

Perivascular adipose tissue modulates aortic reactivity in Marfan syndrome mice in a sex- and region-specific manner: Redox-dependent anticontractile effects in the female ascending aorta

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ABSTRACT

Marfan syndrome (MFS), caused by fibrillin-1 (FBN1) mutations, leads to thoracic aortic aneurysm formation through mechanisms in which redox imbalance plays an important role. Perivascular adipose tissue (PVAT) modulates vascular tone, but its role in aortic reactivity in MFS is unknown. To address this, we examined thoracic aortic reactivity using wire myography in ascending and descending aortic segments from 2 to 3-month-old male and female wild-type and *Fbn1*^{C1041G/+} mice with or without PVAT. PVAT-mediated modulation of α_1 -adrenergic phenylephrine-induced contractions was preserved in the ascending aorta of male MFS mice. In contrast, in the descending thoracic aorta, PVAT from MFS mice exerted an anticontractile effect irrespective of sex. In the female ascending aorta, PVAT induced a genotype-dependent attenuation of contractile responses that persisted after endothelial denudation but was lost with aging. Despite comparable nitric oxide synthase (NOS) expression and NO levels, pharmacological NOS inhibition with L-NAME or selective inducible NOS blockade with 1400 W abolished the PVAT-induced anticontractile effect. Molecular, histological, and synchrotron

Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; BSA, bovine serum albumin; cDNA, complementary DNA; Cq, quantification cycle; Cy3, cyanine 3; DHE, dihydroethidium; E_{max} , maximal response; EC_{50} , half-maximal effective concentration; eNOS, endothelial nitric oxide synthase; FeTPPS, 5,10,15,20-tetrakis (4-sulfonatophenyl)porphyrinato iron(III) chloride; *FBN1*/*Fbn1*, fibrillin-1 gene; FTIR, Fourier transform infrared; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H_2O_2 , hydrogen peroxide; iNOS, inducible nitric oxide synthase; KCl, potassium chloride; L-NAME, N ω -nitro-L-arginine methyl ester; MFS, Marfan syndrome; mRNA, messenger RNA; NADPH, nicotinamide adenine dinucleotide phosphate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NADPH oxidase; NOX4, NADPH oxidase 4; *Nos1*, neuronal nitric oxide synthase gene; *Nos2*, inducible nitric oxide synthase gene; *Nos3*, endothelial nitric oxide synthase gene; *Nox1*, NADPH oxidase 1 gene; *Nox2*, NADPH oxidase 2 gene; *Nox4*, NADPH oxidase 4 gene; OCT, optimal cutting temperature compound; OMIM, Online Mendelian Inheritance in Man; ONOO⁻, peroxyntirite; PBS, phosphate-buffered saline; pEC_{50} , negative logarithm of the half-maximal effective concentration; Phe, phenylephrine; PVAT, perivascular adipose tissue; qPCR, quantitative polymerase chain reaction; RNA, ribonucleic acid; ROS, reactive oxygen species; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SEM, standard error of the mean; SR- μ FTIR, synchrotron radiation-based Fourier transform infrared microspectroscopy; TGF- β , transforming growth factor β ; *Tgfb1*, transforming growth factor β 1 gene; *Tgfb2*, transforming growth factor β 2 gene; U46619, 9,11-dideoxy-9 α ,11 α -methanoepoxyprostaglandin F $_{2\alpha}$; WT, wild-type.

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radiation-based Fourier-transform infrared microspectroscopy analyses showed increased *Nox4* expression and oxidative-nitrosative stress in MFS PVAT, without evidence of lipid oxidation or changes in protein secondary structure. Catalase (an H_2O_2 scavenger) or FeTPPS (a peroxynitrite decomposition catalyst) abolished the PVAT-dependent attenuation of contractility, supporting an integrated redox signaling mechanism. These findings identify PVAT as a sex- and region-specific modulator of aortic reactivity in MFS. In female mice, PVAT-dependent attenuation of α_1 -adrenergic contractility involves redox-sensitive pathways, highlighting PVAT-derived reactive oxygen/nitrogen species signaling as a potential therapeutic target in MFS-associated aortopathy.

1. Introduction

Marfan syndrome (MFS, OMIM #154700) is an autosomal dominant connective tissue disorder caused by mutations in the fibrillin-1 (*Fbn1*) gene, encoding the extracellular matrix glycoprotein fibrillin-1 [1]. The most serious cardiovascular manifestation is thoracic aortic aneurysm formation, typically originating at the aortic root and extending into the ascending aorta, with more distal segments affected with aging or following proximal aortic surgery [2]. Progressive aortic dilation markedly increases the risk of dissection and rupture. Mouse models carrying *Fbn1* missense mutations, including *Fbn1*^{C1041G/+}, recapitulate early dilation of the aortic root with extension into the ascending aorta [3]. Studies using these models show that altered aortic reactivity accompanies aneurysm development and that pharmacological interventions slowing aneurysm growth often improve vascular function [4–6].

Although MFS affects both sexes with similar prevalence, clinical outcomes differ. Men exhibit a higher overall risk of aortic complications, whereas pregnancy represents a well-recognized risk factor for acute aortic events in women [7]. Experimental studies in animal models are consistent with these observations, showing more severe aortic pathology in male mice [8], while female mice display increased vulnerability under pregnancy-like conditions [9]. At the functional level, aortic reactivity in *Fbn1*^{C1041G/+} mice is not uniform but varies according to biological sex and aortic region [8], suggesting that mechanisms regulating vascular tone contribute to disease expression beyond structural remodeling alone. These functional differences likely reflect systemic and local biological factors, including hormonal influences [9,10]. Consistent with this view, aortic dissection can occur at smaller diameters in women [7], indicating that determinants of aortic vulnerability extend beyond luminal dilation. In this context, previous studies assessing aortic reactivity in MFS were performed after removal of perivascular adipose tissue (PVAT), thereby overlooking a potentially sex-dependent extravascular modulator of vascular function.

PVAT is an extravascular compartment surrounding the aortic wall that functions as an active paracrine and endocrine tissue, releasing mediators that modulate vascular contraction in a location-, sex-, and disease-dependent manner [11–13]. Among these mediators, nitric oxide (NO), which is implicated in MFS-associated aortic pathology [14–16], is a well-established contributor to the anticontractile effects of PVAT. Under physiological conditions, PVAT-derived anticontractile signaling is considered protective. However, in the context of a dilated and mechanically compromised MFS aorta, further reductions in smooth muscle contractile responsiveness could impair the ability of the vessel wall to withstand hemodynamic load, independently of additional changes in luminal diameter.

Thoracic PVAT represents a potential source of reactive oxygen species (ROS), partly due to the high mitochondrial content of its brown adipocytes [17]. Under pathological conditions, PVAT can develop oxidative imbalance and inflammation, changes that may affect both the adipose compartment and the adjacent vascular wall [13,18]. MFS itself is characterized by premature vascular smooth muscle cell senescence and oxidative stress [16,19], with NADPH oxidase-derived ROS, particularly NOX4, playing a central role [4,5]. These observations raise the possibility that PVAT in MFS undergoes disease-relevant redox

alterations that influence adjacent vascular function. This concept is supported by recent evidence of periaortic and PVAT inflammation in patients with MFS and in *Fbn1*^{C1041G/+} mice [20,21], as well as by experimental data showing that exacerbation of perivascular inflammation accelerates aortic dilation in this model [21]. Because PVAT-derived redox mediators are established modulators, often negative regulators, of smooth muscle contraction [22–24], disease-associated alterations in PVAT redox signaling could impair contractile responsiveness in MFS. However, whether PVAT differentially modulates aortic function in MFS according to sex or aortic region has not been previously investigated.

In this study, we hypothesized that MFS-associated alterations in PVAT redox signaling impair aortic smooth muscle contractility. We demonstrate that PVAT modulates α_1 -adrenergic-mediated phenylephrine (Phe)-induced contractions in a sex- and region-specific manner in MFS mice. In the female ascending aorta, PVAT attenuates α_1 -adrenergic contractile responses through an endothelium-independent mechanism that is lost with aging and is associated with oxidative and nitrosative stress. Collectively, these findings identify PVAT as a previously overlooked regulator of aortic contractile function in MFS and suggest that redox imbalance within this tissue contributes to the vascular phenotype.

2. Materials and methods

2.1. Animals

We used 2–3-month-old male and female wild-type (WT; male: $n = 9$; female: $n = 45$) and *Fbn1*^{C1041G/+} (MFS; male: $n = 9$; female: $n = 47$) mice, as well as 12-month-old female WT ($n = 6$) and MFS ($n = 7$) mice. The difference in animal numbers between males and females reflects the sequential experimental design of the study, in which an initial sex- and region-dependent functional screening was followed by focused mechanistic experiments in the most relevant experimental condition. All animals were on a C57BL/6J background. Mice were housed according to institutional guidelines (constant room temperature of 22 °C, 12/12-h light/dark cycle, 60% humidity, and access to food and water *ad libitum*). Experiments were conducted in accordance with Spanish (RD35/2013) and European (2010/63/EU) legislation, and procedures were approved by the Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH 3041-CEEA-UAB) and the Generalitat de Catalunya (11517).

2.2. Aortic tissue preparation

Segments of the ascending and descending thoracic aorta were carefully dissected to obtain paired aortic rings with (PVAT +) or without (PVAT –) PVAT. Segments were placed in ice-cold physiological salt solution (composition in mM: NaCl 112.0; KCl 4.7; CaCl₂ 2.5; KH₂PO₄ 1.1; MgSO₄ 1.2; NaHCO₃ 25.0, and glucose 11.1; Sigma-Aldrich, St. Louis, MO, USA), gassed with 95% O₂ and 5% CO₂ (AL Air Liquide España, S.A., Madrid, Spain). Fat harvested from the ascending aorta of female mice was rapidly stored at –80 °C for subsequent nitrite and mRNA analyses. Ascending aortas from female mice were fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 1 h,

Table 1

Primer sequences used for real-time quantitative PCR studies.

Gene	Forward (5'-3')	Reverse (5'-3')
<i>Nos1</i>	ACCCAAGTCATTTCTGTCC	AAGGTGGTCTCCAGGTGT
<i>Nos2</i>	GGCCAGCCTGTGAGACCTTT	TTGGAAGTGAAGCGTTTCG
<i>Nos3</i>	CTGCCACCTGATCCTAACTTG	CAGCCAAACACCAAAGTCATG
<i>Nox1</i>	CCCAGCAGAAGGTCGTGATT	GCTAAAGCCTCGCTTCCTCAT
<i>Nox2</i>	CAGGAACCTCACTTCCATAAGAT	AACGTTGAAGAGATGTGCAATTGT
<i>Nox4</i>	CCGGACAGTCTGGCTTATCT	TGCTTTTATCCAACATCTTCTTGT
<i>Tgfb1</i>	CCGCAACAACGCCATCTATG	CCCGAATGTCTGACGTATTGAAG
<i>Tgfb2</i>	CGAGGAGTACTACGCCAAGG	GCGGACGATTCTGAAGTAGG

Nos1, neuronal nitric oxide synthase; *Nos2*, inducible nitric oxide synthase; *Nos3*, endothelial nitric oxide synthase; *Nox*, nicotinamide adenine dinucleotide phosphate oxidase; *Tgfb*, transforming growth factor β .

rinsed repeatedly with phosphate-buffered saline, embedded in Tissue Tek OCT® (Sakura Finetek Europe, Zoeterwoude, the Netherlands), frozen in liquid nitrogen, and stored at -80°C for immunofluorescence detection and synchrotron radiation-based Fourier transform infrared microspectroscopy (SR- μ FTIR). For dihydroethidium (DHE) staining, the same protocol was followed without fixation.

2.3. Aortic reactivity

Segments (1–1.5 mm in length) of the ascending and descending thoracic aorta, PVAT + and PVAT –, were mounted in an isometric wire myograph (model 410 A; Danish Myo Technology, Aarhus, Denmark) filled with physiological salt solution (37°C ; 95% O_2 and 5% CO_2), as previously described [8]. Vessels were stretched to 6 mN, a resting tension previously established for mouse thoracic aorta based on maximal KCl-induced force generation [25], washed, and allowed to equilibrate for 45 min. Tissues were then contracted twice with 100 mM KCl at 10-min intervals. After washing, vessels were equilibrated for an additional 30 min before starting the experiments. Endothelium-dependent vasodilation to acetylcholine (ACh; Sigma-Aldrich; St. Louis, MO, USA; 10^{-9} – 10^{-4} M) were assessed in 9,11-Dideoxy-9 α ,11 α -methanoperoxystroglanidin F $_{2\alpha}$ (U46619; Calbiochem-Merck KGaA, Darmstadt, Germany) to achieve 70–100% of the KCl (Sigma-Aldrich, St. Louis, MO, USA)-induced contraction. Vessels relaxing $> 50\%$ to ACh were considered endothelium intact. In some experiments, the endothelium was intentionally removed by gently rolling the preparation back and forth with a plastic cannula. Endothelium removal was confirmed when vessels relaxed $< 20\%$ to ACh. Contractile responses mediated by α_1 -adrenergic stimulation were evaluated using Phe (Sigma-Aldrich; St. Louis, MO, USA; 10^{-9} – 3×10^{-5} M).

To investigate the role of NO signaling, vessels were incubated with the non-selective NO synthase (NOS) inhibitor N ω -nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich; St. Louis, MO, USA; 3×10^{-4} M) or the selective inducible NOS (iNOS) inhibitor 1400 W (Sigma-Aldrich, St. Louis, MO, USA; 10^{-5} M) [26]. To assess oxidative and nitrosative mechanisms, preparations were additionally exposed to the hydrogen peroxide (H_2O_2 -degrading enzyme catalase (Sigma-Aldrich, St. Louis, MO, USA; 1000 U/mL) [4], the reactive nitrogen species donor peroxynitrite (Sigma-Aldrich, St. Louis, MO, USA; 10^{-5} M), or the specific peroxynitrite scavenger 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron(III) chloride (FeTPPS; #341492; Sigma-Aldrich, St. Louis, MO, USA; 10^{-5} M) [27]. Peroxynitrite was obtained as a commercially prepared solution stabilized in NaOH (4.7%; Sigma-Aldrich, St. Louis, MO, USA), and corresponding vehicle controls were run in parallel. The peroxynitrite concentration was selected based on preliminary concentration–response experiments in non-transgenic mice that produced reproducible alterations in Phe-induced aortic contractions (data not shown). All treatments were applied 30 min before ACh- or Phe-induced responses. For immunofluorescence analyses of nitrotyrosine following peroxynitrite scavenging, ascending aortic segments from female MFS mice treated with FeTPPS (10^{-5} M) during the functional experiments were immediately fixed at the end of

the experiment for subsequent immunofluorescence processing.

To assess peroxynitrite-induced changes in *Nox4* expression, ascending aortic segments with intact PVAT were incubated *ex vivo* with peroxynitrite (10^{-5} M) or corresponding vehicle (NaOH 4.7%) for 2 h at 37°C in oxygenated (95% O_2 and 5% CO_2) physiological salt solution, followed by separation of PVAT and aortic wall for subsequent RNA extraction and RT-qPCR analysis.

