Clinical Science (2020) **134** 3–13
https://doi.org/10.1042/CS20190577

PORTLAND PRESS

Review Article

Potential role of perivascular adipose tissue in modulating atherosclerosis

Samah Ahmadieh, Ha Won Kim and Neal L. Weintraub

Department of Medicine, Vascular Biology Center, Medical College of Georgia at Augusta University, Augusta, GA, U.S.A.

Correspondence: Neal L. Weintraub (nweintraub@augusta.edu)

Perivascular adipose tissue (PVAT) directly juxtaposes the vascular adventitia and contains a distinct mixture of mature adipocytes, preadipocytes, stem cells, and inflammatory cells that communicate via adipocytokines and other signaling mediators with the nearby vessel wall to regulate vascular function. Cross-talk between perivascular adipocytes and the cells in the blood vessel wall is vital for normal vascular function and becomes perturbed in diseases such as atherosclerosis. Perivascular adipocytes surrounding coronary arteries may be primed to promote inflammation and angiogenesis, and PVAT phenotypic changes occurring in the setting of obesity, hyperlipidemia etc., are fundamentally important in determining a pathogenic versus protective role of PVAT in vascular disease. Recent discoveries have advanced our understanding of the role of perivascular adipocytes in modulating vascular function. However, their impact on cardiovascular disease (CVD), particularly in humans, is yet to be fully elucidated. This review will highlight the complex mechanisms whereby PVAT regulates atherosclerosis, with an emphasis on clinical implications of PVAT and emerging strategies for evaluation and treatment of CVD based on PVAT biology.

Introduction

Over the past several decades, the prevalence of obesity has doubled, with a concomitant increase in risk of associated cardiovascular complications. Understandably, obesity-related cardiovascular disease (CVD) risk is often attributed to concurrent risk factors such as hypertension, diabetes mellitus, and dyslipidemia [1,2]. However, longitudinal studies have demonstrated an independent risk association between obesity and CVD that is not fully accounted for by these traditional risk factors [2–4]. Moreover, some obese patients are metabolically healthy, suggesting that the quality and distribution of adipose tissue is a fundamental determinant of cardiometabolic disease, which highlights the complexity of adipose tissue biology. The extent of inflammation of adipose tissue, which promotes insulin resistance and systemic metabolic disease, appears to confer obesity-related risk; in this regard, attention has turned to the local impact of perivascular adipose tissue (PVAT) on vascular disease. PVAT expansion and chemokine production near the adventitia of large arteries have been detected early in the course of hyperlipidemic, atherosclerosis-prone animal models, and in human arteries, resulting in a heightened state of inflammation that likely plays a fundamental role in the pathogenesis of CVD [1,2,5].

The term PVAT is applied to the adipose tissue that juxtaposes the outer adventitial regions of most large arteries, irrespective of location [2,6]. Studies suggest that as an anatomically separated adipose tissue, PVAT arises from unique progenitor cells, giving rise to its distinctive functional characteristics [7]. Perivascular tissue surrounding coronary arteries is considered to be a part of the epicardial adipose tissue since there are no clear anatomical boundaries separating the two [2]. However, functional differences have been described. For example, *in vitro* differentiated human coronary perivascular adipocytes were reported to secrete more monocyte chemoattractant protein 1 (MCP-1) as compared with epicardial adipocytes derived from the same healthy humans [8]. Although human coronary PVAT exhibits a morphology similar to white adipose tissue, the adipocytes are smaller in size, heterogenous in shape, and

Received: 04 October 2019
Revised: 16 December 2019
Accepted: 23 December 2019

Version of Record published:
03 January 2020

Clinical Science (2020) **134** 3–13
https://doi.org/10.1042/CS20190577

Table 1 Mediators released from PVAT or PVAT resident immune cells along with their functions and major cellular sources

Mediator	Function	Major sources	References
Pro-atherogenic			
MCP-1	Increases chemotaxis and transendothelial migration of monocytes. Proangiogenic	Adipocytes, inflammatory cells	[1,6,8,11,22,28,30,31,45,46,48,53]
TNF-α	Activates M1 macrophages, chemoattractant for neutrophils	Macrophages and other inflammatory cells	[6,11,18,22,28,50]
Leptin	Increases TNF-α IL-6, IL-12, reactive oxygen species (ROS), enhances macrophage phagocytosis, increases proliferation, and migration of monocytes and VSMCs	Adipocytes	[54]
Resistin	Promotes endothelial cell activation by inducing endothelin-1 release and by up-regulating endothelial adhesion molecules (i.e., VCAM-1, MCP-1)	Adipocytes, macrophages	[21,22,55]
Visfatin	Increases IL-6, IL-8, TNF-α, VSMC proliferation and migration Decreases apoptosis of neutrophils	Adipocytes, macrophages	[56–58]
Osteoprotegerin	Both pro-inflammatory and anti-inflammatory	Adipocytes	[8,59]
PAI-1	Fibrinolysis inhibitor, decreases plasminogen activation	Adipocytes, platelets, vascular endothelium,	[60,61]
IL-6	Decreases adiponectin section, lipoprotein lipase activity. Increases lipolysis, suppressor of cytokine signaling type-3 (SOCS-3), proangiogenic	Macrophages, fibroblasts, endothelial cells, adipocytes	[6,18,22,28,45,46]
IL-8	Increases chemotaxis of monocytes, neutrophils, T lymphocytes, ROS production	Macrophages	[6,46]
IL-1β	Pro-inflammatory	Macrophages	[18,62]
Complement 3	Stimulates adventitial fibroblast migration and differentiation	Adipocytes	[63]
Complement 7	Complement systemic factor	Adipocytes	[8]
Complement H	Complement systemic factor	Adipocytes	[60]
GM-CSF	Regulates and promotes growth and population of monocytes and macrophages	Macrophages and T cells	[45]
VEGF	Pro-angiogenic	Macrophages	[1,38,46]
Anti-atherogenic			
Adiponectin	Suppress synthesis of TNF-α, IFN-γ, NF-κB, phagocytosis, induce production of IL-10, IL-1 receptor antagonist	Adipocytes	[54,64]
Adrenomedullin	Decreases inflammation	Adipocytes	[62,65]
TAIP6	Anti-inflammatory	Adipocytes	[8]
SOCS2	Anti-inflammatory	Adipocytes	[60]
IL-4	Stimulation of activated B-cell and T-cell proliferation, and differentiation of B cells into plasma cells. Decreases production of Th1 cells, macrophages	T cells, Basophils, Eosinophils	[37]
IL-10	Down-regulates expression of molecules that activate T cells and macrophages. Enhances B-cell survival. Inhibits synthesis of pro-inflammatory cytokines	Monocytes, T cells, Eosinophils	[66]
TGF-β	Anti-inflammatory. Stimulates differentiation of PV-ADSCs into VSMCs	T cells, B cells, Eosinophils	[37,38]
Omentin	Anti-inflammatory	Adipocytes	[67]

undergo less differentiation and maturation [8]. In contrast, PVAT surrounding the upper thoracic aorta of lean, healthy humans may exhibit a morphology similar to brown adipose tissue; however, the majority of studies report that white adipocytes predominate in human PVAT depots [9]. Conversely, PVAT surrounding the thoracic aorta of rodents exhibits a predominant brown phenotype, whereas PVAT surrounding the abdominal aorta is phenotypically a mixture of white and brown [10].

