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RESEARCH ARTICLE

Does Functionality of Perivascular Adipose Tissue Decrease in the Adult Rat Thoracic Aorta?

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SUMMARY

Perivascular adipose tissue (PVAT) regulates vascular tone with anticontractile effects by releasing paracrine factors. Aging is considered an independent risk factor that impairs vascular function in humans and rodents. There are limited studies examining the effects of aging on PVAT. In this study, we investigated the contractions to noradrenaline and the relaxations to acetylcholine in the presence and absence of PVAT in isolated thoracic aortas of 10-12-week-old young and 52-week-old adult Wistar rats. Noradrenaline contractions were lower in aortas with PVAT compared to rings without PVAT in both young and adults. The amplitude of contractions in adult rat aorta in the absence of PVAT was significantly higher compared to young rats. However, when PVAT was left intact, the amplitude and sensitivity of noradrenaline contractions were low in young and adults, indicating continued anticontractile effect of PVAT. Noradrenaline contractions were significantly potentiated by L-NAME, a nitric oxide (NO) synthase inhibitor, in young rat aortas, and this increase was lower in the presence of PVAT. However, the potentiating effect of L-NAME in the adults did not change with PVAT. Acetylcholine-induced endothelium-dependent relaxations were reduced in the adult rats compared with young. Acetylcholine relaxations were not affected by PVAT in young rats, whereas the decreased acetylcholine relaxations were further reduced in the aged aortas with PVAT. As a result, the anticontractile function of PVAT continues in adult rats and limits the contraction with a tonic inhibitory effect. However, PVAT does not seem to protect endothelium-dependent relaxations that decrease with aging.

Key Words: *Anticontractile, aorta, endothelium, NO, PVAT, rat.*

Erişkin Sıçan İzole Torasik Aortunda Perivasküler Adipoz Doku Fonksiyonu Azalır mı?

ÖZ

Perivasküler adipoz doku (PVAD) parakrin faktörler salıvererek kasılma-karşıtı etki ile damar tonusunu düzenler. Yaşlanma, insanlarda ve kemirgenlerde damar yapısını ve işlevini bozan bağımsız bir risk faktörü olarak kabul edilir. Yaşlanmanın PVAD işlevi üzerine etkilerini inceleyen sınırlı sayıda çalışma vardır. Biz bu çalışmada, 10-12 haftalık genç ve 52 haftalık erişkin Wistar erkek sıçanların izole torasik aortasında PVAD varlığında ve yokluğunda noradrenalin kasılmalarını ve asetilkolin ile elde edilen endotele bağlı gevşemeleri inceledik. Noradrenalin kasılmaları hem genç hem de erişkin gruba ait PVAD'ı sağlam torasik aorta halkalarında PVAD'ı sıyrılmış olanlara göre düşüktü. PVAD yokluğunda erişkin sıçan torasik aortunda elde edilen noradrenalin kasılmalarının boyu genç sıçan aortuna kıyasla anlamlı derecede yüksekti. Ancak bu arterlerde PVAD sağlam bırakıldığında, noradrenalin kasılma boyu ve duyarlılığı genç ve erişkin grupta benzerdi, bu da PVAD'ın erişkin sıçanlarda kasılma-karşıtı işlevinin devam ettiğini gösterdi. Genç sıçan torasik aortunda noradrenalin kasılmaları, nitrik oksit (NO) sentaz inhibitörü L-NAME varlığında güçlenerek arttı ve bu artış oranı PVAD varlığında daha düşüktü. Ancak, L-NAME'in erişkin grupta noradrenalin -aracılı kasılmalardaki güçlendirici etkisi PVAD varlığında değişmedi. Asetilkolin-kaynaklı ve endotel-bağımlı gevşemeler erişkin grupta genç gruba kıyasla önemli ölçüde azaldı. PVAD'ın sağlam bırakılması genç sıçan aortunda endotele bağlı gevşemeleri etkilemezken, yaşlı sıçan aortunda azalmış asetilkolin gevşemeleri daha da azaldı. Sonuç olarak, PVAD'ın kasılma-karşıtı işlevinin erişkin sıçanlarda devam ettiği ve tonik inhibitör etkiyle damardaki kasılmayı sınırladığı belirlenmiştir. Ancak PVAD'ın yaşlanmayla birlikte azalan endotele bağlı gevşemeleri geri çeviremediği söylenebilir.

Anahtar Kelimeler: *Kasılma-karşıtı, aorta, endotel, NO, PVAT, sıçan.*

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INTRODUCTION

Perivascular adipose tissue (PVAT) covers the outer surface of the vascular wall where it is located without being separated by any elastic lamina from the tunica adventitia (Siegel-Axel & Haring, 2016) (Gil-Ortega, Somoza, Huang, Gollasch, & Fernandez-Alfonso, 2015). Previously, PVAT was thought to be only a barrier that protects the vessel from mechanical injury; further studies have revealed that bioactive molecules released from PVAT can also affect neighboring vascular smooth muscle by endocrine and paracrine functions (Rajsheker et al., 2010). Similarly, molecules released from the vascular components such as endothelium can also diffuse into PVAT and modulate its function on vascular homeostasis (Margaritis et al., 2013). Healthy PVAT participates in a number of modulatory processes including anti-inflammatory properties, anti-oxidative defense, vasodilation or anticontractile action (Hu, Garcia-Barrio, Jiang, Chen, & Chang, 2021).

