Inhibitory Effects of Specific Apolipoprotein C-III Isoforms on the Binding of Triglyceride-rich Lipoproteins to the Lipolysis-stimulated Receptor*

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Apolipoprotein (apo)C-III§ has been shown to modulate the plasma triglyceride (TG) concentrations. ApoC-III is a 79-amino acid polypeptide in the plasma that readily exchanges between the different lipoprotein fractions (1). In subjects with normal plasma lipid levels, 30% of apoC-III is distributed among triglyceride-rich lipoproteins (TGRL), i.e. chylomicrons and very low density lipoprotein (VLDL), and about 60% is bound to high density lipoprotein (1).

Biochemical studies showed that apoC-III is an inhibitor of lipase activity (2–4), and it was established in the early 1980's that supplementation of small chylomicrons or apoE-containing triglyceride emulsions with apoC-III inhibited their uptake in perfused rat livers (5–7). An inhibitory effect was also observed with apoC-I and C-II, but was less pronounced and not consistently observed (5, 6). Genetic linkage map studies suggest that polymorphism of apoC-III is associated with hypertriglyceridemia (8–11). Human subjects with defects of apoC-III and apoA-I genes had low plasma TG levels, attributed primarily to an accelerated clearance of TGRL (12, 13). In transgenic mice, suppression of apoC-III expression by homologous recombination increased the removal rate of chylomicron remnants and thus reduced the postprandial triglyceridemia (14). In contrast, overexpression of apoC-III increased the plasma concentrations of TG in a dose-dependent manner, and thereby caused a reduction of the catabolic rate of TGRL (15, 16).

The mechanisms through which apoC-III regulates the catabolism of TGRL are complex and involve both lipolysis and cellular uptake of these particles. Tissue lipase activities of apoC-III overexpressor mice were not different when compared with those of normal mice, nor were their VLDL defective as substrates for lipolysis (16). The hypertriglyceridemia of transgenic apoC-III overexpressors was corrected when these animals were cross-bred with mice overexpressing apoE, consistent with the hypothesis that apoC-III interferes with an apoE-mediated process (17). ApoE has been shown to mediate the binding of TGRL to heparan sulfate proteoglycans (for review, see Ref. 18), and it was recently reported that the VLDL isoforms of apoC-III regulate the binding of heparin-Sepharose, suggesting that the excess apoC-III inhibits their binding to heparan sulfate proteoglycans on endothelial cells (19). Because most of the plasma lipolytic enzymes, i.e. lipoprotein and hepatic lipases, are bound to heparan sulfate proteoglycans, this docking defect of apoC-III enriched TGRL could subsequently reduce the rate of lipolysis.

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§ The abbreviations used are: apo, apoprotein; BSA, bovine serum albumin; FFA, free fatty acid(s); LDL, low density lipoprotein; LRP, LDL-receptor related protein; LSR, lipolysis-stimulated receptor; TG, triglyceride; TGRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.
It is also widely accepted that apoE serves as the primary ligand for the cellular uptake of TGRL. It is therefore possible that apoC-III’s hypertriglyceridemic effect is due in part to a decreased rate of cellular uptake of TGRL by liver cells. ApoC-III has been shown to decrease the binding of VLDL to the low density lipoprotein (LDL)-receptor expressed in human fibroblasts (20, 21). This inhibitory effect was observed not only with apoC-III, but also with apoC-I and C-II, apoC-I being the most effective. In addition, apoC-I, but not apoC-III, inhibits the binding of apoE-enriched β-VLDL to the LDL-receptor related protein (LRP) (22).

