Mechanisms of Inhibition by Apolipoprotein C of Apolipoprotein E-dependent Cellular Metabolism of Human Triglyceride-rich Lipoproteins through the Low Density Lipoprotein Receptor Pathway*

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The mechanism of inhibition by apolipoprotein C of the uptake and degradation of triglyceride-rich lipoproteins from human plasma via the low density lipoprotein (LDL) receptor pathway was investigated in cultured human skin fibroblasts. Very low density lipoprotein (VLDL) density subfractions and intermediate density lipoprotein (IDL) with or without added exogenous recombinant apolipoprotein E-3 were used. Total and individual (C-I, C-II, C-III-1, and C-III-2) apoC molecules effectively inhibited apoE-3-mediated cell metabolism of the lipoproteins through the LDL receptor, with apoC-I being most effective. When the incubation was carried out with different amounts of exogenous apoE-3 and exogenous apoC, it was shown that the ratio of apoE-3 to apoC determined the uptake and degradation of VLDL. Excess apoE-3 overcame, at least in part, the inhibition by apoC. ApoC, in contrast, did not affect LDL metabolism. Neither apoA-I nor apoA-II, two apoproteins that do not readily associate with VLDL, had any effect on VLDL cell metabolism. The inhibition of VLDL and IDL metabolism cannot be fully explained by interference of association of exogenous apoE-3 with or displacement of endogenous apoE from the lipoproteins. IDL is a lipoprotein that contains both apoB-100 and apoE. By using monoclonal antibodies 4G3 and 1D7, which specifically block cell interaction by apoB-100 and apoE, respectively, it was possible to assess the effects of apoC on either apoprotein. ApoC dramatically depressed the interaction of IDL with the fibroblast receptor through apoE, but had only a moderate effect on apoB-100. The study thus demonstrates that apoC inhibits predominantly the apoE-3-dependent interaction of triglyceride-rich lipoproteins with the LDL receptor in cultured fibroblasts and that the mechanism of inhibition reflects association of apoC with the lipoproteins and specific concentration-dependent effects on apoE-3 at the lipoprotein surface.

Regulation of the uptake and degradation of very low density lipoproteins (VLDL)* and their remnants by cellular receptors determines the amount of LDL that is generated by the apoB-100 VLDL → LDL cascade (1-3). Although these lipoproteins contain apoB-100, it appears that their interaction with cell receptors depends on the presence of functional apoE molecules (E-3 and E-4) at the surface of the lipoprotein particle (4-8). Indeed, enrichment of VLDL and IDL particles from either normolipidemic human subjects (9, 10) or patients with hypertriglyceridemia (11) with apoE-3 has recently been shown to cause a manyfold enhancement of their cellular metabolism. This mechanism is presumably responsible for the findings that injection of apoE in Watanabe and cholesterole-fed rabbits causes a dramatic reduction in the VLDL and IDL levels of the animals (12, 13).

Some lipoproteins that are naturally rich in apoE, e.g. β-VLDL and HDL, from cholesterol-fed animals, exhibit exceptionally high affinity toward cell receptors, including the B/E (LDL) receptor (2, 8, 14-16). Yet, neither human plasma VLDL (9, 11) nor IDL (10, 17), even when maximally enriched with apoE-3, are taken up and degraded by cultured human skin fibroblasts or HepG2 cells at rates higher than those of LDL, a lipoprotein that contains only apoB-100. One possible explanation for this unexpected observation is that apoE-3 molecules, when associated with triglyceride-rich lipoprotein particles, are not fully expressed in receptor-binding processes. Alternatively, it can be speculated that the apoE-mediated uptake of triglyceride-rich particles by cell receptors is partially inhibited by other apoproteins that are associated with these lipoproteins. Specifically, apoC molecules that were shown to interfere with the uptake of chylomicrons and nascent hepatic VLDL during perfusion of rat liver (18, 19) may also inhibit the interaction of human VLDL with the B/E (LDL) receptor. This possibility has been explored in this study.

EXPERIMENTAL PROCEDURES

Preparation of Lipoproteins. 125I-Lipoproteins, and 125I-Apoproteins—VLDL, VLDL density subfractions (I-III), IDL, and LDL were prepared from the plasma of healthy normal volunteers as recently described (9). Plasma was isolated from 1 unit of blood taken in the morning hours after a 12-14-h fast from subjects with apoE profile E-3/3 or E-4/3. VLDL was separated by ultracentrifugation at 1,006 g/ml and washed once, and density subfractions were prepared on NaCl gradients following the procedure of Lindgren et al. (20). LDL and IDL were separated by density intervals of 1,006-1,019 and 1,019-1,063 g/ml, respectively, as described (9, 10). Rabbit β-VLDL was isolated at d <1,019 g/ml from plasma obtained 7-10 days after the initiation of a cholesterol-enriched diet (1 g of cholesterol/100 g of chow). Lipoproteins were dialyzed against 0.9% NaCl, 20 mM Tris (pH 7.4), and 0.01% EDTA buffer, radiolabeled (21) to a specific activity of 200-400 dpm/ng, and sterilized by passage through a 0.45-μm Millipore filter. The distribution of radioactivity among apoB, apoC, and apoE was identical to that recently described (9, 10). The lipoproteins were kept at 4 °C and used within 14 days of preparation. 125I-Labeled recombinant apolipoprotein E-3 was prepared following...
Results are expressed as nanograms of apoE II-lipoprotein bound, associated, or degraded per milligram of cell protein after subtraction of blank and nonspecific values.

