# Hydrolysis of guinea pig nascent very low density lipoproteins catalyzed by lipoprotein lipase: activation by human apolipoprotein C-II

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**Abstract** Very low density lipoproteins isolated from guinea pig liver perfusate (VLDLp) lack the equivalent of human apolipoprotein C-II (apoC-II), the activator of lipoprotein lipase (LpL). These lipoproteins are therefore ideal substrates with which to investigate the mechanism by which apoC-II activates the enzyme. VLDLp binds apoC-II, and apoC-II associated with VLDLp markedly increases the rate of lipoprotein lipase-catalyzed hydrolysis of VLDLp-triglycerides. The activator potency of apoC-II is independent of the method of enrichment of VLDLp with apoC-II: delipidated human apoC-II and apoC-II transferred from human high density lipoproteins activate lipoprotein lipase to equal extents. ApoC-II causes pH-dependent changes in both apparent $K_m$ and $V_{max}$ of LpL-catalyzed hydrolysis of VLDLp-triglycerides. At pH 7.4–7.5, the major effect of apoC-II is to decrease the apparent $K_m$ by 3.3–4.0 fold. The apparent $V_{max}$ is increased 1.3-fold. At pH 6.5 and 8.5, the decrease of apparent $K_m$ is less marked, 1.6-fold and 1.4-fold, respectively. At pH 6.5, apoC-II increases the apparent $V_{max}$ by 1.3-fold, while at pH 8.5 the primary effect of apoC-II is a 1.6-fold increase of apparent $V_{max}$. Based on a simple kinetic model, the data suggest that apoC-II favors direct interaction between enzyme and triglyceride within the lipoprotein particle, as well as subsequent catalytic turnover.—**Fitzharris, T. J., D. M. Quinn, E. H. Goh, J. D. Johnson, M. L. Kashyap, L. S. Srivastava, R. L. Jackson, and J. A. K. Harmony.** Hydrolysis of guinea pig nascent very low density lipoproteins catalyzed by lipoprotein lipase: activation by human apolipoprotein C-II. *J. Lipid Res.* 1981. **22:** 921–933.

**Supplementary key words** triglyceride hydrolysis · VLDL catabolism

Very low density lipoproteins (VLDL) synthesized in the liver are the primary vehicles in plasma for the transport of endogenously synthesized triglycerides (1). VLDL catabolism proceeds in at least two phases: initial hydrolysis of VLDL-triglycerides catalyzed by lipoprotein lipase (LpL) situated at the capillary endothelium, and disappearance of the resulting triglyceride-poor VLDL-remnants from the circulation via conversion to plasma LDL and/or uptake by the liver (1). The importance of LpL in initiating triglyceride clearance is dramatically illustrated in subjects with type I hyperlipoproteinemia, a disorder characterized by the absence of the capillary endothelial LpL. In these subjects, the plasma triglyceride levels exceed 2000 mg/dl compared to 30–135 mg/dl in normal subjects (2).

In vivo and in vitro, LpL is activated by apolipoprotein C-II (3), a 78 amino acid residue protein (4). Although VLDL may contain apoC-II when secreted from the liver, it is more likely that nascent VLDL acquires the protein cofactor in the plasma by transfer of the apolipoprotein from HDL (1). ApoC-II is required for normal triglyceride catabolism in the plasma compartment. Breckenridge et al. (5), Yamamura et al. (6), and Cox, Breckenridge, and Little (7) have correlated severe hypertriglyceridemia in human subjects with the absence of plasma apoC-II. Plasma from these individuals, deficient in apoC-II, failed to activate LpL in an in vitro assay, suggesting that hypertriglyceridemia was a direct result of the absence of the peptide.

In addition to apoC-II, the surface of plasma

Abbreviations: VLDL, very low density lipoproteins (d > 1.006 g/ml); VLDLp, VLDL isolated from guinea pig liver perfusate; LDL, low density lipoproteins (d 1.019–1.063 g/ml); DPE, dansyl-phosphatidylethanolamine; LpL, lipoprotein lipase; BSA, bovine serum albumin; SDS, sodium dodecylsulfate; TLC, thin-layer chromatography; TMU, tetramethylurea.

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VLDL consists of apoE, apoB, apoC-I, and apoC-III. The role of these apolipoproteins in triglyceride hydrolysis is controversial. ApoE is reported to inhibit LpL (8). ApoC-I and apoC-III also appear to inhibit, particularly in assays performed with artificial substrates (9). Generally, inhibition in vitro does not occur unless apoC-I or apoC-III is present in large excess relative to apoC-II, suggesting that the inhibitory apolipoproteins alter the lipid structure of the substrate or compete with apoC-II for binding sites at the substrate surface. In this regard, the reports of Lukens and Borensztajn (10) and Kotlar and Borensztajn (11) are particularly significant: apoC-I and apoC-III inhibited apoC-II-activated LpL-catalyzed hydrolysis of rat chylomicrons in vitro but had no influence on triglyceride hydrolysis when the chylomicrons were perfused through isolated rat hearts.

The rate-limiting step in VLDL catabolism has not been identified, nor is the mechanism by which apoC-II activates LpL completely understood. The experiments reported herein were designed to answer three important questions. What is the minimum amount of apoC-II required for maximum activation of LpL? Does purified apoC-II added directly to VLDL have the same activator potency as apoC-II transferred to VLDL from HDL? Which step in the catalytic sequence is influenced by apoC-II? The experimental system consisted of bovine milk LpL and guinea pig liver perfusate VLDLp. LpL has been prepared from a variety of sources, but is most conveniently purified from bovine milk. Antiserum raised against the bovine milk enzyme cross-reacts with LpL released from human endothelium by heparin (12), suggesting that the enzymes are similar if not identical. The rationale for using guinea pig VLDLp is that VLDLp is deficient in the human equivalent of apoC-II and is therefore an ideal substrate with which to study the relationship between apoC-II content of the VLDL and LpL activity. The data reported herein support the hypothesis that apoC-II facilitates the formation of the enzyme-triglyceride complex and enhances the rate of LpL-catalyzed turnover of VLDLp-triglycerides to products.

## METHODS

### Materials

Radioisotopes and Aquasol-2 were obtained from New England Nuclear Corp.; sodium Nembutal was from Abbott Laboratories. Oleic acid was obtained from Nu-Chek-Prep, Inc. Dansyl-phosphatidylethanolamine (DPE) was purchased from Molecular Probes, Inc. Triglycerides and Cholesterol Test Combination Kits were purchased from Boehringer-Mannheim. Silica gel 1B2 thin-layer chromatography strips were obtained from J. T. Baker Chemical Co. Bovine serum albumin (Fraction V, Sigma Chemical Co.) was delipidated at 4°C by extraction with methanol (10 vol/wt) prior to use. ApoC-II was purified (13, 14) from the plasma VLDL of subjects with Type IV hyperlipoproteinemia. The apolipoprotein was homogeneous as determined by isoelectric focusing in 8 M urea. LpL was purified from bovine skim milk by affinity chromatography on heparin-Sepharose as described by Kinnunen (15); the enzyme was stored at −70°C in 0.05 M Tris-HCl, pH 7.6, containing 50% glycerol and 1 M NaCl. LpL was homogeneous as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (SDS). The apparent molecular weight of LpL was 55,000 daltons in agreement with the value reported by Kinnunen, Huttunen, and Ehnholm (16). The activity of the purified enzyme, 36 mmol fatty acids released/hr per mg protein, was assessed with an artificial substrate of [carboxyl-$^{14}$C]triolein emulsified in the presence of Triton X-100 (17).

### Perfusion of guinea pig livers

Adult female albino guinea pigs (300–400g) were maintained ad libitum on standard laboratory guinea pig chow and water under a constant 12 hr light/dark cycle. Livers (10–12g), surgically removed from the animals under sodium Nembutal (1 mg/kg body weight) anesthesia between 7:30 and 9:30 AM, were perfused in a recycling system (18) with 65 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3% (w/v) delipidated bovine serum albumin and 100 mg/dl glucose (19). The perfusate was gassed continuously at 37°C with 95% $O_2$/5% $Co_2$. After a 20-min equilibration period, 10 ml of an oleic acid-albumin complex was added to the perfusate (t = 0). Addition of oleic acid-albumin was repeated at t = 1 hr, t = 2 hr, and t = 3 hr. The oleic acid-albumin complex contained 0.3 g delipidated albumin and 150 $\mu$mol oleic acid in 10.0 ml 0.9% NaCl (pH 7.4). To obtain perfusate lipoproteins with endogeneously radiolabeled triglycerides, 25 $\mu$Ci of [$^3$H]oleic acid were included in the albumin-oleate complex. After 4 hr, the perfusion was terminated and VLDLp was isolated from the perfusate.

