Adipose tissue function in the insulin-resistance syndrome

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Abstract
Insulin resistance is often seen as a consequence of obesity and there are several possible links between adipose tissue function and insulin resistance determined in other organs such as skeletal muscle or liver. One such link is the regulation of NEFA (non-esterified fatty acid) delivery to the systemic circulation. Simplistically, an expanded adipose tissue mass delivers more NEFA to the systemic circulation and these fatty acids compete for substrate utilization in skeletal muscle, which in turn reduces glucose utilization. This increases blood glucose concentration and provides the stimulus for increased insulin secretion and hyperinsulinaemia is a key feature of the insulin-resistance syndrome. However, there is abundant evidence that adipose tissue is exquisitely insulin sensitive and hyperinsulinaemia may therefore lead to a constant lipolytic inhibition in adipose tissue. Consequently, the main function of adipose tissue, to rapidly switch between fat uptake and fat release, will be hampered. Adipose tissue blood flow is the conveyor of signals and substrates to and from the adipose tissue. In healthy people adipose tissue blood flow is much enhanced by food intake, whereas in insulin-resistant subjects this response is blunted. This is another facet of unresponsiveness of adipose tissue in the insulin-resistance syndrome.

Introduction
The prevalence of obesity, along with its associated co-morbidities like cardiovascular disease and Type II diabetes, is increasing, and it is important to understand the pathophysiological role played by adipose tissue in the insulin-resistance syndrome.

Not all adipose tissue depots behave identically. Increased upper body fat is particularly strongly associated with the insulin-resistance syndrome and cardiovascular risk factors [1]. Paradoxically, increased lower body fat does not confer the same risk of cardiovascular disease and insulin resistance as the same amount of fat in the upper body; in fact, lower body fat appears to exert a protective effect [2–6].

This review will deal with two specific aspects of adipose tissue function: [1] the delivery of NEFAs (non-esterified fatty acids) from adipose tissue to the rest of the body; and [2] the regulation of blood flow in adipose tissue. Clarification of these processes may help to explain the relationship between obesity and insulin resistance.

Regional adipose tissue depots and their contribution to systemic NEFA concentrations
Fasting as well as postprandial NEFA concentrations are elevated in obesity, but the source of these NEFAs is unclear. Do these NEFAs originate mainly from lower body fat stores, upper body intra-abdominal fat stores or upper body subcutaneous fat stores? To quantify the delivery of NEFAs from different adipose tissue depots to the systemic circulation, Jensen [7] used the measurement of the isotope dilution of intravenously infused labelled fatty acids combined with regional adipose tissue cannulation. To quantify the NEFA delivery from each depot in the fasting and postprandial states, Jensen and co-workers measured the systemic and regional flux (both uptake and release) of NEFAs before and after a standardized meal [8]. They concluded that the increased postprandial NEFA concentrations observed in the upper body of obese women is mainly derived from non-splanchnic upper body fat depots, and only to a lesser extent from the intra-abdominal and visceral fat depots [8]. Thus visceral fat mass may be a marker for, but not the source of, high postprandial systemic NEFA concentrations in upper-body obesity. However, because venous blood from visceral adipose tissue drains directly into the liver, the delivery of NEFAs to the liver from this depot may be disproportionately high compared with other depots. In order to study the contribution of intra-abdominal fat to the accumulation of liver triacylglycerols, further studies examined the delivery of NEFA from this depot to the liver [9]. In this in vivo study in humans, the contribution of the lipolysis of visceral adipose tissue to NEFA delivery to the liver increases as the visceral fat content increases, and this effect was more pronounced in women compared with men. Typically, 20–50% of plasma NEFAs originated from the visceral fat depot, with the remainder coming from the systemic circulation (presumably via the hepatic artery).

