Regulation of the synthesis of lipoprotein lipase in rat adipose tissue

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Studies involving the incubation of rat epididymal fat-bodies in vitro have shown that insulin and glucocorticoids can promote a protein-synthesis-dependent increase in the activity of lipoprotein lipase in the tissue, and it has been proposed that these hormones are responsible for the increase in the activity of the enzyme which occurs in vivo after the onset of feeding (Ashby & Robinson, 1980).

In order to investigate the mechanism of these hormonal effects, we have devised a novel method for measuring the synthesis of lipoprotein lipase in rat adipose tissue. Epididymal fat-bodies from 24 h-starved rats were incubated for 1 h at 37°C in Krebs-Henseleit bicarbonate buffer solution, supplemented with amino acids at the concentrations defined by Eagle (1955), glucose (10 mM), casein (2% w/v) and $\text{t-[4,5}^{\text{H}}\text{]leucine (10} \mu\text{Ci/ml})$. At the end of the incubation period, groups of four fat-bodies were removed from the incubation flasks, and homogenized in 10 ml of 2% (w/v) casein, pH 7.2. The homogenate was delipidated by extraction with acetone and diethyl ether as previously described (Ashby et al., 1978). Each delipidated tissue residue was homogenized in 15 ml of 5 mM-sodium barbital, pH 7.5, containing 20% (w/v) glycerol, 0.1% Triton X-100 and 50 mM-NaCl, and the suspension was centrifuged at 60000 g for 30 min. Lipoprotein lipase was isolated from the resultant supernatant in a single step by affinity chromatography on heparin-Sepharose as described by Parkin et al. (1982). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of this $^{3}$H-labelled lipoprotein lipase preparation revealed a single sharp peak of radioactivity corresponding to a polypeptide of M, 56000. This polypeptide has previously been identified as lipoprotein lipase (Ferriol et al., 1982). No other peaks of radioactivity were detected on the gel and losses of lipoprotein lipase during this procedure were minimal. Thus the $^{3}$H-labelled enzyme can be rapidly and quantitatively isolated free from other radioactive adipose tissue proteins.

In the presence of insulin (2 m\text{i.u./ml}), the incorporation of $[^{3}\text{H}]$leucine into total adipose tissue protein and into lipoprotein lipase was increased by 1.3±0.2 fold and 2.6±0.6 fold respectively (mean±s.d., n=7), compared with controls. Thus the insulin-induced stimulation of lipoprotein lipase synthesis is partly due to a general increase in total protein synthesis in addition to a specific effect on the synthesis of the enzyme. Similar results have been reported by Vydelingum et al. (1983).

The regulation of lipoprotein lipase by glucocorticoids was investigated by preincubating fat-bodies in the presence or absence of dexamethasone (400 nM) for 3 h before the addition of $[^{3}\text{H}]$leucine, in order to allow for any lag in the glucocorticoid-induced effects. $[^{3}\text{H}]$Leucine (10 $\mu$Ci/ml) was then added and the incubation was continued for a further 1 h. Insulin (2 m\text{i.u./ml}) was present in all the incubations. The rate of synthesis of lipoprotein lipase was increased by 1.99±0.72 (mean±s.d., n=5) fold in the presence of dexamethasone plus insulin, compared with insulin alone. This effect represents a specific induction of lipoprotein lipase synthesis since total protein synthesis was not affected by dexamethasone.

These observed effects of insulin and glucocorticoids on the synthesis of lipoprotein lipase are consistent with the changes in enzyme activity which occur under these hormonal conditions. It thus seems likely that both hormones regulate the activity of lipoprotein lipase at the level of enzyme synthesis. Although the effect of insulin is partly explained by a non-specific increase in general protein synthesis, the effect of glucocorticoids apparently represents a specific induction of the enzyme.

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Role of apoproteins B and E in the receptor-mediated uptake of very-low-density lipoprotein remnant particles by the perfused rat liver

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Two distinct receptors are concerned in the binding and removal of remnants of plasma triglyceride-rich lipoproteins by the liver. The 'LDL receptor' is believed to be primarily concerned with the removal of VLDL remnant lipoproteins of hepatic origin containing apoprotein B$_{100}$ whereas the 'chylomicron remnant receptor' appears to be specific for apoprotein B$_{48}$-containing remnant particles, which may originate either from the intestine or the liver.

