



Sex-Dependent Differences in Pulmonary Vascular Resistance, Impedance, and Distensibility Revealed by Impaired Shear Stress Mechanotransduction

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Sex-dependent differences in pulmonary vascular mechanics have been reported in both healthy and diseased populations, yet the mechanisms contributing to these differences remain poorly defined. Differences in shear stress responsiveness by the pulmonary endothelial surface may contribute to these differences in mechanics. This study investigated sex differences in shear stress responsiveness by measuring pulmonary pressure-flow relationships in isolated perfused lungs from male and female C57BL/6J mice. Pressure-flow relationships were measured under steady and pulsatile flow conditions before and after treatment with heparinase I to impair shear stress mechanotransduction. Pulmonary vascular resistance (PVR), vascular impedance, wave reflectance (R_w), and pulmonary vascular distensibility (α) were quantified from pressure-flow data. Prior to treatment, male and female lungs exhibited comparable pulmonary vascular mechanics. Following heparinase I treatment, female lungs demonstrated significantly greater increases in PVR, 0-Hz impedance (Z_0), and R_w than male lungs. In contrast, characteristic impedance (Z_c) remained unchanged in both sexes post-treatment compared to pretreatment, indicating preservation of proximal conduit arterial mechanics. Female lungs also exhibited lower post-treatment distensibility than males, suggesting reduced pulmonary vascular adaptation to increasing flow. Collectively, these findings indicate that impaired shear stress mechanotransduction had a greater impact in females than males and predominantly affected distal pulmonary vascular function while preserving proximal arterial properties. Our findings reveal significant sex differences in pulmonary vascular function with impaired shear stress mechanotransduction. These findings highlight the importance of considering sex as a biological variable in studies of pulmonary vascular biomechanics and vascular adaptation to flow. [DOI: 10.1115/1.4072671]

Introduction

The pulmonary circulation is a low-pressure, high-compliance vascular network that accommodates the entire cardiac output while maintaining efficient gas exchange. Alterations in pulmonary vascular mechanics contribute significantly to right ventricular afterload and are central to the development and progression of pulmonary vascular diseases, including pulmonary hypertension [1]. While pulmonary vascular resistance (PVR) remains a commonly used index of vascular function, it captures only the steady-flow component of right ventricular afterload dominated by small distal vessels [2]. Pulmonary vascular impedance provides a more comprehensive assessment by characterizing both steady and pulsatile hemodynamic properties, allowing evaluation of vascular resistance, compliance, and wave reflection throughout the pulmonary circulation [2]. Understanding the mechanisms that regulate pulmonary vascular mechanics is therefore essential for

identifying contributors to pulmonary vascular dysfunction and disease progression.

The endothelial surface layer serves as a critical interface between flowing blood and the vascular wall, contributing to endothelial barrier function, vascular homeostasis, and shear stress mechanotransduction [3–5]. Atop the endothelial cell membrane sits a critical regulator of these functions, termed the glycocalyx, composed of membrane-bound proteoglycans, including syndecans and glypican, together with glycosaminoglycan chains such as heparan sulfate, chondroitin sulfate, and hyaluronic acid. Among these components, heparan sulfate-rich structures have been identified as key mediators of endothelial shear stress sensing, participating in the transmission of mechanical forces to intracellular signaling pathways involved in vascular homeostasis, vasodilation, and vascular remodeling [3–5]. Consequently, perturbation of the glycocalyx may alter pulmonary vascular responses to flow and contribute to changes in pulmonary vascular mechanics.

Although glycocalyx dysfunction has been implicated in pulmonary vascular injury and endothelial dysfunction, its influence on pulmonary vascular mechanics remains incompletely understood

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[5–7]. Previous investigations have primarily focused its impact on endothelial permeability, inflammatory signaling, and barrier integrity, whereas comparatively little is known regarding how perturbation of the glycocalyx affects shear stress sensing and thus PVR, vascular impedance, wave reflection, and vascular adaptation to changes in flow. Because endothelial mechanotransduction links hemodynamic loading to nitric oxide production, vascular tone, and vascular remodeling [3,4,6,7], alterations in these pathways may have important consequences for pulmonary vascular function that are not captured by traditional assessments of vascular injury alone.

The endothelial glycocalyx has been identified as a critical mechanosensor that mediates shear stress-induced endothelial signaling through pathways involving mechanosensitive ion channels, intracellular calcium signaling, phosphoinositide 3-kinase (PI3K), endothelial nitric oxide synthase (eNOS), and nitric oxide production [3–6]. While these signaling pathways have been extensively studied in relation to endothelial function and vascular tone, considerably less is known about how glycocalyx-mediated mechanotransduction influences integrated pulmonary vascular mechanical properties, including vascular resistance, impedance, wave reflection, and distensibility [3–7]. Moreover, whether glycocalyx-dependent regulation of pulmonary vascular mechanics differs between males and females remains largely unexplored [8–11].