Key words: adipose tissue, fatty acid, insulin resistance, non-esterified fatty acid (NEFA), thiazolidinedione, Type II diabetes.
Abbreviations used: HSL, hormone-sensitive lipase; NEFA, non-esterified fatty acid; PKB, protein kinase B.
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There are very few studies demonstrating direct quantification of lipolysis from specific fat depots. The small contribution of fat in the lower body to the systemic NEFA concentrations has recently been corroborated [10]. This study used direct measurements of NEFA delivery from veins draining subcutaneous abdominal adipose tissue depot in healthy men [10]. These measurements enabled the calculation of the rate of action of HSL (hormone-sensitive lipase) in both abdominal and subcutaneous adipose tissues. The rate of HSL lipolysis in gluteal fat was only an eighth of that in abdominal fat, a finding consistent with those of Jensen and co-workers [8], which suggested that lower body fat depots contribute only a small amount to the total systemic NEFA delivery.

It is difficult to obtain blood from splanchnic fat depots in humans to allow direct measurements of lipolysis. However, Samra et al. [11] recently sampled blood from veins draining omental fat tissue during abdominal surgery. It is interesting to note that the NEFA concentrations found in this vein (presumably draining almost exclusively visceral fat) were very similar to that in veins which drain subcutaneous abdominal fat (~1500 µmol/l) [12]. Assuming that the adipose tissue blood flow is similar between the two depots, the release of NEFAs would be similar per unit of fat mass. These findings corroborate the studies of Jensen and co-workers because the extra-abdominal fat depot is almost invariably greater in mass compared with the intra-abdominal fat depot. In vitro, the degree of inhibition of lipolysis and stimulation of fatty acid re-esterification seen when adipocytes are exposed to insulin depends on the source of adipocytes: those from visceral adipose tissue show a blunted response to insulin compared with those from subcutaneous adipose tissue [13,14]. In contrast with these in vitro studies, the in vivo studies of Samra et al. [11] found that the suppression of lipolysis in the intra-abdominal fat depot in response to glucose-stimulated hyperinsulinaemia was almost complete, suggesting that the intra-abdominal fat depot behaves in an identical fashion with other fat depots.

**The anti-lipolytic effect of insulin**

Mobilization of fatty acids from adipocyte triacylglycerol stores is mediated by the enzyme HSL [15]. Because HSL knockout animals have an apparently normal adipose tissue distribution and superficially preserved functional lipolytic signals, it was postulated that there were additional lipases in adipose tissues. One such lipase, the ATGL (adipose tissue triacylglycerol lipase), was recently characterized [16]. The activation of HSL is regulated by the reversible phosphorylation of serine residues together with phosphorylation of perilipins, which allows the translocation of HSL from the cytoplasm to the lipid droplet within the adipocyte. The master regulator of the lipolytic signal is the elevation of the intracellular cAMP concentration. Insulin provides a powerful inhibitory control of fat mobilization from adipocytes. This is mediated through a signal chain involving the insulin receptor, via PI3K forming PtdIns(3,4,5)P3, which in turn activates PKB/Akt (where PKB stands for protein kinase B), PKB then phosphorylates and activates cAMP-PDE3B (phosphodiesterase 3B), which hydrolyses cAMP to AMP, thereby reducing adipocyte cAMP concentrations.

