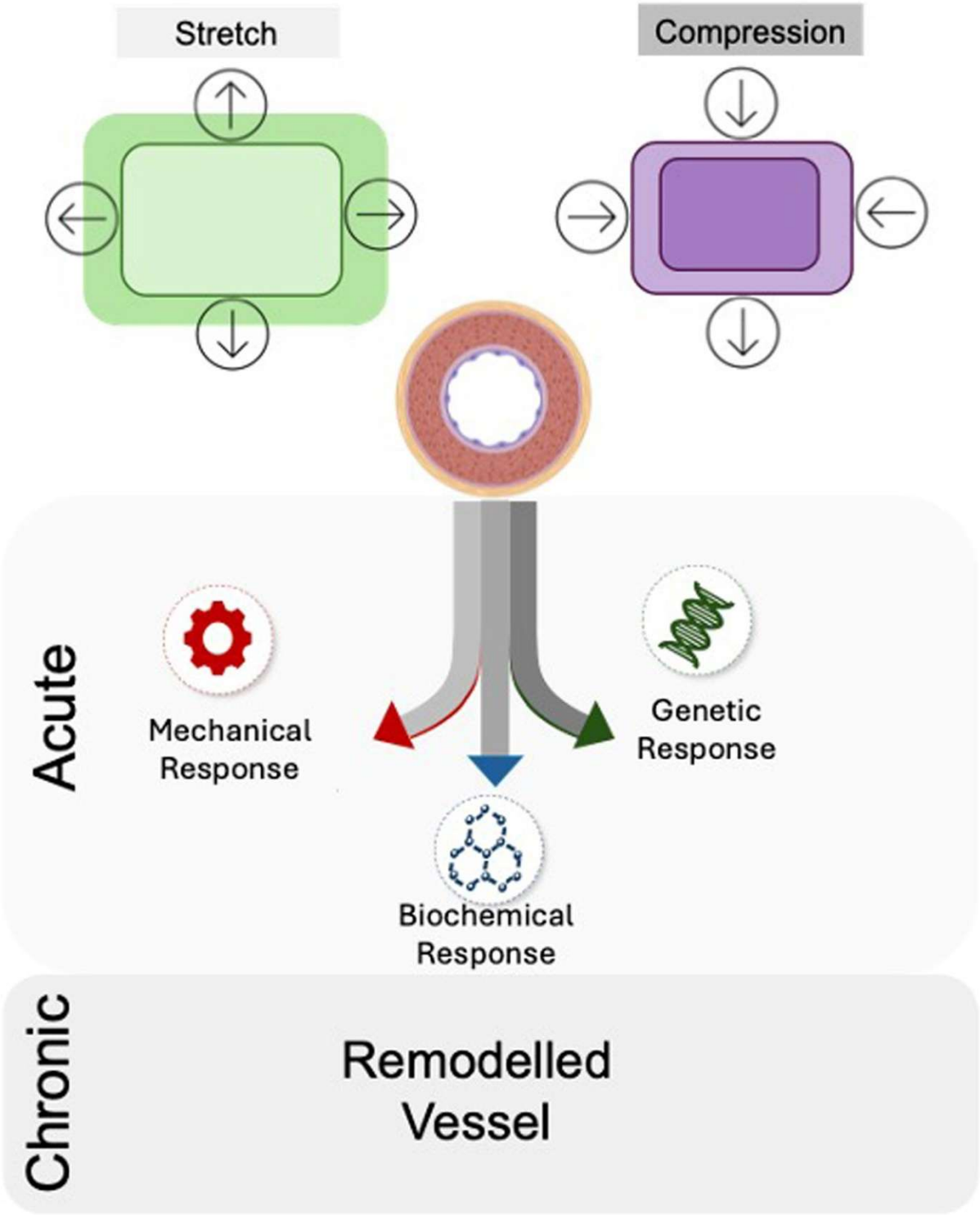
Abstract

Perivascular adipose tissue is of compelling interest when considering tissue mechanotransduction. Because of its location around a vessel, perivascular adipose tissue experiences from high (artery) to low (vein) pressures, pressures that are cyclical in nature. With blood pressure change, such as the elevation of pressure in hypertension, the question has been raised as to whether perivascular adipose tissue senses such changes, evidenced by a response that can be genetic, structural, or mechanical in nature. Here, we briefly review the following knowledge and data that support the ability of perivascular adipose tissue to both (mechano)sense and (mechano)respond.

