INCREASED RED BLOOD CELL STIFFNESS INCREASES PULMONARY VASCULAR RESISTANCE AND PULMONARY ARTERIAL PRESSURE

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Running Title: Increased RBC stiffness increases PVR and mPAP

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Abstract

Patients with sickle cell anemia (SCD) and pulmonary hypertension (PH) have a significantly increased risk of sudden death compared to patients with SCD alone. Sickled red blood cells (RBCs) are stiffer, more dense, more frequently undergo hemolysis, and have a 6-fold shorter lifespan compared to normal red blood cells. Here, we sought to investigate the impact of increased RBC stiffness, independent of other SCD-related biological and mechanical RBC abnormalities, on the hemodynamic changes that ultimately cause PH and increase mortality in SCD. To do so, pulmonary vascular impedance (PVZ) measures were recorded in control C57BL6 mice before and after ~50 μL of blood (Hct=45%) was extracted and replaced with an equal volume of blood containing either untreated RBCs or RBCs chemically-stiffened with glutaraldehyde (Hct=45%). Chemically-stiffened RBCs increased mean pulmonary artery pressure (13.5±0.6 mmHg at baseline to 23.2±0.7 mmHg after the third injection), pulmonary vascular resistance (1.23±0.11 mmHg*min/ml at baseline to 2.24±0.14 mmHg*min/ml after the third injection), and wave reflections (0.31±0.02 at baseline to 0.43±0.03 after the third injection). Chemically-stiffened RBCs also decreased cardiac output, but did not change hematocrit, blood viscosity, pulmonary arterial compliance or heart rate. The main finding of this study is that increased RBC stiffness alone affects pulmonary pulsatile hemodynamics, which suggests that RBC stiffness plays an important role in the development of PH in patients with SCD.

Keywords: cardiopulmonary hemodynamics; sickle cell anemia; pulmonary vascular impedance, red blood cell stiffness, pulmonary hypertension

Introduction

Sickle cell anemia or sickle cell disease (SCD) is one of the most common heritable monogenetic diseases in the world. The World Health Organization estimates there are ~275,000 births per year world-wide with SCD [1]. SCD is characterized by a single amino acid substitution of hydrophobic valine for hydrophilic glutamic acid in the β-chain of hemoglobin. This substitution modifies the quaternary structure of the protein, predisposing it to polymerization of hemoglobin in the deoxygenated state which deforms the red blood cell (RBC) into the characteristic sickled shape [2, 3]. Sickled RBCs are biologically and mechanically quite different from normal RBCs, and these differences lead to clinically important consequences. For example, clinically, SCD is associated with ischemia, thrombotic events, oxidative stress and reperfusion injury in multiple organs [4]. Biologically, sickled RBCs have significantly shortened lifespan and more frequently undergo intravascular hemolysis, which releases free hemoglobin into the plasma, which in turn scavenges nitric oxide [5]. The scavenging of nitric oxide can acutely impair vasodilation and chronically cause endothelial cell dysfunction, vascular cell proliferation, and inflammatory stress [4]. Mechanically, sickled RBCs have increased stiffness [6], which leads to trapping in the microcirculation and downstream ischemia. All of these consequences of SCD can and often do have significant effects on the vasculature including increasing pulmonary vascular resistance (PVR) [4] and systemic arterial wave reflections [7].

The World Health Organization has found that of the chronic cardiopulmonary effects of SCD, pulmonary hypertension (PH) is the major cause of morbidity and mortality [4]. In fact, patients with SCD-related PH have a substantially increased risk of sudden death compared to those with SCD alone, as high as a 50% within 2 years [8, 9]. SCD is associated with a high prevalence of

PH--about 30% [10, 11]. Higher still is the percentage of SCD patients with evidence of PH via lung histology at the time of death--75% [12]. While mortality in patients with SCD-related PH is high, right heart catheterization studies have shown only moderate increases in mean pulmonary artery pressure (mPAP) (30-40 mmHg with SCD compared to 15-20 mmHg in healthy adults [13]) and pulmonary vascular resistance (PVR) (2.24 Wood units with SCD compared to 1 Wood unit in healthy adults [13]). Additionally, patients with SCD-related PH have shown little to no improvement with therapies for PH alone [14-16].

Therefore, mild PH in patients with SCD, which does not respond to traditional therapies, dramatically increases mortality via mechanisms that are not well understood. The mechanical changes in RBCs with SCD are a likely contributor to abnormal pulmonary vascular hemodynamics. Mechanical changes in RBCs are particularly significant in the low oxygen pulmonary arterial vasculature as RBCs are most likely to sickle in this decreased oxygen environment. How mechanical changes to RBCs in SCD, such as increased stiffness, which has been shown to affect myocardial infarction, essential hypertension, and modify whole blood viscosity [17, 18], affect right ventricular (RV) afterload has yet to be investigated.

In this study, we sought to investigate the impact of increased RBC stiffness, independent of other SCD-related biological and mechanical RBC abnormalities, on the hemodynamic changes that ultimately cause PH and increase mortality in SCD. We hypothesized that SCD-like RBC rigidity would increase mPAP, PVR and wave reflections in the pulmonary circulation of otherwise healthy lungs. To test this hypothesis, we replaced normal RBCs with chemically-stiffened RBCs in mice and measured pulmonary vascular impedance (PVZ) *in vivo*. PVZ is the

most comprehensive measure of RV afterload since it measures opposition to both steady and oscillatory flow in the pulmonary vasculature. Our results confirmed that RBC stiffness dramatically increases mPAP, PVR and wave reflections, and likely contributes to poor outcomes in SCD-related PH.

Materials and Methods

Mice

Male C57BL6/J mice, 12-14 weeks-old, were obtained from Jackson Laboratory (Bar Harbor, ME) and used either as donors for RBCs or for *in vivo* experiments. Mice used for *in vivo* experiments were divided into two separate groups: one injected with control, untreated RBCs (n=6) and the other injected with chemically stiffened RBCs (n=11). The University of Wisconsin Institutional Animal Care and Use Committee approved all procedures.

