The effect *in vitro* of high-density lipoprotein on hydrolysis of triacylglycerol by lipoprotein lipase

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In an incubation system *in vitro* with fully activated Intralipid as substrate, rat high-density lipoprotein inhibits the hydrolysis of triacylglycerol by lipoprotein lipase from rat adipose tissue, but does not inhibit hydrolysis by the enzyme from bovine milk. The pattern of inhibition suggests that substrate and high-density lipoprotein may compete for association with rat adipose-tissue lipoprotein lipase.

HD lipoprotein participates in triacylglycerol hydrolysis by lipoprotein lipase in at least two ways. First, it acts as a reservoir for apoCII, the activator for lipoprotein lipase, which is transferred between HD lipoprotein and VLD lipoprotein or chylomicrons (Schaefer *et al.*, 1978). Secondly, HD lipoprotein accepts the polar components (phospholipid, cholesterol and protein) that leave the surface of the VLD lipoprotein and chylomicrons as hydrolysis of the triacylglycerol by lipoprotein lipase proceeds (Nicoll *et al.*, 1980). It was therefore decided to investigate the effect of HD lipoprotein on the rate of hydrolysis of triacylglycerol *in vitro* by lipoprotein lipase (Hutchinson & Rogers, 1981). Jackson *et al.* (1979) reported that the addition of human HD lipoprotein to a system containing bovine milk lipoprotein lipase and human VLD lipoprotein resulted in a decrease in the amount of lipase associated with the VLD-lipoprotein remnants, but did not alter the amount of fatty acid released during hydrolysis. Bovine milk lipoprotein lipase is often used because it can be obtained partially purified in relatively large amounts. In this study the effect of HD lipoprotein on hydrolysis of triacylglycerol by lipoprotein lipase from rat adipose tissue was investigated and the results were compared with the findings when bovine milk was the source of the enzyme.

Materials and methods

The rats used were male Porton Wistar maintained on BP Nutritional no. 3 diet [BP Nutrition (U.K.), Witham, Essex, U.K.].

Abbreviations used: HD lipoprotein, high-density lipoprotein; VLD lipoprotein, very-low-density lipoprotein; apoC, apoE, apoAI etc., apolipoproteins C, E, AI etc.

HD lipoprotein (*d* 1.063–1.21) was isolated from rat serum by the procedure of Havel *et al.* (1955). When required, subfractions HD lipoprotein₂ and HD lipoprotein₃ were isolated from the density ranges 1.063–1.125 and 1.125–1.21 respectively. The lipoproteins were dialysed exhaustively against 0.15 M-NaCl solution containing 1 mM-EDTA, pH 7.6. The concentration of HD lipoprotein is expressed as protein determined by the method of Lowry *et al.* (1951). Serum free of VLD lipoprotein was prepared by ultracentrifugation of the serum, as above, at *d* 1.006 and removal of the VLD lipoprotein.

Acetone/diethyl ether-dried powders of fresh unpasteurized bovine milk (Egelrud & Olivecrona, 1972) and rat epididymal adipose tissue (Ashby *et al.*, 1978) were prepared. Portions of the powders were extracted with 0.025 M-NH₃/NH₄Cl, pH 8.1, and the supernatants obtained after centrifugation at 2500 rev./min for 10 min at 4°C were used as the source of lipoprotein lipase.

To obtain Intralipid with a full complement of lipoprotein lipase activator ('activated Intralipid'), the Intralipid was incubated for 30 min at 27°C with VLD-lipoprotein-free serum in the ratio 200 mg of triacylglycerol/4 ml of VLD-lipoprotein-free serum. The mixture was layered with an equal volume of saline and centrifuged for 1 h at 100 000 *g*, as above, and the fully activated Intralipid removed and redispersed through a 25-gauge needle before use.

Triacylglycerol hydrolysis was measured *in vitro* over a period of 1 or 2 h at 27°C in an assay medium, pH 8.1, containing albumin and Tris/HCl at final concentrations of 60 mg/ml and 0.1 M respectively (Rogers *et al.*, 1976). The substrate was the triacylglycerol emulsion Intralipid (A.B. Vitrum, Stockholm, Sweden) activated as above. Lipoprotein lipase and HD lipoprotein were added as

indicated; 0.15M-NaCl solution containing 1mM-EDTA, pH 7.6 (the solution used for dialysing HD lipoprotein), was added, as necessary, to give an equal volume of this solution in all incubations being compared. Non-esterified fatty acids were measured at the beginning and end of the incubations by titration with 2.5mM-tetra-n-butylammonium hydroxide in methanol (Ashby *et al.*, 1978). The rate of hydrolysis (which was linear over the time period measured) is expressed as μmol of non-esterified fatty acid released/h per ml of medium.

Triacylglycerol was determined by the method of Fletcher (1968).

