Further Observations on the Activation and Inhibition of Lipoprotein Lipase by Apolipoproteins

By Ronald M. Krauss, Peter N. Herbert, Robert I. Levy, and Donald S. Fredrickson

ABSTRACT

ApoC-II was the only apolipoprotein from human very low density lipoprotein that activated rat adipose tissue lipoprotein lipase. Activation was blocked by anti- serum against apoC-II. Addition of increasing amounts of activator did not alter the apparent \( K_m \) of lipoprotein lipase (0.32 mM triolein), but it did produce a progressive increase in the apparent \( V_{max} \) from 0.8 to 2.2 \( \mu \)moles free fatty acid/mg hour\(^{-1} \). Substrate concentrations above 1.27 mM triolein diminished activation by 0.25—5.0 \( \mu \)g/ml of apoC-II as much as 20%. Reversal of this apparent substrate inhibition was achieved by increasing the activator concentration to 50.0 \( \mu \)g/ml. Each of five nonactivating apolipoproteins—apoC-I, C-III-1, C-III-2, A-I, and A-II—inhibited lipoprotein lipase up to 85—100%. ApoC-II also produced less inhibition under appropriate conditions. Inhibition was dependent on apoprotein concentration, inversely related to substrate triglyceride concentration, and unobserved with nonlipoprotein proteins. The inhibitory capacity of the nonactivating apolipoproteins was about the same, was independent of apoC-II concentration, and occurred when the ratio of nonactivator apoprotein to triglyceride exceeded 3% (w/w). It is possible that these apoproteins function partly to modulate the hydrolysis of very low density lipoprotein triglyceride by lipoprotein lipase.

KEY WORDS

rat adipose tissue

high density lipoprotein

enzyme kinetics

very low density lipoprotein

immunoprecipitation

The hydrolysis of triglyceride by lipoprotein lipase (glycerol-ester hydrolase E.C.3.1.1.3) in vitro requires the presence of a lipoprotein activator in the assay medium (1, 2). The activator requirement can be met by high density or very low density lipoproteins (1—3) and by high density apolipoproteins recombinated with phospholipid (4). Although the major high density apolipoproteins have no activating effect (5, 6), two of the lower molecular weight apoproteins found in both high density and very low density lipoproteins, apoC-II and apoC-III, have been reported to be activators of lipoprotein lipase from rat adipose tissue (5, 7), human adipose tissue (7), bovine milk (8—8), and human milk (7). ApoC-II consistently acts as a potent enzyme activator, but apoC-III has a significantly lower, more variable capacity for activation (5—8). Recently, it has been suggested that the activation obtained with certain preparations of apoC-III may be due to contamination with small quantities of apoC-II (9).

ApoC-I, another low molecular weight apolipoprotein, has also been reported to activate lipoprotein lipase from several sources (7, 8). Ganesan et al. (7) observed activation by apoC-I with enzyme purified from human, dog, and rat post-heparin plasma but not with enzyme from adipose tissue or milk. The activating effect of apoC-I in their study exceeded that produced by apoC-II. In contrast, Havel et al. (8) observed activation by apoC-I with lipases from plasma, adipose tissue, and bovine milk; this activation was much weaker than that produced by apoC-II. These discrepant results may relate to differences in methods of enzyme preparation or enzyme assay (8).

Apolipoproteins have been shown not only to activate but also to inhibit the activity of lipoprotein lipase (6, 8, 9). Such an effect has been

1The nomenclature of the apolipoproteins used is that suggested by Alaupovic (10). In the alternative classification based on carboxy-terminal amino acids (11) these apolipoproteins are identified as follows: apoC-I = apoLP-Ser, apoC-II = apoLP-Glu, apoC-III = apoLP-Ala, apoA-I = apoLP-Gln-I, and apoA-II = apoLP-Gln-II.
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described for apoC-III with bovine milk (6, 8, 9) and for apoC-I and apoC-III with purified enzyme from bovine milk or rat postheparin plasma (9). Some authors have also found high concentrations of apoC-II to be inhibitory (5, 6, 8, 9).