Red blood cell preparation

Whole blood was drawn from donor mice and centrifuged at 500g for 15 minutes. Then, the plasma, buffy coat and top layer of cells were removed. RBCs were resuspended to a Hct of ~45% with osmolality and PH balanced saline and centrifuged three more times to remove all non-RBC components. To create SCD-like rigidity, RBCs were treated with 0.016% glutaraldehyde for 30 minutes, which has been shown to stiffen RBCs without altering cell shape [19]. Chemically-stiffened RBCs were centrifuged at 500g for 15 minutes 8 times with isotonic saline and then were resuspended to 45% Hct with isotonic buffered saline.

In vivo Hemodynamic Measurements

Mice were instrumented for PVZ measurements as previously described [20, 21]. In brief, mice were anesthetized with urethane (2mg/g body weight), intubated and placed on a ventilator using a tidal volume of ~225 μL and respiratory rate of ~200 breaths/min of room air. A 1.2F catheter-tip pressure transducer (Scisense, Inc., London, Ontario, Canada) was inserted into the right carotid artery to monitor systemic arterial pressures and a 1.0F pressure-tip catheter (Millar Instruments, Houston, TX) was inserted into main pulmonary artery through the right ventricle to measure pulmonary arterial pressures. Pressure tracings were recorded at 5 kHz on a custom hemodynamic workstation (Cardiovascular Engineering, Norwood, MA). Measurement of blood flow velocity and main PA inner diameter were performed via ultrasound (Visualsonics, Toronto, Ontario, Canada) with a 40 MHz probe during the surgery and recorded with our custom system as done previously; volumetric flow rate (Q) was calculated using velocity time integral and main PA inner diameter measurements obtained via long axis view [20, 21].

After all measurements were completed at baseline, ~50 μL of blood was extracted and replaced with an equal volume of saline containing chemically-stiffened RBCs or control RBCs at 45% by volume (Hct=45%). After a five-minute stabilization period, all hemodynamic measurements were repeated, and this cycle was repeated 2 more times, for 3 total blood replacements and 4 total PVZ measurements. Hematocrit was measured with each 50-μL blood collection. Before euthanasia, a 500-μL sample of blood was taken for viscosity analysis. Each sample was drawn into a closed tube for oscillatory tube viscometry (Vilastic-3 Viscoelasticity Analyzer). Samples were sheared with a constant 2 Hz frequency and continually increasing shear rate. The viscometer was calibrated before testing the first sample of the day; at most four samples were

tested per day and the duration of testing each sample was 25 minutes. Measurements were performed within 12 hours of collection.

In vivo experiments with chemically-stiffened RBCs and control RBCs were performed by different small animal surgeons trained by one of the authors (TAH).

In vivo Hemodynamic Calculations

Data obtained during our experiments was analyzed as previously described [20, 21] using our custom software and hemodynamic workstation (Cardiovascular Engineering, Norwood, MA). In brief, our software used ECG as a fixed standard of reference for the Q and P waveforms, which then processed and analyzed our data accordingly. All equations used henceforth can be found in these previous publications [20, 21], including but not limited to pulmonary vascular resistance Z_0, characteristic impedance Z_C, pulmonary arterial compliance Ca, wave reflections RQ, and pulse wave velocity PWV.

Statistical Analysis

For each group, the significances of the overall changes in parameters with blood replacement were assessed using a one-way analysis of variance (ANOVA) for condition (chemically-stiffened or control RBCs) or generalized least squares for repeated measurements (with subsequent blood replacements). Due to the different surgical personnel and consequently different baseline pressure and flow values, comparisons were not performed between control and chemically-stiffened RBC groups. Also, data are presented in figures normalized to the mean baseline (pre- blood replacements) value for each condition; non-normalized data are

presented in Table 1. When the ANOVA reached statistical significance, Tukey multiple comparisons were used for post hoc analysis. Data were considered significant for P-values less than 0.05. All data are presented in terms of means ± standard error. Statistical analysis was performed using R software (Foundation for Statistical Computing, USA, version 2.14.0).

Results

Morphometric effects of blood replacement

The average body weight for the mice in each experimental group was not different (Table 1 and 2). Hematocrit did not change significantly with blood replacement using either chemically-stiffened RBCs or control RBCs (Table 1 and 2).

Hemodynamic effects of blood replacement with chemically-treated RBCs. Mean pulmonary arterial pressure (mPAP) increased significantly after the first and second blood replacements with chemically-stiffened RBCs and remained elevated after the third blood replacement; mPAP did not increase with blood replacement using control (untreated) RBCs (Figure 1; Table 1 and 2). Systolic pulmonary arterial pressure (sPAP) and diastolic pulmonary arterial pressure (dPAP) changed in the same ways as mPAP (Table 1 and 2).

Cardiac output (CO) decreased continuously after the first two blood replacements of chemically-stiffened RBCs and reached a plateau after the third; CO did not decrease with blood replacement using control RBCs (Figure 2; Table 1 and 2). Due to increases in mPAP and decreases in CO, total pulmonary vascular resistance (Z_0) increased after the first two blood replacements and reached a plateau after the third blood replacement using chemically-stiffened

RBCs, Z_0 did not change with blood replacement using control RBCs (Figure 3; Table 1 and 2). The same pattern was evident in wave reflections (RQ) (Figure 4; Table 1 and 2).

Characteristic impedance (Z_C) tended to decrease with one blood replacement using chemically-stiffened RBCs and significantly decreased after the second blood replacement; Z_C did not decrease with blood replacement using control RBCs (Figure 5; Table 1 and 2).

The duration of systole decreased with all three blood replacements using chemically-stiffened RBCs; no decrease occurred with blood replacement using control RBCs (Table 1 and 2).

Pulse wave velocity (PWV), pulmonary arterial compliance, systemic systolic pressure, main pulmonary artery diameter and pulse pressure did not change after any blood replacement using either chemically-stiffened RBCs or control RBCs (Table 1 and 2). Heart rate did not change after any infusion of chemically stiffened RBCs and did not decrease after the 1^{st} or 3^{rd} infusion of control RBCs, however it did significantly decrease after the 2^{nd} infusion of control RBCs (Table 1 and 2).

Blood viscosity was not changed after three blood replacements with chemically-stiffened RBCs compared to whole blood from donors (Figure 6).

Discussion

The major, novel contribution of this study is demonstrating that RBC stiffening plays a key role in altering pulmonary vascular hemodynamics and increasing RV afterload, including increasing mPAP, PVR and wave reflections.

