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Themed Section: Fat and Vascular Responsiveness

REVIEW

Human obesity and endothelium-dependent responsiveness

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Obesity is an ongoing worldwide epidemic. Besides being a medical condition in itself, obesity dramatically increases the risk of development of metabolic and cardiovascular disease. This risk appears to stem from multiple abnormalities in adipose tissue function leading to a chronic inflammatory state and to dysregulation of the endocrine and paracrine actions of adipocyte-derived factors. These, in turn, disrupt vascular homeostasis by causing an imbalance between the NO pathway and the endothelin 1 system, with impaired insulin-stimulated endothelium-dependent vasodilation. Importantly, emerging evidence suggests that the vascular dysfunction of obesity is not just limited to the endothelium, but also involves the other layers of the vessel wall. In particular, obesity-related changes in medial smooth muscle cells seem to disrupt the physiological facilitatory action of insulin on the responsiveness to vasodilator stimuli, whereas the adventitia and perivascular fat appear to be a source of pro-inflammatory and vasoactive factors that may contribute to endothelial and smooth muscle cell dysfunction, and to the pathogenesis of vascular disease. While obesity-induced vascular dysfunction appears to be reversible, at least in part, with weight control strategies, these have not proved sufficient to prevent the metabolic and cardiovascular complication of obesity on a large scale. While a number of currently available drugs have shown potentially beneficial vascular effects in patients with obesity and the metabolic syndrome, elucidation of the pathophysiological mechanisms underlying vascular damage in obese patients is necessary to identify additional pharmacologic targets to prevent the cardiovascular complications of obesity, and their human and economic costs.

LINKED ARTICLES

This article is part of a themed section on Fat and Vascular Responsiveness. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.165.issue-3

Abbreviations

ADMA, asymmetric dimethylarginine; AMPK, AMP-activated PK; AT, angiotensin; BH4, tetrahydrobiopterin; BMI, body mass index; CVD, cardiovascular disease; DDAH, dimethylarginine dimethylaminohydrolase; eNOS, endothelial NO synthase; ET-1, endothelin 1; ET_A, endothelin receptor type A; FFA, free fatty acids; FMD, flow-mediated dilation; GLUT-4, insulin-responsive glucose transporter; H_2O_2, hydrogen peroxide; HSP90, heat shock protein 90; IRS, insulin receptor substrate; L-NMMA, N^G-monomethyl-L-arginine; MAP, mitogen activated protein; MCP-1, monocyte chemoattractant protein; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; $ONOO^-$, peroxynitrite; PI3K, phosphoinositide 3 kinase; PPARγ, peroxisome proliferator-activated receptor-γ; PVAT, perivascular adipose tissue; RAS/MAPK, rat sarcoma/MAPK; ROS, reactive oxygen species; Ser^{1177}, Serine 1177; SIRT1, sirtuin-1; TZD, thiazolidinediones; VENIRKO, vascular endothelial cell insulin receptor knockout; VLDL, very-low-density lipoprotein; XO, xanthine oxidase

Adult obesity, defined as a body mass index (BMI; calculated as weight in kilograms divided by height in metres squared) of 30.0 or higher, is a recognized worldwide epidemic (Bornstein *et al.*, 2008). Until a few years ago, overweight and obesity were considered a problem limited to high-income countries. However, they are now dramatically on the rise also in low- and middle-income nations, particularly in urban settings.

One of the most dramatic aspects of the epidemic is that obesity is not only a medical condition in itself but also a risk factor for the development of metabolic and cardiovascular disease (CVD) in adults (Haslam and James, 2005; Mokdad *et al.*, 2003) and in children (Berenson *et al.*, 1998; Goran and Gower, 1998; Arslanian, 2002; Caprio, 2002). Obesity, particularly when presenting with a central distribution, is frequently associated with dyslipidaemia (raised triglycerides and lowered high-density lipoprotein cholesterol), elevated BP, and abnormal fasting glucose levels. This cluster is now well recognized and is referred to as the metabolic syndrome (Grundy *et al.*, 2004; Alberti *et al.*, 2009). Even if the definition of the metabolic syndrome does not contain many of the factors that determine absolute risk, such as age, sex, cigarette smoking and low-density lipoprotein cholesterol, its presence is associated with twice the risk of developing CVD over the next 5–10 years and a fivefold increase in risk for type 2 diabetes mellitus compared with individuals without the syndrome.

Several pathophysiological mechanisms have been postulated as a link between central adiposity and the metabolic syndrome. In particular, insulin resistance and hyperinsulinaemia have increasingly been recognized to play a pivotal role in the development of the various components of the metabolic syndrome phenotype (Kahn and Flier, 2000). Even if the precise mechanisms underlying the vascular abnormalities and the increased cardiovascular risk associated with obesity have not been fully elucidated, an impaired vasodilation might affect both peripheral vascular resistance and the delivery of substrates to metabolically active tissues, thereby contributing to both hypertension and the metabolic derangements. A thorough understanding of these mechanisms could have important clinical implications, leading to the development of therapeutic strategies aimed at reducing the metabolic and cardiovascular risk.

Therefore, in this article we will review the effects of obesity on adipose tissue function, including adipocytokine secretion; we will touch upon the available evidence concerning putative mechanisms whereby obesity and insulin resistance might negatively affect vascular function and increase cardiovascular risk; and, finally, we will discuss available therapeutic approaches, including their limitations and potential side effects.

