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Impact of increased hematocrit on right ventricular afterload in response to chronic hypoxia

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Chronic hypoxia causes chronic mountain sickness through hypoxia-induced pulmonary hypertension (HPH) and increased hematocrit. Here, we investigated the impact of increased hematocrit and HPH on right ventricular (RV) afterload via pulmonary vascular impedance. Mice were exposed to chronic normobaric hypoxia (10% oxygen) for 10 (10H) or 21 days (21H). After baseline hemodynamic measurements, ~500 µl of blood were extracted and replaced with an equal volume of hydroxyethylstarch to normalize hematocrit and all hemodynamic measurements were repeated. In addition, ~500 µl of blood were extracted and replaced in control mice with an equal volume of 90% hematocrit blood. Chronic hypoxia increased input resistance (Z₀) increased 82% in 10H and 138% in 21H vs. CTL; P < 0.05) and characteristic impedance (Zₐ) increased 76% in 10H and 109% in 21H vs. CTL; P < 0.05. Hematocrit normalization did not decrease mean pulmonary artery pressure but did increase cardiac output such that both Z₀ and Zₐ decreased toward control levels. Increased hematocrit in control mice did not increase pressure but did decrease cardiac output such that Z₀ increased. The paradoxical decrease in Zₐ with an acute drop in hematocrit and no change in pressure are likely due to inertial effects secondary to the increase in cardiac output. A novel finding of this study is that an increase in hematocrit affects the pulmonary vascular impedance (PVZ). Whereas resistance represents the opposition to steady flow in a vascular bed, which is largely generated by friction in small diameter vessels, impedance represents the opposition to pulsatile flow, which can be generated by stiff vessels that do not accommodate pulsations, branching, and tapering vessels that generate wave reflections, and other phenomena. The impedance to steady flow, i.e., the resistance, is represented by Z₀, the impedance to high frequency pulsations generated by narrow, stiff vessels, i.e., the characteristic impedance, is represented by Zₐ; and the degree of pulse wave reflections is represented by the index of wave reflection Pp/Pp. In PAH, pulmonary arterial stiffness, which is related to Zₐ, is an excellent predictor of mortality from RV failure (11, 14, 18, 22, 26).

Here, we sought to investigate the impact of hematocrit on RV afterload by quantifying changes in PVZ in response to chronic hypoxia. We hypothesize that increasing hematocrit due to chronic hypoxia would be a significant contributor to Z₀ and a mild or nonexistent contributor to Zₐ. To test this hypothesis, we measured PVZ in live mice in situ with and without exposure to chronic hypoxia. Then, we normalized hematocrit to control levels in hypoxic mice and again measured pulmonary vascular impedance. Our results demonstrate that hematocrit is an important contributor to increased RV afterload in chronic hypoxia. To the best of our knowledge, we are the first to quantify how increased hematocrit contributes to RV afterload independent of pulmonary vascular remodeling.

CHRONIC MOUNTAIN SICKNESS (CMS), also known as Monge’s disease, occurs after chronic exposure to hypoxia at high altitudes and is characterized by increased pulmonary artery pressures and pulmonary vascular resistance (29) as well as increased hematocrit (19). Chronic hypoxia also contributes to worse outcomes in lung diseases such as chronic obstructive pulmonary disease, sleep apnea, and pulmonary fibrosis (1, 10, 15). In preclinical animal models of pulmonary arterial hypertension (PAH), chronic hypoxia is often used to generate hypoxia-induced pulmonary hypertension (HPH). PAH is a debilitating disease with a low median survival of 2.8 yr (6, 17) and is characterized by remodeling throughout the pulmonary vasculature, including distal arterial narrowing and proximal and distal pulmonary artery stiffening, leading to right ventric-ular (RV) dysfunction that progresses to RV failure as the cause of death (28). HPH in rodents recapitulates the pulmonary vascular remodeling and RV hypertrophy that occur in patients with PAH but also increases hematocrit. Indeed, the increase in hematocrit can be dramatic, from ~40 to ~70% in only a few days (12).