2.4. Quantitative real-time PCR

RNA was extracted using the Quick-RNA MiniPrep Kit (#R1055; Zymo Research; Irvine, CA, USA) following the manufacturer's protocol. RNA concentration was determined using a NanoDrop spectrophotometer. A total of 350 ng of RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (#4368813; Applied Biosystems, Thermo Fisher Scientific; Waltham, MA, USA) according to the manufacturer's instructions. RT-qPCR reactions were performed in a 10 μL reaction volume containing 0.5 μL of cDNA template, 0.5 μL of each primer at 10 μM (Table 1), 5 μL of iTaq Universal SYBR Green Supermix (#1725124; Bio-Rad; Barcelona, Spain), and 3.5 μL of RNase-free H_2O . mRNA expression levels of NOS isoforms (*Nos1* [nNOS], *Nos2* [iNOS], and *Nos3* [eNOS]), NADPH oxidase (NOX) isoforms (*Nox1*, *Nox2*, and *Nox4*), as well as *Tgfb1* (TGF- β 1) and *Tgfb2* (TGF- β 2) were assessed. Cqs of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to normalize the expression of the target sequences, and relative expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method.

2.5. NO levels

Nitrite levels, used as a surrogate marker of NO production, were determined using the Griess reaction, as previously described [28,29]. Briefly, PVAT from the ascending aorta was homogenized and centrifuged to obtain the supernatant. Then, 100 μL of the supernatant was added to a 96-well microtiter plate, followed by 100 μL of Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) and 100 μL of vanadium(III) chloride (Sigma-Aldrich, St. Louis, MO, USA). Sodium nitrite (1–10 μM ; Sigma-Aldrich, St. Louis, MO, USA) was used to generate a standard curve. Plates were incubated in the dark for 30 min, and absorbance was measured at 540 nm using a Gen5 plate reader (Biotek Instruments; Winooski, VT, USA). Nitrite concentrations were calculated from the standard curve.

2.6. In situ oxidative stress

ROS production was assessed *in situ* using the oxidative fluorescent dye DHE (Sigma-Aldrich; St. Louis, MO, USA), as previously described [29]. Briefly, fourteen- μm -thick frozen aortic sections were incubated with DHE (2 μM), coverslipped, and maintained in a humidified chamber at 37°C for 30 min, protected from light. Parallel sections were incubated with the antioxidant Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin (1.434 mg/mL; Sigma-Aldrich, St. Louis, MO, USA), a cell-permeable superoxide dismutase mimetic, to confirm signal

Table 2

Contractile responses (mN) to KCl (100 mM) in thoracic ascending and descending aortas from young male and female WT and MFS mice, with (+) and without (−) PVAT.

	Male WT PVAT−	PVAT+	MFS PVAT−	PVAT+	Female WT PVAT−	PVAT+	MFS PVAT−	PVAT+
Ascending aorta	2.06 ± 0.31 (7)	5.67 ± 1.23 (9) **	2.16 ± 0.20 (9)	2.02 ± 0.28 (7) ##	1.79 ± 0.36 (9)	2.43 ± 0.52 (10)	2.15 ± 0.69 (8)	2.47 ± 0.52 (10)
Descending aorta	2.39 ± 0.46 (7)	4.78 ± 0.86 (6)	1.97 ± 0.45 (5)	4.31 ± 0.61 (8)	2.67 ± 0.59 (7)	5.73 ± 0.91 (9)*	1.29 ± 0.28 (7)	2.91 ± 1.02 (6)

Data are shown as mean ± SEM. The number of mice is shown in parentheses. * $P < 0.05$, ** $P < 0.01$ versus PVAT−; ## $P < 0.01$ versus the same group in WT by three-way ANOVA with Tukey's post hoc test.

specificity. Images were captured using an Olympus FV1000 confocal microscope ($\times 40$ objective; Olympus Iberia; Barcelona, Spain) under identical acquisition settings for all experimental conditions. Red fluorescence in PVAT and the arterial wall was quantified in a blinded manner on at least three sections per animal, and the results were expressed in arbitrary units using ImageJ software (NIH, Bethesda, MD, USA).

2.7. SR- μ FTIR

Synchrotron radiation-based Fourier transform infrared microspectroscopy (SR- μ FTIR) was used to assess lipid and protein structural signatures in PVAT and aortic wall sections. Thoracic aortas with intact PVAT were cryosectioned at 5 μ m thickness and mounted onto polished calcium fluoride optical windows (PROQUILAB, S.A., Cartagena, Murcia, Spain). Sections were stored at -20°C until analysis. Measurements were performed at the MIRAS beamline of the ALBA synchrotron (Cerdanyola del Vallès, Spain) using a Hyperion 3000 infrared microscope coupled to a Vertex 70 spectrometer (Bruker, Billerica, MA, USA) [30]. Spectra were collected in transmission mode using a $\times 36$ magnification objective and matching condenser, with a $10 \times 10 \mu\text{m}^2$ aperture, 4 cm^{-1} spectral resolution, and 64 co-added scans per spectrum. The spectral range acquired was $4000\text{--}600 \text{ cm}^{-1}$. Background spectra were recorded every 10 sample positions from a clean area of each calcium fluoride window. For each sample, 50 spectra were acquired for PVAT and adjacent aortic wall, respectively. Spectra exhibiting strong Mie scattering or low signal-to-noise ratio were excluded from analysis. To enhance spectral resolution and minimize baseline contributions, second-derivative spectra were calculated using a Savitzky-Golay algorithm (13-point smoothing, second-order polynomial) followed by unit vector normalization. Residual OCT contamination was screened in the carbonyl region. Spectra showing an OCT-associated band at $\sim 1710 \text{ cm}^{-1}$ above a predefined threshold (based on spectra acquired from OCT-only regions/blank areas) were excluded from subsequent analysis. Lipid carbonylation was assessed as the ratio of the carbonyl stretching band to total aliphatic content ($\text{C}=\text{O}/\text{CH}_2$; $1745 \text{ cm}^{-1}/2854 \text{ cm}^{-1}$). Lipid unsaturation was evaluated using the olefinic band normalized to methylene content ($\text{C}=\text{C}-\text{H}/\text{CH}_2$; $3008 \text{ cm}^{-1}/2854 \text{ cm}^{-1}$). Lipid aliphatic chain organization was estimated by the CH_2/CH_3 ratio ($2854 \text{ cm}^{-1}/2958 \text{ cm}^{-1}$). Protein secondary structure was evaluated by the β -sheet/ α -helix ratio ($1635 \text{ cm}^{-1}/1658 \text{ cm}^{-1}$). Ratios were calculated from peak intensities obtained from the processed spectra. Data analysis was done using the open-source data package Quasar (Version 1.11.1) [31].

2.8. Human tissue collection

Control ascending aortic tissue was obtained from anonymous multiorgan donors ($n = 2$) through the *Banc de Sang i Teixits* (Barcelona, Spain). The ascending aortic aneurysm sample was collected from a patient with MFS who fulfilled the Ghent diagnostic criteria [32] and underwent open surgical repair of aortic aneurysm. No genetic information regarding putative *FBN1* mutation was available. All aortic

tissues were maintained in cold saline or cardioprotective solution prior to delivery to the laboratory for further processing. Use of discarded human tissue was approved by the Ethics and Clinical Research Committee of the *Hospital de la Santa Creu i Sant Pau* (Barcelona, Spain; Ref. IIBSP-MAR-2019-08). All samples are part of collection C.0005273, registered in the *Instituto de Salud Carlos III* (Madrid, Spain). Research was conducted in accordance with the Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. Participation of patients was based on informed consent provided by the patients or their legal representatives. All aortic tissues described in this study were obtained from Spanish donors. Due to the Spanish Data Protection Act (Organic Law 3/2018, of 5 December, on the Protection of Personal Data and Guarantee of Digital Rights), we do not have access to their clinical history.

2.9. Human aorta histology

Histological analysis was performed on paraffin-embedded human aortic sections using picrosirius red (Sigma-Aldrich, St. Louis, MO, USA) staining. Sections (5 μ m thick) were first deparaffinized by washing twice in xylene (Merck KGaA, Darmstadt, Germany) for 5 min each and then rehydrated through a graded ethanol (Merck KGaA, Darmstadt, Germany) series (100%, 95%, 70%) before rinsing in distilled water for 5 min. Sections were subsequently immersed in picrosirius red solution for 1 h, followed by three 3-min washes in acidic water. After staining, samples were dehydrated in 100% ethanol ($3 \times 2 \text{ min}$) and xylene ($2 \times 3 \text{ min}$) and finally mounted in a suitable mounting medium. Images were captured using an Olympus BX51TF microscope with a $\times 40$ objective (Olympus Iberia, Barcelona, Spain).

2.10. Immunofluorescence

For mouse aortas, fourteen- μ m-thick aortic cross sections were obtained using a cryostat and blocked with 5% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA). For human aortas, deparaffinized five- μ m-thick aortic cross sections were rehydrated, boiled for 20 min in 10 mM citrate buffer (pH 6.0; Sigma-Aldrich, St. Louis, MO, USA) for antigen retrieval, and blocked for 2 h with 5% BSA in PBS. Mouse aortic sections were incubated for 1 h at 37°C with rabbit polyclonal anti-iNOS antibody (1:100; #PA1-036; Thermo Fisher Scientific, Waltham, MA, USA), whereas both mouse and human aortic sections were incubated for 1 h at 37°C with rabbit polyclonal anti-3-nitrotyrosine antibody (1:100; #06-284; Merck KGaA, Darmstadt, Germany). After washing, slides were incubated for 45 min with a donkey anti-rabbit Cy3TM secondary antibody (1:200; #711-165-152; Jackson ImmunoResearch Laboratories Inc.; West Grove, PA, USA). The specificity of immunostaining was confirmed by omission of the primary antibody, which abolished the fluorescence signal. Nuclei were counterstained with Hoechst 33,342 (10 $\mu\text{g}/\text{mL}$; Sigma-Aldrich; St. Louis, MO, USA). Sections were mounted using fluorescence mounting medium (#S3023; Dako, Agilent; Santa Clara, CA, USA). Images were acquired using an Olympus FV1000 confocal system ($\times 40$ objective; Olympus Iberia; Barcelona, Spain) under identical acquisition settings

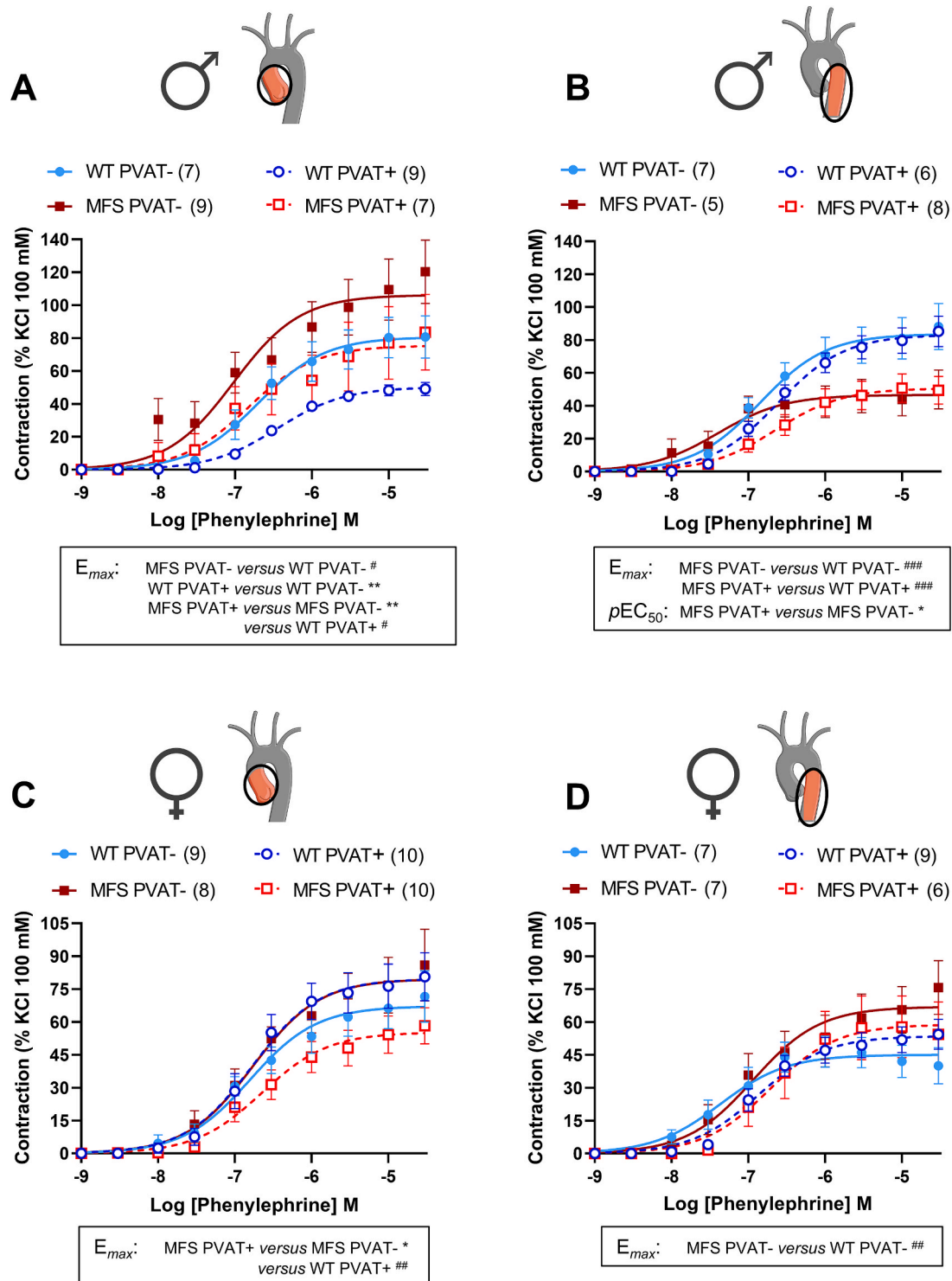


Fig. 1. Influence of aortic region, sex, and PVAT on phenylephrine-induced thoracic aorta contractions. Concentration-response curves to phenylephrine in thoracic ascending (A and C) and descending (B and D) aortas with (+) or without (–) PVAT from young male and female WT and MFS mice. Results are the mean $\pm$ SEM of the number of animals shown in parentheses. * $P < 0.05$, ** $P < 0.01$ versus PVAT–; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the same group in WT by three-way ANOVA with Tukey’s post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC_{50} values obtained from nonlinear regression analysis of the concentration–response curves; the corresponding E_{max} and pEC_{50} values are shown in Table 3.

for all experimental conditions. Mean red fluorescence intensity in PVAT and the arterial wall was quantified in a blinded manner using ImageJ software (NIH, Bethesda, MD, USA), analyzing at least three sections per animal and one section per patient.

2.11. Statistics

All results are expressed as the mean $\pm$ SEM, with the number of mice (n) indicated in the figures and tables. Relaxations to ACh are expressed as the percentage change from the U46619-precontracted level, and contractions to Phe are expressed as the percentage of the

Table 3

PEC₅₀ and E_{max} values for phenylephrine contraction (% of KCl 100 mM-induced contraction) in thoracic ascending and descending aortas with (+) or without (–) PVAT from young male and female WT and MFS mice.