Obesity, adipose tissue distribution and adipose tissue function

In the last decade, a wealth of data has shown that adipocytes possess several roles beyond that of an energy storage depot, including, but not limited to, endocrine and immune functions. Additionally, we have become increasingly aware that body fat distribution varies significantly in lean and obese individuals and may affect the impact of obesity on metabolic and cardiovascular risk. (Bouchard *et al.*, 1993). After the initial recognition that excess fat stored in the trunk (android obesity) could exert more deleterious metabolic effects than fat stored on the limbs (gynoid obesity) (Vague, 1956), several of cross-sectional and prospective studies have confirmed that upper body fat accumulation is linked to the presence of metabolic abnormalities (Bjorntorp, 1991) and to an increased risk of dyslipidaemia (Kissebah *et al.*, 1989), hypertension (Cassano *et al.*, 1990) and type 2 diabetes (Chan *et al.*, 1994; Carey *et al.*, 1997). Even if several fat compartments can be identified in the upper body (i.e. subcutaneous, subfascial and visceral fat, comprising omental, mesenteric and periorgan adipose tissue), the adipose regions that are drained by the portal circulation (omental and mesenteric) appear to exert the most pronounced metabolic effects (Bjorntorp, 1990). Compared with other types of adipose cells, portal adipocytes are exquisitely sensitive to stimuli that mobilize free fatty acids (FFA) due to a high number of β-adrenergic receptors and a relatively weak α-adrenergic inhibition (Rebuffe-Scrive *et al.*, 1989; 1990). This sensitivity leads to high portal FFA concentrations, which, in turn, exert profound actions on the hepatic regulation of energy metabolism. In particular, exposure of the liver to elevated FFA concentrations is followed by an increased synthesis and secretion of very-low-density lipoprotein (VLDL) (Sniderman and Cianflone, 1995), stimulation of gluconeogenesis (Ferrannini *et al.*, 1983), hepatic insulin resistance (Bevilacqua *et al.*, 1987) and reduced hepatic clearance of insulin. Furthermore, the consequent hyperinsulinaemia may lead to the onset of hypertension (Reaven and Hoffman, 1987).

The adipose tissue provides vital information about its own mass and the whole body's nutritional status to the brain and to insulin-sensitive organs and tissues through the synthesis and secretion of a number of adipokines such as leptin, adiponectin and resistin (Lee *et al.*, 2009; Galic *et al.*, 2010). Leptin participates in the physiological regulation of appetite, by signalling the level of satiety to the brain (Ahima and Flier, 2000). Paradoxically, the majority of obese individuals have elevated circulating leptin levels, likely secondary to leptin resistance (Martin *et al.*, 2008). Furthermore, high leptin concentrations correlate with adverse cardiovascular outcomes in obese patients (Sweeney, 2010). Adiponectin is an adipocyte-derived peptide that circulates in high concentrations in plasma (Chiarugi and Fiaschi, 2010) and whose actions include enhancement of insulin-mediated glucose uptake in skeletal muscle, suppression of hepatic glucose production and amelioration of insulin resistance (Yamauchi *et al.*, 2002). Adiponectin's concentrations, unlike most of the other adipokines, are inversely correlated with BMI (Matsubara *et al.*, 2002) and visceral fat (Lara-Castro *et al.*, 2006), and are reduced in patients with obesity and type 2 diabetes (Weyer *et al.*, 2001). Resistin, an adipocyte-specific peptide in rodents, in humans is mainly produced by macrophages. It has been suggested that resistin may play a role in the development of insulin resistance and obesity (Lazar, 2007) and that its levels may be increased in patients with type 1 and 2 diabetes (Fehmann and Heyn, 2002).

The increase of adipose tissue mass in obesity is accompanied by macrophage infiltration, secondary to the production of monocyte chemoattractant protein-1 (MCP-1) and other pro-inflammatory cytokines, such as TNF-α and IL-6, by preadipocytes and endothelial cells (Wellen and Hotamisligil, 2003). It is now clear that inflammation may play an important role in causing both insulin resistance and vascular dysfunction. This concept was originally postulated by the pioneering work of Hotamisligil *et al.* (1996), who demonstrated that TNF-α is expressed in adipose tissue and that its

expression is induced by obesity, hence contributing to systemic insulin resistance. Subsequent studies have largely supported this hypothesis and have also demonstrated that the vasculature is an important target of TNF-α, as discussed later.

Obesity and endothelial dysfunction

The presence of endothelial dysfunction, characterized by decreased availability of NO, in patients with obesity and insulin resistance was first reported by Steinberg and coworkers over a decade ago. These authors demonstrated a blunted increase of leg blood flow in response to graded intra-arterial doses of the muscarinic receptor agonist methacholine in patients with elevated BMI or type 2 diabetes compared with lean controls (Steinberg *et al.*, 1996). Their findings were subsequently replicated by other investigators (Perticone *et al.*, 2001; Van Guilder *et al.*, 2006) and by our group in a study showing that patients with the metabolic syndrome secondary to central obesity have impaired forearm vasodilator response to ACh (Tesauro *et al.*, 2005). More recently, Bigornia *et al.* (2010) have demonstrated that also flow-mediated dilation (FMD; an ultrasound-based non-invasive endothelial function test) of the brachial artery is impaired in severely obese patients and that weight loss leads to an improvement of endothelial function and metabolic parameters in these individuals. Of interest, the improvement correlates most strongly with glucose levels and is independent of weight changes, suggesting that the relationship between obesity and brachial artery endothelial dysfunction is not direct and is likely mediated by obesity-induced metabolic abnormalities (Bigornia *et al.*, 2010). In keeping with these findings, Woo *et al.* (2004b) have reported that mild to moderate obesity is associated with impaired endothelium-dependent dilation of the brachial artery in otherwise healthy young children. Of note, the vascular abnormalities in this group appear to be partially reversible by even a short programme of dietary modification, particularly when in combination with exercise (Woo *et al.*, 2004a). Taken together, the findings of these studies suggest that interventions aimed at reducing weight may lead to a reversal of endothelial dysfunction in obese individuals, thus potentially reducing risk of developing diabetes and CVD.