In the present paper, we examined the effects of purified human apolipoproteins on lipoprotein lipase to define further the nature of the activation and the inhibition of this enzyme.

Methods

APOLIPOPROTEINS

Very low density apolipoproteins (apoC-I, C-II, and C-III) and high density apolipoproteins (apoA-I and A-II) were prepared using previously published techniques (12-14). The homogeneity of these preparations was established by polyacrylamide gel electrophoresis (Fig. 1) and amino acid analysis (12-14). In the separation of very low density apolipoproteins, two forms of apoC-III (C-III-1 and C-III-2) that differ only in content of sialic acid (15) have been isolated.

OTHER PROTEINS

Lysozyme and vasopressin were obtained from Calbiochem, gamma globulin from Pentex, and ribonuclease-A from Sigma Chemical Company.

PREPARATION OF LIPOPROTEIN LIPASE

Lipoprotein lipase was prepared from the epidydimal fat pads of fed 200-350-g Osborn-Mendel rats by the method of Greten et al. (16). The fat pads were either divided immediately after excision or stored at —20°C for up to 2 weeks. Tissue extracts were freshly prepared for each assay. Despite repeated extractions with large volumes of acetone and ether, phospholipids (approximately 15-20% of protein [w/w]) were still detectable in the enzyme extracts by a sensitive assay (17). Delipidation with chloroform and methanol (2:1, v/v) removed all phospholipid but resulted in complete loss of enzyme activity. Protein concentrations were determined by the method of Lowry et al. (18).

LIPOPROTEIN LIPASE ASSAY

Lipoprotein lipase activity was measured using a previously described assay procedure (19). A standard substrate emulsion was prepared by sonification of the following constituents: triolein 113 µmole (100 mg), glyceryl-tri-(14C)-oleate 5-250 µc (30 µc/µmole), fatty acid-free albumin 200 mg, Triton X-100 0.6 ml of 1% aqueous solution, egg lecithin 0.8 mg, heparin 20 units, and Tris-HCl, 0.194M (pH 8.6) containing 0.15M NaCl to a total volume of 12 ml (Tris-NaCl buffer, ionic strength = 0.2). Sonification was performed just before use with a Branson sonifier cell disruptor (model W185) for 1 minute at setting 5 (60 w). Phospholipid concentration was sometimes varied (see Results). The results of thin-layer chromatography in a petroleum ether-diethyl ether-acetic acid solution (36:14:0.3) the radiolabeled triolein appeared to be at least 98% pure and to contain less than 0.2% monoglyceride.

Each assay was performed in a total volume of 1 ml made up of 0.9 ml of the substrate medium, 0.015-0.03 ml of enzyme homogenate in 0.025M NH4OH (pH 8.6) containing 0.1-0.2 mg of protein, 0.070-0.085 ml of Tris-NaCl buffer, and quantities of added protein as described in Results. The substrate concentration was varied by diluting the standard substrate emulsion with Tris-NaCl buffer.

Enzyme and substrate were incubated from 10-60 minutes in a shaking water bath at 27°C. The shorter incubation times were used so that at very low substrate triglyceride concentrations no more than 10% of the substrate ester bonds were hydrolyzed. The reaction was terminated, and free fatty acids (FFA) were extracted by the method of Schotz et al. (20). Labeled FFA were counted in a Packard Tri-Carb scintillation spectrometer. After correction for specific activity, extraction volumes, and efficiency, enzyme activity was expressed as µmole FFA liberated per milligram of adipose tissue protein per hour. All assays were performed in duplicate, and the average difference between duplicate values was less than 5%.

Enzyme specific activity varied among different adipose tissue preparations (see Results). It tended to be inversely related to the size of the animal from which the adipose tissue was obtained.

PREPARATION OF ANTISERA

Apoprotein preparations used as antigens produced a single band on polyacrylamide gel electrophoresis; 1-2 mg (0.5 ml) was emulsified with complete Freund’s adjuvant (0.5 ml) and was injected subcutaneously into the backs of white New Zealand rabbits. The rabbits received an identical booster injection 3 weeks

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After the initial one, and 7 days later they were exsanguinated by cardiac puncture.