We have characterized a third candidate receptor, the lipolysis-stimulated receptor (LSR), which is activated by free fatty acids (FFA), and is distinct from the LDL-receptor and the LRP (23, 24). Similar to the LDL receptor, the LSR binds both apoB and apoE (23, 24). However, unlike the LDL receptor, its affinity is maximal for TGRL (23, 24). Furthermore, the affinity for the LSR of VLDL isolated from subjects with type III hyperlipidemia (apoE-2/2 phenotype) is decreased when compared with that isolated from normal individuals (24). The physiological significance of the LSR as candidate chylomicron remnant receptor has remained controversial. Indeed, during in vitrumaximal activation of the LSR requires the addition of FFA at concentrations that exceed albumin binding capacity and are therefore at levels not normally observed under physiological conditions. It has been shown that FFA bound to albumin at the physiological molar ratio of 1 to 1 are sufficient to consistently activate the LSR. However, under these conditions, the receptor activation is only 10% of the maximal activity. We therefore speculate that under physiological conditions, the LSR is not activated primarily by FFA circulating in plasma bound to albumin, but rather by the FFA generated at the site of lipolysis. When intestinally-derived lipoproteins arrive in the liver sinusoid and are hydrolyzed by hepatic lipase (25) and possibly also lipoprotein lipase (26, 27), it is likely that relatively high local concentrations of FFA are produced. Consistent with the hypothesis that LSR participates in the clearance of chylomicrons is the finding that the apparent number of LSR expressed in rat liver plasma membranes strongly (p < 0.001) and negatively correlates with plasma TG concentrations measured in the physiological postprandial stage of these animals (28). To further examine the potential physiological significance of the LSR, we have now characterized the effect of apoC-III on the binding of the different LSR ligands. Data presented here show that apoC-III is a potent and specific inhibitor of TGRL, but not LDL binding to LSR.

EXPERIMENTAL PROCEDURES

Materials

Na[125I] was purchased from Amersham (Les Ulis, France). Oleic acid (0–1008), bovine serum albumin (BSA) (A2153), neuraminidase from Clostridium perfringens (N2576), leupeptin, and benzamidine were obtained from Sigma (St. Quentin, Fallavier, France). Dulbecco’s modified Eagle’s medium, penicillin-streptomycin, glutamine, and fetal bovine serum were purchased from Life Technologies, Inc. (Eragny, France). Sodium succinate was purchased from FBA Pharmaceutical (Westhaven, CT).

Methods

Animals—Male Sprague-Dawley rats (Janvier Breeding Center, Le Genest-St.-Isle, France) were housed in an animal care facility with ad libitum access to food and water, except for 15 h prior to liver isolation when they were offered only water. For isolation of livers, animals were anesthetized with ether, and the livers were perfused through the portal vein with ice-cold Hepes-buffered saline solution (5 mM Hepes, 150 mM NaCl, 2 mM EDTA, pH 7.4). The livers were then immediately excised, and used for the preparation of liver plasma membranes.

Preparation of Lipoproteins—All plasma samples used for lipoprotein isolation were supplemented with 0.01 volumes of the following inhibitor mixture: leupeptin (1 mg/ml), benzamidine (10 mg/ml), and sodium azide (300 mM) prior to lipoprotein isolation. VLDL (density d < 1.006 g/ml) and LDL (1.025 < d < 1.055 g/ml) were prepared from plasma of overnight fasted human subjects (29). Chylomicrons were prepared by ultracentrifugation (30,000 rpm, 1.5 h, 10 °C). Beckman T50.2 rotor of plasma drawn from human subjects 2 h after a breakfast consisting of 3 croissants with butter and jam, and 250 ml of whole milk. Contaminating albumin was removed from the chylomycin fraction by incubation for 30 min at room temperature with an equivalent volume of swollen Blue Sepharose CL-6B gel (Pharmacia Biotech, Orsay, France). This was verified by SDS-PAGE gel electrophoresis separation of chylomicron protein on 4–18% gradient gels. Image analysis (ImageMaster, Pharmacia Biotech) of Coomassie Blue-stained gels indicated that after this treatment, albumin represented less than 1% of total protein. All lipoproteins were stored in the dark at 4°C under N2 for use within 4 days of their isolation.

Isolation of ApoC Peptides—Human apoC-III was synthetically prepared using solid phase methodology (30) as described previously (31), and provided the source of non-sialylated apoC-III. The apoC-III was stored at −20°C as a lyophilized powder and was dissolved into 0.1 M phosphate-buffered saline, pH 7.4, on the day of use. Human apoC-III preparations containing 1 and 2 sialyl residues were purified from plasma obtained from the local blood bank, as described (32), except that the sialidase digestion was conducted by gel chromatography using a Superdex 75 HR10/30 column (Pharmacia Biotech) for gel filtration, and a Bio-Scale Q20 column (Bio-Rad, Ivry-Sur-Seine, France) for anion-exchange.

