Acceleration of uptake of LDL but not chylomicrons or chylomicron remnants by cells that secrete apoE and hepatic lipase

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Abstract ApoE is a ligand for the low density lipoprotein (LDL) receptor as well as for the LDL receptor-related protein (LRP). The enzyme hepatic lipase (HL) may also affect the uptake of lipoproteins by modifying their composition. We have tested the hypothesis that hepatic lipase and apoE can function as co-factors to alter the rate of lipoprotein uptake. Chinese hamster ovary (CHO) cells were transfected with cDNAs for rat hepatic lipase, human apoE or both HL and apoE. The secreted recombinant proteins were thoroughly characterized and had properties identical to the native proteins. Hepatic lipase and apoE were secreted at 0.17 and 1.25 μg/mg cell protein per hour, rates comparable to those in normal liver. ^{125}I-labeled LDL, chylomicron remnants, or chylomicrons were added to media at concentrations near their K_d. In cells that secreted either apoE or hepatic lipase, or both apoE and hepatic lipase, LDL binding was significantly greater than with control cells (2.2-, 2-, 2-fold greater, respectively). Similar enhancement of LDL degradation was observed. In the presence of anti-LDL receptor antibodies, these values were reduced to control levels; thus, the enhanced uptake was mediated by the LDL receptor and not the LRP. The amount of LDL receptor protein, as judged by Western blotting, was similar in the various cell types. Incubation of control CHO cells with media from secreting transfected cells also increased the uptake of ^{125}I-labeled LDL. Kinetic studies indicated that, in apoE-secreting cells, increased LDL binding is associated with a lower K_d and an unchanged V_{max}, as compared to the control cells; furthermore, when LDL were reisolated by column chromatography (but not by ultracentrifugation) from the incubations where apoE was being secreted, apoE was identified adherent to the LDL particles. Together, these results suggest that the effect is due to alteration of the lipoprotein and not the cell. In contrast, the uptake of ^{125}I-labeled chylomicron remnants, and ^{125}I-labeled chylomicrons was not greater in the transfected cells. Thus, in the amounts secreted by these cells, hepatic lipase and apoE do not convert chylomicrons to chylomicron remnants or alter the uptake of chylomicron remnants by either the LDL receptor or the LRP. The enhancement of LDL removal in cells that secrete hepatic lipase or apoE may help determine the amount of LDL removed by a particular tissue.—**Choi, S. Y., M. C. Komaromy, J. Chen, L. G. Fong, and A. D. Cooper.** Acceleration of uptake of LDL but not chylomicrons or chylomicron remnants by cells that secrete apoE and hepatic lipase. *J. Lipid Res.* 1994. **35:** 848–859.

Supplementary key words lipoprotein • Chinese hamster ovary cells • LDL receptor-related protein • transfected cells

Since the discovery of a strong positive correlation between plasma low density lipoprotein level (LDL) and the incidence of coronary heart disease (1), the determinants of the level of LDL in the serum have been studied intensively. The pioneering studies of Brown and Goldstein identified a receptor on the surface of most cells (2) that initiates the uptake of intact plasma LDL particles into cells via endocytosis and thus plays a major role in maintaining cholesterol homeostasis.

The composition of lipoproteins in terms of the apolipoproteins present, and possibly in terms of the lipid content, can play an important role in determining the affinity for the receptor. Although the receptor recognizes both apoB and apoE, it has a much greater affinity for apoE than apoB (3). ApoE can be secreted by the liver (4), the primary site of LDL uptake, as well as a number of other sites of avid lipoprotein uptake, such as the adrenal glands and the ovary (4–6). Interestingly, these tissues are also sites of hepatic lipase activity. The number of LDL receptors expressed on the surface of a cell is regulated by the cellular cholesterol content. LDL receptors are downregulated when substantial amounts of cholesterol are delivered to the cells, thus preventing cells from accumulating excess cholesterol (7). Quantitatively, the major site of LDL receptor-mediated lipoprotein degradation is the liver; however, even in the complete absence of LDL receptors, a large fraction of LDL continues to be taken up by hepatocytes (8), indicating that mechanisms other than the LDL receptor can play a role in the catabolism of LDL. Other lipoproteins, including chylomicron remnants, also seem to have both LDL receptor-dependent

Abbreviations: LDL, low density lipoprotein; CHO, Chinese hamster ovary; β-VLDL, β-migrating very low density lipoprotein; HRP, horseradish peroxidase; LRP, low density lipoprotein receptor-related protein; HL, hepatic lipase.

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and -independent mechanisms of transport. The low density lipoprotein receptor-like protein (LRP) (9) has been shown to mediate the uptake of β-VLDL cholesterol ester when additional apoE is present (10) and thus may be a remnant receptor (11).

Lipases have long been recognized as playing a critical role in the uptake of lipoproteins. The enzyme lipoprotein lipase is responsible for depleting triglyceride from the core of chylomicrons and VLDL (12). This lipolysis is accompanied by changes in the apolipoprotein composition of the particles and results in the formation of remnant particles that are rapidly removed from the circulation by the liver. The role of hepatic lipase is less well defined. The enzyme is structurally related to lipoprotein lipase, and has the ability to hydrolyze both triglycerides (13) and phospholipids (14). Congenital absence of this enzyme leads to profound abnormalities in the serum lipoproteins (15). It is synthesized in liver parenchymal cells (16) and bound to the surface of liver endothelial cells after being secreted (17). The enzyme is also present in the steroid-producing ovary and adrenal gland (18–21). Depletion of the core triglyceride content of LDL by hepatic lipase enhances degradation of LDL by macrophages (22). Treatment of chylomicrons with phospholipase increases their clearance by the liver (23). The mechanisms by which hepatic lipase facilitates the transport of plasma lipoproteins have not been established. It has been reported that the enzyme can modify the interaction of LDL with the LDL receptor by changing the chemical and physical properties of lipoproteins (22). It has also been suggested that binding of high density lipoprotein, an apoA-rich lipoprotein, to hepatic lipase on hepatic endothelial cells can lead to endocytosis, selective loss of lipid, and retroendocytosis of a lipid-depleted particle (24); moreover, cholesteryl ethers incorporated into rat HDL are taken up into hepatocytes almost entirely independently of apolipoprotein (25), indicating a non-endocytic transfer of lipid by an LDL receptor-independent pathway. Whether this enzyme can facilitate the LDL receptor-independent uptake of lipid from apoB- or apoE-containing lipoproteins has not been determined.