### Isolation of lipoproteins

Lipoproteins were obtained by sequential ultracentrifugation in KBr (20). A Beckman L5-65B preparative ultracentrifuge was employed throughout

the investigation; the temperature was maintained at 15°C. Guinea pig VLDLp was isolated in two steps. Initially, the perfusate was centrifuged at perfusate density (d 1.006 g/ml) in a Beckman type 50 Ti rotor for 18 hr at 48,000 rpm. The top layer containing the VLDLp was collected. The density of this fraction was increased to 1.019 g/ml by addition of KBr, the density-adjusted solution was layered under a solution of KBr, d 1.006 g/ml, and the mixture was centrifuged at 48,000 rpm for 18 hr. The purified VLDLp (top layer) was removed and dialyzed exhaustively against 0.05 M Tris-HCl, pH 7.4, containing 0.9% NaCl. Human plasma VLDL and guinea pig plasma VLDL were isolated by ultracentrifugal flotation at plasma density (d 1.006 g/ml) in a Beckman type 50.2 Ti rotor operating for 18 hr at 48,000 rpm. HDL, d 1.063–1.21 g/ml, was isolated from the plasma of normal fasting human volunteers by ultracentrifugal flotation employing a Beckman type 50.2 Ti rotor operating at 48,000 rpm for 24 hr. Where indicated, lipoproteins were delipidated by the tetramethylurea method of Kane (21).

### Incorporation of apoC-II into VLDL

Two methods of apoC-II enrichment were utilized: the purified apolipoprotein was added directly to VLDLp, or the VLDLp was incubated with human HDL, a lipoprotein source of the apoC peptides. Both methods have been shown by Kashyap et al. (22) to result in incorporation of radioimmunoassayable apoC-II into human plasma VLDL. In the direct addition procedure, VLDLp or [$^3$H-oleate]-labeled VLDLp (usually 1 mg of triglyceride) was incubated for 1 hr at 37°C with 0.5–50.0 $\mu$g of human apoC-II. Alternatively, VLDLp or [$^3$H-oleate]-labeled VLDLp was incubated for 1 hr at 37°C with HDL (0.5–50.0 $\mu$g of apoC-II protein). In each case, the incubation mixtures were layered over KBr solutions of density 1.019 g/ml, and the VLDLp was floated by centrifugation in a Beckman type 50 Ti rotor operating for 18 hr at 35,000 rpm. ApoC-enriched VLDLp was dialyzed against 0.05 M Tris-HCl, pH 7.4, containing 0.9% NaCl. The reisolated VLDLp was analyzed as follows: triglyceride concentrations were determined after scintillation counting of lipid extracts (radioactivity of [$^3$H-oleate]-labeled VLDLp added was 16,000 cpm/mg triglyceride) or by chemical analysis; the amount of apoC-II incorporated into VLDLp was measured by radioimmunoassay (23).

### Hydrolysis catalyzed by LpL

LpL-catalyzed hydrolysis was assessed by the fluorescence method of Johnson et al. (24), and by quantitation of release of free fatty acids. The latter method was employed for determination of the apparent kinetic parameters, $K_m$ and $V_{max}$. For the fluorescence assay, the fluorescence probe DPE in absolute ethanol was added to VLDLp at a molar ratio of 1:40 DPE to VLDLp-triglyceride. The DPE-VLDLp solutions were vortexed gently for 15 sec, then sonicated in a Cole-Parmer Model 8846-50 bath sonicator for 6–8 min at room temperature. After sonication, the DPE-VLDLp was dialyzed against 0.05 M Tris-HCl buffer, pH 7.4. Fluorescence studies were conducted with a Perkin-Elmer 650-10S ratio recording spectrofluorometer. Excitation was at 340 nm and emission was monitored at 490 nm. The temperature was maintained at 25°C by a Lauda K2R circulating water bath and monitored by a Bailey instruments cryo-thermometer.

Reaction mixtures typically contained 0.6 mg of VLDLp-triglyceride, 30 mg of defatted BSA, and 5 $\mu$g of LpL per ml in a buffer of 0.05 M Tris-HCl, pH 7.4, containing 0.9% NaCl. VLDLp was added to the reaction cuvette at t = 0, delipidated BSA was added at t = 1 min, and LpL was added at t = 4 min. First order rate constants were determined by plotting the natural log of $[F_\infty/F_0 - F_t/F_0]$, the difference between the final relative fluorescence and the relative fluorescence at time t, as a function of time in min. Alternatively, the release of [$^3$H]oleate was assessed. Reactions were quenched by immersion in an ice bath or by addition of Dole and Meinertz extraction reagent (25) at a sample–reagent ratio of 1:5 (v/v).

Reactions obeyed first-order kinetics for at least four half-lives. In experiments in which initial rates of hydrolysis were assessed, the data represent $\leq$10% reaction. Observed rate constants were determined by linear regression analysis of the data. The kinetic parameters $K_m$ and $V_{max}$ were calculated from initial rate versus substrate concentration data ($V_i$ *vs* S) transformed according to the Lineweaver-Burk equation. The data were analyzed by weighted linear-least squares, where weighing factors directly proportional to $V_i^4$ were used to prevent artificial biasing of the linear fit due to skewing of the residuals of $1/V_i$ at low $V_i$. This skewing results from transforming the native data set, $V_i$ *vs* S, to $1/V_i$ *vs* 1/S. The rationale for weighting Lineweaver-Burk linear-least squares analyses of enzyme kinetic data has been presented by Cleland (26).

### Electron microscopy

For electron microscopic observation, lipoprotein samples were negatively stained with 2% phosphotungstic acid, pH 7.4. A Phillips EM 300 microscope operating at an accelerated voltage of 60 KV was employed. The instrument was calibrated with a car-

TABLE 1. Composition of human plasma VLDL, guinea pig VLDL, and guinea pig perfusate VLDLp

| Constituent | Human Plasma VLDL | Guinea Pig Plasma VLDL | Guinea Pig VLDLp |
|---|---|---|---|
| | | *weight %* | |
| Protein | 11.0 | 10.7 | 10.4 |
| Glyceride | 47.0 | 61.4 | 66.2 |
| tri- | 43.3 | 56.8 | 65.0 |
| di- | 2.3 | 2.9 | 1.2 |
| mono- | 1.4 | 1.7 | ND[a] |
| Unesterified fatty acid | 3.4[b] | 3.7[c] | 3.0 |
| Phospholipid | 17.1 | 12.6 | 11.2 |
| Cholesterol | 21.5 | 11.7 | 7.2 |
| unesterified | 6.5 | 7.9 | 5.1 |
| esterified | 15.0 | 3.8 | 2.1 |
| Cholesteryl esters: cholesterol | 2.31 | 0.48 | 0.41 |

[a] ND, not detected.
[b] Taken from reference 45.
[c] Taken from reference 46.
VLDL was isolated and the chemical composition was determined as described in Methods. Each reported value represents the mean for five different preparations; the standard deviation is $<\pm 10\%$. Values are reported as weight %.

bon grating replica (28,800 lines/inch, E. F. Fullam, Inc., New York). Preparations were initially observed at a magnification of 42,000×; particle size was determined by examination of the original negatives and of the prints processed to a final magnification of 105,000×. In both cases, results were the same. One hundred particles were counted and measured.

