Receptor-mediated uptake of hypertriglyceridemic very low density lipoproteins by normal human fibroblasts

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Abstract Our previous studies showed that very low density lipoproteins, S_f 60–400 (VLDL), from hypertriglyceridemic subjects, but not VLDL from normolipemic subjects, suppress HMG-CoA reductase activity in normal human fibroblasts. To determine if this functional abnormality of hypertriglyceridemic VLDL resulted from differences in uptake of the VLDL by the low density lipoprotein (LDL) receptor pathway, we isolated VLDL subclasses from the d < 1.006 g/ml fraction of normal and hypertriglyceridemic plasma by flotation through a discontinuous salt gradient for direct and competitive binding studies in cultured human fibroblasts. VLDL from the plasma of subjects with hypertriglyceridemia types 4 and 5 were at least as effective as normal LDL in competing for ^{125}I-labeled LDL binding, uptake, and degradation when compared either on the basis of protein content or on a particle basis. By contrast, normolipemic S_f 60–400 VLDL were ineffective in competing with the degradation of ^{125}I-labeled LDL, and S_f 20–60 VLDL ($VLDL_3$) were less effective in reducing specific ^{125}I-labeled LDL degradation than were LDL, consistent with their effects on HMG-CoA reductase activity. In direct binding studies, radiolabeled VLDL from hypertriglyceridemic but not normolipemic subjects were bound, internalized, and degraded with high affinity and specificity by normal fibroblasts. Uptake and degradation of iodinated hypertriglyceridemic VLDL S_f 100–400 showed a saturable dependence on VLDL concentration. Specific degradation plateaued at approximately 25 μg VLDL protein/ml, with a half maximal value at 6 μg/ml. The most effective competitor of hypertriglyceridemic VLDL uptake and degradation was hypertriglyceridemic VLDL itself. LDL were effective only at high concentrations. Uptake of normal VLDL by normal cells was a linear rather than saturable function of VLDL concentration. By contrast, cellular uptake of the smaller normal $VLDL_3$ was greater than uptake of larger VLDL and showed saturation dependence. After incubation of normal VLDL with ^{125}I-labeled apoprotein E, reisolated ^{125}I-E-VLDL were as effective as LDL in suppression of HMG-CoA reductase activity, suggesting that apoE is involved in receptor-mediated uptake of large suppressive VLDL. We conclude that *1*) hypertriglyceridemic VLDL S_f 60–400 are bound, internalized, and degraded by normal fibroblasts primarily by the high affinity LDL receptor-mediated pathway; *2*) by contrast, normal VLDL S_f 60–400 are bound, internalized, and degraded by normal fibroblasts primarily by nonspecific, nonsaturable routes; and *3*) of the normal VLDL subclasses, only the smallest S_f 20–60 fraction is bound and internalized via the LDL pathway.—**Gianturco, S. H., F. B. Brown, A. M. Gotto, Jr., and W. A. Bradley.** Receptor-mediated uptake of hypertriglyceridemic very low density lipoproteins by normal human fibroblasts. *J. Lipid Res.* 1982. **23:** 984–993.

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Some forms of hypertriglyceridemia are associated with premature atherosclerosis (1). Elevated levels of functionally abnormal triglyceride-rich lipoproteins may contribute to this pathologic condition. We have found that very low density lipoproteins, S_f 60–400, (VLDL) from hypertriglyceridemic but not from normolipemic subjects suppress the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase in cultured normal human fibroblasts and endothelial cells (2–4). Reductase suppression and the concomitant inhibition of cholesterol synthesis would hardly seem deleterious if these were the only consequences of abnormal cellular uptake of the hypertriglyceridemic VLDL. However, we have also found that low levels of the hypertriglyceridemic VLDL, but not normal VLDL, are toxic to endothelial cells (4) and produce massive triglyceride accumulation in macrophages (5, 6). These effects observed in vitro represent potentially atherogenic consequences of abnormal cellular uptake if similar processes occur in vivo. The functional abnormality in hypertriglyceridemic VLDL is present throughout the VLDL spectrum, so that even the largest S_f 100–400 VLDL ($VLDL_1$) from hypertriglyceridemic subjects have the ability to suppress HMG-CoA reductase activity in normal human fibroblasts (3) and endothelial cells (4). By comparison,

Abbreviations: VLDL, very low density lipoproteins S_f 60–400; LDL, low density lipoproteins; HDL, high density lipoproteins; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HTG, hypertriglyceridemic; $VLDL_1$, S_f 100–400 VLDL; $VLDL_2$, S_f 60–100 VLDL; $VLDL_3$, S_f 20–60 VLDL; ^{125}I-LDL, ^{125}I-labeled LDL; LPDS, lipoprotein-deficient serum; TMU, tetramethylurea; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA.

only the smallest S_f 20–60 normolipemic VLDL ($VLDL_3$) have any ability to suppress HMG-CoA reductase (3, 4).

Our previous observation that hypertriglyceridemic VLDL, like LDL, do not suppress the enzyme in mutant receptor negative cells (2, 3) suggested that the suppression of HMG-CoA reductase activity by hypertriglyceridemic VLDL in normal fibroblasts is mediated by the LDL cell surface receptor (7, 8). However, the question remained as to whether the differences in effects of normal and hypertriglyceridemic VLDL on reductase activity were indeed due to differences in mechanisms of uptake of the VLDL by the cells. The results of direct and competitive binding studies, presented herein, show that hypertriglyceridemic VLDL but not normal VLDL are bound, internalized, and degraded via the LDL receptor pathway in cultured normal fibroblasts.

MATERIALS AND METHODS

Na ^{125}I was obtained from Iso Tex, Inc.; heparin was from Sigma; glycerol tri[9,10(n)-^{3}H]oleate was from Amersham; other materials and tissue culture supplies were obtained as previously reported (2, 3).

