) adipocyte membranes were incubated with 40 $\mu\text{g}/\text{ml}$ 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 ¹²⁵I-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.

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

	Specific binding	
	$\mu\text{g HDL}/\text{mg membrane}$	% Inhibited by LDL
Normal HDL ₂ (n = 11)	3.50±1.21*	61.5±10.6
Normal HDL ₃ (n = 4)	1.95±0.72	57.6±8.8
Abeta HDL ₂ (n = 3)	2.76±1.05	78.0±5.2
Apo E ₀ HDL ₂ (n = 3)	3.83±1.16	74.0±1.7
Apo E ₀ HDL ₃ (n = 7)	2.78±1.21	61.3±8.3

Specific binding of the various normal and mutant HDL particles was measured at 40 $\mu\text{g}/\text{ml}$ ^{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 E₀ HDL particles and all were partially inhibited by LDL.

* Mean±SD.

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 simulta-

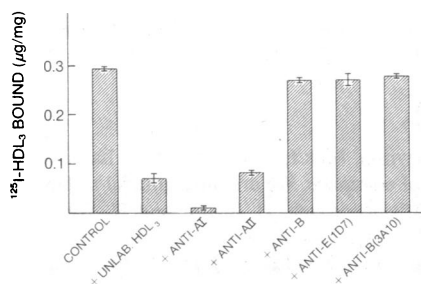


Figure 5. Effect of various anti-apoprotein antibodies on ^{125}I -HDL₃ binding to human adipocyte membranes. Adipocyte plasma membranes were purified from properitoneal fat of a 55-yr-old male as described in Methods. ^{125}I -HDL₃ binding was carried out by incubating 10 μg (protein) of the purified membranes with 0.27 $\mu\text{g}/\text{ml}$ at 0°C (control). Parallel assays contained unlabeled HDL₃ (100-fold excess) or different anti-apoprotein antibodies as indicated. Anti-AI, anti-AII, and anti-B were sheep anti-human apoprotein antisera obtained from Boehringer Mannheim Biochemicals. 1D7 and 3A10 were monoclonal antibodies directed against apo-E and apo-B, respectively.

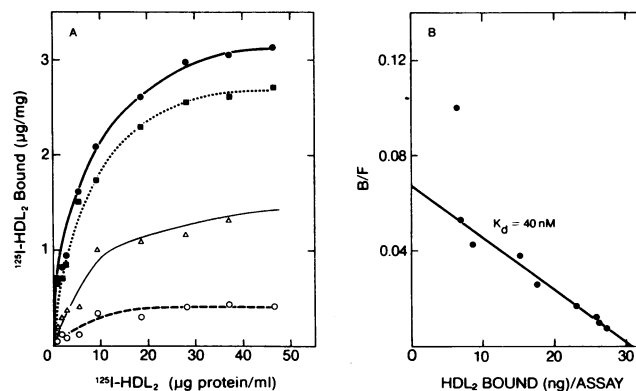


Figure 6. ^{125}I -HDL₂ binding to human adipocyte plasma membranes. Adipocytes were isolated from properitoneal fat of a 67-yr-old male by collagenase digestion and adipocyte plasma membrane were purified. (A) Purified plasma membranes (10 μg protein) were incubated with varying concentrations of ^{125}I -HDL₂ in the absence (●) or presence of 100-fold excess unlabeled HDL₂ (○) or in the presence of 100-fold protein excess unlabeled LDL (Δ). Specific binding (■) was calculated by subtracting nonspecific (○) from total binding (●) and was saturable. Excess unlabeled LDL partially inhibited ^{125}I -HDL₂ specific binding. Each point represents the average of duplicate assays. (B) Scatchard plot of ^{125}I -HDL₂ specific binding. B/F represents the amount of specifically bound ^{125}I -HDL₂ (nanograms protein) divided by the amount of unbound (nanograms protein) in the assay. Abscissa shows the amount of ^{125}I -HDL₂ specifically bound (nanograms protein) to the membranes per assay. The data were best fitted to a straight line by computer analysis. The intercept of the line at the x axis represents maximum binding. The K_d equals $-1/\text{slope}$ of the plot and measures the affinity of the HDL₂ binding. To convert from nanograms of HDL₂ protein to moles, the molecular weight of 3.6×10^5 (40% protein) was used in the calculation.