### Analytical procedures

Triglycerides and cholesterol-cholesteryl esters were analyzed using the Triglycerides and Cholesterol Test Combination Kits, respectively. The triglyceride assay is based on the enzymatic (glycerol-kinase, pyruvate kinase, lactate dehydrogenase) determination of glycerol. The cholesterol assay is based on the enzymatic (cholesterol oxidase) determination of unesterified cholesterol after cholesteryl esterase-catalyzed hydrolysis of cholesteryl esters. Phospholipids were determined by the Bartlett microphosphorus method (27). Protein concentrations were determined by a modification of the method of Lowry et al. (28) using 1% SDS to clarify the samples; bovine serum albumin was the standard. Lipids, extracted from VLDL in ether–ethanol 1:3 (v/v), were separated into classes by thin-layer chromatography on Silica Gel 1B2 in a solvent system of petroleum ether–ether–acetic acid 85:15:1 (v/v). To resolve unesterified fatty acids, mono-, di-, and triglycerides, a solvent system of heptane–ether–formic acid 90:60:4 (v/v) (29) was employed. Lipids were extracted from the TLC bands for quantitation of lipid concentrations by chemical and enzymatic methods. Alternatively, to assess lipid radioactivity, the lipids were scraped from the TLC plates into scintillation vials, mixed with 10 ml of Aquasol-2, and counted in a Packard Tricarb Scintillation Spectrometer. The apolipoprotein composition of the lipoproteins was determined by gel electrophoresis in 8 M urea as described by Kane (21) and in 0.1% SDS by the procedure of Weber and Osborn (30). Protein bands were visualized by Coomassie blue or Amido black staining.

## RESULTS

The chemical compositions of human plasma VLDL, guinea pig plasma VLDL, and VLDLp secreted by perfused guinea pig livers are compared in **Table 1.** The composition of guinea pig plasma VLDL agrees well with that reported by Chapman and Mills (31) and by Sardet, Hansma, and Ostwald (32). Guinea pig plasma VLDL is enriched in glycerides and slightly deficient in phospholipids and total cholesterol relative to human plasma VLDL. The most striking difference between human and guinea pig plasma VLDL is the ratio of cholesteryl esters–cholesterol, 2.3 for human VLDL and 0.5 for guinea pig VLDL. The lipid composition of VLDLp resembles that of guinea pig plasma VLDL, although VLDLp contains slightly more triglycerides and slightly less total cholesterol than guinea pig plasma VLDL. The glyceride fraction in VLDLp is 98% triglycerides, whereas guinea pig plasma VLDL and human plasma VLDL carry an appreciable fraction of their glycerides as di- and monoglycerides (7.9 and 7.4%, respectively). The ratios of total lipid–protein for human VLDL, guinea pig plasma VLDL, and guinea pig VLDLp are approximately the same, 9:1 (wt/wt).

When guinea pig livers are infused with [$^3$H]-oleate, the fatty acid is taken up and incorporated into secreted VLDLp lipids. Of the [$^3$H]oleate incorporated into VLDLp in a typical perfusion, 86.7% is in triglycerides, 5.4% is in phospholipids, and 1.5% is in cholesteryl esters. About 3–5% of the VLDL-associated radioactivity is present as unesterified [$^3$H]oleate. These values correspond to the following radioactivities (cpm/$\mu$g lipid): triglycerides, 145; diglycerides, 118; unesterified fatty acids, 109; phospholipids, 52; and cholesteryl esters, 78.

The apolipoproteins of human plasma VLDL, guinea pig plasma VLDL, and VLDLp as determined by polyacrylamide gel electrophoresis in the presence of SDS or urea are compared in **Fig. 1.** As indicated in Fig. 1A, all the VLDL samples contain a high molecular weight apolipoprotein which, based on its in-

solubility in tetramethylurea (21), is designated apoB. This apolipoprotein does not enter a 7.5% polyacrylamide gel. In addition, human plasma VLDL and guinea pig VLDLp contain an apolipoprotein which has an apparent molecular weight of 33,000 daltons. The latter protein is depleted in guinea pig plasma VLDL. Guinea pig VLDLp contains an additional apolipoprotein of apparent molecular weight 38,000 daltons. The primary difference between peptides detected in human VLDL and guinea pig VLDLp, however, is the absence of low molecular weight apoC peptides in VLDLp; this difference is evident in the urea gels shown in Fig. 1B. Moreover, VLDLp does not contain the equivalent of human apoC-II as determined by a specific radioimmunoassay for human apoC-II (23), an assay sufficiently sensitive to detect 100 ng of apoC-II per mg VLDLp protein. Guinea pig plasma VLDL has proteins which migrate in urea gels in the apoC region, but these do not correspond exactly to human apoC-II or apoC-III.

The data of Fig. 1B further indicate that VLDLp can be enriched with human apoC-II. VLDLp reisolated after incubation with HDL contains apoC-II and apoC-III. Incubation of VLDLp with purified apoC-II results in incorporation of only apoC-II into

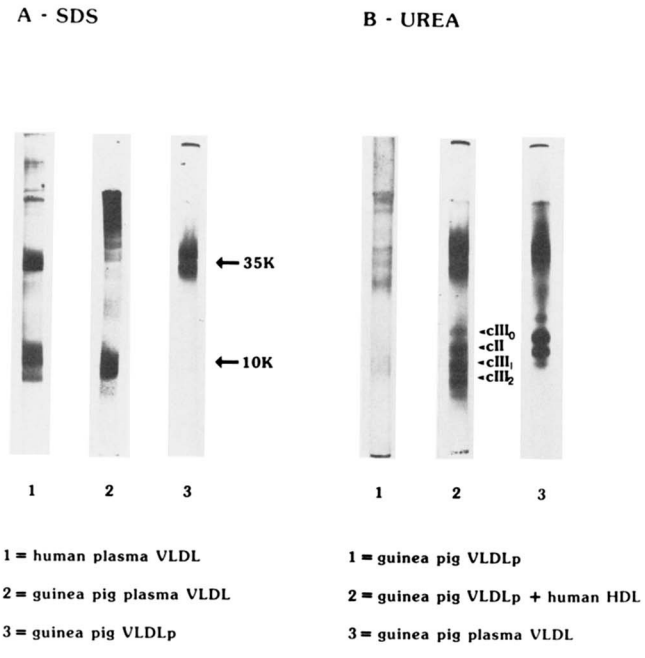


**Fig. 1.** Apolipoproteins of human plasma VLDL, guinea pig plasma VLDL, and guinea pig VLDLp. The apolipoproteins were subjected to polyacrylamide gel electrophoresis in (A) 0.1% sodium dodecylsulfate (200 $\mu$g protein/gel) or (B) 8 M urea (100 $\mu$g protein/gel) as described in Methods. Protein bands were visualized with Coomassie blue (SDS gels) or with Amido black (urea gels). Assignment of molecular weight was based on calibration with standard proteins.

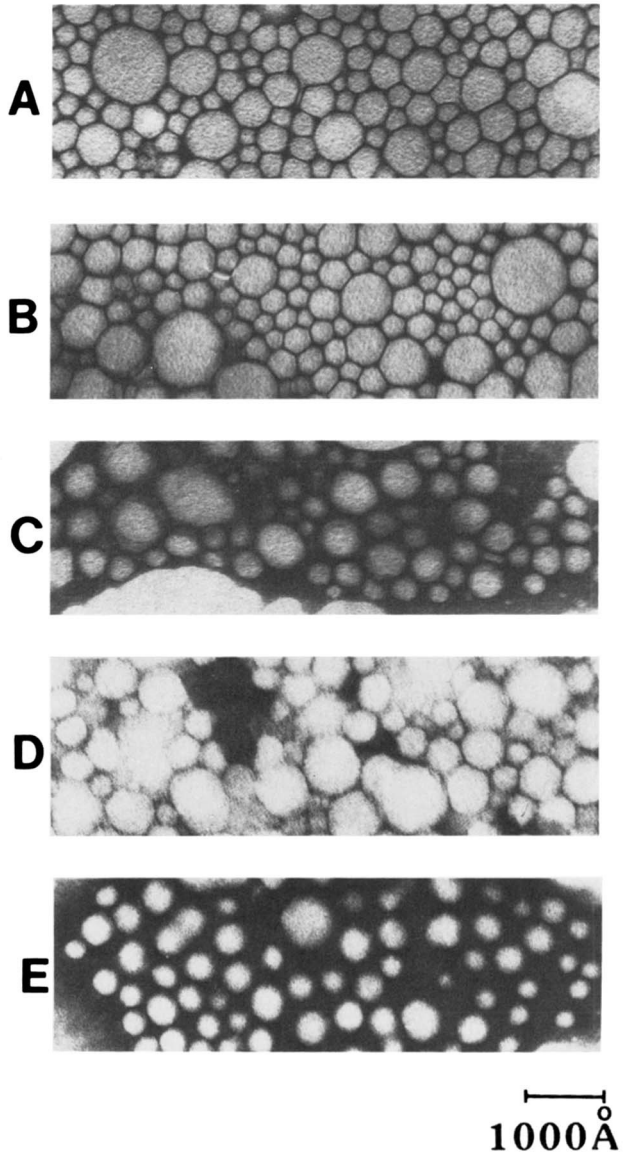


**Fig. 2.** Electron micrographs of very low density lipoproteins: (A) human plasma VLDL, (B) guinea pig plasma VLDL, (C) guinea pig perfusate VLDLp, (D) guinea pig VLDLp incubated with apoC-II (5 $\mu$g apoC-II per 0.6 mg VLDLp-triglyceride), and (E) guinea pig VLDLp incubated with human HDL (5 $\mu$g HDL apoC-II per 0.6 mg VLDLp-triglyceride). Lipoprotein samples were negatively stained with 2% phosphotungstic acid, pH 7.4. The magnification is 105,000×.