In some experiments, monoclonal antibody 1D7 or 4G3 was added to the medium containing the 125I-lipoproteins prior to the addition of apoC and/or apoE-3, and the medium was incubated at 37 °C for 60 min. Thereafter, apoC and/or apoE-3 was added, and the samples were treated as described above.

Incorporation of [14C]Acetate into Sterols—Sterol synthesis from [14C]acetate was determined in up-regulated fibroblasts after a 6-h incubation with unlabeled lipoproteins in 1 ml of LPDS medium as previously described (8). The lipoproteins were eluted at the void volume and 1-5 ml of the column, while free lipoproteins in tubes 10-14. The lipoproteins were recovered; concentrations of recombinant apoE-3 to the culture medium (Table I).

The nature of the fibroblast receptor responsible for the uptake and degradation of VLDL and apoE-3-enriched VLDL was investigated under conditions that discern between the LDL receptor and the LDL receptor-related protein (LRP). The results for the proteolytic degradation data are shown in Table II. In up-regulated fibroblasts, apoE-3 (but not apoE-2) enhanced the degradation of VLDL by 8-9-fold. Down-regulation of the LDL receptor was achieved by growing the cells in the presence of LDL (80 μg of protein/ml). The degradation of VLDL and apoE-3-enriched VLDL in down-regulated fibroblasts was depressed by 80%. Yet, the enhancing effect of apoE-3 (but not apoE-2) on VLDL metabolism was evident in the down-regulated cells. In both up- and down-regulated cells, an excess of unlabeled LDL effectively depressed the degradation of apoE-3-enriched VLDL. Finally, in LDL receptor-negative fibroblasts, neither VLDL nor apoE-3-enriched VLDL was degraded to any appreciable extent. Similarly, neither rabbit β-VLDL nor apoE-3-enriched VLDL was degraded in these cells. All these observations are consistent with the uptake and degradation of VLDL and apoE-3-enriched VLDL through the LDL receptor pathway, and not the LRP.

The effects of individual C apoproteins (C-I, C-II, C-III-I, and C-III-II) and total apoC on the binding and proteolytic degradation of apoE-enriched VLDL-I and VLDL-III and of LDL are shown in Fig. 1. In this experiment, 10 μg of VLDL or LDL protein were included in the culture medium without or with 4 μg of apoE-3 and 4 μg of total or individual C proteins. The C proteins caused a 50-90% reduction in the apoE-3-stimulated VLDL metabolism, but had no effect on LDL metabolism. Whereas all C proteins were effective in reducing the binding and degradation of apoE-3-enriched VLDL by the cells, apoC-I was considerably more effective.

**RESULTS**

**Studies with Exogenous apoE-3—**The chemical composition and apoprotein profile of VLDL-I, -II, and -III and of IDL and LDL were similar to those recently published (9, 10). VLDL and IDL contained apoB-100, apoE, and apoC (data not shown). LDL contained only apoB-100. In agreement with our previous reports, we observed a manyfold increase in the binding, association, and degradation of VLDL and LDL labeled proteins upon the addition of optimal concentrations of recombinant apoE-3 to the culture medium (Table I).

ApoE-3 and apoE-2 enhanced the degradation of VLDL by 8-9-fold. Down-regulation of the LDL receptor was achieved by growing the cells in the presence of LDL (80 μg of protein/ml). The degradation of VLDL and apoE-3-enriched VLDL in down-regulated fibroblasts was depressed by 80%. Yet, the enhancing effect of apoE-3 (but not apoE-2) on VLDL metabolism was evident in the down-regulated cells. In both up- and down-regulated cells, an excess of unlabeled LDL effectively depressed the degradation of apoE-3-enriched VLDL. Finally, in LDL receptor-negative fibroblasts, neither VLDL nor apoE-3-enriched VLDL was degraded to any appreciable extent. Similarly, neither rabbit β-VLDL nor apoE-3-enriched VLDL was degraded in these cells. All these observations are consistent with the uptake and degradation of VLDL and apoE-3-enriched VLDL through the LDL receptor pathway, and not the LRP.