Cells

Monolayer cultures of normal human fibroblasts were established from a preputial specimen obtained from a healthy newborn. Cells were maintained in 100-mm dishes in a humidified incubator (5% CO_2) at 37°C and used between the third and ninth passage. Detailed descriptions of the growth conditions and the subculture method have been presented (2, 3). For experimental purposes, cells from stock plates were combined and approximately 5×10^4 cells/dish were seeded into dishes (60 × 15 mm) in 5 ml of complete medium. After 3 days of growth, when the cells were approximately 75% confluent, the medium was removed; the cells were washed with 3 ml of saline and 2 ml of medium containing 5% human lipoprotein-deficient serum (LPDS) added for 36–48 hr, to derepress the LDL pathway. Indicated quantities of lipoproteins (in 0.2 ml) and ^{125}I-LDL (or radiolabeled VLDL) were added to duplicate or triplicate dishes and incubated at 37°C or 4°C for 2–6 hr. Surface binding of ^{125}I-LDL was measured as the heparin-releasable radioactivity from cells washed with buffer containing albumin (9). Internalized ^{125}I-LDL was measured by determining the cell-associated radioactivity remaining after surface-bound lipoproteins were released by treatment with heparin (9). The heparin wash was omitted in the studies with labeled VLDL; in these studies cell-associated radioactivity represents both bound and internalized VLDL. Protein content was determined by the method of Lowry et al. (10). The amount of non-iodide, non-lipid, trichloroacetic acid-soluble radioactivity in the medium was used as a measure of iodinated lipoprotein degradation (11); these values were corrected for nonspecific degradation by subtracting the amount degraded in control dishes that did not contain cells. HMG-CoA reductase activity was determined by a modification (3) of published methods (12, 13).

Lipoproteins and lipoprotein-deficient serum (LPDS)

Normal lipoprotein fractions and LPDS ($d > 1.21$ g/ml) were isolated from the plasma of fasting adult normolipemic males. Hypertriglyceridemic VLDL were obtained from the plasma of fasting patients with hyperlipoproteinemia types 4 and 5. The diagnoses were based on commonly used criteria (1) as described in detail elsewhere (2, 3). Blood was collected in 0.1% EDTA after a 12-hr fast; red cells were removed by low speed centrifugation. Lipoprotein fractions were isolated from plasma containing 1 mM NaN_3 and 10 μM phenylmethylsulfonyl fluoride according to standard techniques by sequential flotation in a 60 Ti rotor at 45,000 rpm and 14°C for indicated times in a Beckman preparative ultracentrifuge with KBr for density adjustment (14). After chylomicrons were removed (0.5 hr at 35,000 rpm), total VLDL were then isolated in a second centrifugation (18 hr) without adjusting the density of plasma ($d < 1.006$ g/ml); LDL were isolated (18 hr spin) at d 1.006–1.063 g/ml. $VLDL_1$ (S_f 100–400), $VLDL_2$ (S_f 60–100), and $VLDL_3$ (S_f 20–60) subclasses were isolated from the total VLDL fraction ($d < 1.006$ g/ml) by flotation in a density gradient (d 1.006–1.05 g/ml) in a SW-41 rotor [23°C, 35,000 rpm for 144 min ($VLDL_1$), 108 min ($VLDL_2$), and 18 hours, 38 min ($VLDL_3$)] as described by Lindgren, Jensen, and Hatch (15). Before subfractionation, normolipemic VLDL were concentrated in dialysis tubing against dry Sephadex G-75. Characteristics of VLDL subclasses so isolated are detailed elsewhere (3).

Lipoproteins and LPDS (16) were dialyzed against at least three changes of 50 volumes of 0.15 M NaCl containing 50 mM Tris–HCl, pH 7.4, and 0.3 mM EDTA at 4°C for 36–48 hr. LDL and VLDL were iodinated by a modification of the iodine monochloride method of McFarland (17). The iodinated lipoproteins were desalted by gel filtration and extensive dialysis and were filtered (0.45 μm Millex) immediately before use; specific activities ranged from 42 to 200 cpm per ng protein with less than 13% of VLDL label or 5% of LDL label extractable into organic solvent. Lipoproteins were sterilized by filtration through a 0.45 μm Millex filter; LPDS, prepared from lipoprotein-deficient plasma (16), was sterilized by filtration through a 0.22 μm filter. LPDS was stored at −20°C and lipoproteins were stored

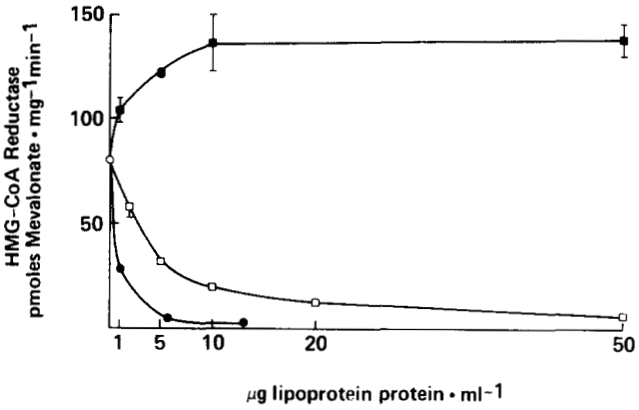

Fig. 1. Effects of normal VLDL, hypertriglyceridemic VLDL, and LDL on HMG-CoA reductase activity in normal human fibroblasts. Cells were grown to approximately 75% confluency in complete medium containing 10% fetal calf serum, washed, and placed on 2 ml of medium containing 5% LPDS for 24 hr. Indicated amounts of lipoproteins, in 0.2 ml, were added to duplicate dishes for 16 hr before the cells were washed and harvested. Each data point is an average of duplicate determinations of HMG-CoA reductase activity in cells from duplicate dishes. Hypertriglyceridemic VLDL were from the plasma of a patient with type 4 hyperlipoproteinemia. The VLDL were purified by flotation through a density gradient d 1.006–1.05 g/ml. Normal LDL (● —— ●); hypertriglyceridemic S_f 60–400 VLDL (□ —— □); normolipemic S_f 60–400 VLDL (■ —— ■).

at 4°C. For reconstitution of suppressive apoE-enriched VLDL, apoprotein E was isolated from the d < 1.006 g/ml fraction of plasma as described by Weisgraber, Mahley, and Assmann (18). ^{125}I-apoE (100 μg) or saline were incubated with normal S_f 100–400 VLDL (450 μg of protein) at 37°C for 2 hr. The VLDL were reisolated by flotation through a discontinuous salt gradient (15) and tested for their effects on HMG-CoA reductase in normal fibroblasts.

