Perivascular adipose tissue: more than just structural support

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ABSTRACT
PVAT (perivascular adipose tissue) has recently been recognized as a novel factor in vascular biology, with implications in the pathophysiology of cardiovascular disease. Composed mainly of adipocytes, PVAT releases a wide range of biologically active molecules that modulate vascular smooth muscle cell contraction, proliferation and migration. PVAT exerts an anti-contractile effect in various vascular beds which seems to be mediated by an as yet elusive PVRF (PVAT-derived relaxing factor(s)). Considerable progress has been made on deciphering the nature and mechanisms of action of PVRF, and the PVRFs proposed until now are reviewed here. However, complex pathways seem to regulate PVAT function and more than one mechanism is probably responsible for PVAT actions in vascular biology. The present review describes our current knowledge on the structure and function of PVAT, with a focus on its role in modulating vascular tone. Potential involvements of PVAT dysfunction in obesity, hypertension and atherosclerosis will be highlighted.

INTRODUCTION
Before the 1980s, the importance of the endothelium in vascular biology was largely ignored. The discovery of EDRF (endothelium-derived relaxing factor) and subsequent identification of NO (nitric oxide) revolutionized the field, and today we recognize the crucial role of the endothelium in modulating the function of underlying VSMCs (vascular smooth muscle cells) via complex pathways mediated by a multitude of factors, such as ET-1 (endothelin-1), NO, prostacyclin and EETs (epoxyeicosatrienoic acids), as well as yet unidentified EDHF (endothelium-derived hyperpolarizing factor) and EDCF (endothelium-derived contracting factor). Additionally, endothelial dysfunction is now widely accepted as an important component of various cardiovascular diseases and pharmacological attempts to restore endothelial function are used in the treatment of these diseases.

A similar trajectory can be described for PVAT (perivascular adipose tissue). After simple observations of the effects of PVAT on vascular function, the term ADRF (adventitium-derived relaxing factor), now PVRF (PVAT-derived relaxing factor), was introduced. Several

Key words: adipokine, atherosclerosis, hypertension, obesity, perivascular adipose tissue, vascular contraction.
Abbreviations: ACE, angiotensin-converting enzyme; ADRF, adventitium-derived relaxing factor; AMPK, AMP-activated protein kinase; Ang-(1–7), angiotensin-(1–7); AngII, angiotensin II; BAT, brown adipose tissue; BMI, body mass index; C3, complement 3; CAD, coronary artery disease; DOCA, deoxycorticosterone acetate; ET-1, endothelin-1; HFD, high-fat diet; 5-HT, 5-hydroxytryptamine; IL, interleukin; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; mTOR, mammalian target of rapamycin; NEFA, non-esterified fatty acid; NMN, nicotinamide mononucleotide; NOS, NO synthase; cNOS, endothelial NOS; PAI-1, plasminogen-activator inhibitor-1; PKC, protein kinase C; PPARγ, peroxisome-proliferator-activated receptor γ; PVAT, perivascular adipose tissue; PVRF, PVAT-derived relaxing factor; RAS, renin-angiotensin system; ROS, reactive oxygen species; SHR, spontaneously hypertensive rat; SNS, sympathetic nervous system; SOD, superoxide dismutase; TNFα, tumour necrosis factor α; UCP-1, uncoupling protein-1; VSMC, vascular smooth muscle cell; WAT, white adipose tissue; WKY, Wistar–Kyoto.
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candidate PVRFs have been proposed and reports of dysfunctional PVAT in obesity and cardiovascular diseases have appeared. As we are beginning to uncover a wide range of PVAT mediators that influence vascular function in physiology and disease, we may envision a future PVAT-centred revolution in vascular biology to parallel the one kindled by the endothelium 30 years ago.

**GENERAL CONSIDERATIONS ON ADIPOSE TISSUE**

Adipose tissue is usually classified as either white or brown. BAT (brown adipose tissue) is traditionally associated with non-shivering thermogenesis and is composed of metabolically active adipocytes equipped with numerous UCP-1 (uncoupling protein-1)-expressing mitochondria, which give the tissue its characteristic brown colour. WAT (white adipose tissue) is traditionally seen as a lipid storage site. Generally, WAT is less vascularized, less innervated and less metabolically active in comparison with BAT [1]. Both BAT and WAT receive innervation from the SNS (sympathetic nervous system), whereas parasympathetic innervation is still under dispute [2]. Typical WAT adipocytes are composed of a single large lipid vesicle, whereas BAT adipocytes contain smaller multilocular lipid vesicles. BAT and WAT adipocytes derive from different precursors. Thus BAT adipocytes share origins with skeletal myocytes, whereas WAT adipocytes have a different, less clear, origin [3,4]. Interestingly, transdifferentiation appears possible, for example in response to β3 adrenergic or PPARγ (peroxisome-proliferator-activated receptor γ) agonist treatment. Thus BAT adipocytes sometimes found inside WAT (mixed adipose tissue) share progenitors with BAT adipocytes and not with ‘classical’ BAT adipocytes [3,4]. Several older concepts on adipose tissue have been overturned in recent years, such as the restriction of brown fat to particular ages or anatomical locations [5]. Most importantly, the idea that adipose tissue is an inert structure with no functional significance other than connective support and lipid storage has now been definitively disproven. The current prevailing opinion is that the various fat depots (to which some have referred collectively as the ‘adipose organ’) are structures with important endocrine and paracrine functions regulated by complex mechanisms, and that possess plastic properties in response to nutritional or temperature stimuli [1,6,7].