SCD is known to cause increased RBC stiffness [22, 23] through mechanisms that remain poorly understood but likely involve abnormal RBC membrane properties, abnormal intracellular hemoglobin polymerization, and increased intracellular density. While the impact of each of these RBC abnormalities on blood flow has been tested *in vitro* [24-28], no studies to date have investigated their physiological impact. Here we investigated how RBC stiffening acutely affects pulmonary pulsatile hemodynamics for the first time *in vivo*. Furthermore, while SCD often affects both RBC mechanics and morphology [29], there is a separate class of RBCs in sickle cell anemia with altered mechanics but apparently normal morphology [30]. Hence, our results obtained with glutaraldehyde-treated RBCs that have increased stiffness and unchanged morphology [19] are especially relevant to the clinical impact of these SCD RBCs in sickle cell anemia.

To isolate how changes in RBC stiffness affect the pulmonary vasculature, we replaced 50 μL of whole blood with 50 μL of a solution containing chemically-stiffened RBCs (45% by volume, or 45% Hct) three separate times. We demonstrate for the first time that stiffened RBCs increase the static component of RV afterload, PVR, continuously, evidenced by both continual increases in mPAP and continual decreases in cardiac output.

We also demonstrate for the first time that stiffened RBCs alter the pulsatile component of RV afterload. Stiffened RBCs acutely increased wave reflections, which is suggestive of increased arterial stiffening. The increase in mPAP could be a possible explanation for acutely increased pulmonary arterial stiffness due to strain-induced arterial stiffening [31]. Additionally, increased backward wave reflection traveling to the heart (RQ; Figure 4) could be caused by trapping of the stiffened RBCs in the lung capillaries. We also found that stiffened RBCs altered the pressure waveform such that the duration of systole was shorter, which may have been caused by increased wave reflections. This finding also suggests that the right ventricle was not able to fully compensate for the effects of chemically stiffened RBCs. However, we did not measure RV function in this study.

We observed a statistically significant decrease in Z_C, which would suggest decreased, not increased, pulmonary arterial stiffening. However, as discussed in [20], Z_C depends not only on proximal artery stiffness (with an inverse square root relationship) but also on blood inertance in the proximal arteries (with a square root relationship). The decrease in CO found here decreases inertance, which likely explains the decrease in Z_C. Viscous losses along the artery walls and Womersley's number can also affect the longitudinal impedance Z_L [32, 33], which contributes to the characteristic impedance, but neither viscosity nor heart rate (or MPA ID) changed with stiffened RBCs. We did not observe significant differences in pulse wave velocity or pulmonary arterial compliance with stiffened RBCs, but both tended to decrease following the same pattern as Z_C.

Hematocrit did not change after infusions of stiffened or control RBCs, which was expected given our experimental design. Whole blood viscosity also did not change after 3 infusions of stiffened RBCs at any shear rate measured. This finding is likely due to the relatively small amount of stiffened RBCs infused. If we assume that the total blood volume of a mouse is 1.8 mL [34] and Hct is 45%, then the replacement of ~ 67.5 μL normal RBCs (i.e., 150 μL whole blood at 45% RBCs by volume) with ~ 67.5 μL stiffened RBCs leads to blood with at most 8.3% of the RBCs in the blood stiffened. Furthermore, stiffened RBCs are more likely to be trapped in both the pulmonary and systemic microcirculation, and therefore may be underrepresented in our samples. That said, even in SCD patients, viscosity of whole blood can be normal when tested ex vivo in a fully oxygenated state [35]. We did not measure the viscosity of a solution of 100% stiffened RBCs. A benefit of the constancy of both hematocrit and whole blood viscosity with our blood replacements is that it does not confound the interpretation of results. That is, the significant increases in mPAP, PVR and wave reflections found here must be due to increased RBC stiffness, as opposed to how changes in RBC stiffness alter viscosity and thereby alter pulmonary pulsatile hemodynamics.

There are four main limitations of this work. First, we did not measure the stiffness of our glutaraldehyde-stiffened RBCs. Sickled RBCs, when tested with micropipette aspiration, have an elastic shear modulus of 21.1×10^{-3} μdyn/cm, compared to normal RBCs, which have an elastic shear modulus of 11.0×10^{-3} μdyn/cm [36]. Exposure of normal RBCs to 0.010% and 0.019% glutaraldehyde solution for one hour results in RBCs having an elastic shear moduli 22.0×10^{-2} μdyn/cm and 32.0×10^{-2} μdyn/cm, respectively [37], which are an order of magnitude larger than both sickled and normal RBC moduli. We exposed to normal RBCs to 0.016%

glutaraldehyde for 30 minutes, which should cause less stiffening, but we cannot directly relate our treatment to the clinical disease state in the absence of direct measurements. Second, glutaraldehyde-stiffened RBCs may deliver less oxygen than untreated, control RBCs, which could cause vasoconstriction and subsequent increases in total PVR independent of RBC stiffening. Previous literature has shown that glutaraldehyde-fixed hemoglobin has a saturation curve with P50 at 20 mmHg, close to the normal hemoglobin value of 26.6 mmHg and far from the myoglobin value of 2.8 mmHg [38], so we anticipate this potential effect is small. Third, glutaraldehyde-treated RBCs are potentially cytotoxic, which could increase PVR due to platelet activation, injury-induced vasoconstriction and inflammation. Previous literature has reported that washing glutaraldehyde-treated RBCs 3 times over 2 hours, removes all traces of glutaraldehyde from solution [39], so that upon blood replacement free glutaraldehyde in solution doesn't initiate a clotting cascade. To be conservative, we chose 8 washes over 3 hours to minimize this potential effect. Fourth, two different small animal surgeons performed the *in vivo* measurements and obtained different baseline pressure and flow values, which precluded a quantitative comparison of PVZ metrics between control and stiffened RBC groups. The absence of changes in PVZ metrics with multiple blood replacements using control RBCs gives us confidence in our interpretation of results despite this limitation.

The major novel finding of this study is that chemically-stiffened RBCs affect both static and dynamic components of right ventricular afterload. The effects of other SCD-related RBC changes on pulmonary vascular hemodynamics and right ventricular function merit further investigation.

