Right Heart Pulmonary Vascular Interactions in Adults Born Preterm

By

Arij G Beshish

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This dissertation is approved by the following members of the Final Oral Committee:

Marlowe W Eldridge, MD, Professor, School of Medicine and Public Health

Kara N Goss, MD, Assistant Professor, School of Medicine and Public Health

Luke J Lamers, MD, Assistant Professor, School of Medicine and Public Health

Garry M Diffee, PhD, Professor, School of Education

Christopher Francois, MD, Associate Professor, School of Medicine and Public Health

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Abstract:

Premature birth, defined as birth prior to 37 weeks gestation, affects 10% of live births in the United States. Advances in neonatal care have resulted in the increased survival of infants born at extremely low gestational ages. Infants born preterm are at higher risk of developing pulmonary arterial hypertension that can ultimately lead to right ventricular (RV) dysfunction with subsequent RV failure. However, little is known about the long-term impact of preterm birth on the RV and pulmonary vasculature.

Our goal is to determine the long-term effects of prematurity on RV and pulmonary vascular function and hemodynamics.

Preterm subjects (n=11) were recruited from the Newborn Lung Project, with very low birth weight (≤1500g; average gestational age 28 weeks) between 1988 and 1991. Control subjects from the same birth years (n=10) were recruited from the general population. Right heart catheterization was performed to assess RV and pulmonary vascular hemodynamics at rest and during exercise in normoxia and hypoxia. Magnetic resonance imaging was performed to determine RV function at rest.

We found that preterm subjects had higher mean pulmonary arterial pressures (mPAP), with 36% meeting criteria for borderline pulmonary. (RV) afterload was higher at rest among preterm subjects. During exercise, preterm subjects had higher total pulmonary vascular resistance and lower compliance, and demonstrated less ability to augment stroke volume (SV). Utilization of two physiological stressors (hypoxia and hypoxic exercise) unmasked altered RV function in preterm subjects. When looking at the percent change from rest to hypoxic exercise, preterm subjects exhibited an attenuated mPAP, sRVP, CI,SV index and HR response compared to age matched

controls suggesting that the RV is inappropriately compensating for the demand during hypoxic exercise. RV end-diastolic volume, SVI and ejection fraction were significantly lower in preterm subjects. Preterm subjects had higher global RV strain compared to controls. However, RV-PV coupling was lower in preterm subjects compared to controls suggesting they have a less efficient RV. Taken together, Young adults born preterm demonstrate early pulmonary vascular disease, and altered right ventricular function suggesting an increased risk to develop overt pulmonary hypertension and right heart failure.

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Introduction:

Preterm birth is defined as a live birth that occurs prior to 37 weeks gestation. An estimated fifteen million infants are born preterm every year (1). Advances in neonatal care, such as antenatal steroids, artificial surfactant, and improved ventilator strategies have resulted in the increased survival of infants born at extremely low gestational ages (2-4). Although the mortality rate for preterm infants and the gestational age-specific mortality rate have dramatically improved over the last 3 to 4 decades, infants born preterm remain vulnerable to many complications, including respiratory distress syndrome, chronic lung disease, and cardiovascular disorders.(5, 6) Infants born at the lower limit of viability have the highest mortality rates and the highest rates of morbidity.(7) Recurrent health problems such as asthma, recurrent respiratory infections (2, 8-11) and decreased exercise tolerance(12) are common sequelae in early childhood of survivors born premature and have been found to persist into adulthood. Prematurity has been associated with increased risk for pulmonary,(13-16) metabolic,(17, 18) and systemic cardiovascular disease into adulthood.(19-21). Although, there is a high risk of numerous co-morbities associated with preterm birth in the neonatal and early childhood stages, less is known of the long-term sequelae associated with prematurity.

Pulmonary function in individuals born preterm:

Ventilation in preterm infants is compromised by anatomic immaturity of the lungs, impaired or delayed surfactant synthesis, underdeveloped chest wall anatomy, and inefficient clearing of lung secretions (22). These factors may cause pulmonary edema,

disruption of alveolar capillary membranes, damage of the alveolar spaces and inadequate gas exchange immediately after birth (22). Prolonged mechanical ventilation and/or oxygen supplementation treatment may contribute to irreversible damage of lung parenchyma and small airways (23). Recurrent wheezing is markedly increased in infants born before 33 weeks of gestational age when compared to infants born at term. Rate of readmission to the hospital for complications of respiratory tract infection was found to be as high as 50% in the first year of life (24). It has been noted that healthy preterm infants have a moderately reduced FRC and a more pronounced impairment of gas mixing efficiency when compared with infants born at term (25).

