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REVIEW

Microvascular responsiveness in obesity: implications for therapeutic intervention

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Obesity has detrimental effects on the microcirculation. Functional changes in microvascular responsiveness may increase the risk of developing cardiovascular complications in obese patients. Emerging evidence indicates that selective therapeutic targeting of the microvessels may prevent life-threatening obesity-related vascular complications, such as ischaemic heart disease, heart failure and hypertension. It is also plausible that alterations in adipose tissue microcirculation contribute to the development of obesity. Therefore, targeting adipose tissue arterioles could represent a novel approach to reducing obesity. This review aims to examine recent studies that have been focused on vasomotor dysfunction of resistance arteries in obese humans and animal models of obesity. Particularly, findings in coronary resistance arteries are contrasted to those obtained in other vascular beds. We provide examples of therapeutic attempts, such as use of statins, ACE inhibitors and insulin sensitizers to prevent obesity-related microvascular complications. We further identify some of the important challenges and opportunities going forward.

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
BMI, body mass index; SNP, sodium nitroprusside; ROS, reactive oxygen species; RNS, reactive nitrogen species; MCP-1, monocyte chemotactic protein-1; EDHF, endothelium-derived hyperpolarizing factor; K_{Ca}, Ca^{2+} activated potassium channel; sGC, soluble guanylate cyclase, HIF-1α, hypoxia inducible factor 1α; TZD, thiazolidinedione; BH_4, tetrahydrobiopterin; ARB, angiotensin receptor blocker; NEP, neutral endopeptidase; RAS, renin-angiotensin system

Introduction

Morphological changes in microvessels are quite rare in obesity prior to the development of hyperglycaemia and type 2 diabetes mellitus. Obesity-related pathological alterations, including atherogenic dyslipidaemia, insulin resistance and hyperinsulinaemia, impair the vasomotor function of arteries. It has been the view that blood flow to various organs is rarely impaired in obesity, unless occlusive atherosclerosis of the larger arteries develops. Throughout life, organs receive normal or even greater than normal blood flow in uncomplicated obesity (Hall *et al.*, 1999). Recent studies using non-invasive imaging techniques revealed that myocardial perfusion and skeletal muscle blood flow are compromised in obese subjects especially when vessels are pharmacologically or metabolically challenged. Such abnormalities are primarily due to the reduced vasodilator capacity of microvessels, which in some instances represents important markers of cardiovascular risk or may even contribute to the pathogenesis of obesity.

Although the importance of microvascular vasomotor dysfunction in contributing to the morbidity and mortality of obese patients was appreciated many years ago, no effective therapeutic strategies are currently available to prevent

abnormalities of resistance arteries. Recent studies raise the prospect that therapeutically targeting the microcirculation may not only prevent vascular complications but via interfering with adipose tissue vascularization may reduce obesity. This review aims to examine studies that focus on alterations in vasodilator function of resistance arteries in obesity. A description is also provided about the underlying cellular mechanisms in endothelial, vascular smooth muscle cells and adipocytes that are believed to be responsible for altered microvascular responsiveness. Moreover, recent interventional studies of obesity are summarized, in which the primary focus was to reverse microvascular dysfunction by using various pharmacological treatments.

Obesity and altered microvascular responsiveness – the nature of underlying mechanisms

In humans, obesity is associated with reduced hyperaemia-induced forearm blood flow (Hashimoto *et al.*, 1998; Vigili de Kreutzenberg *et al.*, 2003). Obese children already exhibit impaired brachial artery relaxation to hyperaemic flow (Kapiotis *et al.*, 2006). In their study Karpoff *et al.* have found that mild-to-moderate obesity [body mass index (BMI) 23.9 ± 2.6] in prepubertal boys without insulin resistance is associated with blunted flow-mediated dilation in the brachial artery (Karpoff *et al.*, 2009). In normal-weight adults, an average weight gain of 4.1 kg impaired flow-mediated dilation, which was restored to baseline when subjects shed the gained weight (Romero-Corral *et al.*, 2010). These observations provide evidence that obesity, without any co-existing cardiovascular diseases, is associated with impaired vasomotor function of conduit arteries (Table 1).

Forearm resistance arteries also possess a reduced endothelium-dependent, ACh- and endothelium-independent, sodium nitroprusside (SNP)-induced dilations in obese individuals (Sivitz *et al.*, 2007), suggesting microvascular involvement. An early study demonstrated that peripheral vascular resistance inversely correlate to BMI, whereas elevated waist/hip ratio is associated with increased systemic vascular resistance (Jern *et al.*, 1992). This example implies that visceral obesity is associated with increased total peripheral vascular resistance. It has been proposed that an increase in body fat mass and its visceral localization are responsible for the impaired vasodilation of resistance vessels (Hashimoto *et al.*, 1998) and consequently elevated peripheral vascular resistance in obesity (Jern *et al.*, 1992). This scenario was also supported by a theoretical analysis using physiological measurements obtained in obese patients (Ferrannini, 1992). Thus, obesity impairs the vasomotor function of microvessels that determine peripheral resistance, but the exact mechanisms are largely unknown in obese patients.

Diminished vasodilator function of resistance arteries develops in animals with experimental obesity, making it feasible to study the underlying pathology, which usually is very difficult to perform in humans. In evaluating results obtained from animal models of obesity, it is important to bear in mind that similar to humans, experimental obesity is associated with co-morbid conditions, such as elevated systemic blood pressure. Also, it is important to note that even in the absence of fasting hyperglycaemia, animals with experimental insulin resistance develop elevated postprandial glucose levels. Thus, the pathological role of high blood pressure and transient hyperglycaemia should be taken into account when evaluating the impact of obesity in these animal models. Moreover, in commonly used animal models, obesity develops on the basis of mutations in the leptin gene or the leptin receptor, genetic constellations that are relatively rare in humans with obesity.

Resistance arteries from the mesentery (Oltman *et al.*, 2006) and skeletal muscle vascular beds (Frisbee and Stepp, 2001) of obese Zucker rats exhibit impaired endothelium-dependent vasodilation, similar to microvascular dysfunction in obese patients. It has been found that in mesenteric arterioles endothelium-dependent relaxation to ACh is preserved at 20 weeks of age, but is reduced in older (32 week) obese Zucker rats, suggesting age-dependent progression of vasomotor dysfunction (Subramanian and MacLeod, 2003). In obese JCR:LA-cp rats, impaired endothelium-dependent dilations of mesenteric arteries to ACh has been reported (O'Brien *et al.*, 1998). Reduced mesenteric (Naderali *et al.*, 2001a) and skeletal muscle (Erdei *et al.*, 2006) arteriolar dilation to ACh was also found in rats fed a high-fat diet. These studies demonstrated microvascular dysfunction in experimental models of obesity and concluded that the reduced dilation of resistance arteries is primarily due to impaired function of microvascular endothelium (Table 1).

The vascular endothelium produces and secretes numerous compounds that regulate a variety of physiological functions, including vasomotor tone, coagulation, inflammation, permeability and cell adhesion (Vane *et al.*, 1990). Among others, NO is considered to be one of the key molecules in maintaining normal vascular homeostasis and is a major contributor to maintaining adequate dilator function of arteries (Loscalzo and Welch, 1995). Experimental evidence indicates that obesity is associated with reduced bioavailability of vascular NO (Frisbee and Stepp, 2001; Naderali *et al.*, 2001b; Erdos *et al.*, 2002). Oxidative stress occurring in response to hyperglycaemia (Ruderman *et al.*, 1992; Bohlen and Lash, 1993; Bagi and Koller, 2003; Bagi *et al.*, 2004b) and hypertension (Jaap *et al.*, 1994; Ungvari *et al.*, 2003) is considered to be one of the key factors leading to the reduced NO availability. Evidence also supports that insulin resistance (Erdos *et al.*, 2002) and obesity (Erdos *et al.*, 2004; Erdei *et al.*, 2006) are associated with an increased vascular production of reactive oxygen species (ROS). In this context, increased production of vascular superoxide anion has been shown to lead to inactivation of endothelial NO, resulting in a reduced agonist-induced dilation of mesenteric and skeletal muscle microvessels of obese mice and rats (Bohlen and Lash, 1993; Frisbee and Stepp, 2001; Bagi and Koller, 2003). Oltman *et al.* have found that a free radical scavenger, tiron, restored the diminished dilations of coronary arterioles in obese Zucker rats (Oltman *et al.*, 2006). Rats fed a high-fat diet also exhibited enhanced vascular production of superoxide anion, which was associated with reduced ACh- and histamine-induced, NO-mediated arteriolar dilations of skeletal muscle arterioles; responses were restored by the ROS scavenger, tiron (Erdei *et al.*, 2006).