Results

HD lipoprotein inhibited the hydrolysis of activated Intralipid by lipoprotein lipase from rat adipose tissue (Fig. 1). The degree of inhibition depended on the concentration of both the substrate and HD lipoprotein. At the lowest triacylglycerol concentration (1mg/ml) the hydrolysis was decreased by 72% and 86% in the presence of HD-lipoprotein concentrations of 0.1 and 0.3 mg/ml respectively. However, at the highest triacylglycerol

Fig. 1. Inhibition by HD lipoprotein of triacylglycerol hydrolysis by lipoprotein lipase from rat adipose tissue. The stated final concentration of fully activated Intralipid triacylglycerol was incubated in the presence of rat adipose-tissue lipoprotein lipase without HD lipoprotein (open columns) or with HD lipoprotein (0.1 mg/ml, hatched columns; 0.3 mg/ml, stippled columns). Hydrolysis is expressed in all figures as μmol of non-esterified fatty acid released/h per ml of medium. For Figs. 1 and 2 the results are means $\pm$ S.D. (indicated by the bars) for three separate experiments.

concentration (4mg/ml) the decreases were 23% and 42% respectively.

These results suggest that HD lipoprotein and substrate may be competing for association with enzyme, perhaps through a common component such as apoC. To test this, HD lipoprotein was depleted of apoC by incubation with unactivated Intralipid. ApoC and other protein components of HD lipoprotein, apoE and apoAI, are known to associate with Intralipid (Carlson, 1980; Connelly & Kuksis, 1981). No apoC bands were visible by gel electrophoresis in urea of the re-isolated HD lipoprotein obtained by removal of the Intralipid after centrifugation (see the legend to Fig. 2). There

Fig. 2. Effect of apoC-depleted HD lipoprotein on triacylglycerol hydrolysis by rat adipose-tissue lipoprotein lipase.

HD lipoprotein depleted of apoC was prepared by incubating 5.2mg of HD lipoprotein with 200mg of Intralipid triacylglycerol for 30 min at 27°C. The Intralipid was removed by centrifugation at 100000g for 1 h. Various concentrations of fully activated Intralipid triacylglycerol (prepared separately) were incubated in the presence of rat adipose-tissue lipoprotein lipase without HD lipoprotein (□), with apoC-depleted HD lipoprotein (0.3 mg/ml; ○) or with normal HD lipoprotein (0.3 mg/ml; ●).

Fig. 3. Effect of HD lipoprotein on the hydrolysis of triacylglycerol by bovine milk lipoprotein lipase

The stated final concentration of fully activated Intralipid triacylglycerol was incubated in the presence of bovine milk protein lipase without HD lipoprotein (open columns) or with HD lipoprotein (0.1 mg/ml, hatched columns; 0.3 mg/ml, stippled columns). Results are means $\pm$ s.d. (indicated by the bars).

was a lower content of apoAI and apoE in this apoC-depleted HD lipoprotein, as judged from gel electrophoresis in sodium dodecyl sulphate. When the apoC-depleted HD lipoprotein was incubated with activated Intralipid and adipose-tissue lipoprotein lipase, no inhibition of triacylglycerol hydrolysis was observed (Fig. 2).

Further evidence that apoC might be involved in the inhibition of enzyme activity by HD lipoprotein came from studies with the subfractions HD lipoprotein₂ and HD lipoprotein₃. In incubations with adipose-tissue lipoprotein lipase and fully activated Intralipid at a triacylglycerol concentration of 3 mg/ml, the percentage inhibition of enzyme activity was 54% and 24% for HD lipoprotein₂ and HD lipoprotein₃ (0.3 mg/ml) respectively. The subfraction HD lipoprotein₂ carries more apoC than does HD lipoprotein₃ (Schaefer *et al.*, 1978).

In contrast with the findings for lipoprotein lipase from rat adipose tissue, when lipoprotein lipase from bovine milk was incubated with activated Intralipid there was only very slight inhibition of hydrolysis in the presence of HD lipoprotein (Fig. 3). Moreover, the degree of inhibition was independent of substrate concentration.

Discussion

This study shows that HD lipoprotein inhibits the hydrolysis of an activated triacylglycerol emulsion by lipoprotein lipase from rat adipose tissue. The pattern of inhibition is similar to that shown by competitive inhibitors, and it is likely that HD lipoprotein and substrate compete for association with the enzyme. There are a number of protein components of HD lipoprotein that could be involved in this association, in particular the apoC proteins, apoE or apoAI. In support of this suggestion, other work (Rogers, 1981) showed that HD lipoprotein decreased the amount of adipose-tissue lipoprotein lipase (as measured by enzyme activity) that could be isolated with the substrate. The results reported here suggest that the presence of apoC is important for HD lipoprotein to associate with the enzyme. In this respect our findings for rat adipose tissue differ from those published by Bengtsson & Olivecrona (1980a), while this work was in progress, on the binding of the hepatic heparin-releasable lipase to HD lipoprotein. These workers reported that the binding was independent of the apoC content of HD lipoprotein.

Bovine milk lipoprotein lipase was only very slightly inhibited by HD lipoprotein. This enzyme binds readily to Intralipid in the absence of apoC (Bengtsson & Olivecrona, 1980b). It could be that the association between enzyme and substrate is tighter for milk lipoprotein lipase than for rat adipose-tissue enzyme and that, therefore, HD lipoprotein does not compete effectively with substrate.

The finding that HD lipoprotein inhibits the hydrolysis of triacylglycerol by rat adipose-tissue lipoprotein lipase is of interest, although the relevance of these results to the situation *in vivo* with physiological substrates has yet to be determined. Moreover, the observation of a differential effect of HD lipoprotein on hydrolysis of triacylglycerol by lipoprotein lipase from dissimilar sources is also of importance, as the bovine milk enzyme is the one most often studied.

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