Alterations in the regulation of lipolysis in brown and white adipose tissue in the diabetic state

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Triacylglycerol mobilization is a central feature in the metabolism of both brown and white adipose tissues. It is generally accepted that the rate-limiting enzyme, the hormone-sensitive lipase, is activated by cyclic AMP-stimulated protein phosphorylation [1]. Hence the regulation by hormone of cyclic AMP synthesis and disposal is important in this system. White adipocytes possess β-adrenoceptors together with receptors for corticotropin and glucagon through which G,-coupled activation of adenylyl cyclase (EC 4.6.1.1) may be exerted. Conversely, paracrine agents, such as adenosine and E-series prostaglandins, inhibit adenylyl cyclase and lipolysis by acting at receptors coupling through G,-. The anti-lipolytic effect of insulin is well documented, although the mechanism of this remains unclear. Clearly, it is pertinent to know the levels of these agents when attempting to interpret changes in adipose tissue lipolysis in diabetes or in any other physiological state. The plasma insulin, glucagon or corticotropin concentrations are readily obtainable, but localized concentrations of noradrenaline, adenosine or prostaglandins are less readily estimated. In addition, the diabetic state may impose relatively persistent changes upon the processes of lipolytic activation and inhibition, thereby resulting in altered responsiveness to the appropriate agents. It is this aspect which is particularly considered here.

The experimental system

Throughout, male rats aged 6 weeks (160–180 g body wt.) were used. These were made diabetic by a single subcutaneous injection of streptozotocin (100 mg/kg body wt.) and used for experimentation after 2 days. Typically, these animals had plasma glucose concentrations in the range 20–30 mM. Adipocytes were isolated from the epididymal (white) and interscapular (brown) depots by collagenase treatment. These cells were then incubated for study of lipolysis or were used as the starting material for estimation of enzyme activities and receptor binding or measurements of G-protein abundance.

Adenosine is present in fat cell incubations either because it is derived in some manner from the cells and/or because it may be a contaminant of the albumin that should be present in incubations to study lipolysis. Since adenosine is a potent anti-lipolytic agent (see above) and responsiveness to this agonist varies with physiological state (see below), it is essential that unknown quantities of endogenous adenosine be removed if meaningful results are to be obtained. This is achieved by addition of adenosine deaminase (EC 3.5.4.4) (typically 0.5–1 unit/ml). Another reason why this precaution is crucial is because G,-coupled inhibitory agonists appear to affect the levels of different stimulatory and inhibitory G-proteins in different ways. Whereas adenosine or prostandil E, (PGE,) simply shift to the right the dose curve for stimulation of lipolysis by noradrenaline, concentrations of N6-L-phenylisopropyladenosine (PIA) below 0.1 μM or of PGE, below 1 μM can virtually abolish glucagon-stimulated lipolysis at any tested concentration of glucagon [2]. Adenosine deaminase appears to produce a more pronounced effect in incubations of white adipocytes [3] compared with brown cells [4], suggesting that the latter operate with lower steady-state levels of adenosine. Actions at adenosine receptors (the A, type) can still be observed experimentally in the presence of adenosine deaminase by using the deaminase-resistant analogue PIA.

Diabetes and white adipose tissue

Enhanced lipolysis in diabetes with the attendant increase in plasma non-esterified fatty acid delivery to the liver assists in the suppression of hepatic fatty acid synthesis and in the enhancement of ketogenesis [5]. The simultaneously increased secretion of very-low-density lipoprotein will contribute to hypertriglyceridaemia and hypercholesterolaemia [6]. Increased fatty acid supply to heart and skeletal muscle results in decreased glucose tolerance [7].