Total protein content of the lipoproteins was determined by modification of the method of Lowry et al. (10); sodium dodecyl sulfate, at a final concentration of 0.1%, was included to prevent interference by opalescence and light scattering (19). ApoB was determined as the difference between the total protein and the protein soluble in 4.2 tetramethylurea (TMU) (20). Soluble apoprotein composition was determined after delipidation with 4.2 M TMU. The apoproteins were separated by electrophoresis on 7.5% polyacrylamide gels containing 8 M urea, stained with Amido-Schwartz, and quantitated by scanning densitometry (20). Cholesterol, free and esterified, was quantified enzymatically (21). Phospholipid phosphorus was assayed by the method of Bartlett (22). Triglycerides were determined in the Clinical Laboratory of the Baylor Lipid Research Clinic (23). Total VLDL (d < 1.006 g/ml) were labeled with [^{3}H]triolein by a procedure described by Fielding (24) which preserves the chemical and physical properties and the biological activity of chylomicrons or VLDL, as judged by their behavior as substrates for lipase or in the perfused rat heart and liver. Briefly, [^{3}H]triolein, 100 μCi, dissolved in 1 ml of dimethyl sulfoxide, was injected into 4 ml of saline containing 2.7 mM EDTA, pH 7.4. The clear solution of triolein (37°C) was injected (500-μl aliquots) into 5 to 10 ml of VLDL (d < 1.006 g/ml) while vortexing. This solution was incubated at 37°C for 2 hr and dialyzed against saline–2.7 mM EDTA, pH 7.4, at 4°C for 16 hr. VLDL subclasses were immediately obtained by cumulative flotation (15), dialyzed and sterilized (0.45 μm filter), and stored at 4°C. In each binding experiment radiolabeled lipoproteins were compared to unlabeled control lipoproteins for their effects on HMG-CoA reductase activity in fibroblasts preincubated in LPDS-containing medium to establish that the labeling procedures had not altered the biological activity of the lipoproteins.

RESULTS

Competition with ^{125}I-LDL

A typical experiment comparing normolipemic S_f 60–400 VLDL, hypertriglyceridemic S_f 60–400 VLDL, and LDL for their effects on reductase activity and their abilities to compete with the specific binding, uptake, and degradation of ^{125}I-LDL by normal human fibroblasts is shown in Figs. 1–3. Normolipemic VLDL stimulated HMG-CoA reductase activity and the hypertriglyceridemic VLDL suppressed the enzyme (**Fig. 1**). The hypertriglyceridemic VLDL were highly effective in competing for LDL binding, giving 50% inhibition of ^{125}I-LDL binding at 1 μg of HTG VLDL protein/ml (**Fig. 2**). The normal VLDL, which failed to suppress reductase activity in the same experiment, nevertheless competed with the binding of ^{125}I-LDL with a potency similar to that of LDL (Fig. 2). Competition of ^{125}I-LDL binding by normal S_f 60–400 VLDL was a general finding. However, several preparations of normal S_f 60–400 VLDL failed to compete with ^{125}I-LDL binding. The hypertriglyceridemic VLDL were just as effective as LDL in competing for ^{125}I-LDL internalization (50% reduction at 9 μg protein • ml^{-1}). However, normal VLDL were less effective, reducing LDL internalization by 45% at 20 to 50 μg protein • ml^{-1}. The differences between normal and hypertriglyceridemic VLDL were more apparent when ^{125}I-LDL degradation was measured. Hypertriglyceridemic VLDL were just as effective as LDL in competing for ^{125}I-LDL degradation whereas the normal VLDL consistently had little or no effect on the degradation of ^{125}I-LDL (**Fig. 3**). The hypertriglyceridemic VLDL used in the experiment shown in Figs. 1–3 was isolated from the plasma of a subject with a type 4 lipoprotein pattern. Similar results were

obtained with VLDL isolated from other normal and hypertriglyceridemic subjects (types 4 and 5 hyperlipoproteinemia).

S_f 60–400 VLDL from normal subjects do not suppress reductase activity; however, the smaller, "remnant" $VLDL_3$ from most normolipemic plasma suppresses HMG-CoA reductase activity in normal fibroblasts to a limited extent (3). Consistent with these effects on reductase activity, neither $VLDL_1$ nor $VLDL_2$ competed with the degradation of ^{125}I-LDL; the moderately suppressive $VLDL_3$ did compete with the specific degradation of ^{125}I-LDL, but less effectively than LDL (**Fig. 4**).

Uptake and degradation of hypertriglyceridemic VLDL

Studies with iodinated $VLDL_1$ from subjects with hypertriglyceridemia demonstrate that this VLDL subclass is taken up and degraded by normal fibroblasts in a manner similar to the uptake and degradation of LDL. Uptake and degradation of hypertriglyceridemic ^{125}I-$VLDL_1$ showed a saturable dependence on VLDL concentration and were inhibited by the presence of excess unlabeled VLDL (**Fig. 5**) or LDL (**Fig. 6**) in the medium. Inclusion of 10 mM EDTA in the medium abolished degradation of hypertriglyceridemic ^{125}I-$VLDL_1$, analogous to LDL uptake and degradation (8).