		Male				Female			
		WT PVAT–	PVAT+	MFS PVAT–	PVAT+	WT PVAT–	PVAT+	MFS PVAT–	PVAT+
Ascending aorta	pEC ₅₀	6.70 ± 0.14 (7)	6.45 ± 0.06 (9)	7.02 ± 0.17 (9)	6.83 ± 0.26 (7)	6.80 ± 0.13 (9)	6.77 ± 0.12 (10)	6.76 ± 0.14 (8)	6.65 ± 0.14 (10)
	E _{max}	80.63 ± 5.08 (7)	50.21 ± 1.34 (9)**	106.20 ± 7.45 (9) [#]	75.41 ± 8.29 (7) **/ [#]	67.22 ± 3.80 (9)	79.66 ± 4.14 (10)	79.62 ± 4.78 (8)	55.31 ± 3.53 (10) */ ^{###}
Descending aorta	pEC ₅₀	6.85 ± 0.13 (7)	6.62 ± 0.08 (6)	7.40 ± 0.22 (5)	6.64 ± 0.16 (8)*	7.37 ± 0.18 (7)	6.87 ± 0.11 (9)	6.72 ± 0.22 (7)	6.94 ± 0.17 (6)
	E _{max}	83.65 ± 4.60 (7)	83.21 ± 3.10 (6)	46.57 ± 3.83 (5) ^{###}	50.63 ± 3.52 (8) ^{###}	45.06 ± 2.99 (7)	53.61 ± 2.50 (9)	66.98 ± 4.60 (7) ^{##}	58.89 ± 5.65 (6)

Data are shown as mean ± SEM. The number of mice is shown in parentheses. **P* < 0.05, ***P* < 0.01 versus PVAT–, [#]*P* < 0.05, ^{##}*P* < 0.01, ^{###}*P* < 0.001 versus the same group in WT by three-way ANOVA with Tukey's post hoc test.

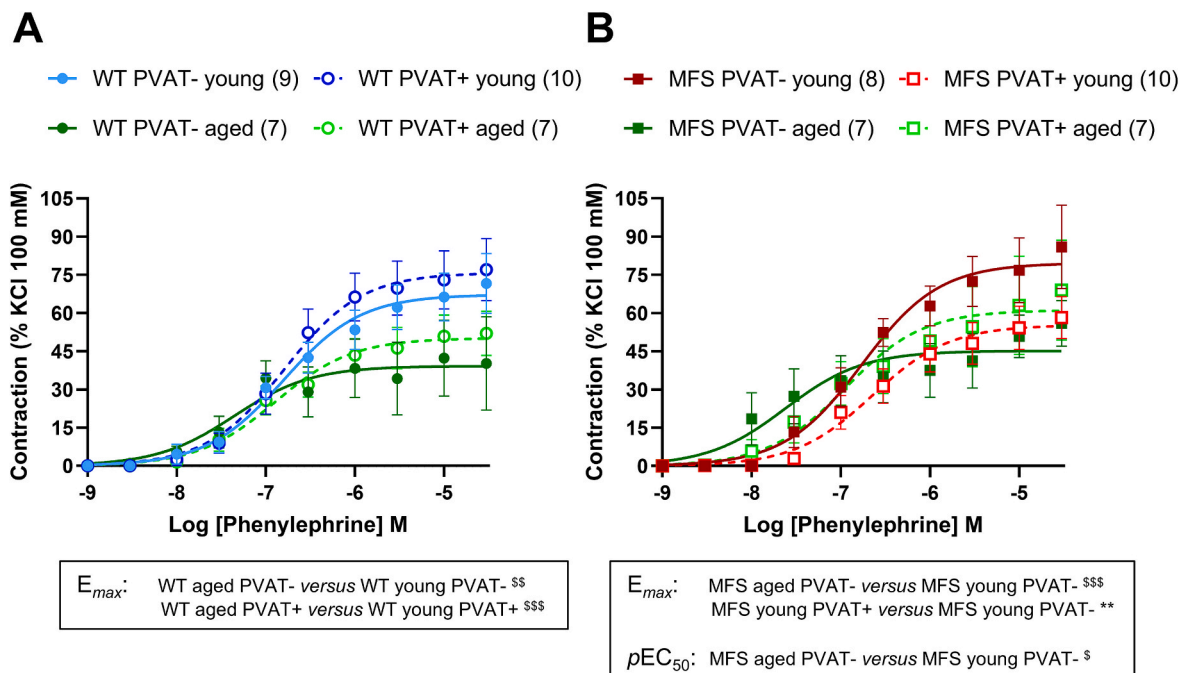


Fig. 2. Influence of aging and PVAT on phenylephrine-induced female ascending aorta contractions. Concentration-response curves to phenylephrine in ascending aortas with (+) or without (–) PVAT from female WT (A) and MFS (B) young (2–3 months of age) and aged (12 months of age) mice. Results are the mean ± SEM of the number of animals shown in parentheses. ***P* < 0.01 versus PVAT–; ^{\$}*P* < 0.05, ^{\$\$}*P* < 0.01, ^{\$\$\$}*P* < 0.001 versus the same group in young by three-way ANOVA with Tukey's post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC₅₀ values obtained from nonlinear regression analysis of the concentration-response curves; the corresponding E_{max} and pEC₅₀ values are shown in Table 4.

Table 4

PEC₅₀ and E_{max} values for phenylephrine contraction (% of KCl 100 mM-induced contraction) in thoracic ascending aorta with (+) or without (–) PVAT from female WT and MFS mice young (2–3 months of age) and aged (12 months of age) mice.

	WT young		WT aged		MFS young		MFS aged	
	PVAT–	PVAT+	PVAT–	PVAT+	PVAT–	PVAT+	PVAT–	PVAT+
pEC ₅₀	6.80 ± 0.13 (9)	6.77 ± 0.12 (10)	7.35 ± 0.33 (6)	6.89 ± 0.15 (6)	6.76 ± 0.14 (8)	6.65 ± 0.14 (10)	7.62 ± 0.25 (7) ^{\$}	6.95 ± 0.25 (7)
E _{max}	67.22 ± 3.80 (9)	79.66 ± 4.14 (10)	39.18 ± 4.87 (6) ^{\$}	50.16 ± 3.07 (6) ^{\$\$}	79.62 ± 4.78 (8)	55.31 ± 3.53 (10) **	45.18 ± 4.05 (7) ^{\$\$}	61.13 ± 6.40 (7)

Data are shown as mean ± SEM. The number of mice is shown in parentheses. ***P* < 0.01 versus PVAT–; ^{\$}*P* < 0.05, ^{\$\$}*P* < 0.01, ^{\$\$\$}*P* < 0.001 versus the same group in young by three-way ANOVA with Tukey's post hoc test.

tone generated by 100 mM KCl. The maximum response (E_{max}) and potency/sensitivity (pEC₅₀) were determined by fitting concentration-response curves using non-linear regression. Data distribution was systematically assessed before selecting the appropriate parametric or non-parametric statistical tests. Differences in mRNA expression and NO levels (single-factor analysis) were evaluated using unpaired

Student's *t*-test for comparisons between two means. Differences in KCl-induced contractions, E_{max}, and pEC₅₀ were analyzed by two- (PVAT × endothelium removal or PVAT × treatment or genotype × treatment) or three-way (sex × genotype × PVAT or genotype × PVAT × age) repeated-measures ANOVA (depending on the number of variables), followed by Tukey's post hoc test. Statistical analyses were performed

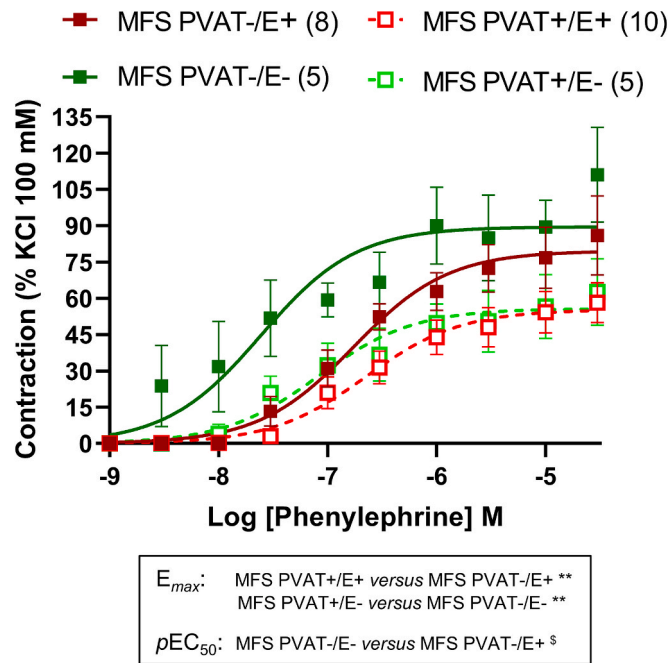


Fig. 3. Influence of endothelium removal and PVAT on phenylephrine-induced female ascending aorta contractions. Concentration-response curves to phenylephrine in ascending aortas with (+) or without (–) endothelium (E) and with (+) or without (–) PVAT from young female MFS mice. Results are the mean $\pm$ SEM of the number of animals shown in parentheses. ** $P < 0.01$ versus PVAT–; § $P < 0.05$ versus the same group in E + by two-way ANOVA with Tukey’s post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC_{50} values obtained from nonlinear regression analysis of the concentration–response curves; the corresponding E_{max} and pEC_{50} values are shown in Table 5.

Table 5

pEC_{50} and E_{max} values for phenylephrine contraction (% of KCl 100 mM-induced contraction) in ascending aortas with (+) or without (–) endothelium (E) and with (+) or without (–) PVAT from young female MFS mice.

	MFS E+ PVAT–	PVAT+	MFS E– PVAT–	PVAT+
pEC_{50}	6.76 $\pm$ 0.14 (8)	6.65 $\pm$ 0.14 (10)	7.61 $\pm$ 0.21 (5) [§]	7.08 $\pm$ 0.21 (5)
E_{max}	79.62 $\pm$ 4.78 (8)	55.31 $\pm$ 3.53 (10)**	89.56 $\pm$ 6.70 (5)	55.76 $\pm$ 4.64 (5)**

Data are shown as mean $\pm$ SEM. The number of mice is shown in parentheses. ** $P < 0.01$ versus PVAT–; § $P < 0.05$ versus the same group in E + by two-way ANOVA with Tukey’s post hoc test.

using GraphPad Prism version 9 (GraphPad Software; La Jolla, CA, USA). A P value < 0.05 was considered statistically significant.

3. Results

3.1. PVAT differentially modulates ascending and descending thoracic aorta contraction in male and female WT and MFS mice

Agonist-independent contractions induced by KCl (100 mM) were similar in PVAT-free ascending and descending aortas, regardless of sex, genotype, or aortic region (Table 2). In contrast, in the presence of PVAT, the ascending aorta of WT male mice and the descending aorta of WT female mice showed increased contractions compared to their respective PVAT-free aortas. This effect was not observed in MFS aortas (Table 2).

In PVAT-denuded aortas, the maximal Phe-induced contraction

normalized to KCl was higher in the ascending aorta of male MFS mice compared with WT mice (Fig. 1A; Table 3), whereas it was lower in the descending aorta of male MFS mice compared with WT mice (Fig. 1B; Table 3). In female MFS mice, Phe-induced maximal contractions were similar to WT in the ascending aorta (Fig. 1C; Table 3) but increased in the descending aorta (Fig. 1D; Table 3).

The presence of PVAT elicited an anticontractile effect on Phe-induced responses (lower normalized E_{max}) in the ascending aorta of male WT mice, which was maintained in MFS mice (Fig. 1A; Table 3). Consequently, MFS-related differences in Phe-induced contractions remained evident in this aortic region. As PVAT significantly increased KCl-induced force in WT but not in MFS male ascending aortas (Table 2), we analyzed absolute (non-normalized) contractions and found that maximal Phe-induced force was significantly reduced in MFS compared with WT arteries in the presence of PVAT (absolute E_{max} , WT: 2.91 ± 0.22 mN; MFS: 1.72 ± 0.24 mN; $P < 0.01$, three-way ANOVA with Tukey’s post hoc test), indicating reduced force-generating capacity in MFS under PVAT-intact conditions. In the male MFS descending aorta, PVAT did not significantly alter maximal Phe-induced contraction, but reduced Phe sensitivity, as shown by the lower pEC_{50} value (Fig. 1B; Table 3).

Notably, PVAT markedly reduced maximal contractions to Phe in the ascending aorta of female MFS mice (Fig. 1C; Table 3). In contrast, in the descending aorta, PVAT did not significantly modify contractions in either group but genotype-related differences in maximal response were abolished (Fig. 1D; Table 3). As PVAT significantly increased KCl-induced force in WT but not in MFS female descending aortas (Table 2), we additionally examined absolute (non-normalized) Phe-induced contractions, which confirmed significantly lower maximal force in MFS compared with WT arteries in the presence of PVAT (absolute E_{max} , WT: 3.31 ± 0.27 mN; MFS: 1.56 ± 0.24 mN; $P < 0.001$, three-way ANOVA with Tukey’s post hoc test), indicating reduced force-generating capacity in MFS under PVAT-intact conditions. The presence of PVAT did not significantly alter ACh-induced relaxations in any group (data not shown). Taken together, these findings demonstrate that the influence of PVAT on α_1 -adrenergic contractility differs by sex and aortic region in MFS.

Given the clinical relevance of the ascending aorta in MFS pathology and the pronounced PVAT-dependent modulation of Phe-induced responses in female MFS mice, subsequent experiments focused on elucidating aging-related and molecular mechanisms in this aortic region.

3.2. Influence of aging on PVAT-induced anticontractile effect in female MFS ascending aortas

To assess whether the PVAT-mediated reduction in Phe-induced responses is maintained with age, we evaluated ascending aorta reactivity in 12-month-old female WT and MFS mice (Fig. 2; Table 4). Endothelium-dependent relaxations to ACh were similar between young and aged female aortas, regardless of genotype or PVAT presence (data not shown). Remarkably, aging reduced overall Phe-induced contractility in both genotypes, which reduced the dynamic range for detecting PVAT-mediated modulation (Fig. 2; Table 4). Thus, whereas PVAT reduced maximal Phe-induced contraction in young female MFS ascending aorta, this PVAT-dependent anticontractile effect was no longer evident in aged female MFS mice.

