Microvascular responsiveness in obesity - implications for therapeutic intervention

‘Review’

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SUMMARY

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 ischemic 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, angiotensin converting enzyme inhibitors and insulin sensitizers to prevent obesity-related microvascular complications. We further identify some of the important challenges and opportunities going forward.

Key words: obesity, microcirculation, coronary flow, nitric oxide, hydrogen peroxide, adipocyte, statin, angiotensin converting enzyme inhibitors, thiazolidinedione, antioxidants
ABBREVIATIONS

BMI, body mass index; ACh, acetylcholine; SNP, sodium nitroprusside; NO, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; H$_2$O$_2$, hydrogen peroxide; IL, interleukin; TNF$\alpha$, tumor necrosis factor $\alpha$; MCP-1, monocyte chemotactic protein-1; EDHF, endothelium derived hyperpolarizing factor; K$_{Ca}$, Ca$^{2+}$ activated potassium channel; PET, positron emission tomography; sGC, soluble guanylate cyclase, HIF-1$\alpha$, hypoxia inducible factor 1$\alpha$; TZD, thiazolidinedione; BH$_4$, tetrahydrobiopterin; ACE, angiotensin converting enzyme; 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 hyperemia-induced forearm blood flow (Hashimoto et al., 1998; Vigili de Kreutzenberg et al., 2003). Obese children already exhibit impaired brachial artery relaxation to hyperemic 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.

Forearm resistance arteries also possess a reduced endothelium-dependent, acetylcholine (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 body mass index (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 man. 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 hyperglycemia, 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 et al., 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 et al., 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.

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, nitric oxide (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 et al., 1995). Experimental evidence indicates that obesity is associated with reduced bioavailability of vascular NO (Erdos et al., 2002; Frisbee et al., 2001; Naderali et al., 2001b). Oxidative stress occurring in response to hyperglycemia (Bagi et al., 2003; Bagi et al., 2004b; Bohlen et al., 1993; Ruderman et al., 1992) 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 (Erdei et al., 2006; Erdos et al., 2004) 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 (Bagi et al., 2003; Bohlen et al., 1993; Frisbee et al., 2001). 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).
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 mitochondrial 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 chemiluminescence-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 hypercholesterolemia (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 hypercholesterolemia, 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 et al., 2004). ROS, derived either from NAD(P)H or xanthine oxidase may reduce the level of NO synthase cofactor, BH₄, which in turn leads to NO synthase uncoupling and consequent generation of superoxide anion by NO synthase (Alp et al., 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 to NO, EDHF-mediated arteriolar dilation is believed to be less sensitive to oxidative stress. In this 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 (Wolffle et al., 2005). Ellis et al. described an augmented, EDHF-mediated dilation of small mesenteric arteries both in wild type and LDL receptor knockout mice fed 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
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, while the magnitude of EDHF-mediated dilation was reduced, due to impaired connexin-dependent cell-to-cell signaling (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 hyperglycemia. Moreover, high fructose containing and atherogenic diet in swine was associated with reduced BK$_{Ca}$ channel-mediated dilation of coronary arteries (Borbouse et al., 2009; Dimitropoulou et al., 2002). These observations suggest a pathological role for high glucose and
atherogenic dyslipidaemia in the development of impaired $\text{BK}_{\text{Ca}}$ channel function and consequently diminished arterial dilation. It is known that $\text{BK}_{\text{Ca}}$ channels are primarily expressed in vascular smooth muscle cells, where they are activated by high concentrations of local $[\text{Ca}^{2+}]_i$ (10-100 μM), also called Ca$^{2+}$ sparks, reviewed in (Jaggar et al., 2000). It is possible that the impaired $\text{BK}_{\text{Ca}}$ 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 $\text{K}^+$ 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 $\text{K}_{\text{Ca}}$ channel could persist early on in obesity, while 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 $\text{K}_{\text{Ca}}$ channels is insufficient to block the EDHF response in small arteries (Dong et al., 2000; Griffith, 2004). Thus, one can speculate that in disease, $\text{K}_{\text{Ca}}$ channels that are less sensitive for harmful stimuli may replace the function of other, likely more susceptible $\text{K}_{\text{Ca}}$ channels to maintain the overall EDHF response and vasodilation. Inhibition of $\text{K}_{\text{Ca}}$ channels may occur via the oxidation of critical SH groups (Cai et al., 1997). It is plausible that $\text{SK}_{\text{Ca}}$, $\text{IK}_{\text{Ca}}$ and $\text{BK}_{\text{Ca}}$ 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 $\text{K}_{\text{Ca}}$ 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 KC$_{Ca}$ 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 et al., 2005; Kadowaki et al., 2005). In obesity, the normal endocrine function of adipocytes is altered and may be manifested as reduced adiponectin (Kadowaki et al., 2005) and elevated circulating and tissue levels of leptin, resistin, interleukin 6 and TNFα (Berg et al., 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/l) attenuated dilation to ACh of coronary arteries in normal dogs, whereas physiological concentrations (250 pmol/l) 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 which was however independent from increased ROS production and was not affecting endothelial production of NO or PGI₂ (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 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 details in other papers in this themed issue and the reader is directed to a more detailed comprehensive recent review (Li et al., 2010).
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 signaling 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 et al., 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 hemodynamic 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, since 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. 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 positron emission tomography (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, pre-menopausal women with similar levels of obesity exhibited a higher myocardial blood flow at baseline when compared to lean subjects, while 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.
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 to lean individuals (Schindler et al., 2006). While 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 hyperemic 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 (Borbouse et al., 2010; Setty et al., 2003). 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 (Chilian, 1997; Jones et al., 1995). 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 man. These aforementioned underscore the need for direct examination of the function of coronary resistance arteries to understand how their vasomotor behavior 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 (Fulop et al., 2007; Szerafin et al., 2006) or from the heart of animals with genetically-induced (Bagi et al., 2004a) or diet-induced, experimental obesity (Feher et al., 2010; Jebelovszki et al., 2008). In this preparation, isolated arterioles develop a spontaneous myogenic tone in response to increases in intraluminal 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 to 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 to 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 nitric oxide (NO) and endothelium-derived hyperpolarizing factor (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$_2$O$_2$ (Matoba et al., 2003). Coronary arterial microvessels from the human heart also generate H$_2$O$_2$ from endothelial cells as a major contributor of coronary arteriolar dilation (Miura et al., 2003). The underlying mechanism of H$_2$O$_2$-mediated dilation varies, but studies show that H$_2$O$_2$ 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$_2$O$_2$, via eliciting K$_{Ca}$ channel activation potentially acts as an EDHF in coronary microvessels (Feletou et al., 2006; Shimokawa et al., 2004). Other studies demonstrate that H$_2$O$_2$-induced vasodilation is mediated through the release of NO from the endothelium (Hirai et al., 2000) or is partially mediated by cGMP formation in vascular smooth muscle cells (Fujimoto et al., 2001). Regardless of the mechanisms of action, H$_2$O$_2$ could maintain dilator function of coronary arterioles in obesity, even if the availability of NO is compromised (Figure 1 and 2). Interestingly, a study by Saitoh et al. indicates that H$_2$O$_2$, released from cardiac myocytes, couples coronary blood flow to myocardial oxygen consumption (Saitoh et al., 2006), providing further importance for H$_2$O$_2$ in the coronary circulation. Whether or not cardiac myocyte derived H$_2$O$_2$ 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, sodium nitroprusside, 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 signaling in obesity. Chronic oxidative and nitrosative stress can lead to inactivation of sGC over time (Munzel et al., 2005) and also could 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_{2}O_{2}-dependent activation of vascular K_{Ca} channels may be able to compensate for the reduced NO-mediated signaling 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_{2}O_{2}, which serves 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, interleukin 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 (Halberg et al., 2009; Trayhurn et al., 2008). HIF-1α may lead to up-regulation of various inflammatory adipokines, such as interleukin-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 et al., 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 protein kinase C 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 A2- 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 to other types of microvessels in the body. Whether these vasoregulatory pathways in adipose tissue microvessels are similarly affected by obesity as compared to 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 to 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 perivascular adipose tissue, 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
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), angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (McFarlane et al., 2003) and insulin sensitizers, metformin and thiazolidinediones (TZDs) (Hill et al., 2009; Jay et al., 2007). 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. 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 coenzyme A (HMG-CoA) reductase, the rate-limiting 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.*, twenty-four obese male subjects were randomly assigned to receive pitavastatin (2 mg/day) 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 *et al.*, 2010). 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 (Mason *et al.*, 2004; Wassmann *et al.*, 2002). In this context, treatment of obese Zucker rats with rosuvastatin (25 mg/kg 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) 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 (BH₄) levels.
Interestingly, the effects of pitavastatin was due to the down-regulation of type 1 angiotensin II (AT1) receptor and NADPH-oxidase subunit, gp91phox expression and also to the upregulation of GTP cyclohydrolase I, the rate limiting enzyme of BH4 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 interleukin-10 and TNFα in these animals (Goodwill et al., 2009). Rosuvastatin treatment (2 mg/kg 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 one-day of treatment (2 mg/kg). 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 signaling 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 et al., 2007). Both ACE-inhibitors and angiotensin receptor blockers (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) 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 mg/kg and 10 mg/kg, 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), 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) in epineuronal arterioles (Davidson et al., 2009). The dual ACE and NEP inhibitor, mixanpril (25 mg/kg) restored femoral blood flow to insulin in obese Zucker rat and was shown to be more effective than captopril treatment (2 mg/kg) alone (Arbin et al., 2001). The beneficial effect of dual ACE/NEP inhibitor, AVE 7688 (30mg/kg) over the ACE inhibitor, ramipril (1 mg/kg) 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 perivascular adipose tissue (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. They found that metformin treatment improved ACh-stimulated and flow-induced dilation of the brachial artery (Mather et al., 2001). Since 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/kg, 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, 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 et al., 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 et al., 2000). As highlighted in this article, 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 et al., 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 beta-carotene as antioxidants (Rapola et al., 1996; Rapola et al., 1998; Virtamo et al., 1998). More concerning yet, an increased harm from supplemental vitamin E, vitamin-A, and beta-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. 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 pharmacologies 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 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 (Gonzalez et al., 2003; Libby et al., 2002). Pro-inflammatory adipokines, such as interleukin-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 H2O2-dependent activation of KCa-channels) undoubtedly requires further investigation. Studies have yet to be performed to clearly distinguish between the vascular bed specific differences of ROS-mediated signaling, 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 signaling may provide further burden to those mechanisms, which are maintaining vascular function in disease.
GRANTS

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CONFLICT OF INTEREST

The authors have nothing to declare.
References


peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. 


TABLE 1: Clinical (a) and animal studies (b) investigating the impact of obesity on vasomotor responses.

**a) Obese Humans**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Vascular bed</th>
<th>Response</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kapiotis et al., 2006</td>
<td>brachial artery</td>
<td>FMD</td>
<td>↓</td>
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<tr>
<td>Hashimoto et al., 1998</td>
<td>brachial artery</td>
<td>FMD</td>
<td>↓</td>
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<tr>
<td>Kreutzenberg et al., 2003</td>
<td>brachial artery</td>
<td>FMD</td>
<td>↓</td>
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<td></td>
<td>forearm res. artery</td>
<td>BK</td>
<td>↓</td>
</tr>
<tr>
<td>Karpoff et al., 2009</td>
<td>brachial artery</td>
<td>FMD</td>
<td>↓</td>
</tr>
<tr>
<td>Romero-Corral et al, 2010</td>
<td>brachial artery</td>
<td>FMD</td>
<td>↓</td>
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<tr>
<td>Sivitz et al., 2007</td>
<td>forearm res. artery</td>
<td>ACh</td>
<td>↓</td>
</tr>
<tr>
<td>Martin et al., 2005</td>
<td>myocardium (PET)</td>
<td>MBF (rest)</td>
<td>↓</td>
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<td></td>
<td></td>
<td>MBF (CPT)</td>
<td>↓</td>
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<tr>
<td>Peterson et al., 2008</td>
<td>myocardium (PET)</td>
<td>MBF (rest)</td>
<td>↑</td>
</tr>
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<td>myocardium (PET)</td>
<td>MBF (rest)</td>
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<td></td>
<td></td>
<td>MBF (MFR)</td>
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</tr>
<tr>
<td>Schindler et al., 2006</td>
<td>myocardium (PET)</td>
<td>MBF (rest)</td>
<td>↔</td>
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<td></td>
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<td>MBF</td>
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<tr>
<td></td>
<td></td>
<td>(MFR/CPT)</td>
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<td>Wang et al., 2006</td>
<td>myocardium (MRI)</td>
<td>MBF (rest)</td>
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<td></td>
<td>MBF (ADO)</td>
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### b) Experimental Obesity

