HORMONAL CONTROL OF ADIPOSE TISSUE CLEARING FACTOR LIPASE ACTIVITY

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1. Introduction

There is now good evidence that the enzyme clearing factor lipase (or lipoprotein lipase) controls the removal of triglyceride from the blood by the extrahepatic tissues of the body [1,2]. Consistent with such a function is the finding that the activity of the enzyme in rat adipose tissue falls on starvation and rises again on refeeding, in parallel with corresponding alterations in the uptake of plasma triglyceride fatty acids (TGFA) by the tissue [2]. Such changes in the activity of clearing factor lipase appear to be hormonally determined. For instance, when adipose tissue from starved rats is incubated in an appropriate medium in vitro, rises in the activity of the enzyme occur and these are promoted by insulin and inhibited by several other hormones, including the catecholamines [3–5].

Though some of the enzyme in adipose tissue is present in association with the fat cells, this fraction is probably not physiologically active in TGFA uptake [6–8]. Infact, the functional enzyme appears to be located in the tissue at the luminal surfaces of the capillary endothelial cells where the plasma chylomicrons and very low density lipoproteins are sequestered by the enzyme and their triglycerides are hydrolysed [1,2]. Moreover, when changes in the total activity of the adipose tissue enzyme occur – as, for instance, on starvation and refeeding – it appears to be only the activity of the functional enzyme that alters significantly. This has, therefore, led to the view that the fat cell enzyme acts as a precursor of that at the endothelial cell surface [4,5].

Recent work by Garfinkel and Schotz has suggested that clearing factor lipase also exists in adipose tissue in two molecular forms, separable by gel filtration chromatography [9]. Furthermore, there is evidence that the form of higher molecular weight (form *a*) is that which is present at the endothelial cell surface and functional in TGFA uptake, while the form of lower molecular weight (form *b*) is that which is associated with the fat cell fraction [10]. The present study was initiated to discover whether the hormonal regulation of the activity of the enzyme involved the interconversion of the forms *a* and *b*. The results suggest, however, that a more complex situation exists. Thus, it appears that there may be at least three forms of the enzyme in the tissue, and that hormonal regulation may be concerned with the interconversion of two forms (*b* and *b'*) of similar molecular weight but different specific activity.

2. Materials and methods

Epididymal fat bodies were taken from male albino rats of the Wistar strain (body weight, 180–200 g) that were maintained on Oxoid modified Diet 86 (H. Styles Ltd., Bewdley, England). Acetone-ether dried preparations of the fat bodies were made as described previously [11], except that the fat bodies were homogenized directly in acetone.

Clearing factor lipase was assayed in extracts of the fat body preparations, as well as in fractions thereof, with Intralipid (Vitrum, Stockholm) as the trigylceride substrate [12], one unit of enzyme activity being equivalent to the release of 1 μmole of free

fatty acid per hour. The extracts were made by homogenizing 200 mg of the preparations in 160 ml of 50 mM NH_4OH–NH_4Cl buffer, pH 8.1 at 4°C. After centrifuging for 30 min at 35 000 *g* and recovering the supernatant, the pellet was rehomogenized in 40 ml of the same buffer and the homogenate was recentrifuged. The supernatants were combined and concentrated for 2–3 hr at 4°C in an Amicon ultrafiltration cell (Model 202) using a PM 30 filter. The concentrated extracts (about 7 ml) contained 45–55% of the clearing factor lipase activity of the original preparations. Suitable portions (2–3 ml) were applied to Sepharose 6B (Pharmacia Ltd., Uppsala, Sweden) columns (110 × 1 cm) that had been pre-equilibrated with the NH_4OH–NH_4Cl buffer. Elution of fractions was carried out with a constant head (25 cm) of the same buffer at a flow rate of 5 ml/hr and 65–85% of the enzyme activity applied was recovered. Rat serum, ovalbumin (Grade 5, Sigma Chemical Co., London) and haemocyanin (molecular weight, 9×10^6) were used to calibrate the column. No significant differences were observed in the protein elution profiles of any of the preparations applied and, therefore, these are not shown in the figures.