The anticontractile function of PVAT has been demonstrated by its inhibiting effect on contraction to various agonists in isolated vascular tissues mounted to organ bath system or wire myograph (Gao et al., 2005). PVAT secretes a wide range of adipocytokines, including adipokines and cytokines, which are involved in the regulation of vascular tone. Adiponectin is the most abundant molecule produced and released by PVAT (Chang, Garcia-Barrio, & Chen, 2020). Adiponectin binds to the receptor of the endothelial cells and leads to the phosphorylation of AMP-activated protein kinase and then to the activation of the endothelial NO synthase, promoting NO production (Cheng et al., 2007). PVAT can act as two sides of the same coin, cardiovascular risk factors as well as vascular aging may alter the structure and functionality of PVAT which leads to the loss of its vascular protective properties, and the release of contractile factors from PVAT affecting the vascular tone (Wang et al., 2024). However, little is known about the impact of age on PVAT and how PVAT modulates the vascular reactivity in aged vasculature.

In this study, we aimed to investigate how PVAT and its paracrine action on vascular reactivity alter in isolated thoracic aortic rings of young and adult rats. Contractile responses to noradrenaline, and endothelium-dependent relaxations to acetylcholine were evaluated in isolated thoracic aortic rings of young (10-12-week-old) and adult (52-week-old) Wistar albino male rats in the absence and presence of PVAT.

MATERIAL AND METHODS

In the current study, Wistar Albino male rats weighing 180-200 g and 8 to 10-week-old were acquired from Kobay DHL Inc. Co. (Ankara, Turkiye). The animals were housed in the laboratory for two weeks to be accustomed to the laboratory environment with unlimited access to standard food and water under a 12-hour light/dark cycle. The rats were included in the experiments when they were 10-12- or 52-week-old. Hacettepe University Animal Experiments Local Ethics Committee authorized all animal procedures (Ethics Committee No: 2022/08-04).

On the day of the experiment, body weights (g) of the rats were measured. After that, rats were stunned with carbon dioxide (CO_2) inhalation and decapitated with a guillotine. Thorax was opened, thoracic aorta was isolated and put into a petri dish containing a cold Krebs-Henseleit Solution (KHS). One group of the vessels isolated from young and adult rats were left PVAT intact (PVAT+) and the other group of the vessels were removed from PVAT (PVAT-). The PVAT was cleaned around the thoracic aorta with small incisions using an appropriate cataract scissor. To ensure that the PVAT volume was the same in all rings, a fat layer of approximately 2 mm was left on the outer surface of the rings when preparing the tissues (Cacanyiova et al., 2021). The volumetric ratio between PVAT and endothelium was kept similar in

all groups. The vessels with or without PVAT were made into 3-4 mm ring preparations and placed in isolated organ baths of 5 mL containing KHS, which was continuously gassed (95% O_2 - 5% CO_2) at 37°C. In some experiments, the endothelium was mechanically damaged with a sterile syringe needle that was rubbed into the vascular lumen. Endothelial function was assessed by relaxations to acetylcholine (10 μM) after phenylephrine pre-contraction. Tissues that relaxed 10% or less were considered endothelium-removed.

At the beginning of the experiment, 2 g of basal tension were applied to the preparations. Immediately after, they were primed with 100 μM phenylephrine and then washed at 15-minute intervals for 1.5 hours. Tension changes were recorded on the computer via the isometric force transducer using the BIOPAC MP35 amplifier and Student Lab program (BIOPAC MP35 Data Acquisition System, Goleta, CA, USA). In the preliminary experiments, contraction responses to KHS containing 80 mM KCL were obtained twice in succession in all preparations. The second contractions to 80 mM KCL were accepted as the maximum contraction of each tissue. After wash out and resting period vascular reactivity was evaluated as mentioned below.

Experimental protocol

To demonstrate the anticontractile action of PVAT in 10-12-week-old young and 52-week-old adult rats, cumulative contraction responses were obtained with α- and β-receptor agonist noradrenaline (0.1 nM-100 μM) in the thoracic aortic rings in which PVAT was left intact or removed. After an hour of wash-out and resting period, the same tissues were incubated for 45 minutes with N^W-nitro-L-arginine methyl ester (L-NAME; 100 μM), a NO synthase inhibitor, and noradrenaline contractions were repeated. In this way, it was investigated the contribution of NO to anticontractile response of PVAT in young rats and how this contribution changes with aging.

In another set of experiments, the possible influence of the endothelium on the anticontractile function of the PVAT was examined by eliciting norepinephrine contractions in the absence of endothelium in aortic rings with or without PVAT in young rats.

