The role of insulin and the adipocytokines in regulation of vascular endothelial function

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ABSTRACT

Vascular integrity in the healthy endothelium is maintained through the release of a variety of paracrine factors such as NO (nitric oxide). Endothelial dysfunction, characterized by reduced NO bioavailability, is associated with obesity, insulin resistance and Type II diabetes. Insulin has been demonstrated to have direct effects on the endothelium to increase NO bioavailability. Therefore altered insulin signalling in the endothelium represents a candidate mechanism underlying the association between insulin resistance and endothelial dysfunction. In recent years, it has become apparent that insulin sensitivity is regulated by the adipocytokines, a group of bioactive proteins secreted by adipose tissue. Secretion of adipocytokines is altered in obese individuals and there is increasing evidence that the adipocytokines have direct effects on the vascular endothelium. A number of current antidiabetic strategies have been demonstrated to have beneficial effects on endothelial function and to alter adipocytokine concentrations in addition to their effects on glucose homoeostasis. In this review we will explore the notion that the association between insulin resistance and endothelial dysfunction is accounted for by adipocytokine action on the endothelium. In addition, we examine the effects of weight loss, exercise and antidiabetic drugs on adipocytokine availability and endothelial function.

INTRODUCTION

Endothelial dysfunction and NO (nitric oxide) bioavailability

Endothelium-derived NO is anti-atherogenic and anti-thrombotic by virtue of its ability to relax the surrounding smooth muscle, inhibit platelet aggregation and smooth muscle cell proliferation and prevent leucocyte adhesion [1]. Endothelial dysfunction occurs when there is an imbalance in which the actions of vasoconstrictors outweigh those of vasodilators. This imbalance manifests itself as impaired endothelium-dependent vasorelaxation and decreased NO bioavailability, either due to decreased NO synthesis or sequestration of NO [2]. Endothelial dysfunction is likely to be the initiating event in atherosclerosis, contributes to ischaemic coronary artery disease and may predict risk of cardiovascular events [3,4]. Reduced NO synthesis can be the result of decreased expression of eNOS (endothelial NO synthase) or decreased intrinsic activity of eNOS due to post-translational modification, reduced concentrations of the essential cofactor tetrahydrobiopterin or increased concentrations of ADMA (asymmetric dimethylarginine), an endogenous inhibitor of NOS [2,5,6]. Each of

Key words: adipocytokine, diabetes, endothelium, insulin, obesity, vascular function.

Abbreviations: ADMA, asymmetric dimethylarginine; AMPK, AMP-activated protein kinase; BMI, body mass index; CRP, C-reactive protein; ET-1, endothelin-1; FasL, Fas ligand; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular cell-adhesion molecule-1; IGF-I, insulin-like growth factor-I; IL, interleukin; IR, insulin receptor; IRS, IR substrate; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acid; NF-κB, nuclear factor κB; NO, nitric oxide; eNOS, endothelial NO synthase; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; ROS, reactive oxygen species; T2D, Type II diabetes; TNF-α, tumour necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

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these mechanisms has been proposed to underlie pathophysiological reductions in NO bioavailability and endothelial dysfunction. Sequestration of NO can occur as a result of reactions with ROS (reactive oxygen species), such as superoxide. Increased generation of ROS is a feature of numerous pathologies, including heart failure, hypertension, atherosclerosis and T2D (Type II diabetes) [2,7,8].

**Insulin resistance, metabolic syndrome and endothelial dysfunction**

Resistance to the action of insulin to promote cellular glucose uptake is associated with a group of metabolic and haemostatic abnormalities, including compensatory hyperinsulinaemia, dyslipidaemia, obesity and hypertension – the ‘metabolic syndrome’, prior to the development of T2D [2,9]. Approximately one quarter of the adult British population is thought to have some degree of resistance to the biological actions of insulin. Insulin resistance is commonly found in overweight individuals, with increased central adipose tissue distribution the most influential risk factor. It has become accepted that insulin resistance is a key association of essential hypertension; there is a clear association between insulin sensitivity and vascular endothelial function in normal subjects, obese individuals and in patients with T2D [10–12]. Indeed, a significant relationship has been proposed to exist between insulin resistance and plasma concentrations of ADMA [13].

Studies performed in vitro and in vivo have demonstrated that insulin has direct physiological effects on the normal endothelium to increase NO availability. Thus, if it is accepted that resistance to the cellular action of insulin is a generalized phenomenon affecting many tissues, altered insulin signalling in the endothelium may represent a common candidate mechanism underlying the association between insulin resistance and endothelial dysfunction. In this review we will explore the notion that this association is accounted for by changes in the production of a number of key molecules released by adipose tissue.

