Evidence that chylomicron remnants and β-VLDL are transported by the same receptor pathway in J774 murine macrophage-derived cells

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Abstract To characterize lipoprotein uptake by macrophages, we studied J774 murine macrophage-derived cells. Uptake of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants was saturable, specific, and of high affinity. Maximal specific uptake and the concentration at which half-maximal uptake occurred were similar for both β-VLDL and chylomicron remnants. Specific uptake of ^{125}I-labeled chylomicrons was only 1/5 that of the other two lipoproteins. Cholesterol loading decreased ^{125}I-labeled chylomicron remnant and ^{125}I-labeled β-VLDL uptake by 25%. Chylomicron remnants and β-VLDL were equipotent in cross-competition studies; acetyl-LDL did not compete, and human LDL was a poor competitor. Although the amounts of cell-associated lipoproteins were similar, β-VLDL and chylomicron remnants had different effects on cellular lipid metabolism. β-VLDL produced a threefold stimulation while chylomicron remnants caused a decrease in [^{3}H]oleate incorporation into cholesteryl ester. β-VLDL had no effect while chylomicron remnants caused a threefold increase in [^{3}H]oleate incorporation into triacylglycerol. β-VLDL produced a 44% suppression and chylomicron remnants produced a 78% increase in HMG-CoA reductase activity. In summary, J774 macrophages express a receptor site that recognizes both β-VLDL and chylomicron remnants; however, these lipoproteins exhibit strikingly different effects on intracellular lipid metabolism. — **Ellsworth, J. L., A. D. Cooper, and F. B. Kraemer.** Evidence that chylomicron remnants and β-VLDL are transported by the same receptor pathway in J774 murine macrophage-derived cells. *J. Lipid Res.* 1986. **27:** 1062–1072.

Supplementary key words lipoprotein receptors • lipid metabolism • HMG-CoA reductase

Lipid-laden cells are a characteristic component of atherosclerotic lesions. Ultrastructural studies have established that these foam cells are derived to a considerable extent from macrophages (1–3). Accordingly, there has been considerable investigation of the mechanisms by which macrophages can accumulate lipid. It has been established that lipoprotein-derived lipids, both cholesterol and triacylglycerol, can enter these cells by several mechanisms, some of which involve cell surface receptors (4, 5).

Macrophages in culture can express receptors for low density lipoproteins (LDL) (6–9); however, since a number of studies have shown that the number of these receptors decreases when the cellular cholesterol content increases (4, 8, 10), lipoprotein transport by this pathway is not likely to cause lipid accumulation. In contrast to native LDL, the uptake of a variety of chemically (4, 6) or biologically modified lipoproteins (11), including acetylated LDL, malondialdehyde-modified LDL, and endothelial cell-modified LDL, by a distinct receptor referred to as the scavenger or acetyl-LDL receptor (4) causes cholesteryl ester accumulation in macrophages.

Additionally, it has been reported that there is a third receptor that mediates the removal of cholesterol-rich, beta-migrating VLDL or β-VLDL (12–15). This receptor pathway also appears to transport VLDL and chylomicrons from patients with hypertriglyceridemia (14, 16, 17) as well as normal VLDL (18–20).

In addition to the receptor-mediated intracellular accumulation of lipoprotein lipid, macrophages secrete lipoprotein lipase (20, 21) which, by hydrolyzing triacylglycerol, allows diffusion of free fatty acids into the cell. Lipid accumulation may then occur by activation of cellular pathways of fatty acid metabolism. The resultant remnant lipoproteins could also become ligands for one of the receptors capable of transporting lipoproteins. Floren and Chait (22) have suggested that chylomicron remnants are transported by the LDL receptor in human monocyte-derived macrophages. Since β-VLDL and chylomicron remnants are rich in apoprotein E and the E apoprotein is a determinant of lipoprotein uptake by the LDL, rem-

Abbreviations: VLDL, very low density lipoprotein; β-VLDL, beta-migrating very low density lipoprotein; LDL, low density lipoprotein; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; LPDS, lipoprotein-deficient fetal calf serum; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; TLC, thin-layer chromatography.

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nant, and β-VLDL receptors (23–27), these lipoproteins can lead to lipid accumulation in a variety of cell types. Accordingly, we investigated the mechanism by which β-VLDL and chylomicron remnants are transported in macrophages.

MATERIALS

Male Sprague-Dawley rats (250–300 g) and retired breeders were obtained from Simonsen Laboratories (Gilroy, CA). Sodium [^{125}I]iodide (13–17 mCi/μg), [9,10-^{3}H]oleic acid (5–7 Ci/mmol), and 3-hydroxyl-3-methyl [3-^{14}C]glutaryl-coenzyme A (57 mCi/mmol) were purchased from Amersham-Searle Corporation (Arlington Heights, IL). Mevalonolactone, RS-[5-^{3}H] (14.9 Ci/mmol) and tri-[^{14}C]oleoylglycerol (103.5 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Radioimmunoassay grade bovine serum albumin and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) were obtained from Sigma Chemical Company (St. Louis, MO). Heparin (sodium salt) and dextran sulfate (potassium) were purchased from Calbiochem-Behring (La Jolla, CA). Bio-Gel A-50m was purchased from Bio-Rad Laboratories (Richmond, CA). Minimal essential medium (Eagle) with Earle's salts and L-glutamine, without sodium bicarbonate (10 × concentrate), Dulbecco's modified Eagle's medium, fetal bovine serum, trypsin (5.0 gm/l)/EDTA (2.0 gm/l) solution and penicillin (10,000 U/ml)-streptomycin (10,000 mcg/ml) solution were obtained from Gibco Laboratories (Grand Island, NY). Six-well tissue culture clusters were obtained from Costar (Cambridge, MA) and 24-well tissue culture plates were purchased from Flow Laboratories, Inc. (McLean, VA). Seventy five-cm^2 tissue culture flasks were obtained from Corning Glass Works (Corning, NY). All other chemicals were obtained as described in a previous publication (18).

EXPERIMENTAL PROCEDURES

Cell culture

Cultures of the J774 murine macrophage-like cell line were obtained from Dr. Jay Unkeless of Rockefeller University. Cells were maintained in medium A that consisted of Dulbecco's modified Eagle's medium supplemented with penicillin (100 U/ml), streptomycin (100 mcg/ml), and 10% fetal bovine serum under a humidified atmosphere of 95% air/5% CO_2. For lipoprotein binding studies, cells were seeded into 6-well plates at a density of 2000–3000 cells/cm^2. Fresh media were added on day 4 and, in general, the cells were used in experiments on the fifth or sixth day of culture. At the time of assay, the cells had formed dense monolayers of 2.6 × 10^6 cells and 560 μg of cell protein. In some experiments, cells were seeded into 24-well plates and grown until the cells had formed dense monolayers of approximately 6.0 × 10^5 cells and 150 μg of cell protein. Cell number was determined by counting an aliquot of cells in a hemocytometer. Cell viability, as determined by the exclusion of 0.04% trypan blue, was always greater than 90%.