VLDLp. The presence of apoC-II and/or apoC-III does not alter the size of VLDLp. The average diameter of VLDLp particles in the absence or presence of the apoC peptides is 480 ± 50 Å as demonstrated by the electron micrographs in **Fig. 2.** The average diameters of guinea pig plasma VLDL and human plasma VLDL are 380 ± 50 Å and 370 ± 50 Å, respectively.

Hydrolysis of [$^3$H-oleate]-labeled VLDLp was assessed by two assay methods: by the increase in fluorescence of dansyl-phosphatidylethanolamine,

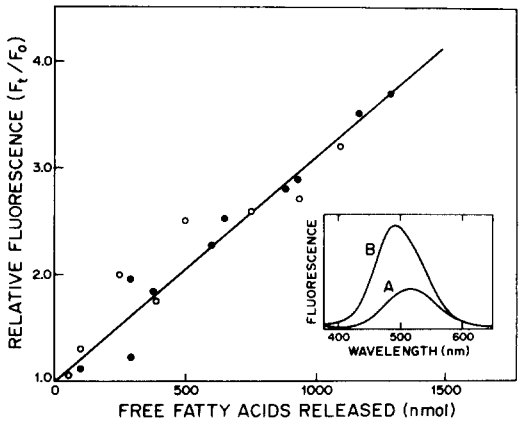


**Fig. 3.** Lipoprotein-lipase (LpL)-catalyzed hydrolysis of VLDLp: the relationship between the increase in relative fluorescence of DPE and release of unesterified fatty acids. The reaction mixture contained 3.0 mg DPE-[$^3$H-oleate]-labeled VLDLp-triglyceride and 3% delipidated BSA in 5 ml 0.05 M Tris-HCl, pH 7.4, containing 0.9% NaCl. The reaction was initiated by the addition of 25 $\mu$g LpL at t = 0. At various times, the relative fluorescence ($F_t/F_0$) was recorded and 0.3-ml aliquots were removed for determination of [$^3$H]oleate as a measure of unesterified fatty acids released. Reactions were performed at 25°C in the absence of apoC-II (○) or with the addition of 25 $\mu$g apoC-II at t = 0 (●). The insert illustrates the change in fluorescence which occurs as DPE (A, $\lambda_{max}$ 515 nm) is hydrolyzed to its corresponding dansyl monoacyl derivative (B, $\lambda_{max}$ 490 nm). Spectrum B was obtained after 75% of the VLDLp-triglyceride and an equivalent amount of DPE were hydrolyzed.

and by release of unesterified fatty acids. The course of LpL-catalyzed hydrolysis determined by both methods is shown in **Fig. 3.** When excited at 340 nm, DPE incorporated into [$^3$H-oleate]-labeled VLDLp has an emission maximum of 515 nm. DPE is converted to the corresponding monoacyl derivative during hydrolysis; the monoacyl derivative and unesterified fatty acids are transferred to albumin, and the relative fluorescence increases by 3.5-fold and the emission maximum shifts to 490 nm (33). The rate of the fluorescence increase at 490 nm is directly proportional to the rate of release of unesterified fatty acids: about 473 nmol of fatty acids are released per $F_t/F_0$ unit. Thus, the data of Fig. 3 establish that the increase in relative fluorescence at 490 nm is an accurate and facile measure of LpL activity. The presence of apoC-II in VLDLp does not compromise the direct correspondence between the increase in relative fluorescence of the danysl moiety and release of fatty acids (Fig. 3).

Guinea pig VLDL-triglycerides are relatively poor substrates for LpL (**Fig. 4A**, open circles; **Fig. 5A**, solid circles), suggesting that these lipoproteins lack an activator of LpL. The TMU-soluble apolipoproteins isolated from guinea pig VLDLp do not enhance the rate of LpL-catalyzed hydrolysis of triglycerides in a triolein emulsion as is indicated in **Table 2.** Under the same conditions, TMU-soluble peptides isolated from guinea pig plasma VLDL also have little effect on the activity of LpL, whereas peptides isolated from human plasma VLDL are potent activators of LpL. These results indicate that guinea pig VLDL does not contain an activator of LpL and therefore corroborate the report of Fidge (34). However, the data contradict the conclusion of Wallinder, Bengtsson, and Olive-

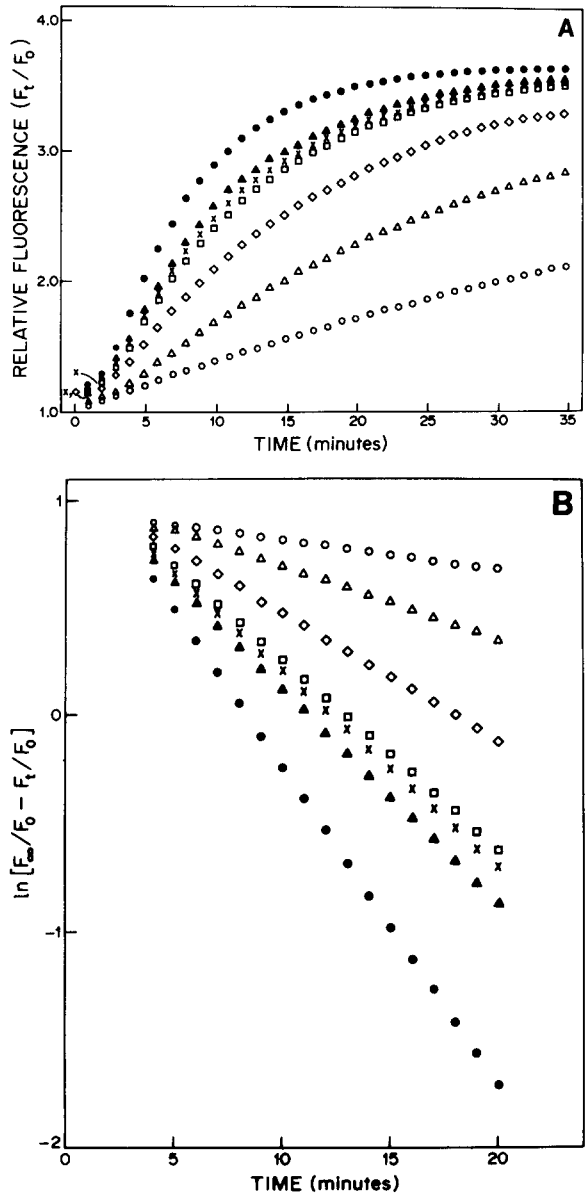


**Fig. 4.** Lipoprotein lipase (LpL)-catalyzed hydrolysis of VLDLp and of apoC-II-enriched VLDLp. Purified human apoC-II was incorporated into VLDLp, the VLDLp was incubated with LpL (5 $\mu$g/ml), and hydrolysis was monitored by the increase in relative fluorescence at 490 nm as described in Methods. The amount of apoC-II incorporated per 680 nmol VLDLp-triglyceride in each reaction was 0 $\mu$g (○), 0.3 $\mu$g (△), 0.4 $\mu$g (◇), 1.4 $\mu$g (□), 2.0 $\mu$g (●), 12.8 $\mu$g (▲), and 25.5 $\mu$g (x). (A) Plots of relative fluorescence versus time; (B) first-order rate profiles.