Purity of apoC-III and C-III preparations was verified by isoelectric focusing on PhastGel IEF polyacrylamide gels (Pharmacia Biotech) using a broad separation range of pI 3–10 expanded at the 3–6 end. Image analysis of these gels stained with Coomassie Blue showed the purity of the apoC-III and C-III preparations to be >96%. Apo-C-II was purified from human plasma as described (32), and stored at concentrations >1 mg/ml at −80°C until the day of use.

Desialylation of ApoC-III—Purified apoC-III was desialylated using a method modified from Morell et al. (33). Briefly, apoC-III was incubated at 37°C for 16 h in a shaking water bath with 2 units/mg neuraminidase in phosphate-buffered saline, pH 7.4, containing 0.1% (w/v) CaCl2 and 0.01% of protease inhibitor mixture. The buffer of this mixture was then exchanged for 10 mM Tris containing 2 mM EDTA, pH 8.0, using a Fast Desalting HR 10/10 column (Pharmacia Biotech), prior to reisolation of the apoC-III by anion exchange employing a Mono Q PC 1.6/5 SMART column (Pharmacia Biotech) using a gradient of 0–250 mM NaCl. Purity of the recovered desialylated apoC-III was verified by isoelectric focusing on PhastGel IEF polyacrylamide gels.

Radiolabeling—Chylomicrons, VLDL, and LDL were radioiodinated by Bilheimer’s modification of McFarlane’s method (34), as described previously (35). 125I-Labeled lipoproteins were used within 2 days of preparation, and 125I-LDL was filtered (0.2 µm filter, Gelman, Ann Arbor, MI) immediately prior to use. Specific activities of 125I-labeled lipoproteins preparations ranged between 102 and 192 cpm/ng protein.

In experiments designed to determine the precise amount of the different apoC-III isoforms associated with TGRL, apoC-III preparations were iodinated using IODO-BEADS (Pierce, Arnnières, France) according to manufacturer’s instructions, and used within 1 week of preparation.

Cells—Primary cultures of rat hepatocytes were prepared as described previously (28), and used 48 h after plating in 36-mm dishes. The cells were precultured at 37°C for 30 min in Dulbecco’s modified Eagle’s medium containing 0.2% (w/v) BSA, 5 mM Hepes, 2 mM CaCl2, and 10 ng/ml recombinant mouse leptin.2 125I-Labeled lipoproteins were used within 2 days of preparation, and 125I-VLDL was filtered (0.2 µm filter, Gelman, Ann Arbor, MI) immediately prior to use. Specific activities of 125I-labeled lipoproteins preparations ranged between 102 and 192 cpm/ng protein.

In experiments designed to determine the precise amount of the different apoC-III isoforms associated with TGRL, apoC-III preparations were iodinated using IODO-BEADS (Pierce, Arnnières, France) according to manufacturer’s instructions, and used within 1 week of preparation.

Rat Liver Plasma Membrane Isolation—Plasma membranes were prepared from livers of fasted rats as described (28, 36), stored in the dark at 4°C under N2 in the presence of the inhibitor mixture, and used within 7 days of their preparation.

2 Results to be reported elsewhere show that recombinant mouse leptin at physiological concentrations (10–20 ng/ml) increase activity of LSR in primary cultures of rat hepatocytes, and is currently used as a supplement in the incubation media (A. A. Troussard, F. T. Yen, and B. E. Bihain).
Preparation of Lipoproteins Supplemented with ApoC—Chylomicrons, VLDL, or LDL (0.15 mg protein) and apoC-II or C-III (0.15 mg) were mixed and incubated at 37 °C for 30 min in phosphate-buffered saline, pH 7.4, containing 2 mM EDTA and 3 mM sodium azide (buffer A) in a volume not exceeding 500 μl. After cooling for 2 min on ice, the sample was applied to a 50 mm × 10-mm gel filtration column containing Sepharose CL-4B (Pharmacia Biotech) pre-equilibrated in buffer A. The sample was eluted at a flow rate of 500 μl/min, and collected in 250-μl fractions. Measurements of the 125I radioactivity and absorbance at 280 μm were used to separate the lipoprotein-bound from the free apoC fractions. All lipoprotein fractions were collected onto ice within 3–7 min, and used immediately. No difference between the 125I to TG ratio of VLDL were found before and after such treatment, indicating that incubation with the apoC peptides did not cause major losses of radiolabeled endogenous apoproteins. Samples were also applied on 4–25% SDS gradient polyacrylamide gels, which were then stained with Coomassie Blue. Analysis of these gels showed that the gel filtration chromatography step did not modify the TGRL apolipoprotein profile. It must be emphasized, however, that the gel filtration step is critical to observe the specificities regarding both: 1) the type of ligand affected, i.e. the lack of effect of apoC-III on LDL binding to LSR, and 2) the type of C apoprotein, i.e. the lack of effect of apoC-II on the binding of TGRL to LSR. Indeed, when the gel filtration chromatography step was omitted prior to testing, all apoC fractions inhibited LDL binding to the LSR. We speculate that this inhibitory effect proceeds through mechanisms distinct from those responsible for the apoC-III specific inhibition of TGRL binding to LSR, and most probably involves the apoC’s that are free in solution.