In some experiments the fat bodies were incubated at 25°C or 37°C before the acetone–ether dried preparations were made. The conditions were similar to those described by Robinson and Wing [4,5,7], except that heparin was always absent from the incubation medium.

Cycloheximide was obtained from Sigma Chemical Co., London, insulin from Burroughs Wellcome Ltd., London, heparin (Pularin) from Evans Medical Ltd., Speke, Liverpool, and adrenaline, glucose and other chemicals from BDH Chemicals Ltd., Poole.

3. Results and discussion

Fig. 1 shows the profiles of clearing-factor lipase activity eluted from Sepharose 6B columns following the application of concentrated extracts of defatted adipose tissue prepared from fed and 24 hr-starved rats. In agreement with the results of Schotz and Garfinkel, obtained with Bio-Gel A-1.5 M [9,10], most of the activity in extracts from fed rats is eluted as a peak of high molecular weight at the void volume. It is this enzyme (form *a*) which the work of Schotz and Garfinkel suggests is the functional form [10,13]. Enzyme activity is also found, however, in a peak of lower molecular weight. This is eluted after the IgG fraction of rat serum (mol. wt. 160 000) and before ovalbumin (mol. wt. 64 000) and has an apparent molecular weight of 100 000–120 000. It represents only a minor proportion of the total enzyme activity in extracts from fed rats, but accounts for most of that in extracts from starved rats. It is this form (form *b*) which Schotz and Garfinkel believe is associated with the fat cell fraction of the tissue [13]. It is noteworthy that, in the elution profile of extracts from fed rats, considerable enzyme activity is also present in the area between the two peaks. In the studies of Schotz and Garfinkel, the peaks were not resolved as completely as in the present work and this intermediate zone of enzyme activity was not observed.

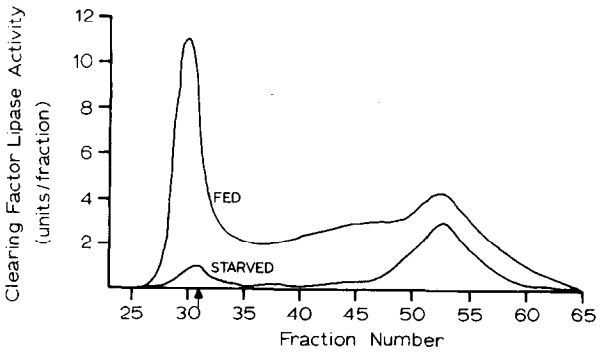

Fig. 1. Elution profiles of adipose tissue clearing factor lipase from fed and 24 hr-starved rats. In each case, extracts of acetone–ether dried preparations were made from 14 fat bodies as described in Materials and methods. Samples of each extract were applied to Sepharose 6B columns and eluted at 4°C. Alternate fractions (1 ml) were assayed for enzyme activity. The column void volume is indicated by the arrow.

Schotz and Garfinkel have shown [13] that, when starved rats are refed for 8 hr, as the total clearing factor lipase activity of adipose tissue increases so does the proportion of that in form *a*. Thus, the elution profile of defatted tissue extracts applied to Bio-Gel columns comes to resemble that observed in extracts from fed rats. The incubation of adipose tissue from starved rats at 37°C in vitro in a medium containing glucose and insulin also causes a rise in the total clearing-factor lipase activity of the tissue [4,5].