Graphical Abstract



Highlights

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All cell types within thoracic aortic perivascular adipose tissue express mechanotransducers.

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Perivascular adipose tissue senses and responds to mechanical stressors, including stretch, and rely, in part, on these mechanotransducers for this response.

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Perivascular adipose tissue should be considered in addressing the mechanisms of arterial disease.

What Is Perivascular Adipose Tissue?

Perivascular adipose tissue (PVAT) is present around a majority of the vasculature, the exceptions being cerebral vessels and some pulmonary vessels.[1,2] PVAT differs from non-PVAT fat in always being the adipose tissue that surrounds a blood vessel. For example, the retroperitoneal or epididymal fat would not be considered a PVAT. However, these tissues—PVAT and non-PVAT fat—are rightly considered an adipose tissue depot. Figure 1A depicts PVAT around the Masson trichrome–stained thoracic aorta (left) and mesenteric resistance artery (right) of the male rat. These 2 fats look different because the PVAT around the thoracic aorta contains dense, multilocular brown adipocytes while that around the abdominal aorta and mesenteric arteries and veins contains primarily a unilocular white adipocyte. Both adipocyte types are set within an extracellular matrix that is rich in multiple types of collagen.[3] The composition of both PVATs (and PVATs in general) is heterogenous and not simple (discussed more below). We refer you to a few of the many existing reviews for a deeper review into the composition of different PVATs.[4–6]