**The adipocytokines**

Adipose tissue is regulated by hormonal, neural and nutrient stimuli. In fasting states, adipocytes release NEFAs (non-esterified fatty acids) as a result of lipolysis, whereas the postprandial increase of glucose and lipids promotes lipogenesis in the presence of insulin. However, it has become apparent that adipose tissue also regulates metabolism in addition to its role in fuel storage. In addition to NEFAs, adipocytes secrete a number of bioactive proteins, collectively termed the adipocytokines (Table 1) [14–29]. Adiponectin, leptin, IL (interleukin)-6 and TNF-α (tumour necrosis factor-α) are among the best characterized of the adipocytokines and are attracting increasing attention due to the important role they are proposed to play in insulin resistance. Secretion of the adipocytokines is altered in obese individuals. Indeed, obesity has been proposed to be a state of chronic inflammation, as indicated by increased plasma concentrations of CRP (C-reactive protein) and the adipocytokine IL-6 [19,30,31]. Furthermore, plasma CRP concentration has been demonstrated to predict cardiovascular disease in apparently healthy individuals [32] and is elevated in insulin-resistant prediabetic subjects [33]. Hepatic synthesis and secretion of CRP is predominantly regulated by the adipocytokine IL-6 [30–32]. Therefore there is currently great interest in the role of the adipocytokines in the pathogenesis of T2D and cardiovascular risk. In this regard, it is known that increased adiposity is a major determinant of insulin resistance and that altered adipocytokine regulation is a key common mechanism. Furthermore, weight loss improves insulin resistance and is associated with improved vascular endothelial NO availability [34]. These data strongly support the notion that signals generated by adipocytes, such as the adipocytokines, influence endothelial cell function. This review will focus on the evidence for regulation of endothelial function by insulin and the adipocytokines adiponectin, leptin, IL-6 and TNF-α. Interactions between the adipocytokines and insulin signalling will be discussed and will relate evidence obtained in vitro to support that from in vivo studies. We shall explore the concept that aberrant adipocytokine secretion in insulin resistance and/or obesity impairs endothelial function and contributes further to reduced insulin sensitivity.

**EFFECTS OF INSULIN ON THE VASCULATURE**

In addition to conventional responsive tissues such as skeletal muscle and adipose tissue, insulin also exerts important biological effects on the vasculature [35–38]. Various hypotheses have been put forward regarding the role

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of insulin action on vascular tissues, including a role in glucose homoeostasis and control of blood pressure and blood flow. It has been widely described that insulin has both vasodilator and vasoconstrictor effects [39–41], with NO being the main mediator of vasodilation and the primary vasoconstrictive mediator being ET-1 (endothelin-1).

**Insulin-stimulated vasodilation**

Insulin-stimulated vasodilation has been attributed to several mechanisms, including an interaction with the sympathetic nervous system at the vascular level, the activation of ion channels [42], the release of adenosine [43] and an increase in the generation of NO by the vascular endothelium [40,44,45]. Endothelium-dependent vasodilation can be assessed by measuring blood flow increases or vascular resistance in response to agents that cause NO release, or by measuring blood flow reductions in response to inhibitors of eNOS [46].

Insulin-stimulated vasodilation has been observed in both humans and animals; however, there is considerable variation in responsiveness [47–49], likely to be attributable to differences in muscularity, physical activity and blood supply.

Studies in isolated vessels and cultured endothelial cells have improved our understanding of the molecular mechanisms by which insulin stimulates vasodilation. Insulin has been demonstrated to stimulate relaxation of isolated vascular strip preparations [50], and blunt the response to a number of vasoconstricting reagents [51].

Insulin binding to the IR (insulin receptor) stimulates the recruitment of IRS (IR substrate) proteins that subsequently recruit and allow activation of the PI3K (phosphoinositide 3-kinase) pathway (Figure 1). In cultured endothelial cells, insulin stimulates NO production, a response inhibited by the PI3K inhibitor wortmannin [45]. Impaired endothelium-dependent vasodilation has been demonstrated in mice lacking IRS-1 [52], and insulin-stimulated NO release was impaired in HUVECs (human umbilical vein endothelial cells) cultured from subjects with a variant (Glu$^{72} \rightarrow$ Arg; G972R) of IRS-1 [53]. Therefore variant insulin signalling proteins such as this may contribute to the genetic predisposition to develop endothelial dysfunction and cardiovascular disease. Insulin-stimulated PI3K activity results in activation of PKB (protein kinase B)/Akt, which has been demonstrated to mediate many metabolic effects of insulin, including glycogen synthesis, increased glucose transport, antilipolysis and suppression of gluconeogenic genes [54]. In cultured endothelial cells, stimulation of PKB by insulin has been proposed to phosphorylate eNOS at a specific residue, Ser$^{1177}$ (human sequence), that has been demonstrated to increase eNOS activity several-fold [55–60]. Intriguingly, proinsulin C-peptide has also recently been demonstrated to enhance eNOS activity in a Ca$^{2+}$-dependent manner in endothelial cells [61].