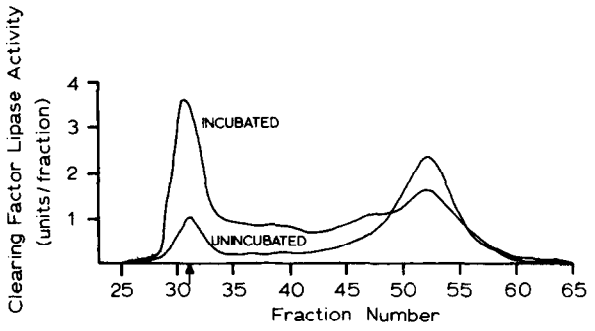

Fig. 2. Elution profiles of clearing factor lipase in adipose tissue from 24 hr-starved rats before and after incubation at 37°C in a medium containing glucose and insulin. Fat bodies were removed from 24 hr-starved rats and paired into two groups, each of 14 fat bodies. One group was incubated for 4 hr in 28 ml of a medium containing glucose (2.4 mg/ml) and insulin (12 mu/ml). After rinsing in 0.85% (w/v) NaCl, an extract of an acetone–ether dried preparation of the fat bodies was made as described in Materials and methods. A similar extract was prepared from the other group of fat bodies immediately after their removal from the animals. Samples of the extracts were chromatographed as in fig. 1.

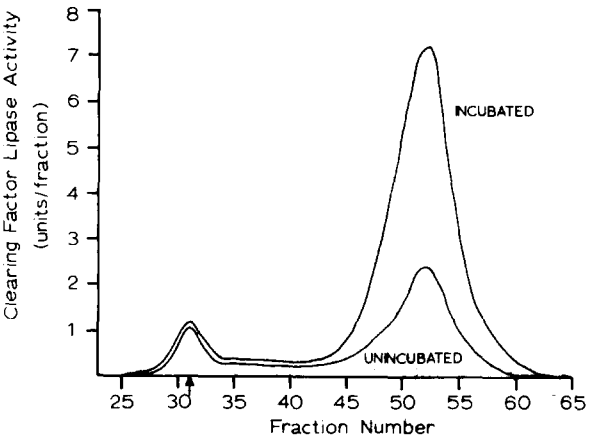

Fig. 3. Elution profiles of clearing factor lipase in adipose tissue from 24 hr-starved rats before and after incubation in a medium containing glucose and insulin. The procedure was as described in the legend to fig. 2, but the incubation temperature was 25°C.

When, after such incubations, extracts of the defatted tissue are applied to Sepharose 6B columns, the elution profile of clearing factor lipase activity is as shown in fig. 2. Evidently, here too there is an increase in the proportion of the enzyme activity that is associated with the peak of high molecular weight (form *a*).

The increase in the form *a* enzyme activity which Schotz and Garfinkel reported on refeeding of starved rats occurred only after a lag period. They were able to show, however, that in rats refed for shorter times there was a rise in the activity of form *b* of lower molecular weight and they suggested that this was later converted to form *a* [13]. We have not observed similar increases in the form *b* peak during incubations at 37°C in vitro. However, the activity of the enzyme in adipose tissue from starved rats also increases in incubations at 25°C [4,14] and, at this temperature, the rise in total activity during a 4 hr-incubation is wholly accounted for by an increase in the form *b* enzyme activity (fig. 3). Similar results have been obtained in incubations lasting 2 hr and 6 hr, as well as in the presence of cycloheximide (10 μg/ml), which previous studies have shown inhibits at least 90% of the protein synthesis in the tissue [14]. The increases in the form *b* activity are markedly reduced when insulin is omitted from the incubation medium, and are abolished when both glucose and insulin are omitted.

The above findings, besides suggesting that the conversion of form *b* to form *a* is temperature dependent, also indicate that hormonal control of the activity of clearing factor lipase in the tissue may occur without protein synthesis and before any conversion of form *b* to form *a*. We have, therefore, carried out experiments to see whether the increase in the activity of form *b* can be reversed in the presence of adrenaline. Two groups of 14 fat bodies were incubated at 25°C in the presence of glucose and insulin for 4 hr, and adrenaline hydrochloride was then added to one group (1 μM, final concn.). After incubation for a further 20 min at 25°C, the medium free fatty acid concentration in the presence of adrenaline was 0.41 μmoles/ml, while that in its absence was 0.07 μmoles/ml, indicating an activation of the adipose tissue mobilizing lipase in the presence of adrenaline. Fig. 4 shows the elution profiles of clearing factor lipase activity after the application to Sepharose 6B columns of extracts of acetone–ether dried preparations made from the two groups of fat bodies. Of particular interest is the reduction in enzyme activity associated with form *b* that occurs