With regard to activation of lipolysis, streptozotocin-diabetes causes a substantial increase in the sensitivity of rat fat cells to glucagon without altering the maximal response to this agent [8]. This effect can be reversed by insulin treatment of the rats. It is important to note that this effect of diabetes on responsiveness to glucagon is only unmasked in the presence of adenosine deaminase [8]. By contrast, under similar experimental conditions, responsiveness of lipolysis to activation by noradrenaline or corticotropin is unchanged by diabetes (D. Saggerson, unpublished work). This selective change in responsiveness to glucagon is similar to that seen in starvation [2, 9, 10] and is accompanied by increased activation of adenylyl cyclase in isolated plasma membranes by glucagon [11]. Diabetes did not alter 11H-glucagon binding to fat cell plasma membranes [11], suggesting that the diabetes-induced change is post-receptor.

With regard to inhibition of lipolysis, diabetes increased the ECO, (concentration that causes 50% of its maximum effect) values by 3-fold for PIA and PGE, acting against noradrenaline-stimulated lipolysis (K. Chatzipanteli & D. Saggerson, unpublished work). Likewise, diabetes also increased ECO, values for PIA and nicotinate acting against forskolin-stimulated lipolysis (J. Shepherd & D. Saggerson, unpublished work). Insulin treatment of rats reversed the effect of diabetes on sensitivity to PIA which again appeared to be post-receptor, since diabetes enhanced rather than decreased 11H-glucagon binding to adipocyte plasma membranes [11]. Similar changes are seen in starvation [12].

Streptozotocin-diabetes causes a substantial decrease in the content of G α-subunit in rat liver plasma membranes [13]. By contrast, hypothyroidism which enhances responsiveness of adipocyte lipolysis to PIA, PGE, and nicotinate increases the fat cell plasma membrane content of G α-subunit [2, 14, 15]. It was therefore surprising to find no appreciable difference in the abundance of G α-subunit in adipocyte plasma membranes from diabetic rats (G. Milligan & D. Saggerson, unpublished work). This does not preclude an alteration in the function of existing G-protein. In summary, diabetes results in some reasonably persistent alterations in the regulation of white fat cell lipolysis. Stimulation by glucagon is enhanced, whereas inhibition by all G,-coupled agonists is diminished by, as yet, unknown mechanisms.

Adapting a more holistic approach, the actual observed release of non-esterified fatty acids by white adipose tissue is not simply a function of the activation state of the hormone-sensitive lipase. Rather, it will be represented by the lipase activity minus the proportion of mobilized fatty acid that is recycled back into triacylglycerol by the adipocyte's very active esterification system. As reviewed in [16, 17], insulin facilitates adipocyte triacylglycerol synthesis in part by

Abbreviations used: PGE, prostaglandin E,; PIA, N6-L-phenylisopropyladenosine; ECO, the concentration of an agonist that causes 50% of its maximum effect.

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stimulating the transport of glucose, the precursor of intracellular glycerolphosphate. Furthermore, insulin plays a more direct acute role in that it prevents/reverses effects of lipolytic hormones to decrease activities of enzymes in the triacylglycerol synthesis pathway [16-20]. Obviously, these short-term effects of the hormone will be diminished in insulin-dependent diabetes. Longer-term decreases in diabetics also occur in the activities of most of the enzymes of the triacylglycerol synthesis pathway in white adipose tissue [21-23] and insulin administration can reverse these changes. In summary, diabetes also decreases the capability of the esterification pathway to re-esterify mobilized fatty acids.

It is to be expected that increased blood flow through adipose tissue should accompany increased lipid mobilization, otherwise deleterious accumulations of fatty acids might occur in the extracellular spaces. It has been shown [24] that a single insulin injection to diabetic rats decreased relative blood flow through epididymal adipose tissue by approx. 60%. This effect was seen 1 h after insulin administration while the animals were still hyperglycaemic. Infusion of insulin has also been shown by Madsen & Malchow-Moller [25] to decrease adipose tissue blood flow, and they proposed that this was mediated by a decrease in the release of the vasodilator adenosine from adipocytes. The reaction catalysed by the 5'-nucleotidase (EC 3.1.3.5) ectoenzyme is a possible source of extracellular adenosine. This activity in white adipocytes is doubled in diabetes [26] and insulin administration over 2 days reverses this change. It is not known how rapidly insulin can exert such an effect. It is noteworthy that 5'-nucleotidase ectoenzyme in some tissues is attached through a phosphatidylinositol-anchored glycosyl-phosphatidylinositol anchor [27] and it has been proposed [27] that insulin might promote the release of phosphatidylinositol-anchored proteins from the cell surface through the action of a specific phospholipase C. Whether this applies to 5'-nucleotidase remains to be established. If it is so insulin might indirectly modify adipose tissue blood flow by decreasing the extracellular production of adenosine. Conversely, increased formation of this paracrine agent could be expected in diabetes. The concomitant decrease in responsiveness to the anti-lipolytic action of adenosine (see above) is a necessary adjunct to increased effects upon the vasculature.

