Hormones and Fatty Acid Metabolism in White Adipose Tissue

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This short review is devoted to considerations of acute regulation rather than longer-term effects achieved through dietary manipulation or massive and prolonged hormone administration in vivo.

Adipose-tissue fatty acid metabolism is conveniently divided into: assimilation of fatty acids from circulating lipoproteins; synthesis de novo of fatty acids, mainly from carbohydrate; assembly of triacylglycerol and phospholipids (esterification); fatty acid oxidation and triacylglycerol degradation (lipolysis). All of these processes can be acutely influenced by hormones in some way. In addition, one should also consider the roles of long-chain fatty acids or their derivatives as possible regulators of metabolism.

Lipolysis

We probably have reasonable knowledge of some of the mechanisms underlying this process. The hormone-sensitive lipase, which may encompass triacylglycerol, diacylglycerol, monoacylglycerol and cholesterol ester hydrolase activities in the same enzyme (Khoo et al., 1976), is rapidly activated on treatment of adipose tissues with a range of hormones. Catecholamines, glucagon and adrenocorticotropin, with species variations in potency, appear to be the main lipolytic agents. It is likely that their actions are transmitted via adenylate cyclase activation, elevation in cell cyclic AMP and protein kinase activation followed, possibly, by phosphorylation of a lipase component. Conversely, though not proved, lipase deactivation may involve the action of a protein phosphatase (Khoo et al., 1976). Anti-lipolytic actions of insulin and prostaglandin E₁ are well documented. Although, under certain conditions, insulin can suppress hormone-induced increases in cyclic AMP, it would now seem untenable to propose this as the sole mechanism for the anti-lipolytic action of insulin (Fain & Rosenberg, 1972; Siddle & Hales, 1974). A role for Ca²⁺ in insulin regulation of lipolysis has been suggested (Siddle & Hales, 1974a), possibly involving activation of a protein phosphatase (Kissebah et al., 1974).

Hydrolysis of plasma lipoproteins

It is generally accepted that lipoprotein lipase activity in the capillary bed of adipose tissue is a major rate determinant in the extraction of fatty acids from very-low-density lipoproteins and chylomicron triacylglycerol. The enzyme has a very short half-life requiring continuous protein synthesis to maintain the concentrations encountered in the fed state. After food deprivation, lipoprotein lipase activity is considerably decreased. Re-establishment of re-feeding correlates well with plasma insulin concentrations (Cryer et al., 1974). Under appropriate conditions insulin can increase activity in vitro in tissues from starved rats, whereas adrenaline, adrenocorticotropin and 6-N,2‘-O-dibutyryl 3′:5′-cyclic AMP have opposing effects (Robinson & Wing, 1970). Lipoprotein lipase exists as two species, extracellular and intracellular. Insulin may be important in promoting intracellular → extracellular interconversion and enzyme secretion (Garfinkel et al., 1976; Nilsson-Ehle et al., 1976). Insulin and adrenaline in vitro have opposing actions on the intracellular form (Davies et al., 1974), although regulation by protein kinase action appears unlikely (Khoo et al., 1976).
Fatty acid synthesis

This is considered elsewhere in this proceedings (Denton et al., 1977).

Esterification

Study of these processes is impeded by poor knowledge of the enzymology of the components. After conversion of fatty acids into CoA thioesters, assembly of glycerides is achieved by acyl-transfer reactions catalysed by enzymes that are predominantly microsomal (Coleman & Bell, 1976; Schlossman & Bell, 1976). Glycerol phosphate, dihydroxyacetone phosphate and monoacylglycerol may all act as initial acyl acceptors, with effectiveness decreasing in that order (Dodds et al., 1976). Possible association of glycerol phosphate and dihydroxyacetone phosphate acyltransferase activities with the same enzyme is at present a matter of controversy.

To avoid futile cycling, triacylglycerol synthesis should be curtailed under mobilizing conditions. It is not sufficient to expect control of lipoprotein lipase alone to achieve this, since non-esterified fatty acids released by lipolysis may circumvent this control point. Other controls over esterification must be involved.

Firstly, in species where insulin exerts considerable control over glucose transport, intracellular provision of this precursor of glycerol and dihydroxyacetone phosphates should be a major regulation site. It is possible that this may be less important in some species, e.g. guinea pig (Chang et al., 1974; Saggerson, 1974).

Secondly, being mindful of analogies between the hormonal control of the glycogen- and triacylglycerol-mobilizing systems, similar analogies between the glycogen-synthesizing and -esterification processes might be predicted. We have investigated the possibility of some kind of rapid direct action on the esterification process in two ways.

When rat adipocytes were incubated with $^{14}$C-fructose and $^3$H-palmitate, insulin stimulated $^{14}$C and $^3$H incorporation into the glyceride glycerol and fatty acyl moieties respectively. The percentage effect of insulin on $^{14}$C incorporation was greater than was expected solely from insulin promotion of sugar uptake (Sooranna & Saggerson, 1975). Under similar conditions slight insulin stimulation of $^{14}$C-pyruvate incorporation into adipocyte glyceride glycerol could be demonstrated. Also, in some instances insulin stimulation of $^3$H$_2$O incorporation into glyceride glycerol could be demonstrated in fat-pads when no exogenous substrates were present. These data gave tentative support to the hypothesis that insulin may promote glyceride synthesis at sites other than lipoprotein lipase and sugar transport. This isotopic-flux approach is obviously limited and cannot be used to investigate the actions of lipolytic hormones, since these very drastically change the pool size and the specific radioactivity of extracellular fatty acyl precursors. It is noteworthy, however, that insulin has also been shown to stimulate incorporation of $^{32}$P$_i$ into adipocyte phospholipids (Stein & Hales, 1974).