Several studies report increased rates of chronic coughing and wheezing among preschool (26) and school aged children born preterm (27-29), especially those in whom bronchopulmonary dysplasia (BPD) developed or prolonged mechanical ventilation was required (30, 31). Impaired lung function and increased respiratory morbidity persist into middle childhood in children born preterm, especially among those with BPD (32). Airway obstruction, hyperactivity, hyperinflation, and abnormal blood gas values were observed in children 6-12 years of age with history of preterm birth (33-35).
Diffused lung capacity for carbon monoxide (DLCO) is a lung function measure that reflects capillary surface area accessible for gas exchange (36). Pulmonary diffusing capacity has been found to be modestly reduced in preterm children compared with term-born control subjects (37).

Cohort studies have demonstrated a significantly greater prevalence of asthma-like symptoms and the use of inhaled asthma medication among adolescence who were born prematurely regardless of whether they had BPD, when compared to age matched

individuals born at term (38-41). Pulmonary gas exchange efficiency is lower in adult survivors of preterm and was accompanied by low arterial blood oxygen tension (42), which may be a result of blunted vascularization and alveolar simplification leading to diffusion limitation or ventilation perfusion mismatch in these individuals. DLCO was also lower in adults born preterm when compared to controls (37). Taken together, these findings suggest that individuals born preterm have altered pulmonary function early in life which continues to be observed well into adulthood.

Exercise Tolerance in Individuals Born Preterm:

Individuals born preterm have impaired exercise capacity. Clinical assessment of children who were born very preterm showed that they had lower exercise capacity compared to age matched controls born at term as measured by a significantly lower peak oxygen consumption despite having normal lung function values (43). Reduced exercise tolerance appears to persist into young adulthood as it has been found that adults born preterm have lower power output at volitional exhaustion Blunted vasculogenesis and alveolar simplification may lead to ventilation-to-perfusion mismatch, diffusion limitations and altered pulmonary vascular function that could in part contribute to the decreased exercise capacity in individuals born preterm.

Right Ventricular and Pulmonary Vasculature Function in Prematurity:

Many factors contribute to the development of PH in preterm infants. The pulmonary vascular bed in preterm infants consists of a smaller overall surface area for gas exchange. That, in combination with injury to the airways and lung parenchyma, results in vasoconstriction, ultimately leading to structural remodeling with intimal hyperplasia and increased muscularization of small pulmonary arteries (14, 44, 45) and increased pulmonary vascular resistance (PVR (44).

The mechanisms by which PVD occurs after preterm birth remain the subject of ongoing investigation. Vascular growth occurs by two distinct mechanisms: angiogenesis (the direct extension of existing vessels), and vasculogenesis (formation of vessels from primitive hemangioblasts) (46). Complex gene–environment interactions, as well as epigenetic influences on gene expression in the setting of prenatal and postnatal factors such as oxidative stress, regional hypoxia, inflammation, and infection, have been shown to impair angiogenesis by disrupting signaling pathways and altering growth factor expression in the developing lung (47). Dysregulation of angiogenic signaling leads to impaired growth, structure, and function of the pulmonary vasculature in part through disruption of local and circulating angiogenic progenitor cells (44).

Pulmonary hypertension (PH) contribute to late morbidity and mortality in premature infants.(48, 49) However, the pathophysiological basis of the pulmonary vascular abnormalities, including, increased pulmonary vascular reactivity, decreased vessel density, and increased vascular stiffness (smooth muscle hypertrophy and increased adventitial thickening) have not been studied in relation to right heart function in adult survivors of prematurity. Young adults born preterm have greater RV mass and lower

resting RV ejection fraction on cardiac MRI than age-matched healthy terms (21), though there was no assessment of the pulmonary vasculature in this study to establish pulmonary vascular disease. Adults born preterm have an increase in pulmonary artery systolic pressure response to incremental exercise when compared to age matched controls (43). Cardiac catheterization has been used to study the pulmonary vascular tone and reactivity in survivors of prematurity. However, these studies were performed in children at rest and thus the long-term impact of premature birth on PV and RV function are not entirely understood.

A current study looked at right ventricular function in a preclinical model of BPD in rats one year of age which corresponds to 30 years in humans, observed an increased RV mass, and a decreased RV ejection fraction. RV pressure overload was secondary to hypertensive pulmonary vascular disease, as well as intrinsic RV dysfunction detected by the inability to maintain contractility in the setting of increased afterload. Altered cardiac mitochondrial function in these rats may be a potential cause for RV dysfunction. Whether adults follow the same course in pulmonary and right ventricular interaction as rats is yet to be determined.

SPECIFIC AIMS

The goal of this study is to determine the long-term sequelae of preterm birth on pulmonary vascular (PV) function and right ventricular (RV) structure and function. **We hypothesize that adult survivors of prematurity have increased PAP and PA stiffness that will lead to altered RV function.**

To test our overall hypothesis, **our specific aims are:**

AIM 1: To determine the long-term impact of prematurity on pulmonary vascular hemodynamics and reactivity.