3.3. The anticontractile effect of PVAT is independent of the endothelium

We next used endothelial removal to determine whether the PVAT-dependent reduction in Phe-induced contraction in young female MFS ascending aorta required an intact endothelium. Concentration-response curves to Phe were performed in PVAT + and PVAT – rings with intact (E +) or denuded (E –) endothelium. In the absence of PVAT, endothelial removal significantly increased the sensitivity of Phe-induced contractions (Fig. 3; Table 5). In contrast, in PVAT-intact rings,

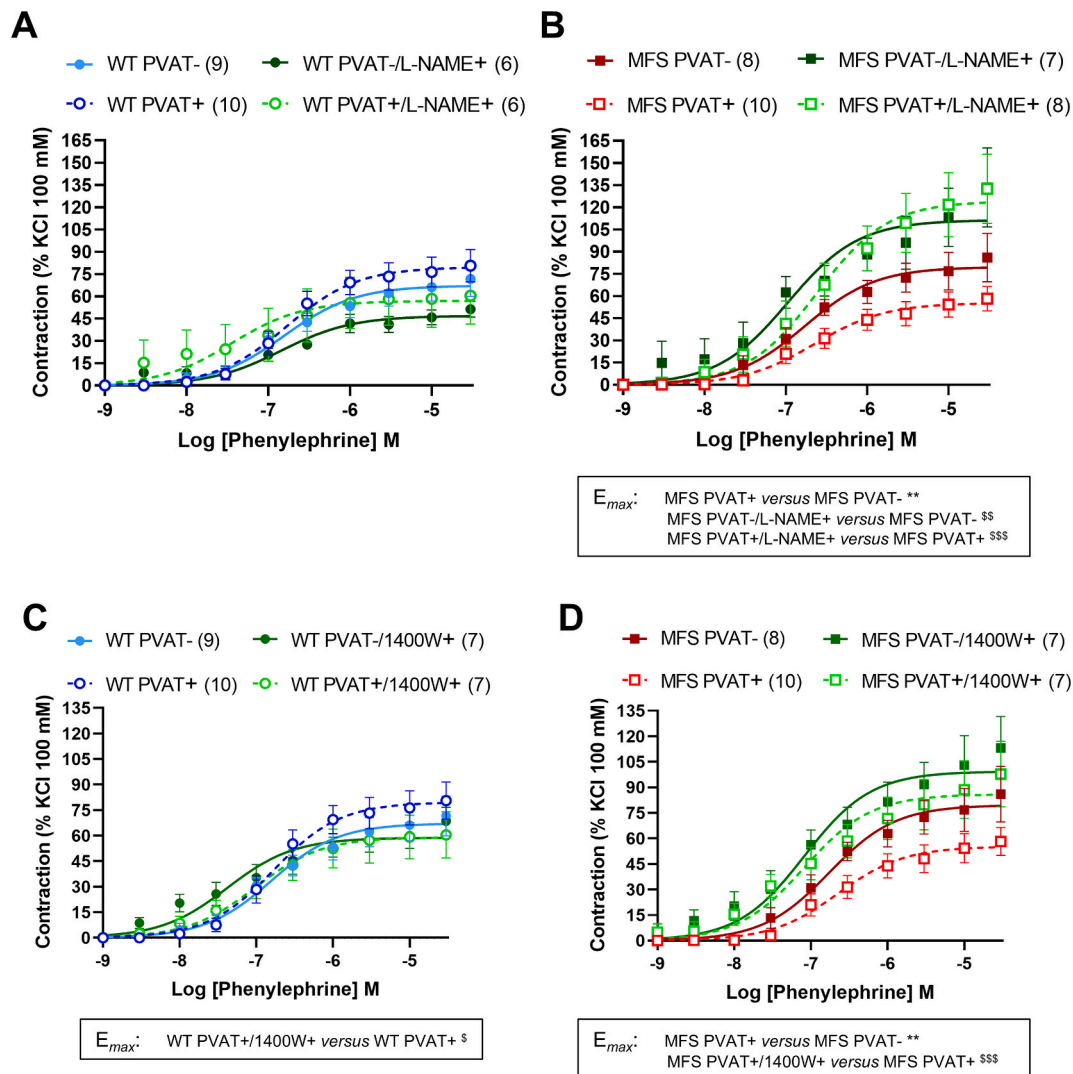


Fig. 4. Influence of NO inhibition on concentration–response curves to phenylephrine in ascending aortas with (+) or without (–) PVAT from young female WT and MFS mice. (A–B) Phenylephrine-induced contractions in the absence and presence of the non-selective NOS inhibitor L-NAME (3×10^{-4} M) and (C–D) the selective iNOS inhibitor 1400 W (10^{-5} M). Results are the mean $\pm$ SEM of the number of animals shown in parentheses. ** $P < 0.01$ versus PVAT–; \$ $P < 0.05$, \$\$ $P < 0.01$, \$\$\$ $P < 0.001$ versus the same group without L-NAME or 1400 W by three-way ANOVA with Tukey's post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC_{50} values obtained from nonlinear regression analysis of the concentration–response curves; the corresponding E_{max} and pEC_{50} values are shown in Table 6.

endothelial removal did not modify Phe-induced contractions. Notably, maximal Phe-induced contractions remained significantly lower in endothelium-denuded arteries with versus without PVAT (Fig. 3; Table 5). These findings indicate that the anticontractile effect induced by PVAT in the ascending aorta of female MFS mice occurs independent of the endothelium.

3.4. NOS activity contributes to the PVAT-induced anticontractile effect

We next investigated the potential involvement of NO in the modulatory effect of PVAT on Phe-induced contractions. To facilitate interpretation of these pharmacological experiments, the effect of NOS inhibition was assessed separately in WT and MFS arteries and compared within each PVAT condition. The non-selective NOS inhibitor L-NAME (3×10^{-4} M) did not alter Phe-induced contractions in ascending aortas from WT mice (Fig. 4A; Table 6). In contrast, in MFS mice, L-NAME significantly enhanced contractions regardless of the presence of PVAT, thereby abolishing the PVAT-induced anticontractile effect (Fig. 4B; Table 6). Incubation with the selective iNOS inhibitor 1400 W (10^{-5} M) reduced maximal Phe-induced contractions in WT

arteries with PVAT (Fig. 4C; Table 6), whereas it increased contractions in PVAT-intact MFS aortas (Fig. 4D; Table 6), abolishing the PVAT-induced anticontractile effect. These findings indicate that NOS activity, and specifically iNOS-sensitive signaling, contributes to the PVAT-dependent attenuation of Phe-induced contractions in female MFS ascending aorta.

3.5. NOS expression and NO levels are not altered

We therefore examined NOS expression and NO availability. Analysis of PVAT mRNA levels for nNOS (*Nos1*), iNOS (*Nos2*), and eNOS (*Nos3*) revealed no differences between genotypes (Fig. 5A). In addition, iNOS protein expression, assessed by immunofluorescence (Fig. 5B), as well as nitrite levels (Fig. 5C), were comparable in the PVAT of WT and MFS female mice. Thus, iNOS activity is required for the PVAT-induced attenuation of contractility, despite unchanged NOS expression or global NO availability.

Table 6

PEC₅₀ and E_{max} values for phenylephrine contraction (% of KCl 100 mM-induced contraction) in ascending aortas with (+) or without (−) PVAT from young female WT and MFS mice in the absence and presence of the non-selective NOS inhibitor L-NAME (3×10^{-4} M) or the selective iNOS inhibitor 1400 W (10^{-5} M).

	WT		WT + L-NAME		WT + 1400 W	
	PVAT−	PVAT+	PVAT−	PVAT+	PVAT−	PVAT+
pEC ₅₀	6.80 ± 0.13 (9)	6.77 ± 0.12 (10)	6.84 ± 0.14 (6)	7.45 ± 0.36 (6)	7.38 ± 0.14 (7)	7.02 ± 0.20 (7)
E _{max}	67.22 ± 3.80 (9)	79.66 ± 4.14 (10)	46.87 ± 2.69 (6)	56.99 ± 7.60 (6)	58.77 ± 3.15 (7)	59.11 ± 4.72 (7) [§]
	MFS		MFS + L-NAME		MFS + 1400 W	
	PVAT−	PVAT+	PVAT−	PVAT+	PVAT−	PVAT+
pEC ₅₀	6.76 ± 0.14 (8)	6.65 ± 0.14 (10)	6.97 ± 0.18 (7)	6.62 ± 0.21 (8)	7.09 ± 0.14 (7)	7.08 ± 0.18 (7)
E _{max}	79.62 ± 4.78 (8)	55.31 ± 3.53 (10) ^{**}	111.34 ± 8.78 (7) ^{§§}	124.19 ± 9.01 (8) ^{§§§}	99.35 ± 6.12 (7)	85.93 ± 6.05 (7) ^{§§}

Data are shown as mean ± SEM. The number of mice is shown in parentheses. ^{**}*P* < 0.01 versus PVAT−; [§]*P* < 0.05, ^{§§}*P* < 0.01, ^{§§§}*P* < 0.001 versus the same group without L-NAME or 1400 W by three-way ANOVA with Tukey's post hoc test.

3.6. Nox4 upregulation and oxidative stress in PVAT of MFS mice

mRNA levels of NADPH oxidase (NOX) isoforms in PVAT revealed no differences in *Nox1* and *Nox2* expression between genotypes, whereas *Nox4* expression was significantly higher in PVAT of MFS females (Fig. 6A). *Tgfb1* and *Tgfb2* mRNA expression levels in PVAT were unchanged (data not shown). We further assessed *in situ* ROS production in both PVAT and the aortic wall using DHE-derived fluorescence. Both PVAT and the aortic wall showed increased ROS-associated fluorescence in MFS mice compared with WT (Fig. 6B). Given that NOX4 predominantly generates H₂O₂, catalase was used to test whether H₂O₂-sensitive signaling contributed functionally to the PVAT-dependent anticontractile effect in female MFS ascending aorta. Incubation with catalase (1000 U/mL) abolished the PVAT-mediated attenuation of Phe-induced aortic contractions (Fig. 6C; Table 7). Together, these findings suggest that NOX4 upregulation in PVAT is associated with oxidative stress and redox-dependent modulation of aortic contractility via PVAT in female MFS mice.

3.7. SR-μFTIR does not reveal accumulation of structural lipid oxidation signatures or changes in protein secondary structure in PVAT and aortic wall of MFS mice

To determine whether increased oxidative stress results in persistent molecular oxidation, we analyzed lipid and protein signatures by SR-μFTIR (Fig. 7A). As expected, based on tissue composition, the absolute methylene (CH₂) band intensity at 2854 cm^{−1}, reflecting the abundance of aliphatic lipid chains, was greater in PVAT than in the aortic wall (data not shown). A genotype-dependent decrease in lipid carbonylation was observed in MFS tissues, assessed as the C=O/CH₂ ratio (1745 cm^{−1}/2854 cm^{−1}; Fig. 7B). In contrast, lipid unsaturation (C=C-H/CH₂; 3008 cm^{−1}/2854 cm^{−1}; Fig. 7C) and the lipid aliphatic integrity index (CH₂/CH₃; 2854 cm^{−1}/2958 cm^{−1}; Fig. 7D) were not altered by genotype. However, both indices were lower in the aortic wall compared with PVAT, irrespective of genotype, likely reflecting tissue-specific differences in lipid organization rather than genotype-driven oxidative modification. The β-sheet/α-helix ratio (1635 cm^{−1}/1658 cm^{−1}; Fig. 7E) showed no genotype-dependent differences but was lower in the aortic wall compared with PVAT, consistent with differences in protein structural composition between these tissues. These findings indicate that oxidative stress in MFS is not associated with accumulation of FTIR-detectable structural lipid oxidation signatures; rather, the carbonyl/aliphatic ratio was modestly reduced in MFS tissues, while other lipid structural indices and protein secondary structure remained unchanged.

3.8. Peroxynitrite-sensitive signaling mediates PVAT-dependent anticontractile effects in MFS

Because NO and superoxide can react to form peroxynitrite [33], a recognized regulator of contractility [34,35], we next assessed peroxynitrite-sensitive signaling using complementary approaches: analysis of protein nitration as an indirect readout of peroxynitrite-related nitrosative stress, pharmacological decomposition of endogenous peroxynitrite with FeTPPS in MFS arteries, exogenous peroxynitrite exposure in PVAT-denuded arteries, and analysis of *Nox4* expression after peroxynitrite incubation. Protein nitration was assessed by immunofluorescence in ascending aortic sections from female mice. These analyses revealed increased protein nitration, indicative of nitrosative stress, in both PVAT and the aortic wall of MFS mice (Fig. 8A). In human samples, picrosirius red staining identified PVAT and revealed collagen accumulation in the ascending aortic wall and PVAT in MFS tissue relative to control donor tissue (Fig. 8B). Consistent with the murine findings, enhanced protein nitration was evident in both PVAT and the ascending aortic wall in human MFS tissue compared with control donor sections (Fig. 8B). Importantly, treatment with FeTPPS (10^{-5} M) abolished the PVAT-induced anticontractile effect in MFS mice (Fig. 9A, Table 8). In contrast, protein nitration was not significantly reduced by FeTPPS, although a trend was observed (*P* = 0.08; treatment factor of two-way ANOVA) (Fig. 9B). In addition, acute peroxynitrite (10^{-5} M) exposure of arteries devoid of PVAT reduced α₁-adrenergic contractility in WT (Fig. 9C; Table 8) but not in MFS (Fig. 9D; Table 8) aortas. To determine whether peroxynitrite modulates PVAT and arterial wall NOX4 expression, tissue was incubated *ex vivo* with peroxynitrite (10^{-5} M). This treatment markedly increased *Nox4* expression in both PVAT and the arterial wall (Fig. 9E). Collectively, these findings support a role for peroxynitrite-sensitive redox signaling in the PVAT-dependent anticontractile phenotype in the ascending aorta of female MFS mice.

4. Discussion

Thoracic aortic aneurysm in MFS reflects progressive alterations in aortic structure and function that contribute to disease progression and adverse outcomes. Within this context, the present study identifies PVAT as an additional and previously underappreciated layer of regulation influencing aortic reactivity in MFS. In the ascending aorta, PVAT-mediated modulation of α₁-adrenergic responsiveness was preserved in males with MFS. In females, PVAT exposure revealed an MFS-specific, age-dependent attenuation of adrenergic contractile responsiveness in the ascending aorta, which was associated with redox-sensitive signaling. In the descending thoracic aorta, MFS PVAT exerted anticontractile effects irrespective of sex. Together, these findings indicate that PVAT contributes to sex- and region-specific heterogeneity in aortic reactivity in MFS and support the concept that determinants of functional vulnerability extend beyond structural dilation alone.

Previous studies in *Fbn1* mutant mice using PVAT-free vessels have reported heterogeneous alterations in aortic reactivity, with both endothelium-dependent relaxations and α₁-adrenergic contractile responses varying according to aortic region, sex, age, and model [4,5,8,16,25,36–41]. The present PVAT-free observations are broadly consistent with this context-dependent pattern. Notably, in female MFS mice, the ascending aorta exhibited no change in α₁-adrenergic contractility under PVAT-free conditions, whereas the descending aorta showed increased contractile responses, a pattern not reported in prior PVAT-free studies of female MFS descending aorta. In this context, the present findings further highlight the need to consider how extravascular factors influence these patterns.