As with all *in vitro* experiments, these data should be interpreted with caution as supraphysiological concentrations of insulin have generally been used, raising the possibility of interaction with the IGF-I (insulin-like growth factor-I) receptor rather than the IR. However, IGF-I is unable to stimulate NO synthesis to the same degree as insulin in HUVECs [45]. It has also been proposed that insulin-stimulated vasodilation is the result of insulin-stimulated release of vasoactive concentrations of ATP, adenosine and serotonin from human platelets in a NO-dependent manner [62], and pronounced insulin-induced vasodilation has been reported to require systemic hyperinsulinaemia [63]. However, the majority of studies demonstrate that insulin stimulates NO production in endothelial cells. In a number of insulin-resistant states, the ability of supraphysiological concentrations of insulin to stimulate blood flow is attenuated [64], indicating that direct effects of insulin NO production is of importance in the vascular endothelium [65].

**Vasoconstrictive actions of insulin**

It has also been suggested that insulin has a vasoconstrictor action, thought to be principally mediated by ET-1 [66]. Insulin stimulates ET-1 production in endothelial cells both *in vivo* and *in vitro*, and this is thought to influence both normal blood flow and changes in blood pressure in patients with hyperinsulinaemia and insulin resistance [67]. During NOS inhibition and hyperglycaemia, insulin has been reported to stimulate vasoconstriction [68], mediated by ET-1 [41]. NO-dependent vasodilation is attenuated in insulin-resistant subjects, whereas acute stimulation of ET-1 release is unaffected [69]. Insulin-stimulated MAPK (mitogen-activated protein kinase) phosphorylation is increased in endothelial cells in response to insulin, and this may influence ET-1 production [70].
protein kinase) activation is thought to be a key step in vasoconstriction and ET-1 activation in skeletal muscle arterioles, and this activation was found to antagonize PI3K-dependent vasodilatation [70].

It is clear therefore that many of the regulatory effects attributed to insulin on endothelial cells, especially the control of vascular tone, depend on the ability to produce NO and ET-1. In vascular endothelial cell IR knockout mice there is a decrease in eNOS mRNA expression, as well as a more significant decrease in ET-1 expression, but normal glucose homeostasis and vascular development [71]. A disruption in the equilibrium between eNOS and ET-1 could subsequently lead to either hypo- or hyper-tension, but the existence of counter regulatory mechanisms in vivo may compensate for the deficiency of insulin signalling [71].

**Other vascular effects of insulin**

In cultured endothelial cells, insulin up-regulates eNOS expression [72,73] and inhibits the expression of the pro-atherogenic transcription factor NF-κB (nuclear factor κB), the adhesion molecules ICAM-1 (intercellular cell-adhesion molecule-1) and E-selectin, in addition to the pro-inflammatory chemokine MCP-1 (monocyte chemoattractant protein-1) [72,74–77]. In human aortic endothelial cells, it has been suggested that increased NO production mediates inhibition of adhesion molecule expression by insulin [72,78]. This implies increased expression of pro-atherogenic adhesion molecules in patients with impaired insulin-stimulated NO production. Insulin also has growth factor activity, effects principally mediated by the MAPK pathway [79,80]. Insulin-stimulated MAPK activation is unaltered when insulin-stimulated PI3K signalling is down-regulated in isolated aorta and microvessels of obese (fa/fa) rats [81]. Furthermore, insulin-stimulated MAPK pathway is up-regulated when PI3K is inhibited in cultured endothelial cells [77]. In the presence of insulin resistance, it is therefore possible for hyperinsulinaemia to be pro-atherogenic. The principal effects of insulin are anti-thrombotic and anti-atherogenic, and these are likely to be impaired under conditions of insulin resistance.

**ADIPOSITY AND THE ADIPOCYTOKINES**

There is a close and dynamic relationship between insulin resistance and visceral adiposity. It has become apparent that insulin sensitivity is modulated by adiposity, and this has led to significant interest in the adipocytokines, which may play a pivotal role in the regulation of insulin signalling and action. Adipose tissue also appears to be important in vascular injury and inflammation [82,83]. The vascular effects of the adipocytokines are discussed further below.