Besides the biological deficit of NO, endothelial dysfunction is characterized by an enhanced bioactivity of vasoconstrictor and proatherogenic factors. In particular, endothelin 1 (ET-1), the most potent vasoconstrictor peptide synthesized by the endothelial cells, appears to play a central role in the pathophysiology of the vasomotor abnormalities associated with endothelial dysfunction and in the formation and progression of the atherosclerotic plaque (Levin, 1995). It is therefore conceivable that an increased activity of the ET-1 system may contribute to the abnormal vascular homeostasis of obese patients. The first evidence in support to this hypothesis was reported by Mather *et al.* (2004), who investigated the interactions of the ET-1 and NO system in obese or type 2 diabetic individuals. These authors observed that antagonism of ET-1 constrictor tone by use of BQ-123, a selective blocker of the endothelin receptor type A (ET_A), corrected the defect in endothelium-dependent vasodilation seen in these patients, thus suggesting an important contribution of ET-1 to their endothelial dysfunction (Mather *et al.*, 2004). In keeping with these findings, we have demonstrated an increased ET-1-dependent vasoconstrictor activity in the forearm circulation of overweight and obese individuals, but not in lean controls, with a linear relationship between the degree of vasodilation induced by ET-1 receptor blockade with BQ-123 and BMI (Cardillo *et al.*, 2004). Furthermore, we have observed that patients with central adiposity and the metabolic syndrome, in addition to enhanced intravascular ET-1 activity, also have impaired vasoconstrictor response to the inhibition of NO synthesis by N^G-monomethyl-L-arginine (L-NMMA), indicating a concomitant decrease of NO-dependent vasodilator capacity (Tesauro *et al.*, 2005). Taken together, these findings clearly suggest an imbalance between the NO pathway and the ET-1 system in the vessels of obese patients, with a shift of the normal prevalence of NO-mediated vasodilator tone towards an enhanced ET-1-mediated vasoconstriction.

Obesity, insulin and endothelium-dependent vasodilation

Several potential mechanisms may explain the development of endothelial dysfunction and of vascular abnormalities in obese patients. Given the impact of obesity on glucose metabolism and the effects of insulin on the vasculature, research has focused on the effects of insulin resistance on vascular homeostasis. Physiologically, insulin stimulates glucose uptake in insulin-sensitive tissues, such as the skeletal muscle and adipose tissue, by causing the translocation of the insulin-responsive glucose transporter (GLUT-4) to the cell surface, which, in turn, allows glucose entrance into the cell. This effect is the final result of the binding of insulin to its receptor on the cell membrane followed by activation of intracellular pathways that involves the insulin receptor substrate (IRS)-1, phosphoinositide 3 kinase (PI3K) and PKB (also known as Akt) (Saltiel and Kahn, 2001). It is now known that insulin receptors are present on endothelial cells, where they activate both the PI3K/Akt pathway and the rat sarcoma/MAPK (RAS/MAPK) cascade leading to NO and ET-1 synthesis respectively (Zeng *et al.*, 2000; Muniyappa *et al.*, 2008). In physiological conditions, the net effect of insulin stimulation on the endothelial cells results in NO-mediated vasodilation, which may contribute to the delivery of insulin and glucose to metabolically active tissues, thereby increasing insulin sensitivity (Baron and Clark, 1997). In contrast, in states associated with endothelial dysfunction, such as obesity, insulin-mediated vasodilation is impaired and may contribute to the pathophysiology of insulin resistance and of the metabolic syndrome (Laakso *et al.*, 1990; Kim *et al.*, 2006).

The first evidence of a possible haemodynamic effect of insulin was reported by Baron *et al.* (1995)., who demonstrated that in healthy individuals, the infusion of the hormone-induced NO-dependent vasodilation of resistance vessels in the leg circulation, which resulted in increased skeletal muscle blood flow and concurrent enhancement of insulin-stimulated glucose uptake (Baron *et al.*, 1995). Even though these findings have been replicated by some investigators (Tack *et al.*, 1996; de Haan *et al.*, 1997), others, includ-

ing our group, have failed to observe a direct vasodilator effect of the hormone (Cardillo *et al*., 1998). The reasons for these diverging findings are not entirely clear; however, the dose of insulin employed and the duration of the infusion may be important factors (Yki-Jarvinen and Utriainen, 1998). In particular, the majority of the studies that reported an increase in limb blood flow following insulin administration achieved supraphysiological concentrations of the hormone (Tack *et al*., 1996) or observed the effect only after several hours of infusion (Zhang *et al*., 2004). Therefore, the role of insulin-mediated vasodilation in the stimulation of glucose uptake in physiological conditions remains uncertain.