Increased hematocrit increases blood viscosity (2, 4, 32, 47), which has been shown to substantially increase both systemic and pulmonary vascular resistance (2, 5, 13, 16, 47). Independent of the increase in blood viscosity, hypoxia increases resistance acutely via hypoxic pulmonary vasoconstriction and chronically via pulmonary vascular remodeling (2, 27, 33, 40). The independent effects of these blood and vessel wall changes on RV afterload have received minimal attention traditionally (16). Investigation into changes in RV afterload, as a result of the increase in hematocrit, in response to chronic hypoxia could potentially lend valuable insight into the role of increased hematocrit in CMS and other lung diseases.

The most comprehensive measure of RV afterload is pulmonary vascular impedance (PVZ). Whereas resistance represents the opposition to steady flow in a vascular bed, which is largely generated by friction in small diameter vessels, impedance represents the opposition to pulsatile flow, which can be generated by stiff vessels that do not accommodate pulsations, branching, and tapering vessels that generate wave reflections, and other phenomena. The impedance to steady flow, i.e., the resistance, is represented by Z₀, the impedance to high frequency pulsations generated by narrow, stiff vessels, i.e., the characteristic impedance, is represented by Zₐ; and the degree of pulse wave reflections is represented by the index of wave reflection Pp/Pp. In PAH, pulmonary arterial stiffness, which is related to Zₐ, is an excellent predictor of mortality from RV failure (11, 14, 18, 22, 26).

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833
METHODS

Materials. Male C57BL/6J mice, 12–13 wk old, were obtained from Jackson Laboratory (Bar Harbor, ME) and exposed to room air (CTL, n = 13) or chronic normobaric hypoxia (10% oxygen) for either 10 (10H, n = 7) or 21 (21H, n = 7) days. Normobaric hypoxia was created in environmentally controlled chambers in which nitrogen was mixed with room air; oxygen levels were measured with a sensor in the chamber (Servoflo, Lexington, MA) that controlled a relay valve on the nitrogen gas inflow line via a custom-built closed loop control system. The chamber was opened for 10–20 min three times per week to clean cages and replenish food and water. All mice were exposed to a 12-h light-dark cycle. The University of Wisconsin Institutional Animal Care and Use Committee approved all procedures.

In vivo hemodynamic measurements. Mice were anesthetized with an intraperitoneal injection of urethane solution (2 mg/g body wt), intubated, and placed on a ventilator (Harvard Apparatus, Holliston, MA) using a tidal volume of 225 μl and respiratory rate of ~200 breaths/min of room air. Mice were then placed supine on a heated pad to maintain body temperature at 38–39°C. A central midline skin incision was made from the lower mandible inferior to the xiphoid process. The thoracic cavity was entered through the sternum, and the chest was carefully removed to expose the right ventricle. To confirm the absence of systemic hypertension, the right carotid artery was cannulated with a 1.2-F catheter-tip pressure transducer (Sciensense, London, ON, Canada) and advanced into the ascending aorta. Hydroxyethyl starch was used to restore vascular volume due to blood loss as done previously (33). Subsequently, the apex of the right ventricle was localized and a 1.0-F pressure-tip catheter (Millar Instruments, Houston, TX) was introduced using a 20-gauge needle leaving the pericardium otherwise intact. After instrumentation was established and pressure was stabilized, the catheter was advanced to the main pulmonary artery for measurement. Pressure tracings were recorded at 5 kHz on a hemodynamic workstation (Cardiovascular Engineering, Norwood, MA). Flow measurement was performed via ultrasound (Visualsonics, Toronto, ON, Canada) with a 40-MHz probe during catheterization and recorded with the same system. Flow was calculated by velocity time integral using spectral analysis of the digitized broadband Doppler audio signal obtained in the main pulmonary artery just distal to the pulmonary valve with the probe in a right parasternal long-axis orientation in the same location as the catheter. The probe was angled until the maximal velocity signal was obtained. Measurement at this point allows for better detection of the main pulmonary arterial inner diameter (MPA ID). Measurement of the MPA ID was taken using the long axis view from leading edge to leading edge during end systole from three different cardiac cycles; we report the average of those three.

We used MPA ID to convert the instantaneous flow velocity signal to instantaneous volume flow rate (Q) assuming a circular cross section and a blunt velocity profile. The signals were visually checked for quality and recorded for later analysis.