Rationale and Approach: Blunted pulmonary vascularization and alveolarization is observed in preterm infants. Age related changes in pulmonary vascular function is poorly understood in adults born preterm. We will use a combination of physiological stressors and right-heart catheterization to comprehensively define PV function in adult survivors of prematurity.

Hypothesis 1a: The underlying decreased pulmonary vascularization in adult survivors of prematurity will lead to increased total pulmonary vascular resistance (TPVR) and proximal artery stiffness leading to an increase in pulmonary arterial pressure.

Hypothesis 1b: PV reactivity to hypoxia and exercise will be abnormal in adult survivors of prematurity.

AIM 2: To determine the long-term impact of prematurity on RV structure and function

Rationale: Infants born preterm that develop cardio-pulmonary complications often develop RV hypertrophy. However, it is unclear whether there are anatomical or functional

anomalies in the right heart that are maintained into adulthood Using cutting edge MRI techniques, we will be able to reveal anatomical and functional RV anomalies which could be used as early stage imaging and physiologic biomarkers to follow RV dysfunction.

Hypothesis: Decrease in pulmonary vascularization in adult survivors of prematurity will lead to increase in proximal arterial stiffness, TPVR and afterload increasing the demand on the RV leading to altered RV function.

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Chapter 1: Early Pulmonary Vascular Disease in Adults Born Preterm

Authors: Arij G Beshish[1], Kara N Goss[1,2], Gregory P Barton[1], Kristin Haraldsdottir [1,4], Taylor S Levin[1,4], Laura H Tetri[1], Therese J Battiola[2], Ashley M Mulchrone[3], David F Pegelow[1], Mari Palta[5], Luke J Lamers[1], Andrew M Watson[6], Naomi C. Chesler[1,2,3], Marlowe W Eldridge[1,3,4]

Authors Affiliations: Departments of Pediatrics[1], Medicine [2], Biomedical Engineering [3], Kinesiology [4], Population Health Sciences and Biostatistics and Medical Informatics [5], Orthopedic and Rehabilitation Medicine[6]. University of Wisconsin, School of Medicine and Public Health, Madison Wisconsin

Introduction:

Premature birth, defined as less than 37 weeks of completed gestation, affects 10% of all live births in the United States (1). Advances in neonatal care, including antenatal steroids, artificial surfactant, and improved ventilator strategies, have resulted in increased survival of infants born at extremely low gestational ages and the vast majority now reach adulthood (2-5). Long term, premature birth is associated with increased risk for pulmonary (6-8), metabolic (9, 10), and cardiovascular disease (11, 12). Young adults born preterm have increased airflow obstruction, lower diffusion capacity, and impaired gas exchange efficiency during exercise, suggesting the potential for pulmonary vascular dysfunction (13-15).

Preterm birth is associated with alveolar simplification and altered pulmonary microvascular development. Although prematurity is a known risk factor for neonatal and childhood pulmonary vascular disease (16-18), little is known about the long-term impact of prematurity on the pulmonary vasculature. A recent registry study identified a 3-fold increased risk for development of adult pulmonary arterial hypertension (PAH) following premature birth (19). Furthermore, a recent cardiac magnetic resonance imaging (MRI) study demonstrated that young adults born preterm have greater right ventricular (RV) mass and lower RV ejection fraction than age-matched term-born controls (12). Whether or not pulmonary vascular disease was present in this study remains unknown. We hypothesized that adults born moderate to extremely preterm would have elevated pulmonary arterial pressures and an abnormal pulmonary vascular response to exercise consistent with decreased recruitable pulmonary vascular surface area and a stiffer pulmonary vascular bed. To test our hypothesis, we measured

pulmonary vascular and right ventricular hemodynamic responses to exercise in young adults born preterm as compared to that of controls born at term within the same time period.

Methods:

Subjects:

Preterm subjects (n=11) were recruited from the Newborn Lung Project (20-23), a prospectively followed cohort established at the University of Wisconsin-Madison. Enrolled individuals were born preterm with very low birth weight (≤1500g) between 1988 and 1991 in Wisconsin and Iowa. Comprehensive neonatal medical records for these individuals are available and include maternal, prenatal, birth, and newborn intensive care data. Furthermore, all of the preterm subjects in this study have been followed serially (15, 20-23). Control subjects (n=10) born at term within the same time period (1988 - 1991) were recruited from the general population in Wisconsin. Term and preterm subjects were free of adult cardiopulmonary disease and were non-smokers, determined by a questionnaire completed at the screening visit. This study was approved by the Institutional Review Board at the University of Wisconsin, School of Medicine and Public Health. Written informed consent was obtained from all subjects.