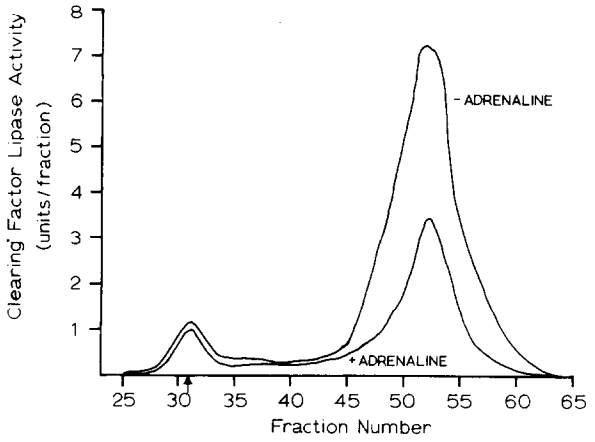

Fig. 4. Elution profiles of clearing factor lipase in adipose tissue from 24 hr-starved rats after incubation for 4 hr at 25°C in a medium containing glucose and insulin, followed by a further incubation for 20 min with or without adrenaline hydrochloride (1 μM, final concn.). Details of the experiment are given in the Results section.

in the presence of adrenaline: further experiments have shown moreover that this reduction is also unaffected by the presence of cycloheximide in the incubation medium.

The enzyme activities shown in figs. 1–4 were all measured in our standard assay [12] in the presence of heparin (0.2 i.v./ml). However, because of evidence that heparin plays an important part in the action of the enzyme [1,2] and that the response of the forms *a* and *b* to it may be different [9, 10, 13], assays were also carried out in its absence. Table 1, which is based on the results of a number of experiments similar to those that have been already described, shows that the activity of form *a* is virtually unaffected by the presence of heparin in the assay, while the activity of form *b* is considerably increased. Of special interest, however, are the variations in the extent of activation of form *b* and, in particular, the finding that the degree of stimulation by heparin increases 7-fold during incubations at 25°C in the presence of glucose and insulin. Moreover, this increase in stimulation is lost again during incubations in the presence of adrenaline. These findings suggest that peak *b* itself contains at least two forms of the enzyme, only one of which is stimulated by heparin, and that insulin and adrenaline bring about the interconversion of these two forms.

On the basis of the above findings we have recently proposed a hypothesis [15] that envisages the initial synthesis in the adipose tissue fat cell of form *b* of the enzyme which is rapidly convertible within the fat cell into form *b'* of lower specific activity but of similar molecular weight. The interconversion of these two forms would appear to be hormonally regulated and, on the basis of evidence which implicates cyclic AMP in the control of the activity of the enzyme [16], it is tempting to speculate that *b'* may be the phosphorylated form of *b*. We believe conversion to form

Table 1

The effect of heparin on the clearing factor lipase activity of peaks *a* and *b*. The peak fractions of highest activity from experiments similar to those shown in figs. 1–4 were assayed in the presence and absence of heparin (0.2 I.U./ml). The heparin concentration was that which produced maximum stimulation.

Adipose tissue system	No. of expts.	Average % stimulation by heparin	
		Peak *a*	Peak *b*
From fed rats	11	7	83
From 24 hr-starved rats	12	5	56
From 24 hr-starved rats. Tissue incubated 4 hr in glucose and insulin at 37°C	3	8	42
From 24 hr-starved rats. Tissue incubated 4 hr in glucose and insulin at 25°C	8	7	305
From 24 hr-starved rats. Tissue incubated 4 hr in glucose and insulin, then in adrenaline for 20 min, at 25°C	4	3	90

a of higher molecular weight may involve protein synthesis [7] and be brought about by the association of form *b* with heparin, or its endogenous counterpart. This, besides leading to a further increase in the specific activity of the enzyme, may commit it to release from the fat cell and transport to its functional site of action at the endothelial cell surface. Further studies to test this hypothesis are in progress.

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