The antisera so prepared were able to activate lipoprotein lipase because of the rabbit lipoproteins they contained. Preparation of a gamma globulin fraction by precipitation with 14% ammonium sulfate did not eliminate all of the capacity for activation from the antisera. Complete removal of this activity was achieved, however, by dialysis of 25 ml of antiserum against 0.1M Tris-HCl buffer (pH 8.0) followed by chromatography on a 1.2 x 45-cm column of DEAE cellulose equilibrated with the same buffer. The column was eluted in batches with 0.1M Tris-HCl buffer (pH 8.0). All elutable antibody activity was found in the effluent between 30 and 60 ml.

CHARACTERIZATION OF ANTISERA

Antisera prepared to apoC-I and apoC-III produced single precipitation lines with their respective antigens but did not react with the other apoproteins of very low density or high density lipoproteins (Fig. 2). The antiserum to apoC-II reacted with this apoprotein and also weakly with apoC-III (not evident in Fig. 2).

Results

CHARACTERIZATION OF LIPOPROTEIN LIPASE ACTIVITY

Enzyme activity in the present assay system was optimal at pH 8.6. At substrate saturation (8.5 mM triolein) the rate of hydrolysis was linear for 60 minutes at 27°C and for 15 minutes at 37°C. Lipolytic activity was enhanced by the addition of serum and was reduced more than 90% by a 30-minute prior incubation with 0.15 mg/ml of protamine sulfate, 0.01M sodium pyrophosphate, or 0.5M NaCl as described by Korn (1) for rat heart lipoprotein lipase (Table 1). Addition of CaCl₂ produced a 10–20% increase in basal activity at an optimal concentration of 3 mM but did not alter the effects of added serum or apolipoproteins.

The enzyme inactivation produced by protamine and by 0.5M NaCl was not reversed by tenfold dilution of the preincubation mixture or by 16 hours of dialysis at 4°C against the Tris-NaCl buffer. Addition of heparin (1.5 units/ml) or albumin (15 mg/ml) to the preincubation mixture failed to reduce the extent of inactivation by salt, protamine, or pyrophosphate. However, when the substrate, enzyme, and inhibitors were immediately mixed without prior incubation, the loss of activity was much less (Table 1). No further decline of enzyme activity resulted from increasing the protamine concentration by a factor of 20 (Table 1). When pyrophosphate concentration was raised twentyfold in the presence of substrate, enzyme activity fell an additional 60%, but this reduction was substantially less than that obtained by a 30-minute prior incubation of the enzyme with pyrophosphate. An increase in NaCl concentration from 0.5M to 5.0M resulted in a paradoxical increase in enzyme activity (Table 1); this effect has previously been described for lipoprotein lipase but remains unexplained (19).

At a substrate concentration of 0.017 mM triolein, the inactivation produced by protamine, pyrophosphate, and 0.5M NaCl was greater than 90% even if

<table>
<thead>
<tr>
<th>Rat serum (0.04 ml)</th>
<th>Other</th>
<th>Lipoprotein lipase activity (umoles FFA/mg protein hour⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td></td>
<td>0.56, 0.81</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>1.56, 1.97</td>
</tr>
<tr>
<td>+</td>
<td>Protamine sulfate (0.15 mg)</td>
<td>0.11, 1.12</td>
</tr>
<tr>
<td>+</td>
<td>Protamine sulfate (3.0 mg)</td>
<td>0.25, 1.40</td>
</tr>
<tr>
<td>+</td>
<td>Na pyrophosphate (0.01 mmole)</td>
<td>0.04, 1.78</td>
</tr>
<tr>
<td>+</td>
<td>Na pyrophosphate (0.2 mmole)</td>
<td>0.05, 0.72</td>
</tr>
<tr>
<td>+</td>
<td>NaCl (0.3 mmole)</td>
<td>0.04, 1.95</td>
</tr>
<tr>
<td>+</td>
<td>NaCl (5 mmole)</td>
<td>2.94, 3.35</td>
</tr>
</tbody>
</table>

*Enzyme was incubated with or without additions for 30 minutes at 27°C in 0.1 ml of the Tris-HCl buffer prior to adding substrate.
the enzyme had been preincubated with inhibitor.

