# The role of PVAT in cardiovascular disease

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**Abstract**

Cardiovascular disease is the primary cause of death in developed countries and is rapidly increasing due to the higher prevalence of obesity worldwide. Obesity exercises its function by altering adipose tissue, affecting its paracrine function and causing cardiovascular disease.

Perivascular adipose tissue (PVAT) is fat which borders most vessels and signals to the vascular wall. In healthy conditions PVAT exercises an anti-contractile effect in the underlying vessels through PVAT-derived relaxing factors. However, in unhealthy conditions such as obesity PVAT is altered into a state known as PVAT dysfunction, negatively influencing cardiovascular health. How PVAT dysfunction affects overall health is dependent on its location as PVAT differs in function based on its location. Common affected factors among different tissues are decreased nitric oxide and adiponectin production, increased leukocyte infiltration and a phenotypical switch to a pro-inflammatory state. Differences in function are mainly attributed to whether PVAT resembles white adipocytes or brown adipocytes. PVAT resembling white adipocyte tissue has been found to be more frequently implicated with negative cardiovascular health compared to PVAT resembling brown adipocyte tissue. Thoracic aortic PVAT, which resembles brown adipocytes, maintains its healthy anti-contractile function in unhealthy conditions. However, abdominal aortic PVAT resembles white adipocytes and consequently is often associated with cardiovascular disease. Other adipose tissues around organs often associated with cardiovascular disease are epicardial adipose tissue and renal perivascular tissue. Both show a switch to a pro-inflammatory state in obesity. Treatment options are diverse ranging from surgery to oral supplements, yet exact treatment mechanisms among different PVAT remains mostly undetermined.

In conclusion PVAT heterogeneity is an important factor in the development of cardiovascular disease, with different sites having differing effects and being differently affected by unhealthy conditions. Although diverse treatment options are available focus should be shifted towards developing treatment specifically for affected PVAT sites.

**Index**

## Introduction

Cardiovascular disease (CVD) is a worldwide problem and is considered to be the primary cause of death in developing and developed countries, even exceeding cancer (Gaziano e.a., 2010). Diabetes and hypertension are important predisposing factors for CVD and their incidence is rapidly increasing as a consequence of a higher prevalence of obesity worldwide (Hossain e.a., 2007). Obesity directly alters adipose tissue mass and phenotype which affects its endocrine and paracrine functions, influencing cardiovascular health (Akoumianakis e.a., 2017).

The endothelium is a key player in cardiovascular health due to its paracrine and endocrine functions. The vascular endothelium releases several vasodilating factors which cause the underlying vascular smooth muscle cells (VSMC) to relax. These vasodilating effects are mainly caused by nitric oxide (NO). However, other factors, collectively called endothelium-derived relaxing factors (EDRFs), can also cause vasomotor changes. When the endothelium is unable to produce adequate amounts of relaxing factors it loses its vasodilating effect, this is called endothelial dysfunction. This may in turn lead to CVD (Vanhoutte e.a., 2009)

There is a growing body of evidence that perivascular adipose tissue (PVAT) has a pathophysiological role in CVD (Lian & Gollasch, 2016). PVAT is fat which borders the adventitia of large vessels where it signals to the vascular wall in an endocrine fashion, as seen in Figure 1. Even though PVAT only constitutes 3% of total body fat it has a strong influence on the cardiovascular system, due to its anti-contractile effects and crosstalk with the underlying endothelium and VSMC, which regulates blood pressure(Siegel-Axel, 2016). PVAT can also release substances into newly forming vasa vasorum, which directly affects the inner vasculature (Gössl e.a., 2009).

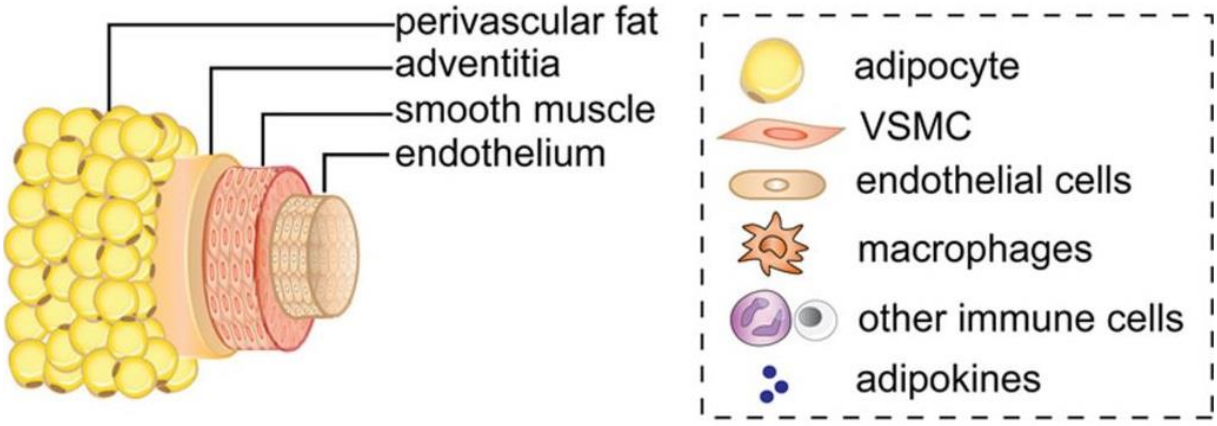


*Figure 1*. The vascular wall and PVAT. (Cao e.a., 2017).