The most effective competitor for the uptake and degradation of hypertriglyceridemic VLDL was hypertriglyceridemic VLDL itself; LDL competed only at much higher concentrations (Fig. 6). In experiments at 4°C, normal $VLDL_1$ at 20 μg protein/ml inhibited the binding of hypertriglyceridemic ^{125}I-$VLDL_1$ (1 μg/ml) somewhat more than did LDL; hypertriglyceridemic $VLDL_1$ was far more effective in competition (**Table 1**). At a higher concentration of labeled hypertriglyceridemic VLDL (4 μg/ml), however, LDL at 20 μg/ml was ineffective in competing with the internalization or degradation of 4 μg/ml of hypertriglyceridemic ^{125}I-$VLDL_1$ while $VLDL_1$ remained effective (Table 1). Low levels of LDL (1–20 μg protein/ml) enhanced and high levels (≥50 μg/ml) inhibited uptake and degradation of hypertriglyceridemic ^{125}I-$VLDL_1$ (1–3 μg/ml) (Fig. 6). The enhanced uptake of radioactivity by cells incubated with ^{125}I-$VLDL_1$ and low levels of LDL may be due to transfer of labeled peptides from VLDL to LDL after which the LDL are internalized via the receptor mechanism. Such an effect would be masked by high levels of unlabeled LDL.

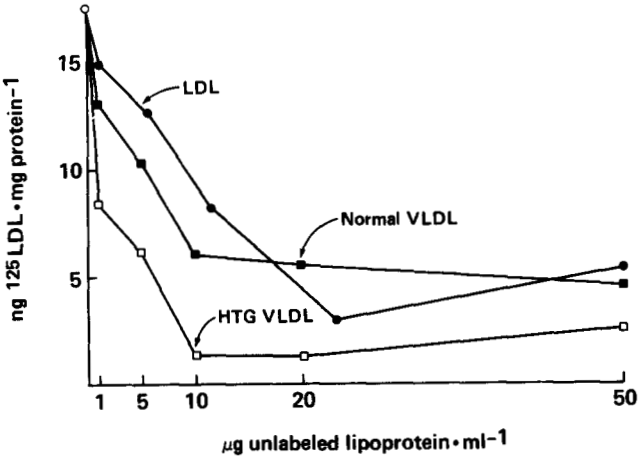

Fig. 2. Competition of ^{125}I-LDL binding by hypertriglyceridemic VLDL, normolipemic VLDL, and LDL. Cells were grown as described in the legend to Fig. 1, except that the cells remained on 5% LPDS for 36 hr. Lipoproteins were the same as those described in the legend to Fig. 1. Indicated amounts of lipoproteins, in 0.2 ml, along with ^{125}I-LDL were added to triplicate dishes (final ^{125}I-LDL concentration, 5 μg/ml, 70 cpm/ng). The dishes were incubated in a humidified CO_2 incubator at 37°C for 4 hr, and washed as described in Methods. Surface binding was measured as the heparin-releasable radioactivity. Each value represents the average of triplicate determinations. Normal LDL (● —— ●); hypertriglyceridemic S_f 60–400 VLDL (□ —— □); normolipemic S_f 60–400 VLDL (■ —— ■).

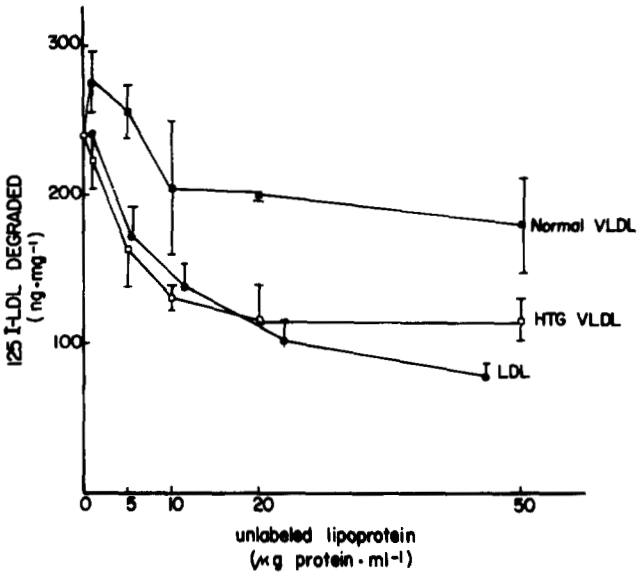

Fig. 3. Effects of hypertriglyceridemic VLDL, normal VLDL, and LDL on the degradation of ^{125}I-LDL. Cell growth, lipoprotein preparation, incubations, and washes are described in the legends to Figs. 1 and 2. The cells were preincubated in LPDS-containing medium for 36 hr. Trichloroacetic acid-soluble, noniodide, nonlipid radioactivity in the medium represents ^{125}I-LDL degradation. Each point represents the mean ± SD of values from three dishes, expressed as ng ^{125}I-LDL acid-soluble material formed per mg of total cell protein. Normal LDL (● —— ●); hypertriglyceridemic VLDL (□ —— □); normal VLDL (■ —— ■).

Uptake and degradation of normal VLDL

Studies with normal, nonsuppressive S_f 60–400 VLDL labeled with [^{3}H]triolein indicate that uptake by normal fibroblasts is a linear function of VLDL concentration

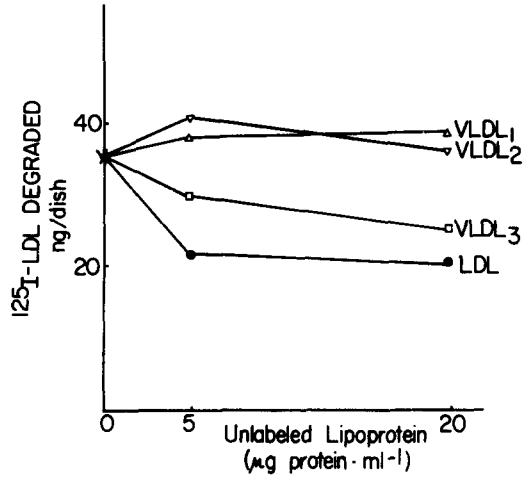

Fig. 4. Effects of normolipemic VLDL subclasses on the degradation of ^{125}I-LDL by normal human fibroblasts. Cells were grown as described in the legend to Fig. 2, preincubated for 36 hr in lipoprotein-deficient medium, and incubated for 4 hr at 37°C with 2 μg of ^{125}I-LDL/ml alone and with the indicated quantities of unlabeled lipoproteins. Trichloroacetic acid-soluble, noniodide, nonlipid radioactivity in the medium represents ^{125}I-LDL degradation. Each point is the average of values from duplicate dishes, which varied by less than 7%. Normal LDL (● —— ●); VLDL$_1$ (S_f 100–400, △ —— △); VLDL$_2$ (S_f 60–100, ▽ —— ▽); VLDL$_3$ (S_f 20–60, □ —— □).

