Mechanisms of Coronary Microvascular Adaptation to Obesity

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ABSTRACT

Metabolic syndrome (MetS) is associated with clustering of cardiovascular risk factors in individuals that may greatly increase their risk of developing coronary artery disease. Obesity and related metabolic dysfunction are the driving force in the prevalence of MetS. It is believed that obesity has detrimental effects on cardiovascular function, but its overall impact on the vasomotor regulation of small coronary arteries is still debated. Emerging evidence indicates that in obesity coronary arteries adapt to hemodynamic changes via maintaining and/or up-regulating cellular mechanism(s) intrinsic to the vascular wall. Among other factors, endothelial production of cyclooxygenase-2-derived prostacyclin and reactive oxygen species, as well as increased NO sensitivity and potassium channel activation in smooth muscle cells have been implicated in maintaining coronary vasodilator function. This review aims to examine studies that have been primarily focused on alterations in coronary vasodilator function in obesity. A better understanding of cellular mechanisms that may contribute to coronary microvascular adaptation may provide insight into the sequence of pathological events in obesity and may allow the harnessing of these effects for therapeutic purposes.

Key words: obesity, coronary, arteriole, H$_2$O$_2$, COX-2
Introduction

Metabolic syndrome (MetS) is associated with a clustering of cardiovascular risk factors in individuals that may greatly increase their risk of developing ischemic heart disease and heart failure. Abnormalities in the vasomotor function of the coronary microvessels occurs in MetS; and in some instances these abnormalities represent important markers of risk or may even contribute to the pathogenesis of myocardial dysfunction. Obesity and its related metabolic dysfunction are the driving force in the prevalence of MetS and the development of type 2 diabetes. The coronary microcirculation is currently being therapeutically targeted aiming to prevent or delay the development of cardiac contractile dysfunction, heart failure and ischemic heart disease, which remains the major challenge in reducing morbidity and mortality in patients with MetS.

However, the impact of obesity on coronary vasomotor regulation and the underlying mechanisms are not completely understood and remain controversial. The first part of this review aims to examine studies that have been focused on alterations in coronary vasodilator function in obesity and MetS, and raises the possibility that during the progression of MetS, obesity and/or obesity related metabolic and hemodynamic changes initiate adaptation of coronary vasodilator function. The second part describes potential, endothelium-, smooth muscle and adipocyte-dependent cellular mechanisms that may contribute to obesity related to coronary vascular adaptation. It is clear that further investigation of the regulation of coronary microvascular function is necessary in view of the limited information in the literature describing changes in the coronary microcirculation and controversial findings in recent clinical studies.
Progression of coronary vasomotor dysfunction in MetS

Although acute and chronic ischemic syndromes are commonly due to coronary flow-limiting atherosclerotic plaques in epicardial coronary arteries, about 10 to 20% of patients with cardiac symptoms undergoing cardiac catheterization are found to have normal coronary angiograms (25). It has been demonstrated that despite the presence of angiographically normal coronary arteries coronary flow reserve is reduced in diabetic patients (117). Thus, it has been suggested that epicardial atherosclerosis may not be the only underlying pathology resulting in abnormal coronary flow reserve in diabetic patients (114, 117). In type 2 diabetic patients impaired acetylcholine (ACh)-induced, endothelium-dependent relaxation of brachial artery (39) and forearm resistance vessels has been reported earlier (56, 102, 140). Performing quantitative angiography, Nitenberg at al has demonstrated impaired coronary dilation using the cold pressor test in type 2 diabetic patients with angiographically normal coronary arteries (116). Using similar methodology, Kaneda et al have performed a study, in which 165 patients underwent intracoronary injection of ACh and found that diabetes was the strongest predictor for ACh-induced vasospasm, as an indicator of endothelial dysfunction (83). Moreover, in coronary arterioles isolated from the heart of diabetic patients, Miura et al demonstrated an impaired hypoxia and ATP-dependent, potassium channel-mediated vasodilation (108). Thus, strong evidence indicates that in MetS development of diabetes mellitus is associated with impaired vasodilator responses of both peripheral and coronary microvessels.

Data obtained in animal models of MetS also indicate that presence of fasting hyperglycemia, hence presence of manifest diabetes, impairs coronary vasodilation to agonists and to increases in intraluminal flow (24, 26, 31, 99, 135). Oltman et al have demonstrated that in Zucker diabetic fatty (ZDF) rats coronary arteriolar dilation to ACh is diminished, whereas dilation in pre-
diabetic, younger (8-12 weeks old) animals is preserved (120). Of note, in pre-diabetic obese Zucker rats or in animals fed a high fat diet, with mild elevation of fasting glucose levels, impaired vasodilator function has been reported in vessels from the mesentery (120), cerebral (43, 44) and skeletal muscle vascular beds (41, 48). In contrast, in the obese Zucker rats and also in animals fed a high fat diet, recent studies found preserved (70, 78, 91) or even augmented coronary dilations (123). Thus, it seems that during the progression of MetS while coronary vasomotor function is protected before the development of type 2 diabetes, peripheral microvessels exhibit impaired vasomotor regulation.