The role of PVAT in CVD is mediated by the balance in secretion of pro- and anti-inflammatory factors- i.e., adipocytokines (Schäfer e.a., 2017). In healthy conditions PVAT has an anti-contractile effect through multiple agents, such as adiponectin and NO. However, in unhealthy conditions, such as obesity, PVAT assumes a state similar to endothelial dysfunction called PVAT dysfunction. This state, likely caused by hypoxia and inflammation, attenuates PVATs anti-contractile effect through adipokine release (Almabrouk e.a., 2018). These factors affect the underlying endothelial and VSMCs. Consequently dysfunctional PVAT, which has an increased expression of inflammatory cytokines, promotes endothelial dysfunction (Villacorta & Chang, 2015).

New evidence suggests that PVAT heterogeneity, the differing locations of PVAT around the body, is a key player in how PVAT affects the vasculature. PVAT is located around large vessels and is also found within organs, such as in the renal sinus of the kidney (Gil-Ortega e.a., 2015). This is shown in Figure 2. This heterogeneity is accompanied by differing PVAT function. For example, coronary PVAT

has been shown to release factors which initiate contraction of coronary arteries while subcutaneous adipose tissue did not have this effect (Owen e.a., 2013). PVAT heterogeneity is coincided by differences in PVAT phenotype. PVAT surrounding the thoracic aorta seems to resemble brown adipose tissue (BAT) (Fitzgibbons e.a., 2011), while PVAT surrounding the abdominal aorta resembles white adipose tissue (WAT) (Police e.a., 2009).

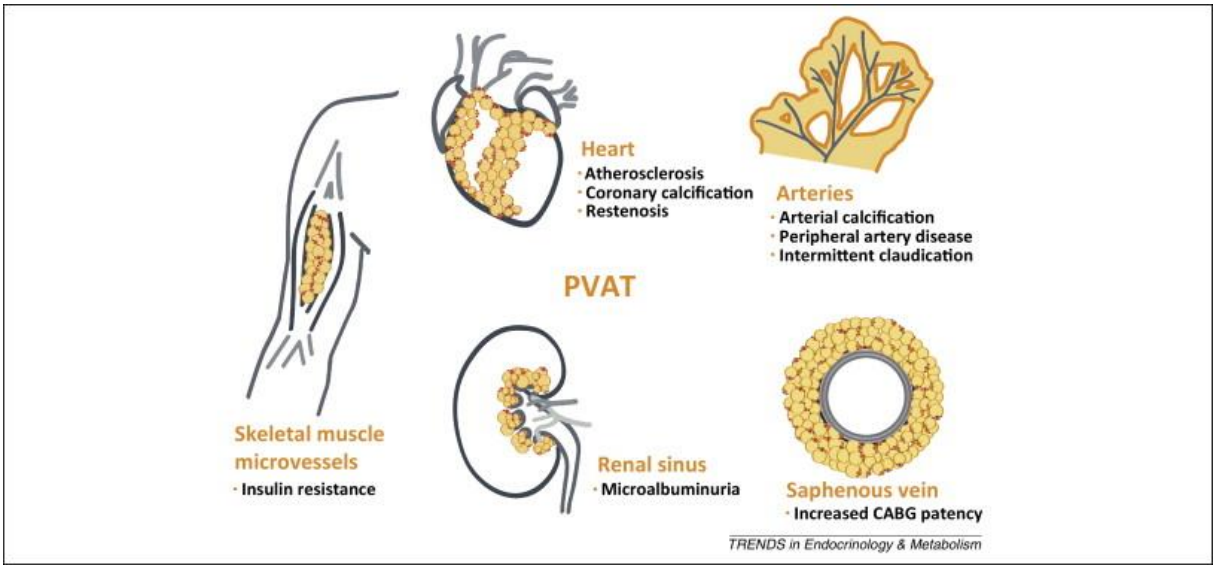


*Figure 2*. PVAT heterogeneity. (Gil-Ortega e.a., 2015).

The exact role of PVAT is still being uncovered. It has been established that PVAT interacts with the endothelium and VSMCs in regulating vascular tone and hence appears to be of great importance to cardiovascular health. While healthy PVAT seems to have positive anti-contractile effects, unhealthy PVAT has negative consequences. The mechanisms with which PVAT causes this remain unclear, yet PVAT heterogeneity seems to play an important role. Hence our research question is as follows;
*What is the role of PVAT heterogeneity in cardiovascular health?*

## Results

### The role of the endothelium and smooth muscle in vessel function

Before describing how PVAT influences the vasculature in detail, we will look at how the endothelium and VSMCs control vascular tone. It has long been established that the endothelium, which lines the inner blood vessels, has an important role in regulating blood flow by locally producing vasoactive substances collectively called endothelium-derived relaxing factors (Griffith e.a., 1987). An important factor in the release of vasoactive substances from the endothelium is shear stress. An increase in blood flow causes an upsurge of frictional forces on the endothelium lining the vessel wall. In response to these forces endothelial cells upregulate vasoactive factors. The most profound reaction is the increase in NO production (Michiels, 2003). NO is one of the main endothelium derived vasodilators and is produced by an enzyme called eNOS (Lamas e.a., 1992). NO diffuses into the adjacent VSMCs, where it activates guanylyl cyclase (Ignarro e.a., 1986). Guanylyl cyclase converts GTP into cyclic GMP, which causes a decrease in muscle tension (Jones e.a., 1999). NO also plays an important part in preventing leukocyte activation and adhesion to the endothelium (Kubes e.a., 1991). In contrast, when shear stress is low a shift will occur towards secretion of factors associated with pathological situations.