and is similar at 4°C and at 37°C after a 2.5-hr incubation (**Fig. 7**). By contrast, cellular uptake of the smaller normal VLDL$_3$ was substantially greater than uptake of the larger VLDL. Moreover, uptake of VLDL$_3$ showed a saturable dependence on VLDL concentration and was greater at 37°C than at 4°C (Fig. 7). These results are consistent with receptor-mediated uptake of VLDL$_3$ but not of the larger normal VLDL. Likewise, uptake of hypertriglyceridemic VLDL$_1$ was greater than uptake of normal VLDL$_1$ (**Fig. 8**). Experiments with ^{125}I-VLDL$_1$ from normal subjects also demonstrate that uptake and degradation of normal nonsuppressive S_f 60–400 VLDL are linear rather than saturable functions of VLDL concentration. Degradation of normal ^{125}I-VLDL was generally observed, but occasionally there was no detectable degradation or uptake of normal VLDL when normal fibroblasts were incubated with 1–5 μg of ^{125}I-VLDL$_1$ for 2–4 hr. The presence or absence of normal VLDL degradation may be a function of different normal VLDL preparations used or metabolic differences in the fibroblasts or both.

ApoE-mediated uptake of VLDL$_1$

The LDL receptor recognizes both apoB and apoE, but the levels of these apoproteins are similar in normal and hypertriglyceridemic VLDL (2, 3). As we have previously reported, the composition of VLDL from normal subjects is similar to the VLDL from hypertriglyceridemic subjects with hyperlipoproteinemia types 4 and 5 (2, 3): the percentage weights of the major components of S_f 100–400 normal VLDL and hypertriglyceridemic type 4 VLDL (in parentheses) were 5.9 ± 0.7 (5.0 ± 1.0), protein; 18.5 ± 1.9 (15.5 ± 1.4), phospholipid; 5.9 ± 1.4 (5.8 ± 1.1), cholesterol; 6.1 ± 1.6 (8.4 ± 1.9), cholesteryl ester; and 63.6 ± 2.6 (65.4 ± 3.4), triglyceride (means ± SD; N = 5, normal; N = 4, hypertriglyceridemic subjects). There were no apparent differences in total apoB, apoE, or C peptide content as judged by tetramethylurea solubility and by polyacrylamide gel electrophoresis (2, 3).

Since hypertriglyceridemic VLDL but not normal VLDL are specifically taken up by the LDL pathway in fibroblasts, subtle differences in surface conformation of critical apoproteins may account for differences in uptake. To see if addition of apoprotein E to normal, nonsuppressive VLDL could reconstitute a particle that is taken up via the LDL pathway, we incubated normal, nonsuppressive S_f 100–400 VLDL alone or with ^{125}I-apoE, reisolated the VLDL by flotation, and tested them for their effects on HMG-CoA reductase activity in cultured normal fibroblasts. In the experiment illustrated in **Fig. 9**, ^{125}I-apoE represented only 2% of the total protein of the reisolated VLDL (approximately one mol

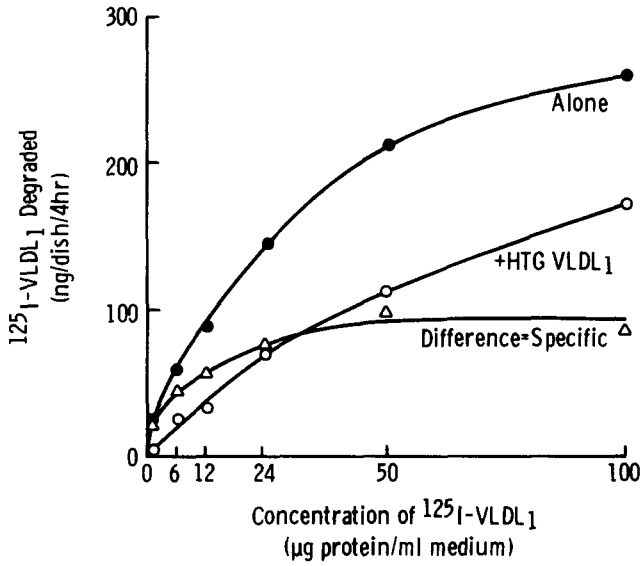

Fig. 5. Degradation of hypertriglyceridemic S_f 100–400 VLDL by normal human fibroblasts. Cells were grown in 60-mm dishes in complete medium for 4 days, placed on 2 ml of medium containing 5% lipoprotein-deficient serum for 48 hr, and incubated for 4 hr at 37°C with the indicated levels of ^{125}I-VLDL$_1$ (49 cpm/ng) in the absence (●) and in the presence (○) of unlabeled homologous VLDL$_1$, 72 μg of protein/ml, added together in 0.6 ml of buffered saline/dish. Trichloroacetic acid-soluble, noniodide, nonlipid radioactivity in the medium represents ^{125}I-VLDL degradation. The S_f 100–400 VLDL were obtained from a subject with endogenous hypertriglyceridemia (type 4). The difference between VLDL degraded in the absence (●) and in the presence (○) of unlabeled VLDL represents specific degradation (△); each value represents the average from duplicate dishes and the average variation between duplicates was 6%.

