Insulin resistance, adipose tissue and coronary heart disease

Keith N. FRAYN and Simon W. COPPACK
Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford, U.K.

INTRODUCTION
In an Editorial Review in this journal in 1989, Brindley & Rolland [1] discussed possible connections between stress, diabetes and obesity, and alterations in lipoprotein metabolism predisposing to atherosclerosis. The intention of this article is to look at one aspect of these inter-relationships that is attracting increasing attention, the link between insulin resistance and coronary heart disease (CHD). The hypothesis which we shall advance, that metabolic changes in adipose tissue underlie this link, is not a new one. Kissesbha et al. proposed it nearly 20 years ago [2, 3]. Since that time, further evidence has accrued in support of this belief. It seems an opportune time to review the evidence in favour of this hypothesis, and in addition to link it to the concept of 'syndrome X' or 'Reaven's syndrome', a group of metabolic changes associated with insulin resistance which has recently attracted considerable interest [4].

The association of insulin resistance with CHD
Many risk factors, or markers, have been identified for CHD. Among the most important of these are levels of lipids in the blood: a high level of low-density-lipoprotein (LDL)-cholesterol, a low level of high-density-lipoprotein (HDL)-cholesterol and a high level of triacylglycerol (TAG; triglyceride) are all interrelated risk factors. The role of these lipid species is not understood in detail, but is at least intuitively reasonable in view of the nature of the atheromatous plaque which is the hallmark of CHD. Hypertension is another risk factor whose relation to CHD is easy to visualize. Other risk factors are not so intuitively linked to the disease. One of these is 'insulin resistance' or, as it is reflected in population studies, the plasma insulin concentration [5–7]. The plasma insulin concentration is, in some groups, a more important risk factor than the blood pressure [8].

Insulin resistance has been demonstrated to be part of the endocrine background to hypertriglyceridaemia in a large number of studies spanning more than two decades [3, 9–12]. Even among normotriglyceridaemic subjects, 'insulin resistance', as measured by glucose disposal, is correlated with plasma levels of TAG [13–16]. More recently, insulin resistance has been recognized as a marker for a complex of interrelated risk factors in otherwise apparently healthy subjects [4, 17–22]. Zavoroni et al. [20], for instance, surveyed 732 factory workers. They identified a sub-group of 32 'hyperinsulinaemic' subjects on the basis of their insulin responses to an oral glucose tolerance test. These subjects were matched for age, sex and body weight with 32 other subjects ('controls'). When compared with the control subjects, the insulin-resistant subjects had mild glucose intolerance; elevated TAG concentrations, decreased HDL-cholesterol levels and hypertension. Reaven [4] has suggested the term 'Syndrome X' for this group of co-existent states (Table 1); this terminology has led to some controversy, and the alternative of 'Reaven's syndrome' has been suggested [23]. Although this constellation of risk factors had long been recognized to co-exist with obesity, Reaven's intention was to draw attention to their existence in otherwise normal subjects. We hope to show that the links between insulin resistance and these other risk factors for CHD may reside in adipose tissue.

The meaning of insulin resistance
The concept of variations among individuals in their sensitivity to the effects of insulin goes back to the obser-
vations of Himsworth [24] in the 1930s. Sensitivity to insulin, or its converse, insulin resistance, has long been viewed in terms of the effects of insulin on glucose metabolism. The precise definition of the terms to be used, and the methods for measurement of sensitivity to insulin, have been thoroughly reviewed many times [25, 26], and these points will not be laboured here. The plasma glucose and insulin concentrations after an overnight fast are broadly related via the whole-body ‘sensitivity to insulin’: the insulin-sensitive individual will have a low fasting plasma insulin concentration; the insulin-resistant individual a slightly higher plasma glucose concentration and perhaps a considerably higher plasma insulin concentration. Hence, in population studies, the fasting insulin concentration is a marker of insulin resistance. The range of ‘glycaemic sensitivity’ to insulin among apparently healthy normal subjects is surprisingly large; for instance, a seven-fold range was found in one study of six male subjects of normal body weight [27]. In that study, despite the relatively narrow range of adiposity, there was a strong negative correlation ($r=-0.95$) between glycaemic insulin sensitivity and percentage body fat.