Table 1

Clinical (A) and animal studies (B) investigating the impact of obesity on vasomotor responses

(A) Obese humans Reference	Vascular bed	Response	Result
Kapiotis *et al.*, 2006	Brachial artery	FMD	↓
Hashimoto *et al.*, 1998	Brachial artery	FMD	↓
Vigili de Kreutzenberg *et al.*, 2003	Brachial artery	FMD	↓
	Forearm res. artery	BK	↓
Karpoff *et al.*, 2009	Brachial artery	FMD	↓
Romero-Corral *et al.*, 2010	Brachial artery	FMD	↓
Sivitz *et al.*, 2007	Forearm res. artery	ACh	↓
Martin *et al.*, 2005	Myocardium (PET)	MBF (rest)	↓
		MBF (CPT)	↓
Peterson *et al.*, 2008	Myocardium (PET)	MBF (rest)	↑
Motivala *et al.*, 2008	Myocardium (PET)	MBF (rest)	↑
		MBF (MFR)	↓
Schindler *et al.*, 2006	Myocardium (PET)	MBF (rest)	↔
		MBF (MFR/CPT)	↓
Wang *et al.*, 2006	Myocardium (MRI)	MBF (rest)	↔
		MBF (ADO)	↔
Fulop *et al.*, 2007	Isolated coronary arteriole	BK	↓
(B) Experimental Obesity Reference	**Model, vascular bed**	**Response**	**Result**
Subramanian and MacLeod, 2003	OZR, mesenteric (~20 weeks)	ACh	↔
	OZR, mesenteric (~30 weeks)	ACh	↓
Oltman *et al.*, 2006	OZR, mesenteric (~20 weeks)	ACh	↓
	OZR, mesenteric (~30 weeks)	ACh	↓
Young *et al.*, 2008	OZR, mesenteric (~12 weeks)	ACh	↓
Ellis *et al.*, 2008	HFD, mesenteric (mouse)	ACh	↔
Naderali *et al.*, 2001a	HFD, mesenteric (rat)	ACh	↓
O'Brien *et al.*, 1998	JCR:LA-cp rat, mesenteric	ACh	↓
Frisbee and Stepp, 2001	OZR, cremaster muscle	ACh	↓
Erdei *et al.*, 2006	HFD, skeletal muscle (rat)	ACh	↓
Erdos *et al.*, 2004	OZR, cerebral (~12 weeks)	ACh	↓
Oltman *et al.*, 2006	OZR, coronary (~20 weeks)	ACh	↔
	OZR, coronary (~30 weeks)	ACh	↓
Katakam *et al.*, 2005	OZR, coronary (~12 weeks)	ACh	↔
Prakash *et al.*, 2006	OZR, coronary (~12 weeks)	ACh	↑
Jebelovszki *et al.*, 2008	HFD, coronary (rat)	ACh	↔
Woodman *et al.*, 2004	HFD, coronary (pig)	BK	↓
Henderson *et al.*, 2004	HFD, coronary (pig)	BK	↔
Setty *et al.*, 2003	HFFD, coronary blood flow	CBF (rest)	↔[a]
Borbouse *et al.*, 2010	HFFD, coronary blood flow	CBF (exercise)	↔[a]

[a]Although no change in CBF was detected the authors found an impaired relationship between CBF and myocardial oxygen consumption, MVO(2). BK, bradykinin, ADO, adenosine; CBF, coronary blood flow; CPT, cold pressor test; FMD, flow mediated dilation; HFD, high-fat diet; HFFD, high-fat, high-fructose diet; MBF, myocardial blood flow; MFR, myocardial flow reserve; OZR, obese Zucker rat.

Excess production of vascular superoxide anion is derived from different ROS-producing systems in the vasculature, including NAD(P)H oxidase, xanthine oxidase, uncoupled NO synthase and mithochondrial complexes (Wolin, 2000). The primary source of microvascular ROS has not yet been identified in obesity. The xanthine oxidase inhibitor, allopurinol partially restored ACh- and histamine-induced dilations in skeletal muscle arterioles of obese but not in lean rats, whereas the NAD(P)H oxidase inhibitor, apocynin had no significant effects on these responses (Erdei *et al.*, 2006). Allopurinol, but not apocynin, also reduced lucigenin enhanced chemiluminescense-detected superoxide anion production in carotid arteries of obese rats (Erdei *et al.*, 2006). Other studies also found an enhanced vascular xanthine oxidase activity in rabbit model of hypercholesterolaemia (Ohara *et al.*, 1993) and demonstrated a crucial role for the circulating form of xanthine oxidase, which contributes to enhanced production of vascular ROS in these animals (White *et al.*, 1996). In humans with hypercholesterolaemia, the xanthine oxidase inhibitor oxypurinol improved ACh-induced dilations of the brachial artery (Cardillo *et al.*, 1997).

Other studies indicated that vascular NAD(P)H oxidase is the major source of superoxide anion production in arteries of obese animals. Both obese, ob/ob mice and obese Zucker rats exhibited increased NAD(P)H oxidase-derived ROS production as assessed by *in vivo* electron spin resonance (Sonta *et al.*, 2004). Obese Zucker rats demonstrate a reduced insulin-induced vasodilation in small coronary arteries, as a result of increased production of ROS by vascular NAD(P)H oxidase (Katakam *et al.*, 2005). Increased superoxide anion generation by NAD(P)H oxidase was associated with enhanced expression of NAD(P)H subunits p22 and p40-phox in coronary vessels of the obese Zucker rat, in which apocynin restored endothelium-dependent dilation (Picchi *et al.*, 2006).

Based on the previous studies, it seems that obesity could lead to activation of vascular xanthine oxidase and NAD(P)H-oxidase, although their relative contribution to enhanced ROS production has yet to be clarified. It is possible that the source of ROS varies in different vascular beds and in various animal models of obesity. More intriguingly, NAD(P)H oxidase-derived superoxide anion could lead to redox-dependent, irreversible activation of xanthine oxidase (Berry and Hare, 2004). ROS, derived either from NAD(P)H or xanthine oxidase, may reduce the level of NO synthase cofactor, BH_4, which in turn leads to NO synthase uncoupling and consequent generation of superoxide anion by NO synthase (Alp and Channon, 2004). This suggests existence of multiple enzymes causing enhanced microvascular production of ROS in obesity. Potential interactions between ROS producing enzymes (also schematically depicted on Figure 1) may exaggerate vascular oxidative stress as obesity progresses. Clearly, further studies are needed to identify the early events that may initiate microvascular ROS production, which in turn leads to inactivation of endothelium-derived NO in resistance arteries in obesity.

In addition to NO, other important mechanisms contribute to dilations of resistance arteries, such as those mediated by endothelium-derived hyperpolarizing factor (EDHF). In comparison with NO, EDHF-mediated arteriolar dilation is believed to be less sensitive to oxidative stress. In this

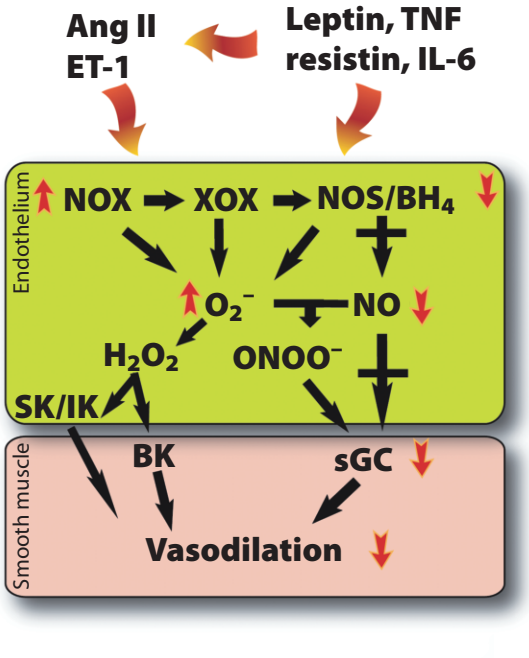

Figure 1

Endothelial and smooth muscle-dependent signalling mechanisms of resistance arteries in obesity. In obesity, pathological alterations of adipocytes develop, which could lead to dysfunction of microvascular endothelium. Adipokines, such as leptin, resistin, TNFα and IL-6 has been directly or indirectly – via increasing endothelin-1 (ET-1) and angiotensin II (Ang II) production – implicated in inducing enhanced reactive oxygen production. Enhanced production of vascular superoxide anion (O_2^{-}.) is due to the activation of various oxidases in the endothelial cells, such as NADP(H)-oxidase (NOX) and xanthine-oxidase (XOX). O_2^{-}. interacts with NO to form peroxynitrate (ONOO-) and to reduce NO availability. O_2^{-}. also reduces the level of NO synthase cofactor, tetrahydrobiopterine (BH_4) and an uncoupled NO synthase further generates O_2^{-}. On the other hand, O_2^{-}. can be converted to H_2O_2 by superoxide dismutase, and it may activate various calcium-activated potassium channels, such as SK, IK and BK channels or the sGC, to maintain dilator function of arterioles, particularly in coronary microvessels.