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Figure Legends

Figure 1. Normalized mean pulmonary arterial pressure (mPAP) for (a) blood replacement with chemically-stiffened RBCs, and (b) blood replacement with control RBCs. *P < 0.05 vs. CTL; †P < 0.05 vs. 1st blood replacement.

Figure 2. Normalized cardiac output (CO) for (a) blood replacement with chemically-stiffened RBCs, and (b) blood replacement with control RBCs. *P < 0.05 vs. CTL; †P < 0.05 vs. 1st blood replacement.

Figure 3. Normalized pulmonary vascular resistance (Z_0) calculated as mPAP/CO for (a) blood replacement with chemically-stiffened RBCs, and (b) blood replacement with control RBCs. *P < 0.05 vs. CTL; †P < 0.05 vs. 1st blood replacement.

Figure 4. Normalized pulmonary arterial wave reflections (RQ) calculated as the ratio of the amplitude of backward traveling components (P_b) to forward traveling components (P_f) for (a) blood replacement with chemically-stiffened RBCs, and (b) blood replacement with control RBCs. *P < 0.05 vs. CTL; †P < 0.05 vs. 1st blood replacement.

Figure 5. Normalized characteristic impedance (Z_C) calculated from dP/dQ when flow reaches 95% of its maximal value for (a) blood replacement with chemically-stiffened RBCs, and (b) blood replacement with control RBCs. *P < 0.05 vs. CTL; †P < 0.05 vs. 1st blood replacement.

Figure 6. Shear stress vs. shear rate for donor whole blood (Donor) and whole blood from mice after 3 blood replacements with chemically-stiffened RBCs (Stiff RBC replacement) obtained using an oscillatory tube viscometer. *P < 0.05 vs. CTL.

Tables

Table 1. Body weight, hematocrit and hemodynamic measurements from pulmonary vascular impedance in mice that underwent blood replacement with chemically-stiffened RBCs.

Stiff RBCs	**Baseline**	**1st**	**2nd**	**3rd**
Body weight (g)	26.8±0.6	N/A	N/A	N/A
Hct (-)	42.8±1.2	42.7±1.1	42.4±1.7	42.1±1.5
sAP (mmHg)	77±4	75±5	72±6	73±3
mPAP (mmHg)	13.5±0.6	18.9±0.4 *	21.8±0.5 *†	23.2±0.7 *†
sPAP (mmHg)	20.0±0.3	25.1±0.6 *	28.4±0.4 *†	29.7±0.7 *†
dPAP (mmHg)	7.9±0.7	13.4±0.5 *	16.4±0.3 *†	18.1±0.6 *†
PVR (mmHg min/ml)	1.23±0.11	1.85±0.16 *	2.22±0.18 *†	2.24±0.14 *†
RQ	0.31±0.02	0.39±0.02 *	0.44±0.01 *†	0.43±0.03 *
Ca (mm^2/mmHg)	1.40±0.08	1.36±0.16	1.28±0.13	1.23±0.14
CO (ml/min)	12.0±0.7	10.7±0.6 *	10.1±0.6 *	9.6±0.8 *
Z_C (mmHg min/ml)	0.26±0.02	0.22±0.01	0.19±0.01 *	0.19±0.01 *
Tes (ms)	52.0±1.0	47.5±0.5 *	46.4±0.8 *	45.4±1.8 *
PWV (mm/ms)	0.34±0.03	0.31±0.02	0.29±0.05	0.29±0.03
HR	551±12	549±9	541±9	530±5
PA diameter (mm)	1.37±0.06	1.39±0.08	1.40±0.07	1.43±0.04
PP (mmHg)	12.3±0.6	12.0±0.5	12.2±0.8	11.4±0.9

Values are means ± SE; *n=11* baseline, 1st, 2nd and 3rd blood replacements. PA, pulmonary arterial, HCT, hematocrit, sAP, systolic aortic pressure, mPAP, mean PA pressure, sPAP, systolic PA pressure, dPAP, diastolic PA pressure, PVR, pulmonary vascular resistance, RQ, PA wave reflections, Ca, PA compliance, CO, cardiac output, Z_C, characteristic impedance, Tes, duration of systole, PWV, pulse wave velocity, HR, heart rate, PP, pulse pressure. *$P < 0.05$ vs. CTL; †$P < 0.05$ vs. 1st blood replacement.

Table 2. Body weight, hematocrit and hemodynamic measurements from pulmonary vascular impedance in mice that underwent blood replacement with control RBCs.

Ctl RBCs	**Baseline**	**1st**	**2nd**	**3rd**
Body weight (g)	26.5±0.9	N/A	N/A	N/A
Hct (-)	41.4±1.4	41.7±1.6	40.8±1.9	41.2±1.3
sAP (mmHg)	67±2	66±4	70±8	63±5
mPAP (mmHg)	15.9±1.0	16.0±1.3	15.8±1.4	15.2±1.8
sPAP (mmHg)	22.3±1.0	22.5±1.4	22.3±1.5	21.5±2.0
dPAP (mmHg)	10.7±0.8	10.8±1.1	10.3±1.2	10.0±1.6
PVR (mmHg min/ml)	2.0±0.3	2.0±0.4	1.9±0.3	1.7±0.3
RQ	0.35±0.02	0.32±0.02	0.34±0.02	0.32±0.01
Ca (mm^2/mmHg)	1.34±0.16	1.40±0.23	1.41±0.24	1.37±0.28
CO (ml/min)	8.6±0.5	8.8±0.6	8.9±0.7	8.7±0.2
Z_C (mmHg min/ml)	0.28±0.02	0.30±0.02	0.28±0.02	0.26±0.03
Tes (ms)	47.8±1.1	48.0±1.2	49.2±1.8	49.0±1.9
PWV (mm/ms)	0.35±0.02	0.37±0.02	0.36±0.03	0.35±0.04
HR	527±25	507±28	492±30 *	521±20
PA diameter (mm)	1.30±0.03	1.30±0.02	1.31±0.02	1.32±0.02
PP (mmHg)	11.8±0.5	11.8±0.6	12.2±0.4	12.0±0.6

Values are means ± SE; *n=6* baseline, 1st, 2nd and 3rd blood replacements. PA, pulmonary arterial, HCT, hematocrit, sAP, systolic aortic pressure, mPAP, mean PA pressure, sPAP, systolic PA pressure, dPAP, diastolic PA pressure, PVR, pulmonary vascular resistance, RQ, PA wave reflections, Ca, PA compliance, CO, cardiac output, Z_C, characteristic impedance, Tes, duration of systole, PWV, pulse wave velocity, HR, heart rate, PP, pulse pressure. **P* < 0.05 vs. CTL; †*P* < 0.05 vs. 1st blood replacement.