There are various ways in which insulin resistance, defined in terms of glucose metabolism, might be thought to influence the risk of CHD. The insulin-resistant individual will have slightly (or, if frankly glucose intolerant, considerably) higher plasma glucose concentrations throughout the day and night than will the insulinsensitive individual. An elevated plasma glucose concentration leads to an increased rate of non-enzymic glycation of proteins. Glycation of apolipoproteins [28] may affect the cellular handling, and thus the atherogenicity, of lipoprotein particles [29, 30]. In addition, an elevated plasma insulin concentration may affect the process of atherosclerosis directly [31–34]. Hypertension, part of the syndrome of susceptibility to CHD discussed earlier, may result directly from insulin resistance through the stimulatory effects of the prevailing high insulin concentration on vascular smooth muscle proliferation [31, 35].

However, much evidence suggests that the links between insulin resistance and heart disease are mediated to a large extent by the alterations in lipid metabolism which accompany the changes in glucose metabolism. First, glucose and insulin are independent risk factors for CHD mortality, and of these insulin is by far the stronger [6, 8]. The severity [36] and duration [37] of glucose intolerance have no simple relationship with subsequent mortality. Glucose intolerance itself is a less powerful predictor of death than lipid abnormalities [38]. Secondly, there is a large body of evidence, reviewed below, that insulin resistance is accompanied by alterations in lipoprotein metabolism which are recognized as harmful. Thirdly, interventions which lower the plasma cholesterol (and in some cases the TAG) concentration, but may not affect the plasma glucose concentration, will reduce the incidence of cardiovascular disease [39, 40].

**ACTIONS OF INSULIN ON ADIPOSE TISSUE**

That insulin affects adipose tissue has been clear since the observation of a dramatic restoration of body weight in wasted diabetic patients who received treatment with early batches of insulin [41]. The major effects of insulin on adipose tissue (Table 2) are, as in other tissues, anabolic: stimulation of glucose uptake and lipogenesis, suppression of fat mobilization, and stimulation and/or activation of lipoprotein lipase (LPL), the enzyme responsible for uptake of TAG fatty acids by the tissue. The stimulation of glucose uptake, via the glucose transporter GLUT4, and the stimulation of lipogenesis mainly via activation of acetyl-CoA carboxylase, are well-documented effects of insulin in adipocytes in vitro, but their role in humans in vivo is not clear [reviewed in [49, 50]]. Here we will focus on the effects of insulin on lipid metabolism. These affect adipose tissue directly, by changing the balance of fatty acid uptake and release, but they also affect the rest of the body.

The supply of non-esterified fatty acids (NEFA) from adipose tissue to other tissues is rapidly and very completely inhibited by an elevation of the plasma insulin concentration. The plasma insulin concentration for half-maximal suppression of NEFA release in normal subjects is about 20 m-units/l [46, 51]. Well below the plasma concentration of insulin giving half-maximal stimulation of glucose uptake either in the whole body or by forearm muscle (approximately 50–100 m-units/l) [52, 53].

The action of insulin in the activation of adipose tissue LPL is essential for the normal clearing of post-prandial lipaemia. The absence of LPL leads to Type I hyperlipoproteinaemia, in which chylomicrons accumulate and plasma TAG concentrations may reach 50–100 mmol/l. Activation of LPL takes several hours [48, 54]; this leads to coincidence in time of post-prandial lipaemia and LPL activation [55]. It is also tissue-specific, insulin decreasing

<table>
<thead>
<tr>
<th>Process</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation of glucose uptake</td>
<td>Exquisitely sensitive to insulin in vitro [42]; relevance in humans in vivo has been questioned [43].</td>
</tr>
<tr>
<td>Stimulation of lipogenesis</td>
<td>Well documented in rat adipose tissue; physiological importance in humans not clear [44, 45].</td>
</tr>
<tr>
<td>Suppression of NEFA release</td>
<td>Half-maximal insulin concentration for suppression within low physiological range [46]. Rapid effect, brought about by inhibition of lipolysis and stimulation of re-esterification.</td>
</tr>
<tr>
<td>Activation of LPL</td>
<td>Activation occurs at multiple sites [47]. Several hours needed for maximal effect [48]. LPL activity also considered essential for normal transfer of cholesterol into HDL (see the text).</td>
</tr>
</tbody>
</table>
the activity of muscle LPL [56]. This leads to the concept of directed storage of lipoprotein-TAG [57].

Each of these actions of insulin on adipose tissue will now be considered with respect to their relevance to risk factors for CHD.