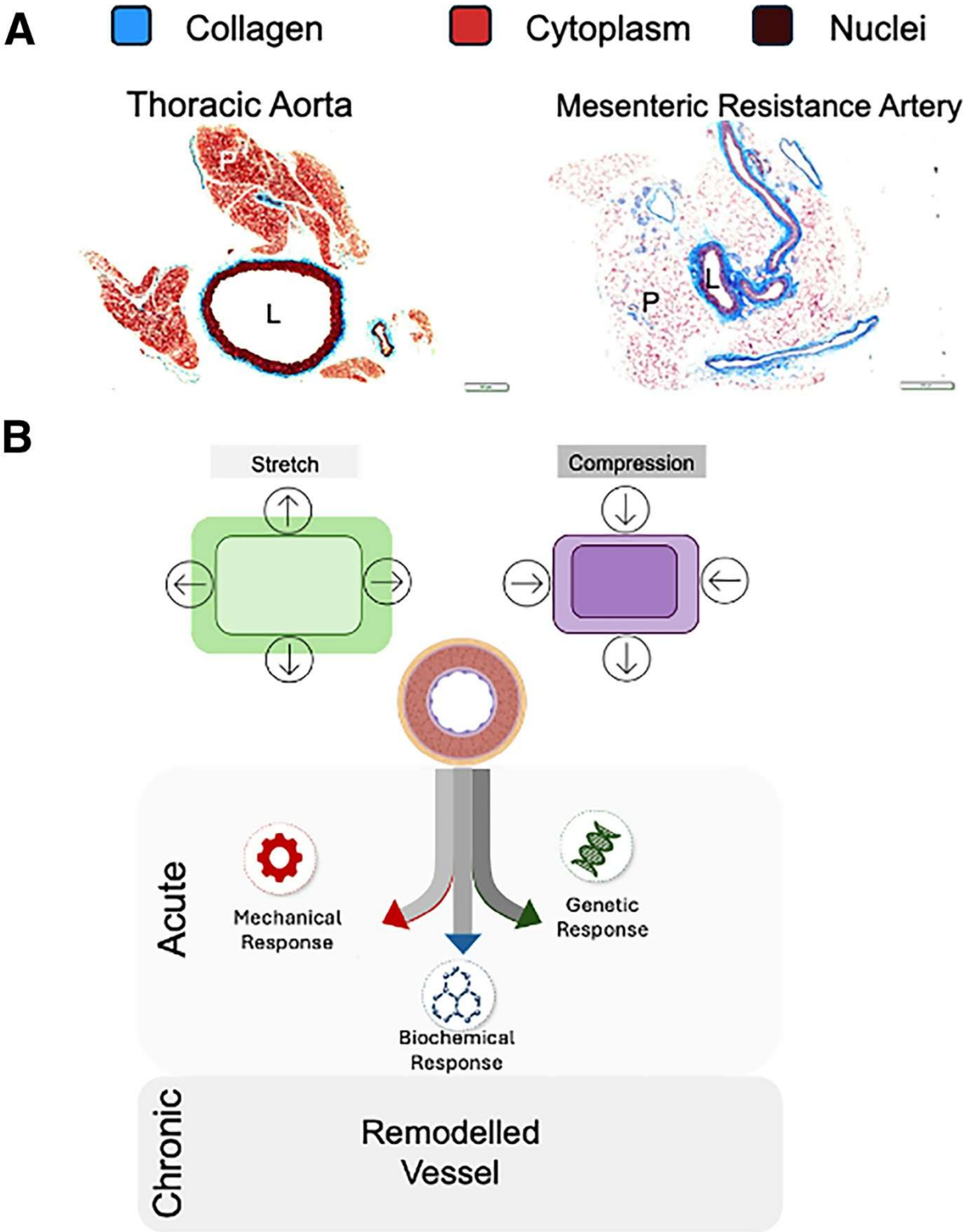

Figure 1. **Perivascular adipose tissue (PVAT) structure and mechanics. A**, Masson trichrome staining of thoracic aortic (**left**) and resistance artery PVAT (**right**) from a Dahl salt sensitive rat. **B**, Considered responses to stretch or compression at acute and chronic time points as an illustration of the basic concept of mechanoresponse.

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Though known to be present for some time,[2] PVAT was rediscovered as a functional vascular layer by Soltis and Cassis in 1991.[7] They discovered that the removal of PVAT enhanced the potency of

norepinephrine-induced contraction of the isolated rat aorta, an event prevented by inhibitors of norepinephrine uptake. Additionally, the indirect sympathomimetic tyramine caused a PVAT-dependent contraction. These studies revealed PVAT to be a tissue that modified vascular responsiveness in an acute and direct way. Eleven years later, Lohn et al[8] published the finding that PVAT produced a relaxing factor that enabled the now well-established anticontractile nature of PVAT. It is for this ability—to be anticontractile—that PVAT is best known. This characteristic is well-established in vessels from both animal models and human.[9,10] We list just 2 reviews here; there are a multitude of studies that support a significant diversity in those molecules considered anticontractile. Our laboratories are dedicated to understanding more about this fourth layer of the blood vessel, functions that likely operate independently of the anticontractile factors produced. We review here a new function of PVAT to be considered, namely its ability to mechanosense and mechanotransduce.

See cover image

What Is Mechanotransduction?

Mechanotransduction is a process by which a cell/tissue converts physical stimuli exerted on it into a biological response. Here, the biological response includes a wide variety of end points. Figure 1B depicts this idea in general terms, using stretch and compression as considered mechanical challenges. Imposed upon a whole blood vessel, observation of a measurable change in any end point means that the tissue sensed the change in the external stimulus and responded to it. A stimulus that is considered physiological is a 5% to 10% change/deformation, while over 20% would be considered a pathophysiological stimulus.[11] Figure 1B considers broad responses to a stretch or compression in direct mechanical, biochemical, or genetic reactions. Other recognized end points include a change in intracellular calcium, signaling pathways, cell shape, and cell/tissue tension, to name a few.[12–14] These responses, as illustrated, can occur acutely/immediately in response to the challenge. Upon a chronic challenge, these various responses likely combine to result in a long-term response to a mechanical challenge. For example, in response to hypertension, both systemic and pulmonary, the vessel structurally remodels in a way that may be irreversible.[15,16] Vascular remodeling also occurs in heart failure and atherosclerosis.[15] Work supports that vascular remodeling is likely the result of interplay between the multiple different cell types and tissue types that compose the whole vessel,[17] and this includes those in PVAT.