A more widely accepted physiological role of insulin is its ability to increase the available capillary surface area in the skeletal muscle circulation, thus augmenting the delivery of insulin itself, glucose and other nutrients to the myocytes (Clark *et al*., 2003). This notion supports a functional coupling between the microvascular effects of insulin, muscle perfusion and glucose utilization. Accordingly, a decrease in muscle perfusion may blunt the delivery of insulin and glucose, and lead to impaired glucose tolerance, whereas a reduced exposure of circulating triglyceride-containing lipoproteins to endothelial lipoprotein lipase may contribute to dyslipidaemia (Lind and Lithell, 1993), suggesting that microvascular dysfunction may precede and contribute to the development of insulin resistance and the metabolic syndrome.

Obesity, insulin and the endothelin system

In addition to stimulating NO production, insulin promotes the synthesis and release of ET-1 by endothelial cells. This *in vivo* effect was originally demonstrated by our group in the forearm circulation of healthy volunteers by using BQ-123 as pharmacological tools. In this experimental setting, the combined infusion of BQ-123 and BQ-788 or the administration of insulin alone in the brachial artery did not affect resting forearm blood flow values. However, the blockade of ET-1 receptors during insulin infusion was associated with a significant vasodilator response, consistent with the presence of an increased ET-1-dependent vasoconstrictor tone. Furthermore, during ET-1 receptor blockade, the vasoconstrictor response to NO synthesis inhibition with L-NMMA was significantly higher after insulin infusion than in the absence of hyperinsulinaemia, indicating an increment in NO bioavailability (Cardillo *et al*., 1999). Based on these findings, we postulated that, in the skeletal muscle circulation under physiological conditions, insulin stimulates the release of both NO and ET-1 from the endothelium, albeit with a neutral haemodynamic response to the hormone due to an equilibrium between these opposing vasoactive stimuli. This hypothesis is supported by the experimental work of Vicent *et al*. (2003) who reported reduced endothelial NO synthase (eNOS) and ET-1 mRNA levels in endothelial cells from a vascular endothelial cell insulin receptor knockout (VENIRKO) mouse model.

A pivotal biochemical abnormality observed in insulin-resistant individuals with obesity or type 2 diabetes is the specific impairment of the PI3K-dependent signalling cascade, while the MAPK pathway is spared (Cusi *et al*., 2000). The potential pathophysiological implications of this abnormality are relevant, since insulin resistance is usually accompanied by a compensatory hyperinsulinaemic state to maintain euglycaemia. Hyperinsulinaemia may overstimulate the normally functioning MAPK-dependent pathways, an effect that cannot be balanced by the abnormal PI3K cascade. At the vascular level, this would lead to an increased endothelial production of ET-1 and to a reduced synthesis of NO, with consequent endothelial dysfunction and increased vascular tone (Figure 1) (Kim *et al*., 2006). This hypothesis is supported by the findings from Mather *et al*. (2002), who demonstrated that insulin-resistant patients with obesity or type 2 diabetes have enhanced ET-1-mediated vascular tone and blunted response to the infusion of methacholine, and that ET-1 importantly contributes to the abnormal endothelium-dependent vasodilation present in these patients.

Additional mechanisms of impaired vascular reactivity in obesity

Adipokines

In animal experiments, hyperleptinaemia significantly impairs coronary endothelial function *in vivo* and *in vitro*, as assessed by response to intracoronary infusion of Ach (Yamauchi *et al*., 2002). Since human endothelial cells also express leptin receptors (Yamauchi *et al*., 2002) and hyperleptinaemia is an independent risk factor for coronary artery disease and a strong predictor of acute myocardial infarction (Soderberg *et al*., 1999; Ren, 2004), it is reasonable to speculate that high circulating leptin levels such as those observed in obese patients may induce endothelial dysfunction in the human vasculature. These effects may be mediated by uncoupling of eNOS with depletion of endothelial NO and increased production of peroxynitrite ($ONOO^-$), as suggested by experiments in obese mice (Korda *et al*., 2008). Ghrelin is a gastric peptide that plays a relevant role in the central regulation of food intake (Kojima *et al*., 1999; van der Lely *et al*., 2004). Recent evidence has indicated that ghrelin exerts favourable cardiovascular effects (Tesauro *et al*., 2010) and that its levels are decreased in obese patients (Tschop *et al*., 2001). In particular, studies conducted by our group have shown that, in patients with obesity-related metabolic syndrome, ghrelin improves endothelial function (Tesauro *et al*., 2005) by restoring the NO/ET-1 balance (Tesauro *et al*., 2009). Furthermore we have demonstrated that ghrelin acutely stimulates production of NO in endothelial cells through a signalling pathway that involves PI3K, Akt and eNOS (Iantorno *et al*., 2007).

In vitro studies have also shown that adiponectin may exert beneficial vascular effects by stimulating NO production by endothelial cells via PI3K-dependent pathways (Chen *et al*., 2003). Furthermore, adiponectin may decrease TNF-α-induced production of asymmetric dimethylarginine (ADMA) (Eid *et al*., 2007) and improve the redox state of the endothelium by suppressing reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived superoxide