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Effects of exogenous recombinant apolipoprotein E-3 on the cellular metabolism of VLDL density subfractions and IDL

Data are means ± S.E. of four to nine different experiments. Binding, cell association, and proteolytic degradation of 125I-lipoproteins were determined as described under "Experimental Procedures." The lipoproteins (10 µg of protein/ml) were incubated with up-regulated human skin fibroblasts for 6 h at 37 °C in the absence or presence of exogenous recombinant apolipoprotein E-3 (4 µg/ml).

| Lipoprotein | Binding | Cell association | Degradation |
|-------------|---------|-----------------|-------------|
| -ApoE-3     | +ApoE-3 | -ApoE-3         | +ApoE-3     |
| VLDL-I      | 12 ± 1  | 31 ± 4          | 18 ± 4      | 91 ± 13 |
| VLDL-II     | 17 ± 6  | 45 ± 6          | 16 ± 4      | 108 ± 19 |
| VLDL-III    | 21 ± 6  | 54 ± 6          | 20 ± 6      | 117 ± 12 |
| IDL         | 26 ± 3  | 45 ± 6          | 81 ± 13     | 162 ± 29 |

Table II

Characteristics of the fibroblast receptor responsible for the cellular metabolism of human plasma VLDL and apoE-3-enriched VLDL proteolytic degradation data.

Data are means of duplicate dishes. The variation between the duplicate dishes was <10%. Up-regulated normal skin fibroblasts were prepared as described under "Experimental Procedures." Down-regulated fibroblasts were prepared by growing the cells for the last 48 h in medium containing human plasma LDL (160 µg of protein/plate). Receptor-negative cells were a gift of Dr. J. Breslow (The Rockefeller University, New York) and were used in previous studies (9, 10). The amount of lipoprotein used was 10 µg/ml, and proteolytic degradation was determined after 6 h of incubation at 37 °C as described under "Experimental Procedures."

| Fibroblasts | Lipoprotein | Additions | Up-regulation | Down-regulation |
|-------------|-------------|-----------|---------------|-----------------|
| Normal      | VLDL-III    | None      | 174           | 30              |
|             | VLDL-III    | apoE-3 (4 µg/ml) | 1335          | 291             |
|             | VLDL-III    | apoE-2 (4 µg/ml) | 133           | 44              |
|             | VLDL-III    | apoE-3 (4 µg/ml) + LDL (400 µg/ml) | 337           | 118             |
|             | LDL         | None      | 1176          | 191             |
| Receptor-negative | VLDL-III | None | 1668          | 191             |
|             | VLDL-III    | apoE-3 (4 µg/ml) | 14            | 18              |
|             | LDL         | None      | 10            | 15              |

Similar results were found for cell-associated labeled apoproteins (data not shown). In an additional experiment, the effects of increasing concentrations of total and individual C apoproteins on the apoE-3 (4 µg/ml)-stimulated VLDL metabolism were determined. Fig. 2 presents the proteolytic degradation data. ApoC and individual C proteins were already effective at a concentration of 1 µg/ml. At concentrations of 4 µg/ml, VLDL proteolytic degradation was reduced to <50% of the original values. With a further increase in apoC concentration in the medium to 8 µg/ml, the degradation of VLDL apoproteins was barely above that found when no apoE-3 was added. Again, apoC-I was more effective than the other C apoproteins.

The effects of mixtures of apoE-3 and total apoC at different protein concentration ratios on VLDL-I and VLDL-III are shown in Tables III and IV. Two observations can be made. First, at any concentration of apoE-3, the addition of increasing amounts of apoC causes higher degrees of inhibition of binding, association (data not shown), and degradation of VLDL. Second, at all different apoC concentrations, including the highest of 8 µg/ml, increasing amounts of apoE-3 cause stimulation of VLDL cell metabolism. This was especially prominent for the proteolytic degradation of VLDL-III; when at a concentration of 8 µg of apoC and 8 µg of apoE-3, the value was 7-8 times higher than that observed with VLDL-III alone. Yet, this value was less than one-tenth that observed with 8 µg of apoE-3 alone. A similar phenomenon was observed with VLDL-I.

In each experiment, labeled human plasma LDL was studied in parallel to VLDL. apoC was without any effect on the binding, cell association, and proteolytic degradation of LDL. Frequently, a slight increase in LDL metabolism was observed.