Several other factors also play an important role in endothelial function. Prostacyclin (PGI2) is synthesized by the enzyme COX-2. PGI2 binds to receptors on VSMCs which causes the cells to relax (Fetalvero e.a., 2007). It has been shown that PGI2 is upregulated when NO is not available, indicating that PGI2 plays a compensatory role (Beverelli e.a., 1997). Another important factor is called endothelium-derived hyperpolarising factor (EDHF). EDHF causes K+ efflux from VSMCs, which results in hyperpolarisation and thus relaxation. The exact mechanism by which EDHF does this remains unclear (Sandoo e.a., 2010). Finally ET-1 is an important vasoconstrictor released by the endothelium. ET-1 binds to receptors located on VSMCs causing calcium channels to open leading to an influx of calcium which creates vasoconstriction. ET-1 and other contracting factors can also cause VSMC hyperplasia, decreasing vascular diameter and increasing blood pressure (Cardillo e.a., 2000).

In endothelial dysfunction there is an imbalance between these vasodilating and vasoconstricting factors. Endothelial cells are susceptible to endothelial dysfunction through chronic hypertension and shift toward a reduced NO and increased ET-1 production, similarly PGI2 is downregulated while contractile factor thromboxane (TXA2) is upregulated. This leads to a shift from vasodilation towards vasoconstriction. This shift causes a reduction in blood-flow in both small and large vessels. Because of this a common complication is heart failure, due to decreased blood flow towards the heart-muscle tissue. In addition to this, the endothelium switches to a pro-adhesion phenotype as well. This causes leukocytes to infiltrate the cellular walls and possibly leads to atherosclerotic plaques, creating more resistance (Herrmann & Lerman, 2001).

The VSMCs are not just under the influence of the endothelium but also under that of adrenergic nerves, metabolites and hormones. For example, the sympathetic nervous system releases norepinephrine which acts on alpha-adrenergic receptors causing contraction. Similarly epinephrine binds to beta-adrenergic receptors causing VSMC relaxation. Circulating metabolites, such as O2 and CO2, also have a prominent effect on VSMC. During exercise these metabolic biproducts are produced, body temperature rises and pH decreases. All these factors mediate smooth muscle contraction and relaxation. Through this mediation VSMC regulates vascular resistance and blood pressure, which in turn plays a big part in CVD (Silfani e.a., 1998).

**What is PVAT?**

PVAT is fat directly adjacent to and has long been seen as a structural support to the surrounding blood vessels. However, recent studies have shown that PVAT is also metabolically active with an important vasocrine function (Siegel-Axel & Häring, 2016). PVAT exercises this vasocrine function through several mediators, such as ROS, leptin, adiponectin (Ketonen e.a., 2010), NO (Gil-Ortega e.a., 2010), H2S (Fang e.a., 2009) and angiotensin 1-7 (Lee e.a., 2011). All of these factors have been shown to have vasocontractile or dilating effects through endothelial and VSMC pathways (Siegel-Axel & Häring, 2016). This indicates that PVAT directly influences vascular tone through its paracrine function by signalling towards the underlying tissue.

Due to PVAT being directly adjacent to the vessel wall, mediators secreted by PVAT can easily access the underlying layers by diffusion. PVAT is also in contact with the vasa vasorum which could potentially transmit mediators to the underlying tissue (Gössl e.a., 2009). In healthy conditions PVAT transmits PVAT-derived relaxing factor (PVRF), which mediates the anti-contractile effect of healthy PVAT by activating the K+ channels of VSMCs thus causing hyperpolarisation (Dubrovska e.a., 2004). A study by Gao et al has shown that PVRFs activity is dependent on NO, indicating that the endothelium and PVAT collectively regulate vascular tone through NO. They also showed that PVAT-derived ROS inhibits VSMC contraction by activating guanylyl cyclase (Gao e.a., 2007).

Healthy PVAT also targets the Kv7 family of K+ channels, which are not targeted by EDRF, by releasing PVRFs which activate Kv7 and causing vasorelaxation through VSMC hyperpolarisation. PVAT-derived H2S seems to activate Kv7 in the same way (Köhn e.a., 2012). This directly shows PVATs role in vascular tone in an endothelium independent matter (Schleifenbaum e.a., 2010). PVAT also releases Ang 1-7 which promotes endothelial NO production (Agabiti-Rosei e.a., 2018). Similarly PVAT produces adiponectin, which stimulates endothelial NO production as well (Cheng e.a., 2007).

Although it is clear that healthy PVAT plays a similar role in cardiovascular health to the endothelium, PVAT dysfunction plays a similar role in CVD as endothelial dysfunction as well. Endothelial dysfunction leads to an increase in blood pressure and thus CVD through an increase in production of vasoconstricting factors (Schächinger e.a., 2000). Similarly PVAT dysfunction, caused by unhealthy conditions such as obesity, upregulates adipokine production which creates a surge in oxidative stress and inflammation (Achike e.a., 2011). This in turn could cause endothelial dysfunction (Ketonen e.a., 2010).

Several key players have been identified to attenuate vascular health as a consequence of PVAT dysfunction. PVAT dysfunction has been shown to decrease NO bioavailability, which reduces PVATs anti-contractile effect while simultaneously causing vasoconstriction through the increase of ROS (Bussey e.a., 2016). PGI2 producing COX-enzymes are upregulated as well and are attributed to an increase in pro-contractile factor TXA2 while reducing PGI2, inducing a contractile effect (Meyer e.a., 2013). Zavaritskaya et al showed that part of regular PVAT function could be restored using Kv7 channel openers in rat arteries. As the name suggests Kv7 channel openers open Kv7 channels, suggesting that K+ activation is lowered in PVAT dysfunction (Zavaritskaya e.a., 2013).