The present studies have been designed to test the hypothesis that these proteins can serve as cofactors to facilitate lipoprotein uptake in a paracrine or autocrine manner. To address this question, we constructed cell lines that were able to secrete apoE or hepatic lipase, singly or together, and examined lipoprotein metabolism by the lines. The results suggest that the ability to secrete apoE or bind hepatic lipase may serve as a paracrine accelerator of LDL uptake and, in the amounts secreted by these cells, hepatic lipase and apoE do not alter the uptake of chylomicron remnants by the LDL receptor.

EXPERIMENTAL PROCEDURES

Materials

Radioiodine (^{125}I-carrier-free) was purchased from Amersham Corp., and tissue culture media were from GIBCO. Fatty acid-poor bovine albumin (BSA) and Nutridoma-NS media suppliment were obtained from Boehringer-Mannheim. All other chemicals were obtained from Sigma or J. T. Baker Chemical Co.

Methods

Construction of hepatic lipase and apolipoprotein E-secreting cell lines. Construction of an expression vector for hepatic lipase and transfection into CHO cells have been described (26). The plasmid pML-E1 (27) encoding human apoE under the control of the MoMLV LTR was obtained from Dr. John Taylor (Gladstone Institute, San Francisco, CA). This was transfected and selected as described into CHO cells (26). Cells secreting apoE were isolated as described (26), using a monoclonal mouse anti-human apoE IgG supplied by Dr. E. Krul (Washington University, St. Louis, MO). For double producers, the cell line D121.3-9F [a cell line over-producing rat hepatic lipase, isolated as described (26)] was co-transfected with pML-E1 and pSV2-*hph* (28, 29), encoding the bacterial gene hygromycin B phosphotransferase. After selection in both G418 and hygromycin B (both at 400 μg/ml), cells producing both hepatic lipase and apoE were cloned as above.

Preparation of lipoproteins. Human LDL (d 1.019–1.063 g/ml) was isolated from ethylenediaminetetraacetic acid (EDTA)-containing plasma by density gradient ultracentrifugation (30) and radiolabeled with Na^{125}I using Iodogen (31). Chylomicrons were prepared by the method previously described (32), and chylomicron remnants were prepared by the modification of the method of Redgrave and Martin (33) previously described (25). Chylomicron remnants were iodinated by a modification (31) of the iodine monochloride method (34). β-VLDL was prepared from plasma of cholesterol-fed rats by density gradient ultracentrifugation (35). Selected batches were analyzed by polyacrylamide gel electrophoresis (PAGE) in a system containing 0.5% sodium dodecyl sulfate with or without autoradiography, and apolipoprotein contents and iodination patterns were similar to previously reported values (36). Triglyceride and cholesterol contents were determined using test kits from Sigma Chemical Co. (St. Louis, MO). Total protein concentration of lipoproteins was determined by the method of Lowry et al. (37).

Anti-LDL receptor antibodies. The anti-LDL receptor antibodies used have been previously described and characterized (38–40). Monospecificity and lack of cross-reactivity with the LRP have been previously documented (40).

Degradation and binding assays of lipoproteins by CHO cells. Control and transfected CHO cells were cultured in DMEM–Coon's 1:1 supplemented with 10% fetal calf serum (FCS) at 37°C in a 5% CO_2 atmosphere until just subconfluent. The medium was then replaced with the same medium containing 30 μM $ZnSO_4$ and 10% FCS and incubated overnight to induce recombinant hepatic lipase. Non-adherent cells were removed by rinsing three times with binding buffer containing 30 μM $ZnSO_4$, 0.5% bovine serum albumin (BSA), and 10 mM N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (pH 7.4). ^{125}I-labeled LDL (10 μg/ml), chylomicrons (1 μg/ml), chylomicron remnants (1 μg/ml), or β-very low density lipoprotein (VLDL) (5 μg/ml) were added to the medium in the presence or absence of unlabeled lipoproteins or anti-LDL receptor antibody at 37°C for 4 h. The extent of lipoprotein degradation was assessed by measuring the amount of trichloroacetic acid (TCA) and silver nitrate soluble radioactivity present in the incubation medium. The small amount of degradation products generated in the absence of cells was also measured and subtracted from the corresponding samples incubated with cells. The cell-associated lipoprotein was determined by dissolving the cells with 0.1 N NaOH after washing the cells three times with PBS. For kinetic analysis, binding was measured at 4°C and binding constructs were determined by the LIGAND method, using the EBDA program from Elsevier. The amount of total cell protein was determined by the method of Lowry et al. (37).

Northern blot analysis. Total cellular RNA was prepared by extraction with guanidinium thiocyanate and phenol as previously described (41). Ten μg of total RNA was applied to 1% formaldehyde-agarose gel electrophoresis and transferred to a nylon membrane. The filter was baked, prehybridized, and hybridized with a [^{32}P]dCTP-labeled probe. The filter was washed, air-dried, and autoradiographed overnight.

Hepatic lipase assay. At the end of incubation, hepatic lipase activity was determined in the medium by measuring triglyceride lipase activity. The assays were done by the method of Nilsson-Ehle and Schotz (42) using a [^{3}H]triolein sonicated emulsion in the presence of 1 M NaCl.

Immunoblotting. Total cellular membranes from control and transfected cells were prepared and solubilized as described earlier (40). The solubilized membranes were separated on 6% polyacrylamide gels by the method of Laemmli (43) under non-reducing conditions. Immunoblotting was performed as previously described (14). Briefly, proteins were transferred to nitrocellulose paper and the portion of the paper containing the molecular weight standards was cut off and stained with 0.1% Amido Black. The remainder of the paper was incubated with anti-serum (1:1000 dilution) for 1 h at room temperature, washed, and then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. The HRP-conjugated IgG was developed with the Bio-Rad ImmunoBlot™ (GAR-HRO) assay kit. The nitrocellulose paper was dried and then scanned with a densitometer (Hoefer Scientific Instruments).

LDL reisolation. LDL (10 μg/ml) was incubated with cells secreting apoE at 37°C for 4 h. The medium was removed and pooled (10 ml total). This was applied to a 50-ml Bio-Rad A-0.5M (1.5 × 30 cm) column and 1-ml fractions were collected. The exclusive molecular weight was 500,000. The samples were then processed for immunoblotting as above, using anti-human apoE.