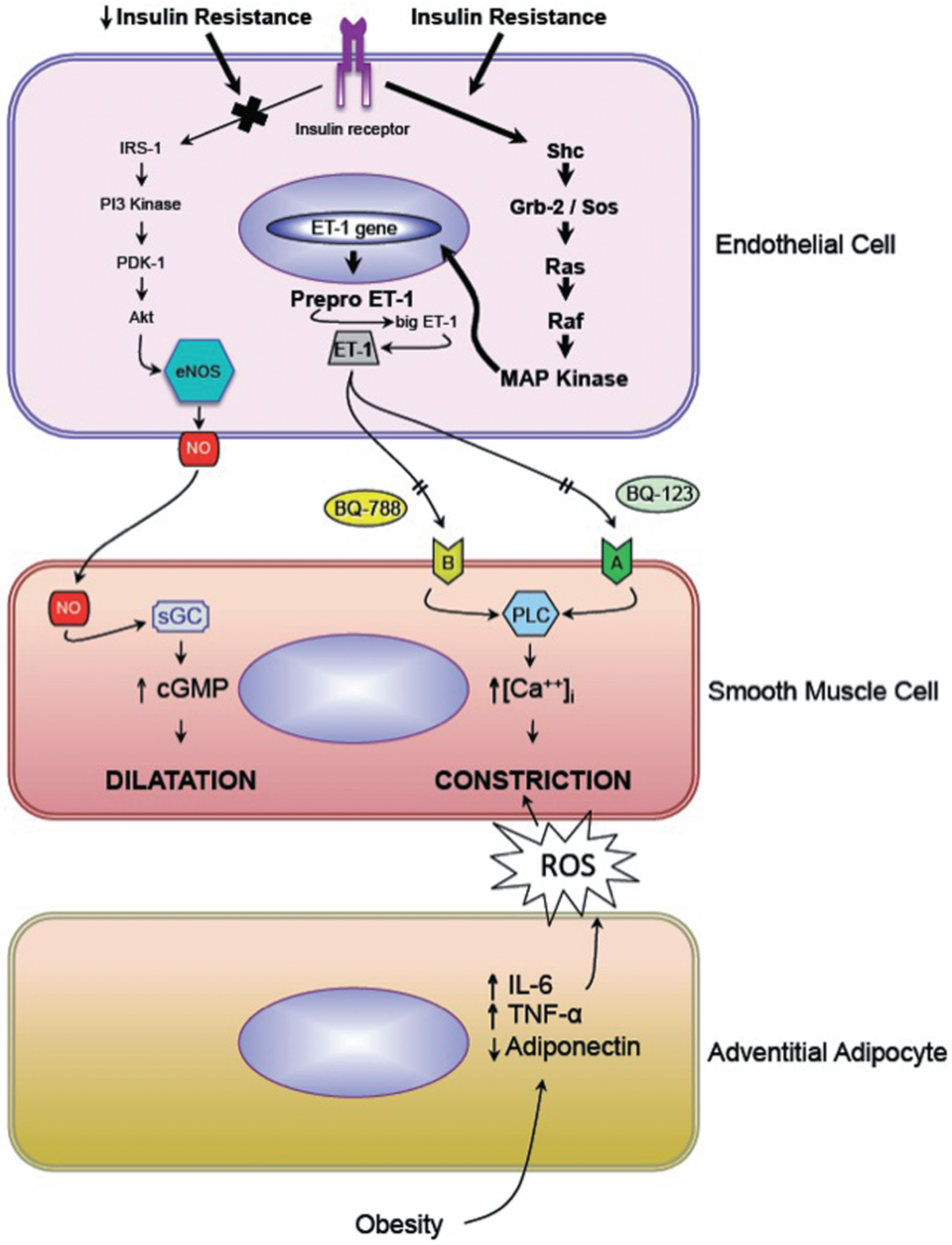

Figure 1

Interactions among endothelial, smooth muscle and adventitial cells in the physiological regulation of vasoactive function. In conditions of insulin resistance, the physiological balance between vasoconstrictor and vasodilator pathways in endothelial cells is shifted towards a predominant vasoconstriction, due to the prevalent activation of the endothelin system. In addition, the impairment of adipocyte function and production of inflammatory cytokines within the perivascular adipose tissue leads to increase ROS production and oxidative stress which, in turn, increases smooth muscle cell contractility.

generation (Motoshima *et al.*, 2004). Therefore, the low levels of adiponectin observed in obese individuals (Weyer *et al.*, 2001) may lead to abnormal endothelial function.

Oxidative stress

Endothelial dysfunction in obesity is a multifactorial condition in which reactive oxygen species (ROS) play a key pathogenetic role. (Siekmeier *et al.*, 2008; Otani, 2010) In particular, ROS generated in the vascular wall have been shown to represent a major determinant of NO synthesis and bioactivity (Siekmeier *et al.*, 2008). A number of intracellular enzymatic activities such as xanthine oxidase (XO), nicotinamide adenine dinucleotide (NADH)/NADPH oxidase, peroxidases, cytochromes P450 system, the mitochondrial respiratory chain, lipoxygenase and COX may generate ROS. However, XO, NADH/NADPH oxidase, and uncoupling of eNOS represent the main sources of these highly toxic compounds in the vasculature (Cai and Harrison, 2000). Endothelial cell generation of ROS is triggered by high circulating levels of pro-inflammatory cytokines generated in the visceral adipose tissue through the action of the local renin–angiotensin system (Dandona *et al.*, 2007) and by low adiponectin levels. The excessive oxidative stress decreases NO bioavailability through multiple mechanisms including generation of $ONOO^-$ anions from a reaction of NO with superoxide; reduction in NO synthesis secondary to uncoupling of eNOS through ROS-induced oxidation and depletion of the eNOS cofactor, tetrahydrobiopterin (BH4) (Bendall *et al.*, 2005); enhanced consumption by high levels of superoxide; inhibition of the enzyme dimethylarginine dimethylaminohydrolase (DDAH), with consequent increase in circulating levels of ADMA, a competitive inhibitor of NO synthesis. (Jia *et al.*, 2006)

FFAs

An additional metabolic feature of obesity is the presence of elevated circulating levels of FFA. A role of these lipids in the pathogenesis of endothelial dysfunction has been suggested by the evidence that the infusion of a solution of FFA in healthy humans impairs endothelium-dependent vasodilation (Steinberg *et al.*, 1997; Watanabe *et al.*, 2005), and pharmacological lowering of FFA with acipimox, a nicotinic acid analogue, leads to an improvement of microvascular function in obese women (de Jongh *et al.*, 2004). Several mechanisms may account for the detrimental effects of circulating FFA on vascular function, including, but not limited to, enhanced oxidative stress due to activation of the renin–angiotensin system (Watanabe *et al.*, 2005) and ET-1 release (Piatti *et al.*, 1996).