This data clearly presents the interaction between PVAT and the vessels, while also indicating its role in cardiovascular health and CVD. However, this role does not seem to be as clear cut as is presented here. Recent clinical studies have shown that it is also largely dependent on both its location and phenotype. Mahabadi et al found that pericoronary fat depots have a local role in atherosclerotic plaque formation in a large number of patients, regardless of total pericardial fat volume (Mahabadi e.a., 2010). And a cross-sectional study by Jeong et al showed that in a large body of patients

epicardial fat thickness was associated with the severity of coronary disease, regardless of total body fat (Jeong e.a., 2007). This suggests that PVAT function varies with its location and therefore is an important part in uncovering the association between PVAT and cardiovascular health.

**What determines PVAT phenotype and function?**

PVAT is not the only adipose tissue associated with cardiovascular health, several other adipose tissues were found to be altered in CVD as well, as seen in Figure 2. The three main adipose tissues are white adipose tissue (WAT), brown adipose tissue (BAT) and beige fat (Berbée e.a., 2015). WAT is the main adipose fat tissue in humans, mainly surrounding the internal organs. Its function is primarily to store lipids in a single big droplet which occupies most of the cell. WAT, just like other adipose tissues, is also a major endocrine organ secreting factors such as leptin and adiponectin (Hildebrand e.a., 2018). BAT is a metabolically very active organ which is activated by noradrenaline when exposed to the cold, inducing thermogenesis (Berbée e.a., 2015). The brown adipocytes in BAT contain many lipid droplets as well as high mitochondrial density, this along with high vascularisation causes BATs brown colour (Hildebrand e.a., 2018). Beige fat lies within certain WATs and becomes more prominent by cold exposure, this is called 'browning'. During browning white adipocytes become more brown like, containing more mitochondria and lipid droplets. Several molecules have been shown to have an important role in browning, namely ANP, catecholamines, NPY and TZDs (Wu e.a., 2013).

As stated earlier PVAT has been shown to resemble WAT or BAT, depending on its location (Fitzgibbons e.a., 2011; Police e.a., 2009). Fitzgibbons et al showed that mice thoracic aortic PVAT resembles BAT, having adipocytes containing multiple lipid droplets. They also showed that this PVAT only differed in 228 genes from BAT. Similarly a study by Matthias et al showed that rat aortic PVAT closely characterises BAT as well (Matthias e.a., 1994). More importantly Chang et al proved that PVAT not only resembles BAT phenotypically but also functionally. By selectively knocking out PVAT they showed that PVAT significantly contributes to the thermogenic effect of BAT (Chang e.a., 2012). Police et al showed that the abdominal aortas in mice were surrounded by a diverse group of cells, predominantly consisting of WAT-like PVAT. Additionally they showed differences in MCP-1 release between brown-like thoracic PVAT and the WAT-like PVAT. WAT-like PVAT also suffered from an increase in macrophage migration compared to brown-like PVAT (Police e.a., 2009). Collectively these studies proved that PVAT phenotype plays an important part in PVAT function.

Yet PVAT cannot be characterised by its adipose cells alone, several different cell types are interspersed within the PVAT affecting its function. A study by Stefanovic-Racic et al studied CD11c+ cells in adipose tissue, which is a marker for dendritic cells (DC). By using electron microscopy they proved that CD11c+ markers in PVAT correspond to DCs. They also showed that these DCs are significantly upregulated in obesity, increasing macrophage recruitment to the tissue and thereby eliciting an inflammatory state (Stefanovic-Racic e.a., 2012). PVAT also houses other immune cells, namely B-cells. Srikakulapu et al used flow cytometry to analyse human coronary artery PVAT. Their analysis showed that PVAT contained not only B-cells but also T-cells. They also found high endothelial venules, which play an important part in lymphocyte recruitment in lymphoid tissues, suggesting PVAT might also function as a lymphoid organ. Furthermore, they proved that B-cells are present in significantly greater numbers within the PVAT compared to the aorta. Many of these B-cells were capable of secreting IgM as well (Srikakulapu e.a., 2017). Finally PVAT also contains stromal cells, important in vascular maintenance, and eosinophils. The eosinophils are directly involved in the anti-contractile effect of PVAT, as shown by Wither et al in eosinophil deficient mice whom showed no anti-contractile PVAT effect (Withers e.a., 2017).

In conclusion, BAT and WAT seem to be important indicators of PVAT function. While the thoracic aortic PVAT closely resembles BAT abdominal aortic PVAT closely resembles WAT. Interestingly the WAT phenotype suffers from higher macrophage infiltration. Along with this PVAT phenotype and function is also largely determined by other cells within the tissue. This proves the importance of the BAT and WAT phenotype in determining local PVAT function as well as the importance of multiple cell types.