Preparation of lipoproteins

Human serum was isolated from blood collected from normolipemic fasting volunteers. The serum was adjusted to 0.04% EDTA, 0.02% NaN_3, and 0.05 mg garamycin/ml, and lipoproteins were isolated by a modification of the procedure described by Havel, Eder, and Bragdon (28), using sequential ultracentrifugation at 120,000 *g* for 18 hr at 4°C in a Beckman (Palo Alto, CA) SW 41 rotor. The lipoproteins were dialyzed at 4°C against a solution of 10 mM sodium phosphate (pH 7.4), 150 mM NaCl, and 0.04% EDTA (PBS), sterilized by passage through a 0.45-μm filter (Millipore Corporation, Bedford, MA) and stored at 4°C until use. The LDLs (d 1.019–1.063 g/ml) were free of apoproteins E, A, and C, as judged by visual inspection of the apoprotein profile on 10% polyacrylamide gels (see below). Acetylated LDL was prepared from LDL as described (29). Lipoprotein-deficient fetal calf serum (LPDS) was prepared by centrifugation at 120,000 *g* for 24 hr at 4°C after adjustment of the density to 1.210 g/ml with KBr. The thin surface film containing the lipoproteins was removed by aspiration, and the lipoprotein-deficient infranatant fraction was collected, dialyzed against PBS, sterile-filtered, and stored at 4°C until use.

Rat hypercholesterolemic VLDL (β-VLDL) was prepared from rats maintained on chow supplemented with 5.0% lard, 2.0% cholesterol, 0.3% cholic acid, and 0.1% propylthiouracil (30). The rats were fasted for 18–24 hr prior to exsanguination. Serum was isolated, and the β-VLDLs (d ≤ 1.006 g/ml) were isolated by ultracentrifugation as described above. Rat β-VLDL contained 1.25 mg of triacylglycerol/mg protein, 5.08 mg of cholesterol/mg protein, and migrated as a single band with beta electrophoretic mobility upon agarose gel electrophoresis. β-VLDL contained 14% apoprotein B-100, 14% apoprotein B-48, 6% 120 kDa protein, 2% albumin, 61% apoprotein E, and 3% C apoproteins, as determined by densitometric scanning of the Coomassie blue-stained gel following electrophoresis (see below). Lipoproteins were radiolabeled by the iodine monochloride procedure as previously described (23). The average specific activities were 308 cpm/ng protein for ^{125}I-labeled LDL and 278 cpm/ng protein for ^{125}I-labeled β-VLDL. With the LDL and β-VLDL, 90–95% of the ^{125}I label was precipitated by an equal volume of 20% trichloroacetic acid; of this amount, 18% was extracted into ethanol–acetone 1:1 (v/v) after overnight incubation at −20°C. The distribution of radioactivity in the apoproteins of β-VLDL is given in **Table 1.** Rat chylomicrons were collected from animals

with a lymph duct cannula, and chylomicron remnants were prepared in eviscerated rats as described previously (31). In general, 2.5 ml of chylomicrons containing 200 mg of triacylglycerol was injected into each retired breeder rat. The animals were exsanguinated, and the lipoproteins were re-isolated as described previously (31). Residual rat serum albumin was removed by chromatography of the chylomicrons or chylomicron remnants on a 2.5 × 50.0 cm column of Bio-Gel A-50m. The lipoprotein-containing void fraction was collected and concentrated by ultracentrifugation at 100,000 *g* for 120 min at 4°C. The chemical composition of the rat chylomicrons and the rat chylomicron remnants prepared as described above has been reported previously (23). The triacylglycerol content of the rat chylomicrons was 124.0 mg/mg protein, and the cholesterol content was 5.2 mg/mg protein. Chylomicrons contained 10% apoprotein B-48, 28% apoprotein A-I, 8% apoprotein A-IV, 18% apoprotein E, and 36% C apoprotein. For rat chylomicron remnants, the values were 41.4 mg of triacylglycerol/mg protein and 3.8 mg of cholesterol/mg protein. Chylomicron remnants contained 13% apoprotein B-48, 3% albumin, 12% apoprotein A-IV, 52% apoprotein E, and 20% an unidentified protein of 120,000 daltons. The chylomicrons and chylomicron remnants were devoid of apoprotein B-100. In most instances, ^{125}I-labeled chylomicron remnants were obtained by iodinating 300 μg of remnant protein with 200 μCi of Na [^{125}I]. The specific activity ranged from 40 to 600 cpm/ng protein with a distribution of radioactivity described in Table 1. In some instances, the chylomicrons (1200 mg of triacylglycerol, 10 mg of protein) were iodinated with 8–10 mCi of Na [^{125}I] as described above. After extensive dialysis to remove unincorporated ^{125}I, these chylomicrons were injected into the eviscerated rat as described above. After re-isolation, the specific activity of these remnants ranged from 50 to 150 cpm/ng protein. For both types of remnants, 80–90% of the radioactivity was precipitated by an equal volume of 20% trichloroacetic acid. In these preparations, amounts of radioactivity ranging from 20% to 80% were extracted by ethanol–acetone 1:1 (v/v). The specific activity of the chylomicron remnant protein was calculated using the trichloroacetic acid-precipitable, non-ethanol–acetone-extractable counts. Despite differences in the extent of lipid labeling, the various batches of chylomicron remnants behaved identically in cell-binding experiments. Chylomicrons and chylomicron remnants radiolabeled with [^{14}C]triolein were prepared using dimethylsulfoxide as described by Fielding (32). The specific activity of triacylglycerol in chylomicrons and chylomicron remnants was 4.3 × 10^4 dpm/mg and 6.4 × 10^4 dpm/mg triacylglycerol, respectively.