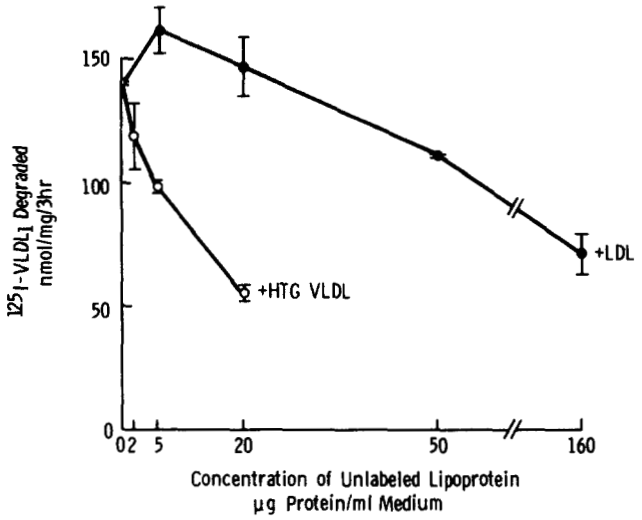

Fig. 6. Relative effects of hypertriglyceridemic VLDL and LDL on the degradation of hypertriglyceridemic VLDL. The fibroblasts were grown and incubated as described in Methods and the legend to Fig. 5, then incubated with ^{125}I-VLDL (1.2 µg of protein/ml, S_f 100–400 VLDL from a primary type 4 subject) alone or with the indicated concentrations of unlabeled homologous VLDL or LDL. Trichloroacetic acid-soluble, noniodide, nonlipid radioactivity in the medium represents ^{125}I-VLDL degradation. Each point is the average of values from duplicate dishes; variations between dishes are indicated by bars. Normal LDL (● —— ●); hypertriglyceridemic S_f 100–400 VLDL (○ —— ○).

of apoE per mol of VLDL), yet the apoE-enriched VLDL were as effective as LDL in reductase suppression, with 50% suppression at only 2 µg protein/ml. By contrast, reisolated control VLDL had no effect on reductase activity.

DISCUSSION

Several lines of evidence indicate that hypertriglyceridemic VLDL can be internalized and degraded in normal human fibroblasts as a direct consequence of binding to the classic LDL receptor. First, hypertriglyceridemic VLDL are at least as effective as LDL in competing for the binding, uptake, and degradation of ^{125}I-LDL; similar results were obtained by Schonfeld and coworkers (25). Second, hypertriglyceridemic ^{125}I-VLDL are taken up and degraded by normal human fibroblasts in a saturable manner with saturation at 25 µg of protein/ml. Third, LDL compete with the uptake of hypertriglyceridemic VLDL, albeit less effectively than hypertriglyceridemic VLDL itself. Fourth, EDTA abolishes the degradation of hypertriglyceridemic VLDL. Since binding to the LDL receptor is calcium-dependent and therefore EDTA-sensitive (8), this provides additional evidence that hypertriglyceridemic VLDL interact with the LDL receptor. Our previous work showed that hypertriglyceridemic VLDL, like LDL, failed to

TABLE 1. Competition of degradation of hypertriglyceridemic ^{125}I-VLDL$_1$[a]

Unlabeled Lipoprotein Added[b]	^{125}I-VLDL$_1$ Concentration 1 µg/ml	4 µg/ml
	Degradation (ng/mg cell protein)	
None	378	836
LDL	290	872
HTG VLDL$_1$	65	461
Normal VLDL$_1$	165	648

[a] After 3 days in culture in complete medium, the cells were incubated for 40 hr in medium containing 5% LPDS. The cells were then incubated with the indicated lipoproteins at 37°C for 2 hr; degradation was measured as acid-soluble noniodide ^{125}I accumulating in the medium. Data represent the average of values obtained from duplicate dishes of cells.

[b] Final concentration of each unlabeled lipoprotein was 20 µg protein/ml. The VLDL were S_f 100–400; the hypertriglyceridemic VLDL were from a subject (L.F.) with endogenous hypertriglyceridemia (primary type 4).

suppress HMG-CoA reductase activity in receptor-negative fibroblasts, indicating that the potent reductase suppression observed in normal fibroblasts was mediated by the LDL receptor (2, 3). Moreover, analogous to LDL and HDL$_c$ (26), modification of the arginyl residues of hypertriglyceridemic VLDL with 1,2-cyclohexanedione abolished the ability of the VLDL to suppress HMG-CoA reductase activity in normal fibroblasts; removal of the adduct with hydroxylamine

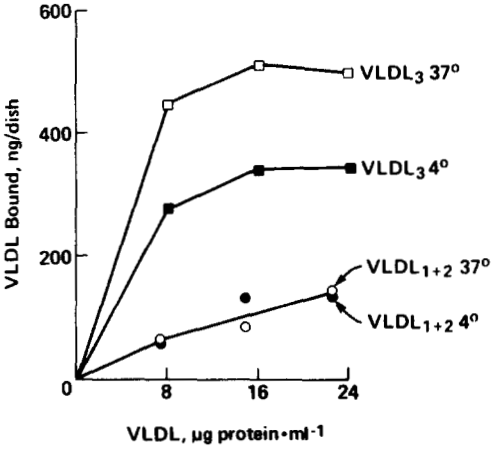

Fig. 7. Uptake of normal VLDL subclasses by normal human fibroblasts. Cells were grown in 60-mm dishes in complete medium for 4 days, preincubated for 48 hr in medium containing 5% lipoprotein-deficient serum, and incubated for 2 hr with the indicated quantities of [^{3}H]triolein-labeled S_f 60–400 VLDL (○, ●) or S_f 20–60 VLDL (□, ■) at 37°C (open symbols) or 4°C (closed symbols). The cells to be incubated at 4°C were precooled for 30 min prior to addition of the lipoproteins. The cells were washed extensively with albumin-containing buffer as described in Methods, dissolved in 1 ml of 1% sodium dodecyl sulfate for liquid scintillation counting. Each point represents the average of values of duplicate dishes (variation ≤ 10%). The average protein content per dish was 424 µg.

