JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 11, NO. 4, 2026
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EDITORIAL COMMENT

Pulmonary Endothelium

A Driver and Therapeutic Target in Pulmonary Hypertension

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Pulmonary hypertension (PH) is a progressive and debilitating cardiopulmonary disorder characterized by vascular remodeling with constriction and narrowing of the small (~100-500 μm in diameter) pulmonary arteries. This vascular remodeling leads to sustained increased pulmonary vascular pressures and resistance often resulting in right ventricular hypertrophy. The clinical consequences are right heart failure, making PH a major and often fatal clinical problem. PH is defined as present when the mean pulmonary arterial pressure (mPAP) is >20 mm Hg, as determined by right heart catheterization. Based on the underlying etiology, PH is classified into 5 clinical groups, with PH associated with left heart disease (Group 2) being the most prevalent, followed by PH associated with lung disease (Group 3), while Groups 1, 4, and 5 remain relatively rare. Despite advances in diagnosis and management, PH remains largely a disease with limited treatment options.[1]

The pulmonary vasculature has multiple cell layers; yet, the vast majority of studies in PH have focused on vascular smooth muscle cells as the primary therapeutic target. Far less attention has been given to the endothelium, where endothelial dysfunction has often been regarded as a secondary consequence of disease progression. Maintaining normal endothelial function and nitric oxide (NO) signaling is well established in regulating vascular tone, inflammation, smooth muscle proliferation, and vascular remodeling in systemic arteries (**Figure 1**). An imbalance between vasodilatory and vasoconstrictive pathways is increasingly recognized as a driver, rather than a byproduct, of PH pathogenesis.[2]

Current treatments targeting soluble guanylate cyclase stimulators (cyclic guanosine monophosphate, riociguat),[3] inhaled NO[4] as a vasodilator or endothelin-1 receptor antagonist as a vasoconstrictor,[5] phosphodiesterase-5 inhibitors (sildenafil and tadalafil)[6] to improve hemodynamics and vascular perfusion in patients with PH. These drugs are administered alone or in combination to attempt to alleviate the symptoms of PH. The efficacy on reducing disease progression has been limited. Furthermore, none of these therapies target vascular remodeling in PAH, which would be crucial for alleviating or halting the progression of PH.

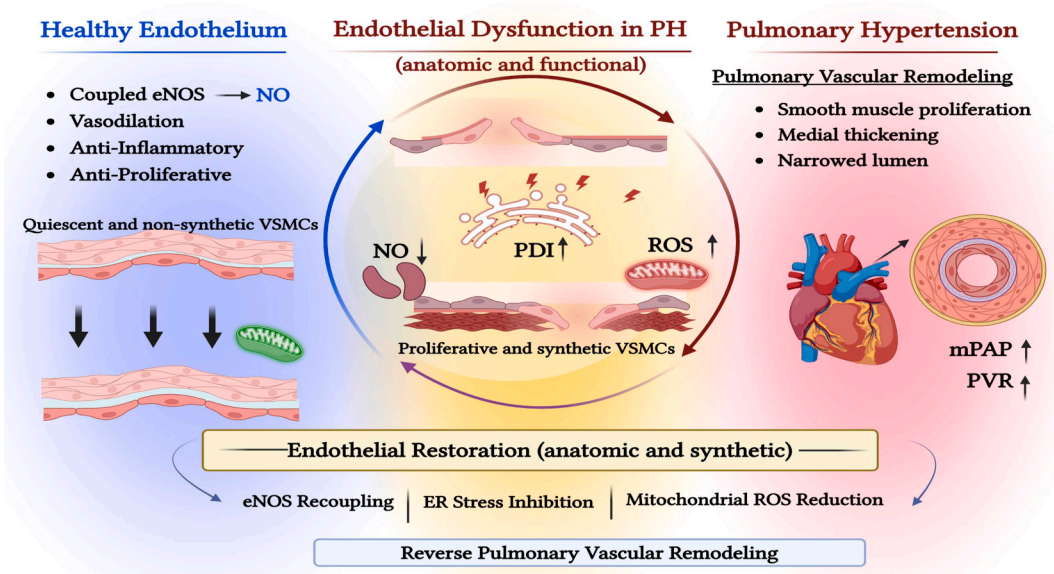
In 2024, the U.S. Food and Drug Administration approved 2 new treatments for PH. Opsynvi, a combination of macitentan (endothelin-1 receptor antagonist) and tadalafil (phosphodiesterase-5 inhibitor) for PH patients with or without cardiac comorbidities, offers convenience, and can improve patients' quality of life.[7] A newly developed drug, sotatercept, which works on a new biological pathway, represents a shift in the treatment of PH, because it targets vascular remodeling.[8] Sotatercept is a first-in-class fusion protein that combines the Fc domain of human IgG with the extracellular domain of activin receptor type IIA, acting as a ligand for transforming growth factor-β superfamily members. Inhibition of the altered transforming growth factor-β signaling by sotatercept can rebalance pulmonary vascular homeostasis by regulating enhanced smooth muscle and endothelial cell (EC) proliferation and diminished apoptosis.[8] Neither of the aforementioned methods specifically targets endothelial function and NO signaling.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2026.101528