To address this discrepancy it has been posited that, as compared to the vascular beds of the periphery, coronary microvessels are more “resistant” to development of vasomotor dysfunction (88). This implies, that coronary vessels either have efficient mechanisms, which protect their vasomotor function, or that coronary vessels have mechanisms that can actively compensate for the loss of vasomotor pathways, as metabolic disease progresses (50, 138). Since in the coronary circulation oxygen extraction is near maximal (141), any impairment in arteriolar dilator function could have significant consequences on myocardial perfusion. As described by Chilian, the coronary circulation matches blood flow with oxygen requirements by coordinating the resistances within different-sized vascular beds, each governed by distinct regulatory mechanisms (28, 80). Such integration in the coronary circulation is believed to be advantageous because the system does not rely on a single mechanism of control, i.e. myogenic, flow or metabolic regulation of vascular resistance (109). An integration of vasomotor regulatory systems in the coronary circulation seems especially important in obesity and MetS, conditions in which metabolic and hemodynamic changes require adaptation of coronary vasomotor regulation.
In MetS there could be several factors that can be implicated necessitating adaptation of coronary vessels. In MetS, the impact of these pathological factors is difficult to discern owing to the close interrelationships between obesity, insulin resistance, type 2 diabetes, hypertension and other known and as yet unidentified pathological factors (34, 35). Yet, several previous and recent studies raised the possibility that the early adaptation of the coronary circulation can be attributed specifically to obesity and/or obesity-related changes in metabolic and hemodynamic regulation. On the other hand, adaptive vasomotor responses in the coronary circulation may decline as MetS progresses and other co-morbid diseases develop, such as, severe insulin resistance, hypertension and fasting hyperglycemia (diabetes). This may lead to limited vasomotor function (both dilator and constrictor functions can be diminished at advanced state of a disease) of coronary microvessels that are primarily responsible for adjusting cardiac perfusion to actual metabolic demand (Figure 1).

**Obesity and cardiovascular regulation**

This review makes no attempt to provide a detailed description of the impact of obesity on complex hemodynamic regulation or the functional and structural changes of the heart but refers to a comprehensive recent review (1). Of particular importance, is the widely accepted view that obesity is independently associated with left ventricular hypertrophy. A large body of evidence indicates that an increase of left ventricular mass, in the long term, leads to diastolic and systolic cardiac contractile dysfunction in obese patients (1). It has been also posited that in “uncomplicated” (lack of co-morbid conditions such as hypertension, diabetes etc.) obesity-associated increases in left ventricular mass can be appropriate for body size (75). Thus, early “physiological” adaptation of cardiac function can be envisioned, which will accommodate for
the higher hemodynamic and metabolic demand in obesity. It is known that any increase in body mass (muscular or adipose tissue) requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements (95). It is also believed that obesity is associated with a hyperdynamic circulation and increased cardiac output (95). Correspondingly, a study by Jern et al has demonstrated that cardiac output and stroke volume were positively related to body mass index (BMI), but inversely to waist/hip ratio. They also found that total peripheral resistance was inversely correlated to BMI, whereas high waist/hip ratio was associated with higher systemic vascular resistance (79). This implies that increased BMI can be associated with increased cardiac output and lower peripheral vascular resistance, but visceral obesity, which is the case in many obese patients, is associated with lower cardiac output and higher total peripheral resistance. Whether these changes can be attributed to an altered cardiac structure or contractile dysfunction or whether they can be related to alterations in the function of coronary and peripheral resistance vessels is not known. The impact of obesity on complex cardiovascular regulation over the course of progression of MetS clearly requires further mechanistic investigations.

Impact of obesity on coronary vasodilator function

Morphological changes in microvessels are quite rare in obesity prior to the development of hyperglycemia. Obesity-related pathological alterations, including atherogenic dyslipidemia, insulin resistance and hyperinsulinemia are believed to impair the vasomotor function of small arteries. However, blood flow to the various organs systems is rarely impaired in obesity, unless atherosclerosis of the arteries develops. Throughout life, organs receive normal or even greater than normal blood flow in obese subjects (66). Yet, convincing evidence of the impact of obesity
on vasomotor regulation of coronary microvessels is lacking at present. Such demonstration is hampered by issues regarding direct investigation of coronary microcirculation both in humans and animal models (28, 124); and also by the impact of several, combined risk factors present in obesity.

Studies on obese patients. Central obesity was found to be associated with reduced bradykinin- or hyperemia-induced forearm blood flow (67, 146). It has been shown that obese children already exhibit impaired brachial artery relaxation to hyperemic flow (84). Forearm resistance vessels also exhibited reduced acetylcholine and NO-donor (sodium nitroprusside)-induced dilations in obese humans (133). Interestingly, it has been posited that body fat distribution, rather than body weight increase is responsible for the impaired brachial artery dilation (67) and elevation of peripheral vascular resistance in obesity (79), an idea, which is further supported by a theoretical analysis using physiological measurements obtained in obese patients (46).

Only a limited number of studies are available that investigated alterations in vasomotor responses of coronary microvessels in obese patients. Because other studies (2, 139) have demonstrated a close association between coronary vasomotor function and relaxation of brachial artery it was speculated that obesity may also adversely affect coronary dilations. Indeed, myocardial blood flow, as measured by positron emission tomography, was found to be significantly reduced in postmenopausal women with obesity, which was negatively correlated with waist/hip ratio (100). Moreover, the study by Fulop et al has found that in isolated coronary arterioles of normotensive patients, obesity was associated with a reduction of agonist-induced coronary dilations (50). These observations are in line with the literature, suggesting detrimental effects of obesity on vasomotor responses (67, 84). However, the study by Fulop et al have found
that in the simultaneous presence of hypertension and obesity, coronary arteriolar dilations to bradykinin and the NO-donor, sodium nitroprusside, were markedly enhanced and also these dilations were positively correlated with BMI in these patients (50). Alterations in coronary arterioles were mirrored in large peripheral arteries in that there was a positive correlation between the flow-mediated and nitroglycerin-induced dilations of the brachial artery (50). In other studies, coronary microvessels dissected from the hearts of diabetic patients also exhibited preserved (108) or even enhanced, endothelium-dependent dilations (138).