TNF-α

Another potential contributor to the metabolic and vascular abnormalities observed in obesity is TNF-α. The circulating levels of this inflammatory cytokine are elevated in obese individuals and are associated with endothelial activation and a blunted response to the infusion of the NO precursor L-arginine (Ziccardi *et al.*, 2002). In keeping with these observations, Rask-Madsen *et al.* (2003) and colleagues have shown that infusion of TNF-α in healthy humans inhibits the stimulatory effects of insulin on glucose uptake and reduces endothelium-dependent vasodilation. A number of studies have investigated the potential mechanisms underlying the detrimental effects of TNF-α on insulin metabolism and vascular function. Incubation of human vascular endothelial cells with TNF-α produces a marked down-regulation of eNOS expression (Yoshizumi *et al.*, 1993; Mohamed *et al.*, 1995) and induces the synthesis of ET-1 and plasminogen activator inhibitor 1 (Mohamed *et al.*, 1995). Furthermore, TNF-α may activate NADH oxidase in vascular smooth muscle cells, thus increasing ROS generation and reducing NO bioavailability (De Keulenaer *et al.*, 1998). In addition to these direct effects, TNF-α may also induce vascular dysfunction indirectly by stimulating lipolysis and FFA release (Pittas *et al.*, 2004). In our laboratory, we investigated the role of TNF-α in the vascular dysfunction associated with the metabolic syndrome by using the TNF-α neutralizing antibody infliximab. We observed that TNF-α blockade enhances the stimulatory effects of insulin not only on endothelium-dependent, but also on endothelium-independent vasodilator reactivity. The effect of infliximab on vascular function was not additive to that observed following the administration of the antioxidant vitamin C, suggesting that TNF-α-related vasculopathy in these patients is likely related to increased oxidative stress (Tesauro *et al.*, 2008). To further characterize the mechanism of abnormal insulin-stimulated vascular reactivity in patients with central obesity, we compared the effects of insulin on vascular responses to vasodilators acting through different mechanisms in healthy individuals and in patients with metabolic syndrome (Schinzari *et al.*, 2010). In the former group, hyperinsulinaemia not only enhanced NO-dependent vasodilation in response to ACh infusion, but also increased the effects of the endothelium-independent vasodilators sodium nitroprusside, an exogenous NO donor, and verapamil, a calcium channel blocker that exerts NO-independent vasodilator actions. In contrast, no enhancement of vasodilator reactivity to any of these agents was observed during hyperinsulinaemia in patients with the metabolic syndrome. Taken together, these findings indicate that the abnormalities of insulin signalling in resistance vessels of patients with central obesity are not limited to the vascular endothelium but involve smooth muscle cell function, suggesting a novel mechanism of insulin action on blood vessels and a potential additional link between obesity, insulin resistance and vascular dysfunction.

Obesity, vascular dysfunction and the perivascular adipose tissue (PVAT)

In addition to the presence of systemic factors linking obesity to impaired vasodilator function, a vasoregulatory role for local deposits of fat around arterial vessels has recently been postulated (Yudkin *et al.*, 2005). Following the initial evidence that PVAT significantly influences vascular responsiveness in rat aortas *in vitro* (Soltis and Cassis, 1991), Gao *et al.* (2007) demonstrated that PVAT exerts anticontractile effects by releasing a diffusible mediator that triggers endothelial NO release and subsequent calcium-dependent potassium channel activation, as well as through an endothelium-independent mechanism involving hydrogen peroxide

Table 1

Effects of weight reduction beneficial to vascular function

Endothelial function	Adhesion molecules	Glucose metabolism	Lipid metabolism	Adipokines	Oxidative stress	Inflammation	Coagulantion
↑ FMD[a,b,c] ↑ ACh Response[b]	↓ ICAM-1[d] ↓ P-Selectin[d] ↓ E-Selectin[d]	↓ FBG[a] ↓ Insulin[c]	↓ Cholesterol[a] ↓ LDL[a] ↓ Triglycerides [a,c]	↓ Leptin[b,c]	↓ LDL_{ox}[b]	↓ CRP[a,b,c] ↓ TNF-R1[c]	↓ vWF[c] ↓ PAI[c]

[a]Bigornia *et al.*, 2010.
[b]Pierce *et al.*, 2008.
[c]Mavri *et al.*, 2011.
[d]Ferri *et al.*, 1999.
CRP, C-reactive protein; FBG, fasting blood glucose; FMD, flow-mediated dilation; ICAM, intercellular adhesion molecule; LDL, low-density lipoprotein; LDL_{ox}, oxidized LDL; PAI, plasminogen activator inhibitor; TNF-R1, TNF receptor type 1; Vwf, von Willebrand factor.