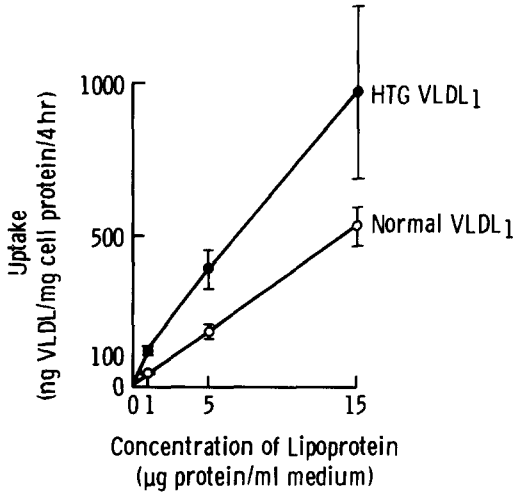

Fig. 8. Uptake of normal and hypertriglyceridemic VLDL. Normal fibroblasts were seeded in 60-mm dishes, grown in complete medium for 4 days, preincubated in 5% LPDS-containing medium for 24 hr, and incubated for 4 hr at 37°C with the indicated concentrations of [^{3}H]triolein-labeled VLDL from a normal subject (○) and a subject with hypertriglyceridemia (●), washed and analyzed as described in Methods. Each data point represents the mean ± SD of determinations on triplicate dishes, expressed as ng of VLDL protein/mg cell protein in 4 hr.

restored the suppressive capacity of the VLDL (3). Considered together, these data show that hypertriglyceridemic VLDL are bound, internalized, and degraded by the classical LDL receptor in normal fibroblasts.

By contrast, normal VLDL S_f 60–400 do not appear to interact specifically with the LDL receptor. Normal VLDL do not suppress the activity of HMG-CoA reductase in normal fibroblasts even at high levels (2, 3). Normal VLDL also fail to compete with the specific degradation of ^{125}I-LDL. The uptake of normal VLDL labeled with either [^{3}H]triolein or ^{125}I on the protein components is nonsaturable, as is degradation of ^{125}I-$VLDL_1$. There is a similar, low-level uptake and degradation of normal VLDL in receptor-negative fibroblasts as in normal cells (data not presented). The uptake and degradation of normal ^{125}I-VLDL are decreased by VLDL, both normal and hypertriglyceridemic, but by LDL only at high concentrations.

VLDL, both normal and hypertriglyceridemic, adsorb to plastic tissue culture dishes (data not presented). This "nonspecific" adsorption of VLDL can be reduced by hypertriglyceridemic VLDL and normal VLDL, in that order of potency; LDL compete only at high levels. Thus it is not surprising that hypertriglyceridemic VLDL are most effective in competing with the nonspecific, nonsaturable uptake of normal, nonsuppressive S_f 60–400 VLDL. The seemingly "specific" adsorption of VLDL to plastic (specific with respect to competition) is reminiscent of the apparently "specific" binding of LDL to glass beads (27). As Dana, Brown, and Goldstein (27) cautioned, care must be exercised in interpreting the results of binding studies. An intracellular endpoint such as specific degradation or HMG-CoA reductase suppression should also be monitored in the same experiment to ensure that observed binding is physiologic. In each binding and degradation experiment we routinely compared a range of concentrations of each lipoprotein to a standard LDL concentration curve for effects on HMG-CoA reductase activity. This intracellular endpoint is extremely sensitive and reproducible with maximal reductase suppression occurring at LDL concentrations of 2–5 μg of protein/ml. Since normal VLDL which do not bind specifically to the LDL receptor do not suppress HMG-CoA reductase activity, whereas hypertriglyceridemic VLDL which do bind are potent reductase suppressors, suppression of HMG-CoA reductase in normal fibroblasts under carefully controlled conditions can be used as a simple and highly sensitive indicator that VLDL are or are not taken up by the LDL receptor pathway.

Most preparations of normal S_f 60–400 VLDL competed with LDL binding; none of the normal VLDL effectively competed with LDL degradation. The association of normal, nonsuppressive VLDL with cells was a linear rather than a saturable function of concentration and was noted at 4°C as well as 37°C. These

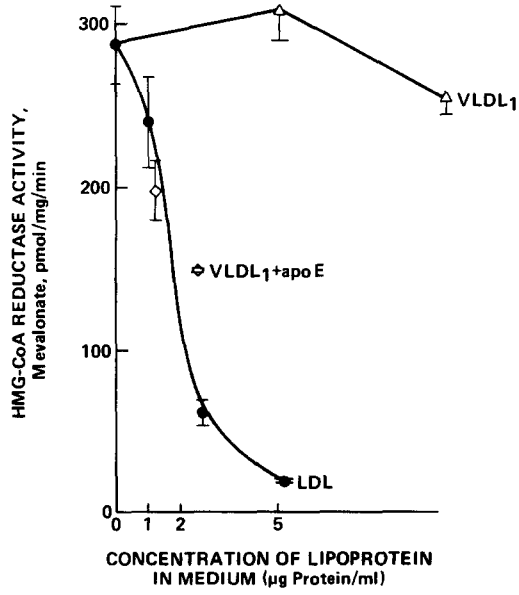

Fig. 9. Reconstituted apoE-$VLDL_1$ but not control $VLDL_1$ suppress HMG-CoA reductase activity. Cells were grown and incubated with the indicated lipoproteins as described in the legend to Fig. 1. Each data point represents the average of HMG-CoA reductase specific activities from duplicate dishes, each assayed in duplicate; the variations between individual dishes are indicated by bars. Normal $VLDL_1$ (450 μg of protein) was incubated in the absence (△) or presence (◇) of 100 μg of ^{125}I-apoE for 2 hr at 37°C, and reisolated by flotation before comparing with LDL (●) for reductase suppression.

observations suggest that normal VLDL can adsorb to the fibroblast surface in a nonspecific manner, similar to nonspecific adsorption to plastic tissue culture dishes. Normal VLDL could then be internalized by endocytosis and by fluid phase pinocytosis. Lack of competition with ^{125}I-LDL degradation suggests that these nonsuppressive VLDL may be degraded in a different intracellular compartment from that in which LDL are degraded. Considered together, these data indicate that normal, nonsuppressive VLDL do not bind with high affinity or specificity to the LDL receptor and that their ability to inhibit LDL receptor binding may be due to steric hindrance of LDL binding by the large, nonspecifically adsorbed VLDL rather than by direct competition for the receptor. These experiments do not completely rule out an alternative explanation, that some preparations of the nonsuppressive VLDL may have the ability to bind to the LDL receptor but may lack some feature necessary for triggering internalization and subsequent degradation.