**Leptin**

Leptin is a 16 kDa protein secreted by adipocytes in proportion to adipocyte mass [84]. Leptin is proposed to act as a signal that informs the hypothalamus of the quantity of stored fat. In normal individuals it is proposed that, as energy is ingested and stored, serum leptin concentrations increase, leptin crosses the blood–brain barrier and ultimately results in anorexigenic stimuli [85–87]. Six forms of leptin receptor have been identified, the best characterized of which is the Ob/Rb receptor [88]. Children with frameshift mutations in the leptin (ob) gene are leptin deficient, display hyperphagia, obesity and hyperinsulinaemia [89]. Leptin receptors have been detected in endothelial cells and atherosclerotic plaques [90–92]. This has led to the hypothesis that leptin interacts with the vascular wall and may contribute to atherosclerotic change and angiogenesis. Leptin concentrations increase with progressive obesity, but the anorexigenic effects of leptin become less pronounced. However, the exact mechanism of this proposed ‘leptin resistance’ is not clear [93,94].

Atherosclerosis is initiated by endothelial cell damage. As the atherosclerotic plaque progresses it develops its own microvascular network (neovascularization), the disruption of which is thought to play a role in plaque destability. Leptin enhances formation of capillary-like tubes and neovascularization in vitro and in vivo. Leptin produced from adipocytes has been proposed to act not only in an endocrine, but also a paracrine manner, promoting an angiogenic response that ensures that there is an appropriate blood supply for fat depot size [95,96].

A recent study of wild-type mice with hyperleptinemia (as a result of a high-fat diet) demonstrated enhanced neointimal and medial thickening of the vascular wall after an induced carotid artery injury [97]. Leptin-deficient (ob/ob) mice on the same diet had increased body weight, glucose and lipid levels, but the vascular changes were not manifested. Daily leptin administration to these mice significantly increased lesion formation, whereas leptin treatment to leptin receptor knockout (db/db) mice did not alter vascular lesion progression, suggesting a direct receptor-mediated effect of leptin on the vascular wall [97]. Studies in normal adolescents with variable BMIs (body mass indexes) found increased leptin concentration to be associated with impaired vascular function independent of the metabolic and inflammatory disturbances associated with obesity [98].

Leptin has also been demonstrated to stimulate activation and proliferation of haematopoietic cells [99]. Recent evidence suggests that this targets progenitor and mature VSMCs (vascular smooth muscle cells) to the site of the vascular injury [100]. Expression of pro-inflammatory markers such as NF-κB and MCP-1 are stimulated by leptin in cultured endothelial cells [90,92]. Furthermore, plasma leptin concentration is associated with soluble thrombomodulin and VCAM-1 (vascular
cell adhesion molecule-1), markers of inflammation, in obese women [101]. In contradiction of this, supplementary leptin administration during weight loss was found to have no effect on pro-inflammatory markers [102]. In cultured human endothelial cells, leptin has been demonstrated to stimulate the production of ROS [90,92]. It has been proposed that this may stimulate atherogenic processes under conditions of chronic hyperleptinaemia [90].

Migration of endothelial cells is a key event in angiogenesis contributing to pathological states such as diabetic vasculopathy. Leptin has been shown to induce cell migration in HUVECs, mediated by the PI3K/PKB/eNOS and MAPK pathways [103]. A number of studies have indicated that leptin stimulates vasodilation [104,105], proposed to be mediated by NO-dependent [106,107] or NO-independent [105] mechanisms. Indeed, leptin stimulates phosphorylation of eNOS at Ser1177 in isolated endothelial cells and human aortic rings [103,108]. This mechanism, however, may be PI3K-independent [108]. Despite this insulin-mimetic action of leptin that may mediate beneficial cardiovascular effects, the majority of studies performed to date indicate that hyperleptinaemia is atherogenic.

**Adiponectin**

Adiponectin, also known as Acrp30 or AdipoQ [16–18], is a 30 kDa protein that exists in plasma as trimeric, hexameric and higher-order polymeric structures [109]. Adiponectin is secreted exclusively by adipose tissue [16–18] with plasma levels negatively correlated with obesity, cardiovascular disease and insulin resistance [110–112]. Variation in serum adiponectin concentrations has been proposed to have a strong heritable component in both a predominantly northern European and Pima Indian populations [113,114]. Adiponectin knockout mice exhibit moderate insulin resistance, glucose intolerance and reduced fatty acid clearance from plasma [115,116]. The exact physiological role of adiponectin is unknown, but such studies suggest that there is close association between hypoadiponectinaemia and insulin resistance [109]. Indeed, retrospective analysis of a Pima Indian population demonstrated that the development of T2D was associated with lower plasma adiponectin many years prior to the diagnosis of T2D [117]. The mechanism by which adiponectin ameliorates insulin resistance has not been identified. Studies of lipoprotrophic mice and rodent models of obesity and T2D have led to the proposal that adiponectin acts to sensitize tissues to insulin by reducing serum NEFA concentrations. This may explain the link between reduced serum adiponectin concentrations and insulin resistance [118,119]. Recently, two putative adiponectin receptors have been identified [120], yet, at the time of writing, the signalling pathways that couple to these receptors remain uncharacterized.