Measurement of LDL oxidation. Human LDL (10 μg/ml) was incubated in induction medium (Coon's DMEM containing heparin, 0.5% BSA, Nutridoma-NS, antibiotics, and 30 μM Zn^{2+}) in the presence or absence of cells at 37°C for 5 h. LDL that had been incubated at 4°C were also included as background controls. The incubation medium was transferred to microfuge tubes and butylated hydroxytoluene was added to 20 μM. The medium was centrifuged and an aliquot of the supernatant (0.25 ml) was assayed for thiobarbituric-reactive substances, using tetramethoxypropane as a standard.

Statistics. Statistical analysis was done by non-paired Student's *t*-test.

RESULTS

Characterization of apoE secreted by CHO cells

The apoE secreted into the medium by the transfected cells was purified by heparin-Sepharose chromatography and subjected to SDS-PAGE. Its molecular mass was approximately 34 kD, a size similar to that of human serum apoE (**Fig. 1A**). When analyzed by immunoblotting, this protein was recognized by an anti-human apoE antibody (Fig. 1B). Northern blot analysis of CHO mRNA probed with an apoE cDNA revealed a single band in the transfected cells and none in the untransfected cells. Furthermore, the amount of mRNA of apoE in the transfected cells was substantially greater than in human HepG2 cells (Fig. 1C). Cells transfected with human apoE constitutively secreted approximately 20 μg/mg cell protein of apoE in 16 h. There was no detectable secretion of apoE by untransfected cells. The hepatic lipase secreted by these cells has been previously characterized (26). The rate of secretion of hepatic lipase activity was determined by measuring hydrolysis of [^{3}H]triolein in the presence of 1 M NaCl. Hepatic lipase mass was measured by ELISA. Two to three μg of hepatic lipase per mg cell protein was secreted in a 16-h induction with $ZnSO_4$. Untransfected, wild-type or transfected cells incubated without $ZnSO_4$ did not produce detectable amounts of the enzyme. Induction of hepatic lipase with $ZnSO_4$ did not affect apoE secretion in double secretors.

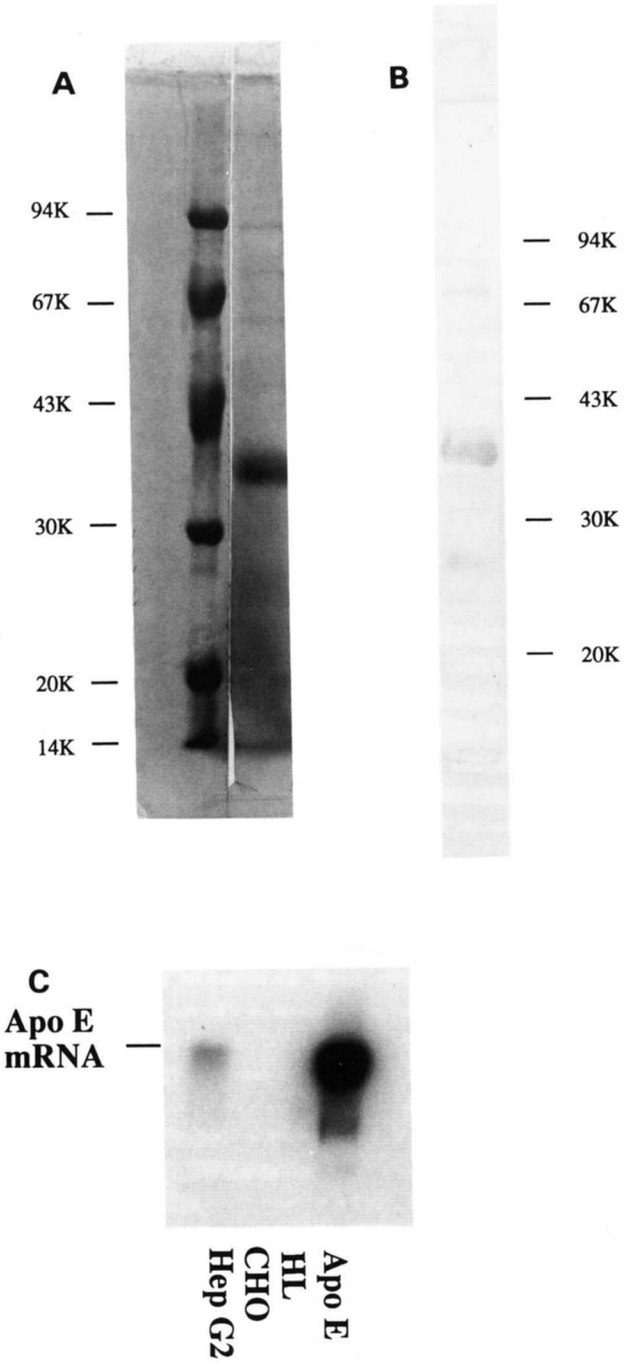

Fig. 1. Characterization of apoE secreted by CHO cells. The medium was collected from the CHO cells transfected with the apoE cDNA, purified by heparin Sepharose and subjected to electrophoresis on 6% SDS-polyacrylamide gels. (A) was stained with Coomassie blue R-250 followed by destaining with 10% acetic acid; (B) was transferred to nitrocellulose paper and immunoblotted with an anti-human apoE antibody; (C) Total cellular RNA was prepared by extraction with guanidinium thiocyanate and phenol. Ten μg of total RNA was run on 1% formaldehyde-agarose and transferred to a nylon membrane. The filter was baked, prehybridized, and hybridized with a 1.2 kb apoE-specific probe labeled with [^{32}P]dCTP. The filter was then washed, air-dried, and autoradiographed overnight. ApoE, cells secreting apoE; HL, cells secreting hepatic lipase.

Degradation and cell association of LDL in transfected as compared to non-transfected CHO cells

^{125}I-labeled LDL was added to the media of wild-type or transfected CHO cells (10 μg/ml) and cell association and degradation were measured. As compared with untransfected CHO cells, cells secreting either hepatic lipase or apoE or both together had substantially more cell-associated LDL (**Fig. 2A**). Specific LDL cell association in transfected cells was more than twofold greater than in the wild-type cells. Interestingly, cotransfection of both hepatic lipase and apoE did not result in further stimulation of uptake. Specific degradation of LDL was also increased in transfected cells by approximately twofold, and cotransfection did not result in further increase in degradation (Fig. 2B).