Figure 1a,b

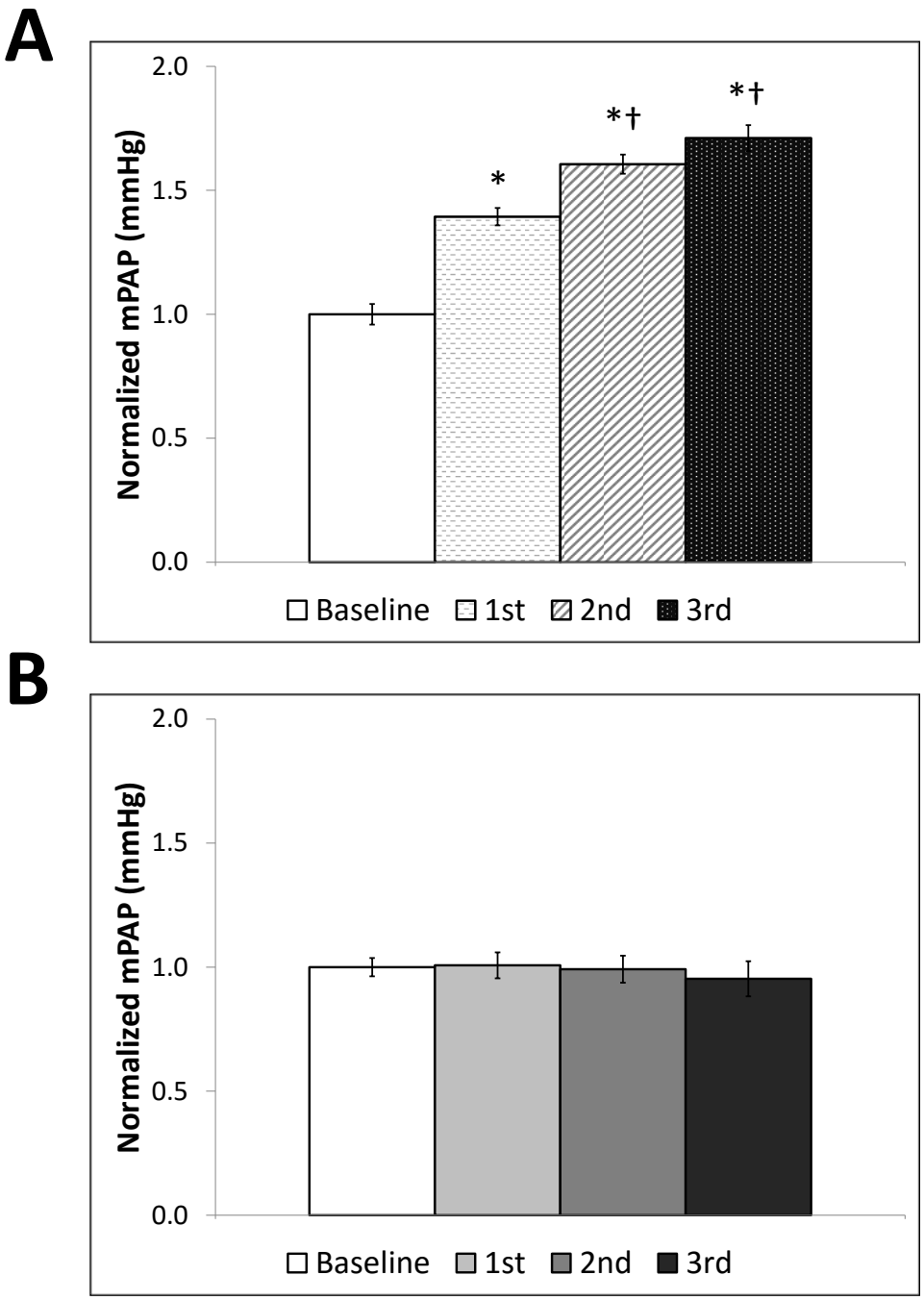

A
2.0
1.5
1.0
0.5
0.0
Normalized mPAP (mmHg)
*
*†
*†
Baseline
1st
2nd
3rd
B
2.0
1.5
1.0
0.5
0.0
Normalized mPAP (mmHg)
Baseline
1st
2nd
3rd