Adiponectin has been suggested to have anti-inflammatory and anti-atherogenic properties. Indeed, hypo-adiponectinaemia is associated with impaired endothelium-dependent vasodilation and reduced blood flow in humans [121,122]. Therefore adiponectin may act to link adipose tissue and the vasculature [121]. Both adiponectin receptor subtypes are expressed in human vascular endothelial cells, indicating a possible direct effect of adiponectin on these cells [123]. Furthermore, there is increased neointimal formation in response to external vascular cuff injury in adiponectin knockout mice compared with wild-type controls [116]. This implies an anti-atherogenic action of adiponectin in vivo.

Recent studies have indicated that AMPK (AMP-activated protein kinase) mediates some of the effects of adiponectin. In cultured endothelial cells, AMPK has been demonstrated to phosphorylate and activate eNOS at the same site as PKB [124]. Furthermore, stimulation of cultured endothelial cells with adiponectin has recently been demonstrated to stimulate eNOS Ser1177 phosphorylation and NO synthesis [125,126]. Both AMPK and PKB signalling pathways have been proposed to mediate adiponectin-stimulated NO production and angiogenesis in cultured endothelial cells [125,127]. Adiponectin has also been shown to inhibit the expression of cell adhesion molecules, including ICAM-1, VCAM-1 and E-selectin [128], in addition to class A1 macrophage scavenger receptors, causing markedly decreased uptake of oxidized LDL (low-density lipoprotein) and inhibition of foam cell formation [129]. Such effects may well underlie the anti-thrombotic and anti-atherogenic effects of adiponectin in vivo, an effect lost as adiponectin levels are reduced in obesity.

**IL-6**

IL-6 is a 26 kDa protein produced by several tissues, including adipose and endothelium [19,130]. Adipose tissue has been postulated to contribute a significant proportion (approx. 30%) of the total circulating IL-6 [19,31]. IL-6 binds to a specific receptor and requires an accessory signal transducer, gp130, to elicit its effects [131]. Endothelial cells lack the IL-6 receptor, yet are able to respond to IL-6 by virtue of a soluble IL-6 receptor, which, when complexed with IL-6, binds endothelial cell gp130 with subsequent stimulation of signalling events [31].

Elevated IL-6 concentrations have been demonstrated to be associated with insulin resistance and obesity [31]. Infusion of IL-6 in mice blunted insulin-mediated suppression of hepatic glucose production [132]. The requirement for co-incubation with soluble IL-6 receptor has limited in vitro studies to determine the direct effects of IL-6 on vascular tissues. IL-6 has been demonstrated to result in impaired NO-mediated relaxation of systemic vessels in pregnant rats [133] and to stimulate endothelial cell proliferation and angiogenesis [134,135].
IL-6 has been demonstrated to stimulate the synthesis and secretion of hepatic CRP, which contributes to atherogenesis [136]. CRP has been demonstrated to have a multitude of pro-inflammatory and pro-atherogenic effects on cultured endothelial cells, including increased expression of adhesion molecules and MCP-1 [137,138], reduced expression of eNOS and reduced eNOS activity [139,140]. Therefore the principal effects of IL-6 on the vasculature may well be indirect, mediated by CRP.

**TNF-α**

TNF-α is expressed as a 26 kDa cell surface transmembrane protein, subsequently cleaved to a 17 kDa active biological form, produced by a broad variety of tissues, including adipocytes [20,141]. It is known to act in an autocrine, paracrine and endocrine manner and has a number of functions, including the promotion of apoptosis, inflammation and cytotoxicity, and it also stimulates the production of other cytokines, including IL-1 and IL-6 [141,142]. Vascular endothelium is a principal target of the actions of TNF-α. TNF-α exerts its biological function via interaction with cognate membrane receptors, comprising the TNF receptor family [141,143].

TNF-α expression is increased in the adipose tissue of obese animals and humans [144,145]. Expression of TNF-α correlates with BMI, percentage body fat and hyperinsulinaemia [20]. In addition, TNF-α gene knock-out obese mice demonstrate improved insulin sensitivity [146]. Infusion of anti-(TNF-α) antibodies has been demonstrated to improve insulin sensitivity in rodents, but is less effective in humans [147,148].