context, dilations mediated by EDHF can persist and may even compensate for the loss of NO-mediated vasodilation in obesity. In support of this scenario, a study has found that ApoE and LDL receptor-deficient mice fed a high-cholesterol diet exhibit a preserved EDHF-mediated dilation to ACh in cremaster muscle arterioles (Wolfle and de Wit, 2005). Ellis *et al.* described an augmented, EDHF-mediated dilation of small mesenteric arteries both in wild-type and LDL receptor knockout mice fed with a high-fat diet (Ellis *et al.*, 2008). A maintained ACh-induced, EDHF mediated dilations have been reported in coronary arterioles of high-fat diet-fed, obese rats (Feher *et al.*, 2010). It is known that activation of Ca^{2+}-activated small (SK_{Ca}) and intermediate (IK_{Ca}) conductance K^+ channels play a crucial role in EDHF-mediated vasodilation (Burnham *et al.*, 2002; Bychkov *et al.*, 2002; Dora *et al.*, 2008). The large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) have been also implicated in EDHF response in porcine coronary vessels (Edwards *et al.*, 2000), although

their contribution to EDHF-mediated dilation seems dependent on the applied pharmacological stimulus (Edwards *et al.*, 2000), and does not occur in some types of arteries, such as mesenteric (Dong *et al.*, 2000) or hepatic vessels (Edwards *et al.*, 1999). In the study by Ellis *et al.*, the augmented, EDHF-mediated dilations to ACh were effectively blocked by K_{Ca} channel inhibitors, apamin and charybdotoxin in mesenteric arteries of obese mice (Ellis *et al.*, 2008). Interestingly, in coronary arterioles of obese, but not in lean rats, the BK_{Ca} channel inhibitor, iberiotoxin exerted a marked inhibitory effect on EDHF-mediated dilations (Feher *et al.*, 2010). No change in K_{Ca} channel function has found in small mesenteric arteries of obese Zucker rats, whereas the magnitude of EDHF-mediated dilation was reduced, due to impaired connexin-dependent cell-to-cell signalling (Young *et al.*, 2008). Thus, the function of SK_{Ca} and IK_{Ca} channels seems preserved and the EDHF response can be maintained early on in obesity.

The function of BK_{Ca} channels, however, can be affected as obesity progresses and atherogenic dyslipidaemia and diabetes develop. Studies by Burnham *et al.* (Burnham *et al.*, 2006) and by Lu *et al.* (Lu *et al.*, 2008) found impaired BK_{Ca} channel-mediated dilations in mesenteric arteries of Zucker Diabetic Fatty rats; animals with insulin resistance and manifest hyperglycaemia. Moreover, high fructose-containing and atherogenic diet in swine was associated with reduced BK_{Ca} channel-mediated dilation of coronary arteries (Dimitropoulou *et al.*, 2002; Borbouse *et al.*, 2009). These observations suggest a pathological role for high glucose and atherogenic dyslipidaemia in the development of impaired BK_{Ca} channel function and consequently diminished arterial dilation. It is known that BK_{Ca} channels are primarily expressed in vascular smooth muscle cells, where they are activated by high concentrations of local $[Ca^{2+}]_i$ (10–100 μM), also called Ca^{2+} sparks, reviewed in Jaggar *et al.* (2000). It is possible that the impaired BK_{Ca} channel function is associated primarily with enhanced, smooth muscle-dependent arteriolar tone in obesity and diabetes. In this context, a study by Mokelke *et al.* has found a decreased Ca^{2+} spark activity and reduced outward K^+ current in coronary microvascular smooth muscle cells of the diabetic dyslipidaemic swine, alterations that were associated with an impaired baseline coronary blood flow (Mokelke *et al.*, 2005).

It still remains unknown how the function of certain K_{Ca} channel could persist early on in obesity, whereas others become impaired, especially as the disease progresses. In obesity, microvessels are exposed to increasing levels of harmful stimuli, such as ROS. Experimental evidence shows that inhibiting individual K_{Ca} channels is insufficient to block the EDHF response in small arteries (Dong *et al.*, 2000; Griffith, 2004). Thus, one can speculate that in disease, K_{Ca} channels that are less sensitive for harmful stimuli may replace the function of other, likely more susceptible K_{Ca} channels to maintain the overall EDHF response and vasodilation. Inhibition of K_{Ca} channels may occur via the oxidation of critical SH groups (Cai and Sauve, 1997). It is plausible that SK_{Ca}, IK_{Ca} and BK_{Ca} channels differ in their susceptibility to ROS-dependent inhibition, providing a potential mechanistic explanation for this phenomenon. Further studies are needed to explore how oxidative stress affects K_{Ca} channel characteristics in microvessels and whether this changes as obesity progresses.

Summary of this section

To conclude this section it is established that obesity is associated with a reduced dilator function of both conduit and resistance arteries. There is a paucity of experimental data in man regarding the exact underlying mechanisms behind the vasomotor dysfunction. On the basis of animal studies it possible that vascular oxidative stress is the main contributor for the development of microvascular dysfunction in obesity, which is characterized by reduced endothelium-dependent, NO-mediated arteriolar dilation (Figure 1). The exact source of ROS production has yet to be elucidated in obesity. Evidence also suggests that EDHF-type dilations of resistance arteries persist over the course of obesity, due to the maintained function of SK_{Ca} and IK_{Ca} channels. The function of BK_{Ca} channels, however, becomes compromised especially when obesity progresses and other co-morbid diseases develop, such as type 2 diabetes, dyslipidaemia and hypertension. Understanding the sequence of pathological events and that could initiate ROS production will provide a rationale for therapeutic interventions to restore NO bioavailability and to prevent dysfunction of K_{Ca} channels in resistance arteries in obese patients.

Role for adipose tissue-derived factors in eliciting increased vascular ROS production

In order to interfere with pathological production of vascular ROS in obesity, it is important to identify mechanisms that are responsible for initiating the sequence of deleterious events. An important role for atherogenic dyslipidaemia, oxidized low-density lipoproteins, free fatty acids in the development of obesity- and insulin resistance-related ROS production is discussed in more detail in a recent comprehensive review (Bashan *et al.*, 2009). Recently, it has been posited that in obesity the altered function of ROS-producing enzymes could arise from the adverse effects of adipocyte- and perivascular adipose tissue (PVAT)-derived factors (adipokines) (Berg and Scherer, 2005; Kadowaki and Yamauchi, 2005). In obesity, the normal endocrine function of adipocytes is altered and may be manifested as reduced adiponectin (Kadowaki and Yamauchi, 2005) and elevated circulating and tissue levels of leptin, resistin, IL-6 and TNFα (Berg and Scherer, 2005). Several recent investigations focused on elucidating adverse vascular effects of secreted adipokines in obesity. In this context, Knudson *et al.* have found that higher, pathological concentration of leptin (625 $pmol \cdot L^{-1}$) attenuated dilation to ACh of coronary arteries in normal dogs, whereas physiological concentrations (250 $pmol \cdot L^{-1}$) were without effect (Knudson *et al.*, 2005). It has been shown that leptin promotes oxidative stress in cultured endothelial cells (Korda *et al.*, 2008). In porcine coronary arteries, exposure to resistin elicited a reduced dilation to bradykinin, via induction of ROS production (Kougias *et al.*, 2005). Dick *et al.* have found a reduced bradykinin-induced dilation of canine coronary arteries exposed to resistin; an effect that was, however, independent from increased ROS production and was not affecting endothelial production of NO or PGI_2 (Dick *et al.*, 2006). Moreover, TNFα via increasing NAD(P)H

oxidase-derived ROS production has been implicated in the development of coronary arterial dysfunction in obese Zucker rats (Picchi *et al.*, 2006).

It still remains unclear how adipokines elicit increased vascular ROS production. In their study of human saphenous vein endothelial cells, Verma *et al.* demonstrated that resistin increases expression of endothelin-1 (Verma *et al.*, 2003). Endothelin-1 is a potent vasoconstrictor, but it is also an important mediator of enhanced ROS production in the vasculature. Over-expression of resistin leads to increased NAD(P)H oxidase activity via increasing the levels of NOX2, NOX4 and p47phox in the rat heart, and results in marked nitrotyrosine formation (Chemaly *et al.*, 2011). Decreased eNOS levels were also observed in human coronary artery endothelial cells incubated with resistin (Chen *et al.*, 2010). The authors also demonstrated resistin's ability to impair mitochondrial respiratory chain function and indicate the mitochondria as a key source of ROS production induced by resistin exposure (Chen *et al.*, 2010). IL-6 has been shown to up-regulate type 1 angiotensin II (AT1) receptor gene expression, which resulted in an increased angiotensin II-mediated ROS production in vascular smooth muscle cells (Wassmann *et al.*, 2004). Recently, Hung *et al.* have shown that IL-6 increased the stability of caveolin-1, a negative regulator of eNOS. As a result of the increased inhibition of eNOS, NO bioavailability was reduced in the presence of IL-6 (Hung *et al.*, 2010). In the study by Payne *et al.*, leptin, likely to be released from PVAT, elicited reduction of bradykinin-induced coronary relaxation in a swine model of metabolic syndrome; a response, which was mediated by activation of PKC (Payne *et al.*, 2010). The importance of PVAT in regulating vascular resistance and its potential role in development of microvascular dysfunction in obesity are discussed in more detail in other papers in this themed issue, and the reader is directed to a more detailed comprehensive recent review (Li *et al.*, 2011).

Summary of this section

Several recent studies indicate that adipokines, such as leptin, resistin, IL-6 and TNFα exert adverse effects on vasodilator function in animals with experimental obesity. These effects in part are mediated by elevated ROS production in the vascular wall. Exact mechanisms by which adipokines increase vascular ROS production are not entirely understood, but can be mediated by adipokine-dependent induction of endothelin-1 and angiotensin II production or could be attributed to direct activation of specific signalling pathways, such as PKC, known to be involved in ROS generation.

Less is known about the pathological role of adipokines in humans. Although higher leptin concentrations were associated with impaired arterial distensibility in healthy adolescents (Singhal *et al.*, 2002), acute subcutaneous administration of leptin unexpectedly increased flow-mediated dilation of the brachial artery (Brook *et al.*, 2007). Furthermore, in obese women, leptin concentrations did not predict the impaired flow-mediated dilation of the brachial artery (Oflaz *et al.*, 2003). The apparent discrepancy between animal and human studies is not known and clearly requires further investigation.

Coronary microvascular responsiveness in obesity – different from that of the periphery?

Increase in body mass, muscular or adipose type, requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements (Lavie and Messerli, 1986). It is widely accepted that obesity is independently associated with left ventricular hypertrophy (Abel *et al.*, 2008). This is considered to be an early adaptation of cardiac function, which accommodates for the higher haemodynamic and metabolic demand in obesity. It has been posited that in 'uncomplicated' obesity – lack of serious co-morbid conditions such as hypertension and diabetes – the increased left ventricular mass can be appropriate for body size (Iacobellis, 2004). Cardiac adaptation in obesity also implies changes in coronary circulation, which aims to provide adequate blood flow to meet the increased metabolic demand. Adaptation in coronary vessels seems particularly important, as in the coronary circulation oxygen extraction is near maximal and any mismatch between blood supply and metabolic demand would deteriorate myocardial contractile function (Tune *et al.*, 2004).

Could adaptation of the coronary circulation meet the increased metabolic demand in obesity? To answer this question several studies set out to investigate whether myocardial blood flow differs in obese subjects (Table 1). Myocardial blood flow can be measured non-invasively in units of milliliters of blood per minute per gram of myocardium at baseline and after pharmacological challenge. Most investigations of the coronary circulation in humans have employed PET for flow measurements. Myocardial blood flow, as measured by PET, has been found to be significantly reduced in post-menopausal women with obesity (Martin *et al.*, 2005). In this study the impaired myocardial blood flow was negatively correlated with waist/hip ratio (Martin *et al.*, 2005). In contrast, premenopausal women with similar levels of obesity exhibited a higher myocardial blood flow at baseline when compared with lean subjects, whereas no difference was detected between lean and obese men (Peterson *et al.*, 2008). Increased myocardial blood flow has also been described in postmenopausal obese women without coronary artery disease, however, the increase in resting blood flow was associated with a significantly reduced coronary flow reserve, as defined by the ratio of coronary flow under maximal dipyridamole-induced vasodilatation to coronary flow under resting conditions (Motivala *et al.*, 2008). A study by Schindler *et al.* compared myocardial blood flow between lean, overweight and obese subjects involving both men and women using PET imaging (Schindler *et al.*, 2006). They found that at baseline, myocardial blood flow did not differ among the three groups, but cold pressor test- or dipyridamole-induced increases in blood flow were significantly reduced in obese patients, when compared with lean individuals (Schindler *et al.*, 2006). Although gender did not correlate with dilator capacity of coronary vessels there was a significant negative impact of age in this study. By using cardiac magnetic resonance imaging neither the resting myocardial blood flow nor the adenosine-induced hyperaemic flow were correlated with obesity in asymptomatic

patients in the Multi-Ethnic Study of Atherosclerosis (MESA), which involved 222 men and women (Wang *et al.*, 2006). The majority of these clinical studies demonstrated that basal myocardial blood flow is not compromised in obese subjects. However, alterations may manifest when the coronary circulation is pharmacologically challenged to mimic the increased metabolic demand in obesity. Thus, it seems, it is the response of myocardial blood flow to pharmacological or physiological stimuli, which could uncover the presence of pathological alterations in the coronary circulation in obesity. The important question, however, remains, whether the basal or stimulated increase in myocardial blood flow is able to meet the elevated metabolic demand in obesity. A series of experiments performed in dogs with experimental obesity and metabolic syndrome show that in spite of unaltered basal and stimulated coronary blood flow rate, there could be a mismatch between myocardial perfusion and metabolism, as estimated by the rate of oxygen consumption (Setty *et al.*, 2003; Borbouse *et al.*, 2010). These experiments suggest that even an elevated coronary blood flow is unable to meet metabolic requirements in obese animals. This phenomenon has yet to be confirmed in obese patients. Furthermore, in order to understand the nature of altered tissue perfusion it seems important to elucidate the cellular mechanisms, which regulate coronary arteriolar diameter and adjust blood flow to enhanced metabolic demand in obesity.

The coronary circulation matches blood flow with metabolic requirements by coordinating the vascular resistance in different-sized coronary vessels, which is governed by distinct mechanisms, such as the myogenic flow or metabolic control of arterial diameter (Jones *et al.*, 1995; Chilian, 1997). The large, conduit coronary arteries exert small, if any, resistance; resistance to blood flow rises as the vessel diameter decreases in arterioles with a diameter of less than 300 μm in humans. These aforementioned underscore the need for direct examination of the function of coronary resistance arteries to understand how their vasomotor behaviour changes and how it relates to coronary blood flow alterations in obese patients.

At present, convincing evidence of the impact of human obesity on vasomotor regulation of coronary arterioles and the exact underlying mechanisms are lacking. Direct investigation of coronary arteriolar vasomotor function and diameter changes can be performed by videomicroscopy of isolated microvessels obtained from the heart of obese patients (Szerafin *et al.*, 2006; Fulop *et al.*, 2007) or from the heart of animals with genetically induced (Bagi *et al.*, 2004a) or diet-induced, experimental obesity (Jebelovszki *et al.*, 2008; Feher *et al.*, 2010). In this preparation, isolated arterioles develop a spontaneous myogenic tone in response to increases in intraluminar pressure. The magnitude of the myogenic tone depends on the level of intra-arteriolar pressure, which can be sensed by vascular smooth muscle cells and can be modulated by factors released from vascular endothelium. It is believed that the spontaneously developed tone in isolated coronary arterioles is comparable in magnitude with those *in vivo*. Studies from our laboratory demonstrated that in coronary arterioles the magnitude of myogenic tone does not differ between lean and obese patients (Fulop *et al.*, 2007). Furthermore, in an animal model of diet-induced obesity, increases in intraluminal pressure elicited similar diameter changes in coronary arterioles from lean and obese rats (Erdei *et al.*, 2006).

The intrinsic myogenic tone also establishes a diameter reserve allowing microvessels to dilate in response to metabolic, neuronal and other vasomotor stimuli. Vasodilator function can be studied in isolated coronary arterioles *in vitro*, where diameter changes in response to agonist stimulation is continuously recorded. Coronary arterioles from the heart of obese patients exhibit a reduced endothelium-dependent, bradykinin-induced dilation (Fulop *et al.*, 2007). Oltman *et al.* have investigated the progression of coronary arterial dysfunction in obese Zucker rats and found that coronary arteriolar dilation to ACh was preserved in 16–24 week old animals, but dilations became reduced in 28–36 week old rats (Oltman *et al.*, 2006). Katakam *et al.* reported that in 12 week old obese Zucker rats ACh-induced dilation of small coronary arteries was preserved, although a reduced vasodilation to insulin was also reported in this study (Katakam *et al.*, 2005). Coronary arterioles from pigs fed a high-fat diet to induce obesity exhibited only modest impairment of dilation to bradykinin (Henderson *et al.*, 2004), whereas coronary dilation to ACh was found to be preserved in high-fat-fed obese rats (Jebelovszki *et al.*, 2008). More intriguing, Prakash *et al.* have reported that ACh-induced dilation of coronary arterioles in obese Zucker rats is markedly enhanced (more than 25% increase in diameter, when compared with lean animals) (Prakash *et al.*, 2006).

Although observations from studies on large conduit arteries suggest detrimental effects of obesity on vascular responsiveness (Hashimoto *et al.*, 1998; Kapiotis *et al.*, 2006), vasomotor tone and agonist-stimulated dilator function of coronary microvessels may remain intact, especially at the early state of the disease. This phenomenon implies that in obesity, vasoregulatory mechanisms intrinsic to the microvascular wall may be protected from those pathological factors that are detrimental to other types of vessels in the periphery. It is known that the dilator function of small coronary arteries is mediated by the release of autacoids from the endothelium including NO and EDHF. Experimental evidence suggests that endothelial availability of NO is reduced in coronary arterioles in obesity; this, however, is compensated for by various other vascular mechanisms. For example, emerging evidence indicates that the same ROS derivates, which otherwise would impair microvascular function in peripheral vessels, may act as prominent vasodilators in the coronary microcirculation. In this context, Matoba *et al.* demonstrated that a major dilator factor released from the endothelium of porcine coronary microvessels is H_2O_2 (Matoba *et al.*, 2003). Coronary arterial microvessels from the human heart also generate H_2O_2 from endothelial cells as a major contributor of coronary arteriolar dilation (Miura *et al.*, 2003). The underlying mechanism of H_2O_2-mediated dilation varies, but studies show that H_2O_2 exerts its vasodilator effects via activating K_{Ca} channels (Hayabuchi *et al.*, 1998; Matoba *et al.*, 2003; Miura *et al.*, 2003). Thus, it has been proposed that H_2O_2, via eliciting K_{Ca} channel activation, potentially acts as an EDHF in coronary microvessels (Shimokawa and Matoba, 2004; Feletou and Vanhoutte, 2006). Other studies demonstrate that H_2O_2-induced vasodilation is mediated through the release of NO from the endothelium (Hirai *et al.*,

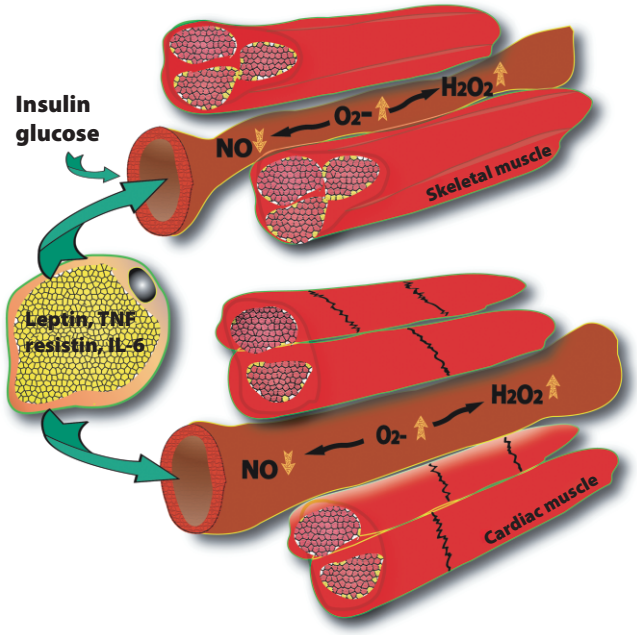

Figure 2

Effects of adipokines on vasomotor function of arterioles. This schemiatic draw illustrates that the degree of vasodilation may vary in various vascular beds in obesity; as shown here in skeletal muscle and coronary resistance arteries, although they are exposed to the same level of circulating adipokines (leptin, resistin, TNFα and IL-6). ROS interact with endothelium-derived NO, which results in a diminished vasodilator response in skeletal muscle arterioles. The inability of insulin to produce NO and thereby elicit vasodilation of skeletal muscle microvessels may also limit the insulin-mediated muscle glucose uptake. On the other hand, the ROS derivate, H_2O_2 serves as a potent vasodilator in the coronary arterioles, which aims to maintain vasodilator responsiveness in the coronary microcirculation.

2000) or is partially mediated by cGMP formation in vascular smooth muscle cells (Fujimoto *et al.*, 2001). Regardless of the mechanisms of action, H_2O_2 could maintain dilator function of coronary arterioles in obesity, even if the availability of NO is compromised (Figures 1 and 2). Interestingly, a study by Saitoh *et al.* indicates that H_2O_2, released from cardiac myocytes, couples coronary blood flow to myocardial oxygen consumption (Saitoh *et al.*, 2006), providing further importance for H_2O_2 in the coronary circulation. Whether or not cardiac myocyte derived H_2O_2 contributes to the regulation of coronary arteriolar diameter in obesity requires further investigation.

A reduced availability of endothelial NO could be associated with enhanced sensitivity to NO in the vascular smooth muscle cells. In this context, studies from our laboratory provided evidence for enhanced NO sensitivity of coronary arterioles isolated from obese rats fed a high-fat diet (Jebelovszki *et al.*, 2008). The enhanced sensitivity of coronary arterioles to NO is mediated by increased activity of soluble guanylate cyclase (sGC) in the coronary arteriolar wall (Jebelovszki *et al.*, 2008). Enhanced dilations of coronary arterioles to the NO donor, SNP, have also been described in female pigs fed a high-fat diet (Woodman *et al.*, 2004) and in mesenteric arterioles of obese Zucker rats (Oltman *et al.*, 2006). Similar results were obtained in humans, showing that NO donor-induced coronary arteriolar and brachial artery dilations were enhanced in patients with obesity (Fulop *et al.*, 2007). Thus, it seems the enhanced sensitivity of smooth muscle cells to NO may also lead to compensation of the impaired NO-mediated coronary signalling in obesity. Chronic oxidative and nitrosative stress can lead to inactivation of sGC over time (Munzel *et al.*, 2005) and could also diminish K_{Ca} channel function (Figure 1). The question that remains to be answered is: to what extent and how long the up-regulation of sGC, and H_2O_2-dependent activation of vascular K_{Ca} channels may be able to compensate for the reduced NO-mediated signalling as obesity progresses?

Summary of this section

Clinical studies have found that basal myocardial blood flow is not compromised in obese subjects, which, however, becomes inadequate when cardiac metabolism is stimulated. Unlike peripheral vessels, coronary microvessels actively adapt to match higher metabolic demand by maintaining their vasodilator function. Evidence indicates that this adaptation involves various cellular pathways, such as the compensatory increased production of H_2O_2, which serves as an important vasodilator factor in the coronary circulation. It is of particular importance that in spite of this compensatory mechanism and maintained vasodilator function, coronary blood flow is unable to meet higher metabolic requirements in obesity. Understanding compensatory mechanisms in coronary arteriolar wall seems important, as they could provide insight into the sequence of pathological events in obesity and could be harnessed for therapeutic purposes.