The issue of resistance in adipocytes or adipose tissue to the antilipolytic effect of insulin is not entirely clear from the literature. It is well known that compared with other cell types, human adipocytes are exquisitely responsive to low concentrations of insulin. Typical half-maximal suppression (ED50) of lipolysis in isolated human adipocytes is achieved with insulin concentrations of around 1 m-unit/l, a concentration only 10–20% of those found in the plasma of healthy fasting humans. Even adipocytes isolated from subjects with insulin resistance such as in obesity or Type II diabetes, have an ED50 for the anti-lipolytic action of insulin similar to adipocytes isolated from normal or control adipose tissue [17,18]. It should be noted that as stated earlier, omental adipocytes appear to be less responsive to insulin than adipocytes derived from subcutaneous tissue when tested in vitro [13,14]. Still, such cells show ED50 values that should render them fully responsive to physiological insulin concentrations. However, it is difficult to extrapolate the effects from isolated adipocytes to that of adipose tissue, perhaps reflecting the limitations of in vitro or ex vivo studies of an organ with multiple nervous and humoral signals that simultaneously up-regulate and down-regulate lipolytic signals [19,20]. We have recently investigated these relationships in vivo in humans with Type II diabetes, who underwent a placebo-controlled, double-blind crossover study for the metabolic effects of the insulin sensitizer rosiglitazone [21]. Rosiglitazone clearly induced insulin sensitization at a whole body level, as demonstrated by a reduction in the fasting plasma glucose concentration alongside a reduction in fasting plasma insulin concentration. In this study, the effects of insulin on adipose tissue lipolysis were investigated in vivo, after cannulation of a vein exclusively draining subcutaneous tissue. These techniques enabled the direct measurements of arterio-venous concentration gradients of NEFAs across the tissue to be made. This is demonstrated in Figure 1, which shows the relationship between systemic insulin concentrations and the output of NEFA from adipose tissue before and after rosiglitazone treatment. The Figure demonstrates the response of adipose tissue NEFA release to insulin concentrations in the fasting and postprandial (hyperinsulinaemic) states. Hyperinsulinaemia was induced by a standardized meal containing 40 g of carbohydrate and 40 g of fat. As expected, when the relatively low concentrations of insulin during fasting increased to the peak insulin concentrations postprandially, adipose tissue output of NEFAs decreased. This decrease seemed to be greater with rosiglitazone treatment. Interestingly, this exaggerated suppression of adipose tissue NEFA output with rosiglitazone is not because the NEFA output is further suppressed at peak insulinemia – in fact, adipose tissue NEFA release appears to be already near-maximally suppressed at 120 min postprandially – instead, the greater suppression is explained...
The relationship between arterial insulin concentration and adipose tissue NEFA output in the fasting state, and 120 min after ingestion of a standardized meal containing 40 g of carbohydrate and 40 g of fat.

The measurement was twice within the same subject, on placebo and on rosiglitazone treatment. The symbols indicate the mean value and the bars the S.E.M., n = 18. Data are derived from Tan et al. [21].

by a higher NEFA release from adipose tissue during the fasting state. This perhaps merely reflects the lower systemic concentrations of insulin. Taken together, it seems as if the anti-lipolytic response of adipose tissue to insulin is merely an appropriate response to ambient insulin concentrations. Thus we suggest that there is no need to attribute a direct insulin sensitizing effect of rosiglitazone on adipose tissue, but rather adipose tissue is merely an innocent bystander to ambient insulin concentrations.

These data are entirely consistent with in vitro studies of adipocytes, which show that the suppression of lipolysis is highly sensitive to insulin [17,18] and that the insulin-induced anti-lipolysis follows a dose–response curve in adipose tissue despite conditions of systemic resistance. It should be noted that the in vivo responses have been examined in a setting where the adipose tissue NEFA release has been normalized and that the effect of an expanded adipose tissue mass on a whole-body level, such as in obesity, will have a major impact on the whole body delivery of NEFA from the tissue.

Another interpretation of the relationship between plasma insulin concentrations and NEFA release from adipose tissue shown in Figure 1 is that the lowering of the systemic insulin concentration induced by rosiglitazone has brought about a left-shift of the dose–response curve between insulin and adipose tissue NEFA output. Due to the exponential nature of the curve, this brings the dose–response relationship into a more responsive or flexible part of the curve, which would fit very well with the notion of metabolic flexibility as a key regulator of the utilization of substrates in the human body [22]. Due to the fact that adipose tissue lipolysis appears to be such an insulin-sensitive process, any elevation of fasting insulin, often seen as a reflection of whole body insulin resistance, will reduce lipolysis. This will inevitably reduce the magnitude of difference between the fasting (lowest possible systemic insulin) and postprandial (highest possible systemic insulin) in terms of lipolytic regulation and thus decrease the amplitude between the fasted and the fed state.