**Differences in function among different PVAT**

PVAT composition, both the adipocytes and other cell types, is of great significance in PVAT function and plays a critical part in the phenotypic differences among different PVAT sites. Coronary and mesenteric arteries significantly alter coronary lean artery tension, P= 0.03 and P=0.04 respectively, compared to subcutaneous PVAT (P=0.67) (Gil-Ortega e.a., 2015). Victorio et al uncovered multiple differing pathways that are of interest. Using phenylephrine to make concentration-response curves from the thoracic and abdominal aorta of 4-month old Wistar rats, they exhibited that an anti-contractile effect is present in the thoracic aorta but not in the abdominal aorta. The abdominal aorta also had a significant reduction in NO production from PVAT while ROS production from both sections of the aorta were similar. Along with this the abdominal aortic PVAT seems to exert no anti-contractile effect at all when excited with phenylephrine, while the thoracic aortas response is exercised by both the endothelium and PVAT. This demonstrates a difference in PVAT function in different sections of the same blood vessel. This difference can be explained by the difference in BAT and WAT phenotype of the thoracic and abdominal aorta respectively (Victorio e.a., 2016). Interestingly the abdominal aorta develops atherosclerosis and aneurysms more frequently (Guo e.a., 2001), highlighting the importance of PVAT heterogeneity in CVD development.

Mesenteric PVAT has been found to exhibit other qualities similar to thoracic aortic PVAT. Mesenteric PVAT can take up norepinephrine (NE), promoting its anti-contractile effect similar to thoracic aortic PVAT. Mesenteric PVAT can also metabolise amines through the SSAO enzyme, further contributing to its function (Ayala-Lopez e.a., 2017). Aghamohammadzadeh et al also showed an anti-contractile effect to NE (Aghamohammadzadeh e.a., 2016). Unlike abdominal aortic PVAT mesenteric PVAT is affected by phenylephrine (Sena Cristina e.a., 2017). Contrastingly mesenteric PVAT resembles WAT compared to the BAT phenotype of thoracic aortic PVAT (Restini e.a., 2018).

Coronary artery PVAT, also known as epicardial adipose tissue (EAT), exhibits traits dissimilar to thoracic aortic PVAT as well. EAT is very susceptible to atherosclerosis, while thoracic aortic PVAT is highly resistant. For this reason thoracic aortic PVAT is often used as an artery bypass graft in coronary artery bypass surgery. Compared to thoracic aortic PVAT EAT possesses 2053 differently expressed genes, mainly upregulating biological processes connected to inflammation. Important to note is that these findings are in CHD patients, non-CHD patients showed changes in 1402 genes with no major alterations in inflammation processes. Still, this indicates the importance of PVAT heterogeneity in the development and progression of CVD (Lu e.a., 2017). Furthermore, H2O2-mediated vasodilation is lower in EAT. This is in contrast with the expected anti-contractile effect of PVAT, suggesting that PVRFs may not be prominent within EAT (Owen e.a., 2013).

The renal arteries are also an important PVAT containing site. Renal PVAT (RPVAT) has been suggested to store a pool of NE used to influence renal vascular function. Normally the sympathetic nervous system alters renal vascular tone through NE release, activating adrenergic receptors. This activation increases vascular resistance and in turn sustains hypertension (Restini e.a., 2018). Restini et al incubated RPVAT with NE antibodies, resulting in an immunohistochemical section with adipocytes containing NE within the cytoplasm. Interestingly RPVAT expresses mixed markers from both BAT and WAT, namely UCP1, Cidea and Pparg from BAT and Tcf21, adipoq and cebpa from WAT. Immunohistochemical and histological results show a mixed phenotype for RPVAT as well. Surprisingly RPVAT did not reduce the contractile potency of adrenergic agonists in vitro, suggesting RPVAT does not serve an anti-contractile purpose. But the internal pool of NE has been found to alter the renal arterial tone independent of the sympathetic nervous system, whether the NE is made within RPVAT cells or solely comes from the sympathetic nerves is unknown. This contrasts the

mesenteric PVAT which is able to produce NE within adipocytes through tyrosine hydroxylase (Restini e.a., 2018).

Concluding, the different PVATs differ widely in function, while also showing many similarities. The abdominal aortic PVAT showed no anti-contractile effect and NO production was significantly reduced. EAT differs from the thoracic aortic PVAT as well and just like the abdominal aorta the coronary arteries are more susceptible to atherosclerosis. RPVAT shows to be a mixture of both BAT and WAT and exercises functions found in both as well. RPVAT seems to have no anti-contractile effect just as abdominal aortic PVAT, yet the internal NE can affect renal vascular tone. These findings suggest that the heterogeneity of PVAT plays an important part in how and where CVD develops.

**PVAT dysfunction and CVD**

Obesity is an ever growing problem in our modern society, because of its critical involvement in the development of CVD. Obesity is characterised by an increase in adipose tissue as well as phenotypical changes in adipose tissue (Akoumianakis e.a., 2017). As we discovered earlier, adipose tissue phenotype is an important predictor of inflammation and CVD, possibly explaining the importance of obesity in CVD. Additionally WAT is expanded in obesity, pairing this fact with the knowledge that WAT-like PVAT has a bigger role in CVD compared to BAT it would appear that WAT is the main contributor in obesity upregulated CVD risk.

Obesity has been found to alter adipose tissue in multiple studies, as summarised in Figure 3. Due to excessive caloric intake adipose tissue expands, driven by adipocyte hyperplasia and eventually adipocyte hypertrophy. Hypertrophic adipocytes die as a consequence of apoptosis or necrosis, which leads to recruitment of inflammatory cells and causes tissue dysfunction. Macrophage-to-adipocyte ratio will increase along with a phenotypical change in the macrophages to an M1 state, being more pro-inflammatory. T cells in adipose tissue are also altered, accumulating more CD8+ effector cells, activating macrophages. Additionally the eosinophils, previously proven to be of importance in the anti-contractile function of PVAT, activate macrophages as well through the production of cytokine IL-4. Furthermore adipose tissue loses vascularisation in obesity, limiting nutrient delivery, increasing necrosis and inducing higher inflammation. All these pathways play an important role in CVD as well as in insulin resistance (Nakamura e.a., 2014). Greenstein et al provided evidence that obesity also plays a major role in PVAT dysfunction. Obesity induced hypoxia and inflammation attenuate PVAT function in human and animal arteries in vitro, mainly caused through downregulating NO by reducing adiponectin production (Greenstein e.a., 2009).