Clinical and experimental studies have identified important sex-dependent differences in pulmonary vascular function and vascular remodeling within the pulmonary circulation [8,9]. Emerging evidence further suggests that males and females differ in endothelial responses to mechanical stimuli and responses to vascular mechanical loading [8,10]. Together, these observations raise the possibility that male and female pulmonary circulations may differ in their responses to perturbations of endothelial flow sensing and vascular adaptation [8–11]. Heparinase I provides a useful experimental tool for perturbing endothelial surface-layer-associated signaling by targeting heparan sulfate-rich components involved in mechanosensing and flow-mediated responses [12,13]. However, sex-specific pulmonary vascular mechanical responses to heparinase I treatment have not been previously measured. Whether perturbation of endothelial shear stress mechanotransduction reveals sex-dependent differences in pulmonary vascular function remains unknown.

Therefore, the objective of the present study was to investigate sex-dependent differences in pulmonary vascular mechanics with and without impaired mechanotransduction. In isolated perfused mouse lungs of male and female mice, PVR, vascular impedance, wave reflection, and distensibility were evaluated before and after treatment. We hypothesized that impaired shear stress mechanotransduction would alter pulmonary vascular function and reveal sex-dependent differences in pulmonary vascular adaptation and flow-mediated regulation.

Methods

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Irvine and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male ($n=6$) and female ($n=6$) C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA), aged 12–14 weeks, were used in this study. Male mice weighed 29.7 ± 1.6 g and female mice weighed 23.3 ± 2.5 g (mean $\pm$ SD), with body weight significantly greater in males than females (unpaired t -test, $p < 0.001$). Hemodynamic analyses were conducted using a paired design, with measurements obtained from each lung before and after heparinase I treatment. Mice were housed under standard conditions with ad libitum access to food and water.

Reagents and Materials. Heparinase I (from *Flavobacterium heparinum*), RPMI-1640 medium, and Ficoll 400 were procured from Sigma–Aldrich (St. Louis, MO, USA). Heparin (1000 IU/mL)

and urethane were obtained from Thermo-Fisher (Waltham, MA, USA). The commercial isolated perfused lung (IPL) system (Size 1, Type 839) was purchased from Hugo-Sachs Elektronik (March-Hugstetten, Germany).

Isolated Perfused Lung (IPL) Preparation. The IPL model was established using a commercially available system, following our previously validated protocols for measuring pulmonary pressure-flow relationships with adaptations for pulsatile flow [14,15].

Mice were anesthetized via intraperitoneal injection of urethane (1200 mg/kg). A tracheotomy was performed, and the animal was mechanically ventilated with room air using a positive-pressure ventilator. Following a midline sternotomy, 100 IU of heparin was injected into the right ventricle to prevent coagulation. The pulmonary artery (PA) and left atrium (LA) were then cannulated with stainless-steel cannulae. The lower half of the body was excised to prevent interference with lung expansion. Successful cannulation was confirmed by visual observation of uniform perfusate flow from the LA into the collection reservoir.

Mice were mechanically ventilated with room air using a positive-pressure ventilator operated in pressure-controlled mode with inspiratory and expiratory pressures of 12 and 2 cm H₂O, respectively, as previously described by Vanderpool and Chesler [14,15]. The ventilation control module was set to a respiratory rate of 90 breaths/min with a 1:1 inspiration-to-expiration ratio (50% inspiratory duty cycle). Ventilation was maintained throughout the entire experiment, including the equilibration, pretreatment, treatment, and post-treatment phases. Because the ventilator was pressure-controlled, tidal volume was not directly prescribed but varied with lung compliance. Pulmonary hemodynamic measurements were obtained during static inflation at an airway pressure of 12 cm H₂O to eliminate respiratory motion and minimize the influence of cyclic ventilation on pressure-flow measurements.

Lungs were perfused at a constant flowrate of 1 mL/min with RPMI-1640 media supplemented with 3.5% Ficoll 400 (as an oncotic agent). The left atrial pressure (P_{LA}) was maintained at 2 mmHg by adjusting the height of the effluent reservoir, with the zero-pressure reference set at the level of the atrium. Baseline pulmonary arterial pressure (PAP) ranged from 10–13 mmHg.

All preparations underwent a 10-minute equilibration period to ensure clearance of blood from the pulmonary vasculature, indicated by a homogeneous blanching of the lung tissue. Preparations exhibiting edema, or nonuniform perfusion were excluded from analysis. Following equilibration, baseline hemodynamic measurements were then obtained during the pretreatment phase. Lungs were subsequently perfused with RPMI-1640/Ficoll containing 10 U Heparinase I for 10 min, followed by a 10-minute re-equilibration period with enzyme-free perfusate before post-treatment hemodynamic measurements were acquired.