NO bioavailability is reduced in cultured endothelial cells incubated with TNF-α [149]. In addition, endothelium-dependent vasodilation is impaired in animals and humans after infusion with TNF-α [150,151]. This has been proposed to be due to reduced eNOS mRNA stability or increased superoxide production [152,153]. Incubation of human endothelial cells with TNF-α has been demonstrated to promote dephosphorylation and inactivation of PKB, an important mediator of cell survival in addition to insulin signalling [154]. Therefore TNF-α may stimulate apoptosis of endothelial cells, an effect counteracted by insulin [154]. TNF-α has also been demonstrated to down-regulate expression of FasL (Fas ligand) in endothelial cells [155]. FasL has recently been shown to stimulate PKB and eNOS phosphorylation in HUVECs [156]. Therefore inhibition of FasL signalling by TNF-α may represent another pathway by which TNF-α reduces NO bioavailability.

TNF-α increases ET-1 concentration in human forearm blood flow models, resulting in decreased endothelium-dependent vasodilation. Despite this, TNF-α was shown to have a vasodilatory effect causing increased forearm blood flow [157]. This latter finding may be of more relevance in septic shock, where increased TNF-α levels have been demonstrated. TNF-α stimulates the expression of adhesion molecules, including ICAM-1, VCAM-1 and E-selectin, in addition to MCP-1 and IL-8, which stimulate leucocyte activation [158].

**ADIPOCYTOKINES AND INSULIN SIGNALLING**

Do the adipocytokines affect insulin sensitivity by modulating the molecular insulin signalling mechanisms? Attenuated activation of PI3K by insulin is a predominant feature of insulin resistance, and subsequent defective activation of eNOS has been implicated in the pathogenesis of hypertension in insulin-resistant states. PI3K has been demonstrated to mediate some of the actions of leptin and adiponectin in various tissues, including the endothelium [125,159]. Similarly, both leptin and adiponectin have been proposed to stimulate NO synthesis in cultured endothelial cells as a result of increased Ser1177 phosphorylation of eNOS [108,125], thereby mimicking insulin-stimulated eNOS phosphorylation and NO synthesis. Leptin has been demonstrated to impair insulin signalling in adipocytes [160], and IL-6 has been shown to reduce IRS-1 expression and insulin-stimulated PKB activation in hepatocyte and adipocyte cell lines [161–163], yet the effects of leptin and IL-6 on insulin signalling in the endothelium remain uncharacterized. TNF-α, on the other hand, has been shown to interfere with intracellular insulin signalling pathways in endothelial cells, and this therefore represents a candidate mechanism by which it leads to impaired insulin action. Incubation with TNF-α has been demonstrated to increase serine phosphorylation of IRS-1 in adipocytes, which reduces its capacity to recruit and activate downstream effectors of insulin [164,165]. In endothelial cells, TNF-α has been demonstrated to inhibit eNOS and IR expression, insulin-stimulated IR phosphorylation, insulin-stimulated NO production and eNOS phosphorylation [55,78,152]. An outline of the molecular mechanisms by which the adipocytokines may impinge on insulin signalling pathways in the endothelium is illustrated in Figure 2.

**PARACRINE AND AUTOCRINE ACTIONS AMONG ADIPOCYTOKINES**

A number of studies have demonstrated that leptin secretion and synthesis is regulated by TNF-α. Chronic incubation with TNF-α has been demonstrated to inhibit leptin secretion in cultured human adipose and isolated rat adipocytes [166–168]. In contrast, acute incubation with TNF-α has been demonstrated to stimulate leptin secretion [167,169,170]. IL-6 had no effect on leptin secretion in cultured human adipose tissue [168].

Similarly, there is evidence to suggest that the secretion of adiponectin is regulated by other adipocytokines, as
antidiabetic strategies and the adipocytokines