FIGURE 1 **Endothelial Dysfunction Drives Pulmonary Vascular Remodeling in Pulmonary Hypertension**



In healthy pulmonary endothelium (left), endothelial cells remain structurally intact and maintain functional integrity, promoting a quiescent, synthetic vascular smooth muscle cell (VSMC) phenotype. Coupled endothelial nitric oxide synthase (eNOS) balances redox signaling by producing nitric oxide (NO), preserving vasodilation, and preventing smooth muscle proliferation. In pulmonary hypertension (middle), endothelial dysfunction is characterized by eNOS uncoupling, endoplasmic reticulum (ER) stress, and increased mitochondrial ROS production, leading to proliferative and synthetic VSMC and vascular remodeling. The downstream consequences (right) include medial thickening and increased resistance of small pulmonary arteries (PVR), elevated pulmonary arterial pressure (mPAP), and right ventricular hypertrophy. Targeting endothelial functional integrity (anatomic and synthetic EC restoration) through eNOS recoupling, reducing ER stress, and mitochondrial ROS can represent a potential therapeutic approach in pulmonary hypertension. Created in BioRender, Nofal M (2026), https://BioRender.com/qz6071y.

In this issue of the *JACC: Basic to Translational Science*, Murugesan et al[9] described a new signaling pathway driven by endothelial nitric oxide synthase (eNOS) uncoupling as a driver and target in PH. Moreover, this abnormality could be potentially targeted by currently available oral treatments further setting the need to examine the innovative approaches within this paper to understand underlying pathophysiological mechanisms of PH. One of the paper's findings that warrants emphasis is that the endothelium itself can indeed be the primary target of therapy in PH. Restoring functional endothelial signaling can then regulate smooth muscle cell proliferation and halt the pathological processes of PH. Thus, endothelial restoration could serve as a central, disease-modifying therapeutic strategy.

In Murugesan et al,[9] the authors identify a previously unrecognized mechanism where the eNOS uncoupling- endoplasmic reticulum (ER) stress-mitochondrial dysfunction signaling axis serves as a central mechanism driving the pathogenesis of PH in both the hypoxia-induced and the 2,4-diamino 6-hydroxypyrimidine (DAHP) model. In the group's previous publication, a new human-like model was described using DHAP, an inhibitor of tetrahydrobiopterin cofactor of eNOS, which stabilizes and regulates the enzyme's coupling and uncoupling and the amount of bioavailable NO, enabling translational studies of PH.[10] In this current study, the authors have proven that uncoupling of eNOS is sufficient to induce PH. Treating the mice with DAHP resulted in a severe PH phenotype, characterized by elevated mPAP and right ventricular systolic pressure, along with right ventricular hypertrophy and pulmonary vascular remodeling. Histopathologic analysis confirmed increased medial and intimal thickening, lesions, and luminal occlusion, resembling hypoxia-induced pulmonary arterial hypertension. In Murugesan et al[9] in both PH animal models, restoring eNOS coupling with folic acid (FA) or attenuating ER stress with 4-phenylbutyric acid (PBA), 2 readily available therapies significantly reduced mPAP and