Screening Visit:

During the initial screening visit, general anthropometric data were collected. A Global Physical Activity Questionnaire (GPAQ) was completed for each subject (24). The GPAQ consists of 16 questions that assess physical activity at work, in transport and leisure time, and how much time is spent in sedentary behavior. The scores from the questionnaire are used to determine metabolic equivalent of task (MET) minutes per week as a measure of general activity level.

Subjects underwent baseline pulmonary function testing, including forced expiratory volume at 1 sec (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and FEF_{25-75} (Desktop

Diagnostics/CPFS; Medical Graphics, St. Paul, MN). Diffusing capacity of the lung for carbon monoxide (DL_{CO}) was measured (MasterScreen PFT; Jaeger, Hoechberg, Germany), and corrected for hemoglobin obtained by venous blood draw (Easy life Hb, London, United Kingdom). Predicted values for pulmonary function tests and DLco were calculated as previously described (25, 26).

Each subject performed a progressive, maximal exercise test on a cycle ergometer (Velotron; RacerMate, Seattle, WA) to determine peak oxygen consumption (VO_{2max}) as well as maximal power output (P_{max}), which were used to establish the workload for the right heart catheterization study visit. VO_{2max} was determined during the last 30 sec of the final stage that was completed. P_{max} was determined as the wattage at the highest completed stage. Stage completion required the subjects to finish at least 30 sec of a given stage. Exercise started at 65 W and resistance was increased by 15 W every minute. Subjects were asked to maintain a cadence of 60-70 revolutions per minute (rpm). The exercise test was concluded when subjects were no longer able to maintain 55 rpm for more than five seconds despite strong verbal encouragement.

Right Heart Catheter Visit:

Utilizing standard sterile techniques and ultrasound guidance, the right internal jugular vein was percutaneously accessed under local anesthesia, with placement of a 40 cm long 8F J-shaped Flexor sheath positioned in the right ventricle. Then via the Flexor sheath, two 3.5 F high-fidelity solid-state pressure sensor catheters (Mikro-Cath; Millar, Houston, TX) were placed, one in the right ventricle and the other in the pulmonary artery under fluoroscopic guidance with position confirmation through transducing appropriate waveforms. A 4F fluid-filled catheter was then passed through

the sheath and positioned into the pulmonary artery for blood sampling. All right heart catheter insertions were performed by the same interventional cardiologist. In addition, a 3F arterial catheter was placed in a radial artery under local anesthesia.

After placement of the catheters, subjects were positioned on a supine ergometer (stepper) (Cardio Step Module, Ergospect Medical Technology, Innsbruck, Austria) with which subjects step against pneumatic pistons, with the exercise power (Watts) determined by the continuously adjustable resistance and the step frequency. Subjects exercised for 5 minutes at 70% P_{max} to achieve steady state heart rate (HR). Exercise data were acquired once steady state was achieved. Mixed venous and arterial blood samples were collected from the pulmonary and radial artery catheters respectively, and blood gases were measured (pHOx Basic; Nova Biomedical, Waltham, MA) in triplicates at rest and during steady state exercise. Expired gases were collected in a breath-by-breath manner (Gemini; CWE, Ardmore, PA) and ventilatory and metabolic parameters were continuously recorded and analyzed in PowerLab (Lab chart version 8 for Windows; ADInstruments; Colorado Springs, CO).

Right Ventricular and Pulmonary Vascular Hemodynamic Measurements:

Resting and exercise hemodynamics including right ventricular pressure (RVP) and pulmonary arterial pressure (PAP) were continuously recorded using a Powerlab data acquisition system (Lab chart version 8 for Windows; ADInstruments; Colorado Springs, CO). Cardiac output (CO) at rest and during exercise was calculated using the direct Fick method (VO_2 divided by arterial-venous oxygen content difference). Stroke volume (SV) was calculated as CO divided by HR. Stroke work (SW) was calculated as systolic right ventricular pressure (sRVP) multiplied by SV. CO, SV and SW were indexed for

body surface area (CI, SVI, and SWI, respectively), which was calculated based on the formula of Mosteller (27). Right ventricular (RV) and pulmonary artery (PA) pressures were averaged over a period of 30 seconds at steady state rest and exercise to ensure measurements over multiple respiratory cycles. Total pulmonary vascular resistance (TPVR) was calculated as mPAP divided by CO. Pulmonary artery pulse pressure (PP) was calculated as the difference between systolic and diastolic pulmonary arterial pressure. Pulmonary vascular compliance (Cp) was calculated as SV divided by PP and arterial elastance (Ea) was calculated as mPAP divided by SV, as previously described (28).