Measurement of LSR Activity in Isolated Rat Liver Plasma Membranes—LSR activity was measured under conditions that maximally activated the receptor (28). All buffers were prepared without Ca2+, and were supplemented with 2 mM EDTA, to suppress the activities of the LDL receptor (37) and the LRP (38). Briefly, aliquots of membranes (100 μg of protein/tube) were incubated at 37 °C for 30 min in the absence or presence of 1000 μg oleate adjusted to a final volume of 250 μl with 0.1 M phosphate buffer containing 350 mM NaCl and 2 mM EDTA, pH 8.0 (buffer B). The membranes were then washed by 3 series of centrifugation (35,000 × g, 15 min, 4 °C) and resuspension into 250 μl of buffer B by brief sonication (power 1.0, 90% pulse, 5 s); at the final wash, the membrane pellets were resuspended in 150 μl of buffer B. After this, the membranes were incubated at 4 °C for 60 min with the different preparations of apoC-supplemented lipoproteins in a final volume of 250 μl. Membrane-bound 125I-lipoproteins were separated from unbound 125I-lipoproteins by layering a 200-μl aliquot over a 600-μl cushion of 5% (w/v) BSA in buffer B, and centrifuging (35,000 × g, 20 min, 4 °C). After careful aspiration of the supernatants, the bottoms of the tubes containing the membrane pellets were cut and counted for radioactivity. LSR activity is expressed as binding in the absence (circles) or presence (squares) of oleate. Each point represents the mean of duplicate determinations.

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FIG. 1. Effect of apoC-III on the binding of 125I-chylomicrons, 125I-VLDL, and 125I-LDL to LSR in rat liver plasma membranes. Aliquots (100 μg of protein) of rat plasma membranes were incubated at 37 °C for 30 min with 1000 μg oleate, and then washed 3 times. Simultaneously, 0.15-mg aliquots of 125I-chylomicrons (specific activity, 145 cpm/ng), 125I-VLDL (specific activity, 102 cpm/ng), or 125I-LDL (specific activity, 121 cpm/ng) were incubated at 37 °C for 30 min in the absence (closed symbols) or presence (open symbols) of 0.15 mg of synthetic apoC-III in buffer A. Unbound apoC-III was removed by gel filtration as described under “Methods.” Lipoproteins not supplemented with apoC-III were treated in the same manner as those containing added apoC-III. The membranes were then incubated at 4 °C for 1 h with the indicated concentrations of 125I-chylomicrons (A), 125I-VLDL (B), or 125I-LDL (C) in buffer B. In all cases, the concentrations represents the endogenous radiolabeled protein and excludes the added apoC-III. Membrane-bound lipoproteins were separated from unbound lipoproteins by sedimenting the membranes through a 5% (w/v) BSA cushion. The bottom of the tube containing the membrane pellet was cut and counted for radioactivity. LSR activity is measured using an enzymatic kit (Boehringer Mannheim, Meylan, France).