**LIPOPROTEIN LIPOASE ACTIVATION**

The effects of very low density apolipoproteins on rat adipose tissue lipoprotein lipase assayed with 8.5 mM triolein are depicted in Figure 3. Similar results were obtained with three enzyme preparations and with very low density apolipoproteins from three different sources. Enzymatic activity was enhanced by the addition of apoC-II; near maximal activation occurred at a concentration of 2-5 μg/ml. The other apolipoproteins produced no significant increase in enzyme activity. Variation of the lecithin concentration from 0.01 to 1.0 mg/ml in the mixture produced no observable effect on enzyme activation in this assay system. No increase in activation occurred after a 30-minute prior incubation of apoC-II with enzyme or with substrate.

**EFFECTS OF ANTISERA ON LIPOPROTEIN LIPOASE ACTIVATION**

Using double immunodiffusion on Ouchterlony plates, we found that 0.075 ml of activator-free antiserum made to apoC-II was required to give an immunoprecipitation line in the equivalence range with 2 μg of apoC-II. When apoC-II and the antiserum in these proportions were incubated for 30 minutes at 27°C before enzyme assay, all capacity for activation was lost. If smaller quantities of antiserum were used, less reduction of activation was observed (Fig. 4). The effect of antiserum was unchanged after apoC-II was first incubated with the enzyme or the substrate for 30 minutes at 27°C. Antisera prepared against either apoC-I or apoC-III (0.075 ml) produced no effect on activation by apoC-II (2 μg). None of the antisera altered the enzyme activity observed in the absence of apoC-II.

**KINETICS OF LIPOPROTEIN LIPOASE ACTIVATION**

Activation of lipoprotein lipase by apoC-II was studied at different concentrations of the substrate emulsion (Fig. 5). In the absence of the activator, enzyme activity followed Michaelis-Menten kinetics. With the addition of apoC-II (0.25, 1.0, and 5.0 μg/ml) the activity increased, but above 1.7 mM triolein a decline in activation was observed (Fig. 5).

The apparent inhibition at higher triolein concentrations was not related to the concentration of any of the other components of the substrate emulsion. A 90% reduction in the concentration of albumin or Triton X-100 in the substrate mixture did not reverse the inhibition and resulted in a significant reduction in basal enzyme activity. Similarly, varying the lecithin concentration from 0 to 0.5 mg/ml had no effect on the inhibition. When the apoC-II concentration was raised to 50 μg/ml, however, an apparent reversal of inhibition was observed (Fig. 5).
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Effect of substrate concentration on activity of lipoprotein lipase in the presence of different concentrations of apoC-II. Samples of enzyme extract (0.03 ml) were incubated with the indicated concentration of apoC-II. Substrate concentration was varied by diluting the standard emulsion with Tris-NaCl buffer (see Methods). LPL = lipoprotein lipase.

The extent of apparent substrate inhibition of activation varied with different preparations of apoC-II but was not influenced by different enzyme preparations or by a fivefold increase in enzyme concentration. Figure 5 shows the maximum inhibition observed among five different apoC-II preparations; in two preparations no inhibition was apparent, but in the remaining three preparations the effect was intermediate. No differences in mobility on polyacrylamide gel, amino acid analysis, or immunochemical behavior were detectable among these preparations, and the variability of inhibition was not explained.

Michaelis constants ($K_m$) and maximal enzyme activities ($V_{max}$) for lipoprotein lipase assayed with different concentrations of apoC-II were calculated from Lineweaver-Burk plots of the data in Figure 5 (Fig. 6). In the absence of activator, the apparent $K_m$ was 0.32 mM triolein and the apparent $V_{max}$ was 0.8 µmoles FFA/mg protein hour$^{-1}$. With addition of up to 50 µg/ml of apoC-II, there was no change in apparent $K_m$. $V_{max}$, however, increased to a maximum of 2.2 µmoles FFA/mg protein hour$^{-1}$. These changes were demonstrated with two different preparations of enzyme and activator.