How is a change in the external environment sensed? By mechanisms that are just as diverse as the response of the tissue: through change in membrane tension, activation of nonmotile cilia, and direct activation of plasma membrane delimited mechanotransducers (eg, Piezo1), etc. Gene ontology (https://amigo.geneontology.org/) identifies at least 85 genes implicated in the first steps of mechanotransduction, reflecting the diversity in mechanisms. Mechanotransduction can work from outside the membrane, to the nuclear YAP/TAZ (yes-associated protein/tafazzin)-dependent mechanotransduction pathways, and back again.[18,19] Vascular mechanotransduction (sensing and responding) has been reviewed by many, including Davis et al.[20] Similarly, adipose tissue depots are recognized to mechanosense and respond to its microenvironment.[21] However, PVAT has not been considered in this way. Does PVAT mechanotransduce? This becomes a more compelling question to answer when one considers the multitude of different responses that could occur, based on the many cell types present in PVAT (below).

Why Would One Think PVAT Should Mechanotransduce?

There is considerably less work done on mechanotransduction in adipose tissue versus the vasculature. The adipocyte itself mechanotransduces[22] with mechanical cues resulting in changes in adipocyte function. During the early stages of hypertension, adipocyte lipolysis increases.[23,24] This is

due, in part, to Piezo1 signaling that activates cAMP and thus canonical lipolysis pathways.[25] Chronic activation of lipolysis can lead to fibrosis development through inflammatory responses that inhibit the formation of new adipocytes,[26] as discussed later. As PVAT fibrosis develops, the depot loses mass, and the secretion of vasorelaxant adipokines, including adiponectin, is reduced. In contrast, the levels of cytokines, fibrotic mediators, and matricellular proteins increase.[27] Knowledge of adipocyte mechanotransduction is important because adipocytes make up the majority of cells in PVAT (60%–80%). The adipocyte progenitor cells (APCs) also respond to mechanical challenges; this is discussed later.

If at least 2 important cell types (adipocytes, APCs) in PVAT can mechanotransduce, it makes sense that PVAT as a whole would mechanotransduce. PVAT surrounds the artery and has a constant exposure to pressure. The thoracic aortic PVAT possesses the additional stressor of being the tethering link to the spine. Cells of PVATs reside in a collagen-based scaffold that likely provides part of the cues for the cells to respond. We review data that support the hypothesis that PVAT can mechanotransduce. Our work has primarily focused on rat PVAT, both the Sprague-Dawley rat and the Dahl salt sensitive (SS) rat. We focus around the thoracic aortic PVAT. This is because this PVAT surrounds only the aorta and is not shared with another vessel (Figure 1A, left). This contrasts with PVAT that is clearly shared by mesenteric arteries and veins (Figure 1B, right). We will consider a few of the potential functions of cells/tissue within PVAT.

Evidence That PVAT Mechanotransduces

PVAT Can Stress Relax in Response to a Mechanical Challenge

Stress relaxation is defined as a decrease in stress associated with constant strain. This response is evidence of mechanotransduction because it is a response to a defined change in an external stimulus. This parameter is important because it is an indication of the health of a tissue, with aged or heated tissue losing the ability to stress relax.[28,29] We discovered that PVAT assisted/enhanced the ability of the isolated thoracic aorta to stress relax over a range of applied tensions.[30] This means the presence of PVAT around the aortic ring made the magnitude of stress relaxation greater than would occur in its absence. However, stress relaxation of the aorta was not enhanced if PVAT was present but not surrounding the aortic ring. Here, the same amount of PVAT was present on the isolated aorta but dissected; so it was at the end of the ring and not around the aorta. This finding supports the physical integrity of the PVAT as a layer/ring around the aorta was essential to this response. The PVAT can be separated from the aorta and, mounted as a ring in a tissue bath, stress relaxes on its own. Isolated PVAT relaxed to a greater magnitude than the aorta it surrounds[31] (Figure 2A). The magnitude of PVAT-assisted stress relaxation was not diminished if the ring of PVAT was physically removed but then placed back on the aorta. This means a direct physical conduit from PVAT to the aortic adventitia was not necessary for this response. These findings are consistent with the idea that PVAT may produce vasoactive substances in response to the stress that is experienced with stretch. This remains to be tested.