To study the role of the LDL receptor in the uptake of LDL in these cell lines, polyclonal anti-LDL receptor antibodies were added to the incubation medium at a concentration of 500 μg/ml. Addition of the anti-LDL receptor antibody eliminated most of the specific cell association and virtually all of the specific degradation of the LDL in both wild type and transfected cells (Fig. 2A and 2B), indicating that most of the increased uptake of LDL was mediated by the LDL receptor.

Evidence that accelerated LDL uptake is due to the media and not the cells

Experiments were done to investigate whether the effect on LDL uptake was occurring at the level of the cell or of the lipoprotein. To determine whether transfection with the foreign genes increased the number of LDL receptors, wild-type and transfected cells were analyzed by immunoblotting using polyclonal anti-LDL receptor antibodies. When the same amount of protein was applied, there was no difference in the amount of LDL receptor detected among these various cell types (**Fig. 3**). This indicates that the ability to secrete hepatic lipase or apoE does not, in itself, increase the number of LDL receptors. Second, kinetic analysis indicated that the affinity for the LDL receptor K_d in apoE-secreting cells was twice that of the wild type cells, whereas the total amount of LDL bound (B_{max}) was the same in transfected and wild-type CHO cells (**Table 1**). In hepatic lipase-secreting cells, the K_m was also reduced by about 40% as compared to the wild-type cells, and B_{max} was virtually the same (Table 1). These results further suggest that the affinity for the LDL receptor, but not the receptor mass, was increased in the transfected cells.

The two preceding experiments suggested that the induction of lipoprotein uptake was not a property of the cells, but of the secretory products. To confirm this, degradation and cell association of LDL were determined in wild-type CHO cells incubated with media collected

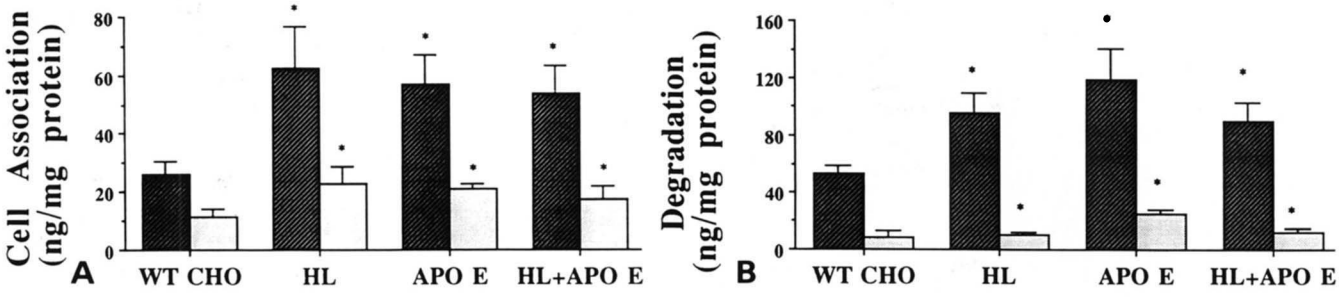

Fig. 2. Cell association (A) and degradation (B) of LDL in transfected and non-transfected (wild type, WT) CHO cells. Cells were cultured in DMEM–Coon's 1:1 supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere until just subconfluent. The medium was replaced with serum-free induction medium containing 30 μM $ZnSO_4$ and incubated overnight to induce hepatic lipase. The medium was removed and fresh serum-free medium, containing ^{125}I-labeled LDL (10 μg/ml) in the presence or absence of unlabeled LDL or anti-LDL receptor antibody, was added to the cells and the incubation continued at 37°C for 4 h. Degradation and cell association of ^{125}I-labeled LDL were determined as described under Methods. Dark bars represent LDL, (n = 4); light bars represent LDL plus anti-LDL receptor antibody. (n = 4); *different from untransfected CHO cells at the 95% confidence level or greater.

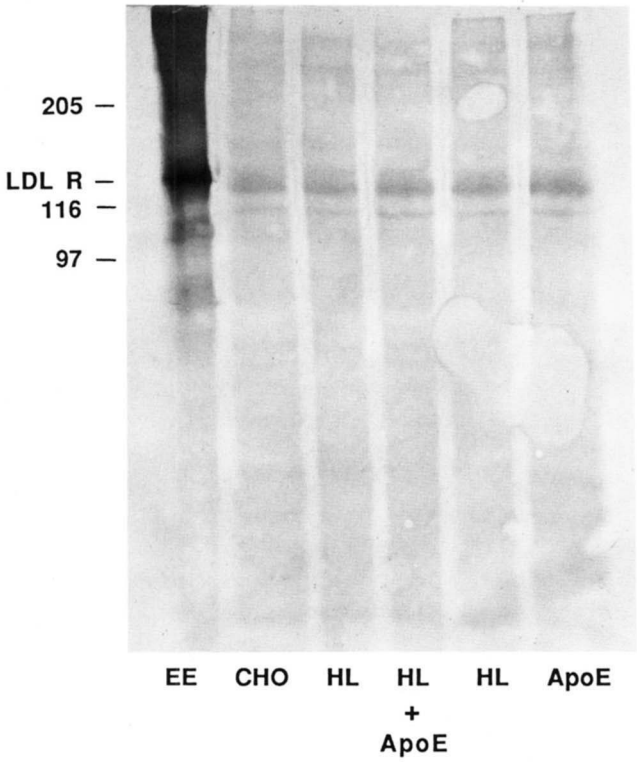

Fig. 3. Western blot analysis of the LDL receptor. Solubilized membranes from control and transfected cells were separated on 6% polyacrylamide gels by the method of Laemmli (43) under non-reducing conditions. Protein (150 μg/lane) was transferred to nitrocellulose paper and the portion of the paper containing the molecular weight standards was cut off and stained with 0.1% Amido Black. The remainder of the paper was incubated with 1 μg/ml of anti-LDL receptor anti-serum for 1 h at room temperature, washed, and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. The HRP-conjugated IgG was developed with the Bio-Rad Immuno-Blot™ (GAR-HRO) assay kit. The nitrocellulose paper was dried and scanned with a densitometer. EE, liver from rats treated with ethinyl estradiol; CHO, wild type CHO cells; HL, hepatic lipase-secreting CHO cells; HL+apoE, hepatic lipase and apoE-screening CHO cells; apoE, apoE-secreting CHO cells.

from the transfected cell lines. Both degradation and binding of LDL in the untransfected CHO cells incubated in this media were increased to the same extent as in transfected cells (**Fig. 4**). These results suggest that the enhanced uptake of LDL is due to the alteration of the lipoprotein particle by the secreted proteins and not due to alterations in the cell.