An alternative approach, which has detected rapid hormone-induced modifications of enzymes in several tissues, is to expose cells or tissues to hormones for short times followed by extraction and enzymic assay. Freeze-stop procedures should be used unless it is established that the enzymic changes relax extremely slowly. This may severely limit investigation of the subcellular distribution of enzyme modification.

By using this approach, $^{14}$C-glycerol phosphate acylation with palmitoyl-CoA was measured in freeze-stopped adipocyte extracts (Sooranna & Saggerson, 1976a). Insulin slightly increased and adrenaline considerably decreased glycerol phosphate acylation in cells incubated without carbohydrate substrates. This pattern accords with the possibility of opposite, but co-ordinated, hormonal control of esterification and lipolysis. Fatty acids did not mimic the action of adrenaline (Sooranna & Saggerson, 1976b). Glycerol phosphate acyltransferase activity represents two acylations by distinguishable enzyme components (Lands & Hart, 1965; Yamashita et al., 1972). In our experiments with adipocyte extracts, two acylations of glycerol phosphate occurred, since the product was identified by t.l.c. as phosphatidate, and palmitoyl/glycerol phosphate incorporation was 2:1 (Fig. 1). This suggests that the first acyl transfer may be rate-limiting. Jason et al. (1976) have also observed rapid insulin stimulation of adipocyte microsomal fatty acyl-CoA ligase activity.
Fig. 1. Time course of glycerol phosphate acylation in adipocyte extracts

Extraction of adipocytes and assay of glycerol phosphate acyltransferase was as described by Sooranna & Saggerson (1976a). Paired incubations contained either [U-14C]glycerol 3-phosphate and unlabelled palmitoyl-CoA (○) or [1-14C]palmitoyl-CoA and unlabelled glycerol 3-phosphate (●). The ratio of palmitoyl/glycerol phosphate incorporation was 2.22, 2.22, 2.15 and 2.14 at 2.5, 4, 5 and 7 min respectively.

Some effects of metabolites on glycerol phosphate acylation were also apparent. Exposure of adipocytes to fructose, but not glucose enhanced this activity. Fructose also reversed the direction of insulin action (Sooranna & Saggerson, 1976b), although this effect was antagonized by palmitate, which itself increased glycerol phosphate acyltransferase activity in the presence of fructose and insulin (Sooranna & Saggerson, 1976b). These effects are not understood at present.

Fatty acid oxidation

Insulin decreases oxidation of endogenous substrates (presumed to be fatty acids) in adipose tissue (Flatt & Ball, 1964) and of exogenous fatty acid when glucose is present (Harper & Saggerson, 1976). Adipocyte mitochondrial fatty acid oxidation is carnitinedependent (Harper & Saggerson, 1975), suggesting that esterification and oxidation may compete for extramitochondrial acyl-CoA thioesters. Since esterification greatly predominates over oxidation, insulin effects on oxidation may be secondary to actions on esterification.

Fatty acids and their derivatives as regulators

Through control mechanisms outlined above, hormones acting on adipose tissue can greatly influence the plasma concentration of unesterified fatty acids, which in turn may have important consequences for the metabolism of many tissues, including adipose tissue itself. Stimulatory and inhibitory feedback effects on adipocyte guanylate cyclase and adenylate cyclase respectively have been reported (Asakawa et al., 1976; Fain & Shepherd, 1975). Under some conditions, fatty acids may greatly decrease adipocyte fatty acid synthesis and over-ride insulin stimulation of this process (Sooranna & Saggerson, 1976b).

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Regulation of Adipose-Tissue Lipid Metabolism in Obese—Hyperglycaemic Mice

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Animal models of obesity have been used extensively to study the mechanism underlying the increased deposition of fat and the changes in metabolism occurring during obesity. The obese—hyperglycaemic (ob/ob) mouse has received particular attention, as it may represent a good model for insulin-resistant obesity in man. The obesity of the ob/ob mouse is inherited as a single-gene homozygous recessive trait and is accompanied by partial resistance to insulin and hyperinsulinaemia. In this review, ob/ob mice will be compared with their lean controls and we will show that the accretion of fat in epididymal adipose tissue of ob/ob mice can be explained by high lipogenesis, high uptake and esterification of fatty acids of hepatic and dietary origin and decreased hormone-sensitive lipolysis.

Lipogenesis in vivo

Studies involving intraperitoneal injection of [1-14C]acetate showed, that although the liver is a major site of lipogenesis in vivo in ob/ob mice, extrahepatic tissues (i.e. the rest of the carcass including epididymal fat-deposits) also accumulate high concentrations of labelled fatty acids (Winand et al., 1973). As the interpretation of the data obtained with [1-14C]acetate was obscured by the fact that acetyl-CoA pools may vary from one organ to another in obese and control mice, this study was repeated with 3H2O. Table I shows that 1 h after the intraperitoneal injection of 10mCi of 3H2O (i.e. at a time when transfer of labelled lipids from the liver was minimal), local hyperlipogenesis occurred in adipose tissue from obese mice; the labelling of glyceride fatty acids was increased by 55%, whereas that of glyceride glycerol was not modified.