Experimental Protocol. Following equilibration, each lung preparation underwent a sequential perfusion protocol (Fig. 1):

- **Pretreatment phase:** lungs were perfused with the RPMI-Ficoll solution under both steady and pulsatile flow conditions. For steady-state pressure-flow measurements, the flow rate (Q) was incremented in a stepwise manner from 1 to 5 mL·min⁻¹. For pulsatile hemodynamic assessment, perfusion was driven by a sinusoidal flow profile of the form $Q(t) = 3 + 2 \sin(2\pi ft)$ mL/min, applied sequentially in ascending order at frequencies (f) of 1, 2, 5, 10, 15, and 20 Hz. To isolate the effects of perfusion from respiratory mechanics, all data were recorded under static inflation at an airway pressure of 12 cm H₂O to eliminate respiratory motion while maintaining a constant lung volume throughout hemodynamic measurements. Pulmonary arterial pressure (PAP) and left atrial pressure (LAP) were recorded continuously.
- **Treatment phase:** lungs were perfused with 10 U of heparinase I dissolved in 50 mL RPMI-Ficoll solution at a flow rate of 5 mL/min. Heparinase I dose was selected based on prior

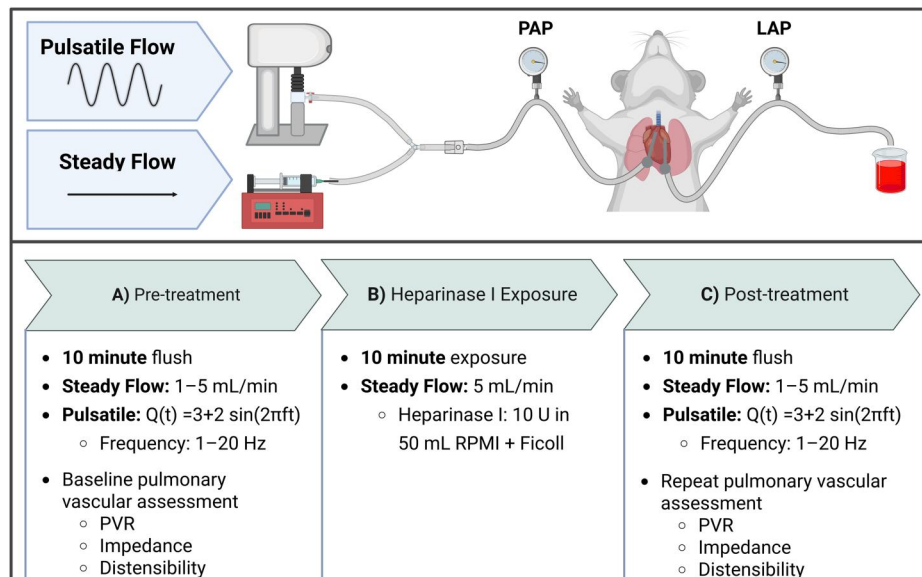


Fig. 1 Experimental protocol for assessment of pulmonary vascular responses to heparinase I exposure in isolated perfused mouse lungs. Following a 10-minute equilibration period, baseline steady-flow and pulsatile-flow measurements were obtained. Lungs were then perfused with 10 U heparinase I in 50 mL RPMI-Ficoll solution for 10 min, followed by a 10-minute re-equilibration period before PVR, impedance, and distensibility measurements were repeated. PAP, pulmonary arterial pressure; LAP, left atrial pressure. Created in <https://BioRender.com>.

studies employing enzymatic degradation of endothelial heparan sulfate in isolated lung and vascular preparations [12,13] and was chosen to produce a measurable physiological perturbation of endothelial surface-layer-associated function based on preliminary studies in male and female mouse lungs.

- **Post-treatment phase:** perfusion was reverted to the standard RPMI-Ficoll solution at the baseline rate of $1 \text{ mL} \cdot \text{min}^{-1}$. After a 10-minute re-equilibration period, all steady-flow and pulsatile hemodynamic measurements were repeated.

Pulmonary Vascular Impedance Analysis. Pulmonary vascular input impedance was determined at oscillatory perfusion frequencies of 1, 2, 5, 10, 15, and 20 Hz using methods previously described by Vanderpool et al. and Vanderpool and Chesler [14,15]. Pressure and flow waveforms were digitized and analyzed in the frequency domain using fast Fourier transform (FFT) analysis. Pulmonary vascular input impedance was calculated as the ratio of the pressure and flow Fourier transforms

$$Z(f) = \frac{P(f)}{Q(f)}$$

where $P(f)$ and $Q(f)$ represent the Fourier transforms of pulmonary arterial pressure and flow, respectively.

The impedance spectrum was characterized using three established metrics. Zero-frequency impedance (Z_0) represents the steady-flow component of the impedance spectrum and is analogous to pulmonary vascular resistance, reflecting the cumulative resistance of the pulmonary vascular bed, which is dominated by the distal resistance vessels. Characteristic impedance (Z_C) was calculated as the arithmetic mean of the impedance magnitudes obtained at the first harmonic of the 5-, 10-, 15-, and 20-Hz oscillatory perfusion conditions, corresponding to the high-frequency plateau of the impedance spectrum, following Vanderpool et al. [14,15]. At these frequencies, the influence of wave reflections is minimal; therefore, impedance primarily reflects the local mechanical properties of the proximal conduit pulmonary

arteries, including vessel stiffness and diameter [14–16]. Wave reflectance (R_W) was calculated as

$$R_W = \frac{Z_0 - Z_C}{Z_0 + Z_C}$$

as previously described by Vanderpool et al. [14,15].