Consistent with this, PVAT is an active regulator of vascular tone under physiological conditions, exerting anticontractile effects through both endothelium-dependent and independent mechanisms [13,23,24]. In the present study, KCl-induced contractions were comparable in

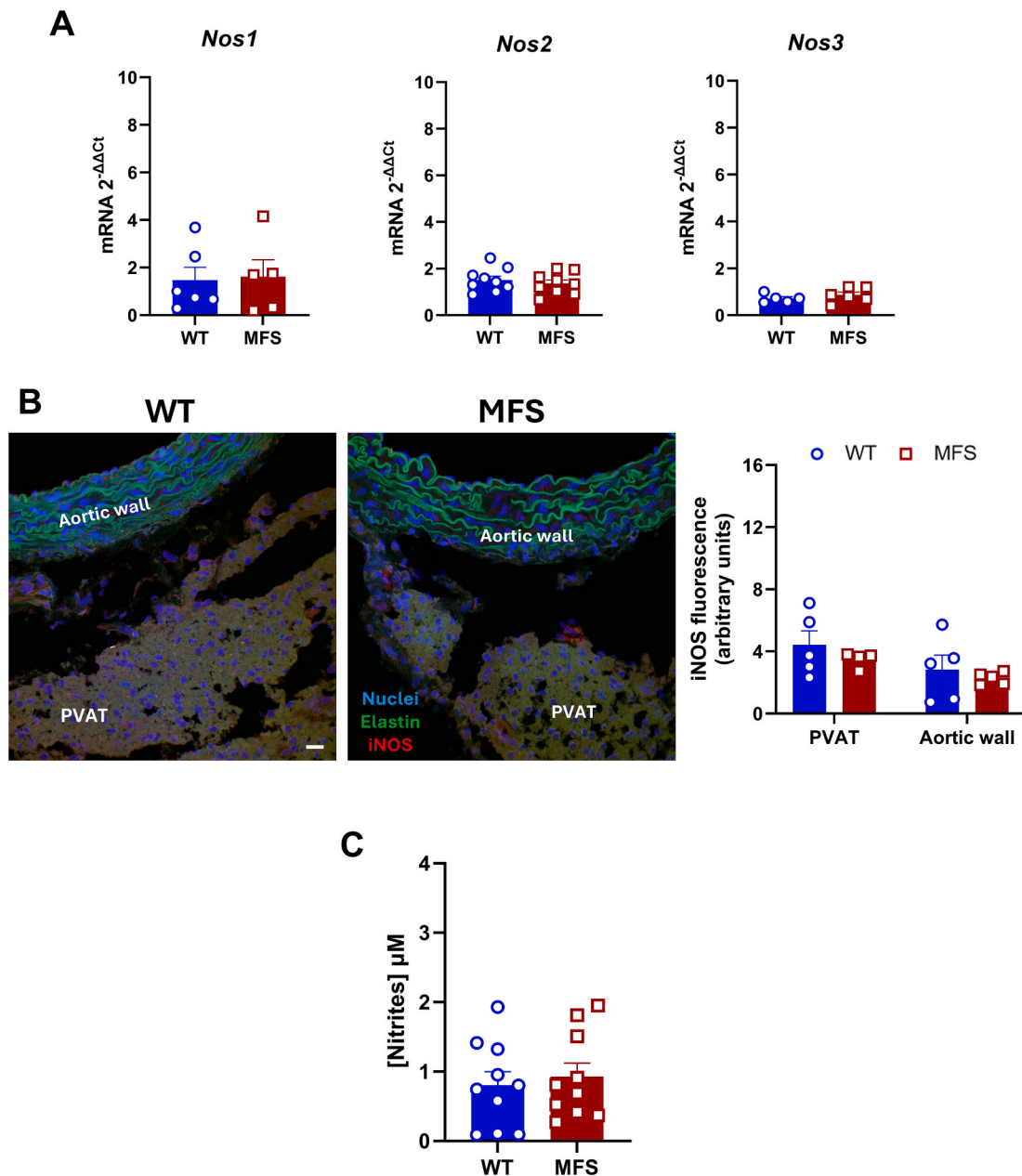


Fig. 5. Analysis of NOS expression and NO availability on ascending aortas from young female WT and MFS mice. (A) Comparative analysis of mRNA levels in ascending aorta PVAT for NOS isoforms: nNOS (*Nos1*), iNOS (*Nos2*), and eNOS (*Nos3*). mRNA levels are expressed as $2^{-\Delta\Delta Ct}$ using GAPDH as a housekeeping gene. Results are the mean $\pm$ SEM from WT ($n = 5-9$) and MFS ($n = 5-9$) mice. (B) Representative photomicrographs and quantification of immunofluorescence (red) for iNOS in confocal microscopic ascending aorta sections. Natural autofluorescence of elastin (green) and nuclear staining (blue) are also shown. Scale bar: 20 μm . Results are the mean $\pm$ SEM from WT ($n = 5$) and MFS ($n = 5$) mice. (C) Concentrations of nitrites in ascending aorta PVAT. Results are the mean $\pm$ SEM from WT ($n = 10$) and MFS ($n = 10$) mice.

PVAT-free aortas from WT and MFS mice, consistent with previous observations [8]. However, PVAT enhanced KCl-induced contractions only in WT vessels, suggesting impaired PVAT-smooth muscle coupling in MFS. This finding supports the notion that PVAT-derived signaling to vascular smooth muscle is altered in MFS, potentially involving changes in adipose-derived mediator release and/or redox-dependent mechanisms. Consistent with this interpretation, PVAT modulated α_1 -adrenergic contractions in a sex- and region-dependent manner. In male mice, PVAT preserved its physiological anticontractile influence on normalized Phe-induced contractions in the ascending aorta irrespective of genotype, while exerting an MFS-associated anticontractile effect in the descending aorta. In contrast, in female MFS mice, PVAT induced a marked attenuation of α_1 -adrenergic contractile responsiveness,

particularly in the ascending aorta, revealing genotype-dependent differences that were not detectable in PVAT-free preparations and thus extending beyond prior PVAT-free observations (e.g., [4,5,8]). This PVAT-dependent reduction in α_1 -adrenergic-mediated contractility may further limit contractile reserve in aortic segments already susceptible to dilation, potentially contributing to the functional vulnerability of the female ascending aorta. Remarkably, PVAT-intact MFS vessels generated lower maximal force than WT arteries in both the male ascending and female descending aorta. In males, this reduction occurred despite preserved normalized Phe-induced α_1 -adrenergic responsiveness, whereas in females it was accompanied by reduced responsiveness. This dissociation suggests that PVAT differentially modulates receptor-mediated signaling and downstream force-generating capacity of the

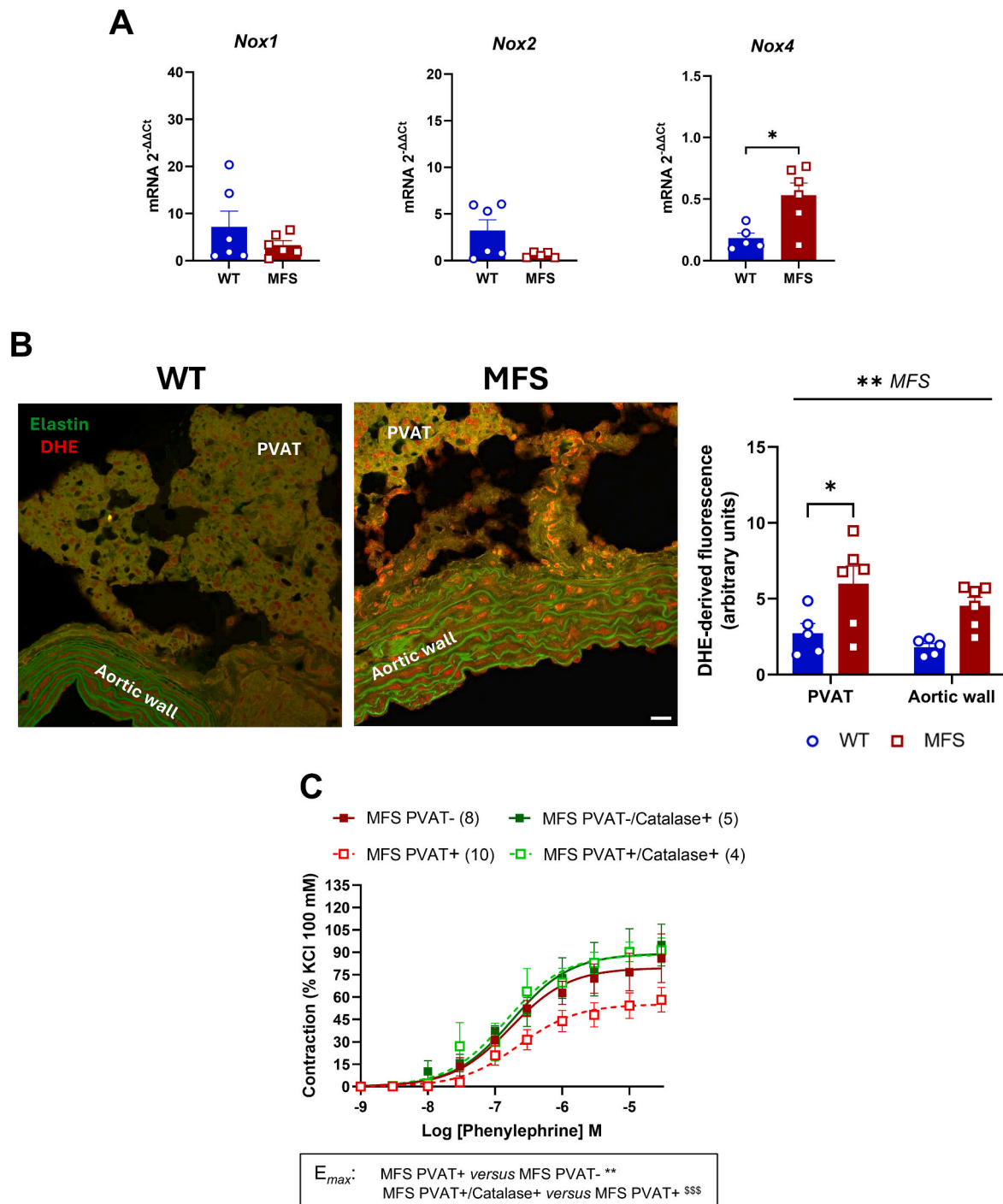


Fig. 6. Analysis of oxidative stress on ascending aortas from young female WT and MFS mice. (A) Comparative analysis of mRNA levels in ascending aorta PVAT for NADPH oxidase (NOX) isoforms (*Nox1*, *Nox2*, *Nox4*). mRNA levels are expressed as $2^{-\Delta\Delta Ct}$ using GAPDH as a housekeeping gene. Results are the mean $\pm$ SEM from WT ($n = 5-10$) and MFS ($n = 5-9$) mice. $*P < 0.05$ by Student's *t*-test. (B) Representative confocal photomicrographs and quantification of dihydroethidium (DHE)-derived fluorescence (red) in ascending aorta sections from female WT and MFS mice. Natural autofluorescence of elastin (green) is also shown. Scale bar: 20 μ m. Results are the mean $\pm$ SEM from WT ($n = 5$) and MFS ($n = 6$) mice. $*P < 0.05$, $**P < 0.01$ by two-way ANOVA with Tukey's post hoc test. (C) Phenylephrine-induced contractions in the absence and presence of catalase (1000 U/mL) in ascending aortas with (+) or without (−) PVAT from young female MFS mice. Results are the mean $\pm$ SEM of the number of animals shown in parentheses. $**P < 0.01$ versus PVAT−; $***P < 0.001$ versus the same group without Catalase by two-way ANOVA with Tukey's post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC_{50} values obtained from nonlinear regression analysis of the concentration–response curves; the corresponding E_{max} and pEC_{50} values are shown in Table 7.

aortic wall. Together, these distinct functional components determine contractile reserve in a biological context-dependent manner. Collectively, these findings underscore marked heterogeneity in PVAT-vessel interactions in MFS and support a role for local PVAT-derived signaling in shaping aortic functional behavior in this disease context.

The anticontractile action of PVAT was no longer detectable in 12-month-old females. While the global decline in Phe-induced contractility with aging may reduce the dynamic range available to detect PVAT modulation, age-related shifts in PVAT phenotype and/or mediator bioavailability could also contribute. We also tested whether the

Table 7

PEC₅₀ and E_{max} values for phenylephrine contraction (% of KCl 100 mM-induced contraction) in ascending aortas with (+) or without (–) PVAT from young female MFS mice in the absence and presence of catalase (1000 U/mL).

	MFS PVAT –	PVAT+	MFS + Catalase PVAT –	PVAT+
PEC ₅₀	6.76 ± 0.14 (8)	6.65 ± 0.14 (10)	6.74 ± 0.15 (5)	6.83 ± 0.14 (4)
E _{max}	79.62 ± 4.78 (8)	55.31 ± 3.53 (10)**	89.22 ± 5.94 (5)	88.60 ± 5.15 (4) ^{sss}

Data are shown as mean ± SEM. The number of mice is shown in parentheses. ***P* < 0.01 versus PVAT–; ^{sss}*P* < 0.001 versus the same group without Catalase by two-way ANOVA with Tukey's post hoc test.

endothelium was required, since PVAT is known to reduce vascular contraction through both endothelium-dependent and endothelium-independent mechanisms [23,24]. Endothelium-denuded vessels retained the PVAT effect, supporting an endothelium-independent mechanism consistent with PVAT-derived signaling acting on adjacent smooth muscle.

MFS is characterized by oxidative stress in both mouse and human aortas [4,5]. We demonstrate here the presence of oxidative stress within PVAT in MFS mice. The predominantly brown phenotype of thoracic PVAT, characterized by high mitochondrial density [17], may, under pathological conditions, predispose this depot to elevated ROS production and contribute to the oxidative imbalance observed in the MFS aortic wall. In addition, *Nox4* mRNA expression was upregulated in MFS PVAT, paralleling findings in the aortic wall of MFS mice and patients [4,5] and supporting a potential contribution to the oxidative stress observed in this tissue. Importantly, to our knowledge, this is the first study to characterize lipid and protein structural signatures in PVAT using SR-μFTIR. Although SR-μFTIR did not reveal evidence of accumulated bulk lipid carbonylation or alterations in global protein secondary structure, the reduced lipid carbonyl/aliphatic ratio raises the possibility that redox-related molecular changes in MFS involve selective lipid remodeling and possibly compensatory responses rather than diffuse oxidative injury. Overall, these findings indicate that oxidative stress in MFS is not associated with detectable large-scale molecular disorganization in PVAT or the arterial wall.

TGF-β signaling may represent an additional upstream or contextual factor linking MFS-associated vascular injury with the PVAT phenotype observed in the present study. In adipose tissue, TGF-β signaling can promote fibrosis, inhibit adipocyte differentiation, and alter adipocyte phenotype [42]. Moreover, TGF-β has been shown to induce NOX4-dependent ROS production in vascular smooth muscle and endothelial cells [43,44]. Given that TGF-β signaling is chronically upregulated in MFS mouse aortas [4,5], it may contribute to shaping the redox-altered PVAT phenotype observed here. Although steady-state *Tgfb1* and *Tgfb2* expression was not increased in PVAT in our experimental conditions, prior, paracrine, or mechanically induced TGF-β signaling cannot be excluded as a contributor to NOX4 upregulation. This interpretation is consistent with recent evidence of perivascular/PVAT inflammation in MFS and with the proposal that PVAT inflammation can amplify TGF-β downstream signaling in the adjacent aortic wall [20,21]. Thus, the PVAT phenotype observed here may reflect a local adipose tissue alteration associated with the MFS background, a secondary adaptation to vascular wall injury, or a combination of both mechanisms.