Both apoB and apoE interact with the LDL receptor when present in small particles (LDL (7), LDL-like remnants from lipolyzed VLDL (25, 28), or apoE-HDL (29)). One or more apoproteins in hypertriglyceridemic VLDL appear to be involved in binding, since we have shown that modification of arginyl residues of hypertriglyceridemic VLDL with 1,2-cyclohexanedione abolished their ability to suppress HMG-CoA reductase activity (3). Therefore, we assume that the interaction of hypertriglyceridemic VLDL with the LDL receptor is via apoB and/or apoE. Several lines of evidence suggest that binding of hypertriglyceridemic VLDL to the cell surface receptor is primarily via apoE. First, apoE has a higher affinity for the LDL receptor than does apoB (29), and we have found that hypertriglyceridemic VLDL bind to the classic LDL receptor with a higher affinity than LDL, as is the case with apoE-HDL (29). In the experiment shown in Fig. 2, the hypertriglyceridemic VLDL were approximately 10 times more effective than LDL in competing for ^{125}I-LDL binding when compared on a protein basis. On a particle basis, hypertriglyceridemic VLDL are approximately 40-fold more effective than LDL. Second, and conversely, the relatively poor ability of LDL to compete with hypertriglyceridemic VLDL uptake and degradation (Fig. 6) is reminiscent of the inefficient competition of LDL with the binding of apoE-HDL. These observations suggest that apoE is more critical in the interaction of hypertriglyceridemic VLDL with the LDL receptor than is apoB. Indeed, the addition of apoE to normal, nonsuppressive S_f 100–400 VLDL results in a particle that can suppress HMG-CoA reductase activity as effectively as LDL (Fig. 9). Moreover, apoE in VLDL is susceptible to proteolytic degradation; the protease inhibitor phenylmethylsulfonyl fluoride protects against apoE degradation and the loss of ability of the hypertriglyceridemic VLDL to suppress HMG-CoA reductase that occurs within 2 to 6 weeks of isolation even when the VLDL are maintained at 4°C under sterile conditions (30). By comparison, normal $VLDL_1$ isolated in the presence of phenylmethylsulfonyl fluoride, like normal $VLDL_1$ isolated in the absence of the inhibitor, fail to suppress reductase activity, indicating that the lack of suppression observed with normal VLDL isolated in the absence of protease inhibitors is not due to proteolytic degradation of critical binding determinants during isolation or storage.

The structural basis for the difference in cellular effects between hypertriglyceridemic VLDL and normal VLDL is not apparent from the present data. Since normolipemic VLDL have amounts of total apoE per particle equivalent to those in hypertriglyceridemic VLDL (2, 3), the ratios of apoE isomorphs and other peptides of hypertriglyceridemic VLDL may be different from those in normal VLDL.

The fact that reconstituted apoE-$VLDL_1$ suppressed reductase activity even though only 2% of the total VLDL protein was ^{125}I-apoE indicates that the appropriate surface configuration of apoE may be more important for efficient receptor-mediated uptake than a dramatic increase in apoE mass.

In addition, as we previously suggested (2, 3), a subpopulation of particles with a higher affinity for the LDL receptor, possibly chylomicron remnants, may be responsible for the cellular effects of the hypertriglyceridemic VLDL. Grundy and Mok (31) have noted delayed chylomicron clearance in most subjects with endogenous hypertriglyceridemia, while Hazzard and Bierman (32) found chylomicron remnants present in the VLDL of subjects with types 3 and 4 hyperlipoproteinemia. The presence in hypertriglyceridemic VLDL subclasses of a subpopulation with high affinity for the LDL receptor would not necessarily be reflected in a dramatic change in overall composition of the subclass.

Our studies described here and elsewhere (2–6) provide evidence that large VLDL from subjects with hypertriglyceridemia, in sharp contrast to VLDL from normal subjects, can be catabolized by the LDL receptor pathway. Poyser and Nestel (33) provided additional evidence that, in this context, hypertriglyceridemic VLDL are functionally abnormal; human mononuclear cells accumulate and degrade hypertriglyceridemic VLDL more avidly than normal VLDL. These studies show that there is a mechanism for direct cellular catabolism of large hypertriglyceridemic S_f 60–400 VLDL that is not functional for normal S_f 60–400 VLDL. Receptor-mediated catabolism of hypertriglyceridemic VLDL could account for observations that, in hyper-

triglyceridemia, a portion of the apoB in VLDL is cleared from the plasma compartment without first appearing in LDL (34). Another study, that showed that normal and hypertriglyceridemic VLDL (unfractionated d < 1.006 g/ml) were equally effective in inducing triglyceride accumulation in fibroblasts, was performed under culture conditions which would maximally suppress the LDL receptor pathway (35). One would not expect to see a difference in the effects of normal and hypertriglyceridemic VLDL under these conditions. Moreover, the triglyceride accumulation induced by both types of VLDL was a linear function of VLDL concentration, which suggests a nonspecific rather than a receptor-mediated uptake mechanism. These results are consistent with our findings: *1*) that purified normal VLDL that do not interact specifically with the LDL receptor can be taken up nonspecifically by both normal and LDL receptor-negative fibroblasts; *2*) that uptake and degradation is a linear rather than a saturable function of normal VLDL concentration; and *3*) that there is a linear component of hypertriglyceridemic VLDL degradation, after receptor saturation at approximately 25 μg VLDL/ml. This is analogous to the nonspecific, low affinity component of LDL degradation by normal fibroblasts observable at higher LDL concentrations (7). Thus, hypertriglyceridemic VLDL can be rapidly accumulated and degraded by cells via the LDL receptor pathway when the receptor is expressed or, less avidly, by nonspecific routes, both fluid phase and adsorptive pinocytosis. Normal S_f 60–400 VLDL are internalized only by a low affinity process. The presence in hypertriglyceridemia of VLDL that can be taken up by cells that possess LDL receptors may be a link between the metabolism of triglyceride-rich particles and the pathogenesis of atherosclerosis.

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