As a metabolically active endocrine tissue, PVAT is ideally positioned to directly govern vascular pathophysiology relative to other fat depots [1,8,11]. In healthy conditions, PVAT appears to play a protective role in regulating metabolism, inflammation, and function of associated blood vessels. In states of chronic caloric excess, perivascular adipocytes undergo hypertrophy; the tissue hypoxia and mechanical stress that ensues in PVAT results in a detrimental change in the secretome profile and the ability to store lipids [5,12]. The spillover of cytokines and fatty acids into the vascular adventitia, which is facilitated by the lack of a connective tissue barrier between PVAT and the adjacent artery, promotes arterial inflammation that may augment atherosclerosis and increase risk of plaque rupture [12]. Indeed, clinical observations suggest that the development of inflamed and dysfunctional coronary PVAT is positively correlated with coronary plaque burden and CVD mortality risk [1,13].

As PVAT's role in the development of CVD is becoming more widely accepted, a PVAT-centered revolution in vascular biology may be on the verge. The focus of this review will be on the growing body of data linking PVAT to the pathogenesis of the most common cause of CVD, atherosclerosis [14].

Adventitial inflammation and the pro-inflammatory phenotype of PVAT

The location of PVAT, abutting the nearby adventitia of blood vessels without a physical anatomical barrier, facilitates its ability to govern the focal vascular milieu via paracrine and vasocrine routes [1,6,8]. The traditional 'inside to outside' model of atherosclerosis pathogenesis centered on endothelial cell dysfunction, inflammation, and intimal foam cell formation as the root cause of atherosclerotic vascular disease [6]. However, most investigators systemically removed PVAT from blood vessels before performing biochemical testing, immunostaining, or functional studies as PVAT was considered to be an inert, non-vascular tissue [15]. More recent evidence suggests that communication between the vascular wall and PVAT may be bidirectional, with an 'outside to inside' inflammatory signaling triggered by dysfunctional PVAT more influential than previously thought [6,9,16]. For example, in hyperlipidemic atherosclerosis-prone apolipoprotein E (ApoE)-deficient mice, the major site of vascular inflammatory cell accumulation was reported to be the adventitia rather than the intima, and in atherosclerotic human aorta, inflammatory cells were observed to be densely clustered in PVAT at the adventitial margin, suggesting that PVAT has the potential to foster vascular inflammation [8,17].

Interest in PVAT biology has been driven in large part by studies examining the phenotype of human PVAT procured from patients undergoing surgical procedures. Human epicardial adipose tissue removed from patients undergoing coronary artery bypass grafting surgery demonstrated significantly higher levels of chemokines (i.e. MCP-1) and inflammatory cytokines [i.e. interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α] in comparison with subcutaneous adipose tissue obtained from the same patients [18]. Conversely, anti-inflammatory adiponectin expression was found to be significantly lower in epicardial fat samples from patients with significant coronary atherosclerosis compared with those without, suggesting an imbalance in PVAT inflammation in the setting of atherosclerosis [19]. Furthermore, macrophage infiltration in human PVAT has been reported to colocalize with resistin, an adipokine that was shown to increase the permeability of endothelial cells *in vitro*, shedding light on the multiple mechanisms whereby PVAT may contribute to the pathogenesis of atherosclerosis [20,21].

The epicardial portion of human coronary arteries is both richly endowed in PVAT and particularly susceptible to atherosclerosis. Chatterjee et al. [6] investigated the phenotype of PVAT and *in vitro* differentiated adipocytes surrounding coronary arteries of healthy humans. Differentiated pericoronary perivascular adipocytes released substantially more IL-8, IL-6, and MCP-1 than adipocytes derived from other fat depots under basal conditions, suggesting that they may be primed to amplify adventitial inflammation and inflammatory cell recruitment. Moreover, osteoprotegerin, a member of the TNF-related family that is correlated with increased atherosclerotic progression and instability, was strongly up-regulated in human coronary perivascular adipocytes [8]. These and other studies suggest that PVAT may be more susceptible to inflammation than other regional adipose depots. The extent of inflammation in PVAT raises the possibility that its expansion could also amplify disease at remote sites in the vasculature. Indeed, transplantation of abdominal aortic white PVAT harvested from high fat fed mice promoted inflammation (elevated TNF-α and MCP-1 expression), endothelial dysfunction, and atherosclerosis remotely in thoracic aorta, properties that were not shared by subcutaneous or visceral (epididymal) adipose tissue [11].

PVAT also contains endogenous anti-inflammatory pathways that may function to limit the extent of local inflammation. Low-density lipoprotein receptor-related protein-1 (LRP1) is a type 1 transmembrane protein receptor that is ubiquitously expressed, particularly in adipose tissue, and plays a role in endocytic and cell signal transduction [22]. LRP1 dysfunction in the liver, smooth muscle cells (SMCs), and macrophages was reported to augment atherosclerosis [23]. Investigating the role of LRP1 in PVAT, Konaniah et al. [22] found that LRP1-deficient PVAT exhibited a pro-inflammatory phenotype and elevated resistin expression. Furthermore, transplantation of LRP1-deficient PVAT to the carotid artery produced a three-fold increase in atherosclerosis development compared with control, associated with increased macrophage recruitment and MCP-1, IL-6, and TNF-α expression. Mechanistically, LRP1-deficient PVAT exhibited impaired lipid storage, suggesting an increase in free fatty acids that may augment lipotoxicity and adventitial inflammatory signaling.

Oxidized lipoproteins are atherogenic and promote vascular inflammation via multiple mechanisms [24–26]. Interestingly, Uchida et al. [27], using immunohistochemistry, showed evidence of oxidized low-density lipoprotein (LDL) and high-density lipoprotein (HDL) stored in human coronary PVAT. This was observed in all coronary PVAT samples, irrespective of the extent of underlying coronary disease, suggesting that the storage of lipoproteins may occur

Clinical Science (2020) **134** 3–13
https://doi.org/10.1042/CS20190577

prior to the formation of atherosclerotic fatty streaks. Meticulous examination of the deposition pattern of oxidized LDL led to the speculation that macrophages engulf oxidized LDL within the coronary PVAT and migrate to the intima via the interstitial space or *vasa vasorum*, while oxidized HDL particles are likely to be supplied from PVAT to the intima principally by the *vasa vasorum*. These findings suggest that PVAT may be an unrecognized supplier of oxidized lipoproteins to the vascular intima.

The renin–angiotensin system (RAS) contributes importantly to vascular inflammation and atherosclerosis. Irie et al. [28] reported that in ApoE-deficient mice fed a high-cholesterol diet (HCD), components of the RAS [i.e. angiotensinogen, angiotensin-converting enzyme, and angiotensin II receptor type 1a (AT_{1a})] were specifically up-regulated in PVAT, in conjunction with increased macrophage markers (i.e. CD68 and CD206); transplantation of PVAT from these mice into ApoE-deficient recipient mice produced a striking increase in atherosclerosis development. Interestingly, inflammation and atherosclerosis were significantly reduced by treating these mice with an angiotensin II receptor blocker or by transplanting PVAT from mice lacking AT_1 receptors, suggesting an AT_1-receptor dependent mechanism of inflammation regulating PVAT phenotype in the pathogenesis of atherosclerosis.