Endothelium-dependent vasodilation was also examined in thoracic aortic rings belong to both age groups in the presence and absence of PVAT. After pre-contraction with phenylephrine (0.1 or 1 μM) cumulative relaxation responses to muscarinic agonist acetylcholine (1 nM-10 μM) were obtained.

At the end of the experiments dry tissue weights of both PVAT-intact or -removed aortas from young and adult rats were measured.

Statistical analysis

Data are seen as Mean ± Standard Error of Mean (SEM). In the organ bath experiments, contractions were shown as a percentage (%) of contractions to 80 mM KCL. Relaxations were shown as % of precontraction induced by phenylephrine. E_{max} is the maximum response (%) of an agonist-induced concentration-dependent response. pD_2 (-logEC_{50}) is the negative logarithm of drug concentration which constitutes half-maximum response of a concentration-response curve. Area Under the Curve (AUC, a.u) was calculated from each response-curve. Analysis of two concentration-response curves was done by 2-way ANOVA. Multiple comparisons (for pD_2, E_{max} and AUC values) were done by one-way ANOVA. All analyses were conducted using GraphPad Prism-9, and $p<0.05$ was considered to be significantly different.

Chemicals and solutions

Acetylcholine chloride, L-NAME, and L-phenylephrine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in distilled water. Indomethacin (Sigma Chemical Co., St. Louis, MO, USA) was prepared in distilled water containing 0.7% Na2CO3 (w/v). KHS had the following composition (mM): NaCL 118.0, KCL 4.7, $MgSO_4$ 1.2, $CaCL_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 11.1. Eighty

mM KCL solution was prepared by substitution of KCL with equimolar NaCL.

RESULTS AND DISCUSSION

Body weights of the rats and dry tissue weights of isolated thoracic aorta rings with and without PVAT are shown in Table 1. Body weights of the adult group and tissue weights of aortic rings having PVAT increased significantly compared to those measured in the young group (p<0.0001). Aortic tissue weights without PVAT were similar in both young and adult rats (Table 1).

Contractile responses to noradrenaline (0.1 nM-100 μM) were evaluated in PVAT-intact and PVAT-removed aortic rings from young (10-12-week-old) and adult (52-week-old) rats. The amplitude of noradrenaline contractions (E_{max}) in the PVAT-intact rings was significantly lower than in PVAT-removed rings in both young and older rats, indicating the persistent anticontractile function of PVAT in both aged rats (Figure 1) (Table 2).

Table 1. Body weights (g) of 10-12-week-old young and 52-week-old adult rats (A), and dry tissue weights (mg/cm) of isolated thoracic aorta rings with and without PVAT in both age groups (B).

A

Wistar Rats	Body Weights (g)	n
10-12-week-old	337.90±11.58	10
52-week-old	620.69±15.96****	13

****p<0.0001 Student's t test (Mean ± SEM).

B

Thoracic Aorta Rings	Tissue Weights (mg/cm)	n
10-12-week-old (PVAT-)	3.17±0.19	10
10-12-week-old (PVAT+)	15.13±1.07	10
52-week-old (PVAT-)	4.72±0.20	7
52-week-old (PVAT+)	28.6±2.92****	6