right ventricular systolic pressure to near-baseline levels. Consistently, the pathologic findings of increased medial thickening, elevated smooth muscle α-actin expression, increased proliferating cell nuclear antigen staining, and enhanced collagen deposition were also completely reversed. Combined FA+PBA treatment did not provide additional benefit, indicating that eNOS uncoupling and ER stress use the same signaling pathway and further supports a common mechanistic axis underlying vascular remodeling. Murugesan et al[9] studies revealed that ER stress occurs after eNOS uncoupling because the increased ER stress marker protein disulfide isomerase (PDI) in pulmonary ECs in both PH models. Restoring eNOS coupling with FA reduced PDI expression, indicating that eNOS uncoupling triggers ER stress. PBA treatment also lowered PDI levels, and again, combining FA and PBA did not further decrease ER stress markers.

Oxidative stress analysis using L-NAME and electron spin resonance demonstrated robust eNOS-derived superoxide production in both PH models, confirming eNOS uncoupling in vivo. Recoupling of eNOS with FA abolished L-NAME-dependent superoxide production, while attenuation of ER stress with PBA similarly reduced eNOS uncoupling, indicating a feed-forward mechanism linking ER stress and eNOS dysfunction. NO bioavailability was markedly reduced in both models and fully restored by FA, PBA, or combined treatment.

Downstream, DAHP and hypoxia induced mitochondrial ROS production, mitochondrial swelling, and cristae disruption, all prevented by FA or PBA. Reduction of mitochondrial ROS with Mito-TEMPO attenuated PH hemodynamics, vascular remodeling, oxidative stress, and restored NO bioavailability, establishing mitochondrial dysfunction as a necessary effector of PH.

As for all studies, there are potential limitations in Murugesan et al.[9] First, they used DAHP to uncouple eNOS but did not consider the possible off-target effects of DAHP. This could include alterations in endothelial metabolism. More studies are needed to exclude or characterize effects of DAHP on other cell types. Are the observed phenotypes only caused by eNOS uncoupling, or do they have a broader effect that was not evaluated? In addition to systemic pharmacologic inhibition, cell-specific (especially EC) genetic modification of eNOS coupling and uncoupling would further strengthen the relevance of the findings.

An additional unanswered question is whether sex-specific differences exist in the endothelial eNOS uncoupling-ER stress-mitochondrial dysfunction pathway, which is especially notable with the authors providing a translation use of the study results. Given well-established sex differences in PH, future analyses are important to determine whether therapeutic modulation of this pathway differs between men and women before assuming that outcomes and safety will be the same.

Finally, the study highlights important differences between anatomic vs functional EC covering of vascular smooth muscle cells. If/when EC coverage is lost/reduced, increasing EC number might be assumed to be good. Experimental and human studies show that vascular endothelial growth factor (VEGF)-driven endothelial proliferation and angiogenesis, however, lead to the formation of functionally impaired, highly permeable vessels with disrupted junctional integrity and reduced nitric oxide signaling. Anatomic coverage does not equal normal vascular function. In the classic sugen/hypoxia model of PH, blocking VEGFR2 in combination with hypoxia induces severe PH caused by increased endothelial apoptosis, whereas preserved VEGF signaling permits endothelial proliferation but often fails to restore barrier integrity or vasodilatory capacity. These observations reinforce the concept emphasized by Murugesan et al[9] that an intact endothelium with normal eNOS coupling with ER and mitochondrial integrity are critical for targeting EC uncoupling as a viable therapeutic option to treat PH, a condition in desperate need of new therapies.

ACKNOWLEDGMENT Figure 1 was created with BioRender.com with a paid subscription.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by HL16492 (Dr Annex Co-I) and 25CVD-3 Leducq Foundation (to Drs Kasa and Annex).

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JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 11, NO. 4, 2026
APRIL 2026:101528

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KEY WORDS endothelial dysfunction, eNOS uncoupling, ER stress, mitochondrial dysfunction, pulmonary hypertension

APPENDIX For a supplemental figure, please see the online version of this paper.