**Suppression of fat mobilization**

Insulin suppresses the release of NEFA from adipose tissue by two mechanisms: direct inactivation of the intracellular enzyme hormone-sensitive lipase (HSL), and increased re-esterification of the fatty acids liberated by this enzyme. The details of how the former is brought about are not clear. HSL is inactivated by phosphorylation, but there is no clear evidence that this results from a decrease in cellular cyclic AMP concentrations. Increased re-esterification results from the increased supply of glycerol 3-phosphate, produced by stimulation of glycolysis. A third possible mechanism is an inhibition of the putative adipocyte membrane fatty-acid transporter [58]. All these mechanisms result from an initial binding of insulin to cell-surface receptors [59].

In insulin-resistant states, there is considerable evidence for both impairment of insulin binding to receptors and post-receptor impairment of metabolic pathways [60–62], although by far the most attention has been devoted to investigation of glucose transport and lipogenesis. Insulin resistance in terms of suppression of fat mobilization is not a universal finding: in early studies there were claims that this process remained normally sensitive to insulin, both in vivo and in vitro, when glucose metabolism was insulin-resistant [63, 64]. It now seems probable that this failure to find a loss of sensitivity to insulin may reflect a lack of understanding of the very low plasma insulin concentrations causing suppression of NEFA release. In more detailed low dose–response studies, loss of sensitivity to insulin-suppression of fat mobilization has been demonstrated in obesity [65–67], non-insulin-dependent diabetes [51, 68, 69], insulin-dependent diabetes [70] and hypertriglyceridaemia [12].

The consequence of an impairment of the suppression of fat mobilization is an increased supply of fatty acids to the liver and other tissues. There is considerable evidence that an increased flux of fatty acids to the liver leads to increased very-low-density lipoprotein (VLDL)-TAG secretion [2, 3, 71, 72]. Insulin resistance in this aspect of the actions of insulin on adipose tissue may thus be a direct cause of hypertriglyceridaemia.

The increased supply of fatty acids may have other systemic effects, albeit somewhat outside the scope of this review. One is an impairment of glucose oxidation in peripheral tissues, particularly skeletal muscle. This is the mechanism described by Randle et al. [73] in 1963 as part of the ‘glucose–fatty acid cycle’. This link between the metabolism of fat and that of glucose has recently been the subject of intense re-investigation (e.g. [74, 75]). Many studies now show a link between increased fatty acid concentrations, turnover or oxidation and ‘insulin resistance’ of glucose disposal, particularly in obesity [76, 77] and in non-insulin-dependent diabetes mellitus [76, 78]. Linked with this is the recent realization that increased hepatic oxidation of fatty acids may stimulate gluconeogenesis [4], and that, in non-insulin-dependent diabetes mellitus in particular, this may reinforce hyper-glycaemia and its unresponsiveness to insulin [74].

A further consequence of increased fatty acid delivery to the liver is of relevance. Insulin is normally extracted to a large extent (around 40%) during its first passage through the liver after secretion from the pancreas [79, 80]. An increased delivery of fatty acids in the portal vein may reduce hepatic insulin clearance by mechanisms not yet well understood [81]. Thus, hyperinsulinaemia is reinforced. In this way, unresponsiveness of adipose tissue lipolysis to insulin may underlie the more commonly observed features of hyperinsulinaemia and insulin resistance of glucose metabolism.

**Stimulation and activation of LPL**

LPL in adipose, and other, tissues is active in the capillary lumen [82], where it is bound to heparan sulphate proteoglycans forming part of the endothelial glycocalyx; in this location, it can act directly on the plasma TAG-rich lipoproteins [83, 84]. The enzyme is synthesized in the adipocytes, and thus is produced in response to insulin stimulation [85]. The enzyme is in some way activated by post-translational modification during transport through the adipocyte. Its own movement is regulated by the structural location of the enzyme in the capillary endothelium and the presence of the ‘membrane anchor’ linking it to the cell-surface membrane, it is subsequently activated by dilution or addition of heparin [90]. The enzyme extractable from adipose tissue. Much cellular LPL protein appears to be ‘cryptic’ or inactive unless activated by dilution or addition of heparin [90]. The possibility that the increased tissue LPL levels seen in obesity reflect an increase in precursor, or cryptic, form has not been investigated. More importantly than measures of fasting activity, most studies of the activation of LPL activity by feeding or by insulin show this to be