To determine whether other apoproteins exert a similar effect on apoE-3-stimulated VLDL cell metabolism, the experiments as detailed in Tables II and III were repeated with apoA-I and apoA-II. Table V presents the results with VLDL-I and VLDL-III (10 µg of protein/ml) incubated with 4 µg/ml exogenous recombinant apoE-3 and increasing concentrations (2-8 µg/ml) of apoA-I. apoA-I had no effect on the binding, cell association, and proteolytic degradation of VLDL. In addition, no effect of apoA-I on the cell metabolism of VLDL-I or VLDL-III was found in incubations without apoE-3 or at apoE-3 concentrations of either 2 or 8 µg/ml (data not shown). Similarly, apoA-II was without any effect on VLDL-I or VLDL-III cell metabolism either in the absence or presence of different concentrations of exogenous apoE-3 (data not shown). Neither apoA-I (Table V) nor apoA-II (data not shown) had any effect on LDL metabolism, although a tendency toward higher metabolic activity was noted.

In the previous experiments, apoC and apoE-3 were added together to incubation media that contained the 125I-labeled
Regulation by ApoE-3 and ApoC of VLDL Cellular Metabolism

Fig. 1. Effects of apoC (total), apoC-I, apoC-111, apoC-111-1, and apoC-111-2 on cellular binding and proteolytic degradation of apoE-3-enriched human plasma VLDL-I, VLDL-III, and LDL. The $^{125}$I-lipoproteins were used at a protein concentration of 10 $\mu$g/ml. Recombinant apoE-3 (4 $\mu$g of protein/ml) alone or in combination with total and individual apoC (4 $\mu$g of protein/ml) was added to aliquots of the lipoprotein-containing incubation medium. The control sample (0; open bars) did not contain added apolipoproteins. The media were transferred to culture dishes with up-regulated human skin fibroblasts for a 6-h incubation at 37 °C. Cellular binding and proteolytic degradation of $^{125}$I-lipoproteins were determined as described under “Experimental Procedures.”

Fig. 2. Effects of increasing concentrations of apoC (total), apoC-I, apoC-111, apoC-111-1, and apoC-111-2 on proteolytic degradation of apoE-3-enriched human plasma $^{125}$I-VLDL-II. Each 1 ml of the incubation medium contained 10 $\mu$g of VLDL-II protein, 4 $\mu$g of recombinant apoE-3, and 0, 1, 2, 4, or 8 $\mu$g of total or individual apoC proteins. Binding, cell association, and proteolytic degradation of $^{125}$I-VLDL were determined after 6 h of incubation with up-regulated human skin fibroblasts at 37 °C as described under “Experimental Procedures.”

Lipoproteins immediately prior to the beginning of the incubation. Since the order of introduction of the apolipoproteins to VLDL may have affected the results, the following experiment was carried out. ApoC or apoE-3 at a concentration of 2, 4, or 8 $\mu$g of protein/ml was added to incubation mixtures containing 10 $\mu$g/ml $^{125}$I-VLDL-II; and the mixtures were preincubated for 30 min at 37 °C in a thermostated water bath. After preincubation, apoE-3 or apoC was added at a concentration of 2, 4, or 8 $\mu$g/ml to mixtures preincubated with the opposing apoprotein (apoC or apoE-3); and the media were applied to duplicate cultured fibroblast dishes. Binding, cell association, and degradation were determined after 6 h of incubation. Fig. 3 presents the proteolytic degradation data. As is evident from Fig. 3, apoC inhibited the stimulated degradation of $^{125}$I-VLDL-II preincubated with apoE-3 at all three concentrations, whereas increasing concentrations of apoE-3 stimulated the activity of $^{125}$I-VLDL-II samples preincubated with different amounts of apoC. In fact, the shape of the curves demonstrates trends similar to those observed with mixtures of apoC and apoE-3. Although the results suggest that the metabolic activity of VLDL was lower when apoC was introduced first to the incubation media, this observation was not consistently seen at all apoC/apoE-3 ratios. The data for binding and cell-associated apoproteins (data not shown) were similar to those described in the legend to Fig. 3 for degradation.

To ascertain that apoC did not merely decrease VLDL-$^{125}$I-apoprotein binding, internalization, and degradation by the cells, we studied the effects of apoC on the ability of apoE-3-enriched VLDL-I, -II, and -III to down-regulate cellular sterol synthesis (Table VI). In agreement with our previous observations (9), VLDL were incapable of down-regulating cellular sterol synthesis in the absence of exogenous apoE-3, but considerable inhibition of the incorporation of [14C]acetate into sterols was observed when apoE-3 was added to the medium. The addition of apoC to the apoE-3-containing incubation medium caused a pronounced decrease in the ability of VLDL to induce down-regulation of cellular sterol synthesis.