<table>
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<td>ACh</td>
<td>↓</td>
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<td>ACh</td>
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<tr>
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<td>OZR, mesenteric (≈30-wk)</td>
<td>ACh</td>
<td>↓</td>
</tr>
<tr>
<td>Young et al., 2008</td>
<td>OZR, mesenteric (≈12-wk)</td>
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<td>↓</td>
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<td>Ellis et al., 2008</td>
<td>HFD, mesenteric (mouse)</td>
<td>ACh</td>
<td>↔</td>
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<td>HFD, mesenteric (rat)</td>
<td>ACh</td>
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<td>JCR:LA-cp rat, mesenteric</td>
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<td>Frisbee et al., 2001</td>
<td>OZR, cremaster muscle</td>
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<td>↓</td>
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<td>ACh</td>
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<td>ACh</td>
<td>↔</td>
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<tr>
<td>Woodman et al., 2004</td>
<td>HFD, coronary (pig)</td>
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<td>Henderson et al., 2004</td>
<td>HFD, coronary (pig)</td>
<td>BK</td>
<td>↔</td>
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<tr>
<td>Setty et al., 2003</td>
<td>HFFD, coronary blood flow</td>
<td>CBF (rest)</td>
<td>↔*</td>
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*Although no change in CBF was detected the authors found an impaired relationship between CBF and myocardial oxygen consumption, MVO(2).
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<td>Nagashima, 2010</td>
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<td>Pitavastatin [2 mg]</td>
<td>Restored FMD after oral fat loading test</td>
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<td>Effect/Mode of Action</td>
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</tr>
<tr>
<td>Oltman, 2008</td>
<td>OZR; epineural arterioles</td>
<td>Enalapril [20 mg/kg]</td>
<td>↑ ACh dilation</td>
</tr>
<tr>
<td>Davidson, 2009</td>
<td>OZR; epineural arterioles</td>
<td>AVE 7688 [30 mg/kg]</td>
<td>↑ ACh dilation</td>
</tr>
<tr>
<td>Arbin, 2001</td>
<td>OZR; femoral artery</td>
<td>Mixanpril [25 mg/kg]</td>
<td>↑ insulin induced blood flow</td>
</tr>
<tr>
<td>Russell, 2004</td>
<td>Obese JCR:LA-cp rat; aorta, coronary artery</td>
<td>AVE 7688 [30 mg/kg]</td>
<td>↑ coronary BK dilation</td>
</tr>
<tr>
<td>Russell, 2004</td>
<td>Obese JCR:LA-cp rat; aorta, coronary artery</td>
<td>Ramipril [1 mg/kg]</td>
<td>↑ coronary BK dilation</td>
</tr>
<tr>
<td>Walker, 1999</td>
<td>OZR; mesenteric artery</td>
<td>Rosiglitazone [50 µM/kg]</td>
<td>↑ ACh and insulin dilation</td>
</tr>
<tr>
<td>Bagi, 2004a</td>
<td>db/db mice; coronary arteriole</td>
<td>Rosiglitazone [3 mg/kg]</td>
<td>↑ ACh, NONOate,</td>
</tr>
</tbody>
</table>

FIGURE LEGENDS

Figure 1. Endothelial and smooth muscle-dependent signaling 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 nitric oxide (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 hydrogen peroxide (H$_2$O$_2$) by superoxide dismutase, and it may activate various calcium-activated potassium channels, such as SK, IK and BK channels or the soluble guanylate cyclase (sGC) to maintain dilator function of arterioles, particularly in coronary microvessels.

Figure 2. Effects of adipokines on vasomotor function of arterioles. This schematic 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). Reactive oxygen species (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$_2$O$_2$ serves as a potent vasodilator in the coronary arterioles, which aims to maintain vasodilator responsiveness in the coronary microcirculation.

**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, tumor necrosis factor $\alpha$ (TNF$\alpha$) and interleukin-6 (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 nitric oxide (NO) and reduces dilator function of microvessels. Altered microvascular responsiveness may provoke hypoxia in the expanded adipose tissue, which via hypoxia inducible factor-1$\alpha$ (HIF-1) would exaggerate pathological changes in adipokine production. Adipocytes may also enhance monocyte chemotactic protein-1 (MCP-1) synthesis, which facilitates macrophage (m$\Phi$) accumulation and activation, to maintain oxidative stress and adipose tissue inflammation. The adipose tissue renin-angiotensin-system (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, angiotensin converting enzyme inhibitors (ACEI) and neutral endopeptidase 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 signaling has yet to be elucidated.
Figure 1

Endothelium:
- Ang II
- ET-1
- Leptin, TNF
- Resistin, IL-6

NOX → XOX → NOS/BH4 → O₂⁻ → NO → H₂O₂ → ONOO⁻ → SK/IK

Smooth muscle:
- BK
- sGC

 vasodilation

↓
Figure 2
Figure 3