These observations indicated that obesity, especially in the presence of co-morbidities, such as hypertension and diabetes, is not necessarily associated with impaired vasodilator function of coronary microvessels. On the contrary, it is possible that the presence of obesity has potentially a key role in maintaining and augmenting vasodilator capacity of coronary microvessels. Interestingly, clinical studies on obese patients with coronary heart disease have found an unexpectedly favorable prognosis on acute cardiovascular outcome, with the worst prognosis associated with either underweight or morbidly obese patients (64, 65, 73). Although obesity is widely accepted as a risk factor for coronary heart disease and heart failure, emerging evidence supports a protective role of obesity once patients have developed cardiovascular disease (65, 73). Whether this protection can be related, at least in part, to the adaptive responses in the coronary vascular beds, also described in this paper, is yet to be tested in future investigations.

**Studies in animal models of obesity.** Before providing a description of animal studies of obesity, it is worth to briefly highlight the models frequently used to study obesity-related non-vascular and vascular pathologies. In evaluating results obtained in animal models of obesity it is also important to bear in mind that similar to the situation in humans, obesity is frequently associated
with co-morbid diseases, such as elevated blood pressure. Also, it is important to note that even in the absence of fasting hyperglycemia, animals with insulin resistance are usually characterized by elevated postprandial glucose levels, thus the potential pathological role of high glucose concentrations cannot be entirely excluded. 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. For instance, in ob/ob mice (leptin gene deficient) obesity and hyperinsulinemia develop shortly after weaning. In db/db mice (leptin receptor deficient) fasting hyperglycemia develops as early as 6-7 weeks of age. The obese Zucker rat has a similar genetic abnormality in leptin signaling and exhibits obesity and insulin resistance with no or relatively mild fasting hyperglycemia, as they age. The JCR:LA-cp rat is also characterized by obesity, insulin resistance, hyperinsulinemia and hypercholesterolemia. Furthermore, high fat feeding has been used to study the vascular effects of obesity in different animals.

Vasomotor dysfunction described in animal models of obesity is similar in characteristics that are observed in obese patients. This similarity also applies to the discrepant findings obtained in various vascular beds, in different models of obesity (Table 1). In the obese, JCR:LA-cp rats an impaired endothelium-dependent relaxation of aorta to A23187 (17) and reduced dilations of mesenteric arteries to ACh (118) have been reported. Reduced mesenteric (112) and skeletal muscle (41) arteriolar dilation to ACh was also found in rats fed a high fat diet. In obese Zucker rats it has been reported that in mesenteric arterioles endothelium-dependent relaxation to ACh was preserved at 20-week old, but was reduced in 32-week old animals (137). In a similar experimental design, Oltman et al have investigated the progression of coronary and mesenteric arterial dysfunction in the Zucker obese rat. They found that coronary arteriolar dilation to ACh was preserved in 16-24 week old animals, but dilations
became diminished in 28-36 week old rats, when compared to lean controls (120). Mesenteric arterioles of Zucker rats exhibited relatively maintained dilations to ACh at both ages (120). Katakam et al reported that in 12-week old obese Zucker rats ACh-induced dilations of small coronary arteries was preserved, although they found a reduced vasodilation to insulin (85). These studies concluded that arteriolar dilations in obese animals can be preserved at younger age (i.e. at the early state of disease), but vascular dysfunction progresses and this progression can be at a different rate in different vascular beds, such as in coronary and mesenteric vessels.

More importantly, the early study by Subramanian et al found that relaxation of aorta to ACh is enhanced at ages of both 20 and 32-week old obese Zucker rats animals (137), results similar to those observed earlier by Auguet et al (4). In mice fed a high fat diet, an enhanced endothelium-dependent, hydroxyl radical-induced relaxation in the femoral artery has been also reported (12). Coronary arterioles from female pigs fed a high fat diet exhibited only modest impairment of dilation to bradykinin (70), whereas coronary dilations to ACh were preserved in the obese Zucker rats (85) and in rats fed a high fat diet (78). More intriguing, Prakash et al have reported that ACh-induced dilations of coronary arterioles from obese Zucker rats were markedly enhanced (more than 25% increase in diameter, when compared to lean animals) (123). These latter observations imply that although coronary dysfunction progresses with obesity, coronary vasodilator function can be preserved or even augmented at early phases of the disease.

Collectively, on the basis of these aforementioned human and animal studies it is likely that coronary microvessels adapt to obesity by maintaining or enhancing their dilator function to increase coronary blood flow to higher metabolic demand. Emerging evidence indicate that hemodynamic adaptation is not a passive phenomenon, but requires active participation of
various cellular pathways, also at microvascular level. Understanding these cellular mechanisms seems important, not only because they provide insight into the sequence of pathological events in obesity, but also because they could be harnessed for therapeutic purposes.

**Cellular mechanisms of vascular adaptation in obesity**

**NO-soluble guanylate cyclase-cGMP pathway.** The vascular endothelium produces and secretes numerous compounds that regulate a variety of physiological functions, including vasomotor tone, coagulation, inflammation, permeability and cell adhesion (144). Among others, nitric oxide (NO) is considered to be one of the key molecules in maintaining normal vascular homeostasis and it is a major contributor to maintain adequate coronary microvascular tone (98). Solid evidence indicates that type 2 diabetes is associated with impaired bioavailability of NO both in conduit vessels and resistance arteries (8, 48, 70, 99, 128, 136, 147). Studies have also demonstrated that obese subjects exhibit a reduced NO-mediated, agonist-induced dilation of cerebral, mesenteric, coronary and skeletal muscle microvessels (42, 48, 113). Interestingly, Brandes et al reported that a reduced endothelial NO production by acute administration of an NO synthase inhibitor in wild type mice or chronic deficiency of NO in eNOS knockout mice increase NO sensitivity of vascular smooth muscle cells in response to exogenous NO donors (15). They have proposed that an increased sensitivity of soluble guanylate cyclase (sGC) to NO may compensate for the reduced NO synthesis (15). Because administration of exogenous NO decreased sGC activity acutely and over time its protein expression (130), it has been posited that NO may play an important, negative feedback regulatory role on the catalytic activity of the sGC, hence any reduction of NO level may lead to an enhancement of the sensitivity of sGC to
NO. To test this hypothesis, Jebelovszki et al provided evidence for an enhanced NO sensitivity of coronary arterioles isolated from obese rats fed a high fat diet (78). They found that the enhanced sensitivity of coronary arterioles to NO was associated with increased activity of sGC in coronary arterioles, in which a reduced NO bioavailability was also detected (78). Similar results were obtained in humans, showing that NO donor-induced coronary arteriolar and also brachial artery dilations were enhanced in patients with obesity and hypertension (50). Enhanced dilations of coronary arterioles to the NO donor, sodium nitroprusside have been also described in female pigs fed a high fat diet (155) and in mesenteric arterioles of obese Zucker rats (120).

Although it seems plausible that the lack of NO release may lead to an enhanced activity of sGC in vascular smooth muscle cells (15), other studies demonstrated that an acute exposure to reactive oxygen species, i.e. H2O2 could also lead to activation of sGC, contributing to the relaxation of the bovine pulmonary artery (18, 154). Moreover, Bauersachs et al have shown that rats with heart failure exhibit increased expression of sGC, which was due to the enhanced vascular superoxide anion production (10). Since obesity is also associated with oxidative stress (41, 120), it is likely that ROS, in addition to their effect in reducing NO availability, may play a role in the activation of sGC, a hypothesis, which has yet to be tested.

Collectively, these data suggest that an impaired endothelial NO availability in coronary arterioles can be associated with an enhanced sensitivity to NO in vascular smooth muscle cells and that this mechanism may lead to compensation of the reduced NO-mediated vascular signaling in obesity. On the other hand, it has been demonstrated that oxidative and nitrosative stress can lead to inactivation of sGC over time (111); thus the question remains to be answered to what extent and how long up-regulation of sGC may compensate for the reduced NO-mediated vascular signaling, as obesity and MetS progress.
**EDHF - potassium channel-activation in obesity.** It is known that in addition to NO, other mechanisms may contribute to dilations of coronary microvessels, such as the endothelium-derived hyperpolarizing factor (EDHF) (108). There is a paucity of data in the literature investigating alterations in EDHF-mediated coronary microvascular responses in obesity. Unlike NO, EDHF-mediated arteriolar dilation is believed to be less sensitive to oxidative stress; and dilations mediated by EDHF can persist and may even compensate for the loss of other vasodilator pathways in obesity. For example a study by Wolfle and de Wit has found that mice even under severe hypercholesterolemic conditions (ApoE and LDL receptor-deficient mice fed a high-cholesterol diet) exhibited a preserved EDHF-mediated, endothelium-dependent dilation to ACh in the cremaster muscle arteriole, in vivo (152). Moreover, the study by Ellis et al has found an augmented, EDHF-mediated vasodilation of small mesenteric arteries both in wild type and LDL receptor knockout female mice fed a high fat diet (38). It is known that opening of the Ca\(^{2+}\)-activated potassium (K\(_{Ca}\)) channels (small, intermediate and large conductance K\(_{Ca}\) channels) plays a crucial role in EDHF-mediated vasodilation (19, 22, 37, 108). Thus, it can be assumed that K\(_{Ca}\) channels function is preserved in obesity. Correspondingly, in the study be 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 high fat diet-treated mice (38). In contrast, in fructose fed, insulin resistant (but not obese) rats Miller et al have reported that ACh-induced small coronary artery dilation was reduced due to decreased responses by K\(_{Ca}\) channels (104). In mesenteric arterioles of type 2 diabetic ZDF rats, Burnham at al demonstrated previously that the large conductance, BK\(_{Ca}\) channel- and also the small conductance, SK\(_{Ca}\) channel-mediated arteriolar relaxation are significantly impaired (20, 21), but no abnormalities in
BK$_{Ca}$ and SK$_{Ca}$ channel function were detected in younger (5-6 week old) animals that already developed insulin resistance. Yet, a very recent study by Young et al has demonstrated that non-diabetic obese Zucker rats exhibit a reduced EDHF-mediated dilation in small mesenteric arteries, but this alteration was due to the impaired connexin-dependent cell-to-cell signaling, rather than changes in K$_{Ca}$ channel function (156). Collectively, limited number of studies indicate that EDHF-mediated, K$_{Ca}$ channel-dependent dilation may be impaired in peripheral vessels in obesity, but future studies are needed to confirm this phenomenon in coronary microvessels, both in humans and animal models.

**Reactive oxygen species in coronary microvascular adaptation.** Oxidative stress occurring in response to hyperglycemia (6-9, 14, 40, 129) and hypertension (76, 142, 143) is considered to be one of the key factors leading to microvascular vasomotor dysfunction in advanced stages of MetS. Evidence supports that even before the development of fasting hyperglycemia and manifest type 2 diabetes, hyperinsulinemia (42) and obesity (41, 43) are also associated with an increased vascular production of reactive oxygen species (ROS). Studies aiming to detect the impact of free radical production on coronary arteriolar dilations, however, yielded conflicting results in animal models of obesity. Oltman et al have found that a free radical scavenger, tiron, restored dilations of coronary arterioles to the level of lean animals (120), suggesting a crucial role for ROS in reducing ACh-induced responses. Rats fed a high fat diet exhibited enhanced vascular production of ROS, which was associated with reduced ACh- and histamine-induced arteriolar dilations of skeletal muscle arterioles; and the responses were restored by the ROS scavenger, tiron (41). In contrast, Katakam et al (85) and Jebelovszki et al (78) were unable to demonstrate impaired coronary dilation of obese Zucker rats and rats fed a high fat diet, in spite
of the presence of enhanced ROS production in these models. Although studies found that ROS-dependent inactivation of NO leads to a reduced agonist-induced dilation of cerebral, mesenteric and skeletal muscle microvessels (6, 14, 48), these latter observations indicated that coronary arteriolar dilations can be resistant to oxidative stress, but the nature of mechanism(s) responsible for this “resistance” remained obscure.

One theory that may explain these discrepant findings is the high subcellular and cellular compartmentalization of ROS production. Normally, effective antioxidant systems (SOD isoforms, catalase and glutathione peroxidase etc.) limit ROS production, for instance, by preventing the interaction between superoxide anion and NO (153). During pathological conditions, such as in obesity, the production of ROS may exceed the capacity of antioxidant mechanisms, which could have detrimental effects on vasomotor regulation, such as reduced availability of NO. The above-described experimental evidence showing that responsiveness of vascular smooth muscle cells to exogenous NO may be preserved or could be augmented in obesity, however, indicated that the excess production of ROS is mainly localized to endothelial, but not vascular smooth muscle cells in coronary microvessels.

On the other hand, emerging evidence indicates that ROS may act as a positive regulator of endothelial signaling pathways, both under normal and pathological conditions (63, 94). For instance, Matoba et al demonstrated that a major dilator factor released from the endothelium of porcine coronary microvessels is the ROS derivate, H$_2$O$_2$ (101). Coronary arterial microvessels from the human heart – likely to be affected by existing disease, such as obesity – also generate H$_2$O$_2$ from endothelial cells, as a major contributor of coronary arteriolar dilation (107). Thus, in addition to inhibitory action of superoxide anion on NO, H$_2$O$_2$ may actively participate in endothelium-dependent vasodilation. 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 (32, 101, 107). Thus, it has been proposed that H$_2$O$_2$, via inducing K$_{Ca}$ channels-mediated endothelium-dependent hyperpolarization, potentially acts as an EDHF (45, 131). Other studies demonstrated that H$_2$O$_2$-induced vasodilation is mediated through the release of NO from the endothelium (72) or is partially mediated by cGMP release in vascular smooth muscle cells (49). Regardless of the mechanisms of action, endothelial production of H$_2$O$_2$ can be one of the key molecules, which may compensate for the loss of dilator function of coronary arterioles and may contribute to vascular adaptation during the progression of obesity (Figure 2).

**Up-regulation of cyclooxygenase-2 and coronary adaptation.** It is clear that several cardiovascular diseases are associated with a state of chronic, low-level inflammation (58, 96). A crucial role of cyclooxygenase (COX)-derived prostaglandins in vascular inflammatory responses has been well established (115). A possible role for prostaglandin-mediated, low-level vascular inflammation has been also described in type 2 diabetes and obesity (52, 69, 70, 134, 150). Recently, a great deal of attention has been devoted to the complexity of molecular events regulating vascular prostaglandin synthesis. For a long time, it was the view that COX-1 was constitutively expressed in most tissues, such as vascular endothelial cells, and was involved in maintenance of cellular homeostasis (105, 106, 145). In contrast, the expression of COX-2 is very low in the endothelium and in smooth muscle cells under normal conditions (33). Importantly, COX-2 can be readily up-regulated by inflammatory, mitogenic and physical stimuli (121). Obesity and type 2 diabetes are associated with low-grade vascular inflammation, thus it is possible that changes in vascular prostaglandin synthesis can be altered in these diseases and the changes can be attributed to alterations in the vascular expression of COX-2.
Correspondingly, recent studies provided evidence that COX-2 protein expression is elevated in the aorta of mice with obesity and type 2 diabetes (5, 62). Also, in diabetic patients vascular expression of COX-2 was found to be markedly elevated in coronary arterioles, which was associated with the enhanced production of dilator prostaglandins, most likely prostacyclin (PGI₂) or PGE₂ (138). These dilator prostaglandins, known to be essential in the mediation of dilator responses elicited by bradykinin, are key vasoactive mediators involved in the regulation of coronary blood flow (29, 60).

Growing body of evidence indicate that COX-2-derived PGI₂ production in endothelial cells is an important homeostatic response in order to accommodate the enhanced aggregability of circulating platelets (59) and vasomotor dysfunction of coronary arterioles (138) (Figure 2). Whether up-regulation of COX-2 and consequently enhanced PGI₂ production contribute to maintained coronary dilations in obesity has yet to be elucidated. Moreover, the potential mechanism(s) responsible for the up-regulation of COX-2 requires further investigation. The key role for high glucose concentrations on COX-2 expression seems to be established (30, 87). It has been found that in high glucose-treated mesangial (87) and endothelial cells (30) increased production of superoxide anion was primarily responsible for the enhanced COX-2 expression. Furthermore, it has been demonstrated that pro-inflammatory cytokines, such as interleukin-1α/β and tumor necrosis factor-α (TNFα) potentially induce COX-2 expression (105) by stabilizing COX-2 mRNA and enhancing transcription through nuclear factor (NF)-κB or peroxisome proliferator activated receptor-γ (PPARγ) (122). In this context, a previous study also identified a region of the COX-2 gene promoter containing a PPAR-response element in human epithelial cells (103). It has been found, that in rat vascular smooth muscle cells in culture, the PPARγ activator, rosiglitazone, increased the expression of phospholipase A₂ and COX-2 (13). In
contrast, in vascular smooth muscle cells in culture PPARγ activation inhibited angiotensin II-
induced COX-2 expression (74). These findings indicate a potential impact of PPARγ activation
on COX-2 expression and prostaglandin formation. Hence, studies have yet to be performed to
elucidate the specific roles of PAPRγ in the regulation of vascular expression of COX-2 and altered synthesis of PGI₂, which may affect platelet aggregation and also coronary vasomotor
responses in obesity. These studies also underline the need for clinical investigations addressing
the possible effects of drugs interfering with COX-2-mediated and/or PPARγ-dependent
prostaglandin synthesis, particularly in obese patients.

