The role of clearing-factor lipase (lipoprotein lipase) in the transport of plasma triglycerides

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The enzyme clearing-factor lipase, or lipoprotein lipase, is responsible for the removal of most of the plasma triglycerides from the bloodstream (Robinson, 1963). The main carriers of such triglycerides are the chylomicrons and the very-low-density lipoproteins and these complexes are believed to be first sequestered by the enzyme at the lumen surfaces of the capillary endothelial cells of the extra-hepatic tissues. Their constituent triglycerides are then hydrolysed at this site and the free fatty acids (FFA) produced are taken up by the tissues either for oxidation or for storage (see Fig. 1).

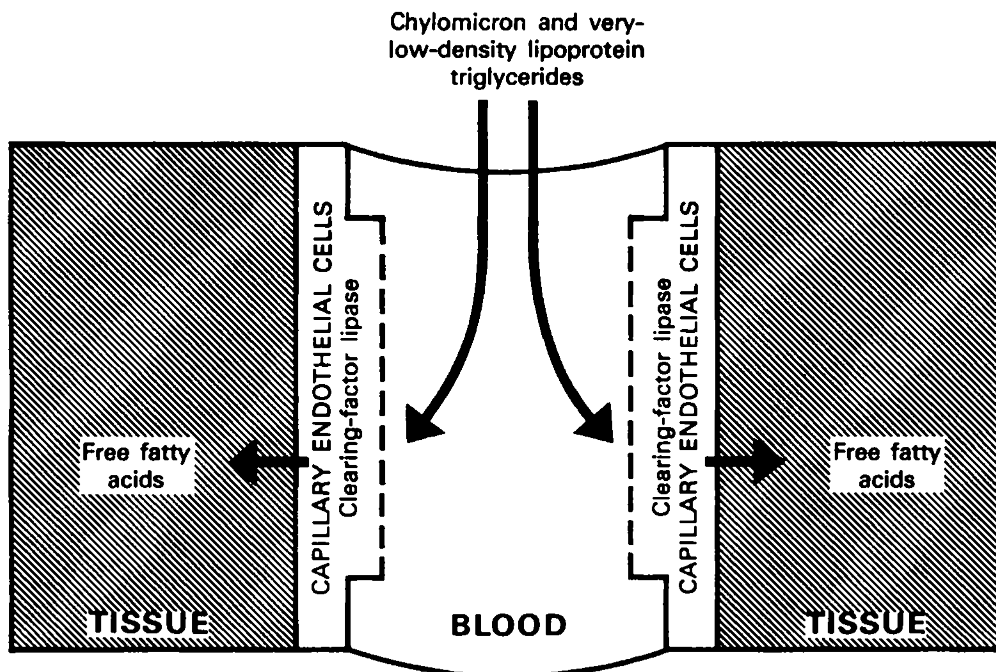

Fig. 1. The role of clearing-factor lipase in the removal of plasma triglycerides from the bloodstream. Adapted from Robinson (1960).

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The activity of clearing-factor lipase in the capillary beds of particular extrahepatic tissues varies with changes in the physiological and nutritional conditions. Thus, in situations of energy excess, as, for example, in animals in the fed state, the enzyme activity in adipose tissue is high while that in muscle is low. As a result a high proportion of the plasma triglyceride fatty acid (FA) are taken up by adipose tissue for storage under such conditions. In energy-deficit situations, on the other hand (during starvation, exercise and cold exposure, for example) the activity in muscle is high while that in adipose tissue is low, and uptake of triglyceride-FA for oxidation by muscle is favoured. These examples, together with others that have been described in the literature, all support the view that the enzyme, besides facilitating the removal of triglyceride-FA from the bloodstream, also controls the pattern of their uptake so as to allow the changing needs of particular tissues to be met (Robinson, 1970).

This regulation of the activity of the enzyme appears to be achieved mainly by hormonal controls. For instance, the activity of the adipose tissue enzyme in the rat in vivo shows a strong positive correlation with the plasma insulin concentration (Cryer, Riley, Williams & Robinson, 1975) and studies in vitro have shown that during incubations carried out at 37° the low activity characteristic of adipose tissue from starved rats increases progressively in the presence of insulin towards that of fed animals. This increase is inhibited by catecholamines and by adrenocorticotrophin and, in a slightly different experimental situation, by glucagon and by thyroid-stimulating hormone (see Robinson & Wing, 1970). High plasma glucagon concentrations, on the other hand, stimulate the activity of the enzyme in cardiac muscle (Borensztajn, Keig & Rubinstein, 1973), whereas plasma insulin concentrations are negatively correlated with its activity in cardiac, diaphragm and skeletal muscle (Cryer, Riley *et al.* 1975). Again there is evidence that the rise in enzyme activity in muscle induced by cold stress may be brought about by changes in the plasma catecholamine concentration (Radomski & Orme, 1971; Rogers & Robinson, 1974). In summary, therefore, it seems that the hormonal balance in the blood supplying a tissue determines the activity of the enzyme in that tissue, and that in this way tissue-specific variations in clearing-factor lipase activity are achieved.