Figure 2 a,b

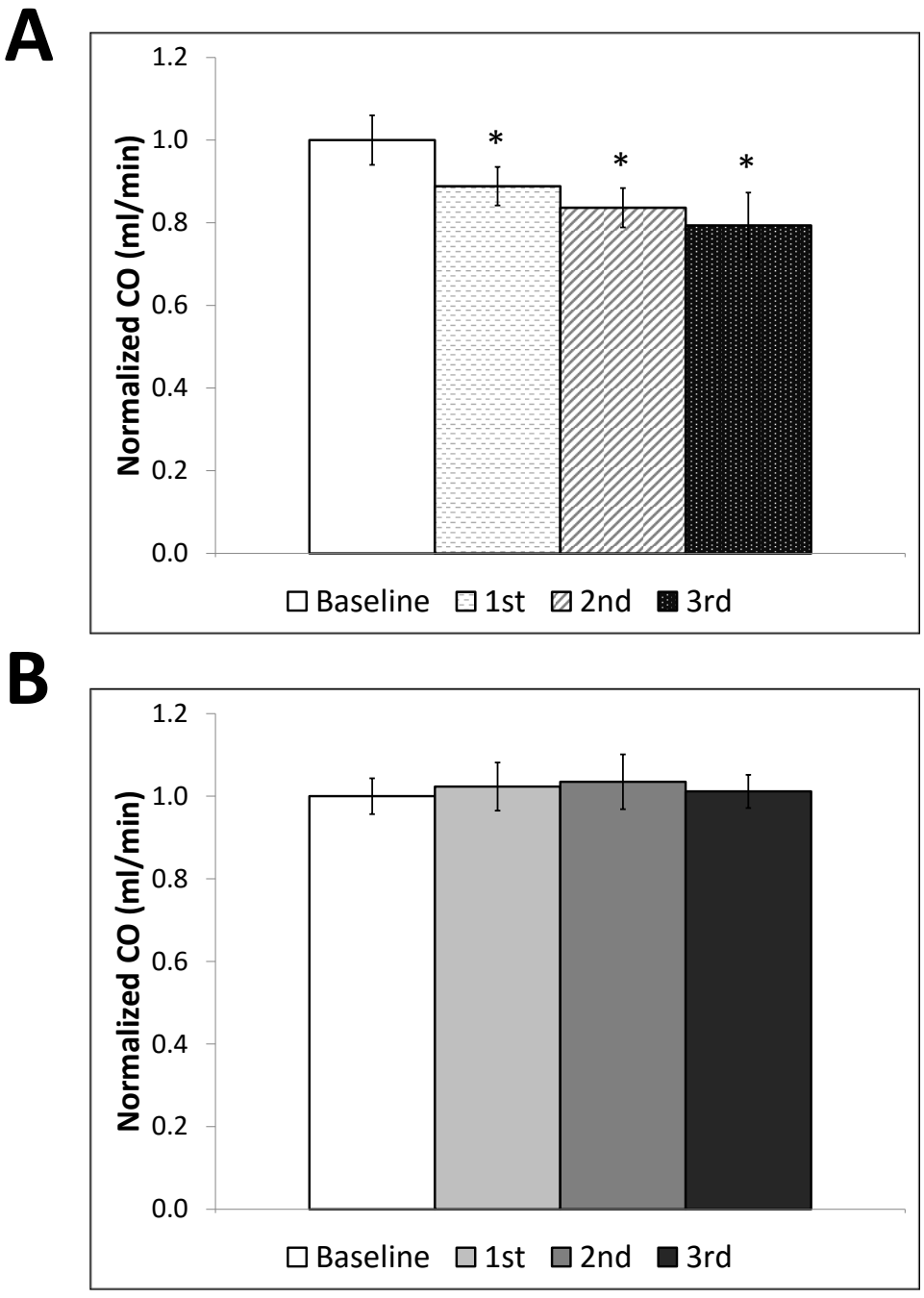

A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized CO (ml/min)
*
*
*
Baseline
1st
2nd
3rd
B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized CO (ml/min)
Baseline
1st
2nd
3rd

Figure 3 a,b

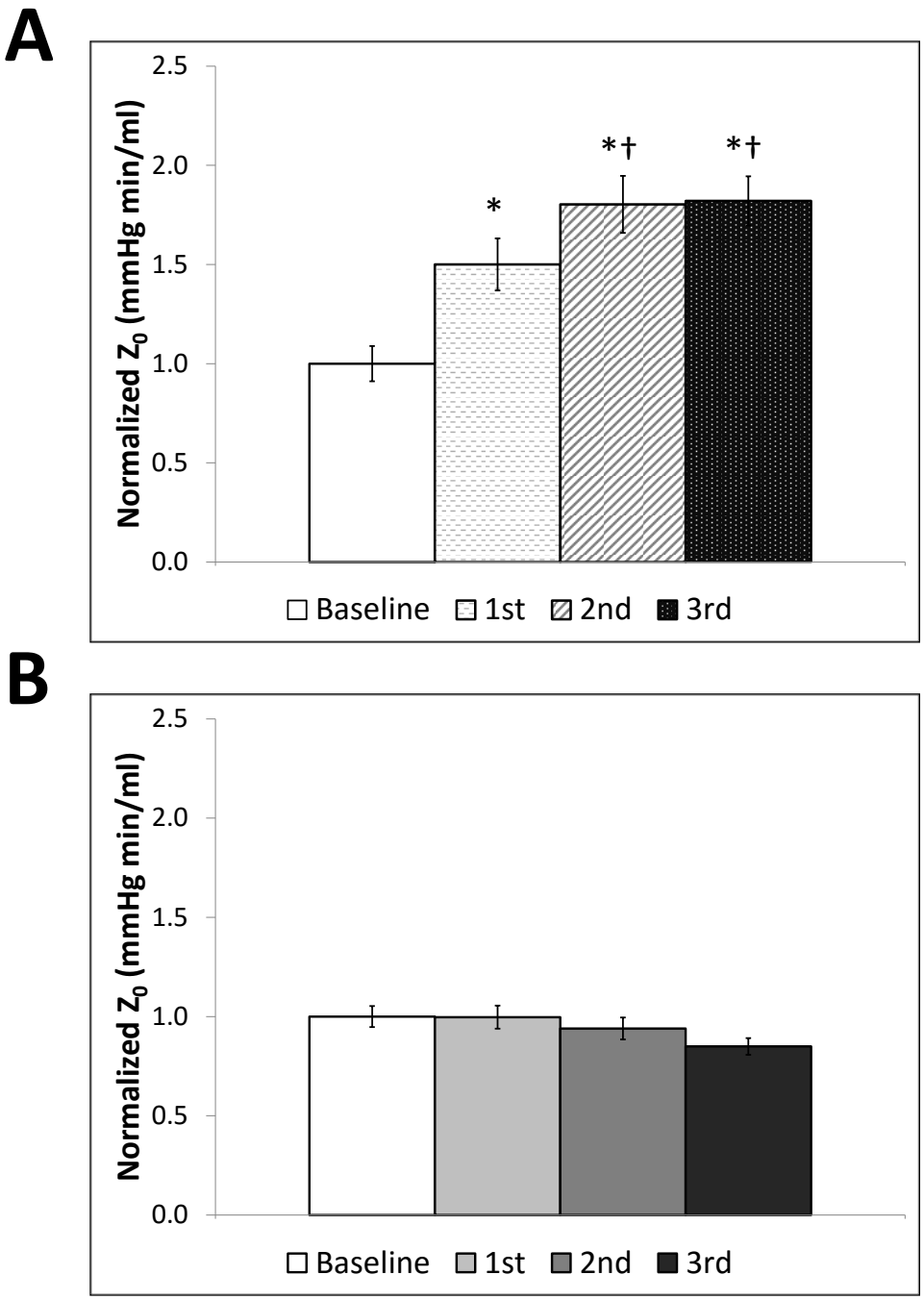

A
Normalized Z_0 (mmHg min/ml)
2.5
2.0
1.5
1.0
0.5
0.0
*
*†
*†
Baseline
1st
2nd
3rd
B
Normalized Z_0 (mmHg min/ml)
2.5
2.0
1.5
1.0
0.5
0.0
Baseline
1st
2nd
3rd