Irrespective of the underlying cause of systemic insulin resistance, the dynamic function of adipose tissue lipolytic regulation appears to be much restricted by the concomitant hyperinsulinaemia. This might be a necessary physiological adaptation in obesity. Otherwise a very large adipose tissue with preserved lipolytic function in the fasting state would induce excessively high systemic NEFA concentrations.

Regulation of adipose tissue blood flow in the insulin-resistance syndrome

Adipose tissue blood flow rapidly changes in response to eating. Typically, 60–90 min after food intake adipose tissue blood flow doubles in normal healthy people. However, this response is blunted in obesity and insulin resistance [23,24]. There is a positive correlation between insulin sensitivity and the magnitude of the increase of the adipose tissue blood flow seen after a standardized oral glucose challenge. It is tempting to speculate that insulin could act as a vasodilator in adipose tissue, partly as there is a very good temporal correlation between the postprandial increase in systemic insulin concentrations and the postprandial increase in adipose tissue blood flow. Figure 2 shows data derived from recent work on the effects of insulin on adipose tissue blood flow [25]. In order to study the effect of insulin itself, a novel technique was developed in which a local infusion of pharmacological or hormonal agents could be delivered into the site of blood flow measurement. The blood flow was measured by the standard 133Xe washout technique [26]. The small distribution area made it possible to restrict the infusion to very small quantities of vasoactive agents, thus avoiding any systemic effects. Using this technique it was shown that local delivery of insulin directly into the tissue, without increasing the systemic insulin concentration, did not change the blood flow within the tissue, and it was therefore concluded that insulin by itself is unlikely to be the mediator of the enhanced blood flow seen in the postprandial state [25]. So what could explain the postprandial and insulin-related increase in adipose tissue blood flow? It is well
established that adipose tissue blood flow is increased both in response to adrenergic agents [27,28] and to mental stress [29]. Acute hyperinsulinaemia causes increased sympathetic activation [30], while the postprandial state leads to an increased sympathetic drive. In order to test this, a series of experiments were conducted to block β- and α-adrenergic receptors in adipose tissue using the local infusion technique described above. Infusion of propranolol, a non-specific β-adrenoceptor blocker, reduced the postprandial increase of adipose tissue blood flow by 50% without any effect on the fasting blood flow [31]. In contrast, fasting blood flow appeared to be largely regulated by nitric oxide, as the specific nitric oxide inhibitor L-NMMA (N(G)-monomethyl-L-arginine) reduced the fasting blood flow, leaving the postprandial response unaltered. It is tempting to speculate that the reduced adipose tissue blood flow seen in obesity is a consequence of reduced nitric oxide function in the tissue. Flow-mediated vasodilatation of the brachial artery, which is a model system for endogenous nitric oxide-mediated vasodilatation, is hampered in insulin resistance and obesity [32]. It is interesting to note that the insulin-sensitizing thiazolidinediones, both enhance resting adipose tissue blood flow [21] and flow-mediated vasodilatation in people with insulin resistance [33,34], whereas rosiglitazone does not appear to improve flow-mediated vasodilatation in coronary patients without diabetes mellitus [35]. This might suggest a common mechanism for increasing nitric oxide bioavailability in response to insulin sensitization.

The lack of postprandial adipose tissue blood flow responsiveness in insulin resistance remains unexplained. One possible explanation is the increased sympathetic drive seen in chronic hyperinsulinaemia. Expression of β-adrenergic receptors in adipose tissue has the propensity to rapidly down-regulate in response to continued stimulation [36] and thus a long-standing increase in sympathetic drive might lead to an unresponsive blood flow pattern.

The physiological importance of other possible regulators of blood flow in adipose tissue such as angiotensin II, endothelin and vasoactive hormones remains to be established.

F.K. is a Wellcome Trust Senior Clinical Research Fellow.

References

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