After all measurements were completed in the 10H and 21H groups, ~500 μl of blood were extracted and replaced with an equal volume of hydroxyethylstarch to normalize hematocrit to CTL levels based on pilot studies. After a 5-min stabilizing period, all hemodynamic measurements were repeated in this normalized-hematocrit state for mice exposed to chronic hypoxia for 10 days (10H-NaCl) and 21 days (21H-NaCl). Before euthanasia, a 500-μl sample of the normalized-hematocrit blood was taken for analysis. Measurements performed on the extracted blood samples included viscosity using a cone and plate viscometer, blood gas and ion concentrations using an I-STAT portable analyzer and CG8+ cartridge, and hematocrit using a centrifuge.

After all measurements were completed in a group of CTL mice, ~500 μl of blood were extracted and replaced with an equal volume of a 90% hematocrit suspension. To create the 90% hematocrit suspension, whole blood was removed from the left ventricle of C57Bl6 donor mice and then transferred to 50-ml conical tube and centrifuged at 500 g for 15 min at room temperature, and blood plasma was removed. The red blood cell pellet was then resuspended at 10 times red blood cell pellet volume in osmolality balanced saline solution and centrifuged again at 500 g for 15 min. This process was repeated four times. Finally, washed red blood cells were resuspended to a target hematocrit of 90%. Five minutes after this high hematocrit blood was administered to CTL mice, all hemodynamic measurements were repeated (CTL-H1) and then, blood extraction and replacement and all measurements were repeated a second time (CTL-H2).

In vivo hemodynamic calculations. The instantaneous volume flow and the pressure waveforms were signal-averaged using the ECG as a fiducial point and then processed and analyzed using custom software (Cardiovascualar Engineering, Norwood, MA). Twenty consecutive cardiac cycles free of extrasystolic beats were selected and averaged. PVZ was calculated using wave intensity analysis as previously described (25, 34). Total pulmonary vascular resistance (Zp) was calculated as mean pulmonary arterial pressure (mPAP) divided by Q averaged over the cardiac cycle (i.e., CO). Total pulmonary arterial compliance was calculated from an exponential fit to the pulmonary arterial pressure decay during diastole (31). Characteristic impedance (Zc) was calculated as the ratio of the change in pressure to the change in flow in early ejection. That is, Zc = ΔP/ΔQ, where ΔP and ΔQ are taken before when Q reaches 95% of its maximum value. An assumption inherent in this calculation is that the system is free from reflections because the reflected waves do not have time to return to the proximal bed so early in the cardiac cycle (25). To allow further comparison of our data to the existing literature, we calculated pulse wave velocity (PWV) as $PWV = \frac{Z_{c}^{*}}{A}$ assuming the density of blood $\rho = 1.060$ kg/m$^3$ and cross-sectional area $A = \pi/4$ (MPA ID)$^2$.

Finally, based on Zc, the pulmonary arterial pressure waveform was separated into forward (Pf) and backward (Pb) traveling components using the linear wave separation method (42). The index of global wave reflections was calculated as the ratio of the amplitude of Pb to Pf.

Statistical analysis. A limitation of the long axis view for calculating MPA ID is the screen resolution of the ultrasound system (80 μm). Therefore, we performed a bootstrap analysis as a nonparametric technique to determine the effects of screen limitation on our calculation of Zc. The application of bootstrap yields a number of resamples of each of our original calculated Zc values per mouse to determine the true error per measurement associated with our technique (8).

For each group, the significances of the overall changes in parameters with exposure to chronic normobaric hypoxia were assessed using a one-way ANOVA for condition or generalized least squares for repeated measurements with normalized hematocrit. When the ANOVA reached statistical significance, Tukey’s multiple comparisons were used for post hoc analysis. Data were considered significant for P values < 0.05. All data are presented in terms of means ± standard error. Statistical analysis was performed using R software (Foundation for Statistical Computing, version 2.14.0).

RESULTS

 Morphometric effects of chronic hypoxia. The average body weight of the CTL mice was higher than the 10H and 21H groups (Table 1). The left ventricular weight normalized by body weight did not change between groups (Table 1). RV hypertrophy measured both by RV mass normalized by body weight and Fulton index (RV/LV + S) increased with 10 days of hypoxia and continued to increase with 21 days of hypoxia (Table 1).