****p<0.0001 52-week-old (PVAT+) vs 12-week-old (PVAT+) Student's t test (mean ± SEM).

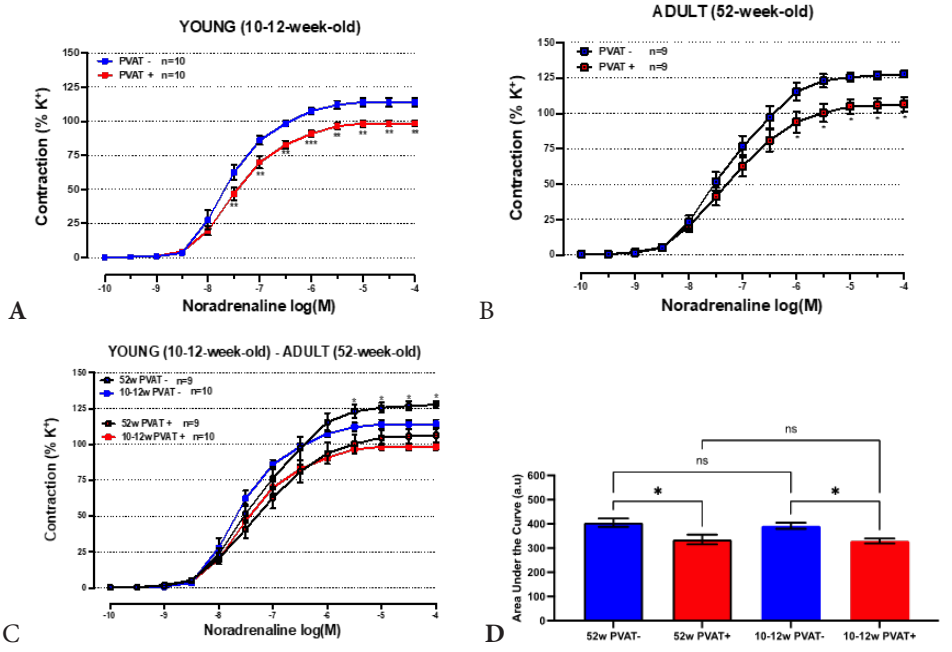

Figure 1. Cumulative contraction responses elicited by noradrenaline (0.1 nM-100 μM) in the presence and absence of perivascular adipose tissue (PVAT) in isolated thoracic aortas of 10-12-week-old young (A and C) and 52-week-old adult rats (B and C), and Area Under the Curves (AUC) of cumulative concentration-response curves of both age groups (D). Values are expressed as a percentage of contraction with 80 mM KCL containing KHS (mean ± SEM) (A and B: *p<0.05; PVAT+ group vs. PVAT- group, two-way ANOVA; C: p*<0.05; PVAT- old group vs. PVAT- young group, two-way ANOVA; D: *p<0.05; PVAT+ group vs. PVAT- group; one-way ANOVA).

To evaluate smooth muscle reactivity changing with aging, noradrenaline contractions in isolated thoracic aortic rings were compared together in young and adult groups. In the absence of PVAT, noradrenaline efficacy slightly but significantly increased in 52-week-old rats compared to 10-12-week-old rats without a significant difference in the sensitivity of noradrenaline contractions (Figure 1 and Table 2). Interestingly, if PVAT was left intact, both efficacy and sensitivity of noradrenaline contractions were similar in the young and older rats (Figure 1C) (Table 2). Contractions obtained with 80 mM KCL did not change in two age groups regardless of the presence of PVAT (Figure 2). These data indicate that increased noradrenaline contractions are suppressed by the presence of reactive PVAT in adult rats.

Table 2. Efficacy (E_{max}) and pD_2 of noradrenaline and acetylcholine in isolated aortic rings with PVAT (PVAT+) or without PVAT (PVAT-) of 10-12-week-old young and 52-week-old adult rats.

		Young Rat Aorta			**Adult Rat Aorta**		
		PVAT- (n=10)	PVAT+ (n=10)	*P* value	PVAT- (n=9)	PVAT+ (n=9)	*P* value
NA	E_{max} (%)	114.12±3.13%	98.52±2.21%**	**p<0.01	127.78±2.55%$^{\#}$	106.44±5.11%*	*,$^{\#}p$<0.05
	pD_2	7.55±0.10	7.42±0.11	ns	7.21±0.15	7.16±0.12	ns
		PVAT- (n=6)	PVAT+ (n=5)	*P* value	PVAT- (n=7)	PVAT+ (n=6)	*P* value
ACh	E_{max} (%)	92.43±1.46%	85.30±2.33%	ns	68.03±4.95%	53.26±4.50%	ns
	pD_2	7.09±0.11	6.94±0.12	ns	7.49±0.09	7.18±0.14*	* p<0.05
L-NAME- (n=5)		**Young Rat Aorta PVAT-**			**Young Rat Aorta PVAT+**		
		L-NAME+ (n=5)	*P* value	**L-NAME- (n=5)**	**L-NAME+ (n=5)**	*P* value	
NA	E_{max} (%)	123.33±4.64%	136.63±8.2%**	p<0.01	108.49±5.29%	113.45±7.56%*	p<0.05
	pD_2	7.56±0.06	8.12±0.13**	p<0.01	7.52±0.14	8.06±0.18*	p<0.05
		Adult Rat Aorta PVAT-			**Adult Rat Aorta PVAT+**		
Noradrenaline (0.1 nM-100 µM)		**L-NAME- (n=5)**	**L-NAME+ (n=5)**	*P* value	**L-NAME- (n=5)**	**L-NAME+ (n=5)**	*P* value
E_{max} (%)		129.18±2.71%	160.19±5.34%***	***p<0.001	114.85±3.34%	140.78±4.76% **	**p<0.01
pD_2		7.25±0.10	7.74±0.08**	**p<0.01	7.09±0.15	7.74±0.11**	**p<0.01

NA: noradrenaline, ACh: acetylcholine, PVAT: perivascular adipose tissue, E*max*: maximum response, pD2: negative logarithm of half-maximum response.

**p<0.01 PVAT+ vs PVAT- in young rat aorta; * p<0.05 PVAT+ vs PVAT- in adult rat aorta; $^{\#}p$<0.05 adult rat aorta vs young rat aorta (PVAT-) *(Student's t test)*.