neously, 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 -HDL₂ and ^{125}I -HDL₃ 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, HDL₂ and HDL₃ 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 HDL₂ and HDL₃ 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

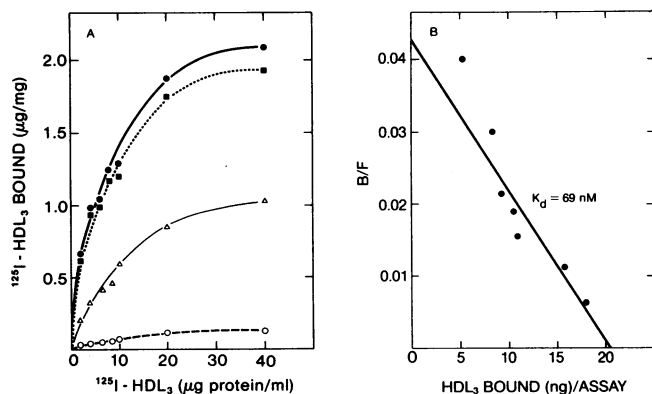


Figure 7. ^{125}I -HDL₃ binding to human adipocyte plasma membranes. Plasma membranes were purified from adipocytes that were isolated from peritoneal fat obtained from a 61-yr-old male. (A) Purified plasma membranes (9.6 μg protein) were incubated with varying concentrations of ^{125}I -HDL₃ in the absence (\bullet) or presence of 100-fold excess unlabeled HDL₃ (\circ) or in the presence of 100-fold protein excess unlabeled LDL (Δ). Specific binding (\blacksquare) was calculated by subtracting nonspecific (\circ) from total binding (\bullet) 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 1.75×10^5 (55% protein) was used in the calculation.

contributed to a nondisplaceable portion of surface-bound HDL particles.

The role of apo E in the binding of HDL to human adipocytes was explored using apo E poor normal HDL₃, apo E₀-HDL₂, and apo E₀-HDL₃ obtained from a patient with genetic apo E deficiency. All these apo E poor or deficient HDL particles bound specifically to human fat cells and to purified plasma membranes with similar affinity (Table VI). This suggests that the presence of apo E is not obligatory for HDL adipocyte interactions. Apo E₀-HDL₂, apo E₀-HDL₃, normal HDL₂, and normal HDL₃ binding were all partially inhibited by unlabeled LDL, which indicated that the cross-competition between LDL and HDL particles is not dependent on an apo E or apo B-E type of recognition (22, 48). This conclusion is further substantiated by our finding that abeta-

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

Experiment	Membrane	HDL	K_d^*	B_{max}
			nM	$\mu\text{g}/\text{mg}$
1	M 67 yr	Normal HDL ₂	40	3.05
2	M 62 yr	Normal HDL ₂	96	7.68
3	M 65 yr	Normal HDL ₃	56	2.76
4	M 61 yr	Normal HDL ₃	69	2.15
5	F 59 yr	Apo E ₀ -HDL ₃	48	1.56
6	M 44 yr	Apo E ₀ -HDL ₃	51	3.47
7	M 64 yr	Apo E ₀ -HDL ₃	67	3.27

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.

HDL₃ and apo E₀-HDL₃ behaved similarly in completely inhibiting ^{125}I -LDL binding to adipocyte membranes.

We conclude that HDL binding to human fat cells is probably related to apo A apoproteins. Indeed, studies on the effects of anti-apo AI and anti-apo AII antibodies on HDL binding suggest that both apo AI 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 lipids 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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