Lipoprotein binding studies

The J774 cells were seeded into 6-well or 24-well tissue culture plates and were grown in medium A as described above. Unless otherwise stated, all binding assays were performed on dense monolayers of cells in the stationary phase of their growth curve. Prior to assay, the plates of cells were removed from the incubator and placed on ice. The cell media were removed, and each monolayer of cells was washed twice with 2.0 ml of ice-cold medium B (minimal essential medium (Eagle's), 0.02 M HEPES, and 40.0 mg/ml bovine serum albumin, pH 7.4). Incubation mixtures contained approximately 2.6 × 10^6 cells (6-well plate) or 4 × 10^5 cells (24-well plate), ^{125}I-labeled lipoproteins, and unlabeled lipoproteins in the amounts described in the legends to the figures. The final volume was 1.0 ml (6-well plates) and 0.3 ml (24-well plates) and was made up to volume with medium B. All unlabeled lipoproteins were dialyzed against medium B for 24 hr prior to assay. Incubations at 37°C or 4°C were started by the addition of the lipoprotein reaction mixture to the cells and terminated by placing the cells on ice. For incubations at 4°C, the cells were left on ice during the entire incubation period. The binding medium was removed, and the cell monolayers were washed three times with 1.0 ml of a wash buffer that consisted of 150 mM NaCl, 0.02 M Tris-HCl, pH 7.4, 0.002 M $CaCl_2$, and 1.0 mg/ml bovine serum albumin and then twice with 2 ml of albumin-free buffer. The cells were solubilized with 1.0 ml of 1.0 N

TABLE 1. Distribution of ^{125}I in ^{125}I-labeled apoproteins

Lipoprotein	Radiolabeled Apoprotein Distribution							
	B-100	B-48	120 kDa	Albumin	A-IV	E	A-I	C
	% of total radioactivity							
Chylomicron remnants (in vivo)[a]	n.d.	40	7	n.d.	6	6	10	31
Chylomicron remnants (in vitro)[a]	n.d.	18	24	n.d.	n.d.	35	n.d.	23
Chylomicrons	n.d.	11	1	6	16	5	22	39
β-VLDL	63	12	n.d.	10	n.d.	8	n.d.	7

Samples of each lipoprotein were prepared and taken for electrophoresis as described in Experimental Procedures. Apoprotein-containing regions of the gel were excised and radioactivity was measured; n.d., not detectable.

[a] In vivo refers to chylomicron remnants prepared from ^{125}I-labeled chylomicrons, whereas in vitro refers to remnants prepared from unlabeled chylomicrons which were then iodinated.

NaOH, and the cell-associated radioactivity was determined in a gamma counter. The measured radioactivity was normalized per mg of cell protein which was determined on an aliquot of the solubilized cell suspension. Lipoprotein degradation was determined by measuring the trichloroacetic acid-soluble, chloroform-insoluble radioactivity that accumulated in the cell medium as previously described (18). To measure cell surface binding of ^{125}I-labeled chylomicron remnants at 37°C, cells were prepared, incubated, and washed as described above. Cell surface binding of ^{125}I-labeled chylomicron remnants was defined as the heparin- (33), dextran sulfate- (33), acid- (34, 35), or trypsin/EDTA- (18) releasable radioactivity. For heparin and dextran sulfate release, 0.5 ml of phosphate-buffered saline (PBS) containing 10 mg/ml of heparin or dextran sulfate was added and each dish was incubated for 60 min at 4°C. The washes were collected and centrifuged at 500 *g* for 3 min at 4°C. Radioactivity in the supernatant and pellet fractions was determined. The cells were solubilized with 1.0 N NaOH, and the cell-associated radioactivity was determined as described above. For acid release of cell-bound lipoprotein, two procedures were used: each cell monolayer was washed with 0.5 ml of a solution of 50 mM glycine, 100 mM NaCl (pH 3.0) for 2 min at 4°C (34), or 0.2 M acetic acid, 0.5 M NaCl for 8 min at 4°C (35). The washes were collected, and the cells were washed quickly with 0.5 ml of the glycine or acetic acid solution. The two washes were combined, and the distribution of radioactivity between the supernatant and pellet fractions was determined as described above. For trypsin/EDTA release, each cell monolayer was incubated for 25 min at 4°C with 0.5 ml of a solution of 0.5 g trypsin, 0.2 g of EDTA/liter. The cells were gently dislodged, placed into separate glass test tubes, and centrifuged at 500 *g* for 3 min at 4°C. The distribution of radioactivity between the supernatant fraction, and the cell pellet was determined as described above.

Assay of HMG-CoA reductase activity in cellular extracts

After incubation with lipoproteins as described in the legends to the figures, cells were washed three times with ice-cold PBS, scraped off the dish with a rubber policeman, suspended in 0.25 M sucrose, 0.3 mM EDTA, 0.1 M potassium phosphate, and 5.0 mM dithiothreitol, and disrupted by sonication. An aliquot of the postnuclear homogenate (1,000 *g* for 10 min at 4°C) was preincubated at 37°C for 10 min and then incubated in a final volume of 0.2 ml of 0.1 M potassium phosphate (pH 7.5), 3.0 mM NADP, 20 mM glucose-6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 10 mM EDTA, 5 mM dithiothreitol, and 4.0 μCi of [3-^{14}C]HMG-CoA at 37°C for 20 min (29). The reaction was terminated by the addition of 30 μl of 5.0 N HCl. [2-^{3}H]Mevalonolactone (40,000 dpm) was added as internal standard to monitor recovery, and 200 μg of mevalonolactone was added as carrier. The mixtures were incubated for 30 min at 37°C to complete lactonization and then plated on Whatman TLC plates with toluene–acetone 1:1 (v/v) as the developing solvent. Mevalonolactone was visualized with I_2 vapor, scraped into scintillation vials, and counted in a toluene-based scintillation mixture. Blank values were obtained from incubation in the absence of protein.

Assay of [^{3}H]oleate incorporation into cholesteryl ester and triacylglycerol

The incorporation of [^{3}H]oleate into cholesteryl ester and triacylglycerol was assayed according to previously published procedures (18). [9,10-^{3}H]Oleic acid was suspended in 50 mM sodium oleate complexed to 5% bovine serum albumin in 0.15 M NaCl. Monolayers of macrophages were incubated in a final volume of 1.0 ml with unlabeled lipoproteins of various concentrations, 850 μl DMEM with 10% LPDS, and 20 μl of the [^{3}H]oleate/albumin solution (approximately 11.0 μCi) at 37°C for 1.5 hr. The incubation was terminated by placing the cells on ice, aspirating the media, and washing the cells as described above. The cellular lipids were then extracted by incubating the monolayers successively with two volumes of hexane–isopropyl alcohol 3:2 for 30 min at room temperature. After the lipids were extracted, the cells were dissolved in 1.0 ml of 0.5 N NaOH, and aliquots were taken for protein determination. An internal standard containing cholesteryl [^{14}C]oleate (40 μg, 5,000 cpm) and tri-[^{14}C]oleoylglycerol (40 μg, 5,000 cpm) was added to the organic solvent extracts. The samples were evaporated to dryness under N_2, resuspended in 100 μl of hexane, and spotted on Whatman TLC plates. The plates were developed in heptane–diethyl ether–acetic acid 75:25:1 (v/v/v); the lipid spots were identified with I_2 vapor, scraped, and counted in 5.0 ml of toluene-based scintillation fluid. Results are expressed as nmol of [^{3}H]oleate incorporated into the indicated ^{3}H-labeled lipid per mg of cell protein.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Samples of lipoproteins were prepared for electrophoresis by delipidation overnight at −20°C with 5.0 ml of ethanol–acetone 1:1 (v/v). Protein precipitates were collected by centrifugation at 500 *g* for 10 min at 4°C and were washed with 2.0 ml of diethylether. The protein precipitates were resuspended in 200 μl of 0.063 M Tris-HCl, pH 6.8, 1% SDS, 1% β-mercaptoethanol, 15% glycerol, and 0.001% bromophenol blue. Prior to electrophoresis, all samples were heated at 60°C for 15 min. Electrophoresis on 10% polyacrylamide gels containing 0.1% SDS was performed as described (36). The stacking gels contained 3% acrylamide. After electrophoresis, the gels were fixed overnight with isopropanol–acetic acid–water 25:10:65