Diabetes and brown adipose tissue

In brown adipose tissue, lipolysis is an important component of the process of thermogenesis, at least during the initial phase of a period of exposure to cold. Fatty acids mobilized in response to noradrenaline release by sympathetic nerves are involved in the activation of the mitochondrial uncoupling protein and also provide the majority of the substrate for respiration (for reviews, see [28, 29]). Brown adipose tissue thermogenesis is impaired in insulin-dependent diabetes in that basal and noradrenaline-stimulated heat production by rat interscapular brown fat is decreased in streptozotocin-diabetic rats [30] and diabetic rats do not exhibit an increase in metabolic rate after consuming a cafeteria diet [31]. It is likely that sympathetic nerve activity in brown adipose tissue is decreased in diabetes [32] together with a decrease in blood flow [24]. However, surprisingly, this is not matched by any decreased capability for lipolysis in brown adipocytes from diabetic rats. In the presence of adenosine deaminase, basal lipolysis was increased and the EC50 for noradrenaline was significantly decreased but with no change in the maximal rate of lipolysis [24]. In addition, diabetes did not appreciably alter the antilipolytic effect of PIA [24]. By contrast, in the same cells maximum rates of noradrenaline-stimulated O2 uptake were decreased by 58% [24]. It therefore appears that lesions in thermogenesis in diabetes must occur subsequent to stimulation of lipolysis. These lesions are found at multiple loci within brown fat mitochondria and encompass decreased abundance of the uncoupling protein (as measured by [14]GDP binding [24]), decreased overt carnitine palmitoyltransferase (EC 2.3.1.21) activity [33] and decreased abundance of cytochromes (a + a,) and total cytochromes b [33]. Although diabetes results in a substantial decrease in the rate of triacylglycerol synthesis by rat brown fat adipocytes [23], this is not matched by a concomitant decrease in the activities of enzymes of the triacylglycerol synthesis pathway [23]. The cause of this decreased triacylglycerol synthesis in diabetes appears to be attributable to decreased conversion of glucose to glycerolphosphate due to a decrease in phosphofructokinase (EC 2.7.1.11) activity (F. Sobrino & E. D. Saggerson, unpublished work).

In summary, several loci are involved in impaired thermogenesis in diabetes but lipolysis does not appear to be one of these loci.

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Triacylglycerol synthesized in the liver from dietary carbohydrate is secreted into the plasma and delivered to peripheral tissues as very-low-density lipoprotein (VLDL). Fatty acids are then derived from the VLDL triacylglycerol by the action of lipoprotein lipase (EC 3.1.1.34) in these peripheral tissues, particularly muscle and adipose tissue. In muscle, the fatty acids may be utilized as a source of energy, whereas in adipose tissue they are re-esterified and stored as triacylglycerol. Insulin plays a key role in the co-ordination of these events, the efficiency of which breaks down under conditions of insulin deficiency or insulin resistance. Some consequences of this breakdown include abnormalities of lipoprotein metabolism which are responsible for the hyperlipidaemia characteristic of the diabetic states. This paper has two objectives. First, to review what is known of the role of insulin in controlling VLDL secretion from the liver. Secondly, to discuss how recent findings in this area could account, in part, for the abnormalities of VLDL metabolism observed in the insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) forms of diabetes. Because these diabetic states differ from each other in their effects on lipoprotein metabolism, each will be dealt with separately below.