Altered microvascular responsiveness in adipose tissue

Adipose tissue can represent 18 and 24% of body weight in normal men and women, respectively; as much as 52 and 74% of body weight in obese man and women respectively (Leibel *et al.*, 1995). Adipocytes perform an important endocrine function by secreting numerous cytokines, hormones and bioactive peptides. Upon secretion into the bloodstream, these adipokines, such as adiponectin, leptin, resistin, IL-6 and TNFα have a key impact on skeletal muscle and liver function to regulate energy homeostasis and metabolism (Kadowaki *et al.*, 2003). In addition to their systemic action adipokines may exert paracrine effects on vasoregulatory mechanisms in adipose tissue microvessels (Figure 2). This does not simply reflect systemic vasomotor dysfunction, but could contribute to the development and also maintenance of adiposity. Expansion in addition to reduction of the fat mass relies on the function of the adipose tissue microcirculation. An important interaction between microvessels and adipocytes has been recently envisioned (Rutkowski *et al.*, 2009). It is known that adipose tissue possesses a dense network of microvessels ensuring adequate tissue perfusion, exchange of nutrients and oxygen. The adipose tissue vasculature delivers lipids to their storage depot in the adipocytes and also exports nutrients in response to metabolic need. It is

plausible that alterations in the responsiveness of adipose tissue microvessels have a significant impact on adipose tissue metabolism and nutrient trafficking. For example, insufficient adipose tissue perfusion may result in local hypoxia, which increases the levels of hypoxia inducible factor, HIF-1α in adipocytes (Trayhurn *et al.*, 2008; Halberg *et al.*, 2009). HIF-1α may lead to up-regulation of various inflammatory adipokines, such as IL-6, TNFα and monocyte chemotactic protein-1 (Halberg *et al.*, 2009). Upon secretion into bloodstream these adipokines will cause damage in systemic microvessels (Trayhurn *et al.*, 2008) and also elicit dysfunction of adipose tissue arterioles, further exaggerating dysregulation of adipose tissue microcirculation.

Given the importance of adipose tissue microcirculation, it is surprising that little attention has been devoted to elucidate alterations in microvascular responsiveness in the adipose tissue. Studies in adipose tissue microvessels are particularly lacking in obese patients. Early *in vivo* reports on autoregulation in the canine inguinal fat pad suggest that the microvessels possess a pressure sensitive myogenic response, which contributes to the regulation of adipose tissue perfusion (Nielsen and Secher, 1971). In isolated adipose tissue arterioles of the rat myogenic tone develops in response to an increase in intraluminal pressure (Undavia *et al.*, 2003). In humans, it has been found that subcutaneous adipose tissue arteries develop myogenic tone in response to an increase in pressure, which is due to the activation of phospholipase C, diacyl-glycerol and PKC pathways (Coats *et al.*, 2001b). In healthy volunteers resistance arteries from gluteal subcutaneous fat exhibit dose-dependent relaxation to histamine, which is mediated by both muscular histamine H2 receptors and endothelial histamine H1 receptors (Van de Voorde *et al.*, 1998). Activation of H1 receptors by histamine was associated with endothelial release of NO in adipose tissue arterioles (Van de Voorde *et al.*, 1998). In another study, ACh elicited EDHF-type, phospholipase A_2- and cytochrome P450-inhibited relaxation of subcutaneous adipose tissue resistance arteries in healthy individuals (Coats *et al.*, 2001a). In subcutaneous fat arteries of healthy subjects it has been shown that increasing concentrations of insulin reduced noradrenaline-induced vascular tone in a dose-dependent manner (McNally *et al.*, 1995). Thus, it seems that adipose tissue microvessels use similar pathways to regulate their diameter compared with other types of microvessels in the body. Whether these vasoregulatory pathways in adipose tissue microvessels are similarly affected by obesity as compared with other types of microvessels is not entirely understood. In the study by Greenstein *et al.*, responses of subcutaneous adipose tissue arterioles from lean or obese patients with metabolic syndrome were studied. Compared with controls, contraction of arterioles to norepinephrine or 60 mM KCl were unaffected by obesity, but relaxation induced by ACh was significantly impaired (Greenstein *et al.*, 2009). The authors suggested that local inflammation in PVAT, mediated by TNFα or IL-6 and oxidative stress, interferes with adiponectin-induced NO release and primarily responsible for vasomotor dysfunction of adipose tissue arterioles in obese patients (Greenstein *et al.*, 2009).

Summary of this section

Several studies demonstrate the feasibility of studying vasomotor function of adipose tissue arterioles in healthy and obesity subjects. Whether vasomotor changes in adipose tissue arterioles, via endothelium- and smooth muscle-dependent cellular pathways contribute to the development of obesity and whether these pathological alterations change as obesity progresses is not known, and clearly requires further investigation. Expanded adipose tissue represents primary therapeutic target to prevent obesity-related vascular complications. Emerging evidence also indicates that specific alteration(s) in adipose tissue microcirculation may contribute to the development of insulin resistance and obesity. Demonstrating that changes in microvascular responsiveness of adipose tissue arterioles could modulate adipose tissue metabolism, and affect lipid and nutrient trafficking, may allow for the discovery of novel therapeutic targets for intervention.

Therapeutic attempts to improve altered microvascular responsiveness in obesity

There is no doubt that physical exercise, balanced diet and weight loss are the primary mediators in the prevention of cardiovascular diseases in obesity. Unfortunately in Western society this simple advice has not resulted in major breakthroughs in fighting obesity and obesity-related cardiovascular morbidity and mortality. Meanwhile, enormous efforts are being made by pharmaceutical companies to search for new targets to treat and prevent life-threatening cardiovascular complications in obese patients. Effective drugs to treat obesity have proven enormously difficult to develop. Many previous (but already withdrawn) and currently available anti-obesity drugs target the brain to modify appetite, but the targeted brain circuits of these drugs unfortunately overlap with those that control other important functions in the body raising the risk of serious side effects. Even if they were safe, patients would have to take a drug for several years. There are several drugs, however, that are frequently prescribed and taken by patients with cardiovascular diseases, those that may also be beneficial – perhaps only indirectly – in targeting microvascular dysfunction in obesity and insulin resistance. These include statins (Grundy *et al.*, 2005), ACE inhibitors, angiotensin receptor blockers (ARBs) (McFarlane *et al.*, 2003) and insulin sensitizers, metformin and thiazolidinediones (TZDs) (Jay and Ren, 2007; Hill *et al.*, 2009). Here, we only provide examples of attempts for pharmacological interventions with these drugs, in which the main focus was to prevent vasomotor abnormalities in obese subjects (Table 2). Convincing evidence for direct beneficial effects of these and other drugs on microvessels in obesity is lacking at present. Such demonstration is hampered by issues regarding to the direct investigation of microcirculation in humans and the obvious impact of these drugs on other pathological factors, such as atherogenic dyslipidaemia, insulin resistance and elevated blood pressure.

Statins

Statins lower plasma lipid levels by inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, the rate-limiting

Table 2

Clinical and animal studies investigating pharmacologic interventions on the vasomotor responses of arteries