834 Impact of Hematocrit on RV Afterload · Schreier DA et al.
Hemodynamic effects of chronic hypoxia. As expected, hematocrit increased with 10 days of chronic hypoxia and remained elevated with 21 days (Table 2). Similarly, mPAP increased with 10 days of chronic hypoxia and remained elevated with 21 days (Table 2). Cardiac output and stroke volume tended to decrease with increasing duration of hypoxia and were significantly decreased by 21 days (Table 2; \( P < 0.05 \) for 21H vs. CTL). Pulmonary vascular resistance \( Z_0 \) increased after 10 days of hypoxia and was further increased after 21 days of hypoxia (Fig. 1). Characteristic impedance (\( Z_C \)) (Fig. 2), systolic pulmonary artery pressure (sPAP), and PWV increased with 10 days of chronic hypoxia and remained elevated with 21 days. Pulmonary arterial compliance decreased with 10 days of chronic hypoxia and remained elevated with 21 days (Table 2). Similarly, mPAP, mean pulmonary arterial stroke volume, pulse pressure, and PWV increased with 10 days of chronic hypoxia and remained elevated with 21 days (Table 2). Heart rate did not change with chronic hypoxia.

Hemodynamic effects of hematocrit. An exchange of \( \sim 500 \) \( \mu l \) of blood with hydroxyethylstarch decreased hematocrit by 39 to \( \sim 43\% \) for 10H-NHct and 21H-NHct mice, which was similar to the control mice (Table 2). The change in hematocrit had no effect on the mPAP, sPAP, or pulse pressure but increased cardiac output and stroke volume (significant at 10 days only) (Table 2); heart rate was also decreased in the 10H-NHct group. Consequently, \( Z_0 \) was lower in the 10H-NHct and 21H-NHct groups compared with the 10H and 21H groups, respectively (Fig. 1). \( Z_C \) was lower in the 10H-NHct and 21H-NHct groups compared with the 10H and 21H groups, respectively, and was similar to the CTL group value (Fig. 2).

Pulmonary arterial compliance increased in the 10H-NHct and 21H-NHct groups compared with the 10H and 21H groups, respectively, but the change was only significant at 21 days (Fig. 3).

One exchange of \( \sim 500 \) \( \mu l \) of CTL mouse blood with 90% hematocrit suspension increased hematocrit by 38 to \( \sim 58\% \); the second exchange of \( \sim 500 \) \( \mu l \) increased hematocrit overall by 66 to \( \sim 70\% \) (Table 3). The increase in hematocrit in CTL mice had no effect on mPAP, sPAP, diastolic (dPAP), diameter of the main pulmonary arterial, stroke volume, pulse pressure, PWV, or \( Z_C \) (Table 3). However, heart rate decreased and therefore cardiac output decreased in the CTL-H1 and CTL-H2 groups. \( Z_0 \) tended to increase with the first exchange and became significantly increased after the second exchange with the high hematocrit suspension in CTL mouse lungs (Fig. 1).

The bootstrap analysis of \( Z_C \) followed identical trends as the original measurements with increased standard error in each exchange.

### Table 1. Body weight, ventricular weights, and Fulton index for combined CTL, 10H, and 21H mice

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>10H</th>
<th>21H</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>26.4 ± 0.7</td>
<td>23.0 ± 0.3*</td>
<td>22.5 ± 0.5*</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.90 ± 0.02</td>
<td>1.06 ± 0.05*</td>
<td>1.31 ± 0.03†</td>
</tr>
<tr>
<td>LV + S/BW, mg/g</td>
<td>3.3 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Fulton index</td>
<td>0.28 ± 0.01</td>
<td>0.34 ± 0.01*</td>
<td>0.40 ± 0.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 13 \) for control (CTL), \( n = 7 \) for chronic normobaric hypoxia for 10 days (10H), and \( n = 7 \) for chronic normobaric hypoxia for 21 days (21H). BW, body weight; RV, right ventricular; LV, left ventricular; S, septum. * \( P < 0.05 \) vs. CTL; † \( P < 0.05 \) vs. 10H.