Figure 4 a,b

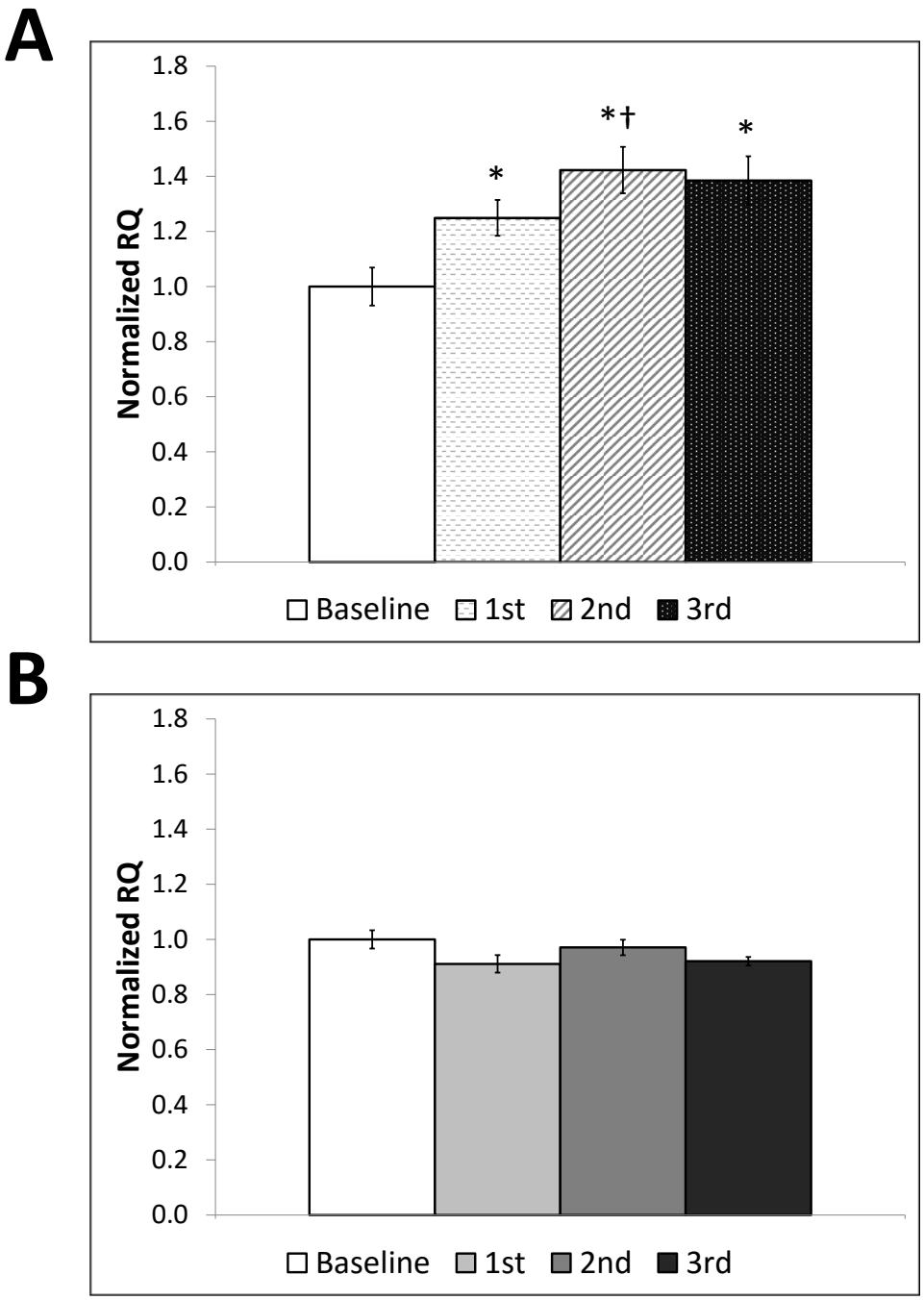

A
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized RQ
*
*†
*
Baseline
1st
2nd
3rd
B
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized RQ
Baseline
1st
2nd
3rd

