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

David A. Schreier,1 Timothy A. Hacker,2 Kendall Hunter,3 Jens Eickoff,2 Aiping Liu,1 Gouqing Song,2 and Naomi Chesler1,2

1Department of Biomedical Engineering University of Wisconsin, Madison, Wisconsin; 2Department of Medicine Medical Science Center, Madison, Wisconsin; and 3Department of Bioengineering University of Colorado, Aurora, Colorado

Submitted 27 January 2014; accepted in final form 20 August 2014

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 ventricular (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 $Z_0$; the impedance to high frequency pulsations generated by narrow, stiff vessels, i.e., the characteristic impedance, is represented by $Z_C$; and the degree of pulse wave reflections is represented by the index of wave reflection $P_r/P_i$. In PAH, pulmonary arterial stiffness, which is related to $Z_C$, 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 $Z_0$ and a mild or nonexistent contributor to $Z_C$. 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.
METHODS

Materials. Male C57BL6/J 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 (Servflow, 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 (Sciensic, London, ON, Canada) and advanced into the ascending aorta. Hydroxyethylstarch 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-NHct) and 21 days (21H-NHct). 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 (Cardiovascular 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 (Zv) 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 from the ratio of the change in pressure to the change in flow in early ejection. That is, $Z_c = \frac{dP}{dQ}$, where dP and dQ 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 $\text{PWV} = \frac{Z_c}{\rho A}$ assuming the density of blood $\rho = 1.060 \text{ kg/m}^3$ and cross-sectional area $A = \pi/4 (\text{MPA ID})^2$.

Finally, also 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 Pf to Pb.

Statistical analysis. A limitation of the long axis view for calculating MPA ID is the screen resolution of the ultrasound system (80 mm). 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 comparison was used for post hoc analysis. Data were considered significant for $P < 0.05$. 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).
Heart rate did not change with 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 Z₀ increased after 10 days of hypoxia and was further increased after 21 days of hypoxia (Fig. 1). Characteristic impedance (Zₐ) (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 (Fig. 3).

Heart rate did not change with chronic hypoxia.

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 Z₀ increased after 10 days of hypoxia and was further increased after 21 days of hypoxia (Fig. 1). Characteristic impedance (Zₐ) (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 decreased with 21 days (Fig. 3).

Heart rate did not change with chronic hypoxia.

Hemodynamic effects of hematocrit. An exchange of ~500 µl of blood with hydroxyethylstarch decreased hematocrit by 39 to ~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, Z₀ was lower in the 10H-NHct and 21H-NHct groups compared with the 10H and 21H groups, respectively (Fig. 1). Zₐ 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 ~500 µl of CTL mouse blood with 90% hematocrit suspension increased hematocrit by 38 to ~58%; the second exchange of ~500 µl increased hematocrit overall by 66 to ~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 Zₐ (Table 3). However, heart rate decreased and therefore cardiac output decreased in the CTL-H1 and CTL-H2 groups. Z₀ 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 Zₐ followed identical trends as the original measurements with increased standard error in each

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 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; Z₀, characteristic impedance; Qₐₑmax, maximum flow; Pₛ/Pₚb, 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 vs. 10H-NHct 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 velocity, mm/ms</td>
<td>0.39 ± 0.03</td>
<td>0.67 ± 0.4*</td>
<td>0.49 ± 0.03§</td>
<td>0.81 ± 0.06*</td>
<td>0.42 ± 0.03†</td>
</tr>
<tr>
<td>Zₐ, mmHg·ml⁻¹·min⁻¹</td>
<td>0.26 ± 0.02</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>Z₀ (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>Qₐₑmax, mm/ms</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>Pₛ/Pₚb</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; Z₀, characteristic impedance; Qₐₑmax, maximum flow; Pₛ/Pₚb, 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 partial pressure of arterial oxygen (pO2) was 65.7 ± 1.0, 58.3 ± 2.0, 58.3 ± 2.0, 58.3 ± 2.0, and 58.3 ± 2.0 mmHg for the CTL, 10H, 10H-NHct, 21H, and 21H-NHct groups, respectively (Table 4). The partial pressure of arterial carbon dioxide (pCO2) was 44.7 ± 1.4, 44.7 ± 1.4, 44.7 ± 1.4, 44.7 ± 1.4, and 44.7 ± 1.4 mmHg for the CTL, 10H, 10H-NHct, 21H, and 21H-NHct groups, respectively (Table 4). The pH was stable for all five groups: CTL, 10H, 10H-NHct, 21H, and 21H-NHct.

The yield stress was 0.085 ± 0.01 dyn/cm² for both 10H and 21H groups, and 0.074 ± 0.01 dyn/cm² 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.

**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/cm² for the CTL group, 0.11 ± 0.01 dyn/cm² for both 10H and 21H groups, and 0.074 ± 0.01 dyn/cm² 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 (pO2) and carbon dioxide (pCO2) and blood oxygen saturation (sO2%) 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 (Z0).

Exchanging ~500 μl 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 Z0, 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 Z0 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 ~70 to ~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 ZC 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 ZC. 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.
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 $\sqrt{\frac{p^*E^*h}{2\pi^2\rho^2r^3}}$, 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 iner-
tance divided by the compliance of the proximal segments of the arterial network (23). When blood iner-tance is constant, the simplistic relationship between $Z_C$ and compliance (inverse stiffness) is valid. However, when blood iner-tance is not constant, such as occurs when stroke volume or cardiac output increase due to hematocrit normalization in hypoxic animals (or when cardiac output decrease due to increased blood viscosity should increase $Z_L$ and thus $Z_C$, and $Z_L$ * $Z_T$. The longitudinal impedance depends on viscosity and Womersley number, which itself depends on radius and the square root of density, heart rate, and inverse of viscosity. Therefore, $Z_C$ increases with the square root of viscosity (24, 43, 45), which is one mechanism by which hematocrit normalization likely decreased $Z_C$. This suggestion is supported by a prior study by Fischer et al. (9) that demonstrated that blood viscosity has a strong influence on arterial wall elasticity in the systemic circulation. Furthermore, $Z_C$ depends on heart rate, which likely explains the lack of in-
crease in $Z_C$ in the high hematocrit control mice. That is, while increased blood viscosity should increase $Z_L$ and thus $Z_C$, decreased heart rate should decrease Womersley number and thus $Z_L$, such that no change in either $Z_L$ or $Z_C$ is a reasonable result.

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>pH</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>pO2, mmHg</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>SO2, %</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>pCO2, mmHg</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>Hemoglobin, g/dl</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>Shear stress, dyn/cm², at $\gamma = 131$ s⁻¹</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>Viscosity, cP at $\gamma = 131$ s⁻¹</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. pO2: partial pressure of oxygen; pCO2: partial pressure of carbon dioxide; SO2: 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 Z_c, PWV, and pulmonary arterial compliance in the 10H-Nlxk and 21H-Nlxk mice suggest that decreasing hematocrit has the beneficial effect of decreasing metrics of pulsatile RV afterload, via increasing blood inertance. 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.

ACKNOWLEDGMENTS
We thank Dr. Ron R. Magness and Jason Austin for assistance in performing viscosity measurements and analysis.

GRANTS
This study was supported by National Institutes of Health Grants R01-HL-086939 and R01-HL-105598 (to N. Chelser), K25-HL-09749 (to K. Hunter), and HL-49210, HD-38843, and HL-117341 (to R. R. Magness).

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

AUTHOR CONTRIBUTIONS

REFERENCES