Yet the exact interplay between PVAT and obesity is still to be fully uncovered. A study by Owen et al found multiple differences in lean versus obese swine EAT responses and effects. In the obese swine an increase in EAT increased coronary artery tension. Furthermore, in obese tissues H2O2-mediated vasodilation was lower when EAT was present, but this effect was more prominent in the lean tissue. Owen et al also studied whether these differences were caused by obesity-induced genotypical changes. As expected obese EAT showed significant alterations in protein expression. Several proteins involved in both cellular proliferation (51 molecules) and cellular action (39 molecules) were altered, along with upregulation of Rhoa (x2.9) and calpastatin (x1.6) both being implicated in progression of hypertension (Owen e.a., 2013).

A different study by Aghamohammadzadeh et al looked at lean versus obese PVAT surrounding mesenteric arteries of Sprague Dawley rats. As expected the obese rats weight more and had raised systolic and diastolic blood pressure, they also showed no anti-contractile effect to NE. Similar to the study by Owen et al the obese PVAT showed altered protein expression, namely in adiponectin and superoxide radical scavenger SOD. This results in higher ROS and lower NO production causing increased VSMC contraction. This is in accordance with earlier studies linking inflammation with obesity and an increase in macrophage-to-adipocyte ratio within the tissue. Interestingly the altered PVAT function is solely caused by obesity-induced damage and independent of the endothelium, despite their integral interplay in vasoactive control (Aghamohammadzadeh e.a., 2016).

Unfortunately little to no research has been conducted on RPVAT and its influence on renal function, let alone RPVAT function in obesity. As a result the following paragraph will be more hypothetical. Early over nutrition has several effects on renal function including enhancement of RAAS, arterial hypertension, glomerulosclerosis and cortical apoptosis (Granado e.a., 2017). Granado et al took 1-day old Sprague Dawley rats and overfed them for 24 days, half of the rats were sacrificed after 21 days and the other half after 5 months. Granado et al showed an increase in COX-2 expression in obese RPVAT, which they suggest downregulates angiotensinogen expression within renal arteries. However, changes in Ang2 production from RPVAT in obesity and as a consequence its effects are unknown. They also found the RPVAT to have upregulated mRNA levels of proinflammatory factors in young overfed rats. Finally, obese RPVAT had increased renin receptor expression. Renin receptors and inflammatory factors are associated with development of hypertension, therefore altered RPVAT may play an important role in CVD (Granado e.a., 2017). Wagner et al also alluded to the importance of RPVAT in CVD development after studying microalbuminuria in 146 patients with high BMI. Microalbuminuria is an important marker of nephropathy and glomerular angiopathy, both linked to CVD. They found that the amount of RPVAT, measured through MRI, was associated with BMI. Additionally increased RPVAT resulted in high albumin excretion (Wagner e.a., 2012). These findings support the idea that obesity alters RPVAT and induces CVD.

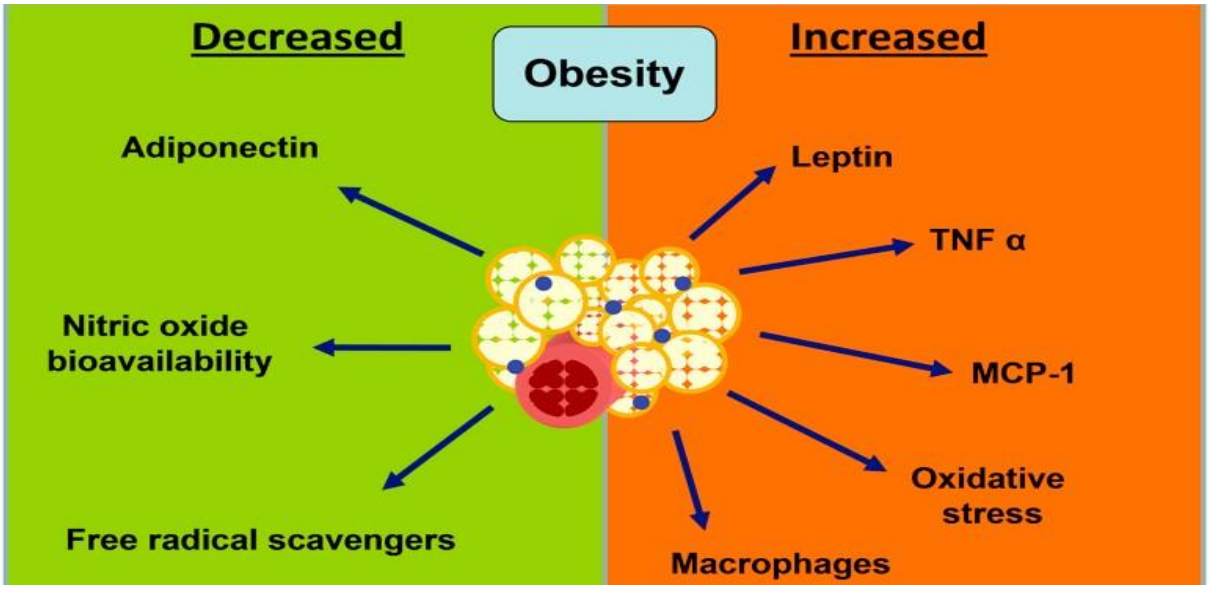


*Figure 3.* Altered PVAT function in obesity. (Aghamohammadzadeh e.a., 2013).