### Table 2. Hemodynamic parameters derived from pulmonary artery pressure and flow waveforms in combined CTL, 10H, 10H-NHct, 21H, and 21H-NHct mice

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>10H</th>
<th>10H-NHct</th>
<th>21H</th>
<th>21H-NHct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>562 ± 10</td>
<td>583 ± 4</td>
<td>548 ± 7§</td>
<td>562 ± 14</td>
<td>557 ± 16</td>
</tr>
<tr>
<td>Hct, %</td>
<td>42.0 ± 1.2</td>
<td>71.3 ± 1.3*</td>
<td>44.3 ± 1.9§</td>
<td>69.9 ± 1.8*</td>
<td>42.3 ± 2.0†</td>
</tr>
<tr>
<td>mPAP, mmHg</td>
<td>15.7 ± 1.6</td>
<td>26.2 ± 1.8*</td>
<td>24.3 ± 1.9</td>
<td>21.3 ± 1.5*</td>
<td>21.3 ± 2.3</td>
</tr>
<tr>
<td>dPAP, mmHg</td>
<td>11.0 ± 1.4</td>
<td>20.4 ± 1.6*</td>
<td>18.0 ± 1.6</td>
<td>15.5 ± 1.5</td>
<td>15.7 ± 2.3</td>
</tr>
<tr>
<td>sPAP, mmHg</td>
<td>22.4 ± 2.0</td>
<td>37.8 ± 2.5*</td>
<td>37.0 ± 2.5</td>
<td>33.4 ± 1.8*</td>
<td>32.4 ± 2.4</td>
</tr>
<tr>
<td>Diameter MPA, mm</td>
<td>1.38 ± 0.02</td>
<td>1.37 ± 0.02</td>
<td>1.39 ± 0.01</td>
<td>1.37 ± 0.01</td>
<td>1.35 ± 0.03</td>
</tr>
<tr>
<td>RV cardiac output, ml/min</td>
<td>11.9 ± 0.5</td>
<td>10.7 ± 0.5</td>
<td>12.8 ± 0.5§</td>
<td>9.5 ± 0.5*</td>
<td>10.7 ± 0.5</td>
</tr>
<tr>
<td>Stroke volume, µl</td>
<td>21.3 ± 1.0</td>
<td>18.3 ± 0.8</td>
<td>23.3 ± 0.9§</td>
<td>17.1 ± 1.2*</td>
<td>19.3 ± 0.8</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>11.4 ± 0.8</td>
<td>17.3 ± 1.6*</td>
<td>19.0 ± 1.0</td>
<td>18.0 ± 0.8*</td>
<td>16.7 ± 0.5</td>
</tr>
<tr>
<td>Pulse wave volume, mm/m</td>
<td>0.39 ± 0.03</td>
<td>0.67 ± 0.4*</td>
<td>0.49 ± 0.05</td>
<td>0.81 ± 0.06*</td>
<td>0.82 ± 0.07*</td>
</tr>
<tr>
<td>Ze (mmHg ml⁻¹ ms⁻¹)</td>
<td>0.26 ± 0.01</td>
<td>0.46 ± 0.03*</td>
<td>0.33 ± 0.04</td>
<td>0.55 ± 0.03*</td>
<td>0.29 ± 0.02†</td>
</tr>
<tr>
<td>Ze (bootstrap method)</td>
<td>0.27 ± 0.07</td>
<td>0.46 ± 0.08*</td>
<td>0.35 ± 0.07</td>
<td>0.56 ± 0.10*</td>
<td>0.28 ± 0.06†</td>
</tr>
<tr>
<td>Qmax, mm/s</td>
<td>0.60 ± 0.02</td>
<td>0.55 ± 0.02</td>
<td>0.60 ± 0.01§</td>
<td>0.41 ± 0.01†</td>
<td>0.59 ± 0.02†</td>
</tr>
<tr>
<td>P/P’</td>
<td>0.28 ± 0.01</td>
<td>0.30 ± 0.03</td>
<td>0.35 ± 0.04</td>
<td>0.32 ± 0.02</td>
<td>0.40 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 13 \) for CTL, \( n = 7 \) for 10H, and 10H-NHct, and \( n = 7 \) for 21H and 21H-NHct. N, normalized; Hct, hematocrit; mPAP, mean pulmonary artery pressure; sPAP, systolic pulmonary artery pressure; dPAP, diastolic pulmonary artery pressure; MPA, main pulmonary artery; RV, right ventricular; Ze, characteristic impedance; Qmax, maximum flow; P/P' backward and forward pressure waveforms. * \( P < 0.05 \) vs. CTL; † \( P < 0.05 \) 21H-NHct vs. 21H; § \( P < 0.05 \) vs. 10H; ‡ \( P < 0.05 \) 10H-NHct vs. 10H.
The pH was stable for all five groups: CTL, 10H, 110H, groups, and 0.074/\text{H}\text{11006}P pulmonary arterial pressure decay during diastole. *\text{not shown}). The yield stress was 0.085 similar shear stress-shear rate curves as the control blood (data hydroxyethylstarch) blood in hypoxic animals demonstrated control levels (Table 4). In addition, the transfused (with exposure. The reduction of hematocrit returned blood viscosity to groups was not affected nor was the significance among the groups, while the significance between 21H and 21H-NHct significant decrease in \( Z_C \) between the 10H and 10H-NHct Fig. 2. Characteristic impedance \( Z_C \) calculated from pressure/flow (\( \text{dP/dQ}\)) when \( Q \) reaches 95% of its maximal value. *\( P < 0.05 \) vs. CTL; §\( P < 0.05 \) 10H-NHct vs. 10H; †\( P < 0.05 \) 21H-NHct vs. 21H.