Pulmonary Vascular Distensibility. Pulmonary vascular distensibility was estimated from steady-flow pressure-flow relationships using the distensible vessel model originally described by Linehan et al. [17]. Distensibility (α) represents the fractional change in vessel diameter per mmHg increase in transmural pressure and provides an index of the pulmonary circulation's ability to accommodate increasing flow through vascular distension.

For each lung, baseline resistance (R_0) was estimated from the lowest flow condition ($1 \text{ mL} \cdot \text{min}^{-1}$), using pulmonary arterial pressure (P_a), left atrial pressure (P_{la}), and flow (Q) measurements.

Distensibility was determined by fitting measured pressure-flow data to the distensible vessel model

$$P_a = \frac{\left[(1 + \alpha P_{la})^5 + 5\alpha R_0 Q \right]^{\frac{1}{5}} - 1}{\alpha}$$

using nonlinear least-square optimization implemented in MATLAB (MathWorks, MA, USA). Distensibility (α) was the only free parameter during optimization, while R_0 remained fixed at the value calculated from the $1 \text{ mL} \cdot \text{min}^{-1}$ flow condition. Distensibility values were determined for each lung before and after heparinase I treatment and compared between treatment conditions and sexes.

Statistical Analysis. Sample size was determined a priori based on power analyses of the primary pulmonary vascular impedance outcomes (Z_0 , Z_C , and R_W). Using effect sizes derived from preliminary studies, six animals per sex provided at least 80% statistical power for the primary impedance analyses. Pulmonary vascular distensibility (α) was evaluated as a complementary

secondary outcome and was not used to determine sample size. Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism. Two-way repeated-measures ANOVA was used to evaluate the main effects of treatment and sex, as well as treatment-by-sex interactions, on pulmonary vascular hemodynamic variables, including PVR, impedance metrics, and distensibility. When significant main effects or interactions were identified, posthoc multiple-comparison testing was performed using Fisher's least significant difference test. Statistical significance was defined as $p < 0.05$.

Results

Baseline Pulmonary Hemodynamics. Prior to heparinase I treatment, male and female lungs demonstrated comparable steady-flow pulmonary hemodynamics across all flow conditions ($1\text{--}5\text{ mL}\cdot\text{min}^{-1}$). In both groups, PVR progressively decreased with increasing flow, consistent with normal pulmonary vascular adaptation to increasing flow through vascular recruitment, distension, and flow-mediated dilation within the pulmonary circulation (Fig. 2). Although female lungs exhibited modestly higher PVR values across flow conditions, no statistically significant sex-dependent differences were observed at baseline.

Sex-Dependent Changes in Pulmonary Vascular Resistance Following Heparinase I Treatment. Heparinase I treatment increased PVR in both sexes, with substantially larger effects observed in female lungs (Fig. 2). In male mice, post-treatment increases in PVR were small and did not reach statistical significance across flow conditions. In contrast, female mice demonstrated significant increases in PVR following treatment across all flow rates ($1\text{--}5\text{ mL}\cdot\text{min}^{-1}$), with the greatest absolute differences observed at lower flow rates. At $1\text{ mL}\cdot\text{min}^{-1}$, PVR increased approximately 32% relative to baseline ($p = 0.014$). In both sexes, the magnitude of the treatment-associated increase in PVR progressively diminished as flow increased. However, the increase in PVR was consistently greater in female lungs, particularly at lower flow rates.

Sex-Dependent Changes in Pulmonary Vascular Impedance Following Heparinase I Treatment. To evaluate sex-dependent pulmonary vascular responses to heparinase I treatment under pulsatile flow conditions, pulmonary vascular impedance spectra

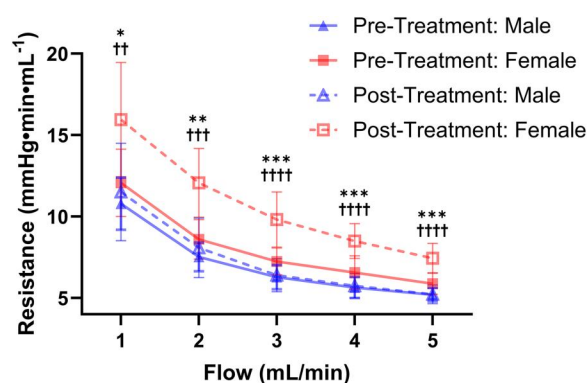


Fig. 2 Effects of heparinase I treatment on steady-flow PVR. Pulmonary vascular resistance was measured across flow rates ranging from $1\text{--}5\text{ mL}\cdot\text{min}^{-1}$ in isolated perfused lungs from male (triangle) and female (square) mice before and after heparinase I treatment. Solid lines and filled symbols represent pretreatment conditions, whereas dashed lines and open symbols represent post-treatment conditions. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus pretreatment female at the same flowrate. †† $p < 0.01$, ††† $p < 0.001$, and †††† $p < 0.0001$ versus post-treatment male at the same flowrate (Fisher's least significant difference test).

were derived from sinusoidal pressure-flow relationships. The impedance magnitude and phase spectra demonstrated preservation of the high-frequency impedance plateau following treatment, consistent with minimal changes in proximal conduit arterial mechanics (Fig. 3(a)).