(v/v/v). The next day, the gels were stained for 2 hr in methanol–acetic acid–water 50:10:40 (v/v/v) containing 0.05% Coomassie brilliant blue-G and destained in isopropanol–acetic acid–water 10:10:80 (v/v/v).

Other methods

Protein was measured by the method of Lowry et al. (37), using bovine serum albumin as standard. After color development, each sample was extracted with 2.0 ml of chloroform to prevent interference by opalescence and light scattering. Lipoprotein triacylglycerol was measured by the glycerol phosphate dehydrogenase enzyme assay (Sigma kit 320-UV, St. Louis, MO). The total cholesterol content of lipoproteins was determined by the cholesterol oxidase/cholesterol esterase enzyme assay (Sigma kit 350-A). Statistical analyses were performed using a two-tailed Student's *t*-test or analysis of variance.

RESULTS

Cell association of ^{125}I-labeled chylomicrons, chylomicron remnants, and β-VLDL by J774 macrophages

In order to minimize the contribution of macrophage-derived lipase to lipoprotein uptake in these studies, we assayed lipoprotein uptake during incubations of short duration, generally 1.5 hr. With 1.5 hr of incubation at 37°C, no lipase-mediated release of radiolabeled fatty acids from triglyceride-labeled chylomicrons or chylomicron remnants could be detected in the culture medium of J774 cells (data not shown). Thus, under the described conditions, lipoprotein uptake should be a direct reflection of cell surface lipoprotein receptor expression.

There was little saturable, specific uptake of ^{125}I-labeled chylomicrons by macrophages (**Fig. 1**). Maximal uptake, achieved at 15 μg of chylomicron protein/ml of incubation medium, was 0.03 μg/mg cell protein. In contrast, the uptake of ^{125}I-labeled chylomicron remnants was five- to tenfold greater (**Fig. 2**). Uptake of ^{125}I-labeled chylomicron remnants was saturable, specific, and of high affinity; half-maximal uptake occurred at an extracellular ^{125}I-labeled chylomicron remnant concentration of 5 μg protein/ml. At an extracellular ^{125}I-labeled chylomicron remnant concentration of 20 μg protein/ml, uptake was approximately 0.6 μg/mg cell protein. Thus, in the absence of significant lipase activity, macrophages express a transport mechanism that distinguishes between chylomicrons and their remnants. Consistent with previous reports (17, 20), J774 cells were capable of high affinity saturable uptake of cholesterol-rich β-VLDL (**Fig. 3**). Specific uptake of ^{125}I-labeled β-VLDL was generally similar to that of ^{125}I-labeled chylomicron remnants. Half-maximal uptake occurred at an extracellular ^{125}I-labeled β-VLDL concentration of 6 μg of protein/ml. At an extracellular ^{125}I-labeled β-VLDL concentration of 30 μg of protein/ml, uptake was approximately 0.4 μg/mg cell protein. Thus, these cells bind and actively transport chylomicron remnants and β-VLDL.

Saturation binding of ^{125}I-labeled chylomicron remnants and β-VLDL to macrophages at 4°C

To further characterize the binding sites for chylomicron remnants and β-VLDL, saturation binding experiments with ^{125}I-labeled lipoproteins were conducted at 4°C. As is shown in **Fig. 4,** cell surface binding of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL to macrophages was saturable, specific, and of high affinity. Analysis of these data by the method of Scatchard (38) (Fig. 4C) was consistent with lipoprotein binding to a single class of high affinity sites. The total number of binding sites, B_{max}, was similar for both lipoproteins: for ^{125}I-labeled chylomicron remnants B_{max} was 56 ng/mg of cell protein and for ^{125}I-labeled β-VLDL, B_{max} was 41 ng/mg of cell protein. The dissociation constant or K_d of each lipoprotein for the macrophage binding sites was approximately 2.0 μg of protein/ml.

Internalization of ^{125}I-labeled chylomicron remnants

In order to determine whether chylomicron remnants are efficiently internalized, macrophages were incubated at 37°C with ^{125}I-labeled chylomicron remnants, in the presence and absence of unlabeled chylomicron remnants, and the cell cultures were washed as described. Cells were then washed under a variety of incubation conditions known to disrupt ligand–receptor interactions. The distribution of radioactivity between the wash medium and the cells was determined. As is shown in **Fig. 5,** none of the wash procedures significantly altered the amount of total or nonspecific cell-associated ^{125}I-labeled chylomicron remnants. These data demonstrate that the chylomicron remnants are not bound to the macrophage surface in a readily releasable form and suggest that the lipoprotein is efficiently internalized.

Competition of lipoproteins for uptake of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL

To determine the relationship between the chylomicron remnant and β-VLDL removal mechanisms, the ability of several unlabeled lipoprotein preparations to compete with the uptake of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL was determined. As is shown in **Fig. 6,** unlabeled chylomicron remnants and β-VLDL were equally potent in competition for uptake of both ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL. At a 10-fold protein excess, unlabeled chylomicron remnants and β-VLDL reduced ^{125}I-labeled lipoprotein uptake by 50%. Human LDL was a poor competitor; at a

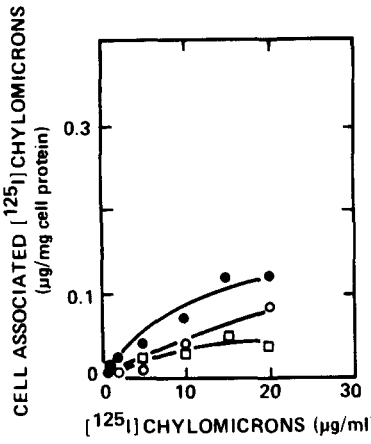

Fig. 1. Uptake of ^{125}I-labeled chylomicrons by macrophages. Each monolayer was incubated at 37°C in 1 ml of medium B for 1.5 hr with the indicated concentration of ^{125}I-labeled chylomicron protein in the presence and absence of unlabeled chylomicrons (60 μg protein/ml). Cell-associated lipoprotein was determined as described. Specific uptake (open squares) was calculated as the difference between total (closed symbols) and nonspecific (open symbols) values. Each point represents the data of a single dish of cells and is representative of two separate experiments.