(H_2O_2) and activation of soluble guanylyl cyclase. In humans, the vasoactive effects of PVAT were initially reported in medium-sized arteries (Gao *et al.*, 2005) and have more recently been confirmed in small arteries (Greenstein *et al.*, 2009). In particular, Greenstein *et al.* (2009) conducted elegant experiments on human arteries 250–350 μm in diameter, with and without PVAT, taken from s.c. gluteal fat biopsies from healthy individuals and from obese patients with the metabolic syndrome. These authors demonstrated that, in physiological conditions, PVAT around small arteries secretes one or more factors that promote vasodilation by increasing NO bioavailability. Among them, adiponectin appeared to be the main candidate, since the anticontractile responses were completely abolished when arteries with PVAT from healthy individuals were incubated with an adiponectin type 1 receptor-blocking fragment (Greenstein *et al.*, 2009). The dilator effect of PVAT was lost in the obese patients with metabolic syndrome, and was accompanied by an increase in adipocyte area and by immunohistochemical evidence of higher expression of TNF-α receptor 1, a marker of inflammation. Of note, the 'obese' phenotype could be reproduced by adding TNF-α or IL-6 to the PVAT around healthy blood vessels, which, in turn, could be blocked by free radical scavengers or cytokine antagonists. Finally, low oxygen or hypoxia stimulated an inflammatory phenotype in PVAT with loss of the anticontractile properties (Greenstein *et al.*, 2009). Since the partial pressure of oxygen is reduced in the adipose tissue of overweight/obese subjects and is associated with lower capillary density and tissue inflammation (Pasarica *et al.*, 2009), these findings further support a prominent role for PVAT-derived ROS and inflammatory cytokines in adversely modulating vascular function. A possible role of epicardial adipose tissue, which directly surrounds the coronary arteries, in the development of coronary atherosclerosis has also been proposed, and a significant relationship between epicardial fat volume and the presence of coronary atherosclerosis measured by multislice computed tomography has been reported (Djaberi *et al.*, 2008; Wang *et al.*, 2009a). Taken together, the findings of these investigations suggest that PVAT may play an important role in the maintenance of normal vascular function and that in obesity and other insulin-resistant states, an abnormal PVAT function may contribute to the pathogenesis of atherosclerosis.

Therapeutic targets of endothelial dysfunction in obesity

Since endothelial dysfunction represents a dynamic response of the endothelium that is potentially reversible, a number of clinical trials have investigated the effect of weight loss on various markers of endothelial function. Despite significant differences in the therapeutic approach employed (diet, surgery, medications and exercise, alone or in various combinations) the majority of these investigations have reported similar beneficial effects on vascular function (e.g. improved NO bioavailability, amelioration of lipid and glucose metabolism, modulation of adipokine release, reduced inflammation and decreased oxidative stress) with these interventions (Table 1).

Exercise and diet

Several studies have examined the effects of diet and/or exercise on obesity-induced endothelial dysfunction both in children (Woo *et al.*, 2004a) and in adults (Ziccardi *et al.*, 2002; Pierce *et al.*, 2008; Bigornia *et al.*, 2010; Mavri *et al.*, 2011), showing consistent improvement in endothelium-dependent dilation. While weight loss through diet and/or exercise would be the most appropriate therapy to reverse the vascular abnormalities present in obese individuals, the long-term efficacy of these interventions is limited (Ayyad and Andersen, 2000). Therefore, several classes of drugs have been tested in patients with obesity with or without the metabolic syndrome. Among the currently approved antiobesity agents, orlistat, a saturated derivative of the natural inhibitor of pancreatic lipases lipstatin, improved FMD in 42 young obese women treated for 12 weeks (Sekuri *et al.*, 2003). However, these findings were not reproduced in another group of patients with uncomplicated obesity receiving orlistat in addition to a calorie-restricted diet (Brook *et al.*, 2004), leaving uncertainty regarding the effects of this medication on vascular function. The most common side effects of orlistat include headache, abdominal pain/discomfort, fecal urgency and fatty/oily stool.

Oral hypoglycemics

Metformin, the most widely prescribed oral agent for the treatment of diabetes, is a biguanide that suppresses endog-

enous glucose production, thus leading to decreased insulin levels (Hundal and Inzucchi, 2003). This agent may exert beneficial effects on cardiovascular risk factors and is associated with improved macrovascular outcomes in overweight patients with type 2 diabetes (UKPDS Group, 1998).