weight loss and exercise

A number of recent studies have addressed the effects of weight loss and exercise on endothelial function and circulating adipocytokines with conflicting results. In both a study of 80 patients (40 with and 40 without the metabolic syndrome) who underwent 4–6 weeks of weight loss [174] and a smaller study of three groups of obese patients who underwent diet and aerobic or anaerobic exercise programmes [175], no change in adiponectin was observed following the intervention period. In 16 middle-age male patients with T2D randomized to 8 weeks of exercise or placebo, no change in adiponectin or leptin was seen despite a 58% increase in insulin sensitivity (without change in weight) in the exercise group [176]. Other lifestyle studies have however demonstrated ‘positive’ effects on adipocytokines. In a study of 120 obese non-diabetic patients randomized to either 2 years of a Mediterranean-style diet or a control diet, reduction in BMI was significantly greater in the intervention arm in association with an increase in adiponectin and a reduction in IL-6 and CRP [177]. The same group had shown in an earlier study that after 1 year of a multidisciplinary programme of weight reduction, obese females losing at least 10% of their body weight had significant reductions in serum TNF-α levels in association with a reduction in adhesion molecules and an improved vascular response to L-arginine [34]. These findings were interpreted as suggesting both an anti-inflammatory and anti-atherogenic effect of weight loss in response to the intervention [14]. In addition, a small study of 24 insulin-resistant obese subjects reported that weight reduction was associated with a significant decrease in leptin and IL-6, whereas adiponectin was only increased in subjects with T2D [178].

The effect of surgical techniques to reduce weight has also been investigated. In a recent study of 15 obese patients, liposuction associated with an approx. 10% reduction in total fat had no effect on circulating CRP, adiponectin, IL-6 or TNF-α; however, following gastric banding surgery in 22 obese patients (mean fall of 21% in BMI), a 46% increase in adiponectin was observed [179, 180].

The variability in the results above is difficult to explain; however, the degree of weight loss and nature of the intervention vary somewhat between studies, also the assays used to measure adiponectin have not all been rigorously validated, and such variation may explain the lack of consistency in these findings.

Pharmacological interventions

Anti-obesity medication

Different anti-obesity medications may have varied effects; modest weight loss (approx. 5% initial body weight) as a result of sibutramine therapy in severely obese non-diabetic women for 6 months was associated with a statistically significant decrease in leptin and an increase in adiponectin, whereas no equivalent effect was seen in those treated with orlistat [181]. Conversely, there was no change in plasma adiponectin concentrations in a small group of obese women after weight loss (approx. 10% initial weight) as a result of sibutramine and a calorie-restricted diet [182]. As no data are available concerning the effects of sibutramine on endothelial function, the role of the adipocytokines in endothelial function as a result of sibutramine therapy remains uncharacterized.
 Sulphonylureas
The principal mechanism of action of the sulphonylurea class of drugs is to improve insulin secretion by the β cells of the endocrine pancreas, and data concerning the effects of sulphonylureas on adipocytokines are limited. The use of sulphonylureas in poorly controlled patients with T2D (on dietary modification only) did not show any effect on TNF-α or IL-6 concentrations, or on markers of endothelial dysfunction [183]; however, leptin levels (normalized to percentage body fat) were increased by glyburide therapy [184].

Metformin
The use of metformin in non-diabetic patients with ischaemic heart disease (obese and non-obese subgroups) showed that in the obese group there was no effect on TNF-α plasma concentration, whereas in the non-obese group there was a 33% increase. This implies that, although metformin does improve insulin resistance, it does not do so through regulation of TNF-α [185]. Although addition of metformin to sulphonylurea therapy had no effect on circulating concentrations of adiponectin or leptin in patients with T2D [186, 187], a reduction in serum leptin has been observed in healthy adults treated with metformin [188] and in diabetic and non-diabetic obese patients treated with metformin in association with a calorie-restricted diet [189].

The diversity of outcomes in these experiments is difficult to explain. It has been suggested recently that differences in treatment with drugs or diet that alter insulin secretion may account for these differences; when leptin was normalized to percentage body fat, levels were reduced in patients with T2D, although were not affected by metformin treatment [184].

In the UKPDS (United Kingdom Prospective Diabetes Study), metformin treatment was demonstrated to reduce vascular risk in obese patients [190]. In addition, 3 months of metformin therapy of patients with T2D has been demonstrated to improve endothelial function, assessed by forearm plethysmography, although no attempt was made to control for glycaemia [191]. It therefore seems unlikely that the beneficial cardiovascular effects of metformin are a result of altered adipocytokine concentrations.

Thiazolidinediones
In a comparison of circulating and adipose tissue adiponectin in patients with T2D treated with metformin or troglitazone, serum adiponectin increased 3-fold in association with an increase in subcutaneous adipose tissue adiponectin protein content and release in those receiving troglitazone, whereas there appeared to be no equivalent effect of metformin [186]. Pioglitazone treatment in patients with T2D resulted in a 3-fold increase in plasma adiponectin levels in association with a decrease in hepatocellular fat content and improvements in hepatic and peripheral insulin sensitivity [192]. Similar results have been shown in other clinical studies of troglitazone and rosiglitazone [193, 194]. Studies in rodents and cultured adipocytes also indicate that thiazolidinediones increase adiponectin secretion, possibly as a result of increased expression of adiponectin [195].