Reisolation of LDL from the media of apoE-secreting CHO cells

It is possible that the apoE secreted by the transfected cells becomes loosely associated with the LDL particle and thus mediates LDL uptake by these cells. To test this, we reisolated the LDL from incubations where apoE was being secreted. LDL was incubated with cells secreting apoE at 37°C for 4 h and then reisolated from the medium by density gradient ultracentrifugation or by gel filtration. The reisolated LDL was then subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis using an anti-human apoE antibody. There was no apoE detected in the LDL reisolated by ultracentrifugation (data not shown). However, when column chromatography was used to isolate the LDL, apoE was identified in the fractions containing LDL particles isolated from incubation medium of transfected cells (**Fig. 5**). Note that the material staining on the top of the gel in lanes 9 and 10 is not apoB, but probably apoE. When medium from the cells to which no LDL had been added was chromatographed on the same columns, there was no apoE in these fractions; nor did the LDL contain any apoE before it was added to the cells in culture. This result supports the hypothesis that at least a subpopulation of LDL may acquire some apoE when in an apoE-rich environment, and this may enhance its uptake.

TABLE 1. Kinetics of LDL uptake by various cell lines

	K_d	B_{max}
	μg/ml	*μg/mg protein*
Wild type	9.0	1.12
Hepatic lipase secretor	4.8	1.40
Wild type	41 ± 14	1.02 ± 0.3
ApoE secretor	24 ± 9	0.95 ± 0.16

Cells were cultured in DMEM–Coon's 1:1 supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere until just subconfluent. The medium was replaced with serum-free induction medium containing 30 μM $ZnSO_4$ and incubated overnight to induce hepatic lipase. The medium was removed and fresh serum-free medium, containing various concentrations of ^{125}I-labeled LDL in the presence or absence of a 20-fold excess of unlabeled LDL was added, and the cells incubated at 4°C. After 4 h the cells were washed and the radioactivity was determined. The binding results were analyzed using the LIGAND program. Each point was run in duplicate. The experiment was carried out twice for HL- and three times for apoE-secreting cells. Different batches of LDL were used for the hepatic lipase and apoE experiments which probably accounts for the differences in absolute values for K_d and between the two experiments.

Degradation and cell association of chylomicrons in transfected as compared to wild type CHO cells

To learn whether cells that secrete apoE and hepatic lipase can convert chylomicrons to particles that can be removed more rapidly by cells, the same types of experiments were conducted with chylomicrons. The results obtained for chylomicron uptake were strikingly different from those with LDL. Neither the ability to secrete hepatic lipase, nor apoE, nor both together, affected the amount of chylomicron binding or degradation (**Fig. 6**). To determine whether the composition of the chylomicron could affect its uptake as mediated by hepatic lipase or apoE, we prepared chylomicrons with a low triglyceride to cholesterol ratio by feeding animals with a lower cholesterol source. The triglyceride-to-cholesterol ratio of low triglyceride chylomicrons was 16, as compared to 21 in our normal chylomicron preparations. There was no significant difference in degradation or binding of these two types of chylomicrons by control and transfected cell lines (data not shown). It should be noted that the absolute amount of chylomicron binding is much less than the amount of chylomicron remnant binding (≈40 vs. 400 ng/mg cell protein). These results suggest that, in the amounts secreted by these cells, hepatic lipase and apoE do not convert chylomicrons to chylomicron remnants. These results are compatible with a primary role for lipoprotein lipase in the initial steps of remnant formation (44, 45), and suggest that, in the experiments of Borensztajn, Kotlar, and McNeill (46) where phospholipase treatment before infusion accelerates the removal of chylomicrons, an unknown step must be occurring in the liver.

Cell association and degradation of chylomicron remnants in transfected as compared to wild type CHO cells

To learn whether the presence of apoE or hepatic lipase could increase remnant uptake by the LDL receptor or induce uptake by the LRP, experiments were carried out with ^{125}I-labeled chylomicron remnants. Degradation and binding of chylomicron remnants (1 μg/ml) were not greater in the transfected cells (**Fig. 7A and B**). This was also observed at concentrations of chylomicron remnants that saturate the LDL receptor (Fig. 7C) and thus could uncover an incremental effect due to the LRP. Moreover, the amount of remnant binding and degradation that was blocked by the anti-LDL receptor antibody was the same among these various cell types, indicating that the amount of LDL receptor specific binding and uptake was not being altered. Thus, secretion of apoE in the amounts present in our cell lines does not appear to increase remnant binding to the LRP or the LDL receptor. Further, addition of apoE to the media of apoE-secreting cells did not enhance remnant uptake. Thus, in the presence of the LDL receptor, the amount of apoE-rich remnants transported by the LRP remains difficult to appreciate.

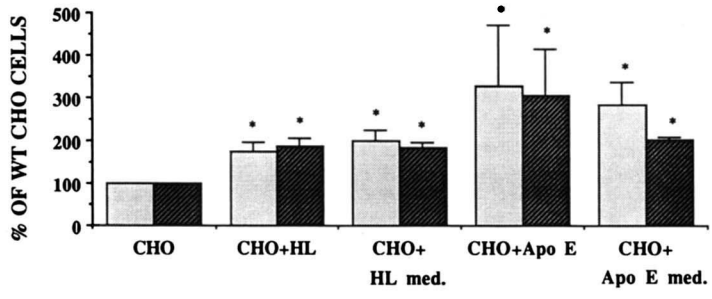

Fig. 4. Cell association and degradation of LDL by wild-type CHO cells after incubation with media secreted by the transfected cells. Induction medium (0.5 ml) was collected from the transfected cells that had been incubated for 4 h with BSA and $ZnSO_4$-containing buffer and added to the untransfected wild-type CHO cells for 4 h incubation. Degradation and cell association of LDL (10 μg/ml) in the untransfected CHO cells incubated with media were determined as described under the Methods (n = 3). Light bars represent degradation; dark bars represent cell association; *different from untransfected cells at the 95% confidence level or greater.