The association of apoE-3 alone, apoC alone, and mixtures of apoE-3 plus apoC with VLDL was investigated by chromatography on agarose columns. In the first experiment, $^{125}$I-VLDL-III was incubated with unlabeled apoC (37 °C, 60 min), and VLDL was separated from unassociated apoC. At the three VLDL/apoC protein concentrations investigated (300 $\mu$g/60 $\mu$g, 300 $\mu$g/120 $\mu$g, and 300 $\mu$g/240 $\mu$g), the amount of radioactivity in VLDL was decreased only slightly by 3.9, 9.0, and 8.8%, respectively, indicating a maximum exchange of labeled endogenous apoC by unlabeled exogenous apoC of 10–20%. Of interest, the small amount of radioactivity associated with apoE in VLDL (3.0% of total radioactivity) remained unchanged after incubation with apoC and was 2.9, 3.2, and 2.8% of total radioactivity, respectively. In the second experiment, VLDL-I and VLDL-II (300 $\mu$g of protein) were incubated for 1 h at 37 °C with 120 $\mu$g of apoE-3 alone, 120 $\mu$g of apoC alone, or a mixture of 120 $\mu$g of apoE-3 plus 120 $\mu$g of apoC. After incubation, VLDL was separated from unassociated protein, and aliquots were taken for SDS-PAGE. As shown in Fig. 4, apoE-3 and apoC became associated with VLDL when incubated separately (lanes 2 and 3) or together (lane 4). Gel scanning yielded the following percent contribution of apoB, apoE, and apoC to total VLDL proteins in the four samples: 53.3, 2.1, and 44.9% for VLDL incubated with 0.9% NaCl (lane 1); 42.5, 18.7, and 32.3% for VLDL incubated with apoE-3 (lane 2); 41.9, 2.5, and 53.7% for VLDL incubated with apoC (lane 3); and 36.9, 10.7, and 45.5% for VLDL incubated with apoE and apoC (lane 4), respectively. Similar results were obtained with other VLDL density subfractions. In another experiment, association of apoA-I with VLDL-I and VLDL-II was determined (data not shown). Association of apoA-I with VLDL was evident, but apoA-I accounted for only 7.1% of total VLDL proteins, about one-third that of apoE-3 or apoC.
The capacity to inhibit cholesterol synthesis was prevented by the gel filtration procedure. The addition of apoC to an incubation system containing VLDL and apoE-3 caused a moderate decrease in the amount of total human apoC fraction, or both, at the protein concentrations indicated. Binding and degradation were determined after 6 h of incubation at 37 °C as described under "Experimental Procedures." Results for cell-associatedapoprotein (data not shown) showed trends similar to those for binding and degradation.

| Lipoprotein | ApoE-3 0 µg/ml | 2 µg/ml | 4 µg/ml | 8 µg/ml |
|-------------|----------------|---------|---------|---------|
|             | Binding: apoC  | Degradation: apoC |
|             | ng protein/mg cell protein | ng protein/mg cell protein/6 h |
| VLDL-I      | 0             | 13 ± 2  | 15 ± 2  | 13 ± 1  | 14 ± 1  | 22 ± ±  | 19 ± 4  | 14 ± 12 | 6 ± 6   |
| VLDL-I      | 2             | 21 ± 5  | 16 ± 1  | 15 ± 1  | 14 ± 1  | 218 ± 55| 67 ± 12 | 28 ± 3  | 15 ± 1  |
| VLDL-I      | 4             | 20 ± 7  | 16 ± 2  | 14 ± 1  | 15 ± 1  | 333 ± 58| 98 ± 17 | 48 ± 10 | 20 ± 4  |
| VLDL-I      | 8             | 36 ± 6  | 20 ± 3  | 17 ± 3  | 15 ± 1  | 436 ± 70| 185 ± 23| 70 ± 6  | 33 ± 7  |
| LDL         | 0             | 38 ± 4  | 34 ± 2  | 44 ± 4  | 42 ± 5  | 1387 ± 20| 1427 ± 24| 1460 ± 22| 1415 ± 110|

**TABLE IV**

Effects of exogenous apoC and apoE-3 on the binding and degradation of VLDL-I by cultured fibroblasts

Data are means ± S.E. of two experiments or of two experiments (Footnote a). Cells were incubated with 125I-VLDL-I (10 µg/ml), and the culture medium was supplemented with either exogenous recombinant apoE-3 or total human apoC fraction, or both, at the protein concentrations indicated. Binding and degradation were determined after 6 h of incubation at 37 °C as described under "Experimental Procedures." Results for cell-associated apoE-3 (data not shown) showed trends similar to those for binding and degradation.