group (Table 2). The increased standard error removed the significant decrease in \( Z_C \) between the 10H and 10H-NHct groups, while the significance between 21H and 21H-NHct groups was not affected nor was the significance among the CTL, 10H, and 21H groups.

Effects of hematocrit on blood viscosity and blood gases. As expected, blood viscosity increased with chronic hypoxia exposure. The reduction of hematocrit returned blood viscosity to control levels (Table 4). In addition, the transfused (with hydroxyethylstarch) blood in hypoxic animals demonstrated similar shear stress-shear rate curves as the control blood (data not shown). The yield stress was 0.085 ± 0.005 dyn/cm\(^2\) for the CTL group, 0.11 ± 0.01 dyn/cm\(^2\) for both 10H and 21H groups, and 0.074 ± 0.01 dyn/cm\(^2\) for both 10H-NHct and 21H-NHct groups in agreement with previous measurements (41). The pH was stable for all five groups: CTL, 10H, 10H-NHct, 21H, and 21H-NHct (Table 4). Similarly, the partial pressures for oxygen (\( \text{pO}_2 \)) and carbon dioxide (\( \text{pCO}_2 \)) and blood oxygen saturation (\( \text{SO}_2\% \)) remained constant for all five groups (Table 4). The hemoglobin increased in the 10H and 21H group and the 10H-NHct and 21H-NHct groups demonstrated decreased hemoglobin compared with the 10H and 21H groups, respectively (Table 4).

### DISCUSSION

The major, novel contribution of this study is the quantification of the impact of the chronic hypoxia-induced increase in hematocrit on the pulsatile components of RV afterload in addition to the steady components of RV afterload (\( Z_0 \)).

Exchanging ~500 \( \mu \)l of blood with hydroxyethylstarch returned hematocrit and hemoglobin levels in chronic hypoxic mice to near control values (Table 2). For the first time, we quantitatively demonstrate that increases in \( Z_0 \), which occur with chronic hypoxia in a mouse model, are decreased with a reduction in hematocrit to control levels. In addition, increasing hematocrit acutely in CTL mice resulted in an increased \( Z_0 \) once a hematocrit level of ~70% (CTL-H2, with hematocrit, comparable to that in the 10H and 21H groups) was achieved (Fig. 1). These findings are consistent with a prior study in rats with CMS in which hemodilution from hematocrit of 40% decreased pulmonary vascular resistance (7). Our results are also consistent with a clinical study in which patients with CMS had increased cardiac output and decreased pulmonary artery pressure with hemodilution (44), suggesting decreased resistance with return to a normal hematocrit.

The effect of hematocrit on the unsteady, time-dependent opposition to blood flow is not as simple, since a return to control hematocrit levels decreased \( Z_C \) in the mice exposed to 10 and 21 days of hypoxia but an increase in hematocrit to 70% in control mice had no effect on \( Z_C \). Similarly, PWV returns to control levels and pulmonary arterial compliance is improved when hematocrit returns to control levels in chronically hypoxic mice, but there were no changes in PWV or compliance when hematocrit was increased in control mice (Table 2 and Fig. 3). Our results confirm that these metrics do not only reflect arterial wall constitutive behavior but rather are also blood rheology and heart rate dependent.

