You Can't Spell Shear without "She": Mechanobiology and Sex Differences in Hypoxic Lung Disease

In a number of lung diseases, pulmonary vascular endothelial cells experience hypoxia. Chronically, hypoxia contributes to vasoconstriction and vascular remodeling, often leading to pulmonary hypertension, which increases morbidity and mortality. When vasoconstriction and vascular remodeling occur, the biomechanical forces that act on pulmonary vascular endothelial cells also change (1). Cyclic stretch and shear stress are two important mechanobiological stimuli that change in pathological conditions and have been shown to act directly on vascular endothelial cells, contributing to cell hypertrophy, hyperplasia, and extracellular matrix deposition (2). Although the effects of these mechanical stimuli are commonly studied *in vitro*, the methods used to expose vascular cells to cyclic stretch and shear vary widely, with inconsistent control conditions, degree and duration of exposure, mode of exposure (e.g., static vs. dynamic), and, thus, physiological relevance. Additionally, hormonal and genetic differences associated with sex have been shown to affect vascular remodeling in multiple pulmonary vascular diseases (3–5). Sex differences in the progression of pulmonary vascular disease remain critically understudied despite clear evidence that estrogen and the female sex play important roles in vascular remodeling and the development of pulmonary hypertension (3). Limitations imposed by these oversights have hindered the translation of pulmonary vascular research to the clinic, especially for female patients (6). Thus, improved understanding of the interactions among hypoxia, mechanobiological stimuli, sex differences, and their sequelae could provide valuable insight into the incidence and progression of hypoxic lung diseases as well as clinical outcomes.

In this issue of the *Journal,* Kostyunina and colleagues (pp. 551–565) directly address this critical knowledge gap by studying the combined effects of hypoxia, shear stress, and sex differences on pulmonary endothelial cells (7). The authors subjected human pulmonary microvascular endothelial cells from healthy male and female donors to shear stress at 10 dyn/cm^2 using an orbital shaker. Shear stress was applied in normoxic conditions for 24 hours and switched to hypoxic conditions or maintained at normoxic conditions for an additional 24 hours. Cells from the same donors cultured under static conditions (i.e., zero shear stress) were used as controls. Gene and protein expression for all cells were assessed using RT-PCR, ELISA, Western blot, mass spectrometry, and immunofluorescent staining. To further assess the gene expression of cells in the disease state, single-cell RNA sequencing was performed on pulmonary endothelial cells from male and female healthy donors and patients with chronic obstructive pulmonary disease (COPD). The authors identified 60 proteins whose expression was altered by hypoxia and shear stress but not by hypoxia alone, many of which had not previously been shown to be hypoxia-responsive. Among the novel findings was the reduction of JAM-A, a protein required for maintaining vascular permeability, by the combination of hypoxia and shear stress. The authors suggest this as a possible mechanism of increased pulmonary vascular permeability in hypoxic lung disease. This result highlights the importance of including mechanobiological stimuli in *in vitro* disease models as well as combining relevant stimuli to more accurately mimic the *in vivo* state. The authors also observed differences in gene expression between cells from healthy female donors and those with COPD that were not observed between cells from healthy male donors and those with COPD, suggesting an important pulmonary vascular cell sex difference in patients with hypoxic lung disease. Although the authors did not further explore the mechanism behind this finding because of the small sample size and lack of statistical power, their attention to sex differences in the context of pathological hypoxia and mechanical stimuli is commendable.

The standardization of mechanical stimuli used *in vitro* and the acknowledgment of sex differences in pulmonary vascular research need to be prioritized to advance knowledge of and develop therapies for hypoxic lung disease and to reduce sex-based health disparities. For future work that builds on the results of Kostyunina and colleagues, we recommend the following modifications. First, the use of an orbital shaker for shear stress application creates a nonhomogenous mechanical stimulus that is dependent on position. Although the authors computed the shear stress exposure with computational fluid dynamics simulations, the magnitude of the stimulus was still a function of radius from the center of the orbital shaker. As an alternative, laminar flow chambers or cone-and-plate viscometers ensure an equal shear stress is applied to the entire surface area (8, 9). Additionally, these devices allow for larger shear magnitudes than the orbital shaker could impose. This is especially relevant for the microvascular endothelium, in which shear stress can reach an order of magnitude higher than in larger vessels, depending on vessel size and/or disease state (10–12). Second, the use of static culture conditions as a control is not physiologically relevant. In the healthy and disease states, the endothelium is always subjected to nonzero shear stress. The magnitude and pulsatility of shear stress vary throughout the circulation and with disease progression, but zero shear is not physiological. A more appropriate comparison is between pathophysiological mechanical cues and physiological ones. Depending on the disease and circulation of interest, pathophysiological shear could be abnormally high or low, abnormally pulsatile or nonpulsatile (13–15). Third, although Kostyunina and colleagues did consider sex-based differences in their analysis, they were limited

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in statistical power because of a low sample size. For future studies, sex should be considered in sample size determination so that any sex-based differences can be uncovered during data analysis.

In conclusion, Kostyunina and colleagues provide a novel and inclusive approach to studying the synergistic effects of hypoxia, mechanical stimuli, and sex differences in hypoxic lung disease. Their findings underscore the importance of considering multiple physiological stimuli in the context of conditions associated with pulmonary hypertension. Moving forward, it will be imperative to investigate the impact of mechanical stimuli on pulmonary vascular disease mechanisms in the most physiologically relevant conditions. Furthermore, understanding the sex-dependent mechanisms of the development and progression of pulmonary vascular disease will be critical to diagnosis and treatment of all patients. ■

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