**SPECIAL CHARACTERISTICS OF PVAT**

First, PVAT is different from adipose tissue in general due to its specific location (surrounding blood vessels). PVAT surrounds most systemic blood vessels, with the notable exception of the cerebral circulation [8]. PVAT lies on the outside of adventitia, without laminar structures or any organized barrier to separate the two. Depending on the vascular bed, PVAT can be mixed adipose tissue (such as aortic PVAT) or WAT (such as mesenteric PVAT) [8]. Vascularization and innervation of PVAT also vary greatly with location, thus helping to explain local variations in the functional characteristics of PVAT. Besides adipocytes and cells associated with penetrating vasa vasorum, PVAT may contain infiltrating macrophages and T-lymphocytes, the activity of which becomes relevant in pathophysiological situations.

Secondly, PVAT may be a functionally specialized type of adipose tissue, with different developmental and secretory properties. Thus PVAT adipocytes appear to be inherently different from adipocytes in other fat depots, as shown in a study by Chatterjee et al. [9] in which perivascular adipocytes from human coronary arteries were compared with adipocytes from perirenal and subcutaneous depots. The study found that PVAT adipocytes are less differentiated and have a secretory profile favouring pro-inflammatory cytokines, such as IL (interleukin)-6, IL-8 and MCP-1 (monocyte chemoattractant protein-1), with contrasting reduced adiponectin secretion, findings which prompted the authors to conclude that perivascular adipocytes contribute to adventitial inflammation and atherosclerosis development. The specialized nature of PVAT is illustrated further by differences in the secretory profiles depending on the vascular bed [10].

Owing to these important differences, knowledge gained from studies focusing on adipose tissue is not necessarily applicable to PVAT. This is true of both the nature of specific molecules being released and of their expression levels.

**RELEASE OF BIOLOGICALLY ACTIVE MOLECULES FROM PVAT**

It is now known that in addition to NEFAs (non-esterified fatty acids; free fatty acids), adipose tissue releases hormone-like substances that disseminate in the blood stream and can act at a distance, for example adipose-tissue-derived leptin acting on leptin receptors in the hypothalamus to regulate appetite. Some researchers have even observed the presence of fenestrated capillaries, a characteristic of endocrine organs, in normal mouse adipose tissue [11]. The extent of endocrine effects of PVAT specifically is as yet unclear; however, PVAT has obvious paracrine effects on vascular structures.

Whether acting in an endocrine or paracrine manner, PVAT releases a wide range of biologically active molecules (Table 1). Adipokines are the specific adipocyte product and judging by their effect on cytokine levels they can be classified as pro-inflammatory (leptin, resistin and visfatin) and anti-inflammatory (adiponectin and adrenomedullin). As emerging modulators of vascular
function, adipocytokines are now under intense scrutiny, and several excellent reviews describe the advances in adipokine research [6,12–14]. Newly identified adipokines (omentin, nesfatin, visfatin, adiponectin) may also play a role in vascular function, as revealed in a recent review [15], although their expression in PVAT has not been proven yet. In addition to adipocytes, adipocytes in PVAT release classical chemokines/cytokines, such as IL-6, IL-8, MCP-1 and PAI-1 (plasminogen-activator inhibitor-1) [14,16]. The infiltrating macrophages and T-lymphocytes present in PVAT or recruited in response to inflammatory mediators, but are likely to be the active partners of adipocytes in maintaining a balance of these factors, as well as in regulating inflammatory responses to external stimuli. PVAT also releases angiotensin peptides, being equipped with an almost complete RAS (renin–angiotensin system), including angiotensinogen, converting enzymes and receptors [10,17]. Release of ROS (reactive oxygen species), such as superoxide and H$_2$O$_2$ (hydrogen peroxide), as well as other gaseous molecules including H$_2$S (hydrogen sulfide), has also been shown in PVAT [18–20]. Additionally, PVAT expresses a complex ROS/RNS (reactive nitrogen species) machinery, containing among others NADPH oxidase, eNOS (endothelial NOS (NO synthase)) and all SOD (superoxide dismutase) isoforms. Finally, steroid hormone production (oestradiol and cortisol) by PVAT is also possible, although not yet fully proven.