Analysis of Z_0 , an index analogous to steady-flow PVR at a mean flowrate of $3\text{ mL}/\text{min}$, revealed significant effects of treatment ($p = 0.0030$), sex ($p = 0.0024$), and a treatment-by-sex interaction ($p = 0.0071$) (Fig. 3(b)). Following treatment, female mice demonstrated significantly greater Z_0 values compared with males ($p < 0.0001$) and exhibited significant increases relative to pretreatment conditions ($p = 0.0004$).

In contrast, Z_C was not significantly altered by treatment or sex. R_W demonstrated a significant overall treatment effect ($p = 0.0178$). Post-treatment R_W was significantly greater in females compared with males ($p = 0.0483$), and female mice exhibited increased R_W relative to baseline conditions ($p = 0.0081$).

Pulmonary Vascular Distensibility. Pulmonary vascular distensibility (α) was estimated from steady-flow pressure-flow relationships using the distensible vessel model. Baseline distensibility did not differ significantly between male and female lungs (female: $0.0097 \pm 0.0023\text{ mmHg}^{-1}$; male: $0.0110 \pm 0.0015\text{ mmHg}^{-1}$; $p = 0.29$). Following heparinase I treatment, female lungs demonstrated a reduction in α to $0.0081 \pm 0.0028\text{ mmHg}^{-1}$, whereas male lungs exhibited minimal change ($0.0113 \pm 0.0017\text{ mmHg}^{-1}$). Although neither the overall treatment effect nor treatment-by-sex interaction reached statistical significance, post-treatment α values were lower in females than males ($p = 0.019$) (Fig. 4).

Discussion

The principal finding of this study is that pulmonary vascular responses to heparinase I treatment differed substantially between sexes. Although male and female lungs exhibited comparable baseline pulmonary vascular mechanics, female lungs demonstrated larger increases in PVR, Z_0 , and R_W following treatment. Z_C remained unchanged in both sexes, while pulmonary vascular distensibility was lower in females than males following treatment. Together, these findings indicate that identical heparinase I exposure produced distinct hemodynamic responses in male and female lungs despite comparable baseline pulmonary vascular mechanics.

Prior to treatment, male and female lungs demonstrated comparable steady-flow hemodynamics across all flow conditions, indicating similar baseline pulmonary vascular function between experimental groups. Although sex-dependent differences in PVR, elastance, and vascular compliance have been reported in human studies, such differences are not consistently observed across populations or experimental conditions [18,19]. In both male and female mouse lungs, PVR decreased progressively with increasing flow, reflecting the normal ability of the pulmonary circulation to accommodate increased perfusion through a combination of vascular recruitment, distensibility, and flow-mediated vasodilation [20,21]. The similar reduction in PVR observed in both sexes suggests comparable baseline pulmonary vascular adaptation to increasing flow. In addition, at baseline, Z_C and R_W were similar between male and female lungs, indicating comparable proximal arterial mechanical properties and impedance matching. Note, because similar mechanics were observed at baseline, we did not normalize metrics of resistance or impedance to body weight. Although male mice weighed significantly more than female mice, matching body weights between sexes was not feasible within this experimental model. Achieving comparable body weights would have required the use of substantially older female mice, introducing age as an additional biological variable. Conversely, using younger, lighter male mice was not practical because the smaller pulmonary artery diameter makes reliable cannulation for the isolated perfused lung preparation technically difficult. Therefore, age-matched animals were used, and the absence of baseline differences in pulmonary vascular mechanics despite the difference in body