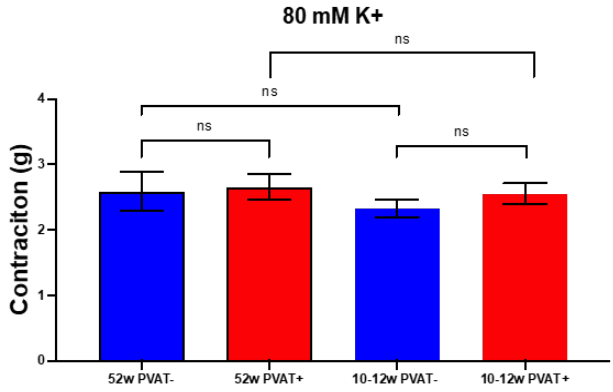

Figure 2. Contractile responses to KHS containing 80 mM KCL in the presence and absence of perivascular adipose tissue (PVAT) in isolated thoracic aortas of 10-12-week-old young and 52-week-old adult rats (mean ± SEM) (one-way ANOVA).

Noradrenaline contractions in isolated thoracic aortas of the young and older rats were repeated after incubation with L-NAME (100 μM), the NO synthase inhibitor. Contractile responses to noradrenaline in the aortas of young rats were significantly potentiated with L-NAME in both PVAT-intact and PVAT-removed groups (Figure 3). When AUC values were examined, the increase rate of noradrenaline contractions with L-NAME was significantly greater in arteries without PVAT than those with PVAT (Figure 3 and Table 2). When the endothelium was damaged in isolated aortic rings with intact PVAT of young rats, the presence of L-NAME (100 μM) did not potentiate noradrenaline contractions (Figure 3).

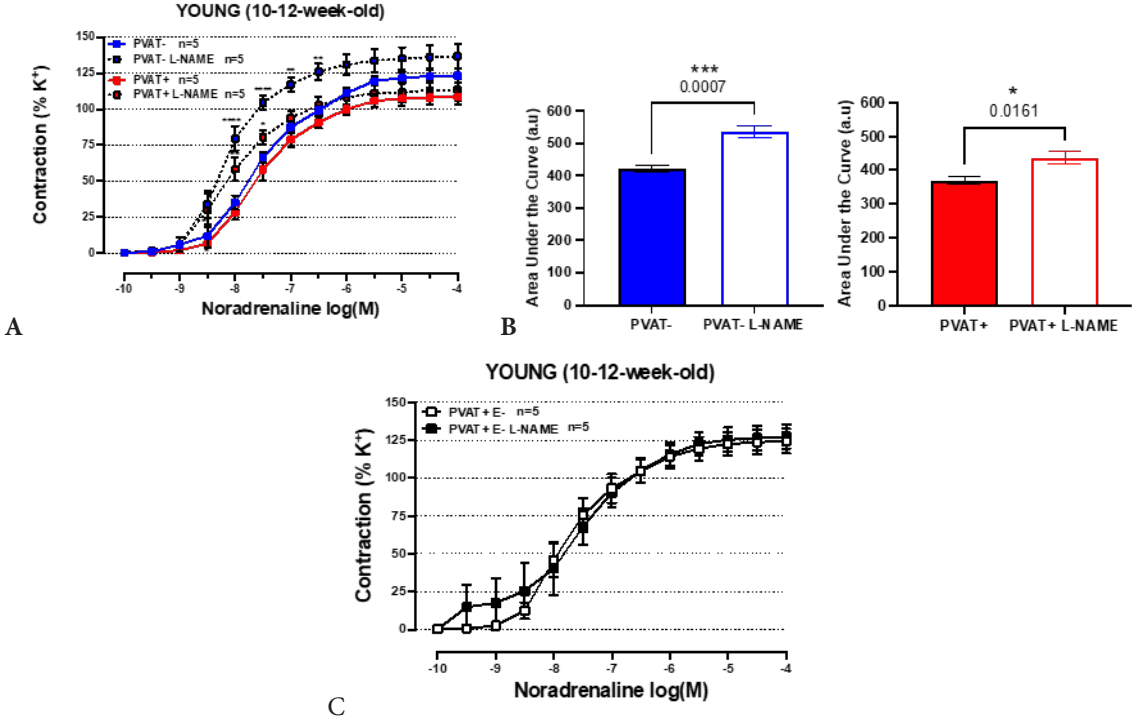

Figure 3. Cumulative contraction-response curves obtained with noradrenaline (0.1 nM-100 μM) and area under the curve (AUC) values in the presence and absence of L-NAME (100 μM) in isolated aortic rings of 10-12-week-old young rats with or without PVAT (A and B), or in endothelium-removed rings with PVAT (C). Values are expressed as a percentage of contraction of 80 mM KCL, and AUC's are expressed as arbitrary units (a.u.) (mean ± SEM) (A: *p<0.05, **p<0.01, ***p<0.001; ****p<0.0001 L-NAME+ vs. L-NAME-, two-way ANOVA) (B: *p<0.05, ***p<0.001 L-NAME+ vs. L-NAME-, Student's t test).

Noradrenaline contractions in the absence and presence of L-NAME were also evaluated in the 52-week-old adult rats. Noradrenaline contractions in both PVAT- and PVAT+ rings were also significantly but similarly potentiated with L-NAME as in young rats (Figure 4). Interestingly, when AUC values were examined, the potentiation obtained with L-NAME was the same in the tissues with and without PVAT (Figure 4 and Table 2).