As suggested by Havel et al. (6), activation produced by increasing concentrations of apoC-II appeared to follow saturation kinetics, permitting analysis by an adaptation of the Lineweaver-Burk plot in which apparent $K_m$ corresponds to the concentration of apoC-II required for half-maximal activation (Fig. 7). At the highest substrate level, 8.5 mM triolein, the apparent $K_m$ in different experiments ranged between 0.3 and 2.5 µg/ml with a mean of $1.2 \pm 0.8$ µg/ml (SD) ($n = 8$, representing seven enzyme preparations and five preparations of apoC-II). This variation was directly related to differences in the extent of substrate inhibition described above. The lowest $K_m$ values were obtained in experiments in which substrate inhibition of activation was the least. For example, in the experiment from which the data in Figure 7 are derived, substrate inhibition was not evident, and the $K_m$ was low. At lower substrate concentrations, the apparent $K_m$ was less variable. With 1.7 mM triolein, $K_m$ values of 0.5, 0.6, and 0.9 µg/ml were obtained with three preparations of enzyme and two of apoC-II. Apparent $K_m$ values of 0.3 and 0.6 µg/ml were obtained with three preparations of enzyme and two of apoC-II. Apparent $K_m$ values of 0.3 and 0.6 µg/ml were obtained with a triolein concentration of 0.48 mM. With 0.17 mM triolein, the $K_m$ in one experiment was 0.8 µg/ml. At lower substrate concentrations, inhibition of lipoprotein lipase activity by apoC-II (Fig. 5) prevented the estimation of $K_m$.

INHIBITION OF LIPOPROTEIN LIPASE ACTIVITY BY APOLIPOPROTEINS

Other very low density and high density apolipoproteins were added to lipoprotein lipase in the presence of apoC-II (1 µg/ml). Enzyme activity was then assayed at different concentrations of the...

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Lineweaver-Burk plot of lipoprotein lipase activation by apoC-II. Enzyme samples (0.010 ml) were incubated with the triolein emulsion 8.5 mM and twelve concentrations of apoC-II ranging from 0.25 to 10 Ìg/ml assay. Lipoprotein lipase activity due to activation was calculated as activated enzyme activity minus basal activity. The reciprocals of these values were plotted on the ordinate, and the reciprocals of the apoC-II concentrations were plotted on the abscissa.

The results are expressed as the percent change in enzyme activity produced by the indicated quantity of added apolipoprotein. Each of the apolipoproteins, including apoC-II, produced progressively greater inhibition of enzyme activity as the substrate concentration was reduced or as the apolipoprotein concentration was raised. The results were the same whether the apoproteins were incubated with apoC-II for 2 hours at 27°C before assay. Inhibition by nonactivating apolipoproteins was much greater than that produced by apoC-II, and no one of the nonactivating apoproteins was a consistently more effective inhibitor than any other. The average reduction produced by 50 Ìg/ml of the nonactivating apoproteins was 9% at 8.5 mM triolein, 22% at 1.7 mM triolein, and greater than 90% at the two lowest substrate concentrations. In experiments not shown, each of these apolipoproteins (50 Ìg/ml) was also incubated with lipoprotein lipase alone or with 50 Ìg/ml of apoC-II. The inhibition produced was the same (+10%) as that observed in the presence of 1 Ìg/ml of apoC-II at each of the four substrate concentrations (Table 2). At 1.7 mM triolein, the inhibition of lipoprotein lipase produced by 50 Ìg/ml of each of the nonactivating apolipoproteins was not reversed by either a tenfold increase or decrease in the concentrations of lecithin, albumin, or Triton X-100 or a fivefold increase in enzyme concentration. A comparable degree of inhibition was observed with at least two different preparations of each of the nonactivating apolipoproteins.

Measurements of lipoprotein lipase activity were also made in the presence of 50 Ìg/ml of five nonlipoprotein proteins of varying molecular weights (albumin, vasopressin, lysozyme, ribonuclease, and gamma globulin). Activity was measured at a substrate concentration of 0.017 mM triolein in the presence of apoC-II (1 Ìg/ml). No inhibition of enzyme activity was observed with any of the proteins.