Measurements of tissue-extractable LPL protein or activity are not necessarily, however, measures of the physiological activity of the enzyme. The enzyme active at the capillary endothelium is only a proportion of the total enzyme extractable from adipose tissue. Much cellular LPL protein appears to be ‘cryptic’ or inactive unless activated by dilution or addition of heparin [90]. The possibility that the increased tissue LPL levels seen in obesity reflect an increase in precursor, or cryptic, form has not been investigated. More importantly than measures of fasting activity, most studies of the activation of LPL activity by feeding or by insulin show this to be
This means that the surface area of individual particles will shrink by about nearly 50% of chylomicron-TAG, for instance, may be shrinking, delipidated particle to other lipoprotein classes. The TAG-rich lipoproteins leads to other changes, especially a transfer of surface components from the concept that the action of LPL leads to the formation of redundant surface material, producing a ‘metastable’ decreased, delayed or ‘blunted’ in insulin-resistant states [84, 86, 89, 91] (Fig. 1).

LPL deficiency, or the presence of LPL-inhibitory activity in plasma, are well-recognized familial causes of hypertriglyceridaemia [92]. The ‘acquired’ defective LPL activity associated with diabetes or insulin resistance may also lead to hypertriglyceridaemia. Eckel [84] summarizes the position by stating that “the more severe the insulin deficiency or resistance, the greater the likelihood that decreases in LPL contribute to the hypertriglyceridaemia” (as opposed to increases in VLDL synthesis).

The effect of LPL is not, however, confined to hydrolysis of lipoprotein-TAG. It is the focal point for a number of lipoprotein transformations. Although the direct action of LPL is lipolytic, the hydrolysis of TAG in the TAG-rich lipoproteins leads to other changes, especially a transfer of surface components from the shrinking, delipidated particle to other lipoprotein classes. Nearly 50% of chylomicron-TAG, for instance, may be removed in a single passage through adipose tissue [55]. This means that the surface area of individual particles will shrink by about 30%. Havel [93] has described the concept that the action of LPL leads to the formation of redundant surface material, producing a ‘metastable’ particle from which surface components dissociate. Some at least of these (including free cholesterol) are transferred to HDL particles where the action of lecithin:cholesterol acyltransferase, esterifying the cholesterol to produce the highly hydrophobic cholesteryl esters which migrate into the core of the particle, results in the formation of stable HDL2 particles [93–95]. This mechanism accounts for the transfer of at least some of the cholesterol into HDL. There is thus a relationship between adipose tissue LPL activity and HDL-cholesterol concentrations [96–98], and disturbances of this mechanism may underlie the association between hypertriglyceridaemia and low HDL-cholesterol concentrations. As the responsiveness of adipose tissue LPL to insulin improves during weight reduction in obese subjects, so the HDL-cholesterol concentration rises [89].

Another related consequence of impaired LPL activity, and particularly of impaired post-prandial activation of LPL by insulin, is a prolonged residence time in the circulation of the remnant particles produced by hydrolysis of the TAG-rich lipoproteins. There is a body of opinion that it is these remnant particles that are responsible for atherogenicity (reviewed in [99, 100]). From this point of view, complete lack of LPL, as in Type 1 hyperlipoproteinemia, would be less harmful than partial impairment as in insulin resistance [100], and this is borne out by the lack of predisposition to atherosclerosis in the former condition.

A link between insulin resistance in the suppression of fatty acid release and impaired activity of LPL may have emerged from the recent demonstration that high concentrations of NEFA can inhibit the action of LPL in vivo [101]. On the basis of studies in vitro, this effect of fatty acids is thought to reflect alterations in the binding of LPL to the target particle, to its cofactor apolipoprotein C-II, and to the endothelium [102–104]. This has been envisaged as ‘product inhibition’ of LPL, and it is thought that the fatty acids involved are those newly released at the endothelial site, before binding to plasma albumin [101]. Since there is evidence that fatty acids released from hydrolysis of circulating TAG form a single pool with those emerging from intracellular lipolysis [105], it would seem that overactive NEFA release from adipose tissue stores might also affect the normal activity of LPL.