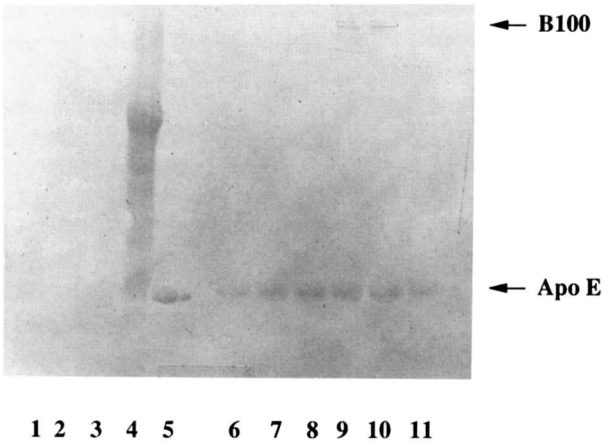

Fig. 5. Reisolation of LDL from the medium of apoE-secreting CHO cells. LDL (10 μg/ml) was incubated with cells secreting apoE at 37°C for 4 h and was reisolated from the medium by column chromatography. The reisolated LDL was subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis using an anti-human apoE antibody. Lanes 1–3, freshly isolated human LDL; lane 4, molecular weight marker; lanes 5–11, column fractions containing LDL reisolated by column chromatography.

Effect of Zn^{2+} on LDL aggregation and uptake by CHO cells

One possible explanation that could account for the enhanced uptake of LDL by the transfected cells is the zinc ion-induced oxidation of LDL. Khoo and coworkers (47) have shown that the mechanical aggregation of LDL by vortexing enhances its uptake several-fold by cells via the LDL receptor. As LDL aggregate formation has also been observed after the oxidation of LDL, it is possible that the added zinc ion required to induce HL production might stimulate LDL metabolism indirectly by promoting its oxidation. To test this, the ability of zinc ion to promote the oxidation of LDL in the presence and absence of CHO cells was measured. LDL that was incubated at conditions identical to those described for the uptake experiments, but in the absence of cells, did not stimulate the oxidation of LDL, as assessed by the measurement of thiobarbituric acid-reactive substances. This was expected, as it has been shown previously that DMEM is unable to support the oxidation of LDL, even in the presence of added metal ions (48). A similar result was also observed when LDL was incubated in the presence of the CHO cells (non-transfected cells, HL, apoE, or HL+apoE-transfected cells). Thus, the induction medium containing zinc ion does not stimulate the oxidation of LDL.

Presence of LRP on CHO cells

As chylomicron remnants are already rich in apoE, the ability of cells to secrete apoE might not be expected to increase remnant binding to the LDL receptor. However, it has been shown that additional apoE is needed to induce binding to the LRP (10); thus, the failure of apoE-secreting cells to have measurable uptake by the LRP was somewhat unexpected. To make certain that the transfected CHO cells continued to express LRP, immunoblotting of CHO cell membranes using an antibody to an extracellular domain of the LRP was carried out. This result showed that comparable amounts of LRP were present in the CHO cell lines used (**Fig. 8**). The LRP was functional, as shown by the ability of CHO cells to bind and degrade activated α_2-macroglobulin (another ligand for the LRP) with a high affinity (49). Thus, in the amounts secreted by these cells, hepatic lipase and apoE do not alter the uptake of remnants by either the LDL receptor or the LRP.

DISCUSSION

Based on the work of others, it was postulated that cells that secreted either apoE, hepatic lipase, or both would take up apoB-containing lipoproteins more rapidly than

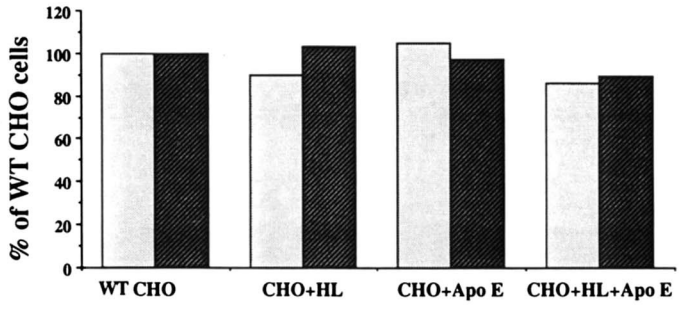

Fig. 6. Degradation and cell association of chylomicrons in transfected and wild-type CHO cells. Cells were treated as in Fig. 2 except that they were incubated with ^{125}I-labeled chylomicrons at 1 μg/ml. Degradation and cell association were determined as described in Methods (n = 3). Light bars represent degradation; dark bars represent cell association.