Statistical Analysis:

Data were initially grouped by birth status (preterm, term) and condition (rest, exercise). Baseline anthropometric data, pulmonary function testing data, and resting and exercise hemodynamic data were compared across birth status using unpaired Wilcoxon Rank Sum Tests. Comparisons of the same individuals across conditions (for example preterms at rest versus exercise) were made using paired Wilcoxon Rank Sum tests. Adjustment was made for multiple pairwise comparisons using the method initially suggested by Holm (29). Among the preterm participants, separate univariable regressions were developed to evaluate the relationship between mPAP and gestational age, number of days on invasive ventilation, number of days on non-invasive ventilation [continuous positive airway pressure (CPAP)], and total days on combined invasive and non-invasive ventilation. Significance level was determined *a priori* at the 0.05 level and all tests were 2-tailed. All data is presented as mean ± SD, unless otherwise noted. All

statistical analyses were performed in R (Foundation for Statistical Computing, Vienna, Austria)(30).

Results:

Subject characteristics:

Preterm subjects had an average gestational age of 28 ± 2 weeks, and birth weight of 1071 ± 310 grams. Adult anthropometric, physical activity, lung function and exercise data for preterm and term subjects are shown in **Table 1**. Subjects were well matched with respect to anthropometric data and baseline physical activity. Spirometric values including FEV_1 and FVC were similar between control and preterm subjects. However, DL_{CO}, DL_{CO} corrected for alveolar volume (DL_{CO}/V_A) and the respective % predicted values were all significantly lower in the preterm subjects as compared to term subjects. P_{max} and VO_{2max} were also significantly lower in preterm subjects. Additional neonatal characteristics of the preterm subjects are shown in **Table 2**.

Hemodynamics:

Rest: Preterm subjects had a higher resting mPAP (18.2 ± 3.9 vs.14.6 ± 1.9 mmHg, P=0.04) and sRVP (28.7 ± 3.7 vs. 22.5 ± 3.6 mmHg, P= 0.001) when compared to controls (**Figure 1**). While PP and TPVR trended higher at rest among preterm subjects, the differences were not statistically significant (P=0.07 and P=0.11, respectively). Pulmonary arterial elastance (Ea) was significantly higher in preterm subjects and vascular compliance (Cp) was lower compared to controls, consistent with a stiffer pulmonary vascular bed. CI and SVI were similar between groups, although preterm subjects had significantly higher resting HR (83.8 ± 11.8 vs. 71.9 ± 10.2 beats per minute, P= 0.02). SWI also trended higher among preterm subjects at rest (P=0.07).

Exercise: Preterm and term subjects demonstrated a similar increase in mPAP and sRVP with exercise (**Figure 1**). During exercise, preterm subjects had higher TPVR,

and Ea remained significantly higher while Cp remained significantly lower. Preterm subjects also had a blunted decline in TPVR during exercise when compared to controls (ΔTPVR: -0.59 ± 0.38 vs. -1.05 ± 0.36 mmHg/L/min, p=0.03). At steady-state exercise, HR, SVI, and CI were similar between groups. However, preterm subjects were less able to augment SVI from rest to exercise (ΔSVI: 12.31 ± 10.45 vs. 27.64 ± 8.42 ml/m^2, p=0.04). This may be in part due to an inability of the RV to augment stroke work in response to exercise, as preterm subjects failed to increase their RV SWI to the same degree as controls (ΔSWI: 920.3 ± 124.91 vs. 1004.82 ± 576.26 mmHg*ml/m^2, p=0.07).

Correlation with Neonatal Clinical Data: In an effort to identify neonatal characteristics that might predict young adult pulmonary vascular dysfunction, we performed separate univariable linear regressions to predict mPAP using gestational age, days on invasive ventilation, days on non-invasive ventilation and total days on combined invasive and non-invasive ventilation. The strongest neonatal predictor of elevated mPAP in adulthood was the number of days on combined invasive and non-invasive ventilation, and was a stronger predictor than gestational age (**Figure 2**).

Discussion:

We hypothesized that moderate to extreme premature birth persistently impairs pulmonary vascular function, such that adults born preterm have elevated resting pulmonary arterial pressures and an abnormal pulmonary vascular response to exercise. To test our hypothesis, we measured the pulmonary vascular response to exercise in young adults born preterm and compared it to that of term-born controls. Here we have identified that young adults born premature, with an average gestational age of 28 weeks and no known history of adult cardiovascular or respiratory disease, have elevated resting pulmonary vascular pressures and evidence of increased right ventricular afterload and pulmonary vascular stiffness, as reflected by increased arterial elastance and decreased pulmonary vascular compliance at rest and during exercise. Although no individuals had evidence of overt pulmonary hypertension, as defined by a mPAP ≥25 mmHg at rest, one third of preterm subjects met criteria for borderline PH, defined by a resting mPAP 19 - 24 mmHg (31). In addition, adults born preterm demonstrated a blunted SV response to exercise, possibly due to the combination of early cardiac dysfunction and increased afterload.