Interplay between inflammation and vascular function: potential atheroprotective effects of PVAT

While much of the interest in PVAT biology centers on its ability to promote inflammation and atherosclerosis in obesity, interestingly, healthy PVAT may be home to immune cells that attenuate atherosclerosis development. B-1 cell-derived IgM has been shown to attenuate pro-inflammatory cytokine production by M1 macrophages in visceral adipose tissue [29]. Srikakulapu et al. [29] demonstrated that in young ApoE-deficient mice, B-1 cells abundantly secreting IgM were present at surprisingly higher numbers in PVAT compared with aorta, and the PVAT B-1/B-2 ratio suggests an anti-inflammatory influence of PVAT. Furthermore, immunohistochemistry data from human coronary PVAT demonstrated that B cells were found aggregated in close proximity to the coronary artery in fat-associated lymphoid clusters. Notably, levels of IgM to oxidation-specific epitopes on LDL were shown to be inversely associated with MCP-1 levels in the plasma and with the development of atherosclerosis in humans [30,31]. More studies are needed to determine if enhancing B1 activity to increase focal IgM production will indeed neutralize the oxidized LDL particles stored in PVAT, and the impact this may have on atherosclerosis. Understanding immune modulation by healthy PVAT may thus improve our knowledge of endogenous atheroprotective pathways.

Healthy PVAT also possesses anti-contractile properties that are abolished in obesity, thus causing increased arterial tone, which is thought to be a key mechanism of obesity-associated hypertension and vascular dysfunction. Interestingly, Withers et al. [32] demonstrated that obesity is accompanied by a significant reduction in the number of resident eosinophils in PVAT, which may lead to the loss of anti-contractile function. Indeed, PVAT from eosinophil deficient-mice lost its anti-contractile effect, which was restored after purified eosinophils were added back to those vessels with intact PVAT. These findings suggest the existence of an eosinophil-derived soluble anti-contractile factor released by PVAT. Mechanistically, this factor was dependent on B3 adrenoreceptors, resulting in downstream signaling of adiponectin and nitric oxide pathways independent of other immune cells. These findings are the first to identify eosinophils as novel therapeutic targets for obesity associated CVD.

Dendritic cells (DCs), professional antigen presenting cells, are found in the border of adventitia and PVAT, where they may promote pro-inflammatory cytokine production from T cells contained in PVAT. The increase in arterial tone due to chronic inflammation and vascular dysfunction was shown to be associated with DC accumulation in PVAT in a murine type 2 diabetes mellitus (T2DM) model, and DC depletion improved both the vascular dysfunction and pro-inflammatory environment, suggesting a critical role of DC in PVAT inflammation associated with atherosclerosis [33]. Expression of adiponectin, an anti-inflammatory adipokine, was decreased in the PVAT of these mice [33], suggesting a possible mechanistic link between adipokine perturbations in PVAT and atherosclerosis progression in T2DM. Interestingly, PVAT inflammation, but not systemic inflammation, was observed in a non-obese and non-hypertensive prediabetic rat model, which was ameliorated by anti-diabetic drugs such as metformin and pioglitazone, also suggesting the potential association of PVAT inflammation and diabetes [34].

Natural killer (NK) cells and invariant natural killer T (iNKT) have also been identified in PVAT. NK cells were reported to regulate visceral adipose tissue inflammation via interferon (IFN)-γ release [35], and iNKT cells promoted adipose tissue inflammation and atherosclerosis in diet-induced obesity mouse models [36]. However, the regulatory role of NK cells or iNKT cells specifically in PVAT, and whether their presence in this niche modulates atherosclerosis, is not clear.

Terada et al. [37] investigated whether PVAT may have anti-atherogenic properties under healthy conditions and pro-atherogenic effects under diseased conditions. Thoracic aortic PVAT from healthy mice was transplanted to the

infrarenal aorta of ApoE-deficient mice, resulting in diminished atherosclerosis in the suprarenal aorta of recipient animals via systemic endocrine mechanisms. Increased transforming growth factor (TGF)-β1 mRNA expression, and positive TGF-β1 immunostaining co-localizing with M2-like macrophages, was detected in the transplanted PVAT. This remote anti-atherogenic effect of transplanted PVAT was neutralized by the administration of TGF-β1 antibody injections, consistent with the finding that patients with advanced atherosclerosis have a significant decrease in circulating active TGF-β1. Furthermore, an accumulation of alternatively activated macrophages was observed in the transplanted PVAT, in association with an increase in percentage of eosinophils and a time-dependent increase in anti-inflammatory IL-4 expression after transplantation. It should be noted, however, that the transplanted PVAT in the present study was phenotypically brown, so the relevance to atherosclerosis in humans is less clear.

Most studies published thus far have attributed the function of PVAT to secreted adipocytokines. However, Gu et al. [38] identified and highlighted the importance of the PVAT-derived mesenchymal stem cells (PV-ADSCs) in vascular remodeling. By conducting a single-cell RNA-sequencing analysis, two unique populations (clusters) of PV-ADSCs were identified with distinct gene expression signatures, signaling pathways, and metabolic profiles. Cluster 1 signaling pathways featured vascular endothelial growth factor (VEGF)-activated receptor activity and peroxisome proliferator-activated receptors (PPAR) signaling that are essential for angiogenesis, while cluster 2 featured platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF) binding, PI3K-Akt signaling, and TGF-β signaling that are essential in SMC differentiation. Using red fluorescent protein-labeled PV-ADSCs, the investigators demonstrated the participation of these cells in vascular remodeling *in vivo* via migration from the adventitia across to the intima. Moreover, *in vitro* studies demonstrated differentiation of PV-ADSCs into SMCs after TGF-β1 stimulation.

The differentiation of resident PV-ADSCs into SMCs is accompanied by metabolic reprogramming of mitochondrial function and lipid metabolism that are thought to drive SMC differentiation [38]. Interestingly, this metabolic reprogramming and differentiation of PV-ADSC was found to be induced by microRNA (miR)-378a-3p. Studies in the literature have demonstrated that in atherosclerosis, adventitial stem cells travel to sites of endothelial injury and differentiate in response to their respective microenvironments; this investigation presents the first data suggesting that PV-ADSCs may participate in pathophysiology in the intima. More studies are needed to validate the role of PV-ADSCs in the pathogenesis of atherosclerosis, and to investigate the efficacy of miR-378a-3p as a potential therapeutic target to regulate differentiation of PV-ADSCs to SMCs.