**Influence of Body Fat Distribution**

Most of the above has been written in terms of changes in adipose tissue without reference to the site of that adipose tissue. It is now clear that this is simplistic view. More than 30 years ago Vague [106] drew attention to different distributions of adipose tissue: the upper-body or ‘android’ distribution typical of men and the lower-body or ‘gynoid’ distribution typical of women. He pointed out that many diseases (diabetes, atherosclerosis, gout and gall stones) were associated with the former fat pattern. We now realize that the upper-body fat distribution represents an accumulation of intra-abdominal fat [107], and that insulin resistance is associated with this type of fat distribution in particular [108]. Again, therefore, the link between insulin resistance, adipose tissue and heart disease appears. The reasons for the particular risk associated with intra-abdominal adipose tissue are becoming clear. First, this tissue appears to have a high

![Fig. 1. Stimulation of LPL activity in adipose tissue: loss of sensitivity to insulin in obese subjects. Control (■—■, n = 5–9) and obese (○—○, n = 6–14) subjects were infused with insulin for 6 h, with the plasma glucose concentration 'clamped' at the fasting level. Adipose tissue biopsies were taken before and at the end of insulin infusion for measurement of LPL activity. The change in LPL activity is plotted against the steady-state serum insulin concentration. There is a shift of the dose-response curve to the right in the obese subjects. Values are means with bars indicating SEM. Reproduced from [86] with the permission of Evener Publishers Inc.](image)
Table 3. Possible consequences of insulin resistance in adipose tissue

<table>
<thead>
<tr>
<th>Primary</th>
<th>Secondary</th>
<th>Subsequent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive NEFA release</td>
<td>Increased hepatic VLDL secretion</td>
<td>Hypertriglyceridaemia</td>
</tr>
<tr>
<td></td>
<td>Decreased hepatic insulin extraction</td>
<td>Hyperinsulinemia</td>
</tr>
<tr>
<td></td>
<td>Impaired peripheral glucose disposal</td>
<td>Glucose intolerance</td>
</tr>
<tr>
<td></td>
<td>Impaired LPL activity in adipose tissue</td>
<td>See below</td>
</tr>
<tr>
<td>Impaired LPL activation</td>
<td>Reduced TAG clearance</td>
<td>Hypertriglyceridaemia</td>
</tr>
<tr>
<td></td>
<td>Impaired transfer of cholesterol into HDL</td>
<td>Low HDL-cholesterol concentration</td>
</tr>
</tbody>
</table>

Fig. 2. Scheme showing the possible consequences of insulin resistance in adipose tissue. Primary changes are the lack of insulin-restraint of NEFA release (right side of adipose tissue in diagram), and an impaired insulin activation of LPL in adipose tissue (left side of adipose tissue in diagram). Secondary consequences of the excessive NEFA flux are: increased hepatic VLDL-TAG secretion, decreased hepatic insulin extraction and glucose intolerance in peripheral tissues. Secondary consequences of the impaired action of LPL are an increased plasma TAG concentration and an impaired transfer of cholesterol into HDL; these may have adverse consequences in terms of predisposition to atherogenesis. For further explanation, see the text.

From the above, it will be seen that defective insulin action on lipid metabolism in adipose tissue may be one important link in the well-known association between elevated plasma insulin concentrations (or insulin resistance), elevated VLDL-TAG concentrations and decreased HDL-cholesterol concentrations (Table 3 and Fig. 2). Impairment of the normal suppression by insulin of fatty acid release from adipose tissue will result in increased NEFA delivery to the liver, and thus accelerated VLDL-TAG secretion. Impairment of the normal responsiveness of adipose tissue LPL activity to insulin will lead to reduced VLDL-TAG clearance, impaired transfer of cholesterol into HDL and possibly prolonged residence in the circulation of atherogenic remnant particles. By other mechanisms increased fatty acid delivery to the liver will lead to reduced hepatic insulin extraction and thus hyperinsulinaemia, and to an impairment of glucose utilization in peripheral tissues. Foster's [17] description of insulin resistance as 'the secret killer' may thus be seen as mediated through changes in adipose tissue metabolism much more directly than through changes in glucose metabolism.

ACKNOWLEDGMENTS

We thank the British Heart Foundation for support of our own research on this topic, and the Oxford Diabetes Trust for support of the Sheikh Rashid Diabetes Unit.

REFERENCES

2. Kissebah, A.H., Adams, P.W. & Wynn, V. Inter-relationships between insulin secretion and plasma free fatty acid and triglyceride transport.