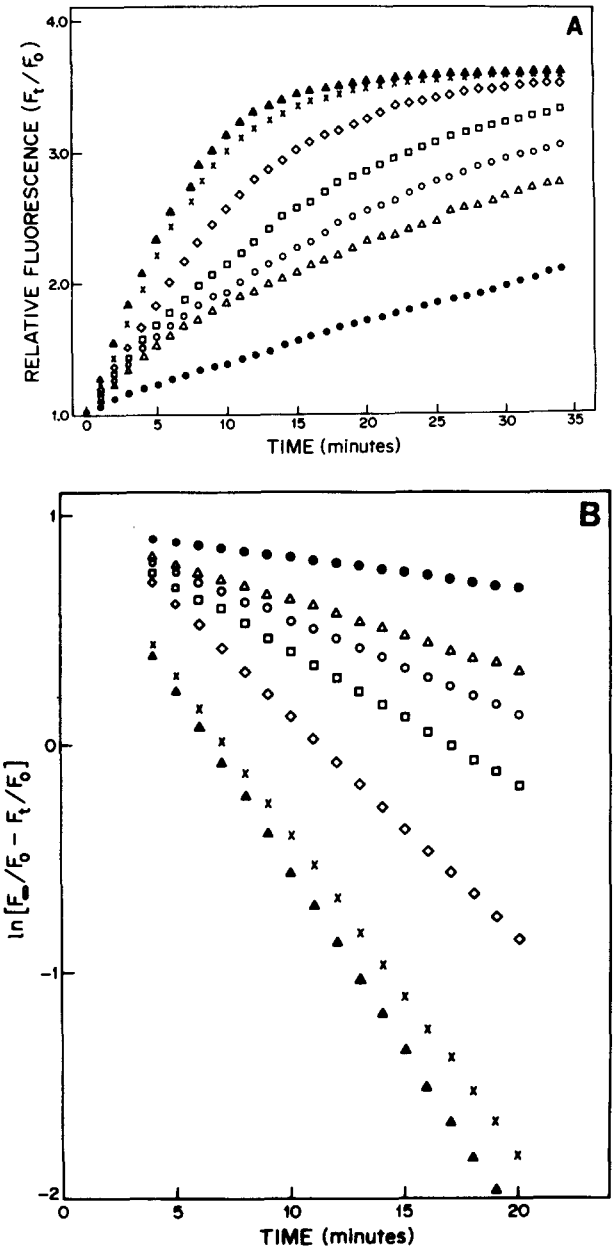


**Fig. 5.** Lipoprotein lipase (LpL)-catalyzed hydrolysis of VLDLp enriched with apoC-II transferred to VLDLp from HDL. Human HDL was incubated with VLDLp, and the rate of hydrolysis of apoC-II-enriched VLDLp was determined after the addition of LpL (5 μg/ml) as described in Methods. VLDLp contained the following amounts of transferred apoC-II per 680 nmol of VLDLp-triglyceride per reaction: 0 μg (●), 0.3 μg (△), 0.4 μg (○), 0.8 μg (□), 2.0 μg (◇), 5.1 μg (▲), and 12.4 μg (x). (A) Plots of relative fluorescence versus time; (B) first-order rate profiles.

crona (35) that guinea pig VLDL has sufficient activator apolipoprotein to support rapid LpL-catalyzed hydrolysis of triglyceride emulsions.

The rate at which hydrolysis of VLDLp-triglycerides occurs is markedly enhanced by the incorporation of μg quantities of purified human apoC-II into VLDLp (Fig. 4A). LpL-catalyzed reactions in the absence and presence of apoC-II are first-order with respect to VLDLp-triglyceride; a kinetic treatment of the data is presented in Fig. 4B. Observed first-order rate constants for hydrolysis of DPE-VLDLp calculated from the data of Fig. 4B are summarized in **Table 3.** ApoC-II transferred from HDL also facilitates LpL-catalyzed hydrolysis of VLDLp-triglycerides (Fig. 5A), and the reactions are first-order with respect to VLDLp-triglyceride (Fig. 5B). The observed first-order rate constants are indicated in Table 3. The data indicate that both methods of apoC-II enrichment—addition of purified apoC-II to VLDLp and transfer of apoC-II to VLDLp by incubation of VLDLp with human HDL—are equally effective in enhancing the rate of LpL-catalyzed lipolysis. This result indicates that apoC-III, which is also incorporated into VLDLp incubated with HDL, does not influence the reaction. The relationship between $k_0$, the observed first-order rate constant for lipolysis, and the apoC-II content of VLDLp is illustrated in **Fig. 6.** Maximum rate enhancement of 9.7- to 10.6-fold occurs at an apoC-II concentration of 2–5 μg which corresponds to 2–5% of the VLDLp protein. There appears to be an optimal activator concentration since activation diminishes with incorporation of 12 μg of apoC-II into VLDLp. The decrease in extent of activation which results when relatively high levels of HDL-

TABLE 2. Activity of lipoprotein lipase: influence of TMU-soluble apolipoproteins isolated from guinea pig plasma VLDL, guinea pig VLDLp, and human plasma VLDL

| Apolipoprotein Source | Amount of Protein Added (μg/ml) | Initial Velocity (mmol [$^3$H]oleate released $h^{-1}$ mg LpL$^{-1}$) |
|---|---|---|
| Guinea pig plasma VLDL | 0 | 1.17 |
| | 20 | 1.31 |
| | 50 | 1.61 |
| | 100 | 1.81 |
| Guinea pig VLDLp | 0 | 1.39 |
| | 20 | 1.20 |
| | 50 | 1.51 |
| | 100 | 1.42 |
| Human plasma VLDL | 0 | 1.24 |
| | 20 | 2.53 |
| | 50 | 4.01 |
| | 100 | 4.80 |

The various VLDL samples were delipidated in tetramethylurea as described by Kane (21). The TMU-soluble apolipoproteins were dialyzed against 0.05 M Tris-HCl, pH 7.4, containing 0.9% NaCl, and the dialyzed proteins were preincubated with [$^3$H-oleate]-labeled triolein (0.68 mM) at room temperature prior to the addition of LpL (10 μg). The final incubation volume was 3.0 ml, and delipidated BSA was added to a final concentration of 3%. The initial velocity of LpL-catalyzed hydrolysis of triolein was assessed by the rate of release of unesterified [$^3$H]oleate.

TABLE 3. Lipoprotein lipase-catalyzed hydrolysis of guinea pig VLDLp: dependence of the observed rate constant, $k_o$, on apoC-II

| Source of ApoC-II | ApoC-II Added[a] | VLDLp-associated[a] ApoC-II | $k_o$ ($min^{-1}$)[b] |
|---|---|---|---|
| | ($\mu g$) | ($\mu g$) | |
| Purified apoC-II | 0 | 0 | 0.015 |
| | 0.5 | 0.3 | 0.035 |
| | 1.0 | 0.4 | 0.060 |
| | 3.0 | 1.4 | 0.088 |
| | 5.0 | 2.0 | 0.145 |
| | 15.0 | 12.8 | 0.100 |
| | 50.0 | 25.5 | 0.091 |
| ApoC-II transfer from HDL | 0 | 0 | 0.015 |
| | 0.5 | 0.3 | 0.031 |
| | 0.9 | 0.4 | 0.042 |
| | 2.3 | 0.8 | 0.059 |
| | 4.5 | 2.0 | 0.097 |
| | 22.7 | 5.1 | 0.159 |
| | 45.4 | 12.4 | 0.140 |

[a] Values are reported as $\mu g$ of apoC-II per 0.6 mg (680 nmol) VLDLp-triglyceride. The accuracy of the data is ±5%.

[b] Variation in $k_o$ is ±5%.

Guinea pig VLDLp was incubated with human apoC-II or with human HDL as described in Methods. The VLDLp, reisolated by ultracentrifugal flotation, was incubated with LpL at 25°C. The rate constants for LDL-catalyzed triglyceride hydrolysis were calculated from the data of Figs. 4B and 5B. The amount of apoC-II associated with VLDLp was determined by radioimmunoassay.

derived apoC-II are incorporated into VLDLp is not quite as pronounced as that which results in the presence of comparable amounts of purified apoC-II.