NOX4 mainly produces H₂O₂ [45]. H₂O₂ can reduce vascular contraction when released from PVAT, including when generated by the mitochondrial electron transport chain in thoracic PVAT [22,23]. More recently, this pathway has been shown to be amplified in female mice through adipose estrogen receptor-α signaling, enhancing the anti-contractile influence of PVAT [46], and estrogen treatment was reported to restore PVAT-mediated anticontractile effects through NOX4-dependent H₂O₂ signaling in ovariectomized hypertensive rats [47]. The abolition of the PVAT-mediated attenuation of Phe-induced

contractions by catalase in female MFS aortas provides functional evidence that an H₂O₂-sensitive mechanism contributes to this effect. In combination with the upregulation of NOX4 in MFS PVAT, these findings support the involvement of NOX4-dependent redox signaling in the modulation of aortic smooth muscle tone in MFS. Beyond its direct vasomodulatory actions, H₂O₂ may also promote further redox imbalance by stimulating additional superoxide generation via NADPH oxidases [48,49], thereby favoring NO-superoxide interactions and peroxynitrite formation [33], which can exert anticontractile effects [34,35]. Thus, these findings raise the possibility that H₂O₂-sensitive PVAT signaling functionally intersects with downstream nitrosative mechanisms regulating aortic contractility in MFS. Although these findings support the involvement of H₂O₂-sensitive redox signaling, the present study does not identify the subcellular source of ROS within PVAT. Given the brown adipose-like phenotype and high mitochondrial density of thoracic PVAT [17], mitochondrial ROS may also contribute to the redox milieu and to PVAT-dependent modulation of aortic tone. This possibility should be addressed in future studies using mitochondrial ROS probes and/or mitochondrial-targeted antioxidants, such as mitoquinone or other mitochondria-targeted superoxide scavengers.

Excessive NO production by smooth muscle iNOS has been linked to thoracic aortic aneurysm progression in MFS [14–16]. Previous work in *Fbn1*^{C1041G/+} mice reported that the absence of contractile differences in the ascending aorta of MFS females was likely due to enhanced negative feedback of iNOS-derived NO on Phe-induced contractions, an effect not observed in males [8]. These observations suggest that iNOS activity plays a key functional role in regulating aortic reactivity in the ascending aorta of female MFS mice. The present findings extend this concept by demonstrating that iNOS activity is required for the PVAT-mediated reduction of Phe-induced contractions, consistent with emerging evidence that PVAT-derived inflammatory signals actively participate in aortic pathology in MFS [21]. Indeed, inhibition of NOS or selective blockade of iNOS abolished this PVAT effect. Although *Nos2* expression and nitrite levels were not increased, the functional sensitivity to 1400 W indicates that iNOS-derived NO is required for the redox-dependent modulation of vascular tone in MFS. Under these conditions, NO is unlikely to act through enhanced bioavailability but rather through interaction with superoxide, leading to peroxynitrite formation [33]. Supporting this interpretation, excessive iNOS-dependent signaling has been associated with increased protein nitration in MFS aortic tissue [14].

In line with increased protein nitration, which is consistent with peroxynitrite formation, we observed elevated protein nitration in MFS mice and, in supportive human tissue evidence, in the available human MFS aortic tissue sample, suggesting a nitrative environment. Although SR-μFTIR primarily detects bulk lipid peroxidation signatures and large-scale protein conformational changes, it would not necessarily capture localized or residue-specific nitrative modifications such as tyrosine nitration. Peroxynitrite modulates vascular tone through effects on smooth muscle signaling and cytoskeletal organization, including actin nitration-dependent reductions in myogenic tone [34], and has been implicated in PVAT-derived regulation of thoracic aortic contractility [35]. To further explore this possibility, we assessed whether exposure to exogenous peroxynitrite could reproduce the PVAT-induced modulation of vascular contractility. Indeed, incubation with peroxynitrite reduced Phe-induced contractions in WT arteries devoid of PVAT, indicating that peroxynitrite is capable of directly influencing smooth muscle reactivity under basal redox conditions. However, peroxynitrite did not fully reproduce the PVAT-associated anticontractile phenotype in MFS aortas under PVAT-free conditions, consistent with altered smooth muscle redox responsiveness and/or context-dependent mechanisms requiring PVAT-derived signaling that may not be replicated by uniform exogenous exposure. Consistently, exogenous peroxynitrite increased *Nox4* mRNA expression in WT PVAT and arterial wall *ex vivo*, suggesting that peroxynitrite can influence *Nox4*-related transcriptional responses. Further supporting a functional role for peroxynitrite

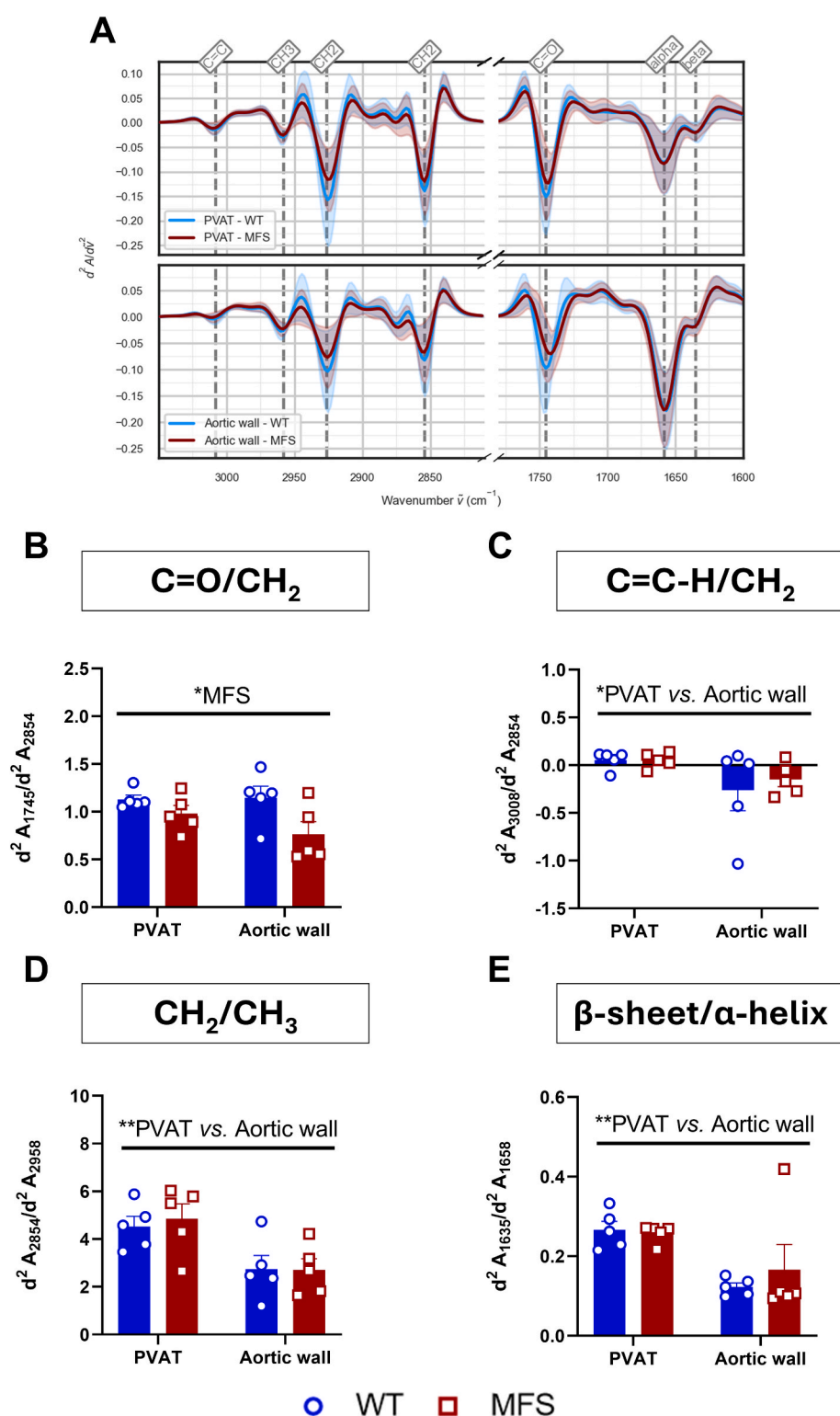


Fig. 7. SR- μ FTIR analysis of lipid structural indices and protein secondary structure in PVAT and aortic wall from young female WT and MFS mice. (A) Mean second-derivative SR- μ FTIR spectra from PVAT (top) and aortic wall (bottom), highlighting spectral regions corresponding to lipid carbonyl C=O ($\sim 1745 \text{ cm}^{-1}$), olefinic C=C-H ($\sim 3008 \text{ cm}^{-1}$), aliphatic methylene CH₂ ($\sim 2854 \text{ cm}^{-1}$), aliphatic methyl CH₃ ($\sim 2958 \text{ cm}^{-1}$), and protein β -sheet ($\sim 1635 \text{ cm}^{-1}$) and α -helix ($\sim 1658 \text{ cm}^{-1}$) bands. Shaded areas represent variability among spectra. Quantification of (B) lipid carbonylation assessed as the C=O/CH₂ ratio ($1745/2854 \text{ cm}^{-1}$), (C) lipid unsaturation index expressed as C=C-H/CH₂ ($3008/2854 \text{ cm}^{-1}$), and (D) lipid aliphatic integrity index calculated as CH₂/CH₃ ($2854/2958 \text{ cm}^{-1}$). (E) Protein secondary structure expressed as β -sheet/ α -helix ratio ($1635/1658 \text{ cm}^{-1}$). Results are the mean $\pm$ SEM from WT ($n = 5$) and MFS ($n = 5$) mice. * $P < 0.05$, ** $P < 0.01$ by two-way ANOVA.

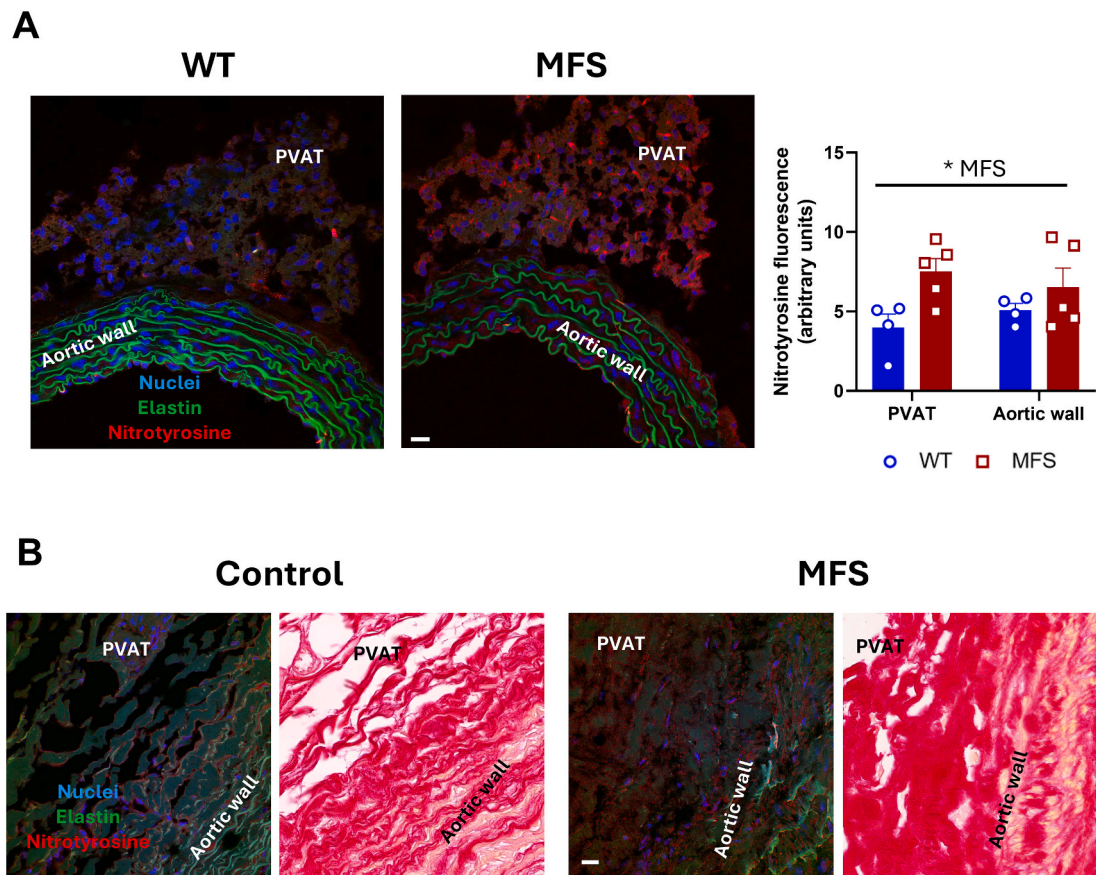


Fig. 8. Analysis of protein nitration on ascending aortas from mice and human. (A) Representative photomicrographs and quantification of immunofluorescence (red) for nitrotyrosine in confocal microscopic ascending aorta sections from young female WT and MFS mice. Natural autofluorescence of elastin (green) and nuclear staining (blue) are also shown. Scale bar: 20 μ m. Results are the mean $\pm$ SEM from WT ($n = 4$) and MFS ($n = 5$) mice. * $P < 0.05$ by two-way ANOVA. (B) Representative photomicrographs of nitrotyrosine immunofluorescence staining (red; left) and picrosirius red histology (right) in ascending aortic sections from control donors ($n = 2$) and an MFS donor ($n = 1$). Natural elastin autofluorescence (green) and nuclear staining (blue) are also shown. Scale bar: 20 μ m.

signaling, scavenging peroxynitrite with FeTPPS abolished the PVAT-mediated anticontractile effect in MFS aortas, without significantly reducing protein nitration. These findings suggest that the anticontractile phenotype depends on ongoing peroxynitrite-sensitive signaling rather than solely on the accumulation of stable nitrated proteins. Taken together, these data support the presence of a localized redox signaling milieu in female MFS aortas, in which *Nox4*-associated and H_2O_2 -sensitive PVAT signaling may functionally interact with peroxynitrite-dependent mechanisms to modulate smooth muscle tone through dynamic and potentially reversible pathways.

Whether the pronounced redox imbalance observed in female MFS PVAT predisposes this tissue to heightened inflammatory activation under pregnancy-like conditions, a clinical context associated with accelerated aortic pathology in MFS [7,9], remains an important question for future investigation. Finally, it will be important to determine whether similar PVAT-mediated effects occur in other genetic models of MFS, including dissection-prone models such as *Fbn1*^{mgR/mgR} [41,50] or *Fbn1*^{G234D/G234D} [51], which represent distinct classes of FBN1 mutations and disease severity.