PVAT and adventitial vasa vasorum

The *vasa vasorum* is a network of microvessels originating primarily in the adventitia of conduit arteries that serves to deliver oxygen, nutrients etc. to medial and outer layers of the arterial wall [39]. In atherosclerosis, thickening of the neointima is thought to limit luminal diffusion of oxygen, thereby causing hypoxia of the deeper layers of the vessel wall, which in turn stimulates angiogenesis of the adventitial *vasa vasorum* [39]. Indeed, animal studies suggested that disruption of the *vasa vasorum* could promote medial necrosis and intimal thickening, leading to the notion that the *vasa vasorum* is atheroprotective [39]. However, more recent studies have challenged this dogma. First, in a pig model of hypercholesterolemia, proliferation of *vasa vasorum* was detected within the first few weeks of high fat feeding, even before the onset of endothelial dysfunction, suggesting that factors other than intimal hypoxia were responsible for its proliferation [40]. Second, in the same pig model, prevention of *vasa vasorum* proliferation using a pharmacological approach attenuated atherosclerosis [40]. Proliferation of *vasa vasorum* in atherosclerotic plaque is also increasingly recognized to contribute to lesion progression and destabilization in humans [41,42]. Postmortem retrospective studies have demonstrated that *vasa vasorum* density is positively correlated with progressive fibrous cap thinning; these fragile neovessels arising at the borders of the of the plaque necrotic core may leak macromolecules, including cholesterol-laden erythrocytes, that are taken up by macrophages, thereby promoting inflammation and rapid plaque growth [5,43,44]. Taken together, these studies suggest that *vasa vasorum* proliferation contributes to plaque vulnerability; thus, understanding mechanisms that promote *vasa vasorum* proliferation and function in atherosclerosis is crucial to devising new approaches to prevent and treat coronary artery disease.

Adipose tissue is inherently angiogenic, suggesting that PVAT could play a previously unrecognized but important role in proliferation of *vasa vasorum* in atherosclerosis. Using a model of PVAT transplantation to the mouse carotid artery, Manka et al. [1] demonstrated a significant increase in adventitial neovascularization compared with sham control. This mechanism was mediated in part by MCP-1, as PVAT from MCP-1 knockout mice did not demonstrate the same potent angiogenic properties. Moreover, in the same study, differentiated human perivascular adipocytes exhibited greater angiogenic potential as compared with subcutaneous and perirenal adipocytes derived from the

Clinical Science (2020) **134** 3–13
https://doi.org/10.1042/CS20190577

same patients. Thus, in addition to being primed to amplify inflammation, perivascular adipocytes may be uniquely poised to promote angiogenesis leading to *vasa vasorum* proliferation.

Ying et al. [45] investigated the potential contributary role of PVAT in plaque vulnerability using a PVAT transplantation model. PVAT from donor wild-type mice fed a high fat diet (HFD) for 4 weeks was implanted next to the carotid artery of atherosclerotic prone ApoE-deficient mice. The transplanted PVAT led to an enhanced plaque vulnerability with a higher intraplaque macrophage number, an increase in lipid core size, elevation in matrix metallopeptidase (MMP)-2 and -9 expression, and a thinner fibrous cap in comparison with transplanted subcutaneous and sham procedure. Furthermore, transplanted PVAT promoted intraplaque angiogenesis along with an elevation in several pro-angiogenic factors, MCP-1, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and up-regulation of anti-angiogenic factor 4. Interestingly, most of these changes were ameliorated by the administration of an endoplasmic reticulum (ER) stress inhibitor to the transplanted PVAT, suggesting that ER paracrine stress may be contributing in PVAT dysfunction. Using *in vitro* models, Ying et al. [45] provided evidence that induction of GM-CSF by ER stress in PVAT may contribute to the pathogenesis of vulnerable plaques via a nuclear factor κ B (NF-κB)-dependent mechanism. GM-CSF is a pro-inflammatory, pro-angiogenetic, hematopoietic growth factor that may play a significant role in plaque destabilization by facilitating macrophage apoptosis and plaque necrosis. Taken together, these findings suggest that ER stress in PVAT may contribute to destabilizing atherosclerotic plaques, and an ER stress inhibitor may serve as a promising therapeutic avenue for treating high risk atherosclerotic plaques.

In addition to promoting neovascularization, VEGF produced by PVAT may augment vascular smooth muscle cell (VSMC) proliferation. Schlich et al. [46] demonstrated that PVAT-conditioned media induced VSMC proliferation via a VEGF-dependent mechanism. Addition of oleic acid produced a synergistic proliferative response, which was significantly greater than that observed with conditioned media from subcutaneous or visceral adipose tissue. Intriguingly, PVAT from obese subjects with type 2 diabetes exhibited a high level of VEGF section, along with elevated expression of VEGF-R1 and -2, and a strong proliferative effect on VSMC. A VEGF-specific antibody only partially attenuated the VSMC proliferation, suggesting that other adipokines, such as activin A, IL-6, IL-8, or MCP-1 may also be contributory. These findings suggest that dysfunctional PVAT may play a particularly important role in promoting atherosclerosis in patients with obesity and diabetes.

Clinical significance of PVAT imaging

Noninvasive detection of vulnerable atherosclerotic plaques has been acknowledged as the 'holy grail' in cardiovascular medicine in the hopes that it would allow for earlier detection of vulnerable plaques and improve cardiovascular risk stratification [47]. Currently, most available methods to evaluate vascular inflammation provide structural information only and cannot specifically discriminate vulnerable atherosclerotic lesions [48]. However, coronary arteries with atherosclerotic plaques appear to have a larger amount of PVAT encroaching into their outer adventitia as detected by computed tomography (CT) [49]. Moreover, atherosclerotic plaque size and complex lipid core composition were positively correlated with PVAT volume and macrophage infiltration in a postmortem study of human subjects [13]. Thus, imaging of coronary PVAT holds promise as a non-invasive method to detect unstable coronary lesions [49,50].

Antonopoulos et al. [47] demonstrated key features of both epicardial and thoracic PVAT that can potentially be monitored via noninvasive imaging. Taking advantage of the fact that PVAT inflammation is linked to impaired differentiation and lower adipocyte lipid content, the authors examined high resolution CT scans and quantified fat attenuation index (FAI), an index of water to lipid ratio, in PVAT. The FAI was demonstrated to exhibit excellent sensitivity and specificity for identifying PVAT inflammation when validated against adipose tissue specimens. Importantly, the PVAT FAI was higher in PVAT surrounding unstable plaques and predictive of cardiovascular mortality in a retrospective patient cohort [47]. The authors concluded that imaging of PVAT can provide spatially localized information regarding the inflammatory microenvironment of human coronary arteries, which might enable early identification of high risk plaques and create an opportunity to pursue more intensive therapies.