The experiments described thus far were performed at a single concentration of VLDLp-triglyceride, 0.6 mg/ml. To attempt to gain further insight into the step in the catalytic sequence that is sensitive to apoC-II, experiments were undertaken to determine whether apoC-II activates by increasing the apparent maximum velocity of the reaction, by decreasing the apparent $K_m$ for VLDLp, or by both mechanisms. The initial rate (≤10%) of LpL-catalyzed hydrolysis of VLDLp was assessed as a function of [VLDLp] and [apoC-II]. The data, illustrated in **Fig. 7A**, are replotted in Fig. 7B to obtain the apparent kinetic parameters $K_m$ and $V_{max}$. For these experiments, VLDLp was enriched in apoC-II by addition of the purified apolipoprotein. It is evident that, at pH 7.4 and within experimental error, low concentrations of apoC-II have a small effect (1.3-fold) on the apparent $V_{max}$ of the reaction, but markedly decrease the apparent $K_m$ for the substrate VLDLp. In the absence of apoC-II, the apparent $K_m$ is 2.8 mM VLDLp-triglyceride; in the presence of 1.9 $\mu g$ apoC-II, the apparent $k_m$ decreases to 1.1 mM VLDLp-triglyceride. The kinetic parameters determined from Fig. 7B are provided in numerical form in **Table 4.** The effect of apoC-II on the apparent $K_m$ is saturated at apoC-II concentrations between 3 and 16 $\mu g$ per mg of VLDLp-triglyceride, as is indicated in **Fig. 8.**

The apparent $K_m$ and $V_{max}$ values were subsequently determined at three pH values: 6.5, 7.5, and 8.5. This experiment was designed to address the possibility that a particular set of reaction conditions determines whether apoC-II activates the enzyme primarily through $K_m$ or $V_{max}$ or both. The concentration of apoC-II, 5 $\mu g$/mg triglyceride, was selected to maximize the effect of the activator (Table 4). The data presented in **Table 5** indicate that, in the absence of added activator peptide, the apparent $V_{max}$ appears to be insensitive to pH while the apparent $K_m$ decreases by 40% from pH 6.5 to 8.5. The incorporation of apoC-II into VLDLp confers pH-sensitivity to the apparent $V_{max}$ term: the $V_{max}$ at pH 8.5 is 1.3 times that at pH 6.5. Moreover, the apparent $K_m$ term decreases by 2.5-fold from pH 6.5 to 7.5, and increases by 2-fold from pH 7.5 to 8.5. At each value of pH, the influence of apoC-II was assessed by comparing the kinetic parameters obtained in the presence of apoC-II to those obtained in the absence of the activator peptide. It is evident in Table 5 that apoC-II influences both the apparent $K_m$ and $V_{max}$ to a limited extent at all pH values, but has a pronounced effect on the apparent $K_m$ at pH 7.5. Two points are relevant. First, the data obtained at pH 7.5 agree well with those obtained at pH 7.4 with different preparations of LpL, apoC-II, and VLDLp (compare Tables 4 and 5). Second, the kinetic parameters may

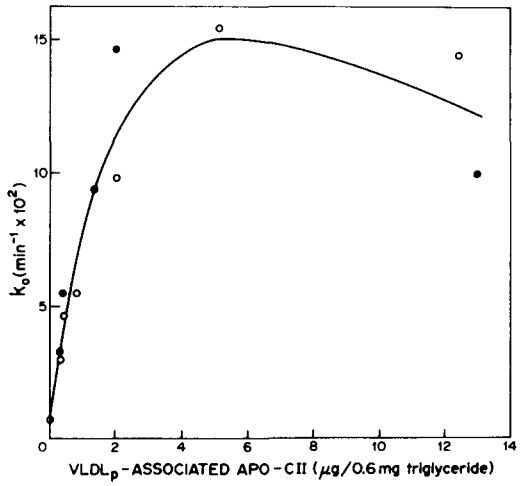


**Fig. 6.** Dependence of the rate of LpL-catalyzed hydrolysis of VLDLp on the amount of VLDLp-associated apoC-II. First-order rate constants determined for the hydrolysis of apoC-II-enriched VLDLp (●) and HDL-apoC-II-enriched VLDLp (○) are plotted as a function of the amount of VLDLp-associated apoC-II. Values are taken from Table 3.

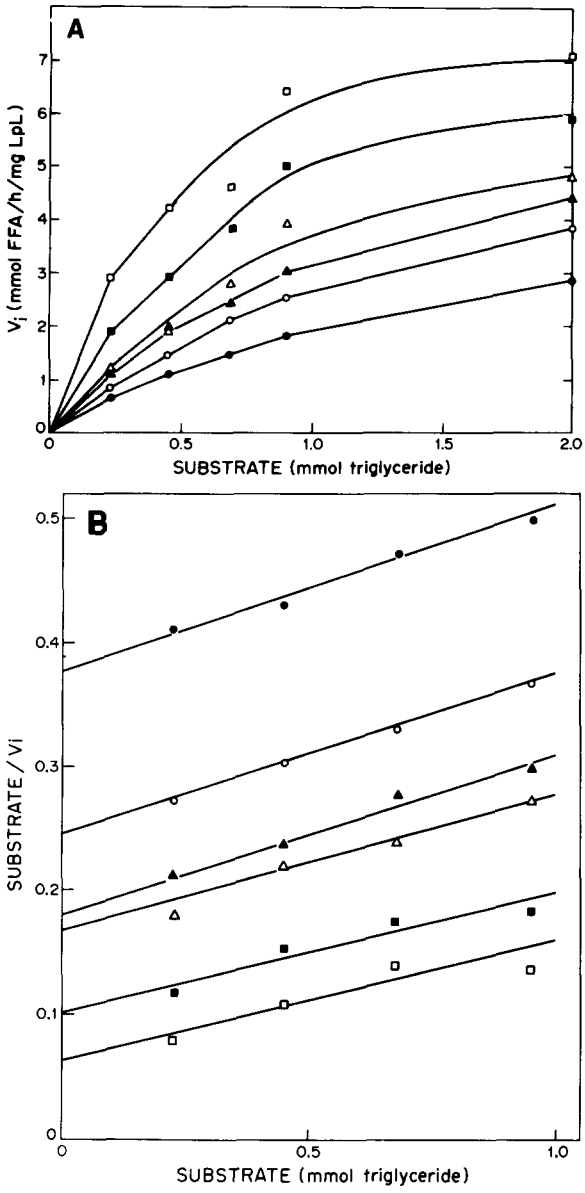


**Fig. 7.** Dependence of the initial rate of lipoprotein lipase (LpL)-catalyzed hydrolysis of [$^3$H-oleate]-labeled VLDLp-triglyceride and of apoC-II-enriched [$^3$H-oleate]-labeled VLDLp-triglyceride on the amount of VLDLp-triglyceride. Purified human apoC-II was incorporated into [$^3$H-oleate]-labeled VLDLp and the initial rates of triglyceride hydrolysis at 24°C were determined by release of [$^3$H]oleate as described in Methods. Each reaction mixture contained BSA (ratio BSA:triglyceride 100:1, wt/wt), VLDLp at the triglyceride concentration indicated, and 5 $\mu$g LpL in 0.05 M Tris-HCl, pH 7.4, containing 0.9% NaCl. The final volume was 1.0 ml. The amount of apoC-II incorporated (as $\mu$g/1133 nmol triglyceride) in each reaction mixture was: 0 $\mu$g (●), 0.3 $\mu$g (○), 1.0 $\mu$g (▲), 1.5 $\mu$g (△), 3.2 $\mu$g (■), and 15.7 $\mu$g (□). (A) Plots of initial rate of triglyceride hydrolysis versus concentration of VLDLp-triglyceride; (B) plots of substrate concentration divided by initial rate versus substrate concentration. The y-intercepts are equal to $K_m/V_{max}$; the slopes are equal to $1/V_{max}$.

increase, decrease, or remain unchanged at pH values intermediate between those chosen for the experiment.

## DISCUSSION

ApoC-II may facilitate LpL-catalyzed hydrolysis by altering the properties of the surface and/or core of VLDL and chylomicrons, by interacting directly with LpL to increase its specific activity, or by a combination of effects. Four systems have been employed to investigate the mechanism of apoC-II activation: artificial emulsions, trypsinized lipoprotein particles, tri- and diglyceride monolayers, and short-chain glyceride substrates. The extent to which apoC-II increases the rate of LpL-catalyzed hydrolysis depends on the form in which the substrate molecules are presented to the enzyme (10, 17, 36, 37). Reduced activation by apoC-II when "pure" substrate is presented to the enzyme relative to that which occurs when the substrate is covered with phospholipid suggests that, in the case of physiological substrates, a primary role of apoC-II is to favor the interaction of LpL with the triglyceride molecules in the lipoprotein surface. The data reported herein are consistent with, but not definitive proof of, this line of reasoning.