### Table 2

Effects of Apolipoproteins on Lipoprotein Lipase Activity

<table>
<thead>
<tr>
<th>Triolein concentration (mM)</th>
<th>Apoprotein concentration (Ìg/ml)</th>
<th>C-I</th>
<th>C-II-1</th>
<th>C-II-2</th>
<th>A-I</th>
<th>A-II</th>
<th>Average</th>
<th>C-II</th>
<th>% Change in lipoprotein lipase activity</th>
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<td>-6</td>
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<td>-3</td>
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<td>-14</td>
<td>-8</td>
<td>-</td>
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</tr>
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</tr>
</tbody>
</table>

*Indicated quantities of each of the apolipoproteins were incubated with apoC-II (1 Ìg) for 2 hours at 27°C in 0.070 ml of the Tris-NaCl buffer. Substrate (0.0 ml) and enzyme (in 0.05 ml of 0.025 M NaCl) were then added, and enzyme assay was performed as described in Methods. The last column shows the percent change in activity produced by the indicated concentrations of apoC-II alone.
ACTIVATION OF LIPOPROTEIN LIPASE

Discussion

Of the well-characterized very low density and high density apolipoproteins, only that designated apoC-II has consistently been found to activate lipoprotein lipase. In the present study, we showed that apoC-II, but not apoC-I or apoC-III, was capable of activating the enzyme extracted from rat adipose tissue. Activation was completely blocked by the addition of activator-free rabbit antiserum directed against apoC-II but not by the addition of antisera against apoC-I or apoC-III. The failure of the antisera to reduce enzyme activity below the nonactivated level suggests that endogenous activator, if present, was not reactive with or was unavailable to the antisera.

The variable activating effects reported by others (5-8) for apoC-I and apoC-III are difficult to explain. Discrepancies may relate, at least partly, to differences in preparation or assay (8). The potent activation by apoC-I of purified plasma lipase (7) may represent an effect on an enzyme distinct from adipose tissue lipoprotein lipase (8, 21). Activation produced by preparations of apoC-III has been ascribed by Brown and Baginsky (9) to contamination with apoC-II, which they have separated chromatographically from the nonactivating apoC-III component. In contrast, Havel et al. (8) have concluded from amino acid analysis of their activating apoC-III preparations that possible contamination with apoC-II is too slight to account for the activation observed. The compositional differences between apoC-II and apoC-III are small, however, and reliable quantification of apoprotein admixtures is difficult. We used immunochemical techniques to characterize an apoC-III subfraction which was found to exhibit the activating property (Herbert and Krauss, unpublished observations). Activation was lost after immunoprecipitation with anti-apoC-II but was not lost after reaction with anti-apoC-III, thus further supporting the exclusive role of apoC-II in the activation process.

The increase in lipoprotein lipase activity produced by apoC-II was shown in the present study to be affected by the concentration of the substrate emulsion. Substrate concentrations above 1.7 mM triolein inhibited the activation of the enzyme by 0.25-5.0 \( \mu \text{g} \)/ml of apoC-II. This effect depended directly on the concentration of the triglyceride emulsion, varied in different experiments with different apoprotein preparations, and was at least partly reversed by increasing the apoC-II concentration to 50 \( \mu \text{g} \)/ml. It is possible that the higher substrate concentrations do not allow for optimal interaction of activator with the enzyme or substrate. The variability of the phenomenon could not be accounted for; no differences were observed in the chemical properties among any of the apoC-II preparations.

The concentration of substrate emulsion also appeared to influence the inactivation of lipoprotein lipase. At 8.5 mM triolein, maximal inactivation by different concentrations of NaCl, protamine, or pyrophosphate was obtained only after incubation of enzyme with these agents prior to addition of substrate. At lower triglyceride concentrations, preincubation was not required to achieve maximal enzyme inactivation.

Quantification of the kinetic parameters for activation of lipoprotein lipase was complicated not only by apparent substrate inhibition but also by the lack of purity of the enzyme preparation and the possibility that endogenous activator was present, thereby accounting for relatively high basal enzyme activity. In addition, enzyme-substrate interaction takes place at an oil-water interface rather than in solution; thus the \( K_m \) may have a significance different from that in classical Michaelis-Menten theory. The apparent \( K_m \) for lipoprotein lipase in the present assay did not change with exogenous activator, but the apparent \( V_{\text{max}} \) increased as the apoC-II concentration was raised. This finding is consistent with the concept that activation is based, at least partly, on production of a more effective substrate for lipolysis by the enzyme (2, 5, 6).