In summary, the present study identifies PVAT as a previously unrecognized extravascular contributor to altered aortic reactivity in MFS, exerting sex- and region-specific effects on aortic α_1 -adrenergic contractility. Our data support a PVAT-dependent redox signaling mechanism in the ascending aorta of female MFS mice, involving iNOS- and peroxynitrite-sensitive components together with a *Nox4*-associated, H_2O_2 -sensitive PVAT signal. However, these findings do not establish a single linear pathway, and future studies are needed to define the spatial and molecular interactions among H_2O_2 , superoxide, NO, and

peroxynitrite-dependent signaling within the PVAT-aortic wall axis. Overall, this study highlights PVAT not merely as a bystander, but as an active determinant of vascular vulnerability in MFS, with potential implications for future sex- and region-specific therapeutic targeting.

Ethics approval and consent to participate

Experiments involving animals were conducted in accordance with Spanish (RD35/2013) and European (2010/63/EU) legislation, and procedures were approved by the Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH 3041-CEEA-UAB) and the Generalitat de Catalunya (11517). Experiments involving humans were approved by the Ethics and Clinical Research Committee of the *Hospital de la Santa Creu i Sant Pau* (Barcelona, Spain; Ref. IIBSP-MAR-2019-08). All samples are part of collection C.0005273, registered in the *Instituto de Salud Carlos III* (Madrid, Spain). Research was conducted in accordance with the Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. Participation of patients was based on informed consent provided by the patients or their legal representatives.

Declaration of Generative AI and AI-Assisted Technologies.

No generative AI or AI-assisted technologies were used in the preparation of this article, except for grammar and spelling checks.

CRediT authorship contribution statement

Jana Hua-Monclús: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Clara Penas:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Belén Pérez:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis,

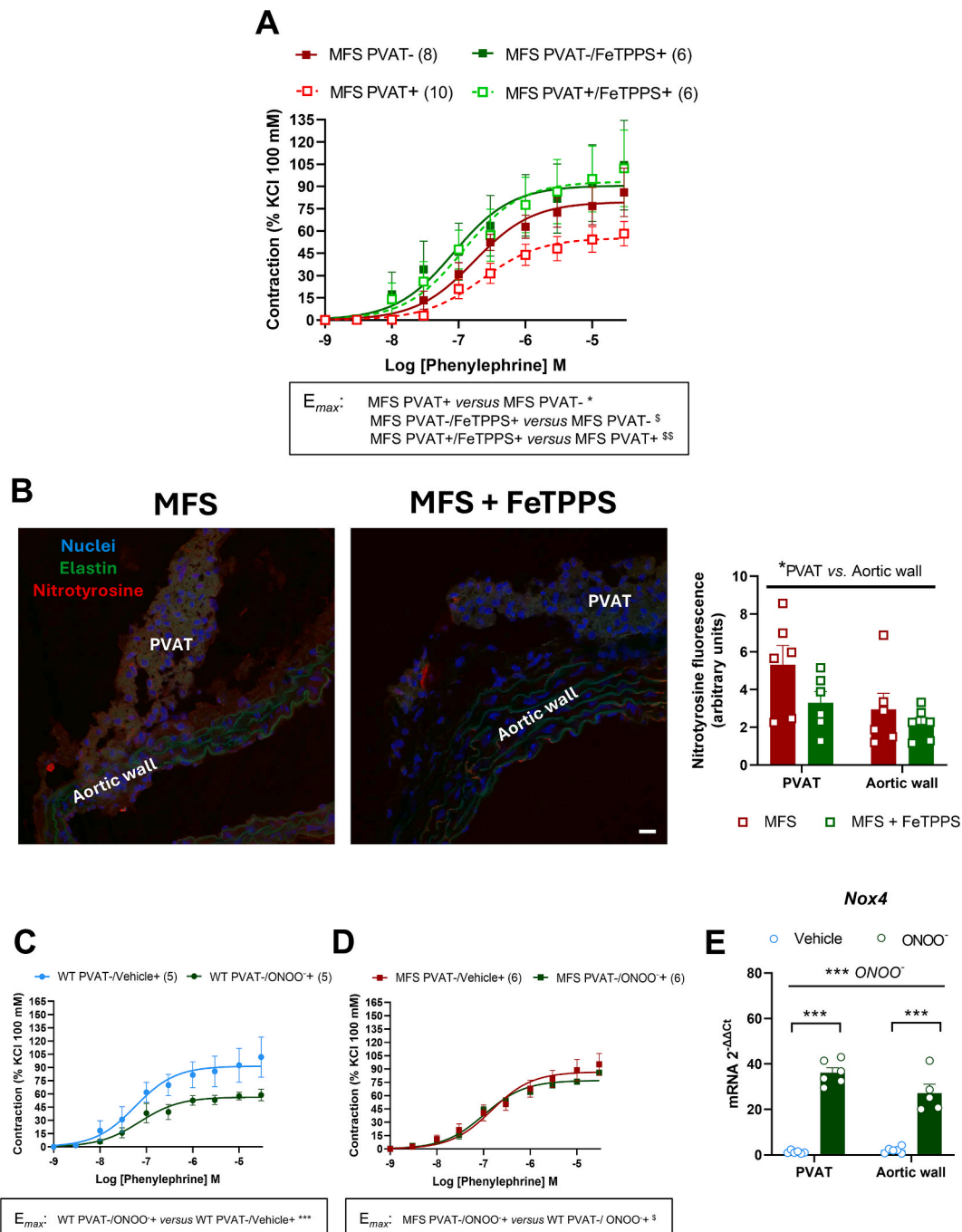


Fig. 9. Influence of peroxynitrite signaling on female ascending aortas. **(A)** Phenylephrine-induced contractions in the absence and presence of the peroxynitrite scavenger FeTPPS (10^{-5} M) in ascending aortas with (+) or without (−) PVAT from young female MFS mice. Results are the mean $\pm$ SEM of the number of animals shown in parentheses. * $P < 0.05$ versus PVAT−; § $P < 0.05$, §§ $P < 0.01$ versus the same group without FeTPPS by two-way ANOVA with Tukey's post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC_{50} values obtained from nonlinear regression analysis of the concentration–response curves; the corresponding E_{max} and pEC_{50} values are shown in Table 8. **(B)** Representative photomicrographs and quantification of immunofluorescence (red) for nitrotyrosine in confocal microscopic ascending aorta sections incubated with peroxynitrite scavenger FeTPPS (10^{-5} M). Natural autofluorescence of elastin (green) and nuclear staining (blue) are also shown. Scale bar: 20 μ m. Results are the mean $\pm$ SEM from MFS ($n = 6$) and MFS incubated with FeTPPS ($n = 6$) mice. * $P < 0.05$ by two-way ANOVA. Phenylephrine-induced contractions in the absence (Vehicle; NaOH 4.7%) and presence of peroxynitrite (ONOO⁻; 10^{-5} M) in ascending aortas with (+) or without (−) PVAT from young female WT **(C)** and MFS **(D)** mice. Results are the mean $\pm$ SEM of the number of animals shown in parentheses. *** $P < 0.001$ versus Vehicle; § $P < 0.05$ versus the same group in WT by two-way ANOVA with Tukey's post hoc test. Statistical symbols indicate significant differences in E_{max} and/or pEC_{50} values obtained from nonlinear regression analysis of the concentration–response curves; the corresponding E_{max} and pEC_{50} values are shown in Table 8. **(E)** Comparative analysis of *Nox4* mRNA levels on ascending aortas from young female WT mice in the absence (Vehicle; NaOH 4.7%) and presence of peroxynitrite (ONOO⁻; 10^{-5} M). mRNA levels are expressed as $2^{-\Delta\Delta C_t}$ using GAPDH as a housekeeping gene. Results are the mean $\pm$ SEM from WT ($n = 5$ –6) mice. *** $P < 0.001$ by two-way ANOVA with Tukey's post hoc test.

Table 8

PEC₅₀ and E_{max} values for phenylephrine contraction (% of KCl 100 mM-induced contraction) in ascending aortas with (+) or without (–) PVAT from young female WT and MFS mice in the absence and presence of the peroxynitrite scavenger FeTPPS (10^{–5} M) or peroxynitrite (ONOO[–]; 10^{–5} M).

	MFS PVAT–	PVAT+	MFS + FeTPPS PVAT–	PVAT+
PEC ₅₀	6.76 ± 0.14 (8)	6.65 ± 0.14 (10)	7.08 ± 0.26 (6)	6.93 ± 0.23 (6)
E _{max}	79.62 ± 4.78 (8)	55.31 ± 3.53 (10)*	90.69 ± 10.00 (6) ^s	93.36 ± 8.75 (6) ^{ss}
	WT PVAT– Vehicle	ONOO [–]	MFS PVAT– Vehicle	ONOO [–]
PEC ₅₀	7.24 ± 0.19 (5)	7.15 ± 0.13 (5)	6.83 ± 0.13 (6)	6.99 ± 0.05 (6)
E _{max}	91.58 ± 6.76 (5)	56.39 ± 2.93 (5)***	86.81 ± 4.69 (6)	77.14 ± 1.69 (6) ^s

Data are shown as mean ± SEM. The number of mice is shown in parentheses. **P* < 0.05, ****P* < 0.001 versus PVAT – or Vehicle; ^s*P* < 0.05, ^{ss}*P* < 0.01 versus the same group without FeTPPS or in WT by two-way ANOVA with Tukey's post hoc test.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on reasonable request.

References

- [1] H.C. Dietz, C.R. Cutting, R.E. Pyeritz, C.L. Maslen, L.Y. Sakai, G.M. Corson, E. G. Puffenberger, A. Hamosh, E.J. Nanthakumar, S.M. Currstin, G. Stetten, D. A. Meyers, C.A. Francomano, Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene, *Nature* 352 (6333) (1991) 337–339, <https://doi.org/10.1038/352337a0>.
- [2] S. Seeburur, S. Wu, D. Hemani, L. Pham, D. Ju, Y. Xie, P. Kata, L. Li, Insights into elastic fiber fragmentation: Mechanisms and treatment of aortic aneurysm in Marfan syndrome, *Vasc. Pharmacol.* 153 (2023) 107215, <https://doi.org/10.1016/j.vph.2023.107215>.
- [3] D.P. Judge, N.J. Biery, D.R. Keene, J. Geubtner, L. Myers, D.L. Huso, L.Y. Sakai, H. C. Dietz, Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome, *J. Clin. Invest.* 114 (2) (2004) 172–181, <https://doi.org/10.1172/JCI20641>.
- [4] F. Jiménez-Altayó, T. Meirelles, E. Crosas-Molist, M.A. Sorolla, D.G. Del Blanco, J. López-Luque, A. Mas-Stachurska, A.-M. Siegfert, F. Bonorino, L. Barberà, C. García, E. Condom, M. Sitges, F. Rodríguez-Pascual, F. Laurindo, K. Schröder, J. Ros, I. Fabregat, G. Egea, Redox stress in Marfan syndrome: Dissecting the role of the NADPH oxidase NOX4 in aortic aneurysm, *Free Radic. Biol. Med.* 118 (2018) 44–58, <https://doi.org/10.1016/j.freeradbiomed.2018.02.023>.
- [5] I. Rodríguez-Rovira, C. Arce, K. De Rycke, B. Pérez, A. Carretero, M. Arbonés, G. Teixido-Turá, M.C. Gómez-Cabrera, V. Campuzano, F. Jiménez-Altayó, G. Egea, Allopurinol blocks aortic aneurysm in a mouse model of Marfan syndrome via reducing aortic oxidative stress, *Free Radic. Biol. Med.* 193 (2022) 538–550, <https://doi.org/10.1016/j.freeradbiomed.2022.11.001>.
- [6] H.C. Yang, J.M. Kim, E. Chum, C. Van Breemen, A.W. Chung, Long-term effects of losartan on structure and function of the thoracic aorta in a mouse model of Marfan syndrome, *Br. J. Pharmacol.* 158 (6) (2009) 1503–1512, <https://doi.org/10.1111/j.1476-5381.2009.00443.x>.
- [7] M.J. Roman, R.B. Devereux, L.R. Preiss, F.M. Asch, K.A. Eagle, K.W. Holmes, S. A. LeMaire, C.L. Maslen, D.M. Milewicz, S.A. Morris, S.K. Prakash, R.E. Pyeritz, W. J. Ravekes, R.V. Shohet, H.K. Song, J.W. Weinsaft, H.C. Dietz, J. Habashi, G. M. Silberbach, B.L. Kroner, Associations of Age and sex with Marfan Phenotype: the National Heart, Lung, and Blood Institute GenTAC (genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions) Registry. *Circulation: Cardiovascular Genetics* 10 (3) (2017) e001647, <https://doi.org/10.1161/CIRCGENETICS.116.001647>.
- [8] F. Jiménez-Altayó, A.-M. Siegfert, F. Bonorino, T. Meirelles, L. Barberà, A.P. Dantas, E. Vila, G. Egea, Differences in the Thoracic Aorta by Region and sex in a Murine Model of Marfan Syndrome, *Front. Physiol.* 8 (2017) 933, <https://doi.org/10.3389/fphys.2017.00933>.
- [9] M. Renard, L. Muñoz-Mosquera, E.C. Manalo, S. Tufa, E.J. Carlson, D.R. Keene, J. De Backer, L.Y. Sakai, Sex, pregnancy and aortic disease in Marfan syndrome, *PLoS One* 12 (7) (2017) e0181166, <https://doi.org/10.1371/journal.pone.0181166>.
- [10] K.K. Dwivedi, J. Rother, J.E. Wagenseil, Age- and sex-specific biomechanics and extracellular matrix remodeling of the ascending aorta in a mouse model of severe Marfan syndrome, *American Journal of Physiology-Heart and Circulatory Physiology* 327 (4) (2024) H1037–H1051, <https://doi.org/10.1152/ajpheart.00391.2024>.
- [11] C.P. Kotanidis, C. Antoniadis, Perivascular fat imaging by computed tomography (CT): a virtual guide, *Br. J. Pharmacol.* 178 (21) (2021) 4270–4290, <https://doi.org/10.1111/bph.15634>.
- [12] C. Agabiti-Rosei, A. Painsi, C. De Ciuceis, S. Withers, A. Greenstein, A.M. Heagerty, D. Rizzoni, Modulation of Vascular Reactivity by Perivascular Adipose Tissue (PVAT), *Curr. Hypertens. Rep.* 20 (5) (2018) 44, <https://doi.org/10.1007/s11906-018-0835-5>.
- [13] J.A. Victorio, R.M. Da Costa, R.C. Tostes, A.P. Davel, Modulation of Vascular Function by Perivascular Adipose Tissue: sex differences, *Curr. Pharm. Des.* 26 (30) (2020) 3768–3777, <https://doi.org/10.2174/138161282666200701211912>.
- [14] A. De La Fuente-Alonso, M. Toral, A. Alfayate, M.J. Ruiz-Rodríguez, E. Bonzón-Kulichenko, G. Teixido-Tura, S. Martínez-Martínez, M.J. Méndez-Olivares, D. López-Maderuelo, I. González-Valdés, E. García-Izquierdo, S. Mingo, C. E. Martín, L. Muñoz-Mosquera, J. De Backer, J.F. Nistal, A. Forteza, A. Evangelista, J. Vázquez, J.M. Redondo, Aortic disease in Marfan syndrome is caused by overactivation of sGC-PRKG signaling by NO, *Nat. Commun.* 12 (1) (2021) 2628, <https://doi.org/10.1038/s41467-021-22933-3>.
- [15] J. Oller, N. Méndez-Barbero, E.J. Ruiz, S. Villahoz, M. Renard, L.I. Canelas, A. M. Briones, R. Alberca, N. Lozano-Vidal, M.A. Hurlé, D. Milewicz, A. Evangelista, M. Salas, J.F. Nistal, L.J. Jiménez-Borreguero, J. De Backer, M.R. Campanero, J. M. Redondo, Nitric oxide mediates aortic disease in mice deficient in the metalloprotease Adamts1 and in a mouse model of Marfan syndrome, *Nat. Med.* 23 (2) (2017) 200–212, <https://doi.org/10.1038/nm.4266>.
- [16] H.H.C. Yang, C. van Breemen, A.W.Y. Chung, Vasomotor dysfunction in the thoracic aorta of Marfan syndrome is associated with accumulation of oxidative stress, *Vasc. Pharmacol.* 52 (1–2) (2010) 37–45, <https://doi.org/10.1016/j.vph.2009.10.005>.
- [17] C.K. Cheng, H. Ding, M. Jiang, H. Yin, M. Gollasch, Y. Huang, Perivascular adipose tissue: Fine-tuner of vascular redox status and inflammation, *Redox Biol.* 62 (2023) 102683, <https://doi.org/10.1016/j.redox.2023.102683>.
- [18] M. Cai, D. Zhao, X. Han, S. Han, W. Zhang, Z. Zang, C. Gai, R. Rong, T. Gao, The role of perivascular adipose tissue-secreted adipocytokines in cardiovascular disease, *Front. Immunol.* 14 (2023) 1271051, <https://doi.org/10.3389/fimmu.2023.1271051>.