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Table 3. Hemodynamic parameters derived from pulmonary artery pressure and flow waveforms in separate CTL, CTL-H1, and CTL-H2 mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTL</th>
<th>CTL-H1</th>
<th>CTL-H2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>563 ± 13</td>
<td>522 ± 14*</td>
<td>464 ± 19*</td>
</tr>
<tr>
<td>Hct, %</td>
<td>44.7 ± 1.4</td>
<td>58.3 ± 2.0*</td>
<td>69.4 ± 1.6*</td>
</tr>
<tr>
<td>mPAP, mmHg</td>
<td>16.4 ± 1.1</td>
<td>15.0 ± 0.9</td>
<td>17.9 ± 2.0</td>
</tr>
<tr>
<td>dPAP, mmHg</td>
<td>11.1 ± 1.0</td>
<td>9.7 ± 1.0</td>
<td>12.8 ± 2.3</td>
</tr>
<tr>
<td>sPAP, mmHg</td>
<td>22.1 ± 1.3</td>
<td>20.9 ± 1.1</td>
<td>23.4 ± 1.6</td>
</tr>
<tr>
<td>Diameter MPA, mm</td>
<td>1.41 ± 0.03</td>
<td>1.41 ± 0.02</td>
<td>1.37 ± 0.03</td>
</tr>
<tr>
<td>RV cardiac output, ml/min</td>
<td>12.2 ± 0.8</td>
<td>9.6 ± 0.9*</td>
<td>8.1 ± 0.6*</td>
</tr>
<tr>
<td>Stroke volume, µl</td>
<td>21.6 ± 1.4</td>
<td>18.3 ± 1.3</td>
<td>17.3 ± 1.6</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>11.1 ± 0.6</td>
<td>11.0 ± 1.0</td>
<td>10.4 ± 1.3</td>
</tr>
<tr>
<td>Pulse wave velocity, mm/ms</td>
<td>0.37 ± 0.04</td>
<td>0.40 ± 0.04</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>( Z_C ), mmHg·min(^{-1})·ml</td>
<td>0.24 ± 0.03</td>
<td>0.26 ± 0.03</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>( Z_C ) (Bootstrap method)</td>
<td>0.24 ± 0.09</td>
<td>0.26 ± 0.05</td>
<td>0.26 ± 0.09</td>
</tr>
<tr>
<td>( Q_{max} ), mm/ms</td>
<td>0.62 ± 0.03</td>
<td>0.61 ± 0.04</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>( P_v/P_f )</td>
<td>0.29 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>0.40 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 7 \) for CTL, \( n = 7 \) for CTL-H1, and \( n = 5 \) for CTL-H2. CTL-H1 and CTL-H2, first and second hemodynamic measurements. *\( P < 0.05 \) vs. CTL.

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Fig. 3. Total pulmonary arterial compliance from an exponential fit to the pulmonary arterial pressure decay during diastole. *\( P < 0.05 \) vs. CTL; †\( P < 0.05 \) 21H-NHct vs. 21H.

Fig. 2. Characteristic impedance \( Z_C \) calculated from pressure/flow (\( \text{dP/dQ}\)) when \( Q \) reaches 95% of its maximal value. *\( P < 0.05 \) vs. CTL; §\( P < 0.05 \) 10H-NHct vs. 10H; †\( P < 0.05 \) 21H-NHct vs. 21H.
Characteristic impedance, $Z_C$, is frequently interpreted as a measure of arterial stiffness. Indeed, in the absence of viscous effects, such as in the artery wall and at the blood-artery interact, $Z_C$ can be approximated as $\sqrt{\frac{p^2E\pi h}{2\pi r^3}}$, where $E$ is arterial elastic modulus, $h$ is arterial wall thickness, and $r$ is arterial inner radius (37). However, it is important to note that $Z_C$ can be calculated as the square root of the blood inertance divided by the compliance of the proximal segments of the arterial network (23). When blood inertance is constant, the simplistic relationship between $Z_C$ and compliance (inverse stiffness) is valid. However, when blood inertance is not constant, such as occurs when stroke volume or cardiac output increase due to hematocrit normalization in hypoxic animals (or when cardiac output decreases due to hematocrit increase due to hematocrit normalization likely decreased $Z_C$. This suggestion suggests that the increases in cardiac output increase cardiac output increase due to hematocrit normalization were a regulatory response.