100-fold protein excess, unlabeled human LDL reduced ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnant uptake by 40%. Acetylated-LDL did not compete with either ^{125}I-labeled β-VLDL or ^{125}I-labeled chylomicron remnants for uptake by macrophages.

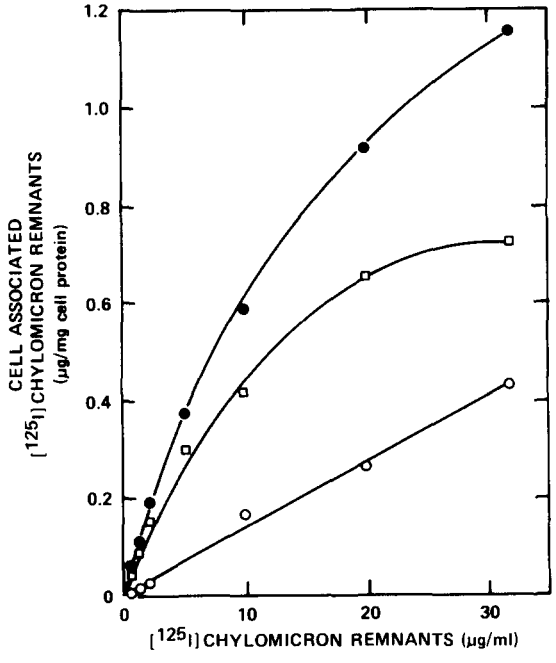

Fig. 2. Uptake of ^{125}I-labeled chylomicron remnants by macrophages. Each monolayer was incubated at 37°C for 1.5 hr in 1.0 ml of medium B, containing the indicated concentration of ^{125}I-labeled chylomicron remnant protein in the absence (●) or presence (○) of unlabeled chylomicron remnants (100 μg protein/ml), and cell-associated lipoprotein was determined. Specific uptake (open squares) was calculated, as described in the legend to Fig. 1. Each point represents the mean value of duplicate dishes of cells from two separate experiments.

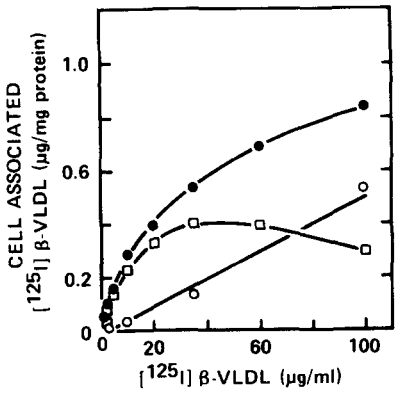

Fig. 3. Uptake of ^{125}I-labeled β-VLDL by macrophages. Each monolayer was incubated at 37°C for 1.5 hr in 1.0 ml of medium B, containing the indicated concentration of ^{125}I-labeled β-VLDL protein in the absence (●) or presence (○) of unlabeled β-VLDL (200 μg protein/ml), and cell-associated lipoprotein was determined. Specific cell associated lipoprotein (open squares) was calculated, as described in the legend to Fig. 1.

Regulation of β-VLDL and chylomicron remnant uptake in J774 cells

To determine whether the expression of the macrophage receptor for β-VLDL and chylomicron remnants is influenced by the cellular cholesterol content, uptake of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants was determined in J774 cells that were cholesterol-loaded by preincubation with acetylated-LDL for 24 hr at 37°C. These conditions were shown to maximally stimulate cholesteryl ester accumulation (data not shown). The uptake of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants was reduced only 25% by cholesterol loading (**Table 2**). Thus, the major portion of β-VLDL/chylomicron remnant uptake proceeds by a receptor pathway that is only partly suppressed by pertubations of the cellular content of cholesterol.

Effect of β-VLDL and chylomicron remnants on lipid accumulation and HMG-CoA reductase

To determine the effects of cholesterol-rich β-VLDL and triacylglycerol-rich chylomicron remnants on cellular lipid metabolism, J774 macrophages were incubated with various concentrations of β-VLDL and chylomicron remnants at 37°C, and [^{3}H]oleate incorporation into cellular lipids was determined. As shown in **Fig. 7A,** incubation of macrophages with increasing amounts of β-VLDL produced a concentration-dependent increase in [^{3}H]oleate incorporation into cholesteryl ester. Maximal stimulation (threefold) was reached at extracellular protein and cholesterol concentrations of 10 μg/ml and 30 μg/ml, respectively. The β-VLDL did not have a significant effect on [^{3}H]oleate incorporation into cellular triacylglycerol (Fig. 7B). The effect of incubating macrophages with various amounts of chylomicron remnants was strikingly different.