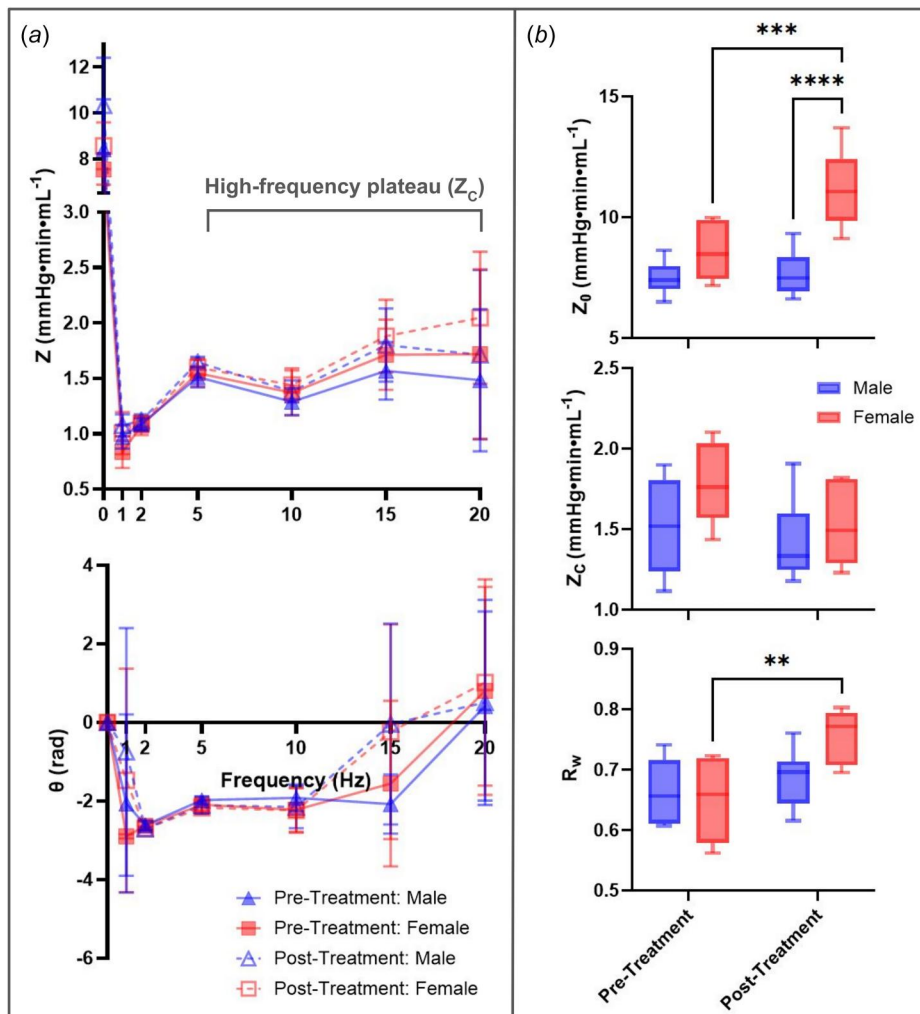


Fig. 3 Pulmonary vascular impedance spectra and derived impedance parameters following heparinase I treatment. (a) Pulmonary vascular impedance magnitude spectrum (left) and phase angle spectrum (right) measured during pulsatile perfusion before and after heparinase I treatment. The 0-Hz impedance (Z_0) corresponds to steady-flow pulmonary vascular resistance, whereas characteristic impedance (Z_c) was calculated as the arithmetic mean of the high-frequency impedance plateau (5–20 Hz). Triangles represent male mice and squares represent female mice; solid lines and filled symbols represent pretreatment conditions, whereas dashed lines and open symbols represent post-treatment conditions. Data are presented as mean $\pm$ SEM. (b) Box-and-whisker plots showing pre- and post-treatment values of Z_0 , Z_c , and R_w in isolated perfused lungs from male and female mice. Within each treatment group, the left and right box plots represent male and female mice, respectively. Boxes represent the interquartile range (IQR) with the median indicated by the horizontal line; whiskers denote the minimum and maximum values. Heparinase I treatment increased Z_0 and R_w with larger post-treatment responses observed in female lungs, whereas Z_c remained unchanged in both sexes.

weight suggests that body size alone is unlikely to explain the differential responses observed following heparinase I treatment.

Following heparinase I treatment, PVR and Z_0 increased significantly in females, particularly at lower flows, whereas males exhibited only small, nonsignificant changes. In contrast, Z_c remained unchanged in both sexes, indicating that proximal conduit arterial mechanics were largely unaffected by treatment. Female lungs also demonstrated increased R_w , indicating greater impedance mismatch within the pulmonary vasculature. The observed increases in Z_0 and R_w in the absence of changes in Z_c are consistent with predominant effects on the distal pulmonary vasculature, as Z_0 primarily reflects the resistance of the distal pulmonary vascular bed whereas Z_c is generally accepted to reflect the mechanical properties of the proximal conduit arteries. This interpretation is based on established pulmonary vascular impedance theory and prior validation studies rather than direct localization of the vascular response [2,22,23].