A systematic review of pulmonary arterial pressures in healthy subjects has previously defined the normal mPAP at rest in a supine position as 14.0 ± 3.3 mmHg. Thus, using standard definitions of normal as mean ± 2 standard deviations, the normal mPAP has been suggested as 7.4 – 20.6 mmHg, and mPAP 21-24 mmHg as borderline PH (32). Although not meeting PH disease threshold criteria, a mPAP of 21-24 mmHg has previously been associated with higher all-cause mortality with a hazard ratio of 4.03 (33). However, a more recent study of borderline pulmonary hypertension among a large cohort of veterans undergoing right heart catheterization suggests a lower

threshold for abnormal. Specifically, they demonstrated an increase in all-cause mortality beginning at a mPAP of 19 mmHg, with an adjusted mortality hazard ratio of 1.23 when borderline PH was defined as mPAP 19-24 mmHg (31). Among our preterm-born cohort, 4 of 11 subjects (36%) presented with resting mPAP 19-24 mmHg, consistent with borderline PH.

The elevated mPAP in adults born preterm is driven by a mild elevation in afterload (Ea). Ea can be elevated by an increase in resistance or a decrease in compliance, both of which were found in the preterm subjects. Preterm subjects had significantly lower vascular compliance when compared to controls, consistent with a stiffer pulmonary vascular bed. Both the increased TPVR and decreased compliance in preterm subjects may be due to a decrease in microvascular surface area, which is consistent with the lower DL_{CO} and DL_{CO}/V_A found in these adults born preterm. In addition to borderline PH as a prognostic indicator of increased mortality, decreased pulmonary vascular compliance maybe a more sensitive predictor of mortality than conventional markers (i.e. pulmonary vascular resistance (PVR) and CI) in pulmonary hypertension patients (34).

In addition to a higher TPVR and decreased compliance when compared to controls during exercise, the decrease in TPVR from rest to exercise (ΔTPVR) was significantly blunted in preterm subjects. This failure to decline with exercise provides further evidence of an inability of the pulmonary vasculature to distend and recruit with exercise in preterm subjects, and may suggest that adults born preterm are using a greater percentage of their total vascular surface area at rest. Of note, an exaggerated increase in pulmonary arterial pressures during exercise among similarly aged adults

born preterm was recently reported (35). Given that our study evaluated pulmonary hemodynamics at 70% of maximal power rather than a graded pulmonary artery systolic pressure response to incremental exercise, our data are not directly comparable. However, our evidence of increased pulmonary vascular stiffness and failure to fully decrease TPVR among adults born preterm is consistent with these prior findings.

Mechanistically, animal models of bronchopulmonary dysplasia (BPD), suggest that preterm birth interrupts pulmonary vascular and alveolar development, resulting in decreased cross-sectional area for blood flow and decreased surface area for gas exchange (36, 37). Vascular simplification and increased vasoreactivity both lead to an increase in pulmonary vascular resistance and result in structural remodeling of the pulmonary arterial circulation (16). Animal models also suggest potential for significant 'catch up' pulmonary vascularization in early life, but ultimately significant vascular pruning and capillary rarefication later in life (38, 39). To what degree subclinical pulmonary vascular disease persists versus re-emerges in humans born premature is unknown. In one study of infants born premature with a birthweight of 500-1250g, echocardiographically diagnosed PH was present in 47% of infants at day 7 of life, but resolved by 36 weeks gestation in 70% of these neonates (17). Whether individuals born preterm have persistent borderline PH throughout childhood and into adulthood is unclear.

At rest, subjects born preterm had higher heart rates but similar SV to controls, resulting in a mild but insignificant increase in resting CO. However, with exercise, preterm subjects failed to augment their SV to the same degree as controls did, suggesting a blunted SV reserve. Importantly, limitations in SV reserve with exercise

have also been identified in adult patients with overt pulmonary hypertension (40, 41). Specifically, since HR is the main mechanism for increasing cardiac output in PH, these patients compensate for the inability to augment SV during exercise by increasing their HR, most likely as a result of increased sympathetic activation (40). In addition, there was a strong trend towards a blunted SWI response to exercise among preterm subjects. Taken together, the perturbed SVI and SWI response from rest to exercise in preterm subjects suggests subclinical cardiac dysfunction. Indeed, a resting cardiac MRI study of a large cohort of adults survivors of preterm birth of similar age to our study population identified clinically relevant RV hypertrophy and decreased RV ejection fraction (12).