Guinea pig liver perfusate VLDLp is an ideal substrate with which to investigate the mechanism by which apoC-II activates LpL. Guinea pig VLDLp does not carry an LpL activator: there is no equivalent of human apoC-II, and the apolipoproteins isolated from VLDLp do not activate the enzyme in vitro. VLDLp does however, bind human apoC-II, and the activator peptide can be added directly to VLDLp or allowed to transfer to VLDLp from human HDL. ApoC-II associated with VLDLp increases the rate of

TABLE 4. Lipoprotein lipase-catalyzed hydrolysis of guinea pig VLDLp: dependence of apparent $K_m$ and $V_{max}$ on apoC-II

| VLDLp-associated ApoC-II ($\mu$g)[a] | Apparent $K_m$[b] | Apparent $V_{max}$[c] |
|---|---|---|
| | *(mM)* | *(mmol fatty acids released $h^{-1}$ mg $LpL^{-1}$)* |
| 0.0 | 2.8 | 7.4 |
| 0.3 | 1.9 | 7.8 |
| 1.0 | 1.6 | 7.3 |
| 1.5 | 1.3 | 7.3 |
| 3.2 | 1.1 | 10.6 |
| 15.7 | 0.7 | 10.6 |

[a] ApoC-II concentrations are reported as $\mu$g of apoC-II per mg (1131 nmol) VLDLp-triglyceride.

[b] $K_m$ values are reported as mM VLDLp-triglyceride; experimental error is <5%.

[c] Experimental error is ±5%.

Guinea pig [$^3$H-oleate]-labeled VLDLp was incubated with human apoC-II and reisolated as outlined in Methods. The [$^3$H-oleate]-labeled VLDLp was incubated with LpL at 25°C, and initial rates of lipolysis were determined by the rate of release of unesterified fatty acids. The amount of lipoprotein-associated apoC-II was assessed by radioimmunoassay.

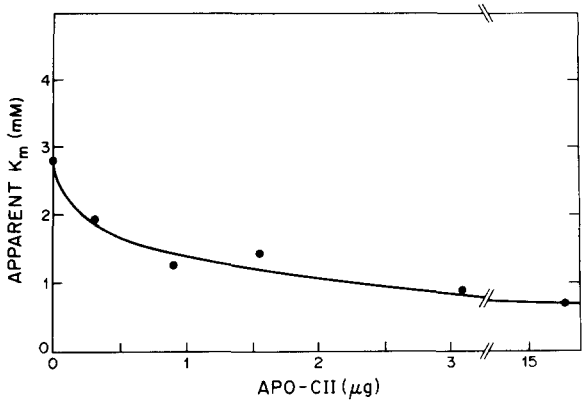


**Fig. 8.** The dependence of apparent $K_m$ of LpL for VLDLp-triglyceride on apoC-II. [$^3$H-Oleate]-labeled VLDLp was enriched in apoC-II by addition of the purified apoprotein as outlined in Methods. The data are taken from Table 4.

LpL catalyzed hydrolysis of VLDLp-triglycerides by as much as 10-fold. The activator potency of apoC-II is independent of the method of enrichment: delipidated human apoC-II and apoC-II transferred from human HDL activate LpL to equal extents. Moreover, the apoC-II-enriched VLDLp employed in this investigation alleviates two shortcomings of conventional emulsion assay systems. First, the VLDLp has been characterized: the lipoproteins are biologically-packaged entities resembling their plasma counterparts in structural organization, they are relatively homogeneous in size and reproducible in composition, and they have intact high molecular weight apolipoproteins. Second, reisolation of apoC-II-VLDLp complexes prior to addition of LpL eliminates the simultaneous presence of both VLDLp-associated and unassociated activator.

At pH 7.4 and over the range of VLDLp-triglyceride from 0.24–2.0 mM, apoC-II decreases the apparent $K_m$ for VLDLp by 4-fold, but has less effect (a 1.3-fold increase) on the apparent $V_{max}$ of the reaction. The apparent $K_m$ obtained at optimum activator concentration, about 0.7 mM triglyceride, is comparable to that obtained for fully-activated chylomicrons and VLDL in perfused adipose tissue (38). The optimum activator concentration is between 3 and 16 $\mu$g of apoC-II per mg VLDLp-triglyceride or 0.34–1.81 nmol of apoC-II per 91 pmol LpL, corresponding to a molar ratio of apoC-II–LpL between 4–20:1. Furthermore, the optimum apoC-II–LpL ratio determined by the method of initial velocity agrees well with the optimum ratio of 2.5–6.2:1 determined by analyzing the increase of observed first-order rate constant for hydrolysis at a single substrate concentration, 0.6 mg/ml VLDLp-triglyceride, with increasing concentrations of apoC-II. The observed first-order rate constant is closely equal to $V_{max}/K_m$ at substrate concentrations well below the apparent $K_m$, consistent with the result that apoC-II decreases the apparent $K_m$.

TABLE 5. Lipoprotein lipase-catalyzed hydrolysis of guinea pig VLDLp: dependence of apparent $K_m$ and $V_{max}$ on pH

| pH | Apparent $K_m$[a] (mM) | | Effect of ApoC-II[c] | Apparent $V_{max}$[b] (mmol fatty acids released $h^{-1}$ mg $LpL^{-1}$) | | Effect of ApoC-II |
|---|---|---|---|---|---|---|
| | −C-II | +C-II | | −C-II | +C-II | |
| 6.5 | 3.6 | 2.2 | 0.6 | 6.8 | 8.9 | 1.3 |
| 7.5 | 2.9 | 0.9 | 0.3 | 7.1 | 9.4 | 1.3 |
| 8.5 | 2.3 | 1.7 | 0.7 | 7.2 | 11.8 | 1.6 |

[a] $K_m$ values are reported as mM VLDLp-triglyceride; experimental error is ±6%.

[b] Experimental error is ±6%.

[c] Ratio of parameter obtained in the presence of apoC-II to that obtained without added apoC-II.

Guinea pig [$^3$H-oleate]-labeled VLDLp was incubated with or without apoC-II as outlined in Methods. The concentration of apoC-II was 5 $\mu$g per mg VLDLp-triglyceride. LpL was added, and initial rates of lipolysis were determined at 23°C by the rate of release of unesterified fatty acids.

The interpretation of an apoC-II-dependent apparent $K_m$ and apparent $V_{max}$ is not straightforward. The data must be considered in view of the simplest scheme for interfacial enzyme kinetics described by Verger and de Haas (39). According to equation *1*), LpL forms two types of complexes with VLDLp: an initial adsorption complex (*A*) in which LpL is associated with the VLDLp surface but not with a triglyceride molecule, and a catalytically competent complex (*B*) in which LpL interacts with the VLDLp particle *and* with triglyceride.

$$S_n \cdot \text{VLDLp} + E \underset{k_d}{\overset{k_a}{\rightleftarrows}} S_n \cdot \text{VLDLp} \cdot E^* \underset{k_2}{\overset{k_1}{\rightleftarrows}} \quad (A)$$

$$S_{n-1} \cdot VLDLp \cdot S \cdot E^* \xrightarrow{k_{cat}} S_{n-1} \cdot VLDLp \cdot E^* + P \quad \text{Eq. } 1)$$

$$(B)$$

E = LpL in solution; E* = adsorbed LpL; S = triglyceride "eligible" for interaction with LpL[6] (*i.e.*, that fraction of total triglyceride accessible to LpL); n = number of molecules; P = lipolysis products.