- [19] C. Fiorillo, M. Becatti, M. Attanasio, L. Lucarini, N. Nassi, L. Evangelisti, M. C. Porciani, P. Nassi, G.F. Gensini, R. Abbate, G. Pepe, Evidence for oxidative stress in plasma of patients with Marfan syndrome, *Int. J. Cardiol.* 145 (3) (2010) 544–546, <https://doi.org/10.1016/j.ijcard.2010.04.077>.
- [20] D. Tuttolomondo, F. Secchi, N. Gaibazzi, N.S. Udugampolage, A. Pini, M. De Filippo, P. Spagnolo, R. Caruso, J. Taurino, Comparative Analysis of Perivascular Adipose Tissue Attenuation on Chest Computed Tomography Angiography in patients with Marfan Syndrome: a Case-Control Study, *Diagnostics* 15 (6) (2025) 673, <https://doi.org/10.3390/diagnostics15060673>.
- [21] H. Sowa, H. Yagi, K. Ueda, M. Hashimoto, K. Karasaki, Q. Liu, A. Kurozumi, Y. Adachi, T. Yanase, S. Okamura, B. Zhai, N. Takeda, M. Ando, H. Yamauchi, N. Ito, M. Ono, H. Akazawa, I. Komuro, Perivascular inflammation in the progression of aortic aneurysms in Marfan syndrome, *JCI Insight* e184329 (2025), <https://doi.org/10.1172/jci.insight.184329>.
- [22] R.M. Costa, F.P. Filgueira, R.C. Tostes, M.H.C. Carvalho, E.H. Akamine, N. S. Lobato, H2O2 generated from mitochondrial electron transport chain in thoracic perivascular adipose tissue is crucial for modulation of vascular smooth muscle contraction, *Vasc.Pharmacol.* 84 (2016) 28–37, <https://doi.org/10.1016/j.vph.2016.05.008>.
- [23] Y.-J. Gao, C. Lu, L. Su, A.M. Sharma, R.M.K.W. Lee, Modulation of vascular function by perivascular adipose tissue: the role of endothelium and hydrogen peroxide, *Br. J. Pharmacol.* 151 (3) (2007) 323–331, <https://doi.org/10.1038/sj.bjp.0707228>.
- [24] R.M. Lee, C. Lu, L.-Y. Su, Y.-J. Gao, Endothelium-dependent relaxation factor released by perivascular adipose tissue, *J. Hypertens.* 27 (4) (2009) 782–790, <https://doi.org/10.1097/HJH.0b013e328324ed86>.
- [25] A.W. Chung, K. Au Yeung, S.F. Cortes, G.G. Sandor, D.P. Judge, H.C. Dietz, C. van Breemen, Endothelial dysfunction and compromised eNOS/Akt signaling in the thoracic aorta during the progression of Marfan syndrome, *Br. J. Pharmacol.* 150 (8) (2007) 1075–1083, <https://doi.org/10.1038/sj.bjp.0707181>.
- [26] F. Jiménez-Altayó, P. Ortiz-Romero, L. Puertas-Umbert, A.P. Dantas, B. Pérez, E. Vila, P. D'Ocon, V. Campuzano, Stenosis coexists with compromised α 1-adrenergic contractions in the ascending aorta of a mouse model of Williams-Beuren syndrome, *Sci. Rep.* 10 (1) (2020) 889, <https://doi.org/10.1038/s41598-020-57803-3>.
- [27] G.X. Zhang, S. Kimura, K. Murao, J. Shimizu, H. Matsuyoshi, M. Takaki, Role of neuronal NO synthase in regulating vascular superoxide levels and mitogen-activated protein kinase phosphorylation, *Cardiovasc. Res.* 81 (2) (2009) 389–399, <https://doi.org/10.1093/cvr/cvn304>.
- [28] L.C. Green, D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok, S. R. Tannenbaum, Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids, *Anal. Biochem.* 126 (1) (1982) 131–138, [https://doi.org/10.1016/0003-2697\(82\)90118-X](https://doi.org/10.1016/0003-2697(82)90118-X).
- [29] G. Manich, B. Pérez, C. Penas, A.P. Dantas, J. Coutinho, P. Sánchez-Bernadó, J. García-Aranda, J. Fraile-Ramos, N. Benseny-Cases, B. Martín-Mur, A. Esteve-Codina, I. Rodríguez-Rovira, L. Giménez-Llort, G. Egea, F. Jiménez-Altayó, Sex- and age-dependent neurovascular abnormalities linked to neuroinflammation lead to exacerbated post-ischemic brain injury in Marfan syndrome mice, *Redox Biol.* 83 (2025) 103662, <https://doi.org/10.1016/j.redox.2025.103662>.
- [30] I. Yousef, L. Ribó, A. Crisol, I. Sics, G. Ellis, T. Ducic, M. Kreuzer, N. Benseny-Cases, M. Quispe, P. Dumas, S. Lefrançois, T. Moreno, G. García, S. Ferrer, J. Nicolas, M.A. G. Aranda, MIRAS: the Infrared Synchrotron Radiation Beamline at ALBA, *Synchrotron Radiat. News* 30 (4) (2017) 4–6, <https://doi.org/10.1080/08940886.2017.1338410>.
- [31] M. Toplak, S.T. Read, C. Sandt, F. Borondics, Quasar: Easy Machine Learning for Biospectroscopy, *Cells* 10 (9) (2021) 2300, <https://doi.org/10.3390/cells10092300>.
- [32] A. De Paeppe, R.B. Devereux, H.C. Dietz, R.C.M. Hennekam, R.E. Pyeritz, Revised diagnostic criteria for the Marfan syndrome, *Am. J. Med. Genet.* 62 (4) (1996) 417–426, [https://doi.org/10.1002/\(SICI\)1096-8628\(19960424\)62:4%253C417::AID-AJMG15%253E3.0.CO;2-R](https://doi.org/10.1002/(SICI)1096-8628(19960424)62:4%253C417::AID-AJMG15%253E3.0.CO;2-R).
- [33] J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall, B.A. Freeman, Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide, *Proc. Natl. Acad. Sci.* 87 (4) (1990) 1620–1624, <https://doi.org/10.1073/pnas.87.4.1620>.
- [34] M.J. Maneen, M.J. Cipolla, Peroxynitrite diminishes myogenic tone in cerebral arteries: Role of nitrotyrosine and F-actin, *American Journal of Physiology-Heart and Circulatory Physiology* 292 (2) (2007) H1042–H1050, <https://doi.org/10.1152/ajpheart.00800.2006>.
- [35] A.B. Ugusman, A. Riddell, S. Kennedy, Effect of Peroxynitrite (ONOO-) on the Function of Murine Perivascular Adipose Tissue, *Pharmacology & Pharmacy* 10 (04) (2019) 194–204, <https://doi.org/10.4236/pp.2019.104017>.
- [36] A.W. Chung, K. Au Yeung, G.G. Sandor, D.P. Judge, H.C. Dietz, C. van Breemen, Loss of elastic fiber integrity and reduction of vascular smooth muscle contraction resulting from the upregulated activities of matrix metalloproteinase-2 and -9 in the thoracic aortic aneurysm in Marfan syndrome, *Circ. Res.* 101 (5) (2007) 512–522, <https://doi.org/10.1161/CIRCRESAHA.107.157776>.
- [37] A.W. Chung, H.H. Yang, C. van Breemen, Imbalanced synthesis of cyclooxygenase-derived thromboxane A2 and prostacyclin compromises vasomotor function of the thoracic aorta in Marfan syndrome, *Br. J. Pharmacol.* 152 (3) (2007) 305–312, <https://doi.org/10.1038/sj.bjp.0707391>.
- [38] A.W. Chung, H.H. Yang, M.W. Radomski, C. van Breemen, Long-term doxycycline is more effective than atenolol to prevent thoracic aortic aneurysm in marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9, *Circ. Res.* 102 (8) (2008) e73–e85, <https://doi.org/10.1161/CIRCRESAHA.108.174367>.
- [39] J. van Langen, P. Franssen, C.E. Van Hove, D.M. Schrijvers, W. Martinet, G.R. De Meyer, H. Bult, Selective loss of basal but not receptor-stimulated relaxation by endothelial nitric oxide synthase after isolation of the mouse aorta, *Eur. J. Pharmacol.* 696 (1–3) (2012) 111–119, <https://doi.org/10.1016/j.ejphar.2012.09.016>.
- [40] T. Curry-Koski, B. Gusek, R.M. Potter, T.B. Jones, R. Dickman, N. Johnson, J. N. Stallone, R. Rahimian, J. Vallejo-Elias, M. Esfandiari, Genetic Manipulation of Caveolin-1 in a Transgenic Mouse Model of Aortic root Aneurysm: Sex-Dependent Effects on Endothelial and Smooth Muscle Function, *Int. J. Mol. Sci.* 25 (23) (2024) 12702, <https://doi.org/10.3390/ijms252312702>.
- [41] A. Cantalupo, K. Asano, S. Dikalov, D. Gordon, F. Ramirez, Fibrillin-1 Deficiency Perturbs Aortic Cholinergic Relaxation and Adrenergic Contraction in a Mouse Model of Early Onset Progressively Severe Marfan Syndrome, *J. Vasc. Res.* 62 (2) (2025) 96–108, <https://doi.org/10.1159/000542481>.
- [42] R. Datta, M.J. Podolsky, K. Atabai, Fat fibrosis: Friend or foe? *JCI Insight* 3 (19) (2018) e122289 <https://doi.org/10.1172/jci.insight.122289>.
- [43] A. Martin-Garrido, D.I. Brown, A.N. Lyle, A. Dikalova, B. Seidel-Rogol, B. Lassègue, A.S. Martin, K.K. Griendling, NADPH oxidase 4 mediates TGF- β -induced smooth muscle α -actin via p38MAPK and serum response factor, *Free Radic. Biol. Med.* 50 (2) (2011) 354–362, <https://doi.org/10.1016/j.freeradbiomed.2010.11.007>.
- [44] F. Yan, Y. Wang, X. Wu, H.M. Peshavariya, G.J. Dusting, M. Zhang, F. Jiang, Nox4 and redox signaling mediate TGF- β -induced endothelial cell apoptosis and phenotypic switch, *Cell Death Dis.* 5 (1) (2014) e1010–e, <https://doi.org/10.1038/cddis.2013.551>.
- [45] K. Bedard, K.-H. Krause, The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology, *Physiol. Rev.* 87 (1) (2007) 245–313, <https://doi.org/10.1152/physrev.00044.2005>.
- [46] T.J. Costa, M.T. Fontes, P.R. Barros, M.C. Hope, R.C. Webb, C.F. Wenceslau, R. T. Enos, C.G. McCarthy, Overexpression of adipose tissue ER α enhances PVAT anticontractility via NOX4-derived H2O2 and is protective against high-fat diet-induced dysfunction, *American Journal of Physiology-Heart and Circulatory Physiology* 328 (5) (2025) H1065–H1072, <https://doi.org/10.1152/ajpheart.00180.2025>.
- [47] B.C. Petroski-Moraes, I.T. Ramos, J.H. Sousa, M.C.T. de Queiroz, A.C. Mendes, D. Rodrigues, P.R. Barros, C.G. McCarthy, R.A.S. Eichler, E.H. Akamine, R. C. Tostes, T.J. Costa, Estrogen treatment restores perivascular adipose tissue-mediated anticontractile effect via NOX4-dependent hydrogen peroxide signaling in ovariectomized hypertensive rats, *J. Hypertens.* 44 (5) (2026) 858–866, <https://doi.org/10.1097/HJH.0000000000004279>.
- [48] C.H. Coyle, L.J. Martinez, M.C. Coleman, D.R. Spitz, N.L. Weintraub, K.N. Kader, Mechanisms of H2O2-induced oxidative stress in endothelial cells, *Free Radic. Biol. Med.* 40 (12) (2006) 2206–2213, <https://doi.org/10.1016/j.freeradbiomed.2006.02.017>.
- [49] P.K. Witting, B.S. Rayner, B.-J. Wu, N.A. Ellis, R. Stocker, Hydrogen Peroxide Promotes Endothelial Dysfunction by Stimulating Multiple sources of Superoxide Anion Radical Production and Decreasing Nitric Oxide Bioavailability, *Cell. Physiol. Biochem.* 20 (5) (2007) 255–268, <https://doi.org/10.1159/000107512>.
- [50] L. Pereira, S.Y. Lee, B. Gayraud, K. Andrikopoulos, S.D. Shapiro, T. Bunton, N. J. Biery, H.C. Dietz, L.Y. Sakai, F. Ramirez, Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1, *PNAS* 96 (7) (1999) 3819–3823, <https://doi.org/10.1073/pnas.96.7.3819>.
- [51] K. Kimura, E. Motoyama, S. Kanki, K. Asano, P. Sips, M.A.A. Sheikh, M.T.R.D. C. Clarin, E. Raja, M. Takeda, R. Ishii, K. Murata, V. Deleuw, L. Muñio Mosquera, J. De Backer, S. Mizuno, L.Y. Sakai, T. Nakamura, H. Yanagisawa, Novel Aortic Dissection Model Links Endothelial Dysfunction and Immune Infiltration, *Circ. Res.* 137 (1) (2025) 26–42, <https://doi.org/10.1161/CIRCRESAHA.125.326230>.