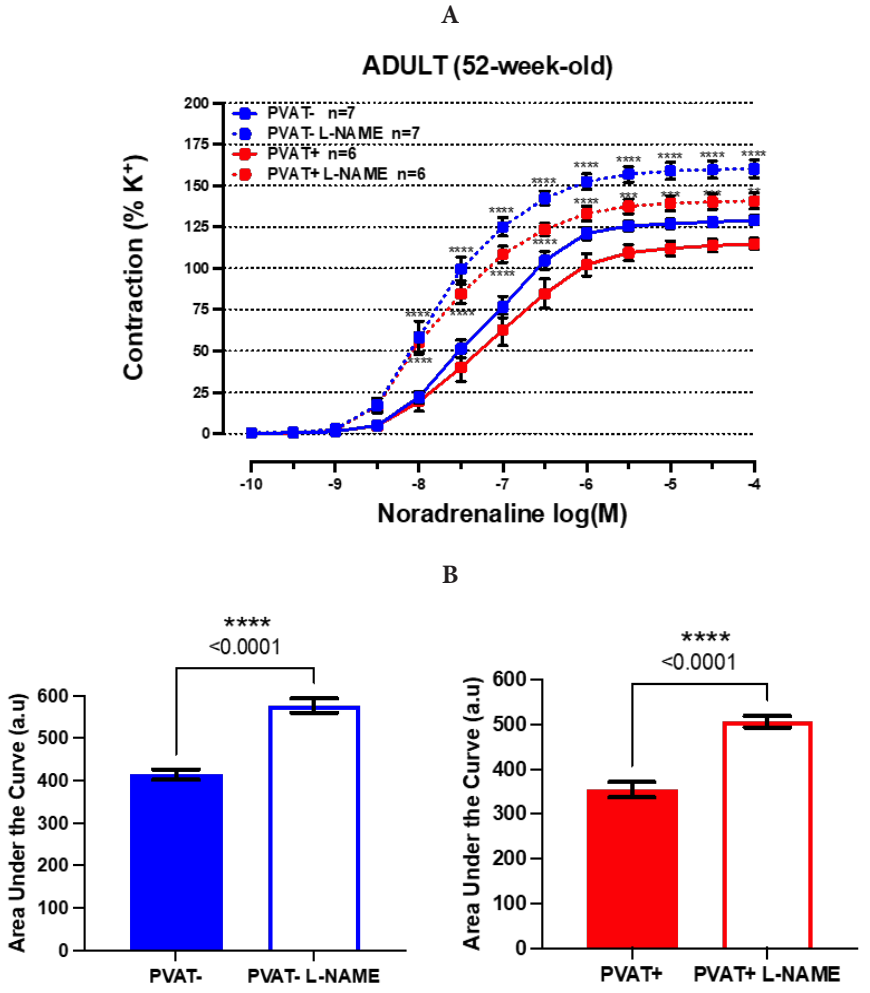

Figure 4. Cumulative contraction-response curves obtained with noradrenaline (0.1 nM-100 µM) (A) and area under the curve (AUC) values (B) in the presence and absence of L-NAME (100 µM) in isolated aortic rings of 52-week-old adult rats with or without PVAT. Values are expressed as a percentage of contraction of 80 mM KCL, and AUCs are expressed as arbitrary units (a.u.) (mean ± SEM) (A: ***p*<0.01, ****p*<0.001, *****p*<0.0001 L-NAME+ vs. L-NAME-, two-way ANOVA) (B: *****p*<0.0001 L-NAME+ vs. L-NAME-, Student's *t* test).

Endothelium-dependent vasorelaxation was also examined in young and adult rats. Concentration-dependent relaxation responses were obtained with the muscarinic agonist acetylcholine (1 nM-10 µM) after precontraction with phenylephrine (0.3 µM or 1 µM) in isolated thoracic aorta rings. Endothelium-dependent relaxations in PVAT-removed arteries of the adult group were significantly decreased compared to the young group (Table 2). While the endothelium-dependent relaxations were preserved in the presence of PVAT in the young group, these relaxations further decreased in the older rats (Figure 5).