| ApoE-3 0 µg/ml | Binding: apoC  | Degradation: apoC |
|---------------|----------------|-------------------|
|               | ng protein/mg cell protein | ng protein/mg cell protein/6 h |
| 0             | 14 ± 5         | 11 ± 6  | 14 ± 6  | 11 ± 3  | 14 ± 4  | 16 ± 3  | 15 ± 6  | 13 ± 1  |
| 2             | 36 ± 10        | 20 ± 5  | 18 ± 6  | 23°     | 551 ± 209| 90 ± 27 | 82 ± 13 | 27°     |
| 4             | 36 ± 19        | 24 ± 7  | 21 ± 7  | 19 ± 7  | 597 ± 230| 370 ± 45| 173 ± 12| 46 ± 7  |
| 8             | 55 ± 10        | 31 ± 11 | 25 ± 8  | 25 ± 11 | 1240°   | 370 ± 19| 309 ± 33| 110 ± 14|
TABLE VI
Effects of apoproteins E-3 and C on regulation of cellular sterol synthesis by VLDL subfractions

| Lipoprotein | Incorporation of [14C]acetate into sterols | Controls | +apoE-3 | +apoE-3 + apoC % of control |
|-------------|-------------------------------------------|---------|---------|-----------------------------|
| VLDL-I      | 105.0 ± 4.0                               | 52.3 ± 4.1 | 93.3 ± 5.2 |                          |
| VLDL-II     | 110.0 ± 1.5                               | 44.7 ± 0.7 | 73.7 ± 0.9 |                          |
| VLDL-III    | 107.6 ± 8.8                               | 27.7 ± 1.2 | 63.3 ± 5.8 |                          |
| LDL         | 24.0 ± 0.6                                | 22.8 ± 1.0 | 20.3 ± 0.7 |                          |

Fig. 4. SDS-PAGE of VLDL-II incubated at 37 °C for 60 min with or without exogenous apoproteins followed by gel filtration on agarose columns. Lane 1, VLDL incubated without exogenous apoproteins; lane 2, VLDL incubated with exogenous apoE-3 (300 μg of protein/120 μg of protein); lane 3, VLDL incubated with exogenous apoC (300 μg of protein/120 μg of protein); lane 4, VLDL incubated with exogenous apoE-3 and exogenous apoC (300 μg of protein/120 μg of protein). The gels were loaded with an equal amount of cholesterol (15 μg).

VLDL-I and VLDL-II (4:10 protein ratio), and VLDL-[125I]-apoE-3 was separated from free [125I]-apoE-3. Then, VLDL-[125I]-apoE-3 was incubated for 1 h at 37 °C without or with unlabelled apoC (total), apoC-I, and apoC-III; and the mixtures were gel-filtered on agarose columns (Fig. 6). ApoC displaced only 1.5% of [125I]-apoE-3 associated with VLDL.

Studies with Endogenous apoE—The possibility that apoC may have different effects on endogenous apoE-3 than those described above for exogenous apoE-3 was evaluated with two apoE-containing lipoproteins that exhibit apoE-dependent cell metabolism: total VLDL from a subject with borderline high plasma triglyceride levels (~200 mg/dl) and IDL from a normotriglyceridemic subject with profile E-4/3. For each lipoprotein, we determined the effects of adding increasing concentrations (2–12 μg/ml) of apoC on the binding, cell association, and degradation of the lipoprotein (10 μg of protein) in the absence or presence of exogenous apoE-3 (4 μg/ml). The results of these experiments are shown in Fig. 7. The data demonstrate an apoC-dependent inhibition of cell metabolic activities for VLDL and IDL that was similar for particles enriched or not enriched with exogenous apoE-3. Yet, for most experimental data points, especially IDL, the effects of apoC were marginally less pronounced for particles not enriched with apoE-3. To ascertain that the effects of apoC on endogenous apoE are not due to displacement of apoE from the particles, IDL was incubated at 37 °C for 60 min without or with apoC (300 μg of protein/240 μg of protein) and then isolated by gel filtration on an agarose column. No apparent displacement of endogenous apoE was found (Fig. 5 and 6).
FIG. 7. Effects of exogenous apolipoprotein C on binding, cell association, and proteolytic degradation of human plasma

\[ ^{125}\text{I}-\text{VLDL}, ^{125}\text{I}-\text{IDL}, \text{apoE-3-enriched}^{125}\text{I}-\text{VLDL}, \text{and} \text{apoE-3-enriched}^{125}\text{I}-\text{IDL}. \]

The \(^{125}\text{I}\)-lipoproteins were used at a protein concentration of 10 \(\mu\text{g/ml}\). apoE-3 (4 \(\mu\text{g of protein/ml}\)) was added to one-half of the samples. The incubation was carried out in the absence or presence of exogenous apoC at the protein concentrations indicated. The effects of apoC are presented as the percent of the values obtained in samples without exogenous apoC. Binding, cell association, and proteolytic degradation of \(^{125}\text{I}\)-VLDL and \(^{125}\text{I}\)-IDL proteins were determined after 6 h of incubation at 37 °C as described under "Experimental Procedures." The values for binding, cell association, and degradation of the different preparations in the absence of exogenous apoC were: VLDL, 53.2, 14.9, and 28.6% of total gel proteins in lane 1 and 42.5, 11.2, and 41.1% in lane 2, respectively. Gels were loaded with 15 \(\mu\text{g}\) of protein for each sample.