This interpretation is consistent with previous isolated perfused lung studies demonstrating that alterations within distal pulmonary vascular segments can substantially influence PVR and pressure-flow relationships without producing proportional changes in proximal vascular mechanics [24,25]. Similar studies of pulmonary vascular impedance have shown that increased R_w arises from impedance mismatches within the pulmonary circulation and can increase right ventricular afterload despite relatively preserved proximal arterial properties [16,26,27]. The pattern of hemodynamic changes suggests that the functional consequences of heparinase I treatment arose predominantly within the distal resistance vasculature rather than the proximal conduit arteries. This interpretation is based on the selective increases in PVR, Z_0 , and R_w together with the absence of changes in Z_c , which is generally accepted to reflect the mechanical properties of the proximal pulmonary arteries. Because enzyme distribution was not

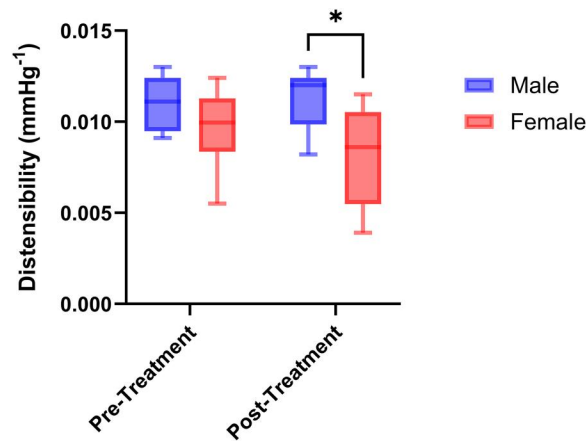


Fig. 4 Pulmonary vascular distensibility before and after heparinase I treatment. Pulmonary vascular distensibility (α) was estimated from steady-flow pressure-flow relationships using the distensible vessel model. Boxes represent the inter-quartile range (IQR) with median indicated by the horizontal line; whiskers indicate minimum to maximum values. Baseline distensibility was similar between sexes. Following heparinase I treatment, female lungs exhibited lower distensibility values than male lungs, suggesting reduced pulmonary vascular adaptation to increasing flow.

directly assessed, these findings should not be interpreted as evidence of preferential distal delivery or accumulation of heparinase I, but rather as evidence that the measured vascular response was dominated by distal vascular segments.

Distensibility analysis provided additional insight into the pulmonary vascular response to heparinase I treatment. Female lungs exhibited lower post-treatment pulmonary vascular distensibility than male lungs following treatment, whereas male lungs exhibited minimal change from baseline. Although post-treatment α values were lower in females than males, neither the overall treatment effect nor the treatment-by-sex interaction reached statistical significance. Therefore, these findings should be interpreted as complementary to the primary resistance and impedance measurements rather than as independent evidence of sex-dependent differences. Because pulmonary vascular distensibility contributes to the normal reduction in resistance with increasing flow [1,17], these findings are consistent with the larger increases in PVR and Z_0 observed in females following treatment and complement the impedance findings suggesting reduced flow-dependent vascular adaptation. Interestingly, while distensibility is often interpreted as reflecting the structural mechanical properties of the pulmonary vasculature [1,17], shear stress responsiveness may be a significant contributor. That is, instead of a decrease in distensibility with disease indicating distal arteriolar stiffening alone [28,29], this decrease may also reflect impaired shear stress-induced vasodilation in addition to changes in passive vascular mechanics.

Because the isolated lung experiments were performed over an acute time course, consisting of a 10-minute heparinase exposure followed by a 10-minute re-equilibration period, the observed changes in pulmonary vascular resistance, impedance, and distensibility are unlikely to reflect structural remodeling of the pulmonary vasculature. Instead, these findings are more consistent with acute alterations in endothelial mechanotransduction and vascular tone following glycocalyx degradation.

Previous studies have demonstrated that disruption of the endothelial glycocalyx can impair shear stress sensing and downstream nitric oxide signaling, providing a plausible mechanism for the altered pulmonary vascular responses observed following heparinase treatment [3–7]. Although baseline pulmonary vascular mechanics were similar between sexes, the present study did not evaluate potential sex-dependent differences in glycocalyx composition, structure, or mechanical properties, which could influence endothelial sensitivity to shear stress despite similar baseline

hemodynamics. Thus, any sex-dependent differences in endothelial mechanosensing would likely become apparent only when the glycocalyx-mediated flow-sensing pathway is challenged, as occurred following heparinase I treatment. Because nitric oxide is a principal mediator of shear stress-induced vasodilation, sex-dependent differences in endothelial nitric oxide production or bio-availability may have contributed to the larger increases in pulmonary vascular resistance and impedance observed in female lungs [3,4,11]. Although circulating sex hormones were absent in the isolated perfused lung preparation, long-term organizational effects of sex hormones on endothelial phenotype and vascular signaling before lung isolation may also contribute to these responses [8,30]. In addition, sex-dependent differences in vascular smooth muscle responsiveness or endothelial-smooth muscle communication may also contribute to the observed hemodynamic responses [10,22,31]. Future studies directly examining glycocalyx integrity, nitric oxide bio-availability, endothelial mechanotransduction pathways, and smooth muscle signaling will help define the molecular basis of these acute functional changes.