Several limitations should be noted. We did not measure blood density but Wang et al. (39) suggest that blood density changes 1.4% between a hematocrit of ~70 and ~40%. Also, the hydroxyethylstarch added to normalize hematocrit has similar density to plasma (1,077 to 1,060 kg/m$^3$). The change in viscosity at a shear rate of 131 s$^{-1}$ is 1.4% between a hematocrit of 40% and 70% and 70 and 80%. Also, the hydroxyethylstarch added to normalize hematocrit has similar density to plasma (1,077 to 1,060 kg/m$^3$). The change in viscosity at a shear rate of 131 s$^{-1}$ is 1.4% between a hematocrit of 40% and 70% and 70 and 80%.

Table 4. Arterial blood gas, shear stress and blood viscosity in CTL, 10H, 10H-NHct, 21H, and 21H-NHct mice

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>10H</th>
<th>10H-NHct</th>
<th>21H</th>
<th>21H-NHct</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>7.33 ± 0.04</td>
<td>7.27 ± 0.03</td>
<td>7.24 ± 0.07</td>
<td>7.26 ± 0.03</td>
<td>7.24 ± 0.01</td>
</tr>
<tr>
<td><strong>pO$_2$, mmHg</strong></td>
<td>83.7 ± 9.8</td>
<td>85.7 ± 19.0</td>
<td>81.5 ± 11.2</td>
<td>69.7 ± 6.4</td>
<td>66.0 ± 10.4</td>
</tr>
<tr>
<td><strong>pCO$_2$, mmHg</strong></td>
<td>92.0 ± 2.0</td>
<td>93.3 ± 4.2</td>
<td>94.5 ± 1.2</td>
<td>90.7 ± 0.8</td>
<td>89.0 ± 4.9</td>
</tr>
<tr>
<td><strong>pCO$_2$, mmHg</strong></td>
<td>27.8 ± 4.4</td>
<td>29.4 ± 4.7</td>
<td>35.2 ± 7.0</td>
<td>25.4 ± 1.5</td>
<td>23.2 ± 2.6</td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dl</strong></td>
<td>14.1 ± 1.0</td>
<td>20.6 ± 0.6*</td>
<td>14.5 ± 0.5§</td>
<td>19.6 ± 0.2*</td>
<td>14.2 ± 0.3†</td>
</tr>
<tr>
<td><strong>Shear stress, dyn/cm$^2$, at $\gamma = 131$ s$^{-1}$</strong></td>
<td>6.6 ± 0.1</td>
<td>8.1 ± 0.5*</td>
<td>5.7 ± 0.5§</td>
<td>8.4 ± 0.3*</td>
<td>6.1 ± 0.2†</td>
</tr>
<tr>
<td><strong>Viscosity, cP at $\gamma = 131$ s$^{-1}$</strong></td>
<td>5.0 ± 0.1</td>
<td>6.1 ± 0.3*</td>
<td>4.4 ± 0.2§</td>
<td>6.4 ± 0.2*</td>
<td>4.6 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$ for CTL, $n = 7$ for 10H, and $n = 7$ for 21H. pO$_2$, partial pressure of oxygen; pCO$_2$, partial pressure of carbon dioxide; sO$\%$, blood oxygen saturation. *$P < 0.05$ vs. CTL; †$P < 0.05$ 21H-NHct vs. 21H; §$P < 0.05$ 10H-NHct vs. 10H.
hemodilution in this study was acute and only occurred once per animal, red blood cell mechanics should not be altered. Finally, there was insufficient blood volume in each mouse to measure viscosity, arterial blood gases, and mixed venous blood gases. In future work, measuring mixed venous blood gas would provide a validation of the catheter-based CO measurement via the Fick equation.

Our results demonstrate for the first time the effects of increased hematocrit on RV afterload during the progression of hypoxic pulmonary hypertension in mice. The significant changes in Zc, PVW, and pulmonary arterial compliance in the 10H-NHct and 21H-NHct mice suggest that decreasing hematocrit has the beneficial effect of decreasing metrics of pulsatile RV afterload, via increasing blood inertia. Clinically, these results suggest that pulmonary vascular impedance studies within the CMS population could lend valuable insight into possible changes in the pulsatile RV afterload. The return of hematocrit to control levels reduced RV afterload toward control levels, suggesting a possible route to alleviate stress on the RV caused by increased hematocrit in patients with CMS and other forms of HPH.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES