Themed Section: Molecular Mechanisms Regulating Perivascular Adipose Tissue – Potential Pharmacological Targets?

REVIEW ARTICLE

The role of perivascular adipose tissue in obesity-induced vascular dysfunction

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Under physiological conditions, perivascular adipose tissue (PVAT) attenuates agonist-induced vasoconstriction by releasing vasoactive molecules including hydrogen peroxide, angiotensin 1–7, adiponectin, methyl palmitate, hydrogen sulfide, NO and leptin. This anticontractile effect of PVAT is lost under conditions of obesity. The central mechanism underlying this PVAT dysfunction in obesity is likely to be an 'obesity triad' (consisting of PVAT hypoxia, inflammation and oxidative stress) that leads to the impairment of PVAT-derived vasoregulators. The production of hydrogen sulfide, NO and adiponectin by PVAT is reduced in obesity, whereas the vasodilator response to leptin is impaired (vascular leptin resistance). Strikingly, the vasodilator response to acetylcholine is reduced only in PVAT-containing, but not in PVAT-free thoracic aorta isolated from diet-induced obese mice, indicating a unique role for PVAT in obesity-induced vascular dysfunction. Furthermore, PVAT dysfunction has also been observed in small arteries isolated from the gluteal/visceral fat biopsy samples of obese individuals. Therefore, PVAT may represent a new therapeutic target for vascular complications in obesity. A number of approaches are currently being tested under experimental conditions. Potential therapeutic strategies improving PVAT function include body weight reduction, enhancing PVAT hydrogen sulfide release (e.g. rosiglitazone, atorvastatin and cannabinoid CB$_1$ receptor agonists) and NO production (e.g. arginase inhibitors), inhibition of the renin–angiotensin–aldosterone system, inhibition of inflammation with melatonin or cytokine antagonists, activators of AMP-activated kinase (e.g. metformin, resveratrol and diosgenin) and adiponectin releasers or expression enhancers.

LINKED ARTICLES

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Abbreviations

ADRF, adipocyte-derived relaxing factor; Ang, angiotensin; BAT, brown adipose tissue; BK$_{Ca}$, large-conductance Ca^{2+}-activated K$^+$ channels; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial NOS; ET-1, endothelin-1; H$_2$S, hydrogen sulfide; HFD, high-fat diet; K$_{ATP}$, ATP-dependent K$^+$ channels; K$_v$, voltage-gated K$^+$ channels; L-NAME, N^G-nitro-L-arginine methyl ester; MCP-1, monocyte chemoattractant protein-1; PAME, palmitic acid methyl ester; PVAT, perivascular adipose tissue; PVRF, perivascular-derived relaxing factors; RAAS, renin–angiotensin–aldosterone system; RAS, renin–angiotensin system; sGC, soluble guanylyl cyclase; VSMC, vascular smooth muscle cells; WAT, white adipose tissue

Tables of Links

TARGETS		LIGANDS
Other protein targets[a]	**Nuclear hormone receptors**[d]	Adiponectin
TNF-α	PPARγ	Angiotensin II
Adipo1 receptor	**Catalytic receptors**[e]	Angiotensin 1-7
GPCRs[b]	Leptin receptor	cGMP
β₃ adrenoceptor	**Enzymes**[f]	ET-1
Ang 1-7 receptor (MAS1)	ACE	IL-6
AT₁ receptor	AMPK	MCP-1 (CCL2)
Voltage-gated ion channels[c]	Akt (PKB)	NO
BK_Ca (K_Ca1.1) channel	CBS	
IK_Ca (K_Ca3.1) channel	eNOS	
K_ATP (K_ir6.1) channel	PKG	
KCNQ (K_v7.1) channel	sGC	
K_V channels		
SK_Ca (K_Ca2.1) channel		

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d,e,f]Alexander *et al.*, 2015a,b,c,d,e,f).

Introduction

The central role of the cardiovascular system is the transportation of oxygen, nutrients, biomolecules and signalling molecules to organs, tissues and cells. In addition, the circulation system is important in host defence by the immune system and in blood haemostasis/coagulation (Daiber *et al.*, 2017). A dysregulation of vascular function may result in increased peripheral vascular resistance and blood pressure. Furthermore, vascular dysfunction promotes atherogenesis, and exacerbates insulin resistance by limiting the nutritive flow downstream in the microcirculation (Yudkin *et al.*, 2005; Greenstein *et al.*, 2009; Fuster *et al.*, 2016).

For decades, the endothelium has been the focus of vascular research. In recent years, however, the importance of other vascular cells has been increasingly recognized, including vascular smooth muscle cells (VSMCs) (Lacolley *et al.*, 2012), adventitia cells (Stenmark *et al.*, 2013; Wu *et al.*, 2015) and cells in the perivascular adipose tissue (PVAT). Moreover, communications exist between the different vascular cells and between the different layers of the vascular wall (Campbell *et al.*, 2012). Therefore, the vascular wall should be regarded as a whole rather than separate layers. The present article focusses on the role of PVAT in regulating vascular function.

PVAT: general aspects

PVAT surrounds large arteries and veins, small and resistance vessels, and skeletal muscle microvessels. Other microvasculatures and the cerebral vasculature are free of PVAT (Brown *et al.*, 2014; Gil-Ortega *et al.*, 2015). In contrast to humans and other large experimental animals (including rabbits and pigs), the murine coronary artery is without PVAT (Brown *et al.*, 2014). Large vessels (such as the saphenous vein) are separated from PVAT by an anatomical barrier that is rich in collagen bundles, elastin networks, VSMCs, fibroblasts, autonomic nerve endings and vasa vasorum (Ahmed *et al.*, 2004). In small vessels and microvessels, however, PVAT is an integral part of the vascular wall without laminar structures or any organized barrier separating PVAT from the adventitia (Szasz and Webb, 2012; Gil-Ortega *et al.*, 2015).

It is proposed that factors released by the PVAT reach the medial and endothelial layer of blood vessels either by direct diffusion or via the vasa vasorum (Gil-Ortega *et al.*, 2015). Another possible way for PVAT-derived factors to reach the inner layers of the vascular wall is via the small media conduits, a dense reticular network of collagenous conduits connecting the medial layer with the underlying adventitia (Grabner *et al.*, 2009; Campbell *et al.*, 2012). These conduits enable soluble molecules to traffic between the PVAT/adventitia and media/intima.

In a murine model with VSMC-specific PPAR-γ deletion, the animals are completely devoid of PVAT in the aortic and mesenteric regions. In contrast, interscapular brown adipose tissue (BAT) and gonadal/inguinal/subcutaneous white adipose tissue (WAT) in these animals remain intact. These results indicate that the origin of the adipocytes in the PVAT is different from those in WAT and BAT (Chang *et al.*, 2012; Brown *et al.*, 2014). This notion is consistent with a previous observation that PVAT is a functionally specialized type of

adipose tissue and PVAT adipocytes differ inherently in developmental and secretory properties from adipocytes in other fat depots (Chatterjee *et al.*, 2009). Indeed, PVAT adipocytes are likely to arise from VSMC progenitors (Brown *et al.*, 2014; Omar *et al.*, 2014; Gil-Ortega *et al.*, 2015).

Importantly, regional phenotypic and functional differences exist among PVAT depots (Brown *et al.*, 2014; Gil-Ortega *et al.*, 2015). Depending on the vascular bed, PVAT can be WAT-like (e.g. murine mesenteric PVAT), BAT-like (e.g. PVAT of the murine thoracic aorta) or mixed adipose tissue (e.g. PVAT of the murine abdominal aorta) and may have different vascularization, innervation and adipokine profiles (Szasz and Webb, 2012; Padilla *et al.*, 2013; Brown *et al.*, 2014; Gil-Ortega *et al.*, 2015; Drosos *et al.*, 2016; Victorio *et al.*, 2016b).

The morphological properties of PVAT in other species are less well-defined than murine PVAT. Human coronary PVAT exhibits a histological appearance and gene expression pattern more consistent with WAT rather than BAT (Chatterjee *et al.*, 2009; 2013).

Anticontractile function of PVAT

PVAT plays a role in intravascular thermoregulation (Chang *et al.*, 2012), vascular inflammation (Chatterjee *et al.*, 2013), VSMC proliferation (Miao and Li, 2012; Ozen *et al.*, 2015), the development of atherosclerosis (Greif *et al.*, 2009; Verhagen and Visseren, 2011; Chang *et al.*, 2012; Kawahito *et al.*, 2013; van Hinsbergh *et al.*, 2015; Gomez-Hernandez *et al.*, 2016) and hypertension (Galvez *et al.*, 2006; Lee *et al.*, 2011; Chang *et al.*, 2013). The present article focuses on the role of PVAT in regulating vascular function under physiological conditions as well as in obesity.

A role for PVAT in vascular function was first indicated by the observation that PVAT decreased the contractile responses to noradrenaline in rat aorta (Soltis and Cassis, 1991). Now, it is known that PVAT attenuates the vascular responsiveness to several (hormonal) agonists, including phenylephrine, angiotensin II (Ang II), 5-HT and endothelin-1 (ET-1) (Gollasch, 2012; Szasz and Webb, 2012).

PVAT may regulate vascular tone by releasing bioactive molecules, which can be regarded as an endocrine-related effect. In addition, PVAT also exerts a more direct, local effect on the vascular wall via paracrine mechanisms. The PVAT is composed mainly of adipocytes and releases a wide range of biologically active molecules that modulate vascular function (Szasz and Webb, 2012). Factors that mediate the anticontractile effects of PVAT are referred to as adipocyte-derived relaxing factor (ADRF) (Lohn *et al.*, 2002; Gollasch, 2012), or perivascular-derived relaxing factors (PVRF) (Lee *et al.*, 2011).

The mechanism of action of some ADRF has been shown to rely on the opening of smooth muscle K^+ channels, especially the KCNQ-type of the voltage-gated (K_v) K^+ channels (Lohn *et al.*, 2002; Gollasch, 2012), whereas ATP-dependent K^+ (K_{ATP}) channels have only minor effects (Schleifenbaum *et al.*, 2010; Gil-Ortega *et al.*, 2015). Indeed, pharmacological openers of KCNQ channels mimic the effects of ADRF in spontaneously hypertensive rats as well as in a mouse model of metabolic syndrome (the New Zealand Obese mouse) (Zavaritskaya *et al.*, 2013; Tano *et al.*, 2014).

The identity of ADRF/PVRF is a matter of ongoing debate and it is likely to be a combination of several different molecules, depending on the stimulus applied, the vascular bed examined, and the phenotypic state of the PVAT (Figure 1).

PVAT-derived vasoregulators

A number of PVAT-derived biologically active molecules with vascular effects have been identified so far; these include adipokines (e.g. leptin, adiponectin, omentin, visfatin, resistin and apelin), cytokines/chemokines [e.g. IL-6; TNF-α; monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor−1], some gaseous molecules (e.g. NO and H_2S), prostacyclin, Ang 1–7, Ang II, methyl palmitate, ET-1 and ROS (e.g. superoxide, H_2O_2) (Gollasch, 2012; Szasz and Webb, 2012; Almabrouk *et al.*, 2014; Tano *et al.*, 2014). Some of these are potential candidates for ADRF/PVRF.

H_2O_2

Bioassay experiments indicate that PVAT can exert its anticontractile effects through two distinct mechanisms: by releasing transferable relaxing factors and by an endothelium-independent mechanism involving H_2O_2 and subsequent activation of soluble guanlylyl cyclase (sGC) (Gao *et al.*, 2007).

PVAT-derived H_2O_2 has been shown to be one of the factors mediating the endothelium-independent anticontractile property of PVAT. Because H_2O_2 is membrane-permeable, PVAT-derived H_2O_2 can easily diffuse to underlying smooth muscle cells and induce vasodilatation by acting as a non-NO sGC activator (Gao *et al.*, 2007; Fernandez-Alfonso *et al.*, 2013). Moreover, H_2O_2 can directly activate cGMP-dependent PKGIα independently of cGMP (Burgoyne *et al.*, 2007).

The PVAT-derived transferable relaxing factors have been suggested by later studies to include Ang 1–7 (Lee *et al.*, 2009; Gil-Ortega *et al.*, 2015), adiponectin (Greenstein *et al.*, 2009; Lynch *et al.*, 2013; Weston *et al.*, 2013) and methyl palmitate (Lee *et al.*, 2011).

Ang 1–7 and Ang II

All components of the renin–angiotensin system (RAS), except renin, are found in aortic and mesenteric PVAT, including angiotensinogen, ACE, ACE2, chymase, Ang I, Ang II, AT_1 and AT_2 receptors (Galvez-Prieto *et al.*, 2008), as well as Ang 1–7 (Lee *et al.*, 2009).

Ang 1–7 produced by PVAT is one of the diffusible factors that induces vasodilatation indirectly through an endothelium-dependent mechanism. It binds to the endothelial Ang 1–7 receptor (Mas) and thereby stimulates endothelial NO production. The latter acts as a hyperpolarizing factor mediated through calcium-dependent K^+ (K_{Ca}) channels to cause relaxation of the blood vessels (Lee *et al.*, 2009; Gil-Ortega *et al.*, 2015).

PVAT also produces the vasoconstrictor Ang II (Huang *et al.*, 2010; Lu *et al.*, 2010). The release of Ang II from PVAT is enhanced in spontaneously hypertensive rats compared with normotensive rats (Lee *et al.*, 2011). PVAT-specific RAS
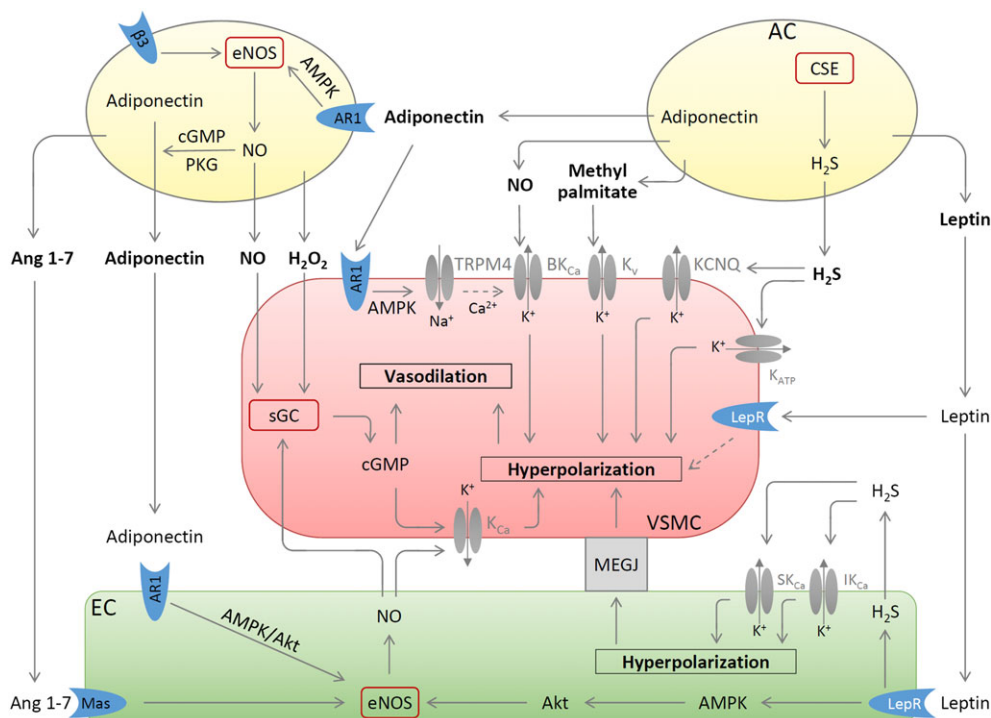
Figure 1

PVAT-derived vasoactive molecules. Methyl palmitate produced by PVAT adipocytes (AC) causes vasodilatation by opening the K_v channels on VSMC. H_2S is synthesized in PVAT by CSE and induces VSMC hyperpolarization by stimulating KCNQ-type K_v or K_{ATP} channels. Leptin induces endothelium-dependent vasodilatation by stimulating leptin receptor (LepR), which leads to activation of eNOS via a pathway involving AMPK and Akt and to H_2S production. This H_2S functions as an EDHF and activates endothelial small (SK_{Ca}) and intermediate (IK_{Ca}) conductance calcium-dependent K^+ channels via autocrine mechanisms. The resulting hyperpolarization of endothelial cells can be transmitted to VSMC by electrical coupling through myoendothelial gap junction (MEGJ). Leptin also causes endothelium-independent vasodilatation by inducing VSMC hyperpolarization through unknown mechanisms. NO and H_2O_2 released from PVAT can elicit vasodilatation by activating sGC leading to the synthesis of cGMP. Adiponectin released from PVAT AC can be enhanced by stimulation of β_3 adrenoceptors (β3) and by the NO-cGMP-PKG pathway. Adiponectin exerts multiple vascular effects: it stimulates NO production from PVAT and from endothelial cells and induces VSMC hyperpolarization by activating TRPM4 channels followed by opening BK_{Ca}; Ang 1–7 produced by PVAT acts on endothelial Ang 1–7 receptor (Mas; *MAS1* receptor) thereby stimulating endothelial NO production. Besides stimulating sGC activity, NO from PVAT and endothelial cells can also induce/potentiate VSMC hyperpolarization through K_{Ca} or BK_{Ca}. Partly adopted from (Beltowski, 2013; Weston *et al.*, 2013; Withers *et al.*, 2014a).

activation has been recently implicated in the development of atherosclerosis associated with chronic kidney disease (Kawahito *et al.*, 2013).

Adiponectin

It is controversial whether adiponectin is an ADRF/PVRF. Aortae and mesenteric arteries from adiponectin-knockout mice have been shown to maintain their anticontractile properties (Fesus *et al.*, 2007). However, recent studies demonstrate that the anticontractile activity of PVAT is significantly reduced in adiponectin-deficient mice (Lynch *et al.*, 2013; Withers *et al.*, 2014b), and the anticontractile function of PVAT can be largely abolished by blocking adiponectin receptors (Greenstein *et al.*, 2009; Lynch *et al.*, 2013). Undisputedly, adiponectin is produced in adipose tissues (also in PVAT) and is a potent vasodilator. Circulating adiponectin has been shown to be directly related to endothelial function in the general population (Saarikoski *et al.*, 2010), although some controversy exists (Ran *et al.*, 2010). Adiponectin is an independent predictor of endothelial function in a well-phenotyped cohort of patients with coronary

artery disease (Margaritis *et al.*, 2013), and genome-wide association studies have revealed that genetically-determined lower circulating adiponectin levels are related to worse endothelial function (Margaritis *et al.*, 2013).

Adiponectin induces vasodilatation through multiple mechanisms. (i) Adiponectin can act directly on VSMC, causing myocyte hyperpolarization by activating TRPM4 channels followed by the opening of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) (Lynch *et al.*, 2013; Weston *et al.*, 2013). (ii) Adiponectin stimulates NO release from adjacent adipocytes through paracrine mechanisms. This adipocyte-derived NO potentiates BK_{Ca} opening in VSMC (Weston *et al.*, 2013). (iii) Adiponectin can enhance endothelial NO production by stimulating the binding of HSP90 to endothelial NOS (eNOS) (Xi *et al.*, 2005), by enhancing eNOS phosphorylation at serine 1177, which is catalysed by either PI3K/Akt (Xi *et al.*, 2005; Cerqueira *et al.*, 2012; Margaritis *et al.*, 2013) or AMP-activated kinase (AMPK) (Withers *et al.*, 2014a), and by increasing the biosynthesis of tetrahydrobiopterin, an essential cofactor of eNOS (Margaritis *et al.*, 2013). Consistently, adiponectin-knockout

mice exhibit reduced eNOS phosphorylation at serine 1177 and impaired endothelial function (Cao *et al.*, 2009).

Moreover, adiponectin produced in PVAT has also been shown to exert a paracrine effect on the underlying arterial wall by suppressing NADPH oxidase activity via a PI3K/Akt-mediated inhibition of Rac1 and down-regulation of p22phox gene expression (Antonopoulos *et al.*, 2015b).

Importantly, adiponectin plays a central role in mediating crosstalk between different vascular cells (Figure 1). Through paracrine mechanisms, adiponectin from PVAT adipocytes regulates NO production in adjacent adipocytes (Withers *et al.*, 2014a,b). As mentioned above, PVAT adiponectin induces vasodilatation by stimulating endothelial cells and VSMC hyperpolarization, and reduces vascular oxidative stress by inhibiting NADPH oxidase activity. As a feedback mechanism, oxidative stress in the vascular wall up-regulates PVAT adiponectin expression by activating PPARγ (Antonopoulos *et al.*, 2015b).

Methyl palmitate

A study using the superfusion bioassay cascade technique has revealed that PVAT releases a lipophilic, heat-stable factor that causes vasodilatation by opening the K_v channels on VSMC (Lee *et al.*, 2011). This factor has been identified as palmitic acid methyl ester (PAME). PAME derives from PVAT adipocytes and is released from PVAT spontaneously in Ca^{2+}-containing solutions. PAME release is inhibited in Ca^{2+}-free conditions and enhanced by the calcium ionophore A23187 (Lee *et al.*, 2011). The anticontractile function of PVAT was found to be reduced in spontaneously hypertensive rats as well as the release of PAME; both mechanisms contributing to the pathogenesis of hypertension (Lee *et al.*, 2011).

H_2S

H_2S is synthesized from L-cysteine by cystathionine-β-synthase (CBS) or cystathionine-γ-lyase (CSE), and is enzymatically metabolized in mitochondria by sulfide:quinone oxidoreductase (Papapetropoulos *et al.*, 2015). In endothelial cells, H_2S is produced by CSE and is considered the most likely candidate for endothelium-derived hyperpolarizing factor (EDHF) (Yang *et al.*, 2008; Wang *et al.*, 2015). In an autocrine mode, endothelial H_2S activates small and intermediate conductance calcium-dependent K^+ channels on endothelial cells (Mustafa *et al.*, 2011). The resulting hyperpolarization of endothelial cells can be transmitted to VSMC by electrical coupling through myoendothelial gap junction or by increased K^+ efflux, which then activates VSMC inwardly-rectifying K^+ channels and/or Na^+/K^+-ATPase (Feletou and Feletou and Vanhoutte, 2009; Jamroz-Wisniewska *et al.*, 2014). In a paracrine mode, endothelium-generated H_2S can directly induce VSMC hyperpolarization by opening K_{ATP} channels in these cells (Wang *et al.*, 2015).

Recent studies indicate that PVAT also produces H_2S via CSE activity, and that PVAT-derived H_2S is involved in the anticontractile effect of PVAT on adjacent vessels (Fang *et al.*, 2009; Schleifenbaum *et al.*, 2010; Kohn *et al.*, 2012). H_2S donors produce a strong vasorelaxation of VSMC by opening the KCNQ-type K_v channels (Kohn *et al.*, 2012), perhaps also partly by opening K_{ATP} channels (Jamroz-Wisniewska *et al.*, 2014). The anticontractile effect of PVAT can be reduced by inhibition of CSE (but not CBS)

in aortas from rats but not from mice, indicating that endogenous H_2S is an ADRF from rat (but not mouse) aortic PVAT (Kohn *et al.*, 2012).

NO

It has been demonstrated that the anti-contractile effects of PVAT cannot be blocked by inhibiting NOS (Lohn *et al.*, 2002). It was, therefore, concluded that that the PVAT-derived relaxing factor was not NO. Now, it is clear that ADRF represents more than one single molecule, and blockade of NO synthesis alone may not be sufficient to prevent the effect of PVAT. Recent studies demonstrate that NO is produced within PVAT and this PVAT-derived NO contributes to the anticontractile effect of PVAT (Dashwood *et al.*, 2007; Gil-Ortega *et al.*, 2010; Withers *et al.*, 2014b; Aghamohammadzadeh *et al.*, 2015; Bussey *et al.*, 2016; Xia *et al.*, 2016; Victorio *et al.*, 2016b).

Immunohistochemistry analysis has shown eNOS staining in adipocytes as well as endothelial cells of the capillaries and vasa vasorum within PVAT (Dashwood *et al.*, 2007). The NO produced in PVAT can be directly visualized *in situ* with fluorescence imaging (Gil-Ortega *et al.*, 2010; Xia *et al.*, 2016; Victorio *et al.*, 2016b). Adipocyte-derived NO is supposed to be released into the interstitial fluid and to diffuse into the capillaries and adjacent arterioles causing vasodilatation (Mastronardi *et al.*, 2002).

In myograph experiments, N^G-nitro-L-arginine methyl ester (L-NAME) induces vasoconstriction, which reflects the amount of basal NO released. The L-NAME-induced vasoconstriction in small arteries isolated from visceral fat of healthy individuals is reduced by the removal of PVAT (Virdis *et al.*, 2015), indicating that PVAT contributes to vascular NO production. In PVAT-intact, endothelium-denuded rat mesenteric arteries, eNOS inhibitors significantly enhance noradrenaline-induced contractions, indicating that PVAT-derived NO contributes to the anticontractile effect of PVAT independently of the endothelium (Aghamohammadzadeh *et al.*, 2015; Bussey *et al.*, 2016).

Multiple mechanisms may mediate the vasorelaxant action of PVAT-derived NO (Figure 1): (i) PVAT NO may diffuse into adjacent smooth muscle cells and induce vasodilatation by stimulating cGMP synthesis; (ii) NO derived from eNOS in adipocytes positively regulates adiponectin release (Withers *et al.*, 2014b); and (iii) adipocyte-derived NO modulates BK_{Ca} in smooth muscle cells and potentiates hyperpolarization (Weston *et al.*, 2013).

Leptin

Leptin is mainly produced by adipocytes and plays important roles in regulating appetite, energy expenditure, inflammation and immune responses (Molica *et al.*, 2015). At the clinical level, its role in coronary heart disease still remains controversial (Antonopoulos *et al.*, 2015a). Leptin is also produced by PVAT (Dashwood *et al.*, 2011; Galvez-Prieto *et al.*, 2012), although it is not considered an ADRF. The anticontractile effect of PVAT is maintained in the Zucker fa/fa rats that lack functional leptin receptors (Lohn *et al.*, 2002).

Under physiological conditions, leptin has no acute effect on blood pressure because it activates both pressor (sympathetic nervous system) and depressor (vasodilatation and

natriuresis) mechanisms in a balanced manner (Jamroz-Wisniewska *et al.,* 2014). Through central mechanisms, leptin increases sympathetic nervous activity to the kidney, the adrenal gland, the hindlimbs and to BAT (Haynes *et al.,* 1997; Mark, 2013). However, leptin also directly induces relaxation of blood vessels. Acute infusion of leptin has no significant effect on blood pressure in rats (Haynes *et al.,* 1997) or in humans (Brook *et al.,* 2007). Interestingly, leptin's dual effect on blood pressure can be revealed by various procedures (Fruhbeck, 1999). When NO synthesis is inhibited, leptin administration to Wistar rats produces a statistically significant increase in blood pressure, whereas a hypotensive response is induced by leptin in the presence of ganglionic blockade (Fruhbeck, 1999).

Leptin may cause vasodilatation in different vessels through different mechanisms. In large arteries (e.g. the aorta), leptin induces an endothelium-dependent vasodilatation that is the result of the release of NO (Lembo *et al.,* 2000). Leptin-stimulated endothelial NO production is mediated by the sequential activation of an AMPKα1-Akt-eNOS pathway leading to eNOS phosphorylation at serine 1177 (Vecchione *et al.,* 2002; Procopio *et al.,* 2009). In small arteries (e.g. mesenteric arteries), both NO and EDHF play a role in leptin-induced relaxation (Lembo *et al.,* 2000; Jamroz-Wisniewska *et al.,* 2014). The leptin-induced vasorelaxation mediated by EDHF, is at least in part, a result of CSE-dependent H_2S production in endothelial cells (Jamroz-Wisniewska *et al.,* 2014). In human saphenous vein and internal mammary artery (Momin *et al.,* 2006), and to a small extent in rat mesenteric artery (Jamroz-Wisniewska *et al.,* 2014), leptin induces an endothelium-independent vasodilatation that is mediated by hyperpolarization of the VSMCs.

In coronary artery bypass surgeries, saphenous vein grafts with PVAT exhibit superior patency rate and better preserved intimal, medial and adventitial architecture compared with those without PVAT (Verma *et al.,* 2014; Kopjar and Dashwood, 2016), demonstrating the beneficial effects of PVAT *in vivo*. Leptin concentrations in the PVAT of saphenous vein grafts are within the concentration range causing relaxation of bypass conduits (Dashwood *et al.,* 2011). It has therefore been proposed that PVAT-derived leptin may play a role in preserving a 'healthy graft' by reducing vasospasm at graft harvesting as well as after its implantation into the coronary circulation (Dashwood *et al.,* 2011; Dashwood and Tsui, 2013). However, there is no evidence for a causal link between PVAT-derived leptin and the superior performance of PVAT-containing grafts. The beneficial effects of PVAT in this regard can also be attributed to other vasoactive molecules released by PVAT.

PVAT dysfunction in obesity

Obesity has numerous adverse effects on the circulation and cardiovascular structure and function (Lavie *et al.,* 2009). Obese patients are more likely to develop hypertension and cardiomyopathy, and have a higher risk of stroke (Lavie *et al.,* 2009). Obesity and atherosclerosis have long been linked in observational studies. The two conditions share similar pathophysiological pathways, including dyslipidaemia and chronic inflammatory processes (Rocha and Libby, 2009).

In humans, the thoracic peri-aortic fat mass correlates with hypertension, diabetes and aortic/coronary calcification, when corrected for body-mass index (but not if corrected for visceral adipose tissue) (Lehman *et al.,* 2010). Clinical studies have shown that abdominal adiposity is associated with vascular dysfunction, as measured by flow-mediated dilation of the brachial artery (Brook *et al.,* 2001; Hamburg *et al.,* 2008). In human small arteries, the anticontractile effect of PVAT is completely lost in obese patients with metabolic syndrome (Greenstein *et al.,* 2009).

In animal models of obesity, the PVAT mass and adipocyte size are increased (Marchesi *et al.,* 2009; Ketonen *et al.,* 2010), which is accompanied by other structural modifications in the PVAT (Szasz and Webb, 2012). The anticontractile effect of PVAT is completely lost in the mouse model of diet-induced obesity (Ketonen *et al.,* 2010) and genetic model of metabolic syndrome (the New Zealand obese mouse) (Marchesi *et al.,* 2009), or significantly reduced in the ob/ob mice (Agabiti-Rosei *et al.,* 2014). In addition, the PVAT of obese animals inhibits the vasodilator responses to acetylcholine (Ketonen *et al.,* 2010; Ma *et al.,* 2010; Xia *et al.,* 2016). Obesity-induced dysfunctions of the PVAT correlate with a rise in blood pressure in rodent models of diet-induced obesity (Aghamohammadzadeh *et al.,* 2015), demonstrating the importance of PVAT function in vascular pathology *in vivo*.

Based on current research results, an 'obesity triad' consisting of PVAT hypoxia, inflammation and oxidative stress can be proposed as the central mechanism in obesity-induced PVAT dysfunction (Figure 2). Cellular hypoxia in PVAT is thought to be due to adipocyte hypertrophy twinned with reductions in capillary density and angiogenesis (Pasarica *et al.,* 2009; Fuster *et al.,* 2016). Hypoxia, in turn, stimulates the production of inflammatory cytokines and chemokines from PVAT adipocytes and infiltrating macrophages (Greenstein *et al.,* 2009). Importantly, PVAT inflammation precedes macrophage infiltration. A short-term high-fat diet (HFD) fed to mice for 2 weeks results in a marked up-regulation of pro-inflammatory leptin and MIP1α (also known as CCL3), and down-regulation of anti-inflammatory PPARγ (Chatterjee *et al.,* 2009; Cheang *et al.,* 2015) and adiponectin (Chatterjee *et al.,* 2009). At this early stage, there are no signs of macrophage infiltration in PVAT, although the infiltration of T cells in PVAT is likely (Chatterjee *et al.,* 2009). Thus, the infiltration of macrophages may be a response to PVAT inflammation, probably triggered by chemokines such as MCP-1. PVAT adipocytes produce 50-fold more MCP-1 than adipocytes from other fat regional depots (Chatterjee *et al.,* 2009), and a HFD further enhances MCP-1 expression in aortic PVAT (Ketonen *et al.,* 2010; Xia *et al.,* 2016). Macrophages, in turn, potentiate the PVAT inflammatory response and enhance the activity of NADPH oxidase, a major source of superoxide anion in the vasculature that is also expressed in PVAT (Gao *et al.,* 2006). Indeed, the expression of p67phox (Ketonen *et al.,* 2010) and Nox2 (Xia *et al.,* 2016), subunits in the NADPH oxidase complex, is increased in the PVAT of obese mice. Moreover, HFD-fed mice also show a reduced SOD3 expression and glutathione levels in their mesenteric PVAT (Gil-Ortega *et al.,* 2014). The resulting oxidative stress
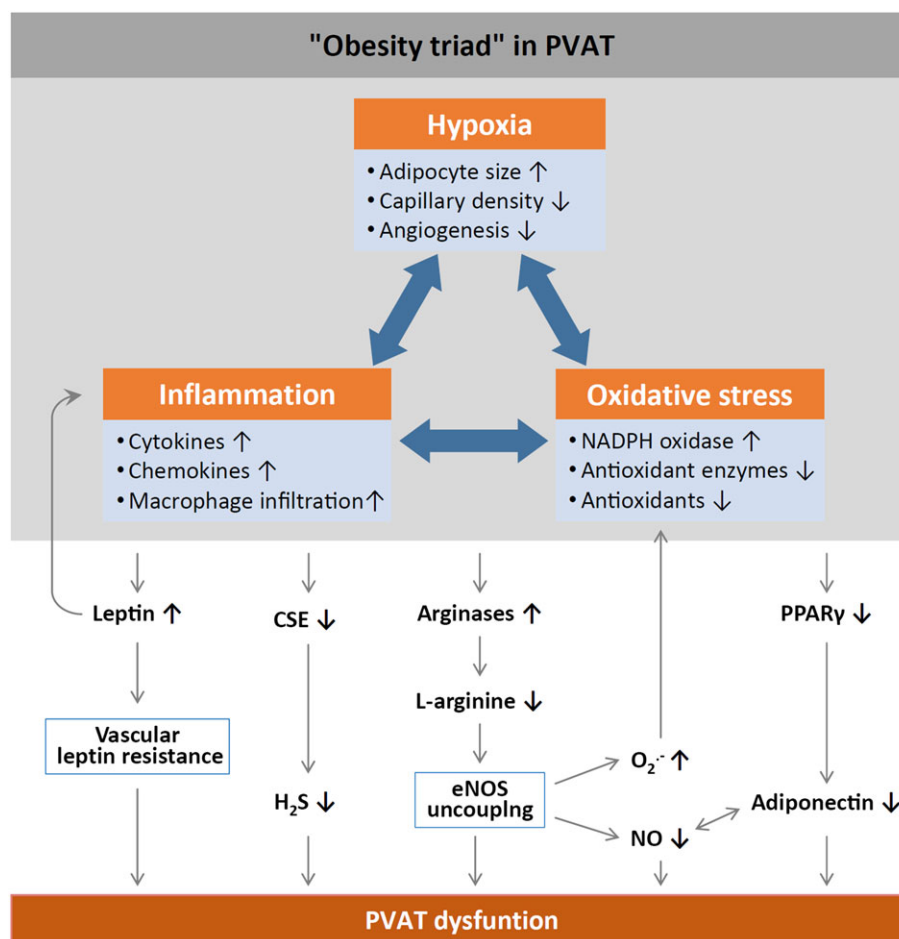
Figure 2

Mechanisms of PVAT dysfunction in diet-induced obesity. HFD-induced adipocyte hypertrophy leads to hypoxia and the production of pro-inflammatory cytokines and chemokines, activation of NADPH oxidase and down-regulation of antioxidant enzymes (e.g. superoxide dismutase and peroxiredoxin-1) and non-enzymatic antioxidants (e.g. glutathione). Infiltrating immune cells potentiate PVAT inflammation and oxidative stress. Chronic hyperleptinaemia leads to vascular leptin resistance (loss of leptin-induced vasodilatation) and potentiation of PVAT inflammation. Long-term obesity decreases PVAT H_2S production by down-regulating CSE expression. The up-regulation of arginases leads to L-arginine deficiency and eNOS uncoupling (enhanced superoxide production and reduced NO production by eNOS). PVAT adiponectin expression is reduced in obesity, very likely due to a down-regulation of PPARγ. Normally, NO stimulates adiponectin secretion and adiponectin increases PVAT NO production. This positive feedback mechanism is impaired in obesity.

further enhances PVAT inflammation, leading to a vicious circle (Figure 2).

In support of this concept, PVAT dysfunction induced by *in vitro* incubation with aldosterone (Withers *et al.,* 2011) or cytokines (Greenstein *et al.,* 2009) can be fully restored using a combination of catalase and superoxide dismutase. *Ex vivo* incubation with superoxide dismutase and catalase also restores the anticontractile function of PVAT from obese individuals (Aghamohammadzadeh *et al.,* 2013, 2015). Similarly, hypoxia-induced PVAT dysfunction can be normalized by *in vitro* incubation with an anti-TNF-α antibody or an anti-IL-6 antibody (Greenstein *et al.,* 2009), confirming the role of inflammation and oxidative stress in PVAT dysfunction. In agreement with this, *ex vivo* incubation with the anti-TNF-α antibody infliximab improves NO production in small arteries isolated from obese patients. Moreover, this effect is more pronounced in PVAT-containing vessels than in PVAT-free arteries (Virdis *et al.,* 2015). The removal of

macrophages prevents hypoxia- and aldosterone-induced PVAT dysfunction (Withers *et al.,* 2011). Also, preventing macrophage infiltration ameliorates PVAT dysfunction in mice with diet-induced obesity (Wang *et al.,* 2012).

Local inflammation in PVAT is potentiated by systemic inflammation and inflammation of other adipose tissues in the obesity state, where adipose tissue mass can range from 30 to 50% of total body mass (Fuster *et al.,* 2016). Adipose tissue is the major source of IL-6, contributing as much as one third of total circulating IL-6 (Mohamed-Ali *et al.,* 1997). Similarly, leptin is an adipose tissue-specific adipokine. It is highly expressed in adipocytes, and circulating leptin levels increase in parallel with adipose tissue mass (Fuster *et al.,* 2016). Thus, IL-6, leptin and other pro-inflammatory factors released from other adipose tissues can reach PVAT via the circulation and contribute to PVAT inflammation.

The obesity triad of hypoxia, inflammation and oxidative stress in PVAT ultimately leads to a dysregulated

production/secretion of adipokines and cytokines. In PVAT of HFD-fed mice, pro-inflammatory adipokines/cytokines (e.g. leptin, IL-6, TNF-α and interferon-γ) are up-regulated, whereas anti-inflammatory adipokines/cytokines (e.g. adiponectin and IL-10) are down-regulated (Chatterjee *et al.,* 2009; Ketonen *et al.,* 2010; Xia *et al.,* 2016). The resulting exacerbation of inflammation and oxidative stress promotes a dysregulation of biomolecule production in the PVAT and vascular dysfunction (Almabrouk *et al.,* 2014; Gil-Ortega *et al.,* 2014; Omar *et al.,* 2014; Molica *et al.,* 2015).

Dysregulation of PVAT-derived vasoregulators in obesity

Under conditions of obesity, the synthesis, secretion or action of PVAT-derived vasoactive molecules are impaired (Figure 2).

H_2S production by PVAT in obesity

Experimental obesity induced by a high-calorie (but not high-fat) 'cafeteria' diet is associated with a time-dependent effect on PVAT-produced H_2S in rats. Feeding for 3 months results in obesity with signs of metabolic syndrome, whereas 1 month feeding leads to adiposity without insulin resistance. Interestingly, short-term obesity (induced by 1 month of cafeteria diet) in rats increases production of H_2S by PVAT and this is associated with an enhanced anticontractile effect of PVAT, whereas long-term obesity (3 months on cafeteria diet) reduces H_2S production and the anticontractile effects of PVAT (Beltowski, 2013). The enhanced H_2S production in the early phase of obesity may represent a compensatory mechanism and result from reduced H_2S oxidation due to hypoxia. In contrast, H_2S production is decreased in long-lasting obesity and metabolic syndrome because of a down-regulation of the CSE enzyme in PVAT (Beltowski, 2013).

Plasma H_2S levels are reduced in overweight people and patients with type 2 diabetes, independently of diabetes. Waist circumference has been shown to be an independent predictor of plasma H_2S and remains an independent predictor of plasma H_2S after adjustment for systolic blood pressure, microvascular function, insulin sensitivity, glycaemic control and lipid profile (Whiteman *et al.,* 2010).

Adiponectin expression in PVAT and its secretion in obesity

In humans, circulating adiponectin levels are decreased with increasing obesity (Weiss *et al.,* 2004) and type 2 diabetes (Antonopoulos *et al.,* 2015b). Hypoadiponectinemia is closely associated with endothelial dysfunction (Shimabukuro *et al.,* 2003).

Reduced adiponectin levels are uniformly found in PVAT of obese subjects (Aghamohammadzadeh *et al.,* 2013) and in PVAT samples from different animal models of obesity, including the ob/ob mice (Agabiti-Rosei *et al.,* 2014), the db/db mice (Meijer *et al.,* 2013) and diet-induced obese mice (Chatterjee *et al.,* 2009).

A recent study has identified PPARγ as a positive regulator of adiponectin expression in PVAT (Antonopoulos *et al.,* 2015b). Interestingly, PPARγ is down-regulated in the PVAT of diet-induced obese mice (Chatterjee *et al.,* 2009); this

may represent a molecular mechanism for the reduced adiponectin expression in PVAT found during obesity.

Furthermore, adiponectin secretion is also reduced during obesity. Normally, adiponectin secretion from PVAT adipocytes is fine-tuned; the mechanisms include β_3-adrenoceptor stimulation (Weston *et al.,* 2013), NO production and PKG activation (Withers *et al.,* 2014b). Under conditions of obesity, adiponectin secretion is decreased, which may be attributable to β_3 adrenoceptor desensitization, reduced NO production and PKG down-regulation in PVAT adipocytes (Withers *et al.,* 2014a).

PVAT NO production in obesity

Although an adaptive overproduction of NO from mesenteric PVAT has been observed at the early phase of diet-induced obesity in C57BL/6J mice (Gil-Ortega *et al.,* 2010), a longer time on a HFD leads to a reduction in PVAT NO production (Aghamohammadzadeh *et al.,* 2015; Xia *et al.,* 2016).

Inhibition of NO synthesis enhances the agonist-induced contraction of PVAT-intact, endothelium-denuded mesenteric arteries from healthy rats. However, this effect of NO synthesis inhibition is lost in PVAT-intact vessel segments from diet-induced obese animals (Aghamohammadzadeh *et al.,* 2015; Bussey *et al.,* 2016). These results indicate that the anticontractile effect of healthy PVAT is partially mediated by PVAT-derived NO, whereas obesity reduces PVAT NO production to a level that is too low to be functionally relevant.

Consistent with these data, basal NO release has been shown to be reduced in small arteries from obese patients compared with non-obese controls. Interestingly, this obesity-induced reduction in basal NO production is only evident in PVAT-containing but not in PVAT-removed arteries (Virdis *et al.,* 2015), supporting the concept that a reduced production of NO by PVAT is involved in the obesity-induced loss of the anticontractile effect of PVAT.

Several molecular mechanisms may be responsible for the reduced PVAT NO production in obesity: (i) A reduced expression of eNOS has been reported in the mesenteric PVAT of diet-induced obese rats (Bussey *et al.,* 2016) and mice (Gil-Ortega *et al.,* 2014). (ii) In the PVAT of thoracic aorta from diet-induced obese mice, however, we did not observe any changes in eNOS expression but have obtained evidence that the function of eNOS is impaired (eNOS uncoupling as well as reduced eNOS phosphorylation at serine 1177 residue) (Xia *et al.,* 2016). (iii) The reduction in PVAT NO can also be partially attributed to a deficiency in PVAT adiponectin that normally stimulates eNOS activity in PVAT adipocytes (Weston *et al.,* 2013).

In addition to PVAT NO, NO production from the endothelium is also reduced in experimental obesity (Korda *et al.,* 2008; Marchesi *et al.,* 2009; Yu *et al.,* 2014).

Vascular leptin resistance in obesity

Hyperleptinaemia in diet-induced obesity leads to selective leptin resistance with preservation of leptin-induced increases in renal sympathetic nerve activity and blood pressure despite resistance to the anorexic and weight-reducing actions of leptin (Rahmouni *et al.,* 2005; Coppari and Bjorbaek, 2012; Mark, 2013). Cerebroventricular administration of leptin antagonists significantly decreases mean

arterial pressure and renal sympathetic nerve activity in diet-induced obese rabbits, indicating that the central actions of leptin play a role in the elevation of blood pressure and renal sympathetic nerve activity induced by a HFD in rabbits (Lim *et al.,* 2013). Similarly, the elevation in blood pressure induced by chronic leptin infusion can be prevented with combined α- and β-adrenoceptor blockade (Shek *et al.,* 1998; Carlyle *et al.,* 2002).

Hyperleptinaemia shows a biphasic effect on vascular function. In the early stage of diet-induced obesity (4 weeks), the NO-mediated component of leptin-induced relaxation of mesenteric artery is impaired, which is compensated for by an up-regulation of EDHF-mediated vasodilatation (Beltowski *et al.,* 2010; Jamroz-Wisniewska *et al.,* 2014). Interestingly, the impaired NO component of leptin-induced vasodilatation in obese rats can be restored by antagonizing the leptin receptors, indicating that hyperleptinaemia induces vascular leptin resistance in diet-induced obesity. Consistently, the impairment of leptin-induced, NO-mediated vasodilatation can also be observed in rats treated for 8 days with exogenous leptin (Jamroz-Wisniewska *et al.,* 2014). In the later phase of obesity (3 months), both the NO- and EDHF-mediated effects of leptin are reduced leading to an increase in blood pressure (Beltowski *et al.,* 2009; Jamroz-Wisniewska *et al.,* 2014). Chronic hyperleptinaemia-induced vascular leptin resistance (loss of leptin-induced vasodilatation) has been shown both *in vivo* in anaesthetised dogs and *ex vitro* in coronary rings isolated from diet-induced obese dogs (Knudson *et al.,* 2005). Mechanistically, leptin-induced endothelial dysfunction is associated with inflammation, oxidative stress and eNOS uncoupling (Korda *et al.,* 2008).

Leptin levels are uniformly increased in diet-induced obesity, not only in the blood but also in PVAT (Ketonen *et al.,* 2010; Schroeter *et al.,* 2013; Gil-Ortega *et al.,* 2014; Xia *et al.,* 2016). In a mouse model of diet-induced obesity, the up-regulation of leptin in aortic PVAT is paralleled by a reduced anticontractile effect of PVAT and correlates with a loss of PVAT-derived NO and PVAT eNOS (Gil-Ortega *et al.,* 2014).

Metabolic syndrome in Ossabaw miniature swine increases epicardial PVAT leptin protein and coronary leptin receptor expression. Coronary arteries from swine with metabolic syndrome display significant endothelial dysfunction that is markedly exacerbated by PVAT. A leptin antagonist reverses the metabolic syndrome effect of PVAT on endothelium-dependent vasodilatation, indicating that epicardial PVAT-derived leptin exacerbates coronary endothelial dysfunction in metabolic syndrome (Payne *et al.,* 2010).

The role of PVAT in obesity-induced vascular dysfunction

In a recent study, we analysed the vascular function of thoracic aorta isolated from diet-induced obese C57BL/6J mice (Xia *et al.,* 2016). Unexpectedly, the endothelium-dependent, NO-mediated vasodilator response to acetylcholine remained unchanged in aortas from mice fed a HFD for 20 weeks (Figure 3A). Even after varying the experimental conditions (60 or 45 kcal% fat; 20 or 12 weeks on a HFD), we did not observe any endothelial dysfunction in the PVAT-free thoracic aorta of obese mice.

Strikingly, when the aortic PVAT was left in place, a clear reduction in the vasodilator response to acetylcholine was observed in the aorta of obese animals as compared with lean controls (Figure 3B). Acetylcholine-induced vasodilatation in the mouse aorta (either with or without PVAT) can be completely blocked by inhibition of NO synthesis (Figure 3 C), indicating that this response is NO-dependent. Thus, the reduced vasomotor function in the aorta of HFD-fed mice (Figure 3B) results from eNOS dysfunction in the PVAT, but not in the endothelium. Indeed, we found evidence for PVAT eNOS dysfunction in diet-induced obese mice (Xia *et al.,* 2016).

Notably, all the pathological changes (arginase induction, L-arginine deficiency, Akt inhibition and reduced eNOS phosphorylation) in diet-induced obese mice were observed only in the PVAT but not in the aorta itself (Xia *et al.,* 2016). These data provide a compelling mechanistic explanation for our initial observation that vascular dysfunction in obese mice is only evident in PVAT-containing aorta but not in PVAT-free aorta. These findings further substantiate our hypothesis that the role of PVAT may be even more important than that of the endothelium in obesity-induced vascular dysfunction under certain experimental settings.

Similar evidence also exists in studies with human samples. In small arteries isolated from obese patients, an ET-1/NO imbalance is evident, which is associated with vascular dysfunction. Interestingly, the reduced NO availability and enhanced ET-1 signalling in arteries from obese patients can be reversed by PVAT removal (Virdis *et al.,* 2015), demonstrating the crucial role of PVAT in obesity-induced vascular dysfunction.

Human studies

The role of PVAT in regulating vascular function in humans has been studied by using either small arteries from gluteal/visceral fat biopsy samples or coronary bypass graft materials such as the internal mammary artery or the saphenous vein.

The anticontractile effect of human PVAT has been shown for the internal mammary artery (Gao *et al.,* 2005; Malinowski *et al.,* 2008; Foudi *et al.,* 2011; Malinowski *et al.,* 2013; Ozen *et al.,* 2013), the saphenous vein (Ford *et al.,* 2006; Dashwood *et al.,* 2007, 2011; Foudi *et al.,* 2011; Ozen *et al.,* 2013) and for small arteries (Aghamohammadzadeh *et al.,* 2013; Greenstein *et al.,* 2009; Virdis *et al.,* 2015).

Currently, PVAT-removed graft materials are mostly used in coronary artery bypass operations. Intimal hyperplasia and vasospasm with a reduction in patency rate are common problems that occur after bypass surgery (Ozen *et al.,* 2015). In this regard, saphenous vein grafts harvested by the 'no-touch' technique with intact adventitia and PVAT have been shown to be superior to those obtained by open vein harvesting or endoscopic vein harvesting with adventitia and PVAT removed (Souza *et al.,* 2001; Dashwood and Tsui, 2013; Dreifaldt *et al.,* 2013; Verma *et al.,* 2014; Kopjar and Dashwood, 2016). Unfortunately, there are yet no such clinical studies addressing the role of internal mammary artery PVAT in coronary artery bypass operations.
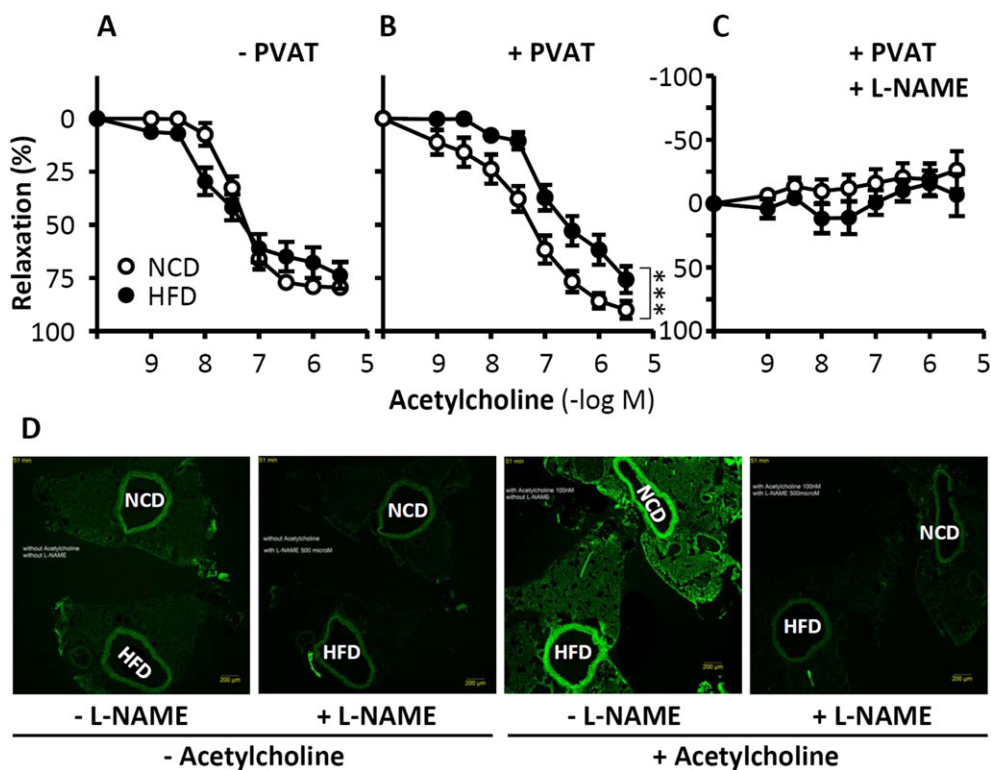
Figure 3

Role of PVAT in obesity-induced vascular dysfunction. C57BL/6J mice were fed a HFD or normal control diet (NCD) for 20 weeks starting at the age of 8 weeks. The vasodilator response to acetylcholine (A–C) was performed in noradrenaline-precontracted aorta with or without PVAT in the absence or presence of the NO synthase inhibitor L-NAME. ***$P < 0.001$, $n = 8$. To detect PVAT NO production, NCD and HFD aorta samples were mounted back-to-back on the same slide to guarantee identical staining conditions for the two samples (D). NO production in PVAT-containing aorta was determined by 4,5-diaminofluorescein diacetate (DAF-2 DA) staining. From Xia *et al.* (2016) with permission of Wolters Kluwer Health, Inc. Copyright © 2016, Wolters Kluwer Health.

Obesity in humans is associated with endothelial dysfunction *in vivo* (Steinberg *et al.,* 1996; Al Suwaidi *et al.,* 2001; Grassi *et al.,* 2010). In human small arteries taken from subcutaneous gluteal fat biopsies, the anticontractile effect of PVAT is shown to be completely lost in obese patients (Greenstein *et al.,* 2009; Aghamohammadzadeh *et al.,* 2013).

In obese patients, PVAT undergoes hypoxia, oxidative stress and inflammation, leading to the up-regulation of pro-inflammatory cytokines (e.g. TNF-α) (Greenstein *et al.,* 2009), enhanced ROS production, a down-regulation of antioxidant enzymes (e.g. SOD1, peroxiredoxin-1) (Aghamohammadzadeh *et al.,* 2015) and reduced release of vasodilating adipokines (e.g. adiponectin) (Greenstein *et al.,* 2009) as well as a decreased NO production from PVAT (Aghamohammadzadeh *et al.,* 2015). *Ex vivo* incubation of arteries from obese individuals with superoxide dismutase and catalase restores the anticontractile effects of PVAT (Aghamohammadzadeh *et al.,* 2013; 2015).

In small arteries isolated from visceral fat of obese patients, the expression of TNF-α is increased both in the vascular wall and in PVAT (Virdis *et al.,* 2015). This increase in TNF-α reduces the production of adiponectin, triggers eNOS uncoupling by activating NADPH oxidase activity and stimulates ET-1 generation. The resulting ET-1/NO imbalance is implicated in obesity-induced vascular dysfunction (Virdis

et al., 2015). Moreover, the reduced NO availability and enhanced ET-1 signalling in arteries from obese patients can be reversed by PVAT removal (Virdis *et al.,* 2015), indicating that the vasorelaxant effect of PVAT under physiological conditions is transformed into an inflammatory pro-contractile phenotype in obesity (Virdis *et al.,* 2015).

Therapeutic approaches to improve PVAT function

Weight loss

Calorie restriction-induced sustained weight loss in obese rats leads to an improvement in PVAT function associated with a reversal in obesity-induced hypertension, the restoration of adipocyte size, PVAT eNOS function, PVAT TNF-α expression and normalization of plasma adipokine levels, including leptin and insulin (Bussey *et al.,* 2016).

In humans, bariatric surgery restores the anticontractile activity of PVAT in severely obese individuals (Aghamohammadzadeh *et al.,* 2013). The normalization of PVAT function after surgery is accompanied by improvements in insulin sensitivity, serum glycaemic indexes, inflammatory cytokines, adipokine profile and systolic blood

pressure together with increased PVAT adiponectin and NO bioavailability (Aghamohammadzadeh *et al.,* 2013). Strikingly, these changes are evident despite the patients remaining obese (BMI declines from 51 before surgery to 38 after surgery) (Aghamohammadzadeh *et al.,* 2013).

Exercise training

In familial hypercholesterolaemic pigs, exercise training significantly reduces the contractile response of left circumflex coronary artery to ET-1. This effect has been shown to be independent of PAVT (Bunker and Laughlin, 2010).

Exercise training of Wistar rats for 8 weeks decreases plasma triglyceride levels with no effect on adipokine profiles. Exercise training up-regulates the expression of eNOS in PVAT but not in the aorta. Exercise reduces the amount of thoracic PVAT without changing the effects of PVAT on relaxation or constriction (Araujo *et al.,* 2015). However, this study was performed in healthy rats on normal chow. Future studies should investigate the effect of exercise training on PVAT function in obesity models.

Improving the function of PVAT eNOS

A major mechanism for the PVAT eNOS dysfunction in experimental obesity is eNOS uncoupling due to a deficiency in L-arginine, which is attributable to an up-regulation of arginase found in PVAT of diet-induced obese mice (Xia *et al.,* 2016). Arginases are major L-arginine-consuming enzymes that metabolize L-arginine to urea and L-ornithine. The up-regulation of arginase limits L-arginine bioavailability for NO production and leads to eNOS uncoupling (Yang and Ming, 2013). An uncoupled eNOS produces superoxide at the expense of NO (Li and Forstermann, 2013; Li *et al.,* 2013) and thus contributes to oxidative stress in PVAT (Xia *et al.,* 2016).

The molecular mechanisms responsible for the increase in arginase and the subsequent L-arginine deficiency observed in diet-induced obesity remain to be determined. We propose that PVAT inflammation plays an important role in this effect. The expression/activity of vascular arginases can be enhanced by a variety of stimuli (Pernow and Jung, 2013), including Ang II (Shatanawi *et al.,* 2011), high glucose (Romero *et al.,* 2008), thrombin (Ming *et al.,* 2004) and oxidized low-density lipoprotein (Ryoo *et al.,* 2006), conditions that inevitably lead to vascular inflammation.

To ascertain that eNOS uncoupling is causally involved in obesity-induced vascular dysfunction, we incubated PVAT-containing aorta with a combination of L-arginine and an arginase inhibitor for 30 min *ex vivo* in organ chambers. This treatment had no effect on acetylcholine-induced vasodilatation in aorta from control mice, but restored the vasodilator effects of PVAT-containing aortas from HFD-fed mice, indicating that L-arginine deficiency is indeed a reason for PVAT eNOS dysfunction (Xia *et al.,* 2016).

Another mechanism for PVAT eNOS dysfunction is the reduced phosphorylation of eNOS at the serine 1177 residue associated with Akt inhibition (one of the upstream kinases for serine 1177 phosphorylation) (Xia *et al.,* 2016). In a recent study, we treated diet-induced obese mice with the standardized *Crataegus* extract WS® 1442, which is known to enhance eNOS phosphorylation at the serine 1177 residue by stimulating Akt activity. The *in vivo* treatment with WS® 1442

improved the phosphorylation levels of Akt and eNOS, and completely normalized the vasodilator response of PVAT-containing aorta from diet-induced obese mice, confirming the functional importance of the Akt-eNOS axis in obesity-induced PVAT dysfunction (Xia N *et al.* unpublished data 2016).

In addition, obesity has been shown to reduce eNOS expression in mesenteric PVAT of diet-induced obese rats (Bussey *et al.,* 2016) and mice (Gil-Ortega *et al.,* 2014). Sustained weight loss in rats restores eNOS expression and improves PVAT NO production (Bussey *et al.,* 2016). In severely obese individuals, bariatric surgery improves NO bioavailability in PVAT of small subcutaneous arteries (Aghamohammadzadeh *et al.,* 2013).

Enhancing PVAT H_2S production

In a rat model of obesity and metabolic syndrome (cafeteria diet, fed for 3 months), treatment with the PPARγ agonist rosiglitazone increases insulin sensitivity, reduces fasting insulin levels and triglyceride concentration, increases CSE expression and activity as well as PVAT H_2S production, and improves the anticontractile effect of PVAT on aortic rings (Beltowski, 2013).

After treatment of rats for 3 weeks with lipophilic atorvastatin (20 mg·kg^{-1}·day^{-1}), but not hydrophilic pravastatin (40 mg·kg^{-1}·day^{-1}), the PVAT H_2S levels were increased as its mitochondrial oxidation was inhibited, and the anticontractile effect of PVAT was augmented (Wojcicka *et al.,* 2011). Inhibition of H_2S metabolism results from the atorvastatin-induced decrease in coenzyme Q, which is a cofactor of H_2S oxidation by sulfide:quinone oxidoreductase. In contrast to H_2S, statins do not impair mitochondrial oxidation of organic substrates (Beltowski and Jamroz-Wisniewska, 2012). The effect of atorvastatin on PVAT H_2S levels is independent of the lipid-lowing action of the drug because both statins at the doses used had comparable effects on the plasma lipid profile. Hydrophilic statins act mainly in the liver whereas lipophilic statins are equally active in hepatocytes and extrahepatic tissues (Shitara and Sugiyama, 2006) and are supposed to accumulate in triglyceride-rich PVAT (Beltowski, 2013). The effect of statins on PVAT function has not yet been verified in obesity models. Atorvastatin has been shown to improve PVAT function in spontaneously hypertensive rats (Zeng *et al.,* 2009). It is not yet clear whether these effects are mediated by H_2S.

In addition, cannabinoid CB_1 receptor agonists elevate PVAT H_2S by inhibiting its mitochondrial oxidation (Beltowski, 2013). The imidazoline I_1 receptor agonist moxonidine has been shown to increase myocardial CSE expression and H_2S production in streptozotocin-induce diabetic rats (El-Sayed *et al.,* 2016).

Sulfhydrylated ACE inhibitors may be of particular interest because of their dual properties: inhibition of ACE and potentiation of the H_2S pathway *in vivo*, as shown for S-zofenopril in spontaneous hypertensive rats (Bucci *et al.,* 2014).

Inhibition of the renin–angiotensin–aldosterone system

In wire myograph experiments with rat mesenteric small arteries, the *in vitro* creation of a hypoxic environment causes

the loss of PVAT's anticontractile function. This hypoxia-induced loss of PVAT's anticontractile effect can be prevented by incubation with the ACE inhibitor captopril or the AT_1 receptor antagonist termisartan (Rosei *et al.*, 2015), or with the aldosterone antagonist eplerenone (Withers *et al.*, 2011), whereas the β-blocker atenolol was without effect (Rosei *et al.*, 2015). Mechanistically, the renin–angiotensin–aldosterone system (RAAS) is thought to play a key role in the exacerbation of the hypoxia-induced inflammation and oxidative stress. Hypoxia stimulates ROS production and may activate RAAS by up-regulating ACE expression and inhibiting ACE2 activity (Zhang *et al.*, 2009). Ang II and aldosterone enhance oxidative stress, macrophage infiltration and inflammation (Withers *et al.*, 2011; Rosei *et al.*, 2015). Both Ang II and mitoROS are potent activators of HIF-1α (Patten *et al.*, 2010). *In vivo* treatment with the AT_1 receptor antagonist losartan improves PVAT function in fructose-induced hypertensive rats (Huang *et al.*, 2010). The aldosterone antagonist spironolactone has also been shown to restore PVAT function in a rat model of β-adrenoceptor overstimulation (Victorio *et al.*, 2016a). Nevertheless, the effect of RAAS inhibitors on PVAT function has not been tested in obesity models *in vivo* so far.

Anti-inflammatory strategies

Chronic administration of melatonin has been shown to reduce body weight, circulating insulin, glucose and triglyceride serum levels in HFD-fed rats (Prunet-Marcassus *et al.*, 2003; Rios-Lugo *et al.*, 2010). In the leptin-deficient ob/ob mice, the anticontractile function of mesenteric PVAT is reduced (Agabiti-Rosei *et al.*, 2014). Treatment with melatonin in the drinking water for 8 weeks improves PVAT function. Importantly, melatonin improves vascular function only in the presence of PVAT, indicating the importance of PVAT in the vascular dysfunction observed in ob/ob mice (Agabiti-Rosei *et al.*, 2014). The improvement of PVAT function by melatonin is likely to be attributable to its antioxidant/anti-inflammatory properties. Melatonin reduces the expression of ET-1, IL-6 and metalloproteases 2 and 9 in the aorta, decreases TNF-α and CD68 levels in visceral fat and increases the expression of adiponectin and adiponectin receptor 1 in PVAT of ob/ob mice (Agabiti-Rosei *et al.*, 2014).

Cytokine antagonists also represent attractive anti-inflammatory approaches. *Ex vivo* incubation with the anti-TNF-α antibody infliximab has been shown to improve PVAT function and PVAT NO production in small arteries isolated from obese patients (Virdis *et al.*, 2015). The effect of *in vivo* anti-TNF-α therapy on PVAT function has not been reported, so far.

AMPK activators

In vitro treatment of PVAT from rat thoracic aorta with AMPK activators AICAR, salicylate, metformin, resveratrol or diosgenin down-regulates the expression of pro-inflammatory factors (TNF-α, IL-6 and MCP-1) and increases anti-inflammatory molecules (adiponectin and PPARγ) in PVAT (Sun *et al.*, 2014; Chen *et al.*, 2016). This is associated with an improvement in eNOS phosphorylation and PVAT function (Sun *et al.*, 2014; Chen *et al.*, 2016).

Fructose-feeding in rats leads to a dysregulation of adipokine/cytokine expression in plasma and PVAT,

accompanied by a vascular dysfunction. Oral administration of fructose-fed rats with resveratrol and metformin improves adipokine/cytokine profiles, enhances AMPK activity and SIRT1 expression in PVAT, and restores PVAT eNOS phosphorylation and acetylcholine-induced vasodilatation (Sun *et al.*, 2014). Importantly, *in vitro* treatment of aortic rings from normal rats with conditioned media of PVAT from fructose-fed rats reduces acetylcholine-induced vasodilatation. This inhibition of vascular function is reversed by conditioned media of PVAT from resveratrol- or metformin-treated rats (Sun *et al.*, 2014), indicating the key role of PVAT in the improvement of vascular function by the two compounds.

In HFD-fed rats, oral administration of diosgenin or resveratrol normalizes PVAT size, restores PVAT expression of TNF-α, IL-6, MCP-1, adiponectin, PPARγ and PVAT eNOS phosphorylation. Diosgenin and resveratrol also restores the effect of PVAT on vascular function (Chen *et al.*, 2016). In contrast to these stimulating effects of diosgenin or resveratrol, another natural compound, genistein, showed no beneficial effects on PVAT despite preventing weight gain in obese ob/ob mice (Simperova *et al.*, 2016).

Adiponectin releasers/expression enhancers

A recent meta-analysis demonstrates a significant increase in plasma adiponectin levels following statin therapy (Chrusciel *et al.*, 2016). Adiponectin release from PVAT can be enhanced by stimulating the eNOS-NO-cGMP-PKG pathway (Weston *et al.*, 2013; Withers *et al.*, 2014b). Thus, the mechanisms of statin-induced adiponectin secretion may involve vascular NO production (Forstermann and Li, 2011; Li and Forstermann, 2014). Theoretically, PVAT adiponectin release may also be stimulated by activators of eNOS, sGC or PKG. As PPARγ plays a role in governing adiponectin expression (Antonopoulos *et al.*, 2015b), PPARγ agonists (e.g. glitazones) and AMPK activators may enhance the expression of adiponectin *in vivo*, in addition to their effects on PVAT H_2S production (see above). However, these concepts remain speculations at this stage. The effect of such approaches on adiponectin expression/release and PVAT function need to be investigated in future studies.

Conclusion

PVAT plays a crucial role in obesity-induced vascular dysfunction. Hypoxia, inflammation and oxidative stress in PVAT lead to an impairment in the release of vasoactive factors from PVAT, and the normal anticontractile function of PVAT is lost in obesity. PVAT may, therefore, represent a new therapeutic target for vascular complications in obesity. Various approaches have been shown to improve PVAT function; however, their therapeutic potential needs to be further verified in obesity models.

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Author contributions

N.X. and H.L. wrote the manuscript. Both authors agreed to its publication.

Conflict of interest

The authors declare no conflicts of interest.

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