Oikonomou et al. [49] analyzed two prospective cohorts who received coronary CT scans and found the PVAT FAI to be a strong predictor of all-cause and cardiac mortality. Moreover, the PVAT FAI was observed to be beneficial in the risk stratification of patients without coronary artery disease who might benefit from intensive primary therapy to reduce their CVD risk. Elnabawi et al. [50] studied the efficacy of novel biologic therapies (i.e. anti-TNF-α, anti-IL-17, and anti-IL12/23 therapy) in psoriasis patients. They demonstrated for the first time that these types of therapies significantly reduced coronary inflammation as measured by the PVAT FAI in patients with and without coronary artery disease. Incorporation of the PVAT FAI into the standard coronary CT analysis may represent a promising

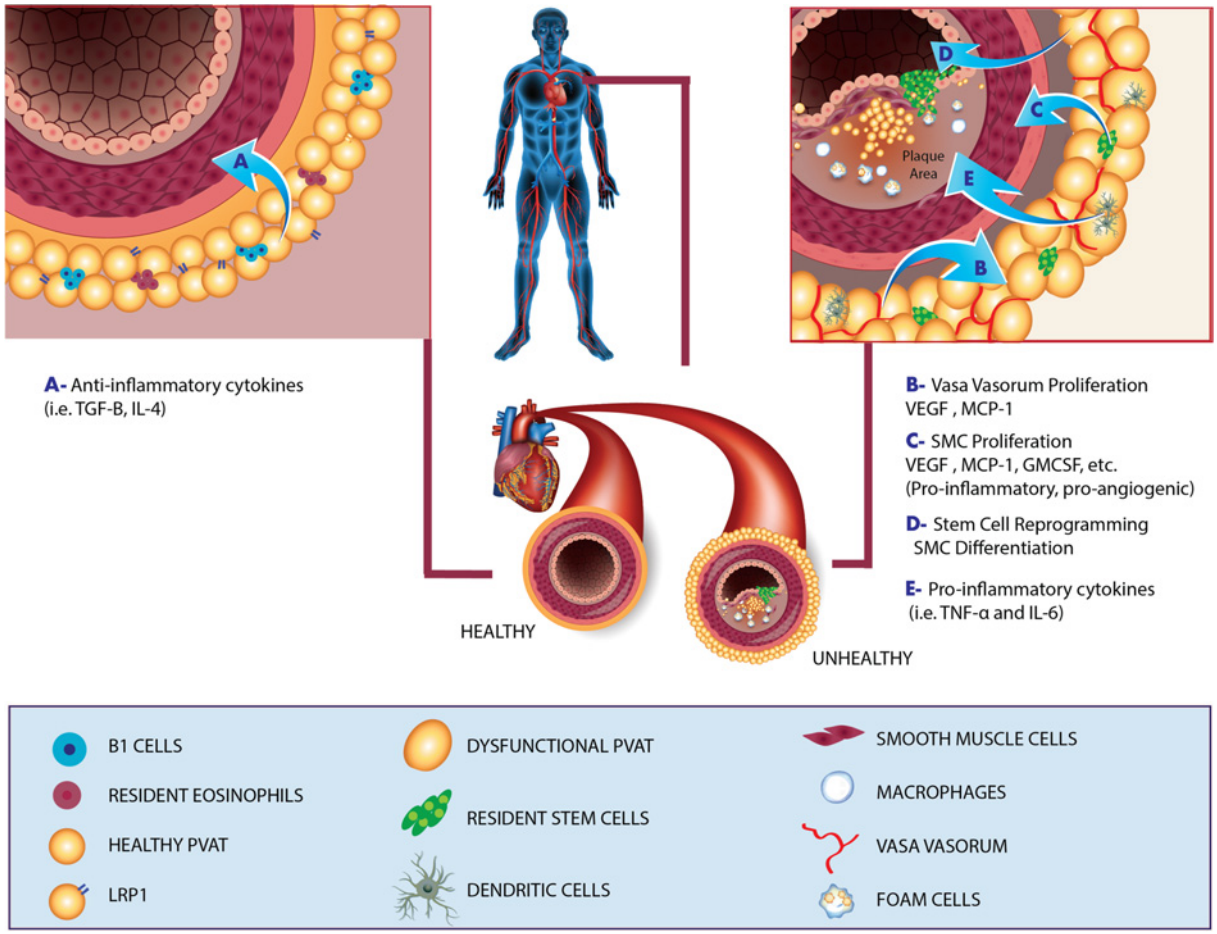

Figure 1. Proposed mechanisms whereby PVAT modulates vascular function and atherosclerotic development

In healthy PVAT, perivascular adipocytes play an important role in regulating vascular functions through releasing anti-atherogenic adipokines (i.e. TGF-β, IL-4 etc.). PVAT-resident immune cells such as eosinophils or subsets of B lymphocytes also serve to down-regulate the inflammatory microenvironment to promote vascular health. On the other hand, unhealthy PVAT augments production of pro-atherogenic and pro-angiogenic adipocytokines (i.e. MCP-1, VEGF, GM-CSF etc.), leading to inflammation, VSMC proliferation and *vasa varosum* neovascularization for development of atherosclerosis. Unhealthy PVAT also induces metabolic reprogramming of PVAT-resident stem cells which facilitates differentiation into VSMCs to promote atherosclerosis.

method to quantify coronary inflammation and mark the beginning of primary or intensive secondary prevention strategies for those patients not identified by traditional CV risk factors [50].

Conclusions

Cross-talk between PVAT and the underlying vasculature occurs bidirectionally and plays a significant role in vascular homeostasis and disease. PVAT dysfunction is suggested to contribute to the pathogenesis of atherosclerosis in both animal and human studies, while vascular inflammation is associated with PVAT phenotype changes that may help to identify vulnerable atherosclerotic lesions. On balance, the evidence suggests an important role of PVAT in regulating the focal inflammatory state and vessel homeostasis via pro-atherogenic or anti-atherogenic mechanisms depending on the PVAT state of health (Table 1 and Figure 1).

Emerging evidence suggests that PVAT may serve as a promising target for atherosclerosis interventions and treatments. For example, targeting pro-inflammatory adipokines secreted by PVAT may reduce the rate of vulnerable plaque rupture, while investigating different ways to enhance anti-atherogenic adipokines, such as TGF-B targeted therapy, may facilitate vascular repair. Meanwhile, enhancing unique resident PVAT immune cells, such as eosinophils or subsets of B lymphocytes, may serve to down-regulate the inflammatory microenvironment and promote vascular

Clinical Science (2020) **134** 3–13
https://doi.org/10.1042/CS20190577

health. Also, utilizing the PVAT FAI may help to assess the efficacy of novel therapeutics to inhibit vascular inflammation and improve cardiovascular mortality and morbidity. Furthermore, studies in mice suggest that exercise and brown adipocyte inducers such as cold exposure and pharmacological β-adrenergic stimulation can enhance the protective effects of PVAT on vascular function through PVAT browning [51,52]. While this is an attractive potential strategy to improve PVAT function, more studies are required to understand the therapeutic implications and to validate these findings in humans.

Acknowledgments

We thank Nassab Ahmadie for her assistance in making Figures.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was supported by the National Institutes of Health [grant numbers HL124097, HL126949, HL134354, AR070029 (to N.L.W)].

Abbreviations

ApoE, apolipoprotein E; AT_{1a}, angiotensin II receptor type 1a; CT, computed tomography; CVD, cardiovascular disease; ER, endoplasmic reticulum; FAI, fat attenuation index; GM-CSF, granulocyte-macrophage colony-stimulating factor; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; LRP1, low-density lipoprotein receptor-related protein-1; miR, microRNA; PVAT, perivascular adipose tissue; PV-ADSC, PVAT-derived mesenchymal stem cell; RAS, renin–angiotensin system; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell; TNF, tumor necrosis factor.