**Role of adipocyte-derived factors in vascular adaptation.** Adipocytes perform an important
endocrine function by secreting numerous cytokines, hormones, and bioactive peptides (81). Upon secretion into the bloodstream, adipocyte-derived signaling molecules (e.g. adiponectin,
leptin, resistin, TNFα etc.) could have an important impact in other tissues such as muscle and
liver to regulate energy homeostasis and metabolism (81). In obesity, this normal function of
adipocytes can be altered and can easily result in reduced adiponectin (82) and elevated levels of
leptin, resistin and TNFα (11). Alterations in adipokine levels have been implicated in the
development of vascular dysfunction in obesity and they may also exert effects on endothelial-
and smooth muscle-dependent vasoregulatory mechanisms in coronary arteries.

In this context, it has been shown that leptin incubation promotes oxidative stress in cultured
endothelial cells (92). Knudson et al have reported that leptin, at higher concentrations (625
pmol/L), significantly attenuated dilation to ACh in isolated coronary rings of normal dogs (90).
Animals fed a high fat diet had elevated leptin levels, but they exhibited a preserved coronary dilatation to ACh (89). Moreover, in porcine coronary arteries exposure of another adipokine,
resistin elicited a reduced dilation to bradykinin, via eliciting oxidative stress (93). Dick et al have also found a reduced bradykinin-induced dilations of dog coronary arteries exposed to resistin, but the effect was independent from free radical production and was not affecting endothelial production of NO or PGI2 (36). These animal studies indicated that circulating adipokines, such as leptin and resistin may exhibit adverse effects on coronary vasodilator responses in obesity. Although higher leptin concentrations were found to be associated with impaired arterial distensibility in healthy adolescents (132), acute, subcutaneous administration of leptin unexpectedly increased flow-mediated dilations of brachial artery in non-obese adults (16). Also, in obese women leptin concentrations did not predict the impaired flow-mediated dilations of the brachial artery (119). Clearly, further studies are needed to solve this existing discrepancy between animal studies and human observations.

A growing body of evidence indicate, on the other hand, that peri-vascular adipose tissue through releasing a transferable non-lipid factor, may induce vasorelaxation and may counteract agonist-induced vasoconstrictor responses (97, 127). In this context, Gao et al have reported that peri-vascular adipose tissue releases a relaxing factor which induces endothelium-dependent relaxation of the rat aorta or the human internal thoracic artery through NO release and KCa channel activation (53, 55). Interestingly, they also proposed a potential involvement of H2O2 in this process, which may also activate sGC in the vascular smooth muscle cells (53). Somewhat contradictory, the same group reported that an increased production of superoxide anion from peri-vascular fat augmented contractile responses of mesenteric arteries (54).

It remained, however, unclear whether phenotypic changes of peri-vascular adipocytes may differently affect vasomotor responses, as obesity progresses. Interestingly, the vasorelaxant effect of peri-vascular adipose tissue was attenuated in spontaneously hypertensive rats, in the
study by Galvez et al (51). Although these aforementioned observations indicate adverse effects of circulating adipokines, such as leptin and resistin on coronary vasomotor function, they raise the possibility that they peri-vascular fat, via activating vasodilator mechanisms in vascular endothelial cells, can be protective (Figure 2). Whether vasomotor effects of peri-vascular fat-derived factors, via activating endothelium- and smooth muscle-dependent cellular pathways, are contributing to coronary microvascular adaptation in obesity and whether this regulatory function changes as obesity progresses, requires further investigation.

**Summary and clinical implications**

Although obesity is widely accepted as a risk factor for coronary heart disease and heart failure, evidence also supports a role for obesity in cardiovascular protection. A growing number of recent reports document a statistically significant survival benefit in obese patients once they have been diagnosed with cardiovascular diseases (64, 65, 73). The conclusion that obesity may both elicit cardiovascular disease and protect from cardiovascular death now clearly requires further investigation at cellular, molecular, and systematic levels. On the basis of the above-described studies it is possible that vascular oxidative stress and low-grade vascular inflammation contribute to coronary microvascular adaptation, which can be attributed specifically to obesity. Understanding the sequence of pathological events in obesity-related microvascular dysfunction and adaptation might provide a rationale for therapeutic interventions and it might well harness these effects for therapeutic purposes.

There are promising pharmacological interventions that may prevent and/or restore coronary arterial dysfunction, such as the use of statins (61) and PPARγ activator insulin sensitizers,
thiazolidinediones (TZDs) early on in obesity (71, 77). On the other hand, it is possible that interfering with adaptive signaling mechanisms in the coronary arteriolar wall may provide further burden to those mechanisms, which are maintaining vascular function in disease. For instance, up-regulation of COX-2 in the endothelium and vascular bed specific production of dilator and antithrombotic prostacyclin may serve to maintain vascular homeostasis in patients with atherosclerosis (3, 47, 59). Recent population-based studies suggest the need for extra caution when using selective COX-2 inhibitors and NSAIDs in patients with cardiovascular risk factors (27, 57, 86). Experimental and clinical studies emphasize the importance of those investigations that strive to elucidate the specific effects of prostaglandin synthesis inhibitors in the regulation of cardiovascular function in obesity. Furthermore, although high level of ROS have been shown to impair vascular function in several pathological condition, and oxidative stress can be considered a negative modulator of vasomotor function (23), recent interventional clinical trials yielded largely negative results, and there has even been some suggestion of harmful effects (151). 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 (68). 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 (125, 126, 148). 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 (149). Further studies are needed to solve the current paradox of pharmacological interventions likely to be affecting prostaglandin metabolism and oxidant status of the coronary arteriolar wall.
GRANTS

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References


37. **Dora KA, Gallagher NT, McNeish A, and Garland CJ.** Modulation of endothelial cell 
KCa3.1 channels during endothelium-derived hyperpolarizing factor signaling in mesenteric 