TNF-α plasma concentration fell following troglitazone therapy in obese patients with T2D [196] and after pioglitazone treatment in fatty rats [197]. Furthermore, in cultured adipocytes, thiazolidinediones antagonize the effect of TNF-α to reduce adiponectin levels [194], whereas the inhibitory effects of TNF-α on insulin signaling proteins are also ameliorated by troglitazone and pioglitazone [198].

The effects of thiazolidinediones on leptin are less well characterized. Incubation with thiazolidinediones dramatically decreased leptin concentrations in a murine adipocyte cell line [199], yet was without effect in cultured human adipocytes [200]. Rosiglitazone therapy in patients with congenital lipodystrophy resulted in a 2-fold increase in leptin levels [201]; however, HIV-positive patients with HAART (highly active anti-retroviral therapy)–induced lipoatrophy did not show any change in leptin concentration following thiazolidinedione therapy [202]. Whether individual drugs, duration of therapy or differences in in vitro and in vivo systems account for these differences is not clear. Thiazolidinediones have also been demonstrated to impair leptin-stimulated PKB and eNOS phosphorylation in cultured endothelial cells [104].

A number of recent studies have identified beneficial vascular effects of the thiazolidinediones in obese patients, patients with T2D or the metabolic syndrome. Rosiglitazone treatment of patients with T2D or the metabolic syndrome has been demonstrated to improve endothelium-dependent vasodilation and reduce blood pressure [203–207]. In addition, pioglitazone or rosiglitazone therapy of obese patients, patients with T2D or the metabolic syndrome results in a reduction in plasma concentrations of CRP [204, 207–209]. One study of Japanese patients with T2D has demonstrated that the anti-atherogenic effect of pioglitazone (as assessed by reduced CRP concentrations and pulse wave velocity) was independent of its antidiabetic effect [207]. Intriguingly, in the same study, pioglitazone therapy increased adiponectin concentrations irrespective of the antidiabetic effect, and the authors speculate that increased adiponectin secretion or synthesis by adipose tissue may well account for the anti-atherogenic effect of pioglitazone [207].

Table 2 summarizes the effects of metformin and the thiazolidinedione insulin-sensitizing drugs on markers of endothelial function and circulating adipocytokine concentrations.
Effects of insulin-sensitizing therapies on circulating adipocytokines and endothelial function

<table>
<thead>
<tr>
<th>Endothelium-dependent vasodilation</th>
<th>Metformin</th>
<th>Thiazolidinediones</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Improved</td>
<td>[191]</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>No change</td>
<td>[186,187]</td>
</tr>
<tr>
<td>Leptin</td>
<td>Reduced</td>
<td>[188]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>No change</td>
<td>[186,187]</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>[185]</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>[196]</td>
</tr>
</tbody>
</table>

FUTURE PROSPECTS AND CONCLUSION

An increased understanding of the pathogenesis of insulin resistance and T2D has highlighted the importance of adipose tissue dysregulation in disease states and has led to the notion of adipose tissue as an endocrine organ. The increase in the prevalence of T2D in the Western world is inextricably linked to the epidemic of obesity, and a greater insight into adipose tissue biology will help to unravel the relationship between obesity and insulin resistance and may also identify novel therapeutic targets. Although the influence of key adipocytokines on endothelial cell biology is accepted, the role of these molecules in regulating vascular risk is less established. TNF-α and adiponectin have opposing effects on the endothelium and decreased adiponectin and increased TNF-α concentrations are good candidate causes of endotheliopathy in insulin resistance and T2D. There is increasing interest in the vascular effects of leptin, which appears to be largely pro-atherogenic, yet the majority of the effects of leptin appear to be mediated by its effect on satiety in the hypothalamus. It also seems likely that the vascular effects of adipose-derived IL-6 are mediated by increased hepatic CRP production. Furthermore, it remains uncertain to what extent the beneficial effects of weight loss and antidiabetic therapies on vascular risk are mediated through changes in adipocytokines. Following the UKPDS finding of a reduction in vascular risk in obese patients treated with metformin, it appears that therapies aimed at improving insulin resistance may offer particular benefits in diabetes; several prospective studies involving thiazolidinediones in a similar setting are underway and their findings are keenly awaited. The contribution of the adipocytokines, particularly TNF-α and adiponectin to the anti-atherogenic effects of the thiazolidinediones, warrants further study and these molecules remain key targets for therapeutic intervention. Elucidation of the mechanisms linking obesity, diabetes and atherosclerosis is fundamental to developing interventions that will reduce vascular disease in large numbers of patients. Further study of adipose tissue and the adipocytokines seems likely to yield important findings in this regard.

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