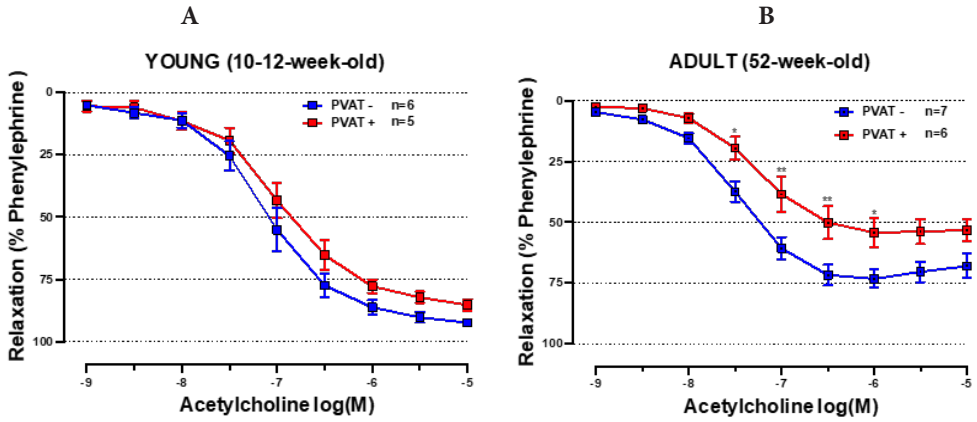

Figure 5. Cumulative relaxation-response curves obtained with acetylcholine (1 nM-10 μM) in PVAT- and PVAT+ isolated thoracic aortas of 10-12-week-old young (A) and 52-week-old adult rats (B). Values are expressed as % of phenylephrine precontraction (mean ± SEM) (*p<0.05, **p<0.01, 52 week PVAT+ vs 52 week PVAT-, two-way ANOVA).

Aging is considered an independent risk factor for cardiovascular and metabolic disorders. The regulatory function of PVAT on the vasculature may be affected by the aging process, contributing to the development of vascular dysfunction. In this study, we examined age-related changes in the anticontractile response of PVAT in thoracic aorta rings isolated from young and adult Wistar Albino rats.

The anticontractile activity of PVAT has been experimentally demonstrated in isolated thoracic aorta rings of rats (Soltis & Cassis, 1991). In this study, we show that vasoconstriction elicited by noradrenaline in isolated thoracic aortas of both 10-12-week-old young and 52-week-old adult rats is reduced in the presence of PVAT. Our data suggest that PVAT is functional in adult rats and that its tonic anticontractile effect contributes to the regulation of vascular tone in this age group.

Fifty-two-week-old adult rats were included in the study to evaluate how PVAT functionality changes with aging. The significant increase in noradrenaline contractions in isolated thoracic aortas of adult rats compared to the young group in the absence of PVAT was a suggested finding consistent with the literature. In general, as arteries age, inflammation and oxidative stress in the adventitia increase, leading to vascular wall remodeling and vasoconstriction (Queiroz & Sena, 2020). However, noradrenaline contractions in isolated aortas of adult rats with intact PVAT were significantly suppressed and they reached a similar level to those in young rat aortas with intact PVAT, suggesting that the age-related increase in noradrenaline sensitivity can be compensated by PVAT.

In humans, PVAT abundance has been associated with decreased insulin sensitivity and decreased vascular reactivity regulation (Rittig et al., 2008). We detected that dry tissue weight with PVAT and body weights increased in the adult rats in comparison to the young group. However, the phenotypic characteristics of PVAT are also an essantial factor in determining the modulation process of PVAT on the vascular structure. Thoracic aorta of rats has a brown adipose tissue phenotype whereas abdominal aorta has mostly a white adipose tissue-like phenotype (Kim, Shi, Winkler, Lee, & Weintraub, 2020; Li, Ma, & Zhu, 2021). While endothelial dysfunction can be observed with age in the isolated thoracic aorta of rats (13-month-old), it has been reported that regional changes in the PVAT phenotype (whitening) occur mainly in the abdominal aorta and more dominantly than in the thoracic aorta (Padilla, Jenkins, Vieira-Potter, & Laughlin, 2013). In other words, although endothelium-dependent vasodilation decreases in

adult rats, PVAT in the thoracic region may continue to exhibit anticontractile function with a brown adipose tissue phenotype. However, we also obtained experimental evidence that the cross-signaling interaction between PVAT and endothelium may be impaired in the advanced age group.