Hepatic VLDL secretion in NIDDM

This form of diabetes is often referred to as type II or maturity-onset diabetes and is characterized partly by tissue resistance to insulin. There is general agreement that this form of diabetes is associated with an increase in the rate of VLDL production (for a review, see [1]). However, controversy exists as to the exact cause of this defect, particularly in regard to the role of insulin, and studies with experimental animals, in vivo, have often led to difficulty in distinguishing between primary and secondary effects. In view of this, and also because the liver is generally believed to be the major source of the plasma VLDL, at least under normal conditions, many investigations in this area have been carried out using isolated liver preparations in vitro. These studies have shown that many of the metabolic abnormalities associated with NIDDM, when simulated in vitro, produce increases in the rate of hepatic VLDL secretion. For instance, mimicking the hyperglycaemia of NIDDM by increasing the glucose content of the medium gave rise to an increase in the rate of VLDL triacylglycerol secretion from hepatocytes in culture [2, 3]. Similarly, elevated concentrations of plasma non-esterified fatty acids are often associated with this diabetic state [4] and it has been known for some time that treatment of isolated liver preparations with increased concentrations of non-esterified fatty acids, particularly oleate, also stimulates VLDL triacylglycerol secretion (e.g. [5–10]). As glucagon rapidly inhibits hepatic VLDL secretion [1, 11, 12], it is also possible that the hypoglycaemia reported to be associated with NIDDM [13] may also contribute to the overproduction of hepatic VLDL in this state.

Short- and longer-term effects of insulin on hepatic VLDL secretion

NIDDM is often associated with an absolute increase in the level of plasma insulin, particularly in the postprandial state [1, 14]. The direct effect of insulin, however, on hepatic VLDL secretion is a matter of some controversy. First, it has been proposed that the hyperinsulinaemia which occurs in insulin-resistant states is directly responsible for the enhanced secretion of hepatic VLDL [4, 15]. Several more recent studies, however, have shown that when insulin was added to isolated hepatocytes for periods up to 24 h, the secretion of VLDL triacylglycerol was diminished [3, 12, 16–20]. There was also a decline in the secretion rate of VLDL-associated phospholipid [16, 18], cholesteryl ester [20], apolipoprotein (apo) B and apo E [16, 17, 19]. The inhibitory effect on the secretion of non-esterified cholesterol was slight compared with that on esterified cholesterol secretion [20] and this gave rise to changes in the ratio of these lipids in the newly secreted VLDL.

It might be argued that the inhibitory effect of insulin on hepatic VLDL secretion is merely an artefact of the in vitro model. However, several studies in vivo have also provided evidence that insulin administration results in a decline in hepatic VLDL production [21–23]. Although a decreased hepatic fatty acid flux resulting from the anti-lipolytic effect of insulin cannot be ruled out, it has been shown, in at least one instance, that the inhibitory effect on VLDL secretion was independent of fatty acid uptake by the liver [22].

Two mechanisms for the inhibitory effect of insulin have been proposed. First, during a 20 h incubation with cultured hepatocytes, insulin, whilst inhibiting the total secretion of apo B and apo E into the medium, also reduced the proportion of these apoproteins which were associated with the VLDL. Concomitantly, the proportion of secreted apoprotein associated with other fractions of the medium such as low-density lipoprotein and albumin increased. It was thus proposed that insulin inhibited hepatic VLDL secretion by interfering with the normal intracellular association of apoprotein and lipid during the assembly of the nascent particles [16]. Second, insulin inhibited the incorporation of $^{14}$C methionine into both the large- and small-molecular-mass variants of apo B [19]. Whether this was a secondary effect of the reduced association of lipid and apoprotein (see above) or whether insulin directly inhibited the synthesis of the apo B variants de novo is not yet known.

The physiological purpose of the inhibitory effect of insulin on hepatic VLDL secretion has been the subject of some debate. During the postprandial period, elevated levels of plasma insulin are coincident with a high rate of hepatic lipid synthesis and an increased entry into the plasma of intestinal.

Abbreviations used: VLDL, very-low-density lipoprotein; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; apo, apolipoprotein.