38. **Ellis A, Cheng ZJ, Li Y, Jiang YF, Yang J, Pannirselvam M, Ding H, Hollenberg MD, and Triggle CR.** Effects of a Western diet versus high glucose on endothelium-dependent 

39. **Enderle MD, Benda N, Schmuelling RM, Haering HU, and Pfohl M.** Preserved 
endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy 

40. **Erdei N, Bagi Z, Edes I, Kaley G, and Koller A.** H2O2 increases production of 
constrictor prostaglandins in smooth muscle leading to enhanced arteriolar tone in Type 2 

41. **Erdei N, Toth A, Pasztor ET, Papp Z, Edes I, Koller A, and Bagi Z.** High-fat diet-
induced reduction in nitric oxide-dependent arteriolar dilation in rats: role of xanthine oxidase-

42. **Erdos B, Miller AW, and Busija DW.** Impaired endothelium-mediated relaxation in 
isolated cerebral arteries from insulin-resistant rats. *Am J Physiol Heart Circ Physiol* 282: 
H2060-2065, 2002.

43. **Erdos B, Snipes JA, Miller AW, and Busija DW.** Cerebrovascular dysfunction in 
Zucker obese rats is mediated by oxidative stress and protein kinase C. *Diabetes* 53: 1352-1359, 
2004.


75. Iacobellis G. True uncomplicated obesity is not related to increased left ventricular mass and systolic dysfunction. *J Am Coll Cardiol* 44: 2257; author reply 2258, 2004.


100. **Martin JW, Briesmiester K, Bargardi A, Muzik O, Mosca L, and Duvernoy CS.**
Weight changes and obesity predict impaired resting and endothelium-dependent myocardial

101. **Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa
L, Mukai Y, Hirakawa Y, Akaike T, and Takeshita A.** Electron spin resonance detection of
hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary

102. **McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry
WR, Andrews JW, and Hayes JR.** Impaired endothelium-dependent and independent
vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:

103. **Meade EA, McIntyre TM, Zimmerman GA, and Prescott SM.** Peroxisome
proliferators enhance cyclooxygenase-2 expression in epithelial cells. *J Biol Chem* 274: 8328-
8334, 1999.

104. **Miller AW, Tulbert CD, and Busija DW.** Rosuvastatin treatment reverses impaired
coronary artery vasodilation in fructose-fed, insulin-resistant rats. *Am J Physiol Regul Integr

105. **Mitchell JA, Larkin S, and Williams TJ.** Cyclooxygenase-2: regulation and relevance

106. **Mitchell JA, and Warner TD.** Cyclo-oxygenase-2: pharmacology, physiology,


120. **Oltman CL, Richou LL, Davidson EP, Coppey LJ, Lund DD, and Yorek MA.**


122. **Pontsler AV, St Hilaire A, Marathe GK, Zimmerman GA, and McIntyre TM.**


### TABLE 1.

**Human (a) and animal studies (b) investigating the impact of obesity on endothelium-dependent and endothelium-independent vasodilations.**

**a. Obese Humans**

<table>
<thead>
<tr>
<th>Study</th>
<th>Vascular Bed</th>
<th>Endo-dependent</th>
<th>Endo-independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kapiotis et al (84)</td>
<td>Brachial artery</td>
<td>FMD ↓</td>
<td>N/A</td>
</tr>
<tr>
<td>Hashimoto et al (67)</td>
<td>Brachial artery</td>
<td>FMD ↓</td>
<td>Nitrate →</td>
</tr>
<tr>
<td>Oflaz et al (119)</td>
<td>Brachial artery</td>
<td>FMD ↓</td>
<td>Nitrate N/A</td>
</tr>
<tr>
<td>Kreutzenberg et al (143)</td>
<td>Forearm res. vessels</td>
<td>BK ↓</td>
<td>SNP →</td>
</tr>
<tr>
<td>Sivitz et al (130)</td>
<td>Forearm res. vessels</td>
<td>ACh ↓</td>
<td>SNP ↓</td>
</tr>
<tr>
<td>Martin et al (97)</td>
<td>Myocardium (PET)</td>
<td>Flow ↓</td>
<td>N/A</td>
</tr>
<tr>
<td>Fulop et al (50)</td>
<td>Coronary arteriole</td>
<td>BK ↑</td>
<td>SNP ↑</td>
</tr>
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</table>

**b. Animal Models**

<table>
<thead>
<tr>
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<th>Vascular Bed</th>
<th>Endo-dependent</th>
<th>Endo-independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subramanian et al (134)</td>
<td>Aorta (20 and 32 wk)</td>
<td>ACh ↑</td>
<td>SNP →</td>
</tr>
<tr>
<td>Auguet et al (4)</td>
<td>Aorta (~20 wk)</td>
<td>Carbach. ↑</td>
<td>SNP →</td>
</tr>
<tr>
<td>Subramanian et al (134)</td>
<td>Mesenteric (~20-wk)</td>
<td>ACh →</td>
<td>SNP →</td>
</tr>
<tr>
<td>Subramanian et al (134)</td>
<td>Mesenteric (~30-wk)</td>
<td>ACh ↓</td>
<td>SNP →</td>
</tr>
<tr>
<td>Young et al (153)</td>
<td>Mesenteric (~12-wk)</td>
<td>ACh ↓</td>
<td>SNP →</td>
</tr>
<tr>
<td>Study</td>
<td>Tissue/Species</td>
<td>Agent</td>
<td>SNP Effect</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------</td>
<td>-------</td>
<td>------------</td>
</tr>
<tr>
<td>Oltman et al (117)</td>
<td>mesenteric (~20-wk)</td>
<td>ACh</td>
<td>→ SNP</td>
</tr>
<tr>
<td>Oltman et al (117)</td>
<td>mesenteric (~30-wk)</td>
<td>ACh</td>
<td>→ SNP ↑</td>
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<tr>
<td>Erdos et al (42)</td>
<td>cerebral (~12-wk)</td>
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<td>↓ SNP</td>
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<td>→ SNP</td>
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<td>Katakam et al (82)</td>
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<tr>
<td>Prakash et al (120)</td>
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<td>ACh</td>
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**High Fat Diet**