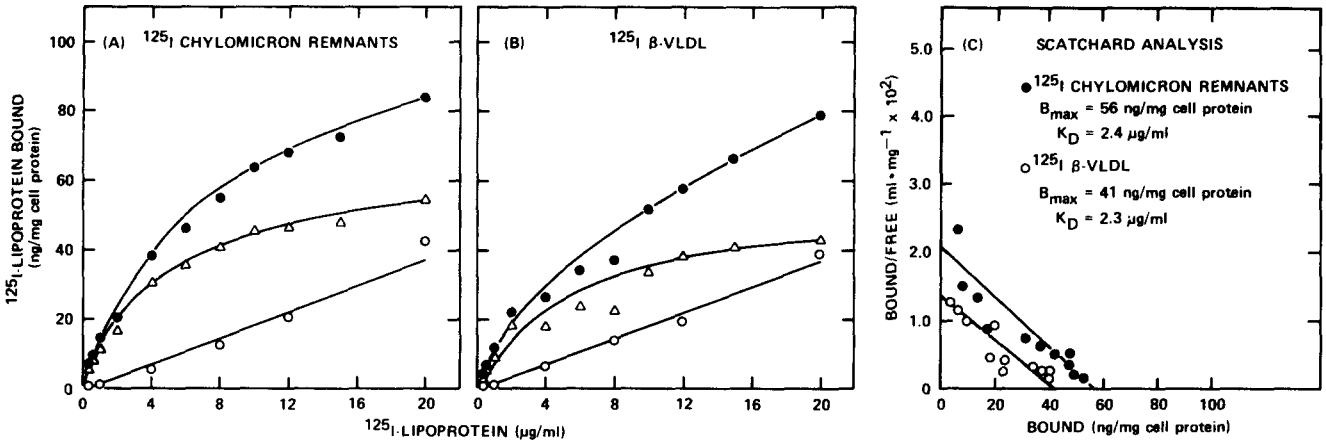

Fig. 4. Saturation binding of ^{125}I-labeled chylomicron remnants and β-VLDL to macrophages at 4°C. Each monolayer was incubated at 4°C in 0.5 ml of medium B containing the indicated concentration of either ^{125}I-labeled chylomicron remnants (A) or ^{125}I-labeled β-VLDL (B) for 2 hr in the presence and absence of the respective unlabeled lipoprotein (100 μg of protein/ml for chylomicron remnants, 200 μg of protein/ml for β-VLDL). Total (●), nonspecific (○), and specific (△) binding were determined as described in Experimental Procedures. Each point represents the mean value of duplicate dishes of cells from two separate experiments. The ^{125}I-labeled chylomicron remnant and ^{125}I-labeled β-VLDL specific binding data from (A) and (B) were taken for Scatchard analysis and plotted as shown (C). "Bound/free" is the lipoprotein concentration specifically bound (nanograms of protein/mg cell protein) divided by the lipoprotein concentration free in the medium (nanograms of protein/ml).

At extracellular protein and cholesterol concentrations identical to those used with β-VLDL, chylomicron remnants produced a dose-dependent decrease in [^{3}H]oleate incorporation into cellular cholesteryl ester, while producing a threefold stimulation of [^{3}H]oleate incorporation into cellular triacylglycerol. It is possible that these rates of incorporation of [^{3}H]oleate into cellular lipids are underestimated when there is appreciable dilution of the pool with newly released fatty acids.

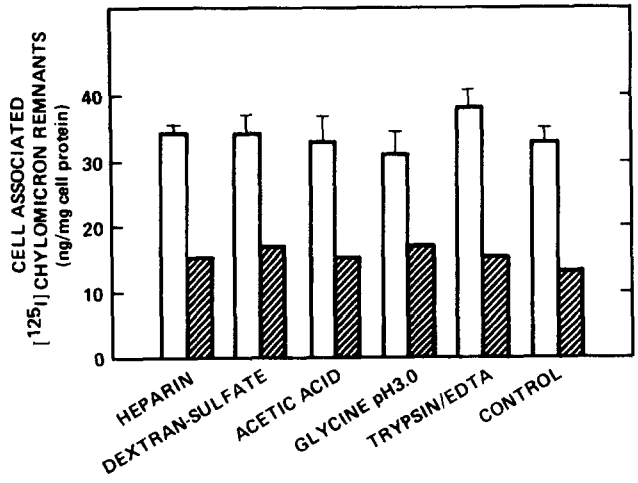

Fig. 5. Internalization of ^{125}I-labeled chylomicron remnants by macrophages. Each monolayer was incubated at 37°C for 1.5 hr in 0.4 ml of medium B containing ^{125}I-labeled chylomicron remnants (1.0 μg of protein/ml) in the presence (striped bars) or absence (open bars) of unlabeled chylomicron remnants (75 μg of protein/ml). The cells were incubated, washed, and treated with the various agents, as described in Experimental Procedures. The specific cell-associated radioactivity was determined. The open bars represent the means ± SD of n = four dishes of cells, and the striped bars represent the mean of duplicate dishes of cells for each incubation condition.

Chylomicron remnants and β-VLDL also had different effects on HMG-CoA reductase activity. At similar extracellular lipoprotein concentrations (expressed as protein or cholesterol), chylomicron remnants produced a 78% stimulation ($P < 0.01$) of ^{14}C-labeled HMG CoA conversion to [^{14}C]mevalonate, while incubation with β-VLDL produced a 44% ($P < 0.05$) suppression of HMG-CoA reductase activity (**Fig. 8**).

Low density lipoprotein metabolism by J774 cells

Human monocyte-derived macrophages (7) and certain human and murine macrophage-like cell lines (9, 39) express high affinity cell surface receptors for native human low density lipoproteins when preincubated in lipoprotein-deficient serum, whereas mouse peritoneal macrophages do not express significant levels of high affinity receptors for unmodified human LDL (4). To determine whether J774 macrophages express a high affinity, saturable LDL-receptor pathway under the conditions used in the present experiments, cell monolayers were incubated at 37°C for 1.5 hr with increasing concentrations of ^{125}I-labeled human LDL in the presence and absence of unlabeled lipoprotein. Monolayers of cells in the stationary (**Fig. 9**, panels A and C) or log (Fig. 9, panels B and D) phase of their growth curve take up or degrade relatively little ^{125}I-labeled LDL over this time period. Uptake of ^{125}I-labeled LDL under these conditions was a linear function of extracellular lipoprotein concentration; however, degradation of ^{125}I-labeled LDL, measured in log phase cultures, was greater than that measured in stationary phase cells (Fig. 9D). Incubation in the presence of a large excess of unlabeled LDL failed to saturate the removal processes. The low levels of specific uptake and degradation of unmodified human LDL by J774 cells dur-

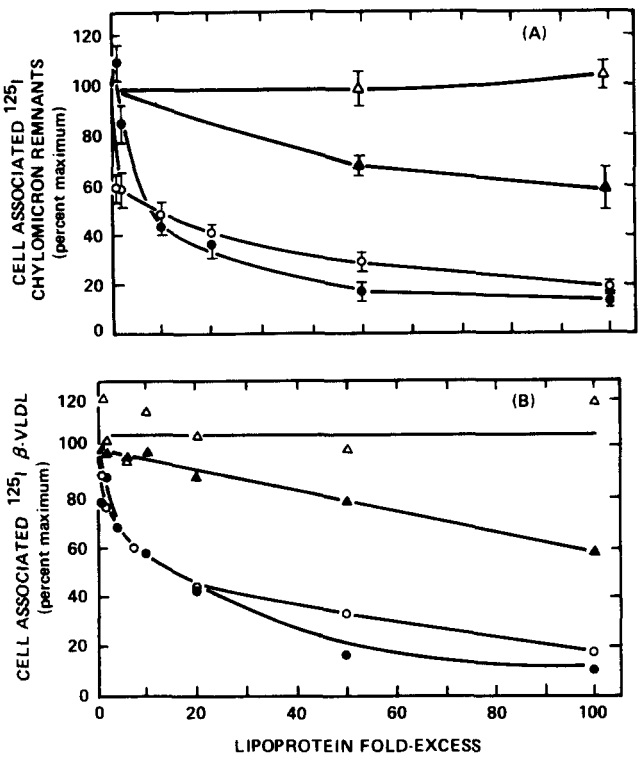

Fig. 6. Effect of nonradioactive chylomicron remnants (●), β-VLDL (○), acetylated LDL (△), and native LDL (▲) on the uptake of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL by macrophages. Monolayers of J774 cells were incubated for 1.5 hr at 37°C in 0.3 ml of medium B containing 1.0 μg protein/ml of ^{125}I-labeled chylomicron remnants (panel A) or ^{125}I-labeled β-VLDL (panel B) and the indicated fold-excess, based on protein, of the unlabeled lipoprotein. The cells were washed and cell-associated radioactivity was determined. For ^{125}I-labeled chylomicron remnant uptake, each point represents the mean ± SD of triplicate dishes of cells. For ^{125}I-labeled β-VLDL uptake, each point represents average of duplicate dishes of cells and is representative of two separate experiments. The 100% value for the uptake of ^{125}I-labeled chylomicron remnants was 65 ng of protein cell-associated/mg cell of protein and for ^{125}I-labeled β-VLDL was 52 ng of protein cell associated/mg cell protein.

ing incubation of short duration are consistent with some previous reports (9, 11), but differ from others (39).