RESULTS

We first tested the effect of apoC-III on the binding to LSR of the different lipoprotein fractions that had previously been identified as potential ligands for this candidate receptor (23, 24). Radiolabeled chylomicrons, VLDL, or LDL were supplemented with synthetic apoC-III, and then separated by gel filtration chromatography to remove unbound apoprotein. Fig. 1 shows that both chylomicrons and VLDL, but not LDL, supplemented with apoC-III (open squares) displayed, in the presence of oleate, a decreased binding to rat liver plasma membranes when compared with those not supplemented with apoC-III (Fig. 1, closed squares). In membranes incubated without oleate, the binding of chylomicrons, VLDL, and LDL was less than 10% of that measured in the presence of oleate. ApoC-III had no detectable inhibitory effect on this nonspecific binding (closed versus open circles). Due to the dilution effect caused by passing the apoC-III supplemented lipoprotein through gel filtration columns, the concentrations of lipoproteins used in these experiments did not exceed 20 μg/ml, a level well below that required to achieve saturation of the receptor (28). Therefore, data of Fig. 1 differ from the typical saturation curves previously reported for the binding of these lipoproteins to LSR (28). We chose not to test at concentrations greater than 20 μg/ml since this would require the addition of a concentration step which would have altered the apoprotein profile of the apoC-III-supplemented TGRL.

We next sought to determine the amount of apoC-III that bound to TGRL or LDL under these experimental conditions. Unlabeled VLDL or LDL were supplemented with 125I-apoC-III...
exactly as described for the experiments shown in Fig. 1. After this, 125I-apoC-III bound to lipoprotein was separated from free 125I-apoC-III by gel filtration chromatography. Under these conditions, a maximum of 10% of added 125I-apoC-III associated with VLDL, while 90% remained in solution. In contrast, only 0.3% of 125I-apoC-III was associated with LDL, while >99% remained unbound (data not shown). This low level of apoC-III bound to LDL could thus explain the lack of significant effect of apoC-III on LDL binding to LSR.

We next tested, in experiments similar to those described in Fig. 1, whether the inhibitory effect of apoC-III on the binding of TGRL to LSR was specific for this apolipoprotein. Fig. 2 shows that supplementation of chylomicrons and VLDL with apoC-II had no significant effect on the binding of these lipoproteins to rat liver membranes incubated with oleate. This lack of effect of apoC-II was not due to a defect of binding of apoC-II to TGRL. Indeed, analysis of Coomassie blue-stained gels showed enrichment of VLDL after incubation with either apoC-II (Fig. 3, third lane from left) or apoC-III (Fig. 3, fourth lane from left).

The next objective was to establish if the specific inhibitory effect of apoC-III on the binding of TGRL to LSR expressed on the plasma membranes caused a decrease of the uptake and degradation of these particles in intact cells. 125I-VLDL supplemented or not with apoC-III was incubated with primary cultures of rat hepatocytes in the presence and the absence of oleate. ApoC-III caused a significant decrease in oleate-induced binding (46%) of VLDL (Fig. 4, panel A). As a consequence, uptake and degradation of 125I-VLDL were reduced to a similar or slightly greater extent (Fig. 4, panels B and C, respectively).

In experiments described thus far, a synthetic nonsialylated form of human apoC-III was used. However, under physiological conditions, apoC-III exists as 3 different isoforms designated as C-IIIα, C-IIIβ, and C-IIIγ, where the subscript indicates the number of sialic residues bound to each of the peptides. We subsequently undertook the purification of the different isoforms of apoC-III to determine which was the most effective inhibitor of LSR. As shown in Fig. 5, apoC-IIIβ showed an inhibitory effect similar to that for nonsialylated apoC-III0 (Fig. 1). However, apoC-IIIγ demonstrated a diminished ability to inhibit VLDL binding to LSR. This decreased inhibitory effect was not caused by a reduction in the binding of apoC-IIIγ to VLDL. Indeed, partitioning experiments showed that the amount of 125I-apoC-IIIγ bound to VLDL was 2-fold greater as compared with the other apoC-III isoforms (Fig. 5, inset). To determine whether the number of sialic acid residues were an important determinant of the degree of inhibition, apoC-III2 was treated with neuraminidase. The top panel of Fig. 6 shows that treatment of apoC-III2 with neuraminidase altered its migration after isoelectric focusing electrophoresis to a level comparable with nonsialylated apoC-III0. This desialylation significantly enhanced the ability of the apoC-III2 to inhibit VLDL binding to a level similar to that observed with apoC-III0 (Fig. 6, bottom panel). Taken together, these data indicate that apoC-III supplementation of TGRL significantly inhibits their binding to LSR, and that this inhibition is dependent on the degree of sialylation of this apoprotein.