**Treating PVAT dysfunction**

In view of the many aspects of PVAT function discussed here, several possible therapeutic targets exist. Considering obesity induces PVAT dysfunction exercise remains one of the main therapies to decrease the negative effects of obesity. Another common factor in CVD and PVAT dysfunction is inflammation, illustrating the importance of altering inflammation in order to induce healthy PVAT function. On a smaller level the importance of adiponectin, NO and other bioactive factors cannot be understated. Additionally, WAT is implicated in development of PVAT dysfunction in several different tissues, while BAT is not. This suggest browning to be a therapeutic goal as well. Finally, EAT and RPVAT are important fat depots due to their location around organs implicated in CVD.

The effect of exercise on obesity is well established, yet the effect on PVAT has not been fully clarified. Lee et al proved that exercise reduced PVAT depots while simultaneously reducing PVAT inflammation in human patients (Lee Sindre e.a., 2016). Similarly, exercise reduces adipocyte size and limits inflammation in mesenteric PVAT in mice (Haczeyni e.a., 2015). By reducing PVAT size the amount of infiltrated inflammatory cells drops as well. Additionally exercise induces a phenotype switch in macrophages from M1 to M2, which is anti-inflammatory. M1 infiltration into adipose tissue was reduced as well. Exercise is also likely to locally increase adiponectin secretion, NO bioavailability and ROS scavenging (Boa e.a., 2017). Another rat study showed similar findings, namely upregulation of the anti-contractile effect, downregulation of inflammatory markers and a decrease in thoracic PVAT mass after aerobic exercise. The study also demonstrated that exercise causes browning of WAT-like PVAT, having a vascular protective effect (Araujo e.a., 2018).

Although WAT can be altered through exercise, other therapies are available as well. WAT and WAT-like PVAT suffer from the same leukocyte infiltration as other adipose tissues. Increasing adiponectin levels or increasing receptor availability seem viable in reducing inflammation (van Dam e.a., 2017). Although as many inflammatory cytokines are upregulated, elevation of adiponectin alone is likely not enough to attenuate this. Salicylates seem a more viable option as they lower overall cytokine production through NF-kB inhibition (Dam e.a., 2015). WAT not only exercises its endocrine function through the excretion of vasoactive factors but also through the excretion of triglycerides by lipolysis. In healthy conditions this serves as energy for metabolically active tissues, in unhealthy conditions this release becomes excessive and has cardiovascular consequences (van Dam e.a., 2017). Lipolysis is inhibited by insulin (Zierath e.a., 1998). However insulin treatment does not seem viable as obesity is the main cause of unhealthy WAT, while also causing insulin resistance. Insulin sensitising drugs such as pioglitazone and metformin are promising in reducing circulating triglycerides. Unfortunately they cause adverse side effects such as myocardial infarction. A final therapeutic approach could be to induce browning. Browning can be accomplished in various ways such as cold, catecholamine stimulation, beta-3-receptor activation and eosinophil upregulation (van Dam e.a., 2017).

Another interesting way of altering PVAT is through bariatric surgery. Aghamohammadzadeh et al followed 15 patients 6 months after bariatric surgery. As expected all patients showed restored anti-contractile gluteal PVAT function 6 months post-surgery. NO bioavailability was significantly increased as well as adiponectin, along with reduced inflammation and adipocyte size (Aghamohammadzadeh e.a., 2013).

In keeping with surgery Manka et al transplanted thoracic aortic PVAT from mice with a high-fat diet to the carotid arteries of lipoprotein receptor knockout mice. They found that transplanted PVAT increased neointima forming, while sham operations did not. Through immunostaining they determined this was caused by VSMC hyperplasia. The PVAT transplant also causes infiltration of macrophages into the adventitia. PVAT was also found to enhance neovascularisation, as

transplanted PVAT became highly vascularised. All of this indicates that healthy PVAT transplantation might not be a viable option in treating PVAT dysfunction (Manka e.a., 2014). However, these findings might not be of clinical significance as these carotid arteries are normally devoid of PVAT, possibly eliciting reactions that wouldn't happen elsewhere in the body.

Another interesting treatment was found by Pelham et al. Gupta et al demonstrated in a previous study that vitamin D deficiency results in an increase in leukocyte infiltration, upregulation of pro-inflammatory cytokines and a decrease in adiponectin production in adipose tissue (Gupta e.a., 2012). Pelham et al expanded on this by studying the effects of vitamin D deficiency in mice mesenteric PVAT. Their findings showed that vitamin D deficient mice had a significantly higher blood pressure as well as a decreased anti-contractile response to serotonin and Ang2 in mesenteric PVAT. They demonstrated the same results in thoracic aortic PVAT. Vitamin D deficiency was also found to activate hypoxia signalling through several different pathways in mesenteric PVAT. Vitamin D supplementation was found to suppress contractile responses to serotonin as well as induce normal levels of hypoxic markers under hypoxic conditions. This suggests that vitamin D can counteract hypoxia and inflammation in mesenteric PVAT while simultaneously restoring its anti-contractile function (Pelham e.a., 2016).