References

1 Manka, D., Chatterjee, T.K., Stoll, L.L., Basford, J.E., Konaniah, E.S., Srinivasan, R. et al. (2014) Transplanted perivascular adipose tissue accelerates injury-induced neointimal hyperplasia: role of monocyte chemoattractant protein-1. *Arterioscler. Thromb. Vasc. Biol.* **34**, 1723–1730, https://doi.org/10.1161/ATVBAHA.114.303983

2 Verhagen, S.N. and Visseren, F.L. (2011) Perivascular adipose tissue as a cause of atherosclerosis. *Atherosclerosis* **214**, 3–10, https://doi.org/10.1016/j.atherosclerosis.2010.05.034

3 Laslett, L.J., Alagona, Jr, P., Clark, III, B.A., Drozda, Jr, J.P., Saldivar, F., Wilson, S.R. et al. (2012) The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. *J. Am. Coll. Cardiol.* **60**, S1–S49

4 Wang, Y., Beydoun, M.A., Liang, L., Caballero, B. and Kumanyika, S.K. (2008) Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring)* **16**, 2323–2330, https://doi.org/10.1038/oby.2008.351

5 Zarzour, A., Kim, H.W. and Weintraub, N.L. (2018) Understanding obesity-related cardiovascular disease: it's all about balance. *Circulation* **138**, 64–66, https://doi.org/10.1161/CIRCULATIONAHA.118.034454

6 Chatterjee, T.K., Stoll, L.L., Denning, G.M., Harrelson, A., Blomkalns, A.L., Idelman, G. et al. (2009) Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding. *Circ. Res.* **104**, 541–549, https://doi.org/10.1161/CIRCRESAHA.108.182998

7 Tchkonia, T., Lenburg, M., Thomou, T., Giorgadze, N., Frampton, G., Pirtskhalava, T. et al. (2007) Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. *Am. J. Physiol. Endocrinol. Metab.* **292**, E298–E307, https://doi.org/10.1152/ajpendo.00202.2006

8 Chatterjee, T.K., Aronow, B.J., Tong, W.S., Manka, D., Tang, Y., Bogdanov, V.Y. et al. (2013) Human coronary artery perivascular adipocytes overexpress genes responsible for regulating vascular morphology, inflammation, and hemostasis. *Physiol. Genomics* **45**, 697–709, https://doi.org/10.1152/physiolgenomics.00042.2013

9 Omar, A., Chatterjee, T.K., Tang, Y., Hui, D.Y. and Weintraub, N.L. (2014) Proinflammatory phenotype of perivascular adipocytes. *Arterioscler. Thromb. Vasc. Biol.* **34**, 1631–1636, https://doi.org/10.1161/ATVBAHA.114.303030

10 Tran, K.V., Fitzgibbons, T., Min, S.Y., DeSouza, T. and Corvera, S. (2018) Distinct adipocyte progenitor cells are associated with regional phenotypes of perivascular aortic fat in mice. *Mol. Metab.* **9**, 199–206, https://doi.org/10.1016/j.molmet.2017.12.014

11 Horimatsu, T., Kim, H.W. and Weintraub, N.L. (2017) The role of perivascular adipose tissue in non-atherosclerotic vascular disease. *Front. Physiol.* **8**, 969, https://doi.org/10.3389/fphys.2017.00969

12 Rogowski, O., Shapira, I., Toker, S., Melamed, S., Shirom, A., Berliner, S. et al. (2005) Obesity-related correlation between C-reactive protein and the calculated 10-y Framingham Coronary Heart Disease Risk Score. *Int. J. Obes. (Lond.)* **29**, 772–777, https://doi.org/10.1038/sj.ijo.0802939

13 Verhagen, S.N., Vink, A., van der Graaf, Y. and Visseren, F.L. (2012) Coronary perivascular adipose tissue characteristics are related to atherosclerotic plaque size and composition. A post-mortem study. *Atherosclerosis.* **225**, 99–104, https://doi.org/10.1016/j.atherosclerosis.2012.08.031

14 Nabel, E.G. and Braunwald, E. (2012) A tale of coronary artery disease and myocardial infarction. *N. Engl. J. Med.* **366**, 54–63, https://doi.org/10.1056/NEJMra1112570

15 Nava, E. and Llorens, S. (2019) The local regulation of vascular function: from an inside-outside to an outside-inside model. *Front. Physiol.* **10**, 729, https://doi.org/10.3389/fphys.2019.00729
16 Mancio, J., Oikonomou, E.K. and Antoniades, C. (2018) Perivascular adipose tissue and coronary atherosclerosis. *Heart* **104**, 1654–1662, https://doi.org/10.1136/heartjnl-2017-312324
17 Moos, M.P., John, N., Grabner, R., Nossmann, S., Gunther, B., Vollandt, R. et al. (2005) The lamina adventitia is the major site of immune cell accumulation in standard chow-fed apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **25**, 2386–2391, https://doi.org/10.1161/01.ATV.0000187470.31662.fe
18 Mazurek, T., Zhang, L., Zalewski, A., Mannion, J.D., Diehl, J.T., Arafat, H. et al. (2003) Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation* **108**, 2460–2466, https://doi.org/10.1161/01.CIR.0000099542.57313.C5
19 Iacobellis, G., Pistilli, D., Gucciardo, M., Leonetti, F., Miraldi, F., Brancaccio, G. et al. (2005) Adiponectin expression in human epicardial adipose tissue *in vivo* is lower in patients with coronary artery disease. *Cytokine* **29**, 251–255
20 Baker, A.R., Silva, N.F., Quinn, D.W., Harte, A.L., Pagano, D., Bonser, R.S. et al. (2006) Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc. Diabetol.* **5**, 1, https://doi.org/10.1186/1475-2840-5-1
21 Langheim, S., Dreas, L., Veschini, L., Maisano, F., Foglieni, C., Ferrarello, S. et al. (2010) Increased expression and secretion of resistin in epicardial adipose tissue of patients with acute coronary syndrome. *Am. J. Physiol. Heart Circ. Physiol.* **298**, H746–53, https://doi.org/10.1152/ajpheart.00617.2009
22 Konaniah, E.S., Kuhel, D.G., Basford, J.E., Weintraub, N.L. and Hui, D.Y. (2017) Deficiency of LRP1 in mature adipocytes promotes diet-induced inflammation and atherosclerosis-brief report. *Arterioscler. Thromb. Vasc. Biol.* **37**, 1046–1049, https://doi.org/10.1161/ATVBAHA.117.309414
23 Ding, Y., Xian, X., Holland, W.L., Tsai, S. and Herz, J. (2016) Low-density lipoprotein receptor-related protein-1 protects against hepatic insulin resistance and hepatic steatosis. *EBioMedicine* **7**, 135–145, https://doi.org/10.1016/j.ebiom.2016.04.002
24 Chen, J.H., Riazy, M., Smith, E.M., Proud, C.G., Steinbrecher, U.P. and Duronio, V. (2009) Oxidized LDL-mediated macrophage survival involves elongation factor-2 kinase. *Arterioscler. Thromb. Vasc. Biol.* **29**, 92–98, https://doi.org/10.1161/ATVBAHA.108.174599
25 Holvoet, P. (2004) Oxidized LDL and coronary heart disease. *Acta Cardiol.* **59**, 479–484, https://doi.org/10.2143/AC.59.5.2005219
26 Takahashi, K., Takeya, M. and Sakashita, N. (2002) Multifunctional roles of macrophages in the development and progression of atherosclerosis in humans and experimental animals. *Med. Electron. Microsc.* **35**, 179–203, https://doi.org/10.1007/s007950200023
27 Uchida, Y., Uchida, Y., Shimoyama, E., Hiruta, N., Kishimoto, T. and Watanabe, S. (2017) Human pericoronary adipose tissue as storage and possible supply site for oxidized low-density lipoprotein and high-density lipoprotein in coronary artery. *J. Cardiol.* **69**, 236–244, https://doi.org/10.1016/j.jjcc.2016.03.015
28 Irie, D., Kawahito, H., Wakana, N., Kato, T., Kishida, S., Kikai, M. et al. (2015) Transplantation of periaortic adipose tissue from angiotensin receptor blocker-treated mice markedly ameliorates atherosclerosis development in apoE$^{-/-}$ mice. *J. Renin Angiotensin Aldosterone Syst.* **16**, 67–78, https://doi.org/10.1177/1470320314552434
29 Srikakulapu, P., Upadhye, A., Rosenfeld, S.M., Marshall, M.A., McSkimming, C., Hickman, A.W. et al. (2017) Perivascular adipose tissue harbors atheroprotective IgM-producing B cells. *Front. Physiol.* **8**, 719, https://doi.org/10.3389/fphys.2017.00719
30 Harmon, D.B., Srikakulapu, P., Kaplan, J.L., Oldham, S.N., McSkimming, C., Garmey, J.C. et al. (2016) Protective role for B-1b B cells and IgM in obesity-associated inflammation, glucose intolerance, and insulin resistance. *Arterioscler. Thromb. Vasc. Biol.* **36**, 682–691, https://doi.org/10.1161/ATVBAHA.116.307166
31 Tsimikas, S., Brilakis, E.S., Lennon, R.J., Miller, E.R., Witztum, J.L., McConnell, J.P. et al. (2007) Relationship of IgG and IgM autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events. *J. Lipid Res.* **48**, 425–433, https://doi.org/10.1194/jlr.M600361-JLR200
32 Withers, S.B., Forman, R., Meza-Perez, S., Sorobetea, D., Sitnik, K., Hopwood, T. et al. (2017) Eosinophils are key regulators of perivascular adipose tissue and vascular functionality. *Sci. Rep.* **7**, 44571, https://doi.org/10.1038/srep44571
33 Qiu, T., Li, M., Tanner, M.A., Yang, Y., Sowers, J.R., Korthuis, R.J. et al. (2018) Depletion of dendritic cells in perivascular adipose tissue improves arterial relaxation responses in type 2 diabetic mice. *Metabolism* **85**, 76–89, https://doi.org/10.1016/j.metabol.2018.03.002
34 Elkhatib, M.A.W., Mroueh, A., Rafeh, R.W., Sleiman, F., Fouad, H., Saad, E.I. et al. (2019) Amelioration of perivascular adipose inflammation reverses vascular dysfunction in a model of nonobese prediabetic metabolic challenge: potential role of antidiabetic drugs. *Transl. Res.* **214**, 121–143, https://doi.org/10.1016/j.trsl.2019.07.009
35 Wensveen, F.M., Jelenčić, V., Valentić, S., Šestan, M., Wensveen, T.T., Theurich, S. et al. (2015) NK cells link obesity-induced adipose stress to inflammation and insulin resistance. *Nat. Immunol.* **16**, 376–385, https://doi.org/10.1038/ni.3120
36 Subramanian, S., Goodspeed, L., Wang, S., Ding, Y., O'Brien, K.D., Getz, G.S. et al. (2018) Deficiency of Invariant natural killer t cells does not protect against obesity but exacerbates atherosclerosis in Ldlr$^{-/-}$ mice. *Int. J. Mol. Sci.* **19**, 510, https://doi.org/10.3390/ijms19020510
37 Terada, K., Yamada, H., Kikai, M., Wakana, N., Yamamoto, K., Wada, N. et al. (2018) Transplantation of periaortic adipose tissue inhibits atherosclerosis in apoE$^{-/-}$ mice by evoking TGF-beta1-mediated anti-inflammatory response in transplanted graft. *Biochem. Biophys. Res. Commun.* **501**, 145–151, https://doi.org/10.1016/j.bbrc.2018.04.196
38 Gu, W., Nowak, W.N., Xie, Y., Le Bras, A., Hu, Y., Deng, J. et al. (2019) Single-cell RNA-sequencing and metabolomics analyses reveal the contribution of perivascular adipose tissue stem cells to vascular remodeling. *Arterioscler. Thromb. Vasc. Biol.* **39**, 2049–2066, https://doi.org/10.1161/ATVBAHA.119.312732
39 Heistad, D.D., Marcus, M.L., Larsen, G.E. and Armstrong, M.L. (1981) Role of vasa vasorum in nourishment of the aortic wall. *Am. J. Physiol.* **240**, H781–H787