It is well established that cells that express an active LDL receptor pathway increase the number of cell surface LDL receptors when the extracellular source of cholesterol is depleted (4). Incubation of J774 macrophages for 48 hr in growth medium containing 5% lipoprotein-deficient serum did not increase ^{125}I-labeled LDL uptake or degradation (data not shown). In other experiments, under the described binding conditions, we were unable to detect appreciable ^{125}I-labeled LDL binding to macrophages at 4°C stationary or log phase cells, cultured either in medium containing complete or lipoprotein-deficient serum. The small amount of specific LDL binding could be due either to a paucity of LDL receptors on the cells or a low affinity of the receptor for human LDL.

DISCUSSION

The present study demonstrates that chylomicron remnants and β-VLDL are transported by the same lipoprotein receptor in mouse macrophage-derived cells. This is not surprising since both lipoproteins are rich in apoprotein E and this apoprotein has been identified as the ligand for the putative β-VLDL receptor (24–27). Moreover, neither the high nor low molecular weight form of the B-apoprotein appears responsible for the lipoprotein uptake observed in the present study; despite similar contents of low molecular weight apoprotein B, chylomicrons were taken up at one-fifth to one-tenth the rate of chylomicron remnants. The striking change in the ability to bind to macrophages when chylomicrons are converted to remnants supports the notion that apoprotein E is an important determinant of the reaction. In addition, despite the absence of the high molecular weight B-apoprotein in the chylomicron remnants, β-VLDL and chylomicron remnants were taken up by J774 cells at similar rates and were equipotent in cross-competition studies.

While Floren and Chait (22) have suggested that chylomicron remnants are removed via the LDL receptor in monocyte-macrophages, the role of the LDL receptor in β-VLDL/chylomicron remnant metabolism in J774 cells is not clear. The strongest evidence that the LDL receptor and the β-VLDL receptor are unique is the fact that macrophages from Watanabe heritable hyperlipemic (WHHL) rabbits, which are defective in LDL receptor activity, express β-VLDL receptors (15, 40). In the present study, LDL competed poorly for β-VLDL and chylomicron remnant uptake, and there was only a slight decrease in rem-

TABLE 2. Effect of preincubation of macrophages with acetyl-LDL on the uptake of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL

Preincubation Conditions	Cell-associated ^{125}I-Labeled Lipoprotein[a]	
	Chylomicron Remnants	β-VLDL
Control	31.2 ± 3.4 (6)	25.6 ± 6.7 (5)
Acetyl-LDL	22.2 ± 3.5 (6)*	16.9 ± 1.2 (4)**

Cultures of J774 cells were incubated for 24 hr at 37°C in a humidified atmosphere of 95% air/5% CO_2 in 0.5 ml of medium A (in the absence of antibiotics) in the presence and absence of acetyl-LDL, 75 μg protein/ml. Prior to assay, the dishes were placed on ice, the media were removed, and the cells were washed twice with 1.0 ml of medium B. Medium B (0.5 ml) was added to each dish and the cells were incubated at 37°C for 30 min. The cells were placed on ice, the medium was removed, and the cells were incubated at 37°C for 1.5 hr in 0.3 ml of medium B containing 1.0 μg protein/ml of ^{125}I-labeled chylomicron remnants or ^{125}I-labeled β-VLDL in the presence and absence of a 100-fold excess of the respective unlabeled lipoprotein. The cells were washed and specific cell-associated radioactivity was determined as described in Experimental Procedures.

[a] The data represent the mean ± SD. The values in parentheses represent the number of dishes of cells used for each incubation condition.

* Differences significant from control $P < 0.01$ by a two-tailed t-test.

** Differences significant from control $P < 0.05$ by a two-tailed t-test.

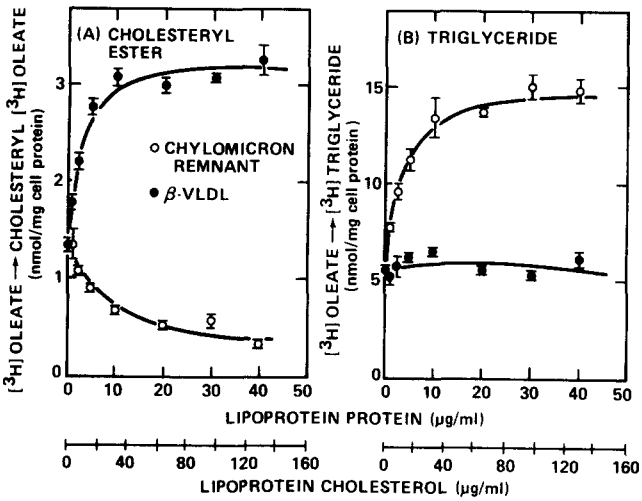

Fig. 7. Effect of β-VLDL and chylomicron remnants on [^{3}H]oleate incorporation into cellular lipids. J774 macrophages were cultured in 35-mm dishes and washed as described in Experimental Procedures. At the time of assay, dense monolayers of cells (900 μg of cell protein) had formed. Each dish received the indicated concentration of β-VLDL or chylomicron remnants, 20 μl of [^{3}H]oleate/albumin mixture, and was incubated for 1.5 hr at 37°C in a final volume of 1.0 ml of medium B. Cells were placed on ice, harvested, and the incorporation of [^{3}H]oleate into cellular lipids was determined as described in Experimental Procedures. Each data point represents the mean ± SD of triplicate dishes of cells and is representative of two separate experiments.

nant and β-VLDL uptake under conditions in which the majority of LDL receptor activity should have been down-regulated (4, 8, 10). However, as shown recently by Tabas, Weiland, and Tall (39), the pathway for uptake of LDL in J774 cells is relatively resistant to down-regulation by high concentrations of either LDL or 25-hydroxycholesterol. Indeed, in the present study, uptake of β-VLDL and chylomicron remnants by J774 cells was only partially suppressed by cholesterol loading with acetylated-LDL. On the other hand, since apoprotein E is a very high affinity ligand for the rodent LDL receptor compared to human apoprotein B (41), it is possible that expression of only a few LDL receptors, as might even occur in the WHHL rabbit or in some patients with familial hypercholesterolemia, could account for the β-VLDL receptor activity. Preliminary studies from our laboratory (42) demonstrate that specific polyclonal antibodies directed against the estrogen-induced LDL receptor of rat liver compete for uptake of β-VLDL and chylomicron remnants in J774 cells and mouse peritoneal macrophages. These data are consistent with the hypothesis that the lipoprotein transport observed in the present study is mediated, at least in part, by an LDL receptor pathway.

Whatever the nature of receptor involved, the fact that macrophages possess a receptor capable of transporting dietary-derived lipoproteins with a high affinity and capacity has potentially important implications for assigning a role to dietary lipoproteins in the atherogenic process (43). In this regard, the dichotomous metabolic effects of β-VLDL and chylomicron remnants in macrophages are of interest. Although β-VLDL and chylomicron remnants achieve similar steady-state levels of cell association, the effects of β-VLDL and chylomicron remnants on cellular lipid metabolism were quite different. While β-VLDL stimulated cholesteryl ester synthesis without affecting triacylglycerol synthesis, chylomicron remnants inhibited cholesteryl ester synthesis and stimulated triacylglycerol synthesis. In addition to the changes in fatty acid incorporation into cellular lipids, the two lipoproteins had different effects on HMG-CoA reductase activity. β-VLDL suppressed this enzyme while chylomicron remnants stimulated it. This divergent response has also been seen in liver (44) where chylomicron remnants stimulate reductase activity and in fibroblasts (45) where chylomicron remnants and β-VLDL, respectively), at saturating concentrations, chylomicron remnants will deliver to the cells approximately one-half as much cholesterol as β-mechanism in fibroblasts is unclear. However, if this is a physiologic effect, one might speculate that the two types of particles would have different effects on net cholesterol accumulation. Because of the differences in lipid compositions (cholesterol/protein ratio 3.8 and 5.08 for chylomicron remnants and β-VLDL, respectively), at saturating concentrations, chylomicron remnants will deliver to the cells approximately one-half as much cholesterol as β-VLDL. However, the metabolic consequences will differ substantially. While β-VLDL will inhibit cellular cholesterol synthesis, chylomicron remnants will increase cellular cholesterol synthesis and, in this way, remnants could actually be a more atherogenic particle.

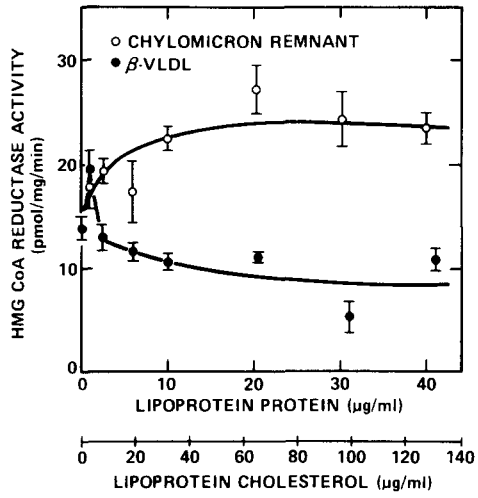

Fig. 8. Effect of chylomicron remnants and β-VLDL on HMG-CoA reductase activity in macrophages. J774 cells were cultured in 35-mm dishes as described in the legend to Fig. 7. At the time of assay, each dish received the indicated concentration of lipoprotein in a final volume of 1.0 ml of medium B and was incubated for 1.5 hr at 37°C. Cells were placed on ice, washed, harvested, and HMG-CoA reductase activity was measured on cellular homogenates as described in Experimental Procedures. Each point represents the mean ± SD of triplicate dishes of cells and is representative of two separate experiments.

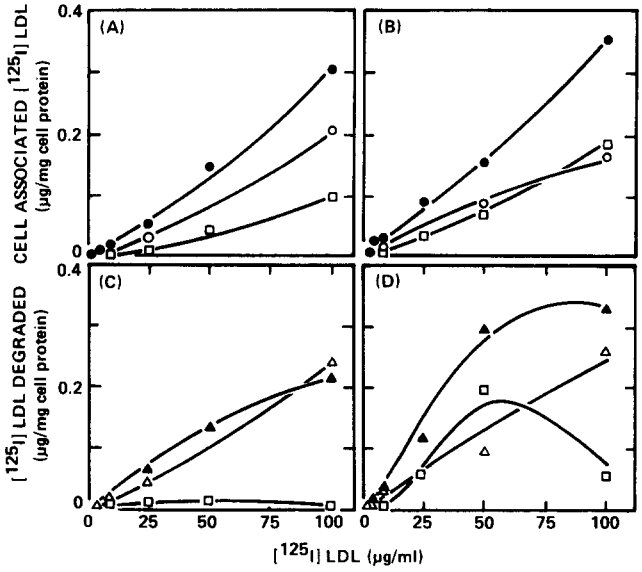

Fig. 9. Uptake and degradation of ^{125}I-labeled LDL by macrophages. J774 cells were incubated for 1.5 hr at 37°C in 1.0 ml of medium B containing the indicated protein concentration of human ^{125}I-labeled LDL, in the absence (total uptake (●) or degradation (▲)) or presence (○,△) of unlabeled LDL (500 μg protein/ml). Cell association and degradation of ^{125}I-labeled LDL was measured in stationary (A,C) and log (B,D) phase cultures. The open squares represent the specific uptake or degradation calculated as the difference between the total and nonspecific values. Each point represents the data from a single dish of cells and is representative of two separate experiments.

Subsequent to the completion of this work, Van Lenten et al. (40) published studies of the metabolism of human chylomicron remnants by human and rabbit macrophages. These investigators reported that chylomicron remnants were removed by a "low affinity high capacity" system that resulted in cholesteryl ester accumulation. Cholesterol pre-loading of the cells with 25-hydroxycholesterol suppressed β-VLDL uptake, but did not suppress chylomicron remnant uptake. Nevertheless, chylomicron remnants and β-VLDL competed equally for uptake. Because of differences in the cell types used, in the lipoprotein compositions, and in the experimental protocols, it is not possible to make more detailed comparisons with our studies; however, both their report and ours point out the potential importance of dietary-derived lipoprotein remnants in inducing lipid accumulation in macrophages. Moreover, the present study emphasizes the variation in biologic effects of different lipoproteins that compete equally for uptake by the same receptor.

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