According to this scheme and assuming that the catalytic step is irreversible, the apparent $V_{max}$ and $K_m$ are defined by equations *2*) and *3*).

$$V_{max} = \frac{k_{cat} \cdot E_0 \cdot S}{S + K_m^*} \quad \text{Eq. } 2)$$

$E_0$ = total LpL concentration; $K_m^*$ = interfacial Michaelis constant = $(k_{cat} + k_2)/k_1$.

$$K_m = \frac{k_d}{k_a} \cdot \frac{K_m^* \cdot S}{S + K_m^*} \quad \text{Eq. } 3)$$

$V_{max}$ is attained when all of the LpL is associated with the VLDLp surface. Since it is not possible to distinguish the fraction of LpL adsorbed to VLDLp (complex A) from that converted to catalytically competent LpL-triglyceride complex (complex B), $V_{max}$ and $K_m$ are only apparent values. In addition, as indicated in equations *2*) and *3*), both kinetic parameters depend on a number of constants. It is conceivable that apoC-II influences all of the parameters: $k_a$, $k_d$, $k_1$, $k_2$, $k_{cat}$, and S (the amount of accessible triglyceride). To help narrow the possibilities, the simplest kinetic situations are presented in **Table 6.**

One situation straightforwardly predicts the observed trends in experimental data, i.e., that apoC-II decreases $K_m^*$ and that, in the absence of apoC-II, $K_m^* \sim S$ (cf. situation IIC, Table 6). Both equations *2*) and *3*) contain a term that is herewith designated the LpL active site occupation factor (equation *4*):

$$\text{Occupation Factor} = \frac{S}{K_m^* + S} \quad \text{Eq. } 4)$$

The occupation factor is the fractional occupancy of enzyme active sites by accessible substrate molecules (S; cf. equation *1*). In equation *2*) the occupation factor multiplies the "intrinsic $V_{max}$" (equal to $k_{cat} \cdot E_0$). In equation *3*) the occupation factor multiplies $(k_d/k_a)K_m^*$, the partitioning ratio between LpL with active sites not bound to substrate molecules (free LpL plus LpL in complex A) and LpL in the complex B form. In equation *4*), if $S \gg K_m^*$ all LpL adsorbed to the VLDLp interface is in the complex B form, and LpL active sites are saturated with triglyceride molecules. The occupation factor is therefore equal to 1. However, if $S \ll K_m^*$, the occupation factor is $S/K_m^*$ and is $\ll 1$, and most of the LpL active sites are free. By decreasing $K_m^*$, apoC-II increases the occupation factor and, from equation *2*), increases the apparent $V_{max}$. The apparent $K_m$ is decreased by apoC-II because $K_m^*$ in the numerator of equation *3*) is decreased, even though this decrease is partly offset by an increase in the occupation factor.

None of the other simple models to explain the role of apoC-II satisfy the experimental data obtained for the pH range 6.5–8.5 ApoC-II most likely does not favor the adsorption of LpL to the VLDLp surface (cf. situation I), nor does apoC-II influence the number of substrate molecules accessible to the enzyme (cf. situations II and IV). Finally, the data indicate that the singular influence of apoC-II cannot be to alter $k_{cat}$ (cf. situations V and VI). Complex kinetic mechanisms in which apoC-II affects more than one parameter are not considered since, in keeping with the principle of

TABLE 6. Possible origin of apoC-II-induced changes in apparent $K_m$ and $V_{max}$ for LpL-catalyzed hydrolysis of VLDLp-triglycerides[a]

| | Situation | Apparent $K_m$ | Apparent $V_{max}$ |
|---|---|---|---|
| I: | $k_d/k_a$ decreased | decreased | unchanged |
| II: | $K_m^*$ decreased | | |
| | a) $S \gg K_m^*$ | decreased | unchanged |
| | b) $K_m^* \gg S$ | unchanged | increased |
| | c) $K_m^* \sim S$ | decreased | increased |
| III: | S decreased | | |
| | a) $S \gg K_m^*$ | unchanged | unchanged |
| | b) $K_m^* \gg S$ | decreased | decreased |
| | c) $K_m^* \sim S$ | decreased | decreased |
| IV: | S increased | | |
| | a) $S \gg K_m^*$ | unchanged | unchanged |
| | b) $K_m^* \gg S$ | increased | increased |
| | c) $K_m^* \sim S$ | increased | increased |
| V: | $k_{cat}$ decreased | | |
| | a) $S \gg K_m^*$ | decreased | decreased |
| | b) $K_m^* \gg S$ | | |
| | i) $k_2/k_1 \gg k_{cat}$ | unchanged | decreased |
| | ii) $k_{cat} \gg k_2/k_1$ | unchanged | decreased |
| | iii) $k_{cat} \sim k_2k_1$ | unchanged | decreased |
| | c) $K_m^* \sim S$ | decreased | decreased |
| VI: | $k_{cat}$ increased | | |
| | a) $S \gg K_m^*$ | increased | increased |
| | b) $K_m^* \gg S$ | | |
| | i) $k_2/k_1 \gg k_{cat}$ | unchanged | increased |
| | ii) $k_{cat} \gg k_2/k_1$ | unchanged | increased |
| | iii) $k_{cat} \sim k_2/k_1$ | unchanged | increased |
| | c) $K_m^* \sim S$ | increased | increased |

[a] Predictions are based on Equations *2*) and *3*). Kinetic situations in which apoC-II influences more than one variable simultaneously are not considered in this table.

[6] $S_n$, the number of triglyceride molecules that can interact with LpL and that can be hydrolyzed, may be equal to or less than the total number of triglyceride molecules present in each VLDLp particle. The term "eligible" does not connote location of the triglyceride; that is, the term is not intended to differentiate between surface and core triglyceride molecules since such a distinction is irrelevant to the kinetic treatment of the data.

least complexity (Occam's razor), they are not justified by the experimental data.

The power of including the concept of the occupation factor in the lexicon of LpL catalysis is that it accommodates various observations in the literature on apoC-II activation of LpL. For example, apoC-II increases the rate of LpL-catalyzed hydrolysis of emulsions and apolipoprotein-depleted lipoproteins by as much as 30-fold (10, 17) but enhances the hydrolysis of diglyceride monolayers by only twofold (36). In emulsions and lipoproteins, triglyceride cores are stabilized by surface layers of phosphatidylcholine or detergent. Accessible substrate in these systems is that fraction of substrate in the surface layer. In lipoproteins, the triglycerides may comprise less than 1% of the surface molecules (40). The occupation factor of equation *4*) is correspondingly small due to the small S term, and the change that apoC-II can cause in the occupation factor by influencing $K^*_m$ is large. However, when "pure" substrate presents to LpL, as in a monolayer film of diglycerides, S will be large and the occupation factor will be close to 1. The change that apoC-II can cause in the occupation factor is small in this case because the upper limit of the occupation factor is 1. The occupation factor can also explain the acid limb of the pH dependence of the influence of apoC-II on $1/K_m$ of purified rat plasma LpL-catalyzed hydrolysis of emulsified triolein reported by Fielding (41). If $1/K_m$ increases with pH because $K^*_m$ decreases with increasing pH, there should be no apoC-II dependence of the apparent $K_m$ at low pH because $K^*_m \gg S$. In this case the occupation factor is small (cf. equation *4*) and equation *3*) reduces to equation *5*).

$$K_m = \frac{k_d}{k_a} S \qquad \text{Eq. } 5)$$

ApoC-II does not change the apparent $K_m$ at low pH because $K^*_m$, which is decreased by apoC-II, drops out of equation *3*) as is shown by equation *5*). However, as the pH increases, $K^*_m$ decreases and eventually becomes approximately equal to S, so that equation *3*) which contains the occupation factor term now holds. ApoC-II will decrease the apparent $K_m$ near the pH optimum because the apparent $K_m$ term contains $K^*_m$. This discussion demonstrates that two seemingly exclusive observations, the acid limb pH dependence of $1/K_m$ and the corresponding pH dependence of apoC-II enhancement of $1/K_m$, can be simply explained by referring to the occupation factor.

In summary, guinea pig VLDLp prepared by perfusion of guinea pig livers is a suitable substrate with which to assess the activation of LpL by apoC-II. The data are consistent with a mechanism of activation whereby apoC-II facilitates formation of a catalytically competent complex in which adsorbed LpL interacts with triglyceride substrate and in which catalytic turnover is more rapid. Since extrapolation to infinite substrate concentration (ratio VLDLp:LpL >>> 1) predicts a decrease of activation by apoC-II (also see ref. 42 and 43), the importance of apoC-II in lipolysis will depend on the amount of triglyceride present. Triglyceride enters the circulation at high concentration in postprandial bursts, suggesting, as did Fielding and Havel in 1977 (44), that apoC-II is most important in dictating further catabolism of remnants of VLDL and chylomicrons that have decreased triglyceride and decreased apoC-II.

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