**DISCUSSION**

The data reported in this study show that the enrichment of VLDL or chylomicrons with apoC-III significantly decreased their ability to bind to LSR. This inhibition was specific for TGRL and was not found with apoC-II. Based on partitioning experiments, up to 10% of the added apoC-III remained associated with VLDL after gel filtration. In normal human subjects, apoC-III represents about 30% of VLDL protein mass.
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Therefore, supplementation experiments described here caused a 30–40% increase in VLDL apoC-III. This led to a 40% inhibition of its binding to LSR. ApoE was present on VLDL supplemented with apoC-III (Fig. 3). Thus, the inhibitory effect was not caused by a massive displacement of apoE, but rather by a masking or a change in the conformation of the apoE that can serve as ligand for LSR (28). The possibility that apoC-III also causes a conformational change in apoB must be considered (40). Indeed, the lack of effect of apoC-III on LDL binding to LSR does not rule out this hypothesis, because it was primarily due to apoC-III inability to associate in significant amounts to purified LDL. It is also possible that apoC-III inhibitory effect occurs through interactions with other apolipoproteins. Among which is apoA-I that is present on most TGRL and remains to be tested for its ability to bind the LSR.

The inhibition of the binding of TGRL to LSR by apoC-III is dependent on the number of sialic residues present on the apolipoprotein. Despite a greater ability of apoC-III to associate with VLDL, this isoform was a less efficient inhibitor as compared with nonsialylated apoC-III or C-III. Therefore we speculate that the charge provided by the additional sialic acid residue interferes with the ability of apoC-III to modulate apoE and possibly apoB binding to LSR. This observation opens a new perspective regarding the regulation of TG removal rate by apoC-III. On the basis of data presented here, one could predict that the least sialylated isoform of apoC-III would be the most efficient inhibitor for the removal of TGRL. The physiological mechanism might, however, be more complex. Indeed, a modulation of the partitioning of apoC-III between TGRL and high density lipoprotein as a function of the degree of sialylation must also be considered. We have shown here that the higher degree of sialylation (i.e. 2 residues/molecule) increased the binding of apoC-III to VLDL. It would be of interest to define whether apoC-III sialylation modulates its affinity for high density lipoprotein in the same or the opposite direction. The degree of sialylation of apoC-III in transgenic animals has thus far not been reported. At this stage, we can only speculate that the degree of sialylation of the apoC-III is an important determinant of its ability to modulate the clearance of TGRL.

**FIG. 5. Effect of apoC-III isoforms on LSR activity in rat liver plasma membranes.** Aliquots of 125I-VLDL (0.15 mg; specific activity, 182 cpm/ng) were incubated at 37 °C for 30 min in the absence of apoC-III ( ), apoC-III ( ), or in the presence of 0.15 mg of nonsialylated apoC-III (apoC-III0, ), apoC-III1, or apoC-III2 (specific activity, 241, 285, and 182 cpm/ng, respectively) in a final volume of 500 µl of buffer A. The VLDL containing bound 125I-apoC-III was then separated from unbound 125I-apoC-III by gel filtration as described under “Methods.” Aliquots of each fraction were counted for 125I content, which revealed two peaks: lipoprotein associated 125I-apoC-III and unbound 125I-apoC-III. The amount of 125I-apoC-III2 (open bar), 125I-apoC-III1 (hatched bar), and 125I-apoC-III0 (solid bar) associated with VLDL is expressed as percentage of total radioactivity eluted from the column. Column recoveries were ≥90%.