Reference	Model/vascular Bed	Therapy	Results
Nagashima and Endo, 2011	Obese man; brachial artery	Pitavastatin (2 mg)	Restored FMD after oral fat loading test
Mather *et al.*, 2001	T2DM man; brachial artery	Metformin (500 mg, twice daily)	↑ ACh dilation and FMD
Pistrosch *et al.*, 2004	T2DM man/woman; brachial artery	Rosiglitazone (4 mg twice daily)	↑ ACh dilation
Regensteiner *et al.*, 2005	T2DM man/woman; brachial artery	Rosiglitazone (4 mg)	↑ FMD
Beckman *et al.*, 2001	Healthy man/woman; forearm	Vitamin C (24 mg·min^{-1})	↑ methacholine dilation
Gazis *et al.*, 1999	T2DM man/woman; brachial artery	Vitamin E (1600 i.u.)	↔ ACh and BK dilation
Wilson *et al.*, 2001	Porcine: high-cholesterol diet; coronary arteries	Simvastatin (80 mg)	↑ BK and Substance P dilation
Oltman *et al.*, 2008	OZR; epineural arterioles	Rosuvastatin (25 mg·kg^{-1})	↑ ACh dilation
Shinozaki *et al.*, 2007	OZR; aorta	Pitavastatin (3 mg·kg^{-1})	↑ ACh dilation, ↓ Ang II constriction
Goodwill *et al.*, 2009	OZR; skeletal muscle arterioles	Atorvastatin Simvastatin	↑ ACh dilation
Erdos *et al.*, 2006	OZR; basilar artery	Rosuvastatin (2 mg·kg^{-1})	↑ ACh and iloprost dilation
Nawano *et al.*, 1999	OZR; liver and skeletal muscle	Imidapril (10 mg·kg^{-1})	↑ blood flow
Duarte *et al.*, 1999	OZR; aorta	Captopril (50 mg·kg^{-1})	↑ ACh and SNP dilation
Duarte *et al.*, 1999	OZR; aorta	Enalapril (10 mg·kg^{-1})	↔ ACh and SNP dilation
Oltman *et al.*, 2008	OZR; epineural arterioles	Enalapril (20 mg·kg^{-1})	↑ ACh dilation
Davidson *et al.*, 2009	OZR; epineural arterioles	AVE 7688 (30 mg·kg^{-1})	↑ ACh dilation
Arbin *et al.*, 2001	OZR; femoral artery	Mixanpril (25 mg·kg^{-1})	↑ insulin induced blood flow
Russell *et al.*, 2004	Obese JCR:LA-cp rat; aorta, coronary artery	AVE 7688 (30 mg·kg^{-1})	↑ coronary BK dilation
Russell *et al.*, 2004	Obese JCR:LA-cp rat; aorta, coronary artery	Ramipril (1 mg·kg^{-1})	↑ coronary BK dilation
Walker *et al.*, 1999	OZR; mesenteric artery	Rosiglitazone (50 μM·kg^{-1})	↑ ACh and insulin dilation
Bagi *et al.*, 2004a	db/db Mice; coronary arteriole	Rosiglitazone (3 mg·kg^{-1})	↑ ACh, NONOate

BK, bradykinin; FMD, flow-mediated dilation; OZR, obese Zucker rat; T2DM, type 2 diabetes mellitus.

enzyme in cholesterol synthesis, and are known to reduce cardiovascular morbidity and mortality (Goldberg *et al.*, 1998). Statins may improve microvascular function both via affecting lipid metabolism and via direct effects on the vasculature (Wilson *et al.*, 2001). In a study by Nagashima *et al.*, 24 obese male subjects were randomly assigned to receive pitavastatin (2 mg·day^{-1}) or placebo for 2 weeks. An oral fat loading test was conducted, which elicited a marked increase of serum triglyceride level and decreased flow-mediated dilation of brachial artery in the placebo group. In the pitavastatin group the increase in postprandial triglyceride was attenuated and the postprandial brachial artery relaxation was restored (Nagashima and Endo, 2011). Thus, statins may improve vascular function due to their effect on dyslipidaemia, but data concerning their direct microvascular effects and the underlying mechanisms in obesity are limited.

Statins may facilitate the synthesis of endothelial NO (Feron *et al.*, 2001) and also could reduce ROS production in the vascular wall (Wassmann *et al.*, 2002; Mason *et al.*, 2004). In this context, treatment of obese Zucker rats with rosuvastatin (25 mg·kg^{-1} daily) for 12 weeks improved ACh-induced relaxation of epineural arterioles by preventing oxidative and nitrosative stress (Oltman *et al.*, 2008). Pitavastatin treatment (3 mg·kg^{-1}) for 8 weeks in the obese Zucker rat also restored the diminished ACh-induced relaxation and normalized angiotensin II-induced contraction of the aorta, which was due to the reduction of endothelial, NADPH-oxidase derived superoxide production and restoration of eNOS cofactor, tetrahydrobiopterin (BH_4) levels. Interestingly, the effects of pitavastatin was due to the down-regulation of AT1 receptor and NADPH-oxidase subunit, gp91phox expression and also to the up-regulation of GTP cyclohydrolase I, the rate-limiting enzyme of BH_4 synthesis (Shinozaki *et al.*, 2007). In the obese Zucker rat 10 week atorvastatin or simvastatin treatments were equally effective at improving the ACh-induced, endothelium-dependent vasodilation of skeletal muscle microvessels via increasing the vascular availability of NO (Goodwill *et al.*, 2009). The beneficial effect of statins was associated with the reduction of inflammatory biomarkers, such as IL-10 and TNFα in these animals (Goodwill *et al.*, 2009). Rosuvastatin treatment (2 mg·kg^{-1} daily) for 4 weeks improved dilator responses to ACh and iloprost in the basilar artery of the obese Zucker rat, which was associated

with the reduction of NAD(P)H-oxidase-derived superoxide production (Erdos *et al.*, 2006). Interestingly, beneficial effects of rosuvastatin were observed after 1 day of treatment (2 mg·kg^{-1}). This study was the first to suggest that statins, independent of their lipid lowering action improve vasomotor function. (Erdos *et al.*, 2006).

Thus, it plausible that statins are beneficial in preventing microvascular dysfunction in obesity. This effect can be attributed to the concomitant reduction of oxidative stress and/or to the limitation of vascular pro-inflammatory signalling mechanisms. Whether the effects are direct on microvascular wall or only due to the systemic effect of statins on atherogenic dyslipidaemia requires further investigations.

ACE inhibitors in obesity

It has been proposed that ACE inhibitors improve insulin resistance in type 2 diabetic patients (Abuissa *et al.*, 2005; Andraws and Brown, 2007). Both ACE-inhibitors and ARBs were associated with reduced incidences of new-onset diabetes among patients with essential hypertension (McFarlane *et al.*, 2003). Orally administered ACE inhibitor, imidapril (10 mg·kg^{-1}) improved insulin sensitivity in obese Zucker rats, which was associated with reduction of systemic blood pressure and increased blood flow in the liver and the skeletal muscle (Nawano *et al.*, 1999). In a study by Duarte *et al.*, obese Zucker rats were treated with captopril or enalapril (50 and 10 mg·kg^{-1}, respectively) and endothelium-dependent relaxation of isolated aortic rings was studied. They found that both captopril and enalapril improved insulin resistance of obese Zucker rat. Interestingly, only the sulfhydryl group containing captopril augmented the impaired endothelium-dependent aortic relaxation in obese animals (Duarte *et al.*, 1999). Treatment of obese Zucker rats with the ACE inhibitor enalapril (20 mg·kg^{-1}), improved ACh-induced relaxation of epineural arterioles by preventing oxidative and nitrosative stress (Oltman *et al.*, 2008). Similar results were obtained when obese Zucker rats were treated with a dual inhibitor of ACE and neutral endopeptidase (NEP), AVE 7688 (30 mg·kg^{-1}) in epineuronal arterioles (Davidson *et al.*, 2009). The dual ACE and NEP inhibitor, mixanpril (25 mg·kg^{-1}), restored femoral blood flow to insulin in obese Zucker rat and was shown to be more effective than captopril treatment (2 mg·kg^{-1}) alone (Arbin *et al.*, 2001). The beneficial effect of dual ACE/NEP inhibitor, AVE 7688 (30 mg·kg^{-1}) over the ACE inhibitor, ramipril (1 mg·kg^{-1}) alone in restoring the impaired insulin sensitivity and endothelial dysfunction of the aorta was also demonstrated in obese JCR:LA-cp rats (Russell *et al.*, 2004). In this study, bradykinin-stimulated coronary flow was also measured in isolated rat hearts. Bradykinin-induced coronary flow was improved in both AVE 7688 and ramipril-treated obese, JCR:LA-cp rats (Russell *et al.*, 2004).

The exact mechanism by which ACE inhibitors without or in combination with NEP inhibitors improve microvascular responsiveness is not clear. Zhang *et al*. have demonstrated that coronary arterioles of high-fat-fed dogs exhibit an increased vasoconstriction to angiotensin II, likely due to the increased vascular expression of AT1 receptors (Zhang *et al.*, 2005). Interestingly, recent studies have demonstrated that the local renin-angiotensin system (RAS) can be up-regulated in PVAT (Cassis *et al.*, 2008). Adipose tissue RAS could be one of the sources of increased angiotensin II production (Lu *et al.*, 2007), and not only contribute to the development hypertension (Engeli *et al.*, 2005), but also could directly impair microvascular function in obesity. To determine whether the effects of ACE inhibitors are solely mediated by improving insulin resistance in obese individuals, or are the results of reduced RAS activity in adipose tissue, or due to the abolished effects of angiotensin II on the microvascular wall, requires further mechanistic insight.