Future studies also should seek to identify the specific mechanotransduction pathways responsible for the sex-dependent vascular responses observed in the present study. While in vivo models are well suited for determining the integrated physiological consequences of altered endothelial flow sensing, they are less capable of isolating glycocalyx-dependent signaling from the influences of circulating hormones, inflammatory mediators, blood cells, and neurohumoral regulation. Complementary *ex vivo* and in vitro approaches, including endothelial cell culture systems with preserved or engineered glycocalyx structures and advanced organ-on-chip platforms, may provide greater mechanistic insight into glycocalyx-dependent signaling under controlled shear stress conditions [32]. These platforms also offer the opportunity to directly compare male- and female-derived endothelial cells under identical hemodynamic conditions [22]. Although direct comparisons of endothelial glycocalyx composition between healthy male and female endothelial cells remain limited, emerging evidence suggests that female sex hormones influence glycocalyx maintenance and integrity [33]. Together, these approaches may help determine whether intrinsic sex-dependent differences in glycocalyx regulation underlie the differential pulmonary vascular responses observed following impaired flow sensing.

Taken together, the present findings suggest that male and female murine pulmonary circulations may differ in their reliance on endothelial regulatory mechanisms involved in flow sensing and vascular adaptation. Prior studies have reported sex-dependent differences in pulmonary vascular function, endothelial signaling, and pulmonary vascular regulation in both human and experimental models [11,18,30,31]. Although baseline differences were not observed in the present study, the larger increases in PVR, Z_0 , and R_w following heparinase I treatment suggest that these regulatory pathways become more apparent after endothelial perturbation. Collectively, these findings support the hypothesis that endothelial shear stress mechanotransduction contributes to sex-dependent regulation of pulmonary vascular function.

Several limitations should be considered when interpreting these findings. First, the study was designed to evaluate functional pulmonary vascular responses to heparinase I treatment and was not intended to directly quantify alterations in endothelial surface-layer structure or verify glycocalyx degradation following heparinase I treatment, nor to identify the specific signaling pathways responsible for the observed sex-dependent responses. Second, the isolated perfused lung preparation excludes systemic influences that may contribute to sex-dependent pulmonary vascular regulation in vivo, including circulating sex hormones, blood cells, inflammatory mediators, and neurohumoral signaling. Although removal of circulating blood components may alter endothelial signaling compared with the in vivo state, all lungs underwent an identical equilibration protocol before baseline measurements. Consequently, any effects associated with perfusate equilibration would

be expected to be consistent across experimental groups and are therefore unlikely to account for the observed sex-dependent differences following heparinase I treatment. Third, measurements from the isolated perfused lung were taken at end-inspiration with positive pressure ventilation. Although ventilation can influence pulmonary vascular hemodynamics through cyclic changes in lung volume, all lungs were ventilated using identical settings throughout the experiment, and all hemodynamic measurements were obtained during standardized static inflation at 12 cm H₂O. Consequently, any influence of ventilation would be expected to be uniform across experimental groups and is therefore unlikely to account for the observed sex-dependent differences. Fourth, estrous cycle stage was not monitored or controlled in the female mice. Although fluctuations in ovarian hormones have been reported to influence vascular physiology in some experimental settings, the extent to which estrous cycle stage affects pulmonary vascular responses in isolated perfused lungs remains unclear [34]. Because our experiments were performed in an *ex vivo* isolated lung preparation, acute effects of circulating ovarian hormones were absent during hemodynamic measurements. Nevertheless, future studies examining pulmonary vascular responses across the estrous cycle or following hormonal manipulation will be important to determine whether ovarian hormone status contributes to pulmonary endothelial mechanotransduction and vascular function. Furthermore, because this study was performed in healthy murine lungs, caution should be exercised when extrapolating these findings to human pulmonary vascular physiology or disease. Future studies in vivo and in human-relevant experimental models will be necessary to determine the translational significance of these observations. Finally, the distensible vessel model assumes a fixed baseline resistance (R_0), which may influence estimated distensibility values. Despite these limitations, the consistent hemodynamic responses observed across resistance, impedance, and distensibility measurements support the presence of sex-dependent differences in pulmonary vascular adaptation following impaired flow sensing.

Conclusion

Heparinase I treatment revealed significant sex-dependent differences in pulmonary vascular function in isolated perfused mouse lungs. Female lungs exhibited greater increases in PVR, Z_0 , and R_W . Lower post-treatment pulmonary vascular distensibility was also observed in females; however, this secondary finding should be interpreted cautiously because neither the overall treatment effect nor the treatment-by-sex interaction reached statistical significance. These findings suggest that pulmonary vascular responses to impaired endothelial flow sensing differ between male and female mouse lungs under the conditions of this isolated perfused lung model. The combination of increased Z_0 and R_W in the absence of changes in Z_C further supports the interpretation that the observed responses arose primarily from altered regulation of distal resistance vessels rather than changes in proximal conduit artery mechanics. These findings highlight the importance of considering sex as a biological variable in studies of pulmonary vascular shear stress mechanotransduction.

Author Contributions Statement

The authors confirm contributions to the paper as follows: study conception and design: FT, NCC; data collection: FT, EA; analysis and interpretation of results: FT, NCC; draft manuscript preparation: FT, NCC. All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest

There are no conflicts of interest.

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