Figure 5 a,b

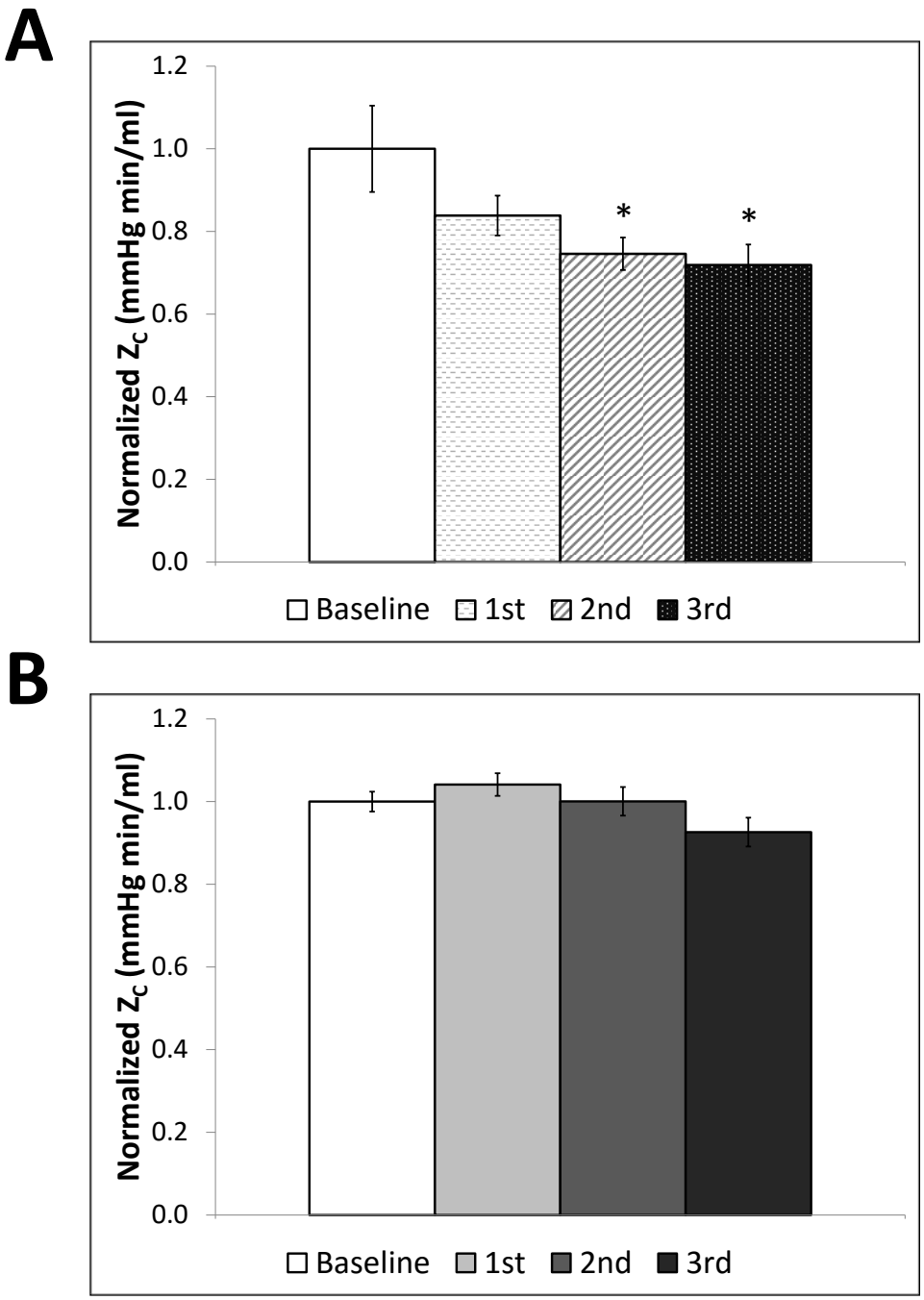
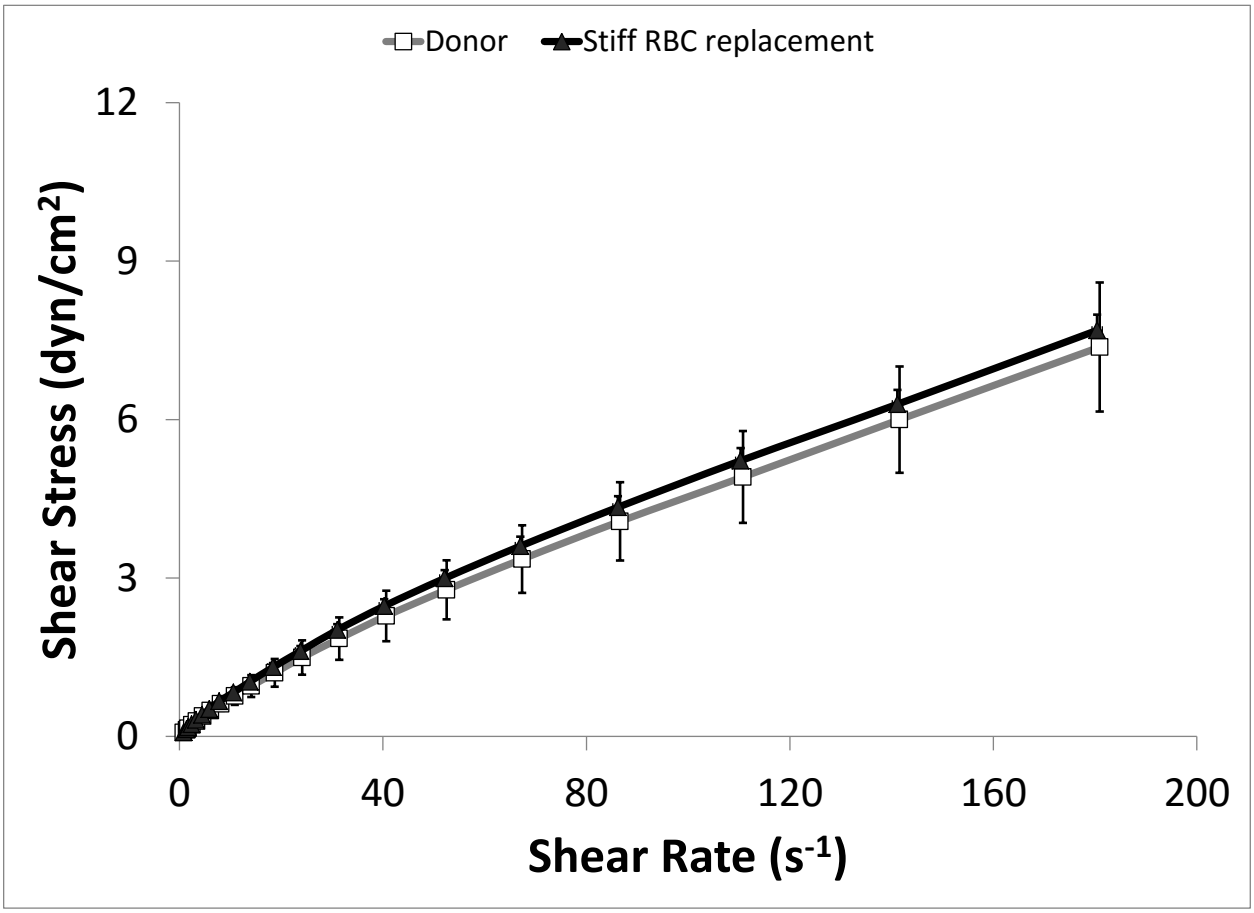

A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Z_C (mmHg min/ml)
*
*
Baseline
1st
2nd
3rd
B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Z_C (mmHg min/ml)
Baseline
1st
2nd
3rd

Figure 6


Donor
Stiff RBC replacement
12
9
6
3
0
Shear Stress (dyn/cm²)
0
40
80
120
160
200
Shear Rate (s⁻¹)