In recent years considerable work concerned with the more detailed molecular mechanism of the control of clearing-factor lipase activity has been carried out. It is now clear that the enzyme that functions at the endothelial cell surface in adipose tissue in vivo is derived from a precursor that exists in the fat cell (Robinson & Wing, 1970). The activity of this fat cell enzyme does not show any marked variations with alterations in nutritional status, and the changes that occur in the enzyme activity of the intact tissue under such circumstances must, therefore, be due to alterations in the activity of the endothelial cell enzyme (Cunningham & Robinson, 1969). The incubation conditions in vitro that lead to progressive increases in the enzyme activity of intact adipose tissue from starved rats also allow increases in activity to occur in fat cells isolated from the tissue (Robinson & Wing, 1970), but, whereas the increase in enzyme activity of intact adipose tissue can take place wholly within the tissue, changes in enzyme activity

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with isolated fat cells occur almost entirely in the incubation medium (Stewart & Schotz, 1974; Cryer, Davies, Williams & Robinson, 1975).

These findings are consistent with the simple concept, shown in Fig. 2, of a precursor fat cell enzyme that can be transported out of the cell to its functional site of action at the endothelial cell surface (Robinson & Wing, 1970). Fig. 2 also implies, however, that the fat cell precursor and the functional enzyme at the endothelial cell surface represent different enzyme forms and that the transfer involves the conversion of the precursor into the functional form and the eventual synthesis of more of the precursor. The results of several studies have provided support for this view. Thus, two forms of the enzyme (*a* and *b*) that differ in their molecular weight have been identified in adipose tissue, and the proportion of the total activity that is contributed by the form of higher molecular weight (*a*) is markedly greater in tissue from rats in the fed state than in that from animals in the starved state, suggesting that it is the form which functions at the endothelial cell surface (Garfinkel & Schotz, 1972; Schotz & Garfinkel, 1972). Again, there is evidence that the rise in activity which occurs when tissue from starved rats is incubated in vitro takes place in two stages. The process is at first independent of protein synthesis (and this is consistent with the activation of a pre-existing enzyme population achieved by the interconversion of forms of different specific activity) but protein synthesis is required for the increases that occur at later times (Wing & Robinson, 1968*a*; Stewart & Schotz, 1971; Cryer, Foster, Wing & Robinson, 1973).

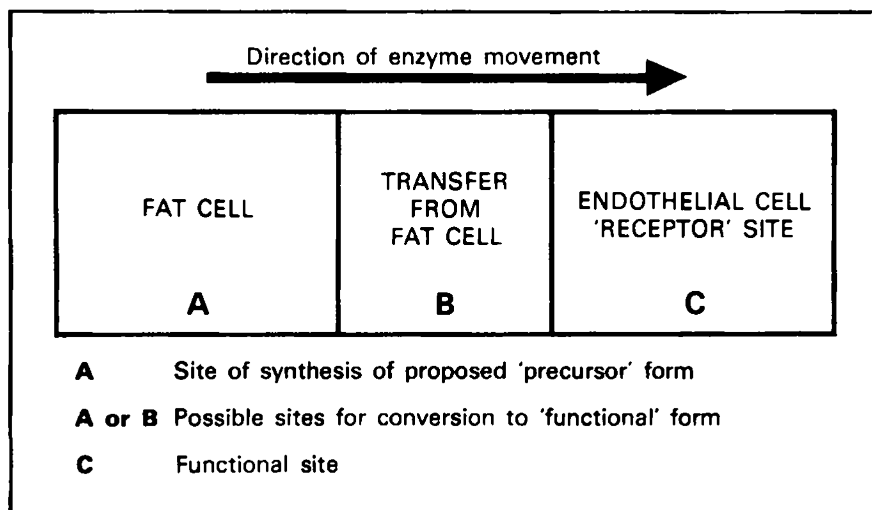

Fig. 2. Possible sites for control of adipose tissue clearing-factor lipase. From Robinson & Wing (1970).