**FIG. 6. Effect of desialylation of apoC-III on its inhibition of 125I-VLDL binding to LSR in rat liver plasma membranes.** Purified apoC-III2 was incubated at 37 °C for 16 h with neuraminidase (2 units/mg apoC-III). The top panel shows isoelectric focusing profiles of nonsialylated apoC-III (apoC-III0), apoC-III2, and neuraminidase-treated apoC-III (apoC-III2N). Image analysis of the Coomassie Blue-stained gel revealed 98% purity. Aliquots of 125I-VLDL (0.15 mg; specific activity, 184 cpm/ng) were incubated at 37 °C for 30 min with 0.15 mg of apoC-III0 (open bar), apoC-III2 (solid bar), or apoC-III2N (hatched bar) in buffer A. The VLDL were then reisolated by gel filtration as described under “Methods.” Rat liver plasma membranes preincubated with oleate and washed were incubated at 4 °C for 1 h with 15 µg/ml of each apoC-III-supplemented VLDL, and the amount of 125I-VLDL bound was determined. Values for oleate-induced 125I-VLDL binding were calculated as described under “Methods,” and are expressed as the percent of inhibition of binding compared with VLDL incubated in the absence of apoC-III. Each bar shows the mean ± S.D. of quadruplicate determinations.
ized on the basis of protein sequences predicted by LSR mRNA. Thus, although performed under nonphysiological conditions, the LSR membrane binding assay is robust and likely to reflect a physiologically significant phenomenon.

ApoC-III inhibition of the LSR was also observed in experiments using primary cultures of rat hepatocytes. In these cells, both the LDL receptor and the LRP are expressed and functional. We do not believe, however, that either of these receptors significantly contribute to the binding, uptake, and degradation of VLDL. Indeed, the LDL receptor has been shown not to bind normal human VLDL (42) and to be inhibited by oleate (43). LRP, on the other hand, recognizes β-VLDL supplemented with large amounts of apoE (44); such high apoE concentrations are unlikely to be found in the hepatocyte incubation media within the short period of time of this incubation. Finally, the oleate-induced binding, uptake, and degradation of lipoprotein in primary cultures of rat hepatocytes was inhibited by more than 85% by a polyclonal anti-LSR antibody.3 The specificity of the inhibitory effect of apoC-III on the binding of TGRL to LSR required experimental conditions in which no free C apoproteins were left in the incubation media. When the apoC/LDL mixture was used directly in the LSR activity assay, apoC-I, C-II, and C-III all inhibited LDL binding to LSR (data not shown). The mechanism of this inhibition is not clear. Partitioning experiments using radiolabeled apoC-III indicated that under these experimental conditions, about 90% of total apoC-III remained free in solution. One possible mechanism is therefore that the hydrophobic residues of the free apoC’s trapped the FFA thus inhibiting the conformational shift needed to activate LSR (28). It is also possible that apoC directly binds to the membrane phospholipid or protein, thereby masking the receptor.

At least three different mechanisms can be proposed to explain the hypertriglyceridemia observed when apoC-III concentrations are moderately increased. First, the increased apoC-III could cause a defective binding of the TGRL to hepatic sulfate proteoglycans and hence a resistance to lipolysis (19). It remains to be determined whether such a defect occurs in the absence of a displacement of the apoE that is responsible for the docking of TGRL to the endothelium (18). The recent finding by Ebara et al. (45) that apoC-III overexpression increases the number of large TG-rich particles in apoE-deficient mice is consistent with the notion that apoC-III is capable of inhibiting the lipolytic process even in the absence of apoE.

Second, the binding of apoC-III-enriched TGRL to the LDL receptor is decreased. This effect accounts for most of the reduced uptake of apoC-III-enriched TGRL observed in cultured fibroblasts or HeLa cells (20, 21). Indeed, in these experiments, the expression of the LDL receptor was maximized by incubation in lipoprotein-deficient serum. Because no FFA were added to the incubation media of these cells, it is unlikely that any LSR activity would account for these observations. Nevertheless, the LDL receptor model is not sufficient to explain all experimental data. Indeed apoC-I is a more potent inhibitor of the LDL receptor than apoC-III. In mice, the LDL receptor is estimated to account for the clearance of LDL and for about 50% of the removal of intestinally-derived chylomicron remnants (46). Consistent with these observations is the finding that apoC-1 causes in transgenic mice an increase not only of plasma TG, but also plasma cholesterol (47), whereas apoC-III overexpression causes a significant increase in plasma TG and only limited changes in cholesterol (15, 16). A recent report has shown that in homozygous LDL receptor knock-out mice, overexpression of apoC-III increased VLDL concentrations 10-fold (48).

Third, apoC-III hypertriglyceridemic effect may be due in part to the inhibition of TGRL binding to the LSR. The finding reported here provides yet another line of circumstantial evidence in support of the notion that the LSR represents a physiological rate-limiting step for the removal of TGRL.

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