Abbreviations used: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; CHD, 1,2-cyclohexanediol.

(Brown & Goldstein, 1983). The apoprotein specificity of the two hepatic receptors remains, however, an area of much interest since all the remnant populations also contain additional apoproteins. HDL$_{4}$, an apoprotein E-enriched lipoprotein, binds to the 'LDL receptor' with a 20-fold greater affinity than a predominantly apoprotein B$_{100}$-containing particle such as LDL (Innerarity et al., 1980). Moreover, HDL will compete with $^{125}$I-labelled chylomicron remnant particles for removal by the perfused liver (Sherrill et al., 1980). These findings have led Mahley and his co-workers to suggest that apoprotein E is the major mediator of the hepatic removal of all the remnant classes (Mahley & Innerarity, 1983). However, the observed differences between the metabolism of apoprotein B$_{100}$ and apoprotein B$_{48}$ remnants cannot be reconciled on the assumption that the receptor-interaction of these particles is mediated through apoprotein E alone (Brown &
Regulation of lipoprotein lipase: starvation-induced changes in the intracellular and intercellular distribution of activity in mammary tissue of lactating rats

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Lipoprotein lipase (EC 3.1.1.34) activity confers on tissues expressing it the ability to hydrolyse triacylglycerols of chylomicrons and very-low-density lipoproteins and consequently to utilize the resulting fatty acids (Cryer, 1981). The physiological site of action of lipoprotein lipase is on the luminal surface of the endothelial cells lining capillary blood vessels serving the tissue in question. The intracellular, and physiologically inactive, component of the total lipoprotein lipase activity of a tissue may be equated with that fraction of activity which is not heparin-releasable and is collagenase-resistant (Cunningham & Robinson, 1969; Cryer et al., 1976; Vanhove et al., 1978; Rajaram et al., 1980).

Lipoprotein lipase is known to be an adaptive enzyme, its activity responding to the nutritional status of the whole animal (Robinson, 1960; Garfinkel & Schotz, 1973; Cryer et al., 1976). These phenomena have been investigated in greatest detail in adipose tissue and muscle: in adipose tissue, activity declines on starvation, whereas in muscle it increases. Alterations in the amount and/or activity of the extracellular component of total tissue lipoprotein lipase are known to be responsible for bringing about these regulatory changes (Cryer, 1981).

In the lactating rat, short-term starvation (24h) greatly diminishes the overall activity of the pathway of lipogenesis de novo in mammary tissue (Robinson et al., 1978). The purpose of the work described here was to determine what parallel effects, if any, such starvation would have on lipoprotein lipase activity in this tissue and hence on its capacity to utilize plasma triacylglycerol.

Total lipoprotein lipase activity of mammary tissue from fed rats in days 9–12 of their first lactation, measured in acetone/ether-treated preparations of tissue homogenates, was 2343 ± 189 (7) munits/g wet tissue wt. (mean ± s.e.m., for the number of observations in parentheses; one unit represents 1 μmol of fatty acid released/min at 37°C). Starvation for 24h caused a decrease (not statistically significant) to 2279 ± 350 (7) munits/g wet tissue wt. Intracellular activity was measured, in experiments paired with those above, by using acetone/ether-treated homogenates of acini prepared by collagenase digestion of mammary tissue (Clegg, 1981a; Robinson et al., 1984). Extracellular lipoprotein lipase was estimated as the difference between the foregoing measurements. Intracellular activity in acini from fed and starved rats was 1329 ± 92 (7) and 775 ± 141 (7) munits/g wet wt. of acini respectively. In the fed animal, therefore, 56% of the total tissue activity was intracellular, whereas on starvation this percentage fell to 36%. Thus the effect of starvation on mammary-tissue lipoprotein lipase was to redistribute an unchanged total tissue activity such that a greater proportion became extracellular, and hence physiologically active. The subcellular distribution of lipoprotein lipase activity