Phenotypic variability exists among preterm subjects born at similar gestational ages, making it difficult to predict which individuals are at highest risk for developing pulmonary vascular disease. Neonatal medical records were reviewed for all preterm subjects to evaluate for correlations between neonatal factors and pulmonary vascular disease. Notably, the strongest predictor of elevated mPAP was the number of total days on combined invasive and non-invasive ventilatory support. Not surprisingly, the subjects with the longest durations of invasive ventilation were those who were diagnosed with BPD, clinically defined as treatment with supplemental oxygen >36 weeks post menstrual age. Our number of subjects is too small to draw firm conclusions, however, a diagnosis of BPD may very well be the primary modifier of long-term pulmonary vascular risk.

It is important to recognize that the preterm subjects who participated in this study were active individuals with no known history of adult respiratory or cardiovascular

disease and who were able to perform maximal exercise testing, resulting in some degree of selection bias. Nevertheless, given that we have demonstrated early pulmonary vascular disease in this relatively healthy young adult population, we suspect we may be underestimating the right ventricular and pulmonary vascular pathology in preterm subjects at large. In addition, the premature subjects in this study were born in an era (1988-1991) of rapidly changing neonatal practice, including the introduction of surfactant and the increasing use of antenatal steroids. Thus, it will require further study to determine whether our findings are applicable to younger populations born preterm as they reach adulthood. Finally, we were unable to reliably obtain pulmonary vascular wedge pressures during exercise in this study, which prevented us from commenting on pulmonary vascular resistance (PVR) and prompted our use of TPVR as a surrogate. We did however measure pulmonary vascular compliance, Cp, which contributes to the pulsatile RV afterload, and Ea, which computes composite RV afterload. Together, these represent a robust measure of RV afterload and were significantly impaired in preterm subjects.

In conclusion, we have identified that young adults born premature with no history of adult cardiovascular or respiratory disease have elevated resting pulmonary vascular pressures and evidence of increased RV afterload including increased pulmonary vascular stiffness. These findings of early pulmonary vascular disease in adult survivors of moderate to extreme premature birth suggest an increased risk for the development of pulmonary hypertension. Given the increased mortality risk associated with both borderline pulmonary hypertension and decreased pulmonary vascular compliance,

further studies will be needed to determine mechanisms and rate of progression of pulmonary vascular disease in this population. Future studies should also address screening methods and treatment options.

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	Term	Preterm
N (males)	10(7)	11(5)
Age (years)	25.8 ± 0.8	26.9 ± 1.1*
Weight (kg)	70.5 ± 11.0	69.9 ± 10.1
Height (m)	1.77 ± 0.08	1.72 ± 0.11
BMI (kg/m^2)	23.2 ± 1.6	22.9 ± 3.0
GPAQ (MET/week)	3631 ± 2556	3703 ± 2402
Pulmonary Function		
FEV_1 (L)	4.4 ± 1.0	3.7 ± 0.7
	(101±17)	(99 ± 21)
FVC (L)	5.4 ± 1.1	4.6 ± 0.8
	(108 ± 11)	(107 ± 19)
FEV_1/FVC	0.9±0.3	0.8±0.1
FEF_{25-75} (L/s)	4.4 ± 1.8	3.4 ± 1.1
	(96.2 ± 33.8)	(81.9 ± 30.9)
DL_{CO} (ml/min/mmHg)	31.0 ± 7.0	23.9 ± 4.2*
	(106 ± 16)	(90 ± 1*)
DL_{CO}/V_A (ml/min/mmHg/L)	5.2 ± 0.6	4.5 ± 0.5*
	(113 ± 13)	(96 ± 11*)
Exercise Test		
VO_{2max} (ml/min/kg)	53.6 ± 13.8	34.6 ± 12.6*
P_{max} (watts)	238 ± 41	186 ± 37*
70% P_{max} (watts)	179 ± 27	134 ± 23*

Table 1. Anthropometric, pulmonary function, diffusion capacity and exercise data for term and preterm subjects. Values are reported as mean ± SD, values in parentheses are % predicted (25, 26). BMI: body mass index, GPAQ: Global Physical Activity Questionnaire, MET: metabolic equivalent of task, FEV_1: forced expiratory volume at 1 sec, FVC: forced vital capacity, FEF_{25-75}: maximum mid-expiratory flow, DL_{CO}: diffusion capacity of the lung for carbon monoxide, DL_{CO}/V_A: diffusion capacity of the lung for carbon monoxide corrected for alveolar volume, VO_{2max}: maximal oxygen consumption, P_{max}: maximal power output. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test. Height, weight, BMI, VO_{2max} and P_{max} were sex adjusted. The sex-adjusted measures were generated as least square means from linear models to predict each outcome variable using birth status and sex as covariates.* indicates a P value ≤ 0.05.