Finally another common oral treatment shown to improve endothelial function has been found to improve PVAT function as well. Resveratrol enhances endothelium-dependent relaxation in multiple rat studies, which used PVAT-free vessels. Resveratrol mediates its function by decreasing oxidative stress in a plethora of ways, thereby preventing NO inactivation. Resveratrol also upregulates endothelial eNOS, increasing NO even further. Studies in rats showed that PVAT dysfunction, induced by a high-fat diet,  is attenuated by oral treatment with resveratrol for 8 weeks. This was discovered through PVAT-derived medium which attenuated vasodilation significantly less compared to PVAT-medium without resveratrol. This indicates that improved PVAT function is indeed mediated by resveratrol, yet its exact mechanisms in PVAT remain unclear (Xia Ning e.a., 2017).

Of importance is that most of these treatments do not look at the effect of the treatment on different PVAT depots, with many studies not declaring which PVAT they studied at all. Overall these studies proved there to be many viable options in treating PVAT dysfunction and therefore reducing the role of PVAT in CVD. However, it is still unclear whether a treatment such as vitamin D supplementation which is viable in mesenteric and thoracic aortic PVAT is also viable in EAT and RPVAT treatment. Hence we suggest further studies be done on the effects of these treatments on differing PVAT depots and focus should be shifted towards developing treatments specifically for affected tissue.

## Conclusion and discussion

Concluding, these results show the importance of PVAT heterogeneity in CVD. PVAT clearly influences vascular tone and the health of the surrounding tissue, implying a twofold role in the development of CVD for PVAT dysfunction. An important determinant of PVAT function seems to be whether PVAT resembles WAT or BAT. Mice thoracic aortic PVAT resembles BAT both phenotypically and functionally, while abdominal aortic PVAT is WAT-like. Dysfunctional abdominal PVAT displayed reduced anti-contractile effect, downregulated NO production and upregulated ROS production, while thoracic aortic PVAT was still able to exert its anti-contractile effect. Mesenteric PVAT, which resembles WAT, showed different responses to both the abdominal and thoracic PVAT. Both EAT and RPVAT showed dissimilar traits as well. EAT is very susceptible to atherosclerosis and expresses highly different genes compared to the relatively close thoracic aortic PVAT. RPVAT resembles both WAT and BAT and interestingly exhibits no anti-contractile response at all. This highlights the importance of the BAT- and WAT-like phenotype in PVAT, while simultaneously proving PVAT differs in function depending on its location.

Healthy PVAT also highly differs in leukocyte composition, altered even further in inflamed conditions which leads to PVAT dysfunction. Obesity induces hypoxia and inflammation in PVAT, altering PVAT function through downregulating NO and adiponectin production. Leukocytes infiltrate PVAT in higher numbers, while also being more pro-inflammatory. The exact mechanisms differ depending on PVATs location. Dysfunctional EAT showed several upregulated inflammatory proteins, mesenteric PVAT showed similar alterations in adiponectin and SOD expression and RPVAT also exhibited increased pro-inflammatory markers along with an increase in renin receptors and microalbuminuria excretion. This proofs both that obesity causes PVAT dysfunction and that this dysfunction is different according to PVAT location.

This dysfunction can be treated in several different ways, the most viable seemingly being exercise as it decreases PVAT mass, induces an anti-inflammatory phenotype switch, attenuates PVAT dysfunction and causes WAT browning in both mesenteric and thoracic aortic PVAT. Bariatric surgery has been shown to restore PVAT function as well, specifically gluteal PVAT. Additionally multiple oral treatments are available, namely vitamin D supplementation, reservatrol, salicytes and insulin sensitising drugs. However, except for exercise none of these treatments have looked at the effects in differing PVAT sites. Due to the proven differences among PVAT sites it cannot be said for certain that the mentioned treatments have the same effect at all sites. Indeed, a treatment which is beneficial in one tissue might be detrimental in another. Therefore further studies are needed in order to examine their exact effects and determine their use in treating PVAT dysfunction.

There are also several apparent problems in multiple studies. Although individual problems will not be discussed we will give an overview of the major problems. One of the biggest problems is a lack of studies on several important tissues, such as RPVAT. Kidney function has important implications in CVD, yet studies on RPVAT are few. The few studies that have been done lack strong conclusions and it is therefore debatable whether these studies represent consistent results.

Also few of these studies were performed on human tissue. Even though different animals such as mice, rats and swine were used, clear data on human PVAT is scarce. And although findings seem consistent among these species it remains unclear whether these findings represent human tissue as well. The studies that were performed in humans mostly focussed on dysfunctional PVAT within a high age-range and with large BMIs. Both are related to PVAT dysfunction but are also implicated in various other unhealthy conditions. Hence results could have been altered due to other unhealthy conditions or due to the conditions being the cause of dysfunctional PVAT instead of BMI or age

themselves. Another problem within the human studies was the lack of using actual tissue. Human studies are mostly cross-sectional, thus using living subjects of which PVAT cannot easily be obtained. This results in the use of mRNA analysis or other means in order to indirectly analyse PVAT alterations. Clearly this is less accurate compared to using actual human tissue.

Another problem is the lack of diverse testing groups. The human and animal studies almost exclusively used male subjects, again putting in question how representable the data is. Along with this several other factors need to be accounted for in further studies: disease history, age, genetic mutations, genotype, medication, waist-to-hip circumference, smoking status and physical activity.

So although PVAT heterogeneity plays an important role in CVD and is heavily altered in obesity, many questions about its exact mechanisms remain to be answered. Extra studies are needed in order to fully uncover these remaining mysteries so effective treatments can be developed for reducing PVAT dysfunction and consequently CVD.

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