Insulin sensitizers in obesity

An increase in insulin sensitivity appears to be associated with an increase in coronary flow reserve. Insulin resistance therefore can be associated with coronary microvascular abnormalities (Dagres *et al.*, 2004). Insulin resistant patients with type 2 diabetes have a lower risk of developing microvascular complications if they are treated with insulin or sulfonylureas (UKPDS, 1998). Insulin sensitizers, such as metformin and PPARγ activator TZDs, improve coronary microvascular function in obesity. In a study by Mather *et al.*, patients with type 2 diabetes but without other manifested cardiovascular co-morbidities were given metformin (500 mg, daily twice) or placebo treatment to evaluate the effect of metformin on endothelial cell function, as measured by forearm plethysmography (Mather *et al.*, 2001). They found that metformin treatment improved ACh-stimulated and flow-induced dilation of the brachial artery (Mather *et al.*, 2001). As there was a significant improvement in insulin resistance with metformin, this study concluded that beneficial effects were likely mediated through the insulin resistance normalizing action of metformin.

Moreover, the TZD rosiglitazone has been shown to improve endothelium-dependent vasorelaxation of the brachial artery in patients with insulin resistance (Pistrosch *et al.*, 2004; Regensteiner *et al.*, 2005). The PPARγ activator, rosiglitazone (50 μM^{-1} kg^{-1}, daily) prevented the development of hypertension and partially protected against impaired endothelial function associated with insulin resistance in the obese Zucker rat (Walker *et al.*, 1999). Rosiglitazone (3 mg·kg^{-1}, daily for 1 week) reduced the enhanced vascular ROS production in coronary microvessels of obese mice with type 2 diabetes (Bagi *et al.*, 2004a). Thus, it seems that PPARγ activators may exert pleiotropic effects, which include increasing insulin sensitivity and direct antioxidant properties in improving microvascular function in obesity. On the other hand, use of rosiglitazone is associated with increased risk for myocardial infarction (Nissen and Wolski, 2007), but the harming effect of rosiglitazone was questioned in the interim analysis of the 'Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes' (RECORD) trial (Home *et al.*, 2007). At present, it seems that there is not enough data available to evaluate the cardiovascular safety and efficacy of these drugs (Woodcock *et al.*, 2010). These studies also underline the need for clinical investigations addressing the possible effects of drugs interfering with PPARγ-regulated microvascular mechanisms in obese patients with insulin resistance.

Antioxidants

Oxidative stress has been shown to impair microvascular function in several pathological conditions (Cai and

Harrison, 2000). As highlighted in this paper, ROS play a key role in the development of microvascular dysfunction in obesity. In patients with diabetes, administration of the antioxidant vitamin C prevented the decreased methacholine-induced, endothelium-dependent brachial artery relaxation (Beckman *et al.*, 2001). In contrast, other studies failed to demonstrate beneficial effects of antioxidant therapy in the prevention of vascular complications (Marchioli *et al.*, 2001; Maxwell and Greig, 2001). For example, vitamin E supplementation for 8 weeks did not improve the reduced ACh- and bradykinin-induced dilations of brachial artery in diabetic patients (Gazis *et al.*, 1999). Recent interventional clinical trials also yielded largely negative results, and there has even been some suggestion of harmful effects (Willcox *et al.*, 2008). For example, the Heart Outcomes Prevention Evaluation (HOPE) trial assessed the antioxidant vitamin E in high-risk patients with cardiovascular disease and diabetes and found no effect on cardiovascular outcomes (Hegele, 2000). Even worse, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial found an increased risk for coronary events in subjects receiving vitamin-E or β-carotene as antioxidants (Rapola *et al.*, 1996; 1998; Virtamo *et al.*, 1998). More concerning yet, an increased harm from supplemental vitamin E, vitamin A and β-carotene is indicated by the meta-analysis of 15 clinical trials on cardiovascular outcomes (Vivekananthan *et al.*, 2003). Further studies are needed to solve the current paradox regarding the adverse effects of antioxidants versus other therapeutics, such as statins and TZDs, the pleiotropic effects of which also include antioxidant activity and exert mostly beneficial effects on microvascular function.

While it is accepted that prescription of the pharmacological agents discussed is intended to treat sequelae of the metabolic syndrome not associated with microcirculatory dysfunction, consideration of their use in targeting the microvessels in the setting of obesity is warranted. The studies highlighted herein not only demonstrate clear pathology and impaired microvessel function as a result of obesity, but also discuss the unintended benefits of certain drugs in improving microvessel function (Table 2). While it is certainly difficult to perform patient follow-up studies on microvessel function, develop drug doses aimed specifically at the microcirculation, and adapt results from animal models (often with very high drug doses) to patient care, implementation of effective pharmachologics presently available to treat microvessel dysfunction as a means of alleviating the deleterious effects of their pathology would be welcomed. However, consideration of the potential side effects, alongside the hopeful benefit of these drugs in the context of obesity induced microvascular dysfunction needs to be evaluated.

Summary

Experimental and clinical studies emphasize the importance of those investigations that strive to elucidate the mechanisms that regulate tissue perfusion in obesity in order to develop novel therapeutics that target the microcirculation in obese patients. Recent studies also raise the prospect that

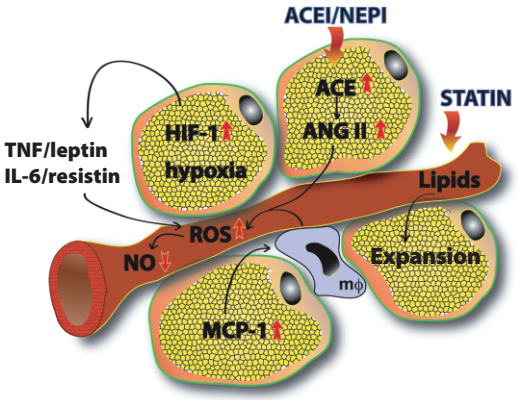

Figure 3

Adipose tissue microcirculation in obesity. In obesity, during expansion of adipose tissue phenotypic changes of adipocytes occurs, which results in increased production of leptin, resistin, TNFα and IL-6. In addition to their systemic actions, these adipokines may locally induce production of superoxide anion (O_2^{-}.) in the adipose tissue arteriole. Superoxide anion interferes with the availability of NO and reduces dilator function of microvessels. Altered microvascular responsiveness may provoke hypoxia in the expanded adipose tissue, which via hypoxia inducible factor-1α (HIF-1) would exaggerate pathological changes in adipokine production. Adipocytes may also enhance monocyte chemotactic protein-1 (MCP-1) synthesis, which facilitates macrophage (mΦ) accumulation and activation, to maintain oxidative stress and adipose tissue inflammation. The adipose tissue RAS could be one of the sources of increased local production angiotensin II, which may contribute to the development of microvascular dysfunction in obesity. Evidence indicates that statins, ACE inhibitors and NEP inhibitors (NEPI) may be effective in restoring arteriolar dilator function in obesity. Whether they affect adipose tissue arterioles and whether their direct effects are via interfering with altered adipocyte signalling has yet to be elucidated.

modulation of adipose tissue angiogenesis could be a potential therapeutic target to reduce adiposity (Cao, 2010), although this possibility requires further investigation and validation. Several cardiovascular diseases are associated with a state of chronic, low-level inflammation (Libby *et al.*, 2002; Gonzalez and Selwyn, 2003). Pro-inflammatory adipokines, such as IL-6 and TNFα, could contribute to the development of microvascular dysfunction in obesity. Adipose tissue-derived inflammatory cytokines and adipokines may elicit ROS production in adipose tissue microvessels and also in other arterioles in the body. The conclusion that ROS may impair vasodilator function (i.e. in skeletal muscle and mesenteric arteries) and contributes to enhanced coronary dilations (through H_2O_2-dependent activation of K_{Ca}-channels) undoubtedly requires further investigation. Studies have yet to be performed to clearly distinguish between the vascular bed specific differences of ROS-mediated signalling, which may affect microvascular responses in obesity. Current studies also underline the need for clinical investigations addressing the possible effects of drugs affecting microvascular responsiveness likely via interfering with adipokine and/or vascular ROS production in obese patients (Figure 3). A growing number of recent reports document a statistically significant benefit of taking statins and ACE inhibitors in

obese patients. On the other hand, it is possible that interfering with vascular signalling may provide further burden to those mechanisms, which are maintaining vascular function in disease.

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Conflict of interest

The authors have nothing to declare.

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