The vascular effects of metformin have not been completely clarified; however, they appear to be mediated by the activation of AMP-activated PK (AMPK), a highly conserved, multisubstrate serine/threonine PK involved in the regulation of cellular and organ metabolism. In vascular endothelial cells, activated AMPK increases phosphorylation of eNOS at Ser^{1179} and promotes eNOS association with heat shock protein 90 (Hsp90), resulting in increased NO synthesis and bioavailability (Davis *et al*., 2006). To test the hypothesis that this drug may improve endothelial function *in vivo*, Vitale *et al*. (2005) randomized 65 obese patients with metabolic syndrome to treatment with either metformin 500 mg twice daily or placebo for 3 months. The authors report that FMD of the brachial artery was significantly improved in the active treatment group, whereas it remained unchanged in patients who received placebo (Vitale *et al*., 2005). In line with these results, de Aguiar *et al*. (2006) showed that metformin also improves microvascular endothelium-dependent vasodilation, measured as the response to intra-arterial infusion of ACh, in obese first-degree relatives of type 2 diabetic patients with metabolic syndrome and normal glucose tolerance. Thus, these results strongly suggest that metformin exerts beneficial vascular effects also in non-diabetic obese patients with metabolic syndrome. However, the frequent occurrence of gastrointestinal side effects, such as diarrhoea and nausea/vomiting reduces the tolerability of this treatment. Another class of oral hypoglycaemic drugs, the insulin sensitizers thiazolidinediones (TZD) (Yki-Jarvinen, 2004), have shown to possess important beneficial vascular effects. Their main mechanism of action is mediated by binding to the peroxisome proliferator-activated receptor-γ (PPARγ), which affects transcription of several genes involved in glucose and lipid metabolism (Schoonjans and Auwerx, 2000). However, recent evidence suggests that TZD may exert acute, gene transcription-independent effects via AMPK-dependent phosphorylation of eNOS at Ser^{1177} (Morrow *et al*., 2003) leading to NO-dependent vasodilation of conduit (Wang *et al*., 2009b), as well as resistance vessels (Bradley *et al*., 2010). Consistent with the experimental evidence, rosiglitazone has been shown to exert beneficial effects on endothelial function in patients with type 2 diabetes independent of glucose lowering (Pistrosch *et al*., 2004). Our group expanded this observation by showing that 8 week treatment with pioglitazone improves the vasodilator response to infusion of bradykinin in obese hypertensive and hypercholesterolaemic patients (Campia *et al*., 2006), suggesting that the improved vascular function secondary to PPARγ stimulation is independent of body fat mass. Thiazolidinediones are overall well tolerated; however, they may cause oedema and may precipitate hospitalization in patients with heart failure. Furthermore, concerns about an increased cardiovascular risk of rosiglitazone have been raised. In particular, even if an open-label, non-inferiority trial was inconclusive about any possible effect of rosiglitazone on myocardial infarction [hazard ratio 1.14; 95% confidence interval (CI) 0.80–1.63 for myocardial infarction] (Home *et al*., 2009), the results of recent meta-analyses indicate that rosiglitazone use is associated with an increased risk for myocardial infarction (odds ratio 1.28; 95% CI, 1.01–1.62; $P = 0.04$), although not for cardiovascular mortality (odds ratio 0.99; 95% CI, 0.75–1.32; $P = 0.96$). (Nissen and Wolski, 2007; 2010)

Angiotensin receptor antagonists and antioxidants

Since endothelial dysfunction in patients with the metabolic syndrome may be due to increased angiotensin (AT)-II activity and enhanced oxidative stress, Sola *et al*. (2005) investigated whether irbesartan, an AT-II receptor blocker, and lipoic acid, an antioxidant, affect endothelial function and inflammation in patients with metabolic syndrome. These investigators reported that short-term treatment with irbesartan, lipoic acid or both increased FMD compared with placebo, suggesting that these agents may be potentially beneficial in improving cardiovascular function in this population.

Resveratrol

Resveratrol is a natural phenolic phytochemical found in the skin of red grapes, red wine, apples, peanuts, blueberries and cranberries (Baur and Sinclair, 2006). The potential beneficial cardiovascular effects of resveratrol have stemmed from epidemiological studies indicating that red wine consumption is inversely related to mortality due to CVDs (Gronbaek, 2002). Its biological effects are mediated, at least in part, through the activation of sirtuin-1 (SIRT1), a NAD-dependent class III histone deacetylase of the sirtuin family (Brooks and Gu, 2009). Several lines of research indicate that SIRT1 plays a pivotal role in endothelial homeostasis by increasing eNOS gene expression while reducing baseline and H_2O_2-stimulated expression of ET-1 gene (Mattagajasingh *et al*., 2007), as well as by regulating pot-translational eNOS acetylation, leading to increased NO synthesis (Mattagajasingh *et al*., 2007). Consistent with this experimental evidence and similar to the effects seen with calories restriction, oral resveratrol supplementation improved FMD in overweight/obese individuals with mildly elevated BP (Wong *et al*., 2011).

Conclusions

Obesity is an ongoing worldwide epidemic that affects both adults and children. Besides being a medical condition in itself, obesity dramatically increases the risk of development of metabolic and CVD. This risk appears to stem from multiple abnormalities in adipose tissue function, leading to a chronic inflammatory state and to dysregulation of the endocrine and paracrine actions of adipocyte-derived factors. These, in turn, disrupt vascular homeostasis by causing an imbalance between the NO pathway and the ET-1 system, with impaired insulin-stimulated endothelium-dependent vasodilation. Importantly, emerging evidence suggests that the vascular dysfunction of obesity is not just limited to the endothelium, but also involves the other layers of the vessel wall (Figure 1). In particular, obesity-related changes in medial smooth muscle cells seem to disrupt the physiological facilitatory action of insulin on the responsiveness to vasodilator stimuli, whereas the adventitia and the perivascular fat

appear to be a source of pro-inflammatory and vasoactive factors that may contribute to endothelial and smooth muscle cell dysfunction and to the pathogenesis of vascular disease. While obesity-induced vascular dysfunction appears to be reversible, at least in part, with weight control strategies, these have not proved sufficient to prevent the metabolic and cardiovascular complication of obesity on a large scale. While a number of currently available drugs have shown potentially beneficial vascular effects in patients with obesity and the metabolic syndrome, elucidation of the pathophysiological mechanisms underlying vascular damage in obese patients is all the more necessary to identify additional pharmacologic targets to prevent the cardiovascular complications of obesity and their human and economic costs.

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Conflicts of interest

The authors declare no conflict of interest.

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