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<th>SNP Effect</th>
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<tbody>
<tr>
<td>Bhattacherya et al (12)</td>
<td>femoral art. (mouse)</td>
<td>ACh</td>
<td>↑ SNP</td>
</tr>
<tr>
<td>Mundy et al (110)</td>
<td>aorta (mouse)</td>
<td>ACh</td>
<td>→ SNP</td>
</tr>
<tr>
<td>Roberts et al (125)</td>
<td>aorta (rat)</td>
<td>ACh</td>
<td>↓ SNP ↓</td>
</tr>
<tr>
<td>Ellis et al. (38)</td>
<td>mesenteric (mouse)</td>
<td>ACh</td>
<td>→ SNP</td>
</tr>
<tr>
<td>Naderali et al (110)</td>
<td>mesenteric (rat)</td>
<td>ACh</td>
<td>↓ SNP ↓</td>
</tr>
<tr>
<td>Erdei et al (41)</td>
<td>skeletal muscle (rat)</td>
<td>ACh</td>
<td>↓ SNP</td>
</tr>
<tr>
<td>Jebelovszki et al (74)</td>
<td>coronary (rat)</td>
<td>ACh</td>
<td>→ SNP ↑</td>
</tr>
<tr>
<td>Woodman et al (152)</td>
<td>coronary (pig)</td>
<td>BK</td>
<td>↓ SNP</td>
</tr>
<tr>
<td>Henderson et al (69)</td>
<td>coronary (pig)</td>
<td>BK</td>
<td>→ SNP</td>
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</table>

**JCR:LA-cp Rat**

<table>
<thead>
<tr>
<th>Study</th>
<th>Tissue/Species</th>
<th>Agent</th>
<th>SNP Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Brien et al (115)</td>
<td>mesenteric</td>
<td>ACh</td>
<td>↓ SNP</td>
</tr>
<tr>
<td>Brunner et al (17)</td>
<td>aorta</td>
<td>A23187</td>
<td>↓ NONO ↑</td>
</tr>
<tr>
<td>Brunner et al (17)</td>
<td>perfused heart</td>
<td>BK</td>
<td>↓ NONO →</td>
</tr>
</tbody>
</table>

FMD: flow mediated dilation, ACh: acetylcholine, BK: bradykinin: NONO: spermine NONOate.
Figure Legends

**Figure 1. Vasomotor adaptation and progression in MetS.** The ability of vasomotor adaptation of coronary microvessels may result in an augmented or maintained vasodilator response at the early phases of obesity and MetS. On the other hand, adaptive vasomotor responses in the coronary circulation may decline as MetS progresses and other co-morbid conditions, such as severe insulin resistance, hypertension and fasting hyperglycemia (diabetes) develop. This may result in a limited dilator and constrictor capacity of coronary microvessels, leading to a limited ability to adjust tissue perfusion to actual metabolic demand.

**Figure 2. Cellular mechanisms of coronary adaptation in obesity.** In obesity, among other pathological alterations in lipid metabolism, changes in adipocyte-derived factors (i.e. reduced adiponectin and elevated levels of leptin, resistin, tumor necrosis factor α (TNFα) and interleukin-6 (IL-6)) lead to enhanced production of superoxide anion (O$_2^-$) by activating oxidases, such as xanthine-oxidase and NADP(H)-oxidase, in the coronary arteriolar wall. Superoxide anion interferes with nitric oxide (NO) availability and reduces coronary dilation, however, its derivate, hydrogen peroxide (H$_2$O$_2$) activates calcium-activated potassium channels (K$_{Ca}$-ch) and/or soluble guanylate cyclase (sGC) to maintain dilator function of coronary arterioles in obesity. Adipocyte-derived relaxing factor (ADRF) released from peri-vascular fat has been also implicated to augment vasodilation. Moreover, superoxide-dependent up-regulation of cyclooxygenase-2 (COX-2) increases prostacyclin (PGI$_2$) production, which reduces platelet aggregation and enhances coronary arteriolar dilations. Peroxisome proliferator-activated receptor-γ (PPARγ) and nuclear factor NFκB have been proposed to play a crucial role.
in the transcriptional regulation of these processes. There are therapeutic interventions, such as the use of statins and PPARγ activator, thiazolidinediones (TZDs) that may prevent/restore coronary vasomotor dysfunction. A potential interference with adaptive signaling mechanisms by selective and non-selective prostaglandin synthesis inhibitors (COXIBs and NSAIDs) and high dose antioxidant vitamins could paradoxically temper the dilator function of coronary arterioles. Black arrows indicate activation or enzymatic conversion of a compound, such as by superoxide dismutase (SOD) or prostacyclin synthase (PGIS).
PROGRESSION OF METABOLIC SYNDROME

HEALTH    OBESITY    DYSLYPIDEMIA

INSULIN RESISTANCE

HYPERGLYCEMIA

VASOMOTOR

ADAPTATION

HEALTH → OBESITY → TYPE 2 DIABETES

coronary arteriole

Figure 1.
Adaptive Dilation

- COXIBs
- NSAIDs
- STATINS
- Antioxidants
- Vitamins
- COXIBs
- NSAIDs
- STATINS
- Antioxidants
- Vitamins

Endothelium

- COX-2
- PGI₂
- H₂O₂
- eNOS
- NO
- PGIS
- K⁺
- Ca-ch
- sGC
- cGMP
- SOD
- 'Oxidases'
- 'ADRF'

Adipose tissue

- Adiponectin
- Resistin
- Leptin
- TNFα/IL-6
- TZDs

Coronary arteriole