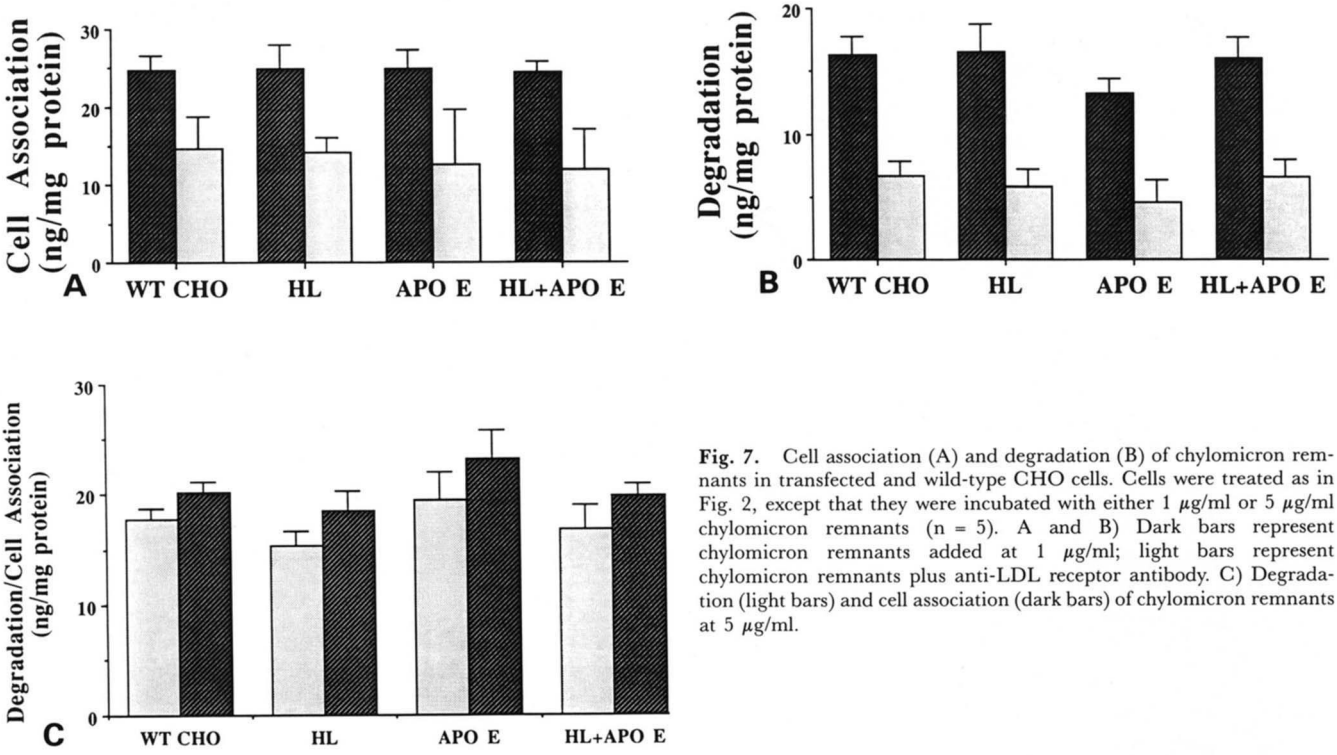

Fig. 7. Cell association (A) and degradation (B) of chylomicron remnants in transfected and wild-type CHO cells. Cells were treated as in Fig. 2, except that they were incubated with either 1 μg/ml or 5 μg/ml chylomicron remnants (n = 5). A and B) Dark bars represent chylomicron remnants added at 1 μg/ml; light bars represent chylomicron remnants plus anti-LDL receptor antibody. C) Degradation (light bars) and cell association (dark bars) of chylomicron remnants at 5 μg/ml.

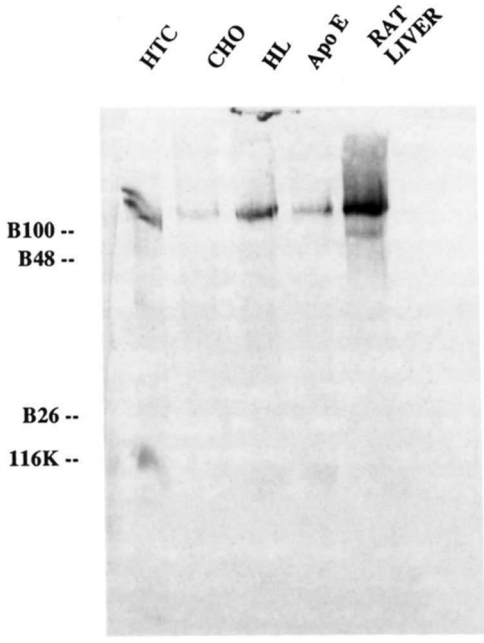

Fig. 8. Western blot analysis of the LRP in CHO cells. Methods were identical to those in Fig. 3 except that the nitrocellulose paper was incubated with 1 μg/ml of anti-LRP anti-serum for 1 h at room temperature. Lane 1, HTC cells; lane 2, wild-type CHO; lane 3, CHO+HL; lane 4, CHO+apoE; lane 5, rat liver membranes. ApoB subspecies were used as molecular weight markers.

cells that did not secrete these proteins. In order to determine whether hepatic lipase or apoE can serve as cofactors to facilitate lipoprotein uptake, we constructed cell lines that were able to secrete apoE or hepatic lipase, alone or together. The results demonstrate that the ability to secrete apoE or hepatic lipase may serve as a paracrine accelerator of LDL uptake, but somewhat surprisingly did not affect the uptake of triglyceride-rich lipoproteins.

Hepatic lipase has both triglyceride and phospholipid hydrolyzing activity. It has been suggested that it catalyzes the hydrolysis of triglyceride in intermediate density lipoprotein to produce low density lipoprotein, converts HDL_2 to HDL_3 (50) via its triacylglycerol and phospholipase hydrolyzing activities, and that, via its phospholipase activity, it renders chylomicrons recognizable as chylomicron remnants (23). Further, its presence in organs with large sterol demands, such as the ovary and the adrenal glands as well as the liver, suggests that it could have a paracrine or autocrine role in sterol transport. In human monocyte-derived macrophages, preincubation of LDL with hepatic lipase led to significant increases in LDL uptake. This was shown to be due to modification of the core lipid composition (22), which affected the chemical and physical properties of the lipoprotein surface (51). This, in turn, modified the interaction of apoB with the LDL receptor. In the present experiments, we

observed approximately a twofold increase in LDL uptake in hepatic lipase-secreting cells, and virtually all of this uptake was mediated by the LDL receptor, as shown with anti-LDL receptor antibodies. Furthermore, the results obtained from the incubation of wild-type CHO cells with media secreted by the transfected CHO cells and from the Western blot analysis of the LDL receptor mass indicate that the enhancement of LDL uptake in hepatic lipase-secreting cells is due to changes in the lipoprotein particles and not in the cells. Kinetic analysis of LDL binding further supported the postulate that the affinity for the LDL receptor, and not the mass of LDL receptor, increased in hepatic lipase-secreting cells. In our experiments the hepatic lipase was in the medium, but in vivo it is anchored to the capillary endothelial surfaces of the liver, adrenal gland, and ovary, where presumably it directs LDL to these cells by creating lipoproteins of higher affinity in the local environment than is present in the general circulation.

ApoE participates in several aspects of lipoprotein metabolism. It has been established that lipoproteins containing apoE have a significantly greater affinity for LDL receptors than those containing only apoB (52–54). Further, chylomicrons contain only apoB-48, which is not a ligand for the LDL receptor; thus, chylomicron remnants cannot be removed by the liver until they acquire apoE. More recently it was found that β-VLDL are not ligands for the LRP-α_2-macroglobulin receptor until they are further enriched in some way with apoE (10). Our data also showed that degradation and binding of LDL in apoE-secreting cells were more than twofold greater than in wild-type cells (Fig. 2A and 2B). Western blot analysis and kinetic analysis indicated that this enhancement of LDL uptake by the apoE-secreting cells was not due to an increase in LDL receptor mass but rather to an increase in affinity of LDL for the LDL receptor.