Although adipocytes constitute the majority of cells in PVAT, the other cell types present (macrophages, T-lymphocytes, fibroblasts and capillary endothelial cells) also possess the capacity to produce biologically active molecules. This is especially true of the infiltrating immune cells, which will secrete the aforementioned cytokines when activated. The cross-talk between adipocytes and the infiltrating immune cells in PVAT is a central feature of PVAT function, which becomes important especially in disease conditions [6,21–23]. Few present studies have attempted to identify the cellular source of cytokines secreted from adipose tissue, which for example is the macrophage for most of the TNFα (tumour necrosis factor α) and IL-6 [23], with the macrophage also being the primary infiltrating cell type in obese adipose tissue, ahead of lymphocytes and neutrophils [24]. No such studies have been performed for PVAT. The relative importance of macrophages in PVAT has been illustrated by a study by Withers et al. [25] using a conditional macrophage ablation to demonstrate the dependence of PVAT function on macrophage infiltration in a mouse model of hypoxia. Additionally, important differences between BAT and WAT adipocyte secretion may also exist. Therefore, until studies are performed to distinguish the exact source of PVRF, it is impossible to ascribe the effects of PVAT to adipocytes, whether white or brown, or any other cell type.

**PVAT Function**

Typical experimental preparations of blood vessels for measurements of contraction begin by ‘cleaning’ the vessel, which largely means removal of PVAT. This practice was justified by the belief that PVAT, as a connective tissue, provides only mechanical support to vessels, but does not play any role in vascular contraction. This view is still illustrated in almost any diagram of vascular structure/function with endothelium, smooth muscle and adventitia as the only blood vessel layers. Additionally, PVAT was also removed because it was believed that its presence would impair diffusion of the pharmacological agents used experimentally. An observation of a PVAT-mediated decrease in contractile responses to noradrenaline (norepinephrine) in rat aorta, made by Soltis and Cassis [26] in 1991, first pointed to the potential role of PVAT in vascular contraction. These studies were continued a decade later by Gollasch and co-workers [27–31], who began analysing the mechanism of the anti-contractile effect of PVAT. Subsequently, more researchers, notably Gao and co-workers [8,17,19,32–38],

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**Table 1** PVAT-derived biologically active molecules with vascular effects

<table>
<thead>
<tr>
<th>Released by PVAT</th>
<th>Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipokines</td>
<td>Lepin, adiponectin, resistin, visfatin, adrenomedullin, omentin (?)</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-1, IL-6, IL-8, MCP-1, PAI-1, TNFα and RANTES</td>
</tr>
<tr>
<td>Gaseous molecules</td>
<td>Superoxide, H$_2$O$_2$, NO and H$_2$S</td>
</tr>
<tr>
<td>Expressed by PVAT</td>
<td></td>
</tr>
<tr>
<td>RAS molecules</td>
<td>Angiotensinogen, angiotensin I, AngII, Ang-(1–7), ACE1, ACE2, (pro)renin receptor and AngII receptors</td>
</tr>
<tr>
<td>ROS/oxidative stress</td>
<td>NADPH oxidase, SODs, eNOS and lipoygenases</td>
</tr>
<tr>
<td>Adipocyte-specific</td>
<td>Adipokines, adipokine receptors, NEFAs, β3-adrenergic receptor, PPARγ and UCP-1</td>
</tr>
<tr>
<td>Inflammatory-cell-specific</td>
<td>Cytokines and cytokine receptors</td>
</tr>
<tr>
<td>Others</td>
<td>Metalloproteases, steroid hormones (?) and C3</td>
</tr>
</tbody>
</table>
Table 2  Potential mechanisms of PVAT action on vascular contraction

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Effect</th>
<th>Condition</th>
<th>Vascular bed and species</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺ channel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KATP</td>
<td>Anti-contractile</td>
<td>Physiological</td>
<td>Rat aorta</td>
<td>[27,28]</td>
</tr>
<tr>
<td>Kᵣ</td>
<td>Anti-contractile</td>
<td>Physiological</td>
<td>Rat aorta</td>
<td>[19]</td>
</tr>
<tr>
<td>Kᵥ</td>
<td>Anti-contractile</td>
<td>Physiological</td>
<td>Human internal thoracic</td>
<td>[33]</td>
</tr>
<tr>
<td>Loss of anti-contractile effect</td>
<td></td>
<td></td>
<td>Rat mesenteric</td>
<td>[29,99]</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Anti-contractile</td>
<td>Physiological</td>
<td>Rat mesenteric</td>
<td>[10,81]</td>
</tr>
<tr>
<td>Leptin</td>
<td>Direct relaxing</td>
<td>Physiological</td>
<td>Rat aorta</td>
<td>[31]</td>
</tr>
<tr>
<td>Superoxide</td>
<td>Contractile</td>
<td>Physiological</td>
<td>Rat aorta</td>
<td>[32]</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Anti-contractile</td>
<td>Physiological</td>
<td>Rat aorta</td>
<td>[19]</td>
</tr>
<tr>
<td>NO</td>
<td>Anti-contractile</td>
<td>Early diet-induced obesity</td>
<td>Mouse mesenteric fat arterioles</td>
<td>[75]</td>
</tr>
<tr>
<td>NO</td>
<td>Anti-contractile</td>
<td>Diet-induced obesity</td>
<td>Mouse mesenteric fat arterioles</td>
<td>[70]</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Blocks anti-contractile effect</td>
<td>Obesity</td>
<td>Mouse mesenteric fat arterioles</td>
<td>[70]</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Blocks anti-contractile effect</td>
<td>Obesity</td>
<td>Human subcutaneous fat arterioles</td>
<td>[59]</td>
</tr>
<tr>
<td>H₂S</td>
<td>Anti-contractile</td>
<td>Physiological hypertension</td>
<td>Rat aorta</td>
<td>[18,99]</td>
</tr>
<tr>
<td>AngII</td>
<td>Contractile</td>
<td>Physiological</td>
<td>Rat mesenteric</td>
<td>[34]</td>
</tr>
<tr>
<td>Ang-(1–7)</td>
<td>Anti-contractile</td>
<td>Physiological</td>
<td>Rat aorta and vena cava</td>
<td>[35,38]</td>
</tr>
<tr>
<td>Mineralocorticoid receptor</td>
<td>Blocks anti-contractile effect</td>
<td>Obesity</td>
<td>Rat aorta</td>
<td>[29]</td>
</tr>
<tr>
<td>AMPK</td>
<td>Endothelial dysfunction</td>
<td>Obesity</td>
<td>Rat mesenteric</td>
<td>[71]</td>
</tr>
<tr>
<td>Infiltrating macrophages</td>
<td>Blocks anti-contractile effect</td>
<td>Obesity</td>
<td>Mouse mesenteric fat arterioles</td>
<td>[25]</td>
</tr>
<tr>
<td>Changes in fatty acid composition</td>
<td>Blocks anti-contractile effect</td>
<td>Metabolic syndrome</td>
<td>Rat aorta</td>
<td>[74]</td>
</tr>
<tr>
<td>Hyponatra</td>
<td>Blocks anti-contractile effect</td>
<td>Anti-contractile via Kᵥ channel</td>
<td>Mouse aorta</td>
<td>[57]</td>
</tr>
</tbody>
</table>

Löhne et al. [28] coined the term ADRF in their elegant article from 2002 in which they showed that the anti-contractile effect of PVAT in rat aorta was transferrable and adipocyte-derived. These authors [28] also proposed the mechanism to be the activation of KATP channels and excluded contributions from NOS and from other K⁺ channels, such as large conductance Ca²⁺ channels, and delayed rectifier and inwardly rectifying K⁺ channels. The same group went on to demonstrate that the release of ADRF is Ca²⁺-dependent and regulated by tyrosine kinase and PKA (protein kinase A)-dependent mechanisms, but was independent of influences from vanilloid, cannabinoid and CGRP (calcitonin gene-related peptide) receptors, suggesting that nerve endings in PVAT are not involved in the anti-contractile effect of PVAT [27]. The anti-contractile effect of PVAT was then observed in the rat mesenteric bed, where the mechanism for ADRF release appeared to be Kᵥ-channel-dependent [29].

The exact identity of PVRF(s) has been chased ever since, with several candidates appearing over time. Gao et al. [19] showed that the anti-contractile effect in rat aorta has both endothelium-dependent and -independent components, involving NO release and Kᵥ.

reproduced this effect and efforts began to identify PVRF and to study the changes in PVAT in various disease states.

**MECHANISMS OF THE ANTI-CONTRACTILE EFFECT OF PVAT**

Several mechanisms (Table 2) have been proposed for the anti-contractile effect of PVAT (Figure 1). As with other common processes in vascular biology, probably no single mechanism would explain all the experimental findings, the relative importance of different factors varying according to species, vascular bed and pathophysiological state [21,30,39].

Soltis and Cassis [26] hypothesized that PVAT increased the uptake of noradrenaline, explaining the observed reduced contractile response in the presence of PVAT. This idea would not explain the subsequent observations of PVAT reducing contraction to a host of agonists, including phenylephrine, AngII (angiotensin II), thromboxane A₂ agonists, serotonin or ET-1, some of which are not subject to uptake or extracellular inactivation.
channel activation for the former and relying on H₂O₂ and sGC (soluble guanylate cyclase) activation for the latter. Variations in the type of K⁺ channels involved in mediating the effects of PVAT are probably also explained not only by species and vascular bed, but also by methodological differences between studies, with Verlohren et al. [29] being the only ones to employ electrophysiological measurements. More of these studies are needed to explore the role of K⁺ channels and this is especially true of small vessels, which are particularly sensitive to the perivascular K⁺ environment [40].

In more recent studies, Gao et al. [35] have proposed Ang-(1–7) [angiotensin-(1–7)] to be (one of) the PVRF released in rat aorta. Thus Ang-(1–7) expression was demonstrated in PVAT, and inhibition of the Mas receptor or ACE (angiotensin-converting enzyme) 2 inhibitor treatment decreased the anti-contractile effect of PVAT [35]. These findings with Ang-(1–7) were later reproduced in the rat vena cava, which displayed the same effect of PVAT [38]. Interestingly, the only report of eNOS expression in PVAT was also in a vein, the human saphenous vein [41].

Adipokines are also obvious candidates for PVRF, although quite a few of them have not yet been tested in vascular contractile experiments. Leptin, probably the longest studied adipokine, exerts a direct vasodilatory effect in the absence of PVAT in canine mesenteric vessels [42], rabbit aorta [43] and the human forearm [44], and leptin receptors are present in endothelium, where they may mediate vasorelaxation via both NO-dependent and -independent mechanisms [43,45,46]. Leptin has been shown to directly inhibit AngII-induced vasoconstriction in rat aorta [47], and this effect was mediated by stimulation of iNOS (inducible NOS) activity in VSMCs [48]. Among the other effects of leptin infusion in vivo, it produces acute hypotension, supporting a direct vasodilatory role. However, chronic leptin infusion leads to endothelial dysfunction [49] and hypertension [50], highlighting the dual role of leptin as a detrimental and beneficial factor in the cardiovascular system, depending on plasma levels and duration of exposure [21]. Other studies were not able to reproduce these findings or had conflicting results, and a final conclusion on the effects of leptin on the vasculature is lacking [46]. Adiponectin was proposed as a candidate PVRF by Gollasch et al. [31], who showed that recombinant adiponectin reduced 5-HT (5-hydroxytryptamine)-mediated contraction of rat aorta. However, contractile responses to 5-HT in the mesenteric bed of adiponectin-deficient mice were not different from wild-type mice, dismissing adiponectin as a PVRF. This does not, however, exclude multiple protective effects of adiponectin on vascular function, including insulin-sensitizing, anti-atherogenic and anti-inflammatory actions. Vascular function is probably influenced by adiponectin via multiple mechanisms, including AMPK (AMP-activated protein kinase)-mediated activation of eNOS [51]. Additionally, expression of adiponectin by endothelium following injury supports the idea of cross-talk mechanisms between PVAT and the endothelium [52]. With two circulating active forms (full length and cleavage-generated globular adiponectin), capable of multimerization, and two recently discovered receptors, adiponectin is probably part of a more complex pathway with roles in immunity, insulin resistance and inflammation [6,12,53]. The less-investigated protein resistin may also play a role in the effect of PVAT on vascular contraction; however, probably only in an indirect manner, such as ROS-mediated, by regulating protein expression of other mediators or by stimulating macrophage infiltration and the release of other cytokines.
[21,54,55]. The full picture of resistin action is still lacking, a putative receptor has not been identified yet and, indeed, even its main cellular source is not entirely clear [6]. Visfatin was excluded from exerting direct effects on vascular contraction [56]; however, this adipokine may play important roles in VSMC proliferation and atherosclerosis pathogenesis (see below). Additionally, newly identified adipokines, such as omentin, chemerin, nesfatin and vaspin, may also have anti-contractile effects; however, their expression in PVAT remains to be demonstrated [15].

A recent report has demonstrated the loss of the anti-contractile effect of PVAT in the presence of aldosterone in rat mesenteric arteries [25], similar to the initial report by Soltis and Cassis [26], where the same effect was observed with DOCA (deoxycorticosterone acetate) in the rat aorta [26]. The same loss of PVAT function was observed during hypoxia, and the authors [25] used a conditional macrophage ablation mouse model to demonstrate that these effects are mediated by infiltrating macrophages in PVAT and that aldosterone receptor antagonism reverses both the effects of aldosterone and hypoxia, restoring PVAT anti-contractile function. Another group published the opposite results with respect to hypoxia, with the relaxing effect of aortic PVAT being enhanced by exposure to hypoxic conditions via KATP-dependent and endothelium-independent mechanisms [57]. One poorly explored mechanism for the effects of PVAT on vascular contraction is SNS activity, which innervates BAT and may be part of a brain–adipocyte axis in which signals from adipose tissue, such as leptin, work to modulate food intake, while at the same time influencing SNS activity, which will in turn regulate vasoconstriction [21].

A few results of the studies performed in animals were reproduced in human vessels in healthy conditions. The anti-contractile effect of PVAT was observed in the internal thoracic artery, acting via KCa channels [33,58]. The protective effects of adiponectin in healthy human PVAT is supported by the results of a study in which an adiponectin receptor fragment blocked the vasorelaxant effect of PVAT in small arteries [59].

Alterations in the mechanisms of PVAT vasoactivity may occur in pathophysiological conditions, such as obesity and hypertension, in which the most consistent finding has been a loss of the anti-contractile effect of PVAT.

**PRO-CONTRACTILE EFFECTS OF PVAT**

Interestingly, some authors have found either opposing or negative effects of PVAT on vascular contraction, thus contesting the now-accepted dogma [60]. The first account of the effects of PVAT on vascular function by Soltis and Cassis [26] included data demonstrating contraction of intact rat aorta to electrical field stimulation that was absent in tissues without PVAT. Additionally, in their first study on PVAT, Gao et al. [32] actually proposed a role for PVAT in promoting vasoconstriction elicited by perivascular nerve stimulation of the rat superior mesenteric artery. This effect was mediated via superoxide production by NADPH oxidase in PVAT adipocytes [32] and was reversed by treatment with an ACE inhibitor or AT1 (AngII type 1) receptor blocker [17]. The role of ROS was emphasized further in subsequent studies by the same group; however, the vasoconstrictor effect was not reproduced, instead the authors studied the already established anti-contractile effect.

In contrast with the study by Gao et al. [19] showing NO-mediated endothelium-dependent mechanisms for the anti-contractile effects of PVAT, Tune and co-workers have demonstrated that canine coronary PVAT decreases endothelium-dependent relaxation via PKC (protein kinase C) β-mediated phosphorylation of eNOS at Thr495 in physiological conditions [61] and this effect may be exacerbated during the metabolic syndrome [62] (see below).

**PVAT FUNCTION IN VSMC PROLIFERATION/MIGRATION**

In addition to its impact on VSMC contraction, PVAT is involved in the proliferative and migration function of VSMCs [63,64] (Figure 2). Secretion of visfatin by PVAT adipocytes was higher in aortic PVAT compared with subcutaneous or visceral fat depots, and it was correlated with phosphoribosyltransferase enzymatic activity and NMN (nicotinamide mononucleotide) production. Although lacking a direct vasoactive effect, visfatin has been shown to act as a VSMC growth factor, stimulating proliferation of VSMC via insulin-independent NMN-mediated activation of the ERK1/2 (extracellular-signal-regulated kinase 1/2) and p38 pathways [56]. Leptin and resistin, also acting via the MAPK (mitogen-activated protein kinase) and/or PI3K (phosphoinositide 3-kinase) pathways, have been shown to increase VSMC proliferation and migration in vitro [6,55]. NEFAs released by PVAT also stimulate VSMC proliferation in vitro [65]. Additionally, although not directly proven yet, other PVAT products, such as ROS, angiotensin peptides and steroid hormones, may stimulate VSMC growth and migration, thus potentially implicating PVAT as a pathogenic factor of atherosclerosis. Adiponectin decreases proliferation of VSMCs [65], probably by similar mechanisms as in endothelial cells [66], thus promoting a beneficial anti-angiogenic effect that may be reduced in pathophysiological conditions in which circulating adiponectin is decreased.
Effects of perivascular adipose tissue on VSMCs

Addressed together.

Of the present review these particular states will be

towards them often lacking, which is why for the purpose

insulin resistance and diabetes, with a clear distinction

continuum of metabolic alterations linking obesity with

In both human pathology and animal models, there is a

Obesity, insulin resistance and diabetes

We will discuss the human data available separately.

PVAT IN PATHOPHYSIOLOGICAL STATES

The vast majority of studies on PVAT have been

performed in animals. Conclusions from these studies

may not necessarily be applicable to human physiology

and pathophysiology; therefore in the following sections

we will discuss the human data available separately.

Insulin resistance and fatty acid composition

being redefined as an increase in adiposity. However,

besides pure mass increase, the changes in adipose

tissue accompanying obesity are also in adipocyte type

(brown compared with white), fatty acid composition of

lipid vesicles, cell size, potentially cell number,

inflammatory cell infiltration, ECM (extracellular matrix)

remodelling and other structural modifications. These are

naturally followed by functional alterations, imbalance

in adipocytokine production and potential oxidative

stress, hypoxia and inflammation [60]. Fulfilling the

predictions from other fat depots [69], a study by

Greenstein et al. [59] showed that total PVAT mass is

also increased in obese humans. In addition, the anti-

contractile effect of PVAT was diminished and signs of

local inflammation and hypoxia observed in PVAT were

mimicked by application of IL-6 or TNFα and rescued

with SOD and catalase, as well as with TNFα antagonists.

Highlighting the importance of PVAT, it has been shown

that PVAT mass in humans was associated with visceral

fat mass and negatively correlated with insulin sensitivity

[72]. Moreover, evidence from the Framingham Heart

Study [73] shows that peri-aortic fat mass correlates with

hypertension, diabetes and aortic/coronary calcification,

even if corrected for BMI (but not if corrected for visceral

adipose tissue).

In animal models of obesity, total PVAT mass and

adipocyte size is also increased [20,70,71]. As PVAT

releases PVRF with anti-contractile effects in normal

conditions and this effect is adipose-mass-dependent

[29], it would be expected that PVAT would confer

a protective effect to vascular function in obesity via

increased PVRF release. However, as mentioned above,

the opposite occurs and the anti-contractile effect of

PVAT is lost in obesity. Various explanations have

been proposed for this puzzling finding, mostly centred

around the idea of impaired adipokine secretion and

activation of detrimental pathways, such as inflammation

and oxidative stress. The influence of PVAT on the link

between inflammation and insulin resistance in obesity

has been recently reviewed extensively elsewhere [74].

Release of leptin is uniformly increased in animal

models of obesity and, when specific PVAT leptin

production was measured in models of HFD (high-

fat diet)-induced obesity, it followed the same trend

[9,20]. In a swine model of the metabolic syndrome,

coronary endothelial dysfunction was exacerbated in

the presence of PVAT, via leptin and PKCα-dependent

mechanisms [62]. The inhibition of VSMC Ca2+-

signalling and vasoconstriction by leptin observed in

normal animals was lost in the Zucker rat [47]. On the

other hand, adiponectin release from PVAT was

reduced in both HFD and genetic models of obesity

[9,70]. In the NZO (New Zealand obese) mouse, in

addition to changes in PVAT mass, there was evidence

of macrophage infiltration, increased ROS formation,

decreased expression of SODs and signs of eNOS

uncoupling [70]. On the contrary, early diet-induced

obesity was correlated with increased NO production

by mesenteric PVAT in a mouse model [75]. The

role of ROS in obesity-induced PVAT dysfunction

was demonstrated in a study by Ketonen et al. [20]
in which impaired endothelium-dependent relaxation in

diet-induced obese mice was restored by PVAT removal

or treatment with ROS scavengers. The same study

found increased leptin, MCP-1 and NADPH oxidase

expression in PVAT from obese mice, and the increased

ROS production was corrected by treatment with

apocynin, suggesting a role for PVAT NADPH-oxidase-

produced superoxide in mediating the obesity-associated
endothelial dysfunction [20]. In a study of chronic (6 months) HFD treatment in rats [71], endothelial dysfunction was similarly improved by removing PVAT. HFD was accompanied by decreases in AMPK and eNOS and the up-regulation of mTOR (mammalian target of rapamycin). By creating a VSMC–adipocyte co-culture, a decrease in AMPK phosphorylation and an increase in mTOR phosphorylation in VSMCs co-cultured with adipocytes from HFD-treated rats were demonstrated [71]. Finally, the role of fatty acid composition was highlighted in a recent fructose-fed rat model of the metabolic syndrome, in which the anti-contractile effect of PVAT was diminished in the aorta and the saturated/mono-unsaturated fatty acid ratio and saturated/polyunsaturated fatty acid ratio was increased, antioxidant enzyme expression was decreased and markers of oxidative stress were increased in PVAT [76]. In another study, the increase in noradrenaline-induced contraction in fructose-fed rats was corrected with losartan in both the absence and presence of PVAT [77]. Contrary to the studies described above, the anti-contractile effect of PVAT was increased in a rat model of Type 1 diabetes [36].

Overall, it appears that alterations in PVAT function in obesity and the metabolic syndrome are indeed accompanied by alterations in the release of adipokines, inflammation and oxidative stress. The initiating factor, as well as the sequence of pathological events is as yet unclear; however, increased NEFA release from hypertrophied adipocytes is a clear candidate hypothesis, leading to the activation of a cascade of inflammatory pathways and oxidative stress that feed-forward each other [78].

Hypertension
The typical vascular dysfunction encountered in hypertension, whether present as one of the causal factors or as a consequence of remodelling in the face of chronically increased pressure, has been studied so far from the point of view of deregulation of the endothelial, smooth muscle and even adventitial layers. Only a handful of studies exist on the function of PVAT during hypertension, and they have all been completed in animals. Two consistent findings are shared by most of these studies: decreased PVAT mass and decreased PVAT anti-contractile effects. PVAT adipocyte size is decreased in several animal models of hypertension: the SHR (spontaneously hypertensive rat) [79], the AngII-induced hypertensive rat [34] and the DOCA-salt rat [80]. Total PVAT mass [79,81] is also reduced in the SHR compared with the control WKY (Wistar–Kyoto) rat. The function of PVAT is impaired in aorta from the SHR [82] and isolated mesenteric arteries, as well as the perfused whole mesenteric bed [79,81], where the anti-contractile effect of PVAT is diminished or lost. This reduced anti-contractile effect is not due to endothelial dysfunction, as it is still observable in endothelium-denuded arteries (therefore the alteration is probably due to changes in the endothelium-independent PVRF) [34]. Interestingly, Gao and co-workers [34] observed potentiation of responses to AngII in the presence of PVAT in control rats, and this contractile effect of PVAT was reduced in aorta from AngII-treated rats. PVAT mass and PVAT adipocyte size were also decreased in AngII-induced hypertension as compared with controls [34]. The same group reported opposite findings in the SHR model, in which PVAT-intact aorta contracted more in response to phenylephrine than the control WKY rats, thus exhibiting the previously published loss of anti-contractile effect compared with control. Relaxation induced by transfer of bath solution from PVAT-intact vessels was also diminished in aorta from SHRs, suggesting that the decreased PVAT mass is not responsible for the loss of function. This relaxation was inhibited by Ang-(1–7) receptor antagonism [37]. Interestingly, the anti-contractile effect of PVAT was restored by systemic atorvastatin treatment [82]. Fetal and neonatal exposure to nicotine induced hypertension in rats and this hypertension was associated with a loss of the anti-contractile effect of PVAT, as well as an increase in periaortic brown adipocytes [83].

Not many studies have explored the changes in adipocytokine secretion during hypertension. In contrast with obesity, leptin secretion is decreased in mesenteric PVAT from SHRs [79]. The leptin-induced decrease in VSMC Ca2+ signalling and AngII-mediated vasoconstriction is also lost in SHRs [84]. In the only published study of PVAT in the DOCA-salt model of hypertension, Ruan et al. [80] performed a different kind of experiment by using LC (liquid chromatography)-MS/MS (tandem MS) to identify the secretome of aortic PVAT. Using this method, the most abundant secretory protein in PVAT was identified as C3 (complement 3). PVAT-conditioned medium and recombinant C3 induced adventitial fibroblast migration and differentiation via JNK (c-Jun N-terminal kinase) activation, which was counteracted by treatment with a C3 antagonist and a neutralizing antibody. The PVAT C3 expression and JNK phosphorylation was increased in DOCA-salt compared with sham rats and this expression was associated with adventitial remodelling observed in the arteries of DOCA-salt rats [80].

Therefore the hypertensive PVAT, although reduced in size, seems to be affected by similar alterations in function as the obese PVAT. The extent of the similarities between PVAT dysfunction in obesity and hypertension has yet to be investigated (Figure 3).

Atherosclerosis
Atherosclerosis is a chronic inflammatory condition and, in view of the list of inflammatory mediators released by PVAT (Table 1), it is understandable why PVAT is
Perivascular adipose tissue: more than just structural support

Figure 3  Alterations of PVAT structure and function during obesity and hypertension lead to decreased anti-contractile effects of PVAT

Although similar imbalances in adipokine release and inflammatory events occur in PVAT during atherosclerosis, there appear to be no changes in contractile function.

being investigated as a link in the pathogenetic events leading to atherosclerosis. The 'outside to inside' view of atherogenesis is supported by a series of studies suggesting that alterations in PVAT function that follow an increase in PVAT mass lead to the release of pro-inflammatory and chemotactic mediators from PVAT, macrophage and inflammatory cell infiltration with ultimate pro-atherogenic consequences [16,85,86].

Because of the clinical manifestations of atherosclerosis, most human studies are focused on epicardial adipose tissue [18,87,88], which contains, although it is not identical with, pericoronary adipose tissue. An increase in epicardial adipose tissue was correlated in several clinical studies with parameters of CAD (coronary artery disease), such as the existence of plaque, the degree of stenosis, clinical CAD or cardiovascular events [85]. Adipokine secretion was shown to be impaired in epicardial adipose tissue from CAD patients, with increases in IL-6, PAI-1, TNFα, adrenomedullin, visfatin and leptin, and decreases in adiponectin [89–94]. Macrophage and T-cell infiltration in PVAT in the vicinity of atherosclerotic plaques was demonstrated in human aorta [22]. However, since increases in human epicardial adipose tissue are also associated with obesity and increases in visceral adipose tissue, the causal relationship is harder to establish between pericoronary PVAT dysfunction and atherosclerosis. A very convincing argument would be the fact that intramyocardial segments of coronary arteries, lacking PVAT, are more likely to be devoid of atherosclerosis [95].

In animal studies, the involvement of PVAT on the vascular tone of the left circumflex artery was excluded in a porcine model of atherosclerosis [96]. However, direct vasomotor effects of PVAT may also play a role in this setting. Pro-inflammatory adipokines were increased and anti-inflammatory adipokines were decreased in PVAT following balloon injury in animal models of endovascular injury [97]. Increased adipokine secretion and inflammatory cell infiltration have also been demonstrated in PVAT from atherosclerotic apoE (apolipoprotein E)−/− mice [98].

CONCLUSIONS

The importance of PVAT as a paracrine modulator of vascular function is becoming increasingly apparent. Complex mechanisms of PVAT action on vascular contraction relying on both secretion of PVRF and plastic properties of PVAT are probably involved in the pathogenesis of vascular dysfunction in obesity/the metabolic syndrome, hypertension and atherosclerosis. Irrespective of the changes in PVAT mass present in these conditions, in all cases PVAT dysfunction seems to contribute to the impairment in the underlying vessel function. The most likely common denominator present in these conditions is inflammation. However, the differences between PVAT and other fat depots are still poorly defined and a clear beneficial/detrimental role of PVAT has not been established. Whether PVAT is a feasible target of therapies designed to restore its function in order to improve vascular function in these diseases is therefore a separate question, and one that will require more complete mechanistic studies.

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