Endothelium and PVAT are considered vital paracrine tissues that regulate the contractile state of smooth muscle through the production of diffusible mediators. Some studies have reported that endothelium-derived NO contributes to the anticontractile response of PVAT in rat isolated thoracic aorta. In contrast, some others have suggested that it is independent from the endothelium. Indeed, agents such as adiponectin and angiotensin 1–7 released from PVAT can relax vascular smooth muscle by direct action, while they can also stimulate NO production when they diffuse to the endothelium (Chen, Montagnani, Funahashi, Shimomura, & Quon, 2003; Sampaio et al., 2007). In our study, the anticontractile response of PVAT was preserved in endothelium-stripped thoracic aortas isolated from young rats, which suggests that the anticontractile response of PVAT is independent of the endothelium. Since PVAT in the thoracic aorta is known to continuously express endothelial and neuronal NO synthase, different physiological stimuli may cause NO release from PVAT and NO-related vasorelaxation even in the absence of endothelial contribution. In the rat mesenteric arteries, it has been suggested that β3-adrenoceptors in perivascular fat cells are stimulated by noradrenaline and that NO derived from fat cells mediates the anticontractile response of PVAT in these arteries (Bussey et al., 2018). However, in our study, noradrenaline contractions obtained in endothelium-removed aortic tissues with intact PVAT were not changed after incubation with L-NAME, indicating that fat cell-derived relaxing factor is not a NO or NO-related substance in rat thoracic aorta. The differential sensitivity of anticontractile responses of PVAT to NO synthase inhibition in different vascular beds suggests that endothelial NO synthase expression in perivascular fat cells varies according to the vascular tissue or anatomical region examined (Victorio, Fontes, Rossoni, & Davel, 2016; Barp, Bonaventura, & Assreuy, 2021). In this study, we did not aim to elucidate the molecular nature of the factor released from PVAT, but evidence in the literature suggests that PVAT-derived hydrogen peroxide (H_2O_2) may be one of the candidate molecules that causes endothelium-independent relaxation in rat isolated thoracic aorta (Gao, Lu, Su, Sharma, & Lee, 2007). Noradrenaline contractions in the aortic rings (with endothelium) of young rats were increased in the presence of L-NAME. The increase in contractions could be explained by inhibition of basal NO release from the endothelium. Interestingly the inhibitory effect of L-NAME was significantly less in the tissues with PVAT than in those without PVAT. This may be due to PVAT acting synergistically with the endothelium to limit vascular contraction, and the presence of PVAT limits NO release from the endothelium. Another possibility is that the anticontractile response of PVAT is potentiated to compensate for the increased noradrenaline contractions and endothelial NO inhibition by L-NAME.

Similar to young rats, noradrenaline contractions in isolated aortic rings with the endothelium of 52-week-old adult rats were sensitive to the inhibitory effect of L-NAME. However, when the AUC levels were evaluated, unlike the young group, the potentiation detected in noradrenaline contractions in the presence of L-NAME was independent of PVAT. This finding indicates that the anticontractile response of PVAT in adult rats could not compensate for the inhibitory effect of L-NAME on endothelial NO release as in the young group. However, the decrease in endothelium-dependent relaxations obtained with acetylcholine in the adult group compared to the young group means that endothelial NO release or bioavailability also decreases with age. Therefore, it can be speculated that adipocyte-derived NO release is also triggered due to the production of various adipokines in PVAT during noradrenaline contractions in adult rats. Although

this view can speculatively explain the increased sensitivity of noradrenaline contractions to L-NAME in the presence of PVAT in the adult group, the effect of L-NAME should also be examined in endothelium-removed tissues in adult rats to support this point of view.

When the literature is reviewed, endothelium-mediated acetylcholine relaxations in isolated rat aortas are either unchanged or reduced in the presence of PVAT (Soltis & Cassis, 1991; Lee, Chen, Tsao, & Wu, 2014). In our study, the relaxations obtained with acetylcholine in the young group were unaffected by the presence of PVAT. However, in adult rats, it was found that endothelium-dependent vasodilation in arteries devoid of PVAT was partially reduced compared to the young group, and interestingly, this was further reduced when PVAT was left intact in adult rats. The findings obtained in the young and adult groups indicate that PVAT does not make an additional contribution to endothelium-dependent relaxations. In addition, the bidirectional signaling interaction between the endothelium and PVAT may have changed in old age. Adipokines including adiponectin and leptin stimulate the activation of endothelial NO synthase and then contribute to vasodilation through the enhanced NO production in endothelial cells. It has been previously shown that perivascular-derived adiponectin partly compensates for age-related decline in NO-mediated vasodilation in rat isolated thoracic aorta (Juttner et al., 2024). However, PVAT also produces PVAT-derived contracting factors, including superoxide anion, prostaglandins, angiotensin II, and resistin, which contribute to counteract the effects of perivascular-derived relaxing factors, primarily reducing NO bioavailability and thus inducing endothelial dysfunction (Cheng, Bakar, Gollasch, & Huang, 2018). Although we did not aim to examine cellular pathways of PVAT-derived molecules affecting endothelial function, any of these contractile agents could be responsible for decreased endothelium-dependent vasorelaxations in adult rats in the presence of PVAT.

The biological age of 52-week-old rats corresponds to the average age range of a human adult (30-35 years) (Sengupta, 2013). Therefore, structural and phenotypic changes associated with cardiovascular aging in 52-week-old rats (such as inflammation, oxidative stress, smooth muscle cell proliferation, loss of elasticity and endothelial dysfunction) may not be fully reflected in vascular function. However, age-related endothelial dysfunction has been previously reported in 52-week-old mice or 59-week-old Sprague-Dawley rats (Nakladal et al., 2022). Although the findings obtained in this study are valuable regarding the regulatory role of PVAT on vascular tone in adult rats, repeating similar experiments in 18-24-month-old rats to make inferences about aging and changes in PVAT function is among our future research goals.

CONCLUSION

These data suggest that the anticontractile function of PVAT persists in adult rats and limits vascular contractions through its tonic inhibitory effect. However, PVAT does not protect endothelium-dependent relaxations that decrease with aging.

AUTHOR CONTRIBUTION STATEMENT

Data collection (GO), Data presentation (GO), supervision (MHO), writing manuscript (MHO).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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