Consistent with this, analysis of reisolated LDL indicated that in apoE-secreting cells, a subpopulation of LDL particles containing loosely associated apoE was generated; thus, the enhanced uptake of LDL can be attributed to the greater affinity of those particles for the LDL receptor. However, our results are in contrast with recent reports in which LDL uptake was not different in CHO cells transfected with rat apoE as compared to the control cells (55). The discrepancy could be attributed to several differences in the experimental protocols. Our cells were transfected with human apoE rather than rat apoE, and human apoE may have a higher affinity for the LDL receptor than rat apoE. Furthermore, in our study, to prevent the depletion of substrate, the incubation was carried out for 4 h at 10 μg/ml of LDL, as compared to 12 h at 2.5 or 5 μg/ml in the other report. Our result is also consistent with the observation that LDL removal is accelerated in transgenic mice that over-express apoE (56).

A possible mechanism for enhanced LDL uptake in the apoE-secreting cell lines could have been via the LRP. It was reported that the LRP mediates uptake of apoE-enriched lipoproteins, such as chylomicron remnants (57) and β-VLDL (10), in the presence of additional apoE. A role for this or another receptor has been suggested by observations in patients with familial hypercholesterolemia (58) and some (59), but not all (60), of the studies in WHHL rabbits, whose LDL receptors are genetically defective. Our Western blot analysis indicates that there are similar amounts of LRP present in all of the cell lines we used. Further, in other studies it was found that CHO cells bind and degrade activated α_2-macroglobulin, another ligand for the LRP (49). In our system, however, uptake of LDL by the LRP did not appear to occur, as virtually all of LDL uptake was abolished by an anti-LDL receptor antibody in all transfected and wild-type CHO cell lines, indicating that in CHO cells almost all LDL uptake is mediated by the LDL receptor, despite abundant LRP.

We also studied the metabolism of chylomicrons and chylomicron remnants by these cells. Somewhat surprisingly, unlike LDL, degradation and binding of these lipoproteins was not enhanced in the hepatic lipase- or apoE-secreting cells or cells that secreted both together. The role of hepatic lipase in the metabolism of lipoproteins is still controversial. It was recently reported that the hydrolysis of phospholipid catalyzed by hepatic lipase could be activated by apoE in apoE-rich HDL (61). Furthermore, phospholipid-depleted chylomicrons and remnants were taken up by the liver more rapidly than the intact chylomicrons or remnants (62). In contrast, apoE-induced uptake of triglyceride emulsions by hepatocytes was inhibited by heparin (63). Sultan et al. (64) reported that hepatic lipase increased chylomicron remnant uptake by rat hepatocytes, and anti-hepatic lipase antibody infused into rats inhibited the accumulation of chylomicron remnants (64). The discrepancies between these reports and our results might have resulted from differences in the composition of chylomicron remnants in terms of triglyceride content, phospholipids, and apolipoproteins. As hepatic lipase has both triglyceride lipase and phospholipase activities, the content of triglyceride and/or phospholipid of the particular remnant preparation could significantly affect the hepatic lipase-mediated remnant uptake.

We anticipated that chylomicron remnant uptake might be enhanced in the cells transfected with apoE because apoE is a high affinity ligand for LDL receptor. Furthermore, it was reported (10) that an artificial enrichment of β-VLDL with excess apoE was required to achieve functional LRP binding, although a relatively large amount of apoE was present in β-VLDL particles. Western blot analysis showed approximately the same amounts of LDL receptor and LRP in all cell lines we used; however, there were no differences in binding or

degradation of remnants between wild-type and apoE-secreting cells at a saturating concentration of 1 μg/ml. This was also true at 5 μg/ml (data not shown). Furthermore, an anti-LDL receptor antibody blocked about the same amount of total uptake in both control and apoE-secreting cells, indicating that additional apoE did not enhance either LDL receptor-dependent or -independent uptake. Similarly, Eisenberg et al. (65) recently showed that enrichment of normal human VLDL with apoE in vitro markedly increased its ability to bind to LDL receptors of normal human fibroblasts, but found no detectable binding to FH homozygote cells. A possible explanation for our data is that our remnants are already rich in apoE; thus, the ability of cells to secrete additional apoE might not increase the interaction with receptors. In in vivo studies using mice or these in vitro studies using CHO cells, we did not observe difference in the plasma clearance or in the degradation and binding by cells between normal and apoE-enriched chylomicron remnants (S. Choi and A. Cooper, unpublished observation). Thus, although in the in vivo study a significant amount of chylomicron remnant uptake was mediated by a non-LDL receptor pathway, secretion of apoE in the amounts present in our cell lines does not appear to increase remnant binding to the LRP to a degree detectable in binding assays. In all of the previously published literature, it appears that, if LDL receptors are present, it is difficult to detect the contribution of the LRP to lipoprotein uptake, at least in non-hepatic cells where the level of LRP may be lower than in the liver. Clearly, when the LDL receptor is absent, the role of the LRP could be primary and the ability of a cell to secrete apoE could then be a key determinant of lipoprotein uptake. Alternatively, it has been suggested that the LRP system may require an additional component, proteoglycans, to capture the particle (66), and these may be lacking, or not of the appropriate type, in the CHO cells we used.

Finally, although in vitro treatment of chylomicrons with hepatic lipase can create a particle that is rapidly taken up by the liver, this does not seem to occur in this test system. The liver contains lipoprotein lipase in addition to hepatic lipase and apoE, and it is possible that lipoprotein lipase activity is necessary for conversion of the particle. This is consistent with older data with isolated liver perfusion (32). It should be noted that CHO cells produce some lipoprotein lipase (67); however, they do not produce apoC-II, and thus either there is not sufficient lipase or it is not active in the absence of an exogenous source of the apolipoprotein cofactor. Lauer et al. (68) have presented preliminary data suggesting that secretion of hepatic lipase in a different cell line can accelerate the uptake of remnant lipoprotein. Again, it is not yet clear whether these differences are due to different cell lines or particles used. Understanding the basis of the differences may help reveal the critical determinants of remnant removal.

In conclusion, the ability to secrete apoE or hepatic lipase may serve as a paracrine or autocrine accelerator of LDL uptake by altering the lipoprotein particles in the local environment where these effectors are present at a high concentration; thus, in the liver, adrenal, and ovary, this may contribute to the avid uptake of LDL by these cell types. In the amounts secreted by these CHO cells, hepatic lipase and apoE do not alter the uptake of remnants by either the LDL receptor or the LRP. Thus, compared to uptake by the LDL receptor, uptake by the LRP is quantitatively relatively insignificant or another factor is necessary to initiate this process.

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