More recent work has suggested that the situation may be more complex than is indicated in Fig. 2. Thus, the increase in adipose tissue enzyme activity that takes place when starved rats are refed appears initially to involve a rise in the proportion of the enzyme form of lower molecular weight (form *b*) (Schotz &

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Garfinkel, 1972; Garfinkel & Schotz, 1973). Moreover, when incubations of tissue from starved rats are carried out at 25° rather than at 37° in vitro, the total enzyme activity of the tissue again increases but there is no increase in the form *a* of higher molecular weight. Instead there is a marked increase in the activity associated with form *b* and this is accompanied by a change in its properties, expressed as an increase in its capacity for stimulation by heparin (Davies, Cryer & Robinson, 1974). This change in the properties of *b* is hormonally controlled, being promoted by insulin and reversed by adrenaline, and is not influenced by the presence of inhibitors of protein synthesis.

These findings suggest that the hormonal control of the activity of clearing-factor lipase is normally exercised in the fat cell through the interconversion of two forms of the enzyme of similar molecular weight (say *b* and *b*′). Moreover, on the basis of evidence that implicates cyclic AMP in the control of the enzyme's activity (Wing & Robinson, 1968*b*), it is tempting to speculate that *b*′ may be the phosphorylated form of *b* and that the formation of *b* is promoted by insulin and inhibited by adrenaline.

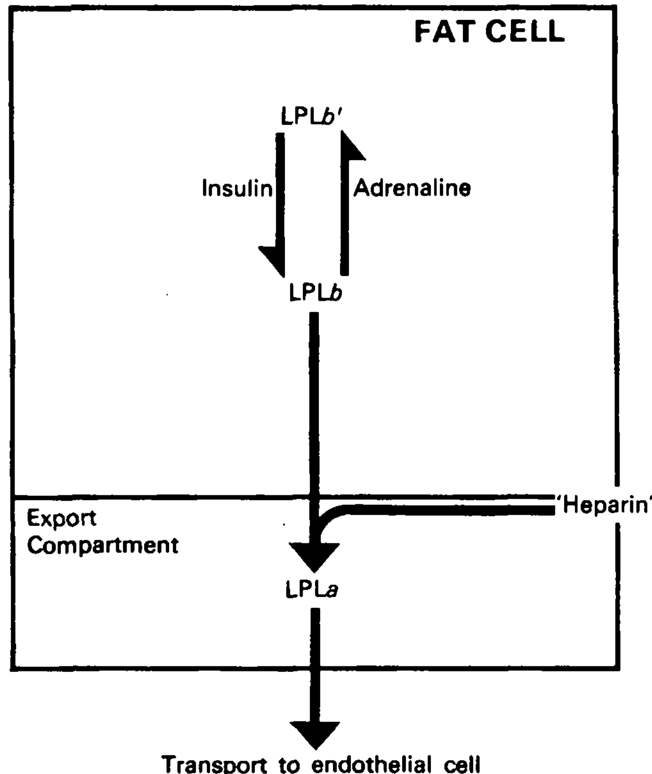

Fig. 3. Schematic representation of possible interconversions of forms of clearing-factor lipase (LPL) involved in the control of the adipose tissue enzyme activity. Form *a* is a polymer of *b* formed in the presence of heparin or its endogenous counterpart. Forms *b* and *b*′ are interconvertible without concomitant enzyme synthesis. Adapted from Cryer, Davies & Robinson (1975).

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What then is the relationship of the forms *b* and *b'* to the form *a* of higher molecular weight? There is already good evidence to suggest that a heparin-like substance plays an important part in the action of clearing-factor lipase and it was suggested many years ago that the functional enzyme at the endothelial cell surface might contain heparin as a prosthetic group (Korn, 1959; Robinson & French, 1960). It seems possible, therefore, that form *b*, but not form *b'*, can associate with heparin, or its endogenous counterpart, and that this association leads to the polymerization of *b* to give *a*, with a concomitant rise in specific activity and a commitment to export from the fat cell (see Fig. 3). We have recently found that colchicine inhibits the increase in enzyme activity that occurs during the incubation of isolated fat cells and that it does this by interfering specifically with the release of the enzyme into the incubation medium (A. Cryer, A. McDonald, E. R. Williams & D. S. Robinson, unpublished results). If, as this suggests, the polymerization of *b* to *a* occurs in association with the microtubular elements of the fat cell, this would provide additional evidence for the association of this process with the export of the enzyme from the cell.

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