The concentration of apoC-II required to achieve half-maximal activation of adipose tissue lipoprotein lipase was comparable to that (0.45-0.60 \( \mu \text{g} \)/ml) reported by Havel et al. (6) for activation of milk lipoprotein lipase. At the highest substrate levels, when substrate inhibition was greater, a higher concentration of apoC-II was required.

Each of six very low density and high density apolipoproteins, including apoC-II, inhibited the activity of lipoprotein lipase. The effect was directly dependent on apoprotein concentration and inversely related to the concentration of substrate triglyceride. Similar findings have been described by Brown and Baginsky (9) for the inhibition of lipoprotein lipase in bovine milk by apoC-III. In the present studies, the extent of inhibition produced by the five nonactivating apolipoproteins was similar. Other nonlipoprotein proteins of differing
molecular weights had no inhibitory effect, suggesting that lipase inhibition is a specific property of apolipoproteins.

Although the mechanism of apolipoprotein inhibition of lipoprotein lipase has not been determined, the present results suggest an effect of apoprotein that decreases the availability of the substrate to the active site of the enzyme. The percent reduction produced by each of five nonactivating apolipoproteins was the same regardless of the apoC-II concentration. From the data in Figure 5, it is apparent that at substrate levels of 1.7 mM triolein and less, such reduction of enzyme activity is consistent with a reduction of effective substrate concentration. At higher triolein concentrations, apoprotein and substrate inhibition appeared to be additive.

In a recent study describing inhibitory effects of apoC-I and apoC-III on lipoprotein lipase from different sources, Havel et al. (8) observed that an increase in apoC-II concentration partially reversed apoprotein inhibition. Brown and Baginsky (9), however, found no such reversal of inhibition with added apoC-II. The apparent discrepancy between these studies might be related to different concentrations of apoC-II in their assays. Our results suggest that apoprotein inhibition is mediated by a reduction in the available substrate concentration. At any substrate concentration (Fig. 5) the increase in activity produced by addition of a given amount of apoC-II varies with the apoC-II concentration: with higher apoC-II concentrations, the increment in activity produced by additional apoC-II is less. Thus, reversal of inhibition by added apoC-II may appear to be large when the apoC-II concentration is relatively low (8) but may not be apparent if the concentration is higher (9).

Comparable degrees of enzyme inhibition were observed in our study with 50 μg/ml of apolipoprotein at a substrate level of 1.7 mM triolein, 5 μg/ml at 0.17 mM triolein, and 1 μg/ml at 0.017 mM triolein. The ratio of apoprotein to triglyceride (w/w) in each case was similar (3–6:100). Near-complete inhibition was achieved with a tenfold increase in this ratio. Brown and Baginsky (9) have reported that apoC-III inhibits milk lipoprotein lipase when the apoprotein concentration exceeds 2% of the substrate triglyceride concentration.

The effects of apolipoproteins demonstrated in these in vitro assay systems may not necessarily be involved in the physiological lipolysis of plasma triglyceride. For instance, the high density apolipoproteins, shown in this paper to inhibit lipoprotein lipase, are present in only trace amounts in triglyceride-rich lipoproteins (10, 12). However, the ratios of apoprotein to triglyceride associated with inhibition are within the range reported for the C-apoproteins in very low density lipoproteins (10, 22). The ratio of C-apoprotein to triglyceride, which may be calculated from the compositional data on very low density lipoprotein subfractions, is greater for particles of smaller size (S, 20–60) than it is for those of larger size (22–24). Thus, at physiological plasma triglyceride concentrations, variation in the apoprotein content of very low density lipoproteins conceivably may influence the effectiveness of hydrolysis by lipoprotein lipase.

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References


ACTIVATION OF LIPOPROTEIN LIPASE


Further Observations on the Activation and Inhibition of Lipoprotein Lipase by Apolipoproteins
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