8). The apoE/apoB absorbance ratio determined by gel scanning was 0.28 in IDL preincubated without apoC and 0.27 in IDL preincubated with apoC. The corresponding values for apoC/apoB absorbance ratios, in contrast, increased from 0.54 to 0.97, indicating considerable association of added apoC to IDL without a measurable decrease in apoE content.

IDL contains two proteins that may serve as ligands for the LDL receptor: apoB-100 and apoE. To determine whether apoC inhibits interaction of IDL with the receptor via apoB-100 or apoE, or both, the effects of apoC on IDL cell metabolism were determined after preincubation (37 °C, 60 min) of the lipoprotein with a monoclonal antibody that specifically blocks receptor interactions by apoE (antibody 1D7) or an antibody that is specific for apoB-100 (antibody 4G3). The results of this experiment are shown in Fig. 9. For comparison, Fig. 9 also includes the results obtained with IDL alone, in the absence of antibodies. In the presence of antibody 4G3, apoC was as effective or more effective as an inhibitor of IDL catabolism by the cells as compared to IDL alone. In contrast, in the presence of antibody 1D7, the inhibition of IDL degradation was only one-fourth to one-third that observed with IDL alone or with IDL and antibody 4G3. The difference was especially pronounced at low, more physiological concentrations of apoC. It thus appears that C apoproteins exert their inhibitory effects on IDL metabolism predominantly through apoE-dependent interactions, but not apoB-100-dependent processes.

DISCUSSION

Lipoprotein particles along the apoB-100 cascade may follow one of two metabolic routes: interaction with endothelium-bound lipases and delivery of triglyceride fatty acids to tissues or interaction with cellular receptors followed by internalization and lysosomal degradation of the lipoproteins (3, 35). The large apoB-100 lipoproteins (VLDL) contain, at their surfaces, protein molecules that are responsible and essential for either metabolic route. These are apoC-II, which are necessary for activation of lipoprotein lipase-mediated triglyceride hydrolysis (36), and apoB-100 and apoE-3 (or apoE-4), which serve as ligands for interactions of lipoproteins with the LDL receptor and other receptors (2, 8, 14–16). Thus, theoretically, the interaction of VLDL with lipoprotein lipase

![Fig. 7: Effects of exogenous apolipoprotein C on binding, cell association, and proteolytic degradation of human plasma lipoproteins.](image-url)

![Fig. 8: SDS-PAGE of human plasma IDL incubated for 60 min at 37 °C without (lane 1) and with (lane 2) exogenous apoC (300 μg of protein/240 μg of protein) followed by chromatography on agarose columns.](image-url)

![Fig. 9: Inhibition of \(^{125}\text{I}\)-IDL proteolytic degradation by apolipoprotein C in presence of monoclonal antibodies 1D7 (○) and 4G3 (□). \(^{125}\text{I}\)-IDL (10 μg of protein/ml) was preincubated with either monoclonal antibody 1D7 or 4G3 (10 μg of protein/ml) for 60 min at 37 °C. ApoC at the indicated protein concentrations was added to aliquots of the two incubation mixtures, and the media were transferred to culture dishes containing up-regulated human skin fibroblasts. Binding, cell association, and proteolytic degradation of the \(^{125}\text{I}\)-IDL proteins were determined after 6 h of incubation at 37 °C as described under "Experimental Procedures." Shown are the degradation data as percent of the activity in samples containing the monoclonal antibodies, but not apoC. For comparison, also included are the effects of apoC on IDL degradation in the absence of antibodies (None). Antibody 4G3 reduced the binding of \(^{125}\text{I}\)-IDL from 69 to 0 ng of protein/mg of cell protein, the cell association from 174 to 0, and the degradation from 749 to 11 ng of protein/mg of cell protein/6 h. Antibody 1D7 was without effect on \(^{125}\text{I}\)-IDL metabolism.](image-url)
or cell receptors should occur at random. Yet, many studies demonstrated that intact VLDL, especially large-size and light VLDL-I and VLDL-II, possess a very limited capacity to interact with cell receptors (4-9). Apparently, the ligand-binding domains of apoB-100 and apoE-3 (or apoE-4) in VLDL are not available for interaction with cellular receptors either because of inadequate exposure of the proteins to the receptor or because of inhibition of receptor interaction by other apoproteins, e.g. apoC. This study was carried out to critically examine the latter mechanism.

Several studies have demonstrated that apoE-dependent uptake of rat chylomicrons and VLDL by perfused rat liver is inhibited by individual and total human and rat apoC molecules (18, 19, 37). In two studies, the apoE/apoC ratio was reported to be without effect on the liver uptake of chylomicrons and their remnants (19, 37). This study was carried out with human lipoproteins in cultured human skin fibroblasts, where the predominant receptor for the uptake of apoB-100-and apoE-containing lipoproteins is the LDL receptor (9, 10). When using VLDL density subfractions from normolipidemic human subjects that lack the capacity to interact with the receptors unless enriched with exogenous apoE-3, we were able to precisely determine the stoichiometry of the effects of combinations of apoC and apoE on the binding, internalization, and degradation of the lipoproteins. Our data unequivocally demonstrate that the ratio of apoE to apoC determines the degree of cell metabolism of VLDL. At any apoE concentration, increasing the apoC concentration in the incubation mixture caused progressive inhibition of the cell metabolism of VLDL. Conversely, increasing apoE-3 concentrations enhanced VLDL metabolism at all apoC concentrations. It is interesting to note, that in the cell culture system, the order of introducing apoC and apoE to VLDL had no or only a minor effect on the resulting metabolic activity. Thus, VLDL that had already acquired apoE was inhibited by apoC, whereas VLDL that acquired apoC was stimulated by apoE.

In experiments on perfused rat liver (18, 19, 37), it was impossible to determine whether the inhibitory effect of apoC is on the LDL receptor or on additional receptor(s) specific for apoE, e.g. LRP (38-40). Previous studies from this laboratory (9, 10) and this study (Table II) show that the metabolism of apoE-3-enriched VLDL and IDL in cultured human fibroblasts occurs through the LDL receptor (9, 10). Therefore, this study proves that apoC inhibits the metabolism of apoE-enriched human VLDL by the LDL receptor pathway. apoC was recently shown to inhibit the interactions of apoE-enriched rabbit β-VLDL with the LRP (41). This effect was especially pronounced with apoC-1 (42). Our results, however, differ from those of Kowal et al. (41) and Weisgraber et al. (42) in several respects. First, we show a very pronounced effect of apoC on VLDL metabolism through the LDL receptor pathway, whereas in the assays used by Kowal et al. and Weisgraber et al., there are minimal effects, if any, of apoC on the binding of apoE-3 to the LDL receptor. Second, in our studies, although apoC-I was most effective, all other C apoproteins including apoC-III-I and apoC-III-2 were also effective. Third, the concentrations of total and individual C apoproteins used by us as well as the concentration of apoE-3 are considerably lower than those used in the other studies. Last, we observed a partial recovery of the cell metabolism of VLDL upon addition of increasing concentrations of apoE-3 to an apoC-inhibited system. These differences perhaps reflect one or more of the following differences between the studies: (a) methodology (uptake and degradation versus cholesterol esterification); (b) lipoproteins (human VLDL as compared to rabbit β-VLDL); (c) cells (normal skin fibroblasts versus LDL receptor-negative cells); and (d) receptors (LDL receptor versus LRP).

Another aspect of this study was the question of whether apoC similarly affects apoE-3 that is incorporated into lipoproteins by an exogenous route as compared to endogenous apoE-3 associated with particles isolated from the plasma. The results reported here demonstrate a similar, although not identical effect of exogenous apoC on both endogenous and exogenous E apoproteins. This observation indicates that the inhibition of cell metabolism of VLDL and IDL by apoC is not an artifact of added exogenous apoE-3, but is of physiological significance. Yet, it should be pointed out that, in this as well as in previous studies (18, 19, 37, 41, 42), apoC is added by an exogenous route. This route of adding apoC to lipoproteins may result in different behavior of the apoC molecules at the lipoprotein surface as compared to endogenous apoC. It is of significance to note in this context that the amounts of apoC necessary to produce a measurable inhibition of cellular metabolism of VLDL and IDL (1-4 μg/10 μg of lipoprotein) are one-fourth or equal to the amounts of apoC present. In contrast, the addition of similar amounts of exogenous apoE-3 without loss of endogenous apoC causes a dramatic stimulation of the same metabolic activities (9, 10).

The mechanisms by which C apoproteins exert their inhibitory effects on apoE-dependent cell metabolism of lipoproteins were partially elucidated in this study. This effect was specific for C apoproteins that associate with VLDL and IDL and was not observed with apoA-I and apoA-II, proteins that poorly associate with VLDL (16). Although apoC-I was most effective, all apoC molecules (C-I, C-II, C-III-1, and C-III-2) were inhibitory. apoC molecules do not associate and had no effect on LDL metabolism in the same cells. Thus, direct interaction of apoC with the receptor can be ruled out as a mechanism of interference with the uptake of lipoproteins. Moreover, the observations with IDL and monoclonal antibod-
indicate that the transfer of apoE-3 and apoC molecules to and from triglyceride-rich lipoproteins exerts profound positive and negative effects, respectively, on their capacity to interact with cell receptors and therefore has a major regulating effect on plasma triglyceride transport and remnant removal processes.

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