40 Herrmann, J., Lerman, L.O., Rodriguez-Porcel, M., Holmes, Jr, D.R., Richardson, D.M., Ritman, E.L. et al. (2001) Coronary vasa vasorum neovascularization precedes epicardial endothelial dysfunction in experimental hypercholesterolemia. *Cardiovasc. Res.* **51**, 762–766, https://doi.org/10.1016/S0008-6363(01)00347-9
41 Sedding, D.G., Boyle, E.C., Demandt, J.A.F., Sluimer, J.C., Dutzmann, J., Haverich, A. et al. (2018) Vasa Vasorum angiogenesis: key player in the initiation and progression of atherosclerosis and potential target for the treatment of cardiovascular disease. *Front. Immunol.* **9**, 706, https://doi.org/10.3389/fimmu.2018.00706
42 Kolodgie, F.D., Gold, H.K., Burke, A.P., Fowler, D.R., Kruth, H.S., Weber, D.K. et al. (2003) Intraplaque hemorrhage and progression of coronary atheroma. *N. Engl. J. Med.* **349**, 2316–2325, https://doi.org/10.1056/NEJMoa035655
43 Ahmadi, A., Leipsic, J., Blankstein, R., Taylor, C., Hecht, H., Stone, G.W. et al. (2015) Do plaques rapidly progress prior to myocardial infarction? The interplay between plaque vulnerability and progression. *Circ. Res.* **117**, 99–104, https://doi.org/10.1161/CIRCRESAHA.117.305637
44 Xu, J., Lu, X. and Shi, G.P. (2015) Vasa vasorum in atherosclerosis and clinical significance. *Int. J. Mol. Sci.* **16**, 11574–11608, https://doi.org/10.3390/ijms160511574
45 Ying, R., Li, S.W., Chen, J.Y., Zhang, H.F., Yang, Y., Gu, Z.J. et al. (2018) Endoplasmic reticulum stress in perivascular adipose tissue promotes destabilization of atherosclerotic plaque by regulating GM-CSF paracrine. *J. Transl. Med.* **16**, 105, https://doi.org/10.1186/s12967-018-1481-z
46 Schlich, R., Willems, M., Greulich, S., Ruppe, F., Knoefel, W.T., Ouwens, D.M. et al. (2013) VEGF in the crosstalk between human adipocytes and smooth muscle cells: depot-specific release from visceral and perivascular adipose tissue. *Mediat. Inflamm.* **2013**, 982458, https://doi.org/10.1155/2013/982458
47 Antonopoulos, A.S., Sanna, F., Sabharwal, N., Thomas, S., Oikonomou, E.K., Herdman, L. et al. (2017) Detecting human coronary inflammation by imaging perivascular fat. *Sci. Transl. Med.* **9**, eaal2658, https://doi.org/10.1126/scitranslmed.aal2658
48 Maurovich-Horvat, P., Kallianos, K., Engel, L.C., Szymonifka, J., Fox, C.S., Hoffmann, U. et al. (2011) Influence of pericoronary adipose tissue on local coronary atherosclerosis as assessed by a novel MDCT volumetric method. *Atherosclerosis* **219**, 151–157, https://doi.org/10.1016/j.atherosclerosis.2011.06.049
49 Oikonomou, E.K., Marwan, M., Desai, M.Y., Mancio, J., Alashi, A., Hutt Centeno, E. et al. (2018) Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. *Lancet* **392**, 929–939, https://doi.org/10.1016/S0140-6736(18)31114-0
50 Elnabawi, Y.A., Oikonomou, E.K., Dey, A.K., Mancio, J., Rodante, J.A., Aksentijevich, M. et al. (2019) Association of biologic therapy with coronary inflammation in patients with psoriasis as assessed by perivascular fat attenuation index. *JAMA Cardiol.*, https://doi.org/10.1001/jamacardio.2019.2589
51 Boa, B.C.S., Yudkin, J.S., van Hinsbergh, V.W.M., Bouskela, E. and Eringa, E.C. (2017) Exercise effects on perivascular adipose tissue: endocrine and paracrine determinants of vascular function. *Br. J. Pharmacol.* **174**, 3466–3481, https://doi.org/10.1111/bph.13732
52 Bussey, C.E., Withers, S.B., Saxton, S.N., Bodagh, N., Aldous, R.G. and Heagerty, A.M. (2018) β3-Adrenoceptor stimulation of perivascular adipocytes leads to increased fat cell-derived NO and vascular relaxation in small arteries. *Br. J. Pharmacol.* **175**, 3685–3698, https://doi.org/10.1111/bph.14433
53 Braunersreuther, V., Mach, F. and Steffens, S. (2007) The specific role of chemokines in atherosclerosis. *Thromb. Haemost.* **97**, 714–721, https://doi.org/10.1160/TH07-01-0036
54 Tilg, H. and Moschen, A.R. (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.* **6**, 772–783, https://doi.org/10.1038/nri1937
55 Jung, H.S., Park, K.H., Cho, Y.M., Chung, S.S., Cho, H.J., Cho, S.Y. et al. (2006) Resistin is secreted from macrophages in atheromas and promotes atherosclerosis. *Cardiovasc. Res.* **69**, 76–85, https://doi.org/10.1016/j.cardiores.2005.09.015
56 Wang, P., Xu, T.Y., Guan, Y.F., Su, D.F., Fan, G.R. and Miao, C.Y. (2009) Perivascular adipose tissue-derived visfatin is a vascular smooth muscle cell growth factor: role of nicotinamide mononucleotide. *Cardiovasc. Res.* **81**, 370–380, https://doi.org/10.1093/cvr/cvn288
57 Cheng, K.H., Chu, C.S., Lee, K.T., Lin, T.H., Hsieh, C.C., Chiu, C.C. et al. (2008) Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. *Int. J. Obes. (Lond.)* **32**, 268–274, https://doi.org/10.1038/sj.ijo.0803726
58 Spiroglou, S.G., Kostopoulos, C.G., Varakis, J.N. and Papadaki, H.H. (2010) Adipokines in periaortic and epicardial adipose tissue: differential expression and relation to atherosclerosis. *J. Atheroscler. Thromb.* **17**, 115–130, https://doi.org/10.5551/jat.1735
59 Van Campenhout, A. and Golledge, J. (2009) Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis* **204**, 321–329, https://doi.org/10.1016/j.atherosclerosis.2008.09.033
60 Bray, G.A., Clearfield, M.B., Fintel, D.J. and Nelinson, D.S. (2009) Overweight and obesity: the pathogenesis of cardiometabolic risk. *Clin. Cornerstone* **9**, 30–40, https://doi.org/10.1016/S1098-3597(09)80003-3
61 Chen, Y., Budd, R.C., Kelm, Jr, R.J., Sobel, B.E. and Schneider, D.J. (2006) Augmentation of proliferation of vascular smooth muscle cells by plasminogen activator inhibitor type 1. *Arterioscler. Thromb. Vasc. Biol.* **26**, 1777–1783, https://doi.org/10.1161/01.ATV.0000227514.50065.2a
62 Shibasaki, I., Nishikimi, T., Mochizuki, Y., Yamada, Y., Yoshitatsu, M., Inoue, Y. et al. (2010) Greater expression of inflammatory cytokines, adrenomedullin, and natriuretic peptide receptor-C in epicardial adipose tissue in coronary artery disease. *Regul. Pept.* **165**, 210–217, https://doi.org/10.1016/j.regpep.2010.07.169
63 Ruan, C.C., Zhu, D.L., Chen, Q.Z., Chen, J., Guo, S.J., Li, X.D. et al. (2010) Perivascular adipose tissue-derived complement 3 is required for adventitial fibroblast functions and adventitial remodeling in deoxycorticosterone acetate-salt hypertensive rats. *Arterioscler. Thromb. Vasc. Biol.* **30**, 2568–2574, https://doi.org/10.1161/ATVBAHA.110.215525
64 Takaoka, M., Suzuki, H., Shioda, S., Sekikawa, K., Saito, Y., Nagai, R. et al. (2010) Endovascular injury induces rapid phenotypic changes in perivascular adipose tissue. *Arterioscler. Thromb. Vasc. Biol.* **30**, 1576–1582, https://doi.org/10.1161/ATVBAHA.110.207175
65 Silaghi, A., Achard, V., Paulmyer-Lacroix, O., Scridon, T., Tassistro, V., Duncea, I. et al. (2007) Expression of adrenomedullin in human epicardial adipose tissue: role of coronary status. *Am. J. Physiol. Endocrinol. Metab.* **293**, E1443–E1450, https://doi.org/10.1152/ajpendo.00273.2007

66 Gruzdeva, O., Uchasova, E., Dyleva, Y., Borodkina, D., Akbasheva, O., Karetnikova, V. et al. (2019) Relationship between epicardial and perivascular fatty tissue and adipokine-cytokine level in coronary artery disease patients. *PLoS ONE* **14**, e0208156, https://doi.org/10.1371/journal.pone.0208156

67 Gaborit, B., Venteclef, N., Ancel, P., Pelloux, V., Gariboldi, V., Leprince, P. et al. (2015) Human epicardial adipose tissue has a specific transcriptomic signature depending on its anatomical peri-atrial, peri-ventricular, or peri-coronary location. *Cardiovasc. Res.* **108**, 62–73, https://doi.org/10.1093/cvr/cvv208