Differences in the processing of chylomicron remnants and β-VLDL by macrophages

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Abstract To gain a detailed understanding of those factors that govern the processing of dietary-derived lipoprotein remnants by macrophages we examined the uptake and degradation of rat triacylglycerol-rich chylomicron remnants and rat cholesterol-rich β-very low density lipoprotein (β-VLDL) by J774 cells and primary cultures of mouse peritoneal macrophages. The level of cell associated ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants reached a similar equilibrium level within 2 h of incubation at 37°C. However, the degradation of ^{125}I-labeled β-VLDL was two to three times greater than the degradation of ^{125}I-labeled chylomicron remnants at each time point examined, with rates of degradation of 161.0 ± 36.0 and 60.1 ± 6.6 ng degraded/h per mg cell protein, respectively. At similar extracellular concentrations of protein or cholesterol, the relative rate of cholesteryl ester hydrolysis from [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate-labeled chylomicron remnants was one-third to one-half that of similarly labeled β-VLDL. The reduction in the relative rate of chylomicron remnant degradation by macrophages occurred in the absence of chylomicron remnant-induced alterations in low density lipoprotein (LDL) receptor recycling or in retroendocytosis of either ^{125}I-labeled lipoprotein. The rate of internalization of ^{125}I-labeled β-VLDL by J774 cells was greater than that of ^{125}I-labeled chylomicron remnants, with initial rates of internalization of 0.21 ng/min per mg cell protein for ^{125}I-labeled chylomicron remnants and 0.39 ng/min per mg cell protein for ^{125}I-labeled β-VLDL. The degradation of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL was dependent on lysosomal enzyme activity: preincubation of macrophages with the lysosomotropic agent monensin reduced the degradation of both lipoproteins by greater than 90%. However, the pH-dependent rate of degradation of ^{125}I-labeled chylomicron remnants by lysosomal enzymes isolated from J774 cells was 50% that of ^{125}I-labeled β-VLDL. The difference in degradation rates was dependent on the ratio of lipoprotein to lysosomal protein used and was greatest at ratios > 50. The degradation of ^{125}I-labeled β-VLDL by isolated lysosomes was reduced 30–40% by preincubation of β-VLDL with 25–50 μg oleic acid/ml, suggesting that released free fatty acids could cause the slower degradation of chylomicron remnants. Thus, differences in the rate of uptake and degradation of remnant lipoproteins of different compositions by macrophages are determined by at least two factors: *1*) differences in the rates of lipoprotein internalization and *2*) differences in the rate of lysosomal degradation. —**Ellsworth, J. L., L. G. Fong, F. B. Kraemer, and A. D. Cooper.** Differences in the processing of chylomicron remnants and β-VLDL by macrophages. *J. Lipid Res.* 1990. **31:** 1399–1411.

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The development of diet-induced atherosclerosis is associated with the accumulation in blood of remnant lipoproteins of intestinal origin as well as cholesterol-rich hepatic lipoproteins. Based on their electrophoretic mobilities, these particles have been termed β-VLDL and are observed in the plasma of a variety of species fed high fat, high cholesterol diets (1). A potential role for these lipoproteins in atherogenesis is suggested by the fact that their appearance is coincident with the formation of cholesteryl ester-laden cells that have been termed foam cells. Ultrastructural evidence has demonstrated that the cholesteryl ester-laden foam cells of the early fatty streak lesion are derived from monocyte-derived macrophages (2–6). As the lesion progresses, the fatty streak expands by the combined attachment and subendothelial migration of monocytes which become macrophages and by the gradual accumulation of smooth muscle cells that proliferate in the intima and also accumulate lipid (7). Although this sequence of cellular changes leading to progression of the atherosclerotic lesion has been elucidated in detail, the biochemical mechanisms responsible for foam cell formation are not yet clear.

Based on studies of macrophages cultured in vitro, several pathways for intracellular lipid accumulation and foam cell formation have been described. Some of these involve specific cell surface receptors (8, 9). One pathway is mediated by the scavenger receptor which initiates the uptake of a variety of negatively charged ligands such as acetyl-LDL, malondialdehyde-modified or oxidatively modified LDL (8). The uptake and degradation of modified lipoproteins by the scavenger receptor can produce massive intracellular deposition of cholesteryl ester (8). Although these studies have shown that macrophage-derived foam cells can be produced in vitro, the naturally occurring lipoprotein ligand for the scavenger receptor, if any,

Abbreviations: β-VLDL, beta migrating very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; PBS, phosphate-buffered saline; LPDS, lipoprotein-deficient fetal calf serum; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; LpL, lipoprotein lipase.

as well as the biological mechanism producing lipoprotein modification and scavenger receptor recognition have not been conclusively identified, although recent data suggest that products of lipid oxidation may be involved (10, 11).

Cholesteryl ester-laden foam cells can also be produced by the incubation of macrophages in vitro with β-VLDL or chylomicron remnants (8). High concentrations of unlabeled human LDL as well as antibodies to the rat liver (12) or bovine adrenal gland (13, 14) LDL receptor compete for uptake and degradation of these lipoproteins by mouse peritoneal macrophages (12, 13), various murine macrophage cell lines (12), and by human monocyte-derived macrophages (14), indicating that transport of these lipoproteins by macrophages is mediated by the LDL receptor. Importantly, the LDL receptor-mediated transport of apoE-rich lipoproteins has been shown to occur in macrophages under conditions in which little demonstrable LDL transport occurs (15). Thus, it is possible that, although transport of native LDL by the LDL receptor does not lead to foam cell formation, transport of apoE-rich lipoproteins by this pathway could cause significant cholesteryl ester deposition, even in cells whose LDL receptors are relatively down-regulated.

In previous studies we demonstrated that β-VLDL and chylomicron remnants bind to the same macrophage receptor and are equipotent in cross-competition studies (15). Nonetheless, they produce divergent effects on cellular lipid metabolism: incubation with β-VLDL inhibited HMG-CoA reductase activity and stimulated cholesteryl ester synthesis, whereas incubation with chylomicron remnants stimulated both HMG-CoA reductase activity and triacylglycerol synthesis (15). In addition, preliminary studies in our laboratory demonstrated that the relative rate of chylomicron remnant apoprotein degradation and the relative rate of hydrolysis of chylomicron remnant cholesteryl ester were significantly less than that of β-VLDL (16), suggesting that the pathways for processing of these lipoproteins by macrophages may be different. In an effort to understand in detail the metabolism of chylomicron remnants by macrophages, in the present study we have examined and compared several parameters of the processing of dietary-derived chylomicron remnants and β-VLDL by cultured macrophages.

EXPERIMENTAL PROCEDURES

Materials

Male Sprague-Dawley rats (250–300 g) and retired breeders and female Swiss Webster mice (27–30 g) were obtained from Simonsen Laboratories (Gilroy, CA). Sodium [^{125}I]iodide (13–17 mCi/μg), [1,2(n)-^{3}H]cholesteryl oleate (32 Ci/mmol), cholesteryl[1-^{14}C]oleate (58 mCi/mmol), and [9,10(n)-^{3}H]oleic acid (5.0 Ci/mmol) were obtained from Amersham-Searle Corporation (Arlington Heights, IL). Iodogen^{R} was obtained from Pierce Chemical Company (Rockford, IL). Pronase was obtained from Calbiochem Corporation (La Jolla, CA). Sodium oleate and radioimmunoassay grade bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO). All tissue culture supplies and other chemicals were obtained as described in a previous publication (15).

Methods

Cell culture. Cultures of the J774 mouse macrophage cell line were maintained in medium A which consisted of Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum under a humidified atmosphere of 95% air, 5% CO_2. For lipoprotein binding studies, the cells were subcultured in multi-well plates at a density of 5–6 × 10^4 cells/cm^2 (day 0). Fresh growth media were added on day 4, and, in general, the cells were used on day 5. At the time of assay, the cells had formed dense monolayers of 2–4 × 10^5 cells/cm^2 and 60–70 μg of cell protein/cm^2. Resident mouse peritoneal macrophages were harvested from female Swiss Webster mice by peritoneal lavage with DMEM. The cells were resuspended in medium A (1.25 × 10^6 cells/ml) and 2.0 ml was added to 12-well tissue culture plates. The cells were incubated at 37°C under a humidified atmosphere of 95% air and 5% CO_2 and were used the following day.

Preparation of lipoproteins. Rat β-very low density lipoproteins were prepared from rats maintained on chow supplemented with 5.0% lard, 2.0% cholesterol, 0.3% cholic acid, and 0.1% propylthiouracil (17). The rats were fasted for 18–24 h prior to exsanguination. Serum was isolated and β-VLDL ($d < 1.006$ g/ml) were isolated by centrifugation as described previously (15). The rat β-VLDL contained 1.3 mg of triacylglycerol/mg protein and 0.9 mg free fatty acid/mg protein. Rat chylomicrons were collected from animals fitted with a lymph duct cannula and chylomicron remnants were prepared as described in a previous publication (15). The chylomicron remnants contained 40 mg of triacylglycerol/mg protein and 3.1 mg free fatty acid/mg cell protein. Chylomicron remnants and β-VLDL were radiolabeled with ^{125}I by either Iodogen^{R} or the iodine monochloride procedure as described (15). Chylomicron remnants were also prepared from ^{125}I-labeled chylomicrons (15). For both ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants, greater than 94% of the ^{125}I radioactivity was precipitable by an equal volume of 20% trichloroacetic acid and between 15–20% and 20–80% of the ^{125}I label was extractable into chloroform–methanol, respectively. Labeling of lipids with ^{125}I was lowest for chylomicron remnants prepared from ^{125}I-labeled chylomicrons. Despite differences in the extent of lipid labeling, the various ^{125}I-labeled chylomicron remnants were indistinguishable in cell binding studies (15).

The apoprotein distribution of ^{125}I incorporation in the radiolabeled lipoproteins was as described previously (15). The average specific activities were 190 cpm/ng protein for ^{125}I-labeled β-VLDL and 100 cpm/ng protein for ^{125}I-labeled chylomicron remnants. Rat chylomicron remnants and β-VLDL dual-labeled with [^{3}H]cholesteryl oleate and cholesteryl[1-^{14}C]oleate were prepared exactly as described by Halperin, Stein, and Stein (18) using heptane-extracted human HDL (1.063–1.210 g/ml) as lipid donor. The chylomicron remnants and β-VLDL were incubated with dual-labeled HDL in the presence of freshly prepared human lipoprotein-deficient plasma as a source of lipid transfer protein activity (19). After 18 h at 37°C, the lipoproteins were reisolated by centrifugation at 105,000 *g* for 18 h at 4°C. Lipids were extracted with chloroform–methanol 2:1 (v/v) and separated by thin-layer chromatography on silica gel G as described (15). For the chylomicron remnants, the specific activities for [^{3}H]-cholesteryl oleate and the cholesteryl[^{14}C]oleate were 4717 dpm/nmol and 468 dpm/mmol, respectively. For the β-VLDL, the values were 2952 dpm/nmol for [^{3}H]cholesteryl oleate and 244 dpm/nmol for cholesteryl[1-^{14}C]oleate.

Negative stain electron microscopy of chylomicron remnants and β-VLDL was carried out as described (20) after overnight dialysis of the lipoproteins against a solution of 0.125 M ammonium acetate, 2.6 mM ammonium carbonate, and 0.26 mM Na_4 EDTA, pH 7.4. The lipoproteins were dropped onto carbon-coated grids and stained with sodium phosphotungstate.

Lipoprotein binding studies. The binding, uptake, and degradation of chylomicron remnants and β-VLDL by J774 cells were assayed as described previously (12,15). Cell-associated (bound + internalized) lipoprotein was calculated from the total specific activities of the ^{125}I-labeled lipoproteins, while the amount of ^{125}I-labeled lipoprotein degraded was calculated from the protein specific activities. Cell-associated and degraded lipoprotein radioactivity was normalized per mg of cell protein which was determined on an aliquot of the solubilized cell suspensions. To monitor the delivery and subsequent hydrolysis of lipoprotein-derived esterified cholesterol, the J774 cells were cultured as described above and were incubated at 37°C in DMEM supplemented with 10% lipoprotein-deficient fetal calf serum in the presence of the indicated concentrations of dual-labeled β-VLDL or chylomicron remnants. At the appropriate time, the cells were placed on ice, washed with 2.0 ml of ice-cold PBS, and were harvested by scraping with a rubber policeman. Radiolabel incorporation into cellular free and esterified cholesterol was determined following lipid extraction as described previously (15).

Lipoprotein internalization was measured as the amount of lipoprotein binding that was resistant to digestion by pronase. J774 cells were incubated with 4.0 μg protein/ml of either ^{125}I-labeled chylomicron remnants or ^{125}I-labeled β-VLDL in 0.5 ml of medium B at 4°C for 2 h. The cells were washed three times with ice-cold medium B to remove unbound lipoprotein and then incubated for the indicated time at 37°C. The cells were immediately chilled on ice, washed three times with ice-cold medium B, and then incubated with 0.5 mg pronase/ml in PBS for 2 h at 4°C to release cell surface-bound lipoprotein. The cell suspension was centrifuged at 1000 *g* for 5 min at 4°C and the supernatant (pronase-releasable lipoprotein) transferred to another tube. The remaining cells were washed twice with PBS and centrifuged as described above. The ^{125}I radioactivity in the combined supernatant fractions was measured and expressed as a percent of the total amount of lipoprotein initially bound. The pronase-releasable lipoprotein was then expressed as a percent of the maximal amount of pronase-releasable lipoprotein (the amount of lipoprotein released from cells not incubated at 37°C).

Isolation of lysosomes and lysosomal degradation of lipoproteins. J774 cells were scraped into ice-cold homogenization buffer (0.25 M sucrose, 2 mM EDTA, 0.01 M HEPES, pH 7.4) and lysed using a Dounce homogenizer as described (21). The lysate was centrifuged for 10 min at 750 *g* at 4°C and the resulting supernatant was centrifuged for 15 min at 10,000 *g* at 4°C to obtain a lysosome-enriched fraction (pellet). The pellet was resuspended in water and freeze-thawed six times to disrupt the lysosomes. The specific activity of the lysosomal enzyme β-glucuronidase was routinely increased threefold in the partially purified lysosomal fraction. To assay lipoprotein degradation by partially purified lysosomes, ^{125}I-labeled lipoproteins were incubated with the lysosomal fraction in 0.1 ml of DMEM buffered with 50 mM sodium citrate (pH 4.5) at 37°C for 4 h in 96-well microtiter plates. The amount of degradation products was then determined by measuring the amount of TCA/$AgNO_3$-soluble radioactivity generated (22).

Other methods. Protein was measured as described previously (23) using BSA as standard. To determine the protein content of cholesterol- and triacylglycerol-enriched lipoproteins, each sample was extracted with 2.0 ml of $CHCl_3$ 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), and the total cholesterol content of the lipoproteins was measured by the cholesterol oxidase/cholesterol esterase enzyme assay (Sigma kit Cholesterol-20). The free fatty acid content of the lipoproteins was measured as described previously (23) after lipid extraction of the lipoproteins and purification of the free fatty acids by thin-layer chromatography on silica gel G. Samples of lipoproteins were prepared for electrophoresis on 7% polyacrylamide gels containing 0.1% SDS and

electrophoresis was carried out as described previously (15). The relative apoprotein composition of the lipoproteins was determined by scanning densitometry of Coomassie blue-stained gels following electrophoresis. Statistical analyses were performed by a matched or unmatched two-tailed Student's *t*-test.

RESULTS

Relative rates of uptake and degradation of apoprotein from β-VLDL and chylomicron remnants by macrophages

In previous work (15), we observed that the binding of β-VLDL and chylomicron remnants to macrophages appeared to be to a single class of high affinity sites ($K_d \approx 2.0$ μg/ml), subsequently identified as LDL receptors (12). In binding studies at 4°C, the maximum amount of β-VLDL and chylomicron remnants bound was nearly identical, and these lipoproteins were equipotent in cross-competition studies (15). However, in spite of these similarities in cell surface binding, these lipoproteins had different effects on cellular lipid metabolism (15). To explore further the mechanism for these differences, the kinetics of uptake and degradation of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL were examined at 37°C. The amount of cell-associated ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants reached an equilibrium level within 2 h of incubation at 37°C (**Fig. 1**, open circles). However, the amount of lipoprotein that was bound + internalized was 30% higher for ^{125}I-labeled chylomicron remnants than for ^{125}I-labeled β-VLDL at the later time points (Fig. 1, inset). In addition to these differences in cell-associated lipoprotein, the amount of $CHCl_3$-insoluble, ^{125}I-labeled degradation products present in the extracellular medium of J774 cells was greater for ^{125}I-labeled β-VLDL at each time point (Fig. 1, solid circles). Consequently, the total processed (cell associated + degraded) ^{125}I-labeled β-VLDL was two- to fourfold greater than that of ^{125}I-labeled chylomicron remnants (Fig. 1, open triangles). The rate of lipoprotein degradation calculated from the data presented in Fig. 1 was 161.0 ± 36.0 ng/h/mg cell protein (mean ± SE) for ^{125}I-labeled β-VLDL and was 60.1 ± 6.6 ng/h/mg cell protein (mean ± SE, $P < 0.01$) for ^{125}I-labeled chylomicron remnants.

The reduction in the relative rate of chylomicron remnant degradation was not an artifact of lipid labeling since the degradation rate of various ^{125}I-labeled chylomicron remnant preparations was independent of the extent of lipid labeling (data not shown). To assure that the reduced rate of ^{125}I-labeled chylomicron remnant degradation was not due to the presence of the ^{125}I label on a subfraction of the chylomicron remnants that did not represent the bulk of the lipid, chylomicron remnants were chromatographed on Bio-Gel A-150m. The ^{125}I radioactivity comigrated with the triacylglycerol as a single peak (**Fig. 2**). Eighty percent of the TCA-precipitable radioactivity and 86% of the chylomicron remnant triacylglycerols were recovered within the peak between fractions 20 to 37.

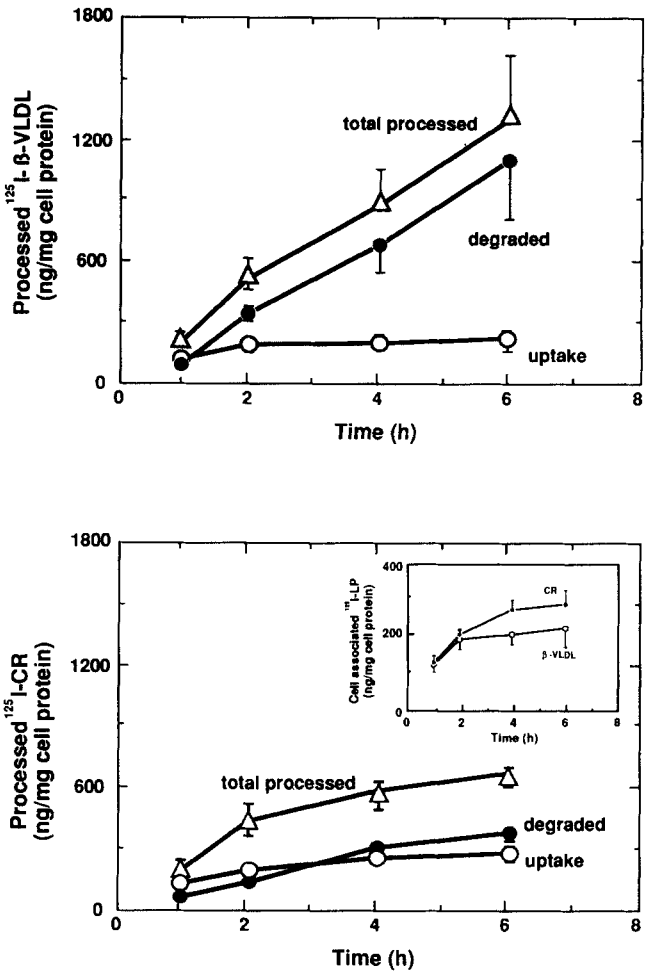

Fig. 1. Kinetics of the uptake and degradation of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL by J774 macrophages. Monolayers of J774 cells (0.2 mg cell protein/dish) were incubated with 0.3 ml of medium B (Eagle's minimal essential medium without sodium bicarbonate, 0.02 M HEPES, pH 7.4, and 40.0 mg/ml bovine serum albumin) containing 3.0 μg protein/ml of ^{125}I-labeled β-VLDL (upper panel) or ^{125}I-labeled chylomicron remnants (lower panel) in the presence and absence of a 60-fold excess of the respective unlabeled lipoprotein for the indicated time at 37°C. The cells were then removed from the incubator, placed on ice, washed and the amount of specific cell-associated (○), degraded (●), and total processed (△) ^{125}I-labeled lipoprotein was determined as described under Experimental Procedures. The inset shows the cell-associated ^{125}I-labeled lipoproteins on an expanded scale. The specific cell-associated or degraded ^{125}I-labeled β-VLDL represented 90% of the total values and for ^{125}I-labeled chylomicron remnants, the specific values were 80% of the total. Each point represents the mean ± SE of three separate experiments of triplicate dishes of cells. Different lipoprotein preparations were used in each experiment.

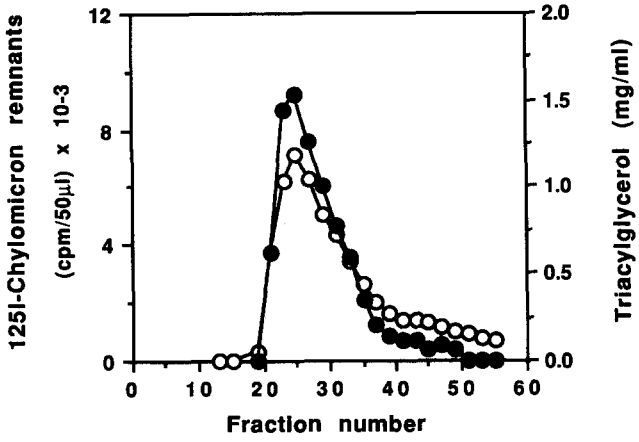

Fig. 2. Chromatography of ^{125}I-labeled chylomicron remnants on Bio-Gel A-150m. A sample of unlabeled chylomicron remnants (0.44 mg protein) was mixed with 0.02 mg protein of ^{125}I-labeled chylomicron remnants in a final volume of 0.2 ml of PBS and the lipoprotein mixture was applied to a 1.0 × 49 cm column of Bio-Gel A-150m equilibrated in PBS at room temperature. The fraction size was 22 drops (0.5 ml) and the flow rate was 5–10 ml/h. The calculated void volume was 13 ml at fraction number 19. A sample of each fraction was taken for determination of trichloroacetic acid-precipitable radioactivity (○) and triacylglycerol content (●) as described under Experimental Procedures. The figure shown is representative of two separate experiments using two different preparations of labeled and unlabeled chylomicron remnants.

The reduction in the relative rate of chylomicron remnant degradation was not specific for J774 cells, but was also observed when lipoprotein degradation was measured in primary cultures of mouse peritoneal macrophages. As is shown in **Table 1**, after 5 h of incubation at 37°C, the amount of cell associated ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL was nearly identical. However, the degradation of ^{125}I-labeled chylomicron remnants by mouse peritoneal macrophages was significantly less than the degradation of ^{125}I-labeled β-VLDL. Although the variation in the degradation rates of ^{125}I-labeled chylomicron remnants was somewhat greater than that observed with J774 cells, in each case, the degradation of ^{125}I-labeled β-VLDL was greater than that for ^{125}I-labeled chylomicron remnants. Taken together, these data demonstrate that in spite of their similarities in cell surface binding, at equivalent extracellular protein concentrations, degradation of ^{125}I-labeled chylomicron remnants by macrophages proceeded at a rate that was about one-third to one-half that of ^{125}I-labeled β-VLDL.

Relative rates of uptake and degradation of cholesterol and cholesteryl ester in β-VLDL and chylomicron remnants by macrophages

To assure that the reduced rate of chylomicron remnant degradation was not due to an alteration induced by the iodination procedure, lipoproteins were labeled with [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate. Incubation of macrophages at 37°C with dual-labeled β-VLDL resulted in a time-dependent increase in the intracellular accumulation of radiolabeled cholesteryl ester that had been internalized, hydrolyzed, and reesterified (**Fig. 3**, panel A). In contrast, at an equivalent extracellular concentration of protein, there was much slower accumulation of radiolabeled cholesteryl ester from dual-labeled chylomicron remnants. Further, when the relative rates of hydrolysis of lipoprotein cholesteryl ester by J774 cells were measured and compared (Fig. 3, panel B), it was observed that cholesteryl esters from β-VLDL were hydrolyzed at a rate two- to threefold faster than cholesteryl esters from chylomicron remnants. Similar results were obtained when the macrophages were incubated with equivalent extracellular concentrations of lipoprotein cholesterol. The cellular accumulation of unesterified (**Fig. 4**, panel A) and esterified (Fig. 4 panel B) cholesterol from

TABLE 1. Uptake and degradation of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL by mouse peritoneal macrophages

Lipoprotein	Cell Associated	Degraded
	ng/mg cell protein per 5 h	
Experiment 1		
^{125}I-labeled β-VLDL	1326.1 ± 179.4	1637.4 ± 15.1
^{125}I-labeled chylomicron remnants	1240.7 ± 134.5	884.3 ± 48.5[a]
Experiment 2		
^{125}I-labeled β-VLDL	1063.3 ± 42.4	1490.5 ± 27.5
^{125}I-labeled chylomicron remnants	959.3 ± 131.1	1089.7 ± 9.0[a]

Monolayer cultures of mouse peritoneal macrophages were incubated with ^{125}I-labeled β-VLDL (5 µg protein/ml) or ^{125}I-labeled chylomicron remnants (5 µg protein/ml) for 5 h at 37°C in 1 ml of buffer B in the presence and absence of a 10-fold excess of the respective unlabeled lipoprotein. The specific cell associated and degraded ^{125}I-labeled lipoprotein was determined as described under Experimental Procedures. The values for each experiment represent the mean ± SD for triplicate dishes of cells. Different lipoprotein preparations were used for each experiment.

[a]Difference significant ($P < 0.01$) between the degradation of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants as determined by a two-tailed Student's *t*-test.

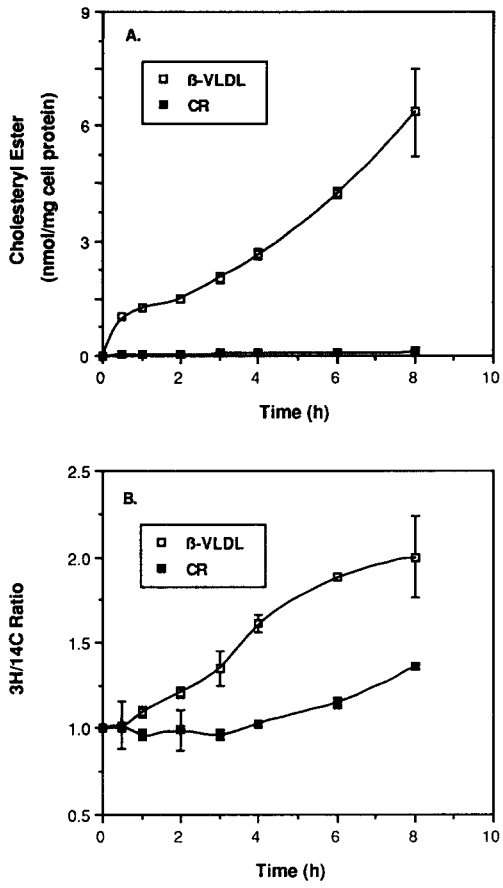

Fig. 3. Metabolism of lipoprotein-derived cholesteryl esters by J774 macrophages. J774 cells were cultured in 12-well plates and washed as described under Experimental Procedures. At the time of assay, confluent monolayers of cells (0.3 mg cell protein) had formed. Each dish received equivalent concentrations of lipoprotein protein (5.0 μg/ml) of [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate-labeled β-VLDL (□) or [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate-labeled chylomicron remnants (■) and were incubated for the indicated time at 37°C in 0.5 ml of medium A. Cells were placed on ice, harvested, and the cellular incorporation of [^{3}H]cholesteryl oleate (panel A) and the hydrolysis of lipoprotein-derived cholesteryl ester (panel B) were determined as described under Experimental Procedures. The total cholesterol:protein ratios of the [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate-labeled chylomicron remnants and β-VLDL were 13.9 and 20.1 μg cholesterol/μg protein, respectively. Each point represents the mean ± SD of triplicate dishes of cells and is representative of two separate experiments.

β-VLDL was greater than that of chylomicron remnants at each time point examined. For both dual-labeled lipoproteins, the rate of unesterified and esterified cholesterol accumulation was linear for the first 4.0 h. For dual-labeled chylomicron remnants, the rate of unesterified and esterified cholesterol accumulation by macrophages was 1.3 pmol/h per mg cell protein and 0.1 pmol/h per mg cell protein, respectively, as compared to the intial rates of 2.7 pmol/ h per mg cell protein and 0.75 pmol/h per mg cell protein, respectively, for dual-labeled β-VLDL. These data indicate that the rate at which macrophages process both the apoprotein and cholesteryl ester components of chylomicron remnants is significantly slower than the processing of the same components of β-VLDL.

Internalization rate of β-VLDL and chylomicron remnants by macrophages

Since the chylomicron remnants and β-VLDL used in the present studies have similar apoB/apoE ratios and, accordingly, exhibit similar binding affinities to the macrophage LDL receptor (15), some post-binding event(s) must account for the differences in processing of the different types of particle. The internalization rate of ^{125}I-labeled chylomicron remnants was measured and compared to that of ^{125}I-labeled β-VLDL. Monolayers of J774 cells were incubated with saturating concentrations of either ^{125}I-labeled β-VLDL or ^{125}I-labeled chylomicron remnants at 4°C to saturate the cell surface LDL receptors. The cells were then washed and incubated at 37°C to allow internalization. At the indicated time, the dishes were again placed on ice and the amount of pronase-releasable (cell surface-bound) lipoprotein was measured.

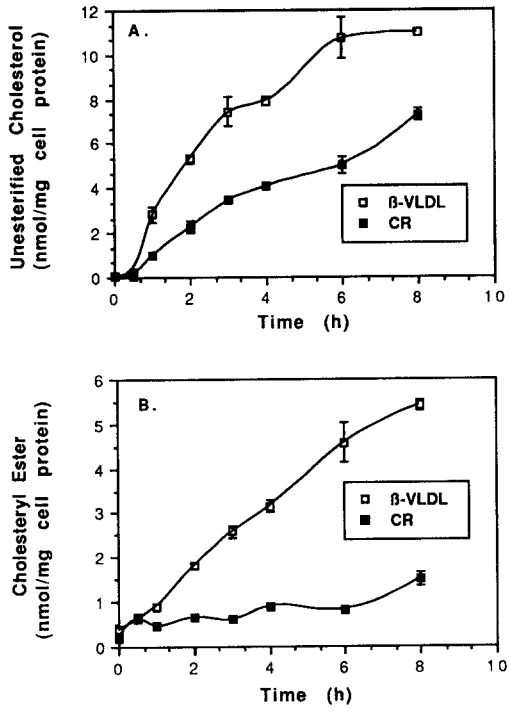

Fig. 4. Delivery of β-VLDL and chylomicron remnant cholesterol to J774 macrophages. Monolayers of J774 cells were cultured as described in the legend to Fig. 2. Each dish received equivalent concentrations of lipoprotein cholesterol (30.0 μg cholesterol/ml) of [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate-labeled β-VLDL (1.5 μg protein/ml) (□) or [^{3}H]cholesteryl oleate/cholesteryl [^{14}C]oleate-labeled chylomicron remnants (2.1 μg protein/ml) (■) and were incubated for the indicated time at 37°C in 0.5 ml of medium A. Cells were placed on ice, harvested, and the incorporation of [^{3}H]cholesteryl oleate into cellular cholesterol (panel A) and cholesteryl ester (panel B) was determined as described under Experimental Procedures. Each point represents the mean ± SD for triplicate dishes of cells and is representative of two separate experiments.

Incubation of macrophages at 37°C resulted in a time-dependent decrease in the cell surface bound lipoprotein (**Fig. 5**). The initial rate at which ^{125}I-labeled β-VLDL were internalized was nearly twice that of ^{125}I-labeled chylomicron remnants; for ^{125}I-labeled β-VLDL, the initial rate of internalization was 0.39 ng/min per mg cell protein compared to a rate of 0.21 ng/min per mg protein for ^{125}I-labeled chylomicron remnants. Thus, one difference for the slower degradation of chylomicron remnants by J774 cells is a 50% slower rate of internalization.

Recycling of LDL receptors after internalization of β-VLDL remnants by macrophages

It remained possible that other steps in the endocytic pathway were also different for the chylomicron remnants. For example, the rate of dissociation of the receptor-ligand complex could be slower for chylomicron remnants, which would lead to disruption of LDL receptor recycling. To evaluate this, J774 macrophages were preincubated for either 20 min or 40 min at 37°C with saturating concentrations of unlabeled β-VLDL or chylomicron remnants. The cells were then washed and the binding of ^{125}I-labeled β-VLDL to the cells was determined at 4°C. Since the LDL receptor recycles back to the cell surface about once every 10 min (24), chylomicron remnant-induced trapping of LDL receptors inside the cell would be evident as a time-dependent reduction in the subsequent cell surface binding of ^{125}I-labeled β-VLDL. At 4°C without lipoprotein preincubation, the binding of ^{125}I-labeled β-VLDL to J774 cells was saturable, specific, and of high affinity (**Fig. 6**, panel A). Preincubation of the cells with β-VLDL or chylomicron remnants for 20 min (Fig. 6, panel B) or 40 min (Fig. 6, panel C) at 37°C had no effect

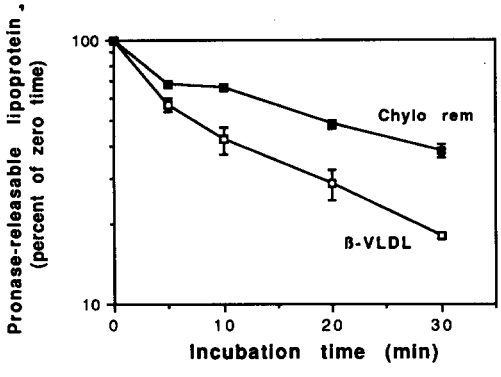

Fig. 5. Rate of internalization of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants by J774 cells. The ratio of internalization of ^{125}I-labeled chylomicron remnants (■) and ^{125}I-labled β-VLDL (□) by J774 cells was determined as described under Experimental Procedures. Each point represents the mean ± SD of triplicate dishes of cells and is representative of three separate experiments.

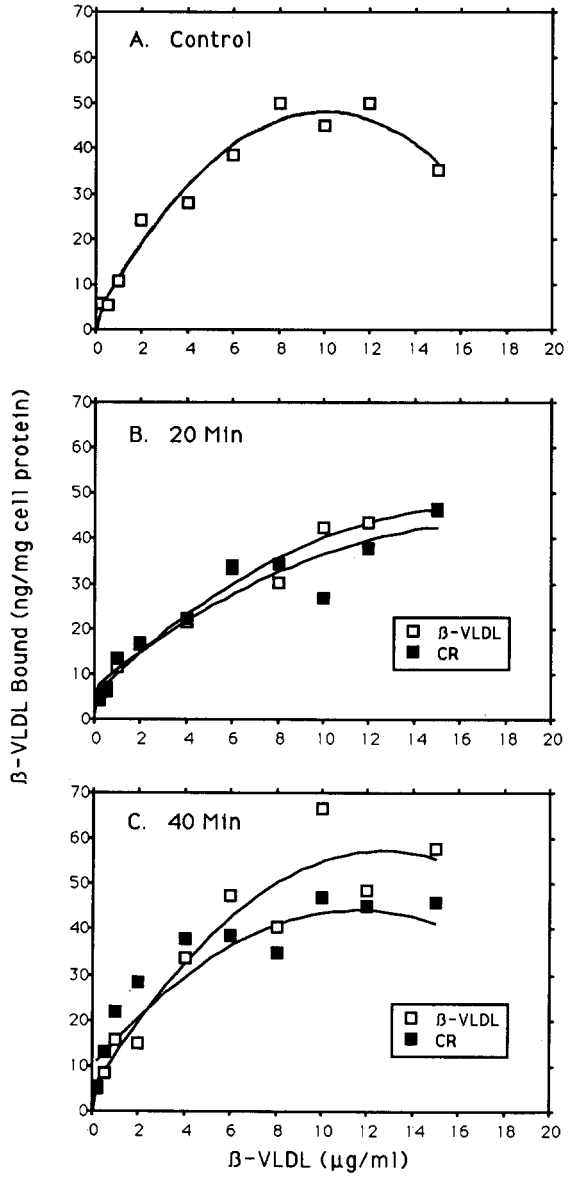

Fig. 6. Effect of preincubation of J774 cells with β-VLDL and chylomicron remnants on the binding of ^{125}I-labeled β-VLDL measured at 4°C. Monolayers of J774 cells were cultured in 12-well plates as described in the legend to Fig. 1. Each dish of cells (0.32 mg protein/dish) was removed from the incubator, placed on ice, and washed with 2.0 ml of ice-cold PBS. The cells were then incubated with 0.4 ml of medium B in the absence of lipoproteins for 40 min at 37°C (panel A, control) or in the presence of 15 μg protein/ml of unlabeled β-VLDL (□) or chylomicron remnants (■) for 20 min (panel B) or 40 min (panel C) at 37°C. After the appropriate time, the cells were placed on ice, washed with 2.0 ml of ice-cold PBS, and reincubated in 0.4 ml of lipoprotein-free medium B. After 1.0 h at 37°C, the cells were placed on ice and washed with 2.0 ml of ice-cold PBS. The binding of ^{125}I-labeled β-VLDL to the cells was then measured by incubating each monolayer with 0.4 ml of medium B containing the indicated protein concentration of ^{125}I-labeled β-VLDL in the presence and absence of 200 μg protein/ml of unlabeled β-VLDL for 3 h at 4°C. The specific binding of ^{125}I-labeled β-VLDL was determined as described under Experimental Procedures. Each data point represents a single dish of cells and is representative of two separate experiments.

on the amount of ^{125}I-labeled β-VLDL subsequently bound at 4°C. Scatchard (25) plots of the binding data were linear and indistinguishable, indicating that short-term preincubation of J774 cells at 37°C, with either β-VLDL or chylomicron remnants, had no effect on the binding affinity or the maximum amount of ^{125}I-labeled β-VLDL subsequently bound to the macrophage LDL receptor (**Table 2**). With more prolonged preincubation (4 h), there was down-regulation of subsequent binding most likely due to sterol-mediated suppression of LDL receptor gene transcription (26). Taken together, these data indicate that chylomicron remnant-induced alterations in LDL receptor recycling were not responsible for the reduction in the relative rate of chylomicron remnant degradation.

Retroendocytosis of β-VLDL and chylomicron remnants by macrophages

Aulinskas et al. (27) demonstrated that smooth muscle cells and fibroblasts incubated with ^{125}I-labeled LDL at 37°C can release trichloroacetic acid-precipitable material into the culture medium by a process termed retroendocytosis. Transport of ^{125}I-labeled LDL through this pathway is enhanced when cells are cultured in the presence of lysosomotropic agents (28), demonstrating that changes in lysosomal function can alter the metabolic disposition of internalized ^{125}I-labeled LDL. To evaluate whether the reduction in the relative rate of chylomicron remnant degradation could be explained by a preferential retroendocytosis of chylomicron remnants, J774 cells were incubated with saturating concentrations of ^{125}I-labeled chylomicron remnants or ^{125}I-labeled β-VLDL for 1.5 h at 37°C, the cells were washed and reincubated for 3 h at 37°C in lipoprotein-free medium. The amount of TCA-soluble and -insoluble ^{125}I radioactivity in the extracellular medium was measured and compared to the cell associated ^{125}I radioactivity. For both ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants 80–90% of the total ^{125}I radioactivity remained associated with the cells after 3 h of incubation at 37°C (**Table 3**). For both ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL, less than 9% of the total cell associated ^{125}I radioactivity was found in the extracellular medium in a form precipitable with 10% TCA, indicating that little retroendocytosis of either lipoprotein occurred. Thus, a preferential retroendocytosis of chylomicron remnants cannot explain the reduction in the relative rate of chylomicron degradation.

Lysosomal processing of β-VLDL and chylomicron remnants by macrophages

To evaluate whether the degradation of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL by J774 macrophages is dependent on the usual endosome-lysosome pathway, and the degradation of each lipoprotein was measured in the presence and absence of the monovalent carboxylic ionophore monensin. Monensin has previously been shown to inhibit the degradation of LDL in human skin fibroblasts (24) and asialoglycoproteins in rat hepatocytes (29) as a result of blocking the pH-dependent dissociation of the ligand-receptor complex by altering lysosomal pH. Preincubation of J774 cells with 50 μM monensin inhibited the degradation of both ^{125}I-labeled chylomicron remnants, and ^{125}I-labeled β-VLDL by greater than 90%. In this experiment, for ^{125}I-labeled chylomicron remnants, the rate of degradation decreased from 143.9 ± 53.8 to 15.1 ± 3.1 ng/mg cell protein per 4 h (mean ± SD, n = 3 wells of cells) in the absence and presence of monensin, respectively. Similarly, for ^{125}I-labeled β-VLDL, the rate of degradation decreased from 431.9 ± 50.6 in the absence of monensin to 21.4 ± 1.0 ng/mg cell protein per 4 h (mean ± SD, n = 3 wells of cells) in its presence. These data demonstrate that the proteolytic degradation of chylomicron remnants and β-VLDL by J774 macrophages is sensitive to lysosomal pH.

To determine whether chylomicron remnants and β-VLDL are degraded at different rates by lysosomal enzymes, lysozomes were isolated from J774 macrophages, lysed by freeze-thawing, and the lysosomal enzymes were incubated in vitro with equivalent protein concentrations

TABLE 2. Equilibrium dissociation constants for the binding of ^{125}I-labeled β-VLDL to macrophages preincubated with unlabeled β-VLDL or chylomicron remnants

Lipoprotein in Preincubation	Duration of Preincubation at 37°C	K_d	B_{max}
		μg/ml	*ng/mg cell protein*
None	40 min	3.7	59.0
Chylomicron remnants	20 min	3.4	50.0
β-VLDL	20 min	3.3	52.1
Chylomicron remnants	40 min	2.8	52.4
β-VLDL	40 min	4.2	73.9

Scatchard plots (25) of the ^{125}I β-VLDL binding data from Fig. 6 were constructed and the equilibrium dissociation constants (K_d) were derived as described under Experimental Procedures.

TABLE 3. Retroendocytosis of ^{125}I-labeled chylomicron remnants and ^{125}I-labeled β-VLDL by J774 macrophages

Lipoprotein	Incubation at 37°C		^{125}I Radioactivity		
				Extracellular	
	1.5 h pulse	3.0 h chase	Cell-Associated	TCA-Soluble	TCA-Insoluble
			cpm/dish		
^{125}I-labeled chylomicron remnants	+	−	45799 ± 3357		
	+	+	41078 ± 1332	1167 ± 33	2335 ± 62
^{125}I-labeled β-VLDL	+	−	35676 ± 1362		
	+	+	30185 ± 2427	3677 ± 355	3251 ± 438

Monolayer cultures of J774 cells were "pulsed" with 10 μg protein/ml of ^{125}I-labeled chylomicron remnants or ^{125}I-labeled β-VLDL in 0.3 ml of medium B for 1.5 h at 37°C. The cells were removed from the incubator, placed on ice, and washed five times with 1.0 ml of wash buffer containing 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.002 M $CaCl_2$, and 1.0 mg/ml BSA. The cell-associated ^{125}I radioactivity was determined on one-half of the cells as described under Experimental Procedures. The other half of the dishes were "chased" by the addition of 0.3 ml of fresh medium B and the cells were returned to the incubator. After 3 h at 37°C, the cells were removed from the incubator and placed on ice. The cell-associated radioactivity and the TCA-precipitable radioactivity in the extracellular medium were determined as described. Each value represents the mean ± SD of triplicate dishes of cells from a representative experiment.

of ^{125}I-labeled chylomicron remnants or ^{125}I-labeled β-VLDL and the production of trichloroacetic acid/silver nitrate-soluble ^{125}I-labeled degradation products was measured. The degradation of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants by isolated lysosomal enzymes was dependent on the pH of the incubation medium (**Fig. 7**, panel A). Little degradation of either ^{125}I-labeled lipoprotein occurred when the assay was conducted at pH 9.0. When lysosomal degradation of ^{125}I-labeled lipoprotein was measured at pH 4.5, the degradation of both ^{125}I-labeled lipoproteins was greatly enhanced. Consistent with the reduced rate of ^{125}I-labeled chylomicron remnant degradation observed with intact macrophages, the degradation of ^{125}I-labeled chylomicron remnants by macrophage lysosomal enzymes was approximately one-third that of ^{125}I-labeled β-VLDL. Furthermore, the relative difference in the degradation rates was dependent on the concentration of ^{125}I-labeled lipoproteins in the assay medium (Fig. 7, panel B).

The dependence of lysosomal degradation of ^{125}I-labeled chylomicron remnants on pH and lipoprotein concentration suggested that a component of the chylomicron remnants or a product of their metabolism may be responsible for the reduced rate of chylomicron remnant apoprotein degradation observed in vitro. Since the chylomicron remnants used in the present studies are triacylglycerol-enriched (triacylglycerol:total cholesterol ratio ~11.0) and since macrophages rapidly hydrolyze the chylomicron remnant triacylglycerols (15), the ability of exogenous free fatty acid to alter lysosomal degradation of ^{125}I-labeled lipoproteins was tested. Preincubation of ^{125}I-labeled β-VLDL with 25–50 μg sodium oleate/ml reduced the degradation of the radiolabeled lipoprotein by isolated lysosomal enzymes (**Fig. 8**). The reduction in ^{125}I-labeled β-VLDL degradation did not appear to be due to a fatty acid-induced decrease in accessibility of the lysosomal proteases to the ^{125}I-labeled β-VLDL since in other experiments, the degradation of ^{125}I-labeled β-VLDL by incubation with trypsin in vitro was unaffected by the addition of 10–50 μg sodium oleate/ml to the incubation media (**Table 4**). These data demonstrate that free fatty acids can decrease lysosomal degradation of lipoproteins in vitro and suggest a mechanism whereby the free fatty acid products of chylomicron remnant triacylglycerol hydrolysis decrease the lysosomal degradation rate of chylomicron remnant apoprotein. Whether this is due to a direct effect of fatty acids on the lysosomal enzymes themselves or is an indirect effect based on buffering of the lysosomal pH by the released free fatty acid remains to be determined.

DISCUSSION

Although there are a number of possible pathways by which cells can accumulate lipids, including cholesterol, the LDL receptor pathway is the best characterized to date (30). The rate-limiting step in this pathway in generally believed to be binding to the LDL receptor. A variety of lipoproteins bind to the LDL receptor of cultured cells and their affinity for the LDL receptor is determined by the presence and relative abundance and accessibility of apoproteins B-100 and E. Most of the above conclusions have been drawn on the basis of studies of the relatively homogeneous lipoproteins, LDL, as a model of the LDL receptor:apoB-100 interaction and HDL_c as a model of the LDL receptor:apoE interaction (30). These

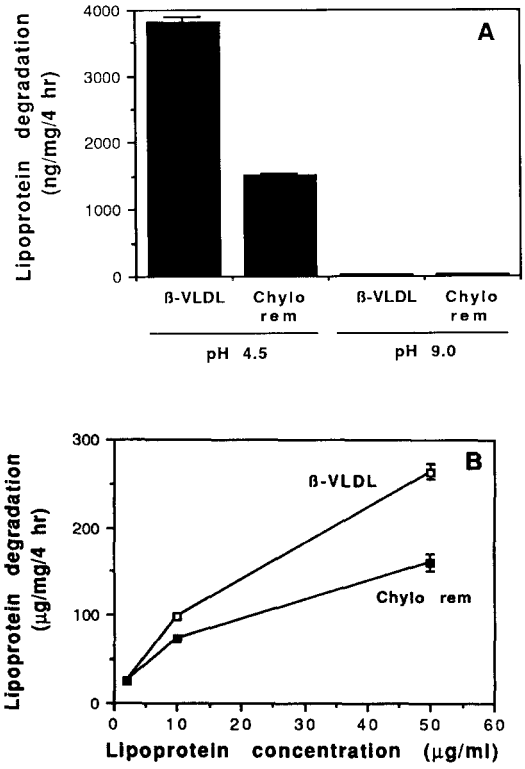

Fig. 7. Degradation of ^{125}I-labeled β-VLDL and ^{125}I-labeled chylomicron remnants by isolated lysosomal enzymes. Panel A: A subcellular fraction enriched for lysosomes was isolated from J774 cells by differential centrifugation as described under Experimental Procedures. The lysosome fraction was resuspended in water and lysed by six cycles of freezing and thawing. Lysosomal enzymes (50 µg protein) were incubated with ^{125}I-labeled β-VLDL or ^{125}I-labeled chylomicron remnants (4.0 µg protein/ml) in 0.1 ml of either citrate-buffered MEM, pH 4.5, or Tris-buffered MEM, pH 9.0, for 4 h at 37°C. The amount of TCA-soluble (non-iodide) ^{125}I radioactivity was measured and the results were expressed as ng degraded/mg of lysosome protein. Panel B: Lysosomal enzymes were further purified after lysis by centrifugation at 100,000 *g* for 1.0 h at 4°C. The partially purified lysosomal enzymes (1.0 µg protein) were then incubated with the indicated concentration of either ^{125}I-labeled β-VLDL (□) or ^{125}I-labeled chylomicron remnants (■) in 0.1 ml of MEM buffered with 0.05 M citrate, pH 4.5, for 4.0 h at 37°C. The amount of lipoprotein degraded was then measured and expressed as µg degraded/mg lysosomal protein. Each point represents the mean ± SD of triplicate dishes of cells and is representative of two separate experiments.

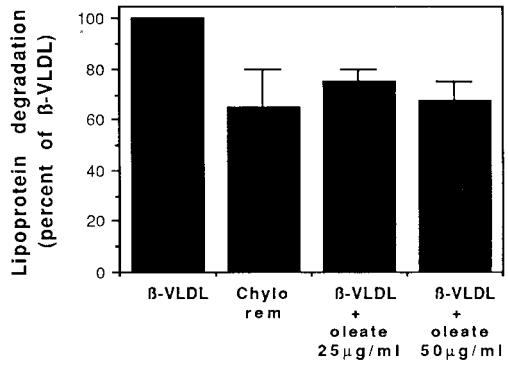

Fig. 8. Inhibition of lysosomal degradation of ^{125}I-labeled β-VLDL by free fatty acid. Lysosomal enzymes (10 µg protein) were incubated with 2.0 µg protein/ml of either ^{125}I-labeled β-VLDL or ^{125}I-labeled chylomicron remnants or free fatty acid-enriched ^{125}I-labled β-VLDL (^{125}I-labeled β-VLDL that had been preincubated with 25 or 50 µg oleic acid/ml in 1.0% ethanol at 37°C for 2 h) in 0.1 ml of MEM buffered with 0.05 M citrate, pH 4.5, for 4 h at 37°C. The amount of TCA-soluble (non-iodide) radioactivity was then measured and the results were expressed as a percent of ^{125}I-labeled β-VLDL degradation. The 100% value for lysosomal degradation of ^{125}I-labeled β-VLDL was 2.44 ± 0.23 µg/mg lysosome protein/4 h (mean ± SE, for three separate experiments). The values represents the mean ± SE of triplicate assays of lipoprotein degradation from three separate experiments.

lipoproteins are of similar size and lipid composition. While these studies have defined the ligand specificity of the LDL receptor, little information is available on post-binding events that may regulate the processing of other more heterogenous lipoproteins, such as chylomicron remnants and β-VLDL. In studies with liver and liver-derived cells from example, we found that chylomicron remnants and β-VLDL had different metabolic effects (31). These could be explained in part by the effects of free fatty acids liberated from the chylomicron remnant triacylglycerol on lipoprotein and bile secretion by the liver. When macrophages and a macrophage-derived cell line were studied, some of these differences, such as a stimulation of HMG-CoA reductase by chylomicron remnants, persisted (15). In addition, the rate of degradation of ^{125}I-labeled apoprotein from the two types of lipoproteins was very different (16). The present study with macrophages is an attempt to elucidate the basis for differential processing of the two particle types.

Two steps in lipoprotein processing were found to be significantly different between chylomicron remnants and β-VLDL and appear to account for the differences in the rates of degradation. First, the rate of internalization of chylomicron remnants, after binding to the LDL receptor, is much slower than that of β-VLDL. Second, the rates of chylomicron remnant apoprotein degradation

TABLE 4. Effect of free fatty acid on the degradation of ^{125}I-labeled β-VLDL by trypsin in vitro

Treatment	^{125}I-labeled β-VLDL Degradation	
	pH 7.0	pH 8.0
	ng degraded/4 h	
Trypsin		
+ No addition	22.2 ± 0.9	19.9 ± 1.0
+ Ethanol	24.0 ± 0.8	19.2 ± 0.9
+ Oleic acid (10 µg/ml)	22.9 ± 0.9	21.7 ± 1.3
+ Oleic acid (25 µg/ml)	23.7 ± 0.8	21.8 ± 0.2
+ Oleic acid (50 µg/ml)	22.8 ± 1.2	20.4 ± 0.7

A sample of ^{125}I-labeled β-VLDL (2.0 µg protein/ml) was incubated with 0.05 µg trypsin in the presence or absence of 10–50 µg oleic acid/ml dissolved in ethanol (final concentration = 1.0%) at 37°C for 4.0 h in a final volume of 0.1 ml of MEM buffered with 0.04 M HEPES (pH 7 or pH 8). The amount of TCA-soluble (non-iodide) radioactivity was then measured and the results were expressed as nanograms lipoprotein degraded after 4.0 h. The values represent the mean ± SD of triplicate samples.

and cholesteryl ester hydrolysis by lysosomal enzymes is less than that of β-VLDL. The regulation of lipoprotein internalization has not been extensively studied in the past and, at least for LDL transport by the LDL receptor of human skin fibroblasts (30), is generally considered to be a relatively constant and not rate-limiting process. However, as shown by the results of the present study, this constancy cannot be assumed when lipoproteins of different composition and size are being studied and such differences in the rate of internalization may affect the overall rate of lipoprotein processing. Although the content of cholesterol is similar for rat chylomicron remnants and rat β-VLDL (15), the chylomicron remnants are larger in size due to their enrichment in triacylglycerol. This was confirmed by negative stain electron microscopy. The β-VLDL formed a relatively homogeneous population of particles with an average particle size distribution of 29.7 ± 6.9 nm (mean ± SD, n = 200 particles). In contrast, the chylomicron remnants comprised a more heterogeneous population of large particles with an average particle size of 97.4 ± 44.8 nm (mean ± SD, n = 200 particles). The size heterogeneity of chylomicron remnants was quite marked even though they eluted from various gel filtration columns as a sharp peak. Whether the different rates of internalization seen in this study are simply physical constraints, due to the large size of the chylomicron remnants or to a difference in a signal transduced by the different particles, remains to be determined. In any event, the reduction in the relative rate of chylomicron remnant degradation coupled with the marked difference in size between chylomicron remnants and β-VLDL is consistent with the notion that large triacylglycerol-enriched chylomicron remnants may undergo additional processing prior to their uptake by tissues.

The need for such processing could contribute to the tissue selectivity of uptake for chylomicron remnants. A likely mechanism for the extracellular processing of large triacylglycerol-enriched lipoproteins is one that involves a lipase-mediated hydrolysis of lipoprotein lipids. The liver, for example, is the principal site of chylomicron remnant uptake and also secretes hepatic triglyceride lipase (see ref. 32 for review). Hepatic lipase hydrolyzes the phospholipid and triacylglycerol components of a variety of lipoproteins including triacylglycerol-enriched VLDL, VLDL remnants, and chylomicrons. Since hepatic lipase does not have an apoprotein cofactor requirement, this enzyme may be capable of hydrolyzing the phospholipid and triacylglycerol components of the apoC-II-deficient chylomicron remnants once these lipoproteins are bound to the hepatocyte cell surface. Another potentially important site of chylomicron remnant uptake is the macrophage. Previous reports from our laboratory (12, 15), have demonstrated that macrophages in culture bind, take up, and degrade the lipid and protein components of chylomicron remnants. Furthermore, a lipase-mediated mechanism may be important for the uptake of triacylglycerol-enriched lipoproteins by this cell type. This is consistent with a number of other observations. First, macrophages in culture secrete lipoprotein lipase (LpL) (33) and VLDL and chylomicrons are a substrate for this enzyme (34, 35). Second, LpL-mediated hydrolysis of VLDL and chylomicron triacylglycerol in vitro accelerates the uptake of the lipid and protein components of the resulting remnant lipoproteins by macrophages (34, 35). A role for macrophage-derived LpL is not yet established, since LpL activity is not required for chylomicron remnant uptake (15, 34, 35) and rat chylomicron remnants are deficient in apoprotein C-II, the cofactor for LpL (15).

Although LpL has been shown to increase the uptake and degradation of triacylglycerol-enriched lipoproteins in a variety of cell types, it is not clear whether increases in lipoprotein internalization were responsible for enhancement of remnant lipoprotein uptake in those studies. Since lipoprotein lipase-mediated hydrolysis of lipoprotein lipids has been shown to alter the relative accessibility of apoB-100 on VLDL (36) and the amount of apoE on chylomicron remnants (37), the lipase-mediated increase in cell-associated and degraded lipoprotein observed in those studies could be accounted for by increased cell surface binding of the remnant lipoproteins. Since the rat chylomicron remnants and rat β-VLDL used in the present study have similar apoprotein B/E ratios and bind with similar affinities and capacities to macrophage LDL receptors, the reduction in the relative rate of chylomicron remnant degradation is not due to differences between chylomicron remnants and β-VLDL in cell surface binding. Furthermore, the difference in the rate of degradation between chylomicron remnants and β-VLDL is not due to differences in the number of particles being processed. Based on the apoB composition of the lipoproteins [13% of the total protein as apoB-48 for chylomicron remnants as compared to 14% apoB-48 and 14% apoB-100 for β-VLDL (15)] one can calculate the amount of lipoprotein bound on an apoprotein B molar basis. One mg of chylomicron remnant and β-VLDL protein is thus equivalent to about 5.5×10^{-4} and 8.7×10^{-4} mol of apoB, respectively and based on previous results of 4°C binding studies with J774 macrophages (15), the B_{max} for chylomicron remnant binding is 30.0 fmol bound/mg cell protein and for β-VLDL is 35.0 fmol bound/mg cell protein. Thus, the binding of these lipoproteins to macrophage LDL receptors on a particle basis, assuming one apoB per particle, is virtually identical. In contrast, when the rates of internalization and degradation of chylomicron remnants and β-VLDL from the present study are calculated on an apoprotein B molar basis, the differences between chylomicron remnants and β-VLDL are actually accentuated.

Once in the cell, receptor-ligand dissociation and recycling proceed at similar rates for chylomicron remnants and β-VLDL. Chylomicron remnants, however, are hydrolyzed by lysosomal enzymes more slowly than β-VLDL. In fact, the reduction in the relative rate of chylomicron remnant degradation resulted in the accumulation of apparently undegraded chylomicron remnants within the cell. Our data are most compatible with the hypothesis that this is due to direct inhibition of the degradative enzymes by liberated free fatty acids: *1*) the degradation of ^{125}I-labeled β-VLDL preincubated with oleic acid is not due to the free fatty acid interfering with the exposure of the lipoprotein to the lysosomal proteases since the degradation of ^{125}I-labeled β-VLDL by trypsin in vitro is unaffected by the presence of oleic acid; *2*) the addition of oleic acid can directly reduce the degradation of ^{125}I-labeled β-VLDL by partially purified lysosomal proteases under conditions in which the pH of the medium is maintained; and *3*) the rat chylomicron remnants are relatively triacylglycerol- and free fatty acid-enriched as compared to rat β-VLDL and macrophages rapidly hydrolyze chylomicron remnant triacylglycerol (15). Thus, the chylomicron remnants can be an abundant source of free fatty acid. Alternatively, the free fatty acids released from the hydrolysis of chylomicron remnant triacylglycerol might buffer the lysosomal pH and thus reduce the activity of lysosomal degradative enzymes. However, in preliminary studies using various pH-sensitive fluorescent dyes to label the lysosomal compartment of J774 cells, no such buffering was seen; although if it occurred in a small subset of lysosomes, it might not have seen apparent. Further, since a single enzyme, lysosomal acid cholesteryl esterase, hydrolyzes lipoprotein-derived triglyceride and cholesteryl ester (38), one would predict that the rate of cholesteryl ester hydrolysis will be relatively slower when delivered to the lysosome in triacylglycerol-rich chylomicron remnants. Such significant differences in the hydrolysis of cholesteryl ester and triglyceride have been reported with the processing of lipid emulsions by smooth muscle cells in culture (39) and in the present study, a lag in the onset of cholesteryl ester hydrolysis was seen for the chylomicron remnants.

The consequences of these differences in processing of two potentially atherogenic lipoproteins, chylomicron remnants and β-VLDL, in terms of LDL receptor down-regulation and net accumulation of cholesterol and whether this could, over the long run, alter the generation of foam cells are subjects worthy of further exploration.

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