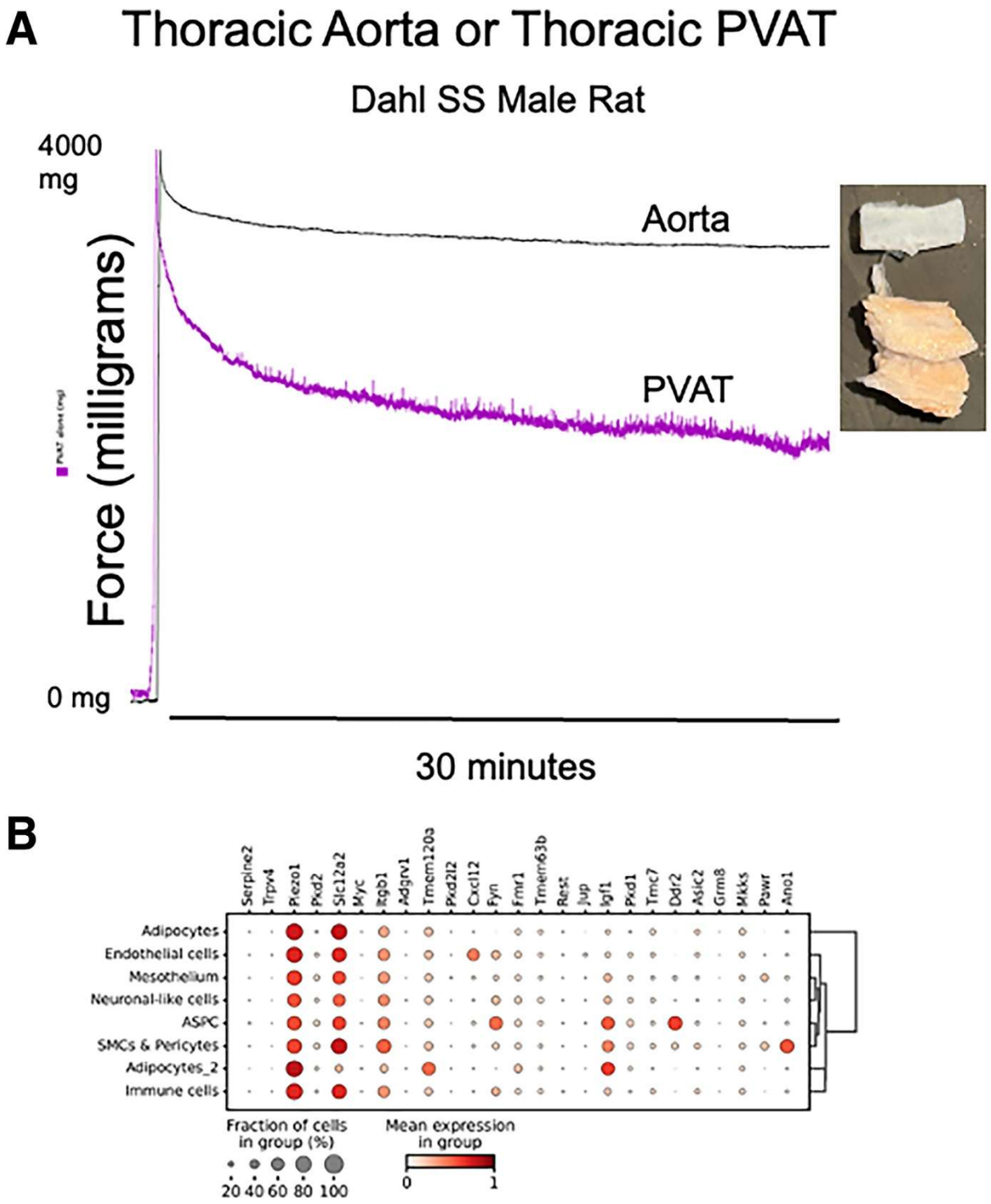

Figure 2. **Perivascular adipose tissue (PVAT) stress relaxation and mechanotransducers. A**, Representative tracing of stress relaxation in an aortic ring or the PVAT removed from it (far right shows the actual tissue). **B**, Dot plot of the most highly expressed mechanotransducers in identified clusters from cells in thoracic aortic PVAT of a Dahl SS rat. Key at the bottom shares that dot size represents the percentage of nuclei expression in the gene, while color intensity represents the expression level. Data derived from Thompson et al.[32]

Another finding supports PVAT as a mechanical structure. Intact PVAT on the isolated thoracic aorta reduced the stiffness of the aorta. Our speculation is that stress relaxation and stiffness are linked, with a stiffer tissue having reduced stress relaxation. The isolated PVAT ring of PVAT itself had a markedly low stiffness[30]; we have yet to determine whether this changes in the condition of

hypertension. Fleenor et al[33] convincingly demonstrated that a greater aortic PVAT density was associated with aging, aortic stiffness, and central blood pressure in humans.

Work by others edifies these above ideas. Liu et al[34] used the healthy, nondiseased Yorkshire pig in vivo to demonstrate that removal of the PVAT around the carotid and femoral arteries resulted in increases in circumferential stress and strain when compared with animals in which PVAT was left intact. PVAT supported part of the load, contributing to the mechanics of the artery. Kim et al[35] developed computational models that emphasize the importance of surrounding tissues on the biomechanics of the thoracic aorta of the Yorkshire pig. They concluded that "surrounding tissues and non-uniform thickness should be included in biomechanical analysis to better understand regional adaptation of the aortic wall during normal physiological conditions or pathological condition." These general ideas were reaffirmed by Ferruzzi et al[36]: perivascular tethering "allows arteries to work at lower values of wall stress." Changes in PVAT function and structure ultimately influence arterial mechanics, such that the vessel would have to adapt in response to maintain stress. Mechanistically, at the tissue and not molecular level, PVAT needs to be taken into account to understand blood vessel function and dysfunction.

Together, these findings place PVAT as mechanically important to the functioning of the artery. PVAT must possess mechanisms that sense and respond to a change in its mechanical environment. What are these mechanisms?

Multiple Cell Types in PVAT Possess Mechanotransductive Elements

The literature on vascular mechanotransduction is rich.[20] We have focused on investigation of 2 mechanisms for mechanotransduction: individual mechanotransducers and interaction of integrins and collagens. We created a cell atlas of the Dahl SS male thoracic aorta using a single-nucleus RNA sequencing study.[32] This has significantly aided our ability to interrogate thoracic aortic PVAT for the identification of cell types/clusters that express mechanotransducers.

The mRNA for multiple mechanotransducers was present in the thoracic aortic PVAT and in a majority of the 8 clusters found in this tissue: adipocytes (2 clusters), endothelial cells, immune cells, adipocyte stem cell precursors, neuronal-like cells, smooth muscle cells/pericytes and mesothelial cells.[32] The adipocyte itself possessed mechanotransducer mRNA (Figure 2B). These data added to the sparse knowledge of the adipocyte's ability to respond to mechanical cues.[22,27] Piezo1 was the most highly expressed mechanotransducer in PVAT, observed both at the level of mRNA and protein and at the greatest level in adipocytes.[32] Piezo1 is functionally important to stress relaxation. Inhibition of Piezo1 by the spider venom peptide GsMTx4 increased the magnitude stress relaxation in isolated PVAT, suggesting the endogenously activated Piezo1 channel helped maintain tissue tone. Importantly, human arterial PVAT expresses Piezo1 protein, allowing for consideration of these same mechanisms within human PVAT.[32] In addition to Piezo1, *Slc12a2* (NKCC1 [Na-K-2Cl cotransporter-1]) and integrin-β_1 were the next highest and most homogeneously expressed in cells of PVAT. Integrins and collagens interact to sustain tone after stress relaxation as their inhibition by the disintegrin RGD (arginine, glycine, and aspartic acid) peptide also increased the magnitude of stress relaxation.[37]

PVAT Responds With a Biological Change to an Elevation in Pressure (Hypertension as a Cardiovascular Disease)

Changes in the Thoracic Aorta

In this section, we will focus on elevated blood pressure/hypertension as a stimulus to which PVAT responds. Whether by coarcting the thoracic aorta in the middle[38] or feeding a high-fat (HF) diet from

weaning to create an elevated pressure,[39] experiments provide evidence that PVAT responds to an elevation in blood pressure. The mid-thoracic aorta coarcted rat (Sprague-Dawley) was used to test directly whether pressure changed function and gene expression in the tissue exposed to a higher (above coarct) versus a lower (below coarct) pressure in the same animal. The PVAT exposed to the higher pressure (above coarct) had a reduced ability to assist stress relaxation. This loss was similarly observed in the Dahl SS rat made hypertensive through HF (60%) diet from weaning.[39] It is tempting to speculate that elevated pressure harms the ability of PVAT to mechanotransduce, leaving it distanced from communication with its environment and thus less likely to be able to respond to a challenge.

Another experiment was performed on the thoracic aorta of control diet–fed (10% fat) and HF diet–fed (60%) Dahl SS rats.[3] It revealed an important difference between PVAT and the tunica that serves as its basement, the tunica adventitia. Dahl SS rats, which develop a hypertension in response to this diet from weaning,[40] were used at a time in which hypertension was not apparent (8 weeks on diet) and a time in which it was established (24 weeks). The adventitia and PVAT were separated, mRNA isolated, and taken through a SuperArray for quantifying genes, the proteins of which are established to contribute to the extracellular matrix. Differentially expressed genes between HF and control diets were measured. The 2 tunicas (adventitia versus PVAT) had a strikingly different change in differentially expressed genes at the 8- and 24-week time point. The adventitia showed a majority of gene changes at the 8-week time point. Twenty-nine genes were upregulated in the adventitia while no genes were modified (HF versus control) in the PVAT. The picture at 24 weeks was quite different. Here, PVAT showed a robust response in differentially expressed genes (47 genes) while the adventitia showed only 3. We interpreted this to mean that an elevation in blood pressure—a mechanical stress—was necessary to incite these changes in differentially expressed genes in the PVAT. The HF diet alone was not sufficient to do so. This is mechanotransduction evidenced at a genetic level.

Changes in Arterial PVAT APCs

Adipocytes are the most abundant cell type in PVAT. The population of PVAT cells is maintained through adipogenesis, the process by which APCs mature into functional adipocytes that secrete adipokines, including the vasoactive adiponectin and apelin. Adipogenesis is influenced by the activation of mechanotransducers on APCs. Our research demonstrated that these cells express Piezo1 and that both pharmacological and mechanical activation of this mechanoreceptor reduced their adipogenic potential, enhanced fibrogenesis, and limited the lipid storage capacity of mature PVAT adipocytes.[41] The distribution of APC populations within PVAT is influenced by anatomic location, sex, and age, which can amplify the effects of mechanotransducer activation under specific conditions.[42] For instance, the PVAT of the abdominal aorta contains fewer APCs than the descending thoracic aorta and mesenteric vessels, potentially leading to reduced adipogenic capacity and increased sensitivity to vascular forces during hypertension. This may also make the abdominal aorta more susceptible to developing lesions that could evolve into aneurysms, more common in the abdominal aorta than in the thoracic aorta. Additionally, aging diminishes the APC pool in PVAT, potentially exacerbating the anti-adipogenic and profibrotic effects of hypertension through mechanotransducers.

Are There Specific Cells That Are Responsible for Mechanotransduction?

This question was one we hoped to answer directly through creation of the cell atlas of the thoracic aorta of the Dahl SS rat. However, all cell types express some type of mechanotransducer or elements of mechanotransduction (Figure 2B). These data suggest that there was no one specific cell type

primarily responsible for mechanotransduction in PVAT. However, cell-cell communication analysis revealed that the APCs and adipocytes had the most chatter between them (the greatest number of potential source/receiver interactions) in the cells of PVAT.[38] Tools such as these will help us refine the key players in this process.

Why Does This Matter in Health and Disease?

The impetus behind these studies was to determine whether new functions of PVAT—independent from formation of anticontractile substances—contribute to vascular dysfunction in disease. Of focus here is the ability of PVAT to mechanosense and respond. Like the dysfunction of the endothelial cell (tunica intima) became a hallmark of vascular disease/dysfunction, it may be that PVAT (tunica adiposa) needs to be considered in the same way. Treatment of hypertension, when the vasculature is considered, never includes PVAT; maybe it should. Video S1 validates the stresses on and movements of PVAT of the descending rat thoracic aorta in vivo. Indeed, a human meta-analysis demonstrated that PVAT amount correlated with the studied cardiovascular risk factors (body mass index, cholesterol, LDL [low-density lipoprotein][43]). The fat attenuation index, or FAI, may be a marker of disease activity in coronary PVAT.[44] This concept may be most important relative to the PVAT around the thoracic aorta. Measures of pulse wave velocity typically do not consider the PVAT as part of the measures made. In other words, the finding of elevated PWV/stiffness does not consider the PVAT as being part of this finding. Clinically, it has been convincingly argued that PVAT needs to be considered in nonatherosclerotic diseases that include aortic aneurysms and vasculitis.[45]

A relevant question is how PVAT mechanotransduction changes in obesity. PVAT burden is increased around the thoracic aorta in Dahl SS rats fed an HF diet. Our laboratories are currently undertaking a study that tests whether PVAT mechanotransducer expression is reduced, whether mechanotransduction is reset, and whether different cells take on the duty of mechanotransduction when moving from a control to a hypertensive condition.

Limitations and Future Directions

How Does PVAT Function Around a Vessel at a Lower Pressure (eg, Veins)

Does it serve similar functions and does it need to mechanotransduce in such a situation? Is that PVAT different than that around an artery given the lower pressure to which it is exposed?

How Does the Thoracic Aorta Compare to the Abdominal Aortic PVAT in Terms of Mechanotransduction?

We have not compared these 2 PVATs (thoracic versus abdominal) in any of our studies. We can appreciate, however, that the functions of PVAT may be different in these 2 aortic sections given that they are composed of different types of fat.[46]

Additionally, the thoracic aorta experiences a higher blood pressure than the abdominal aorta.

How Do Vessels (Small Resistance Arteries or Other Vessels) Mechanotransduce?

A majority of the work around this concept has been done with the thoracic aorta. Thus, the mechanisms described have to be limited to the thoracic aorta at this time. We and many others have focused on the thoracic aorta because there is no question that the PVAT belongs to the thoracic aorta. PVAT does surround the important arteries that control the total peripheral resistance, such as the mesenteric resistance arteries. However, in this bed, the artery and vein share the PVAT. We speculate here, as in the aorta, that PVAT helps dampen the mechanical stimuli that are received. Work is being put toward this effort presently.

How Does PVAT Remain With Low Stiffness in the Face of Constant Pressure?

PVAT around the aorta is exposed continuously to the highest vascular pressure experienced within the body, including in the early decades of life when cardiovascular disease occurs rarely. Why does it not fail in the face of this constant, relentless challenge, and what happens when it does fail?

Does PVAT Remodel in Ways Similar to the Other Arterial Tunicas?

From the standpoint of collagen expression, PVAT remodels in response to a higher pressure.[3] Remodeling work has, to this point, largely considered remodeling a structural event. But what about adipokine remodeling, lipolytic remodeling, and immune cell remodeling? Intriguing work by Jenkins et al[47] supports mesenteric PVAT remodels in immune cell population in an interleukin-10 colitis model of inflammatory bowel disease. Remodeling could also mean a change in the adipocyte type, such as an aortic brown adipocyte remodeling to white when moving from an external environment that is at room temperature to thermoneutrality.[48]

Do Immune Cells Within PVAT Mechanotransduce?

The mRNA for mechanotransducers is present in these cell types, though at a seemingly smaller quantitative level.[32]

Do GPCRs Mechanosense?

This provocative idea has been largely around the angiotensin AT_1 receptor, GPR68, and other adhesion GPCRs (G-protein–coupled receptors).[49] In the data set for the cell atlas of the thoracic PVAT, multiple G proteins were expressed but at low levels that were below the threshold for formal expression.[32] What if the suite of GPCRs expressed within the cells of PVAT enables the tissue to be mechanoresponsive?

Conclusions

PVAT should be firmly considered as a layer that communicates with other layers, functioning in ways to maintain arterial integrity and to be mechanoresponsive in reflecting changes in the environment.