N (males)	11(5)
Gestational age (weeks)	28.2 ± 2.8
Birth weight (grams)	1071 ± 310.4
Delivered by C-section (N)	9
Apgar score at 1 minute	3 ± 2
Apgar score at 6 minutes	6 ± 2
Singleton/multiple birth	5/6
Received antenatal steroids	0
Intubated at 24 hours of age (yes/no)	9/2
Received surfactant (yes/no)	4/7
Days of mechanical ventilation	17 ± 25
Days on supplemental oxygen	96 ± 160
Days in the NICU	64 ± 32
BPD diagnosis at discharge (yes/no)	5/6
Persistent PDA (yes/no)	8/3
Diagnosis of PH by echo in the NICU	0
Discharge home on supplemental O_2 (yes/no)	5/6

Table 2. Birth and neonatal clinical data for preterm subjects. Values are indicated in mean ± SD. NICU: neonatal intensive care unit, BPD: bronchopulmonary dysplasia, PDA: patent ductus arteriosus, PH: pulmonary hypertension.

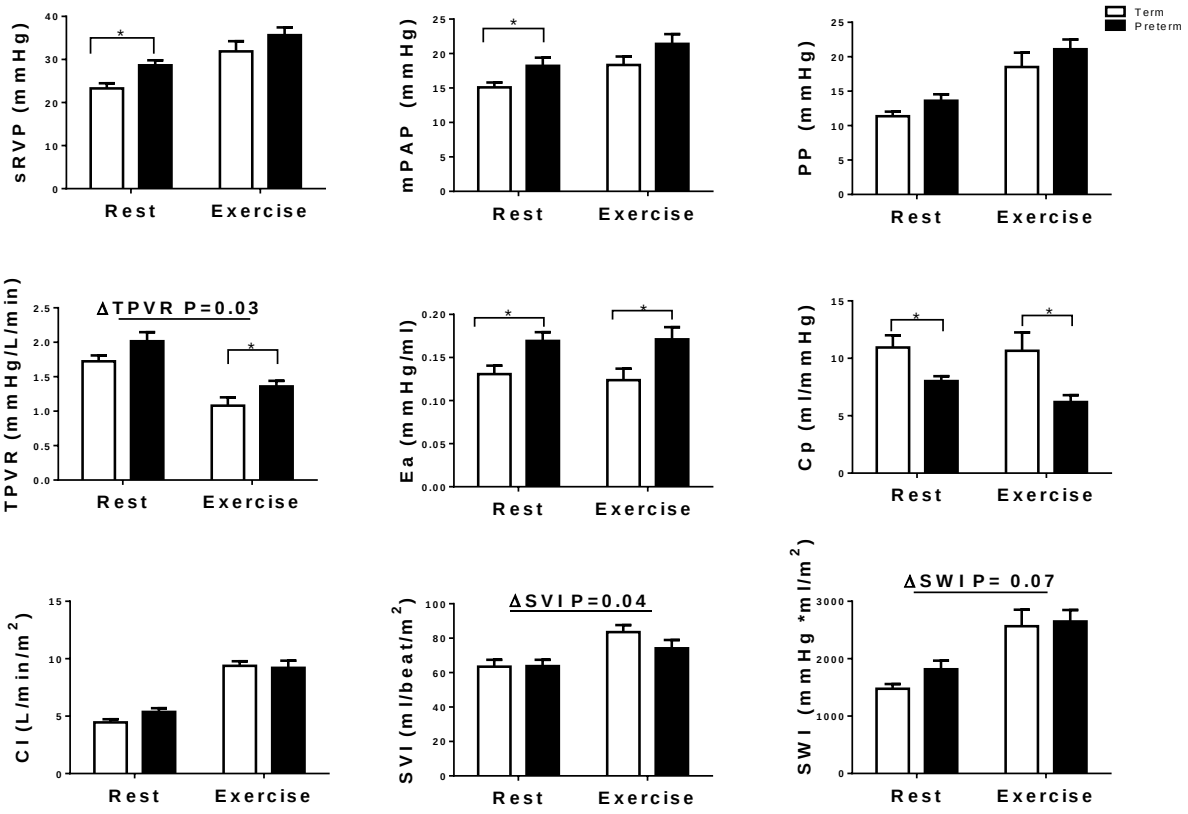

Figure 1: Pulmonary vascular and right ventricular hemodynamics at rest and exercise. Values indicated in mean ± SEM. mPAP: mean pulmonary artery pressure, sRVP: systolic right ventricular pressure, PP: pulmonary arterial pulse pressure, TPVR: total pulmonary vascular resistance, Ea: arterial elastance, Cp: pulmonary vascular compliance, CI: cardiac index, SVI: stroke volume index, SWI: stroke work index. Cardiopulmonary hemodynamic changes from rest to exercise: ΔTPVR: change in total pulmonary vascular resistence from rest to exercise, ΔSVI: change in stroke volume index from rest to exercise, ΔSWI: change in stroke work index from rest to exercise. Unpaired Wilcoxon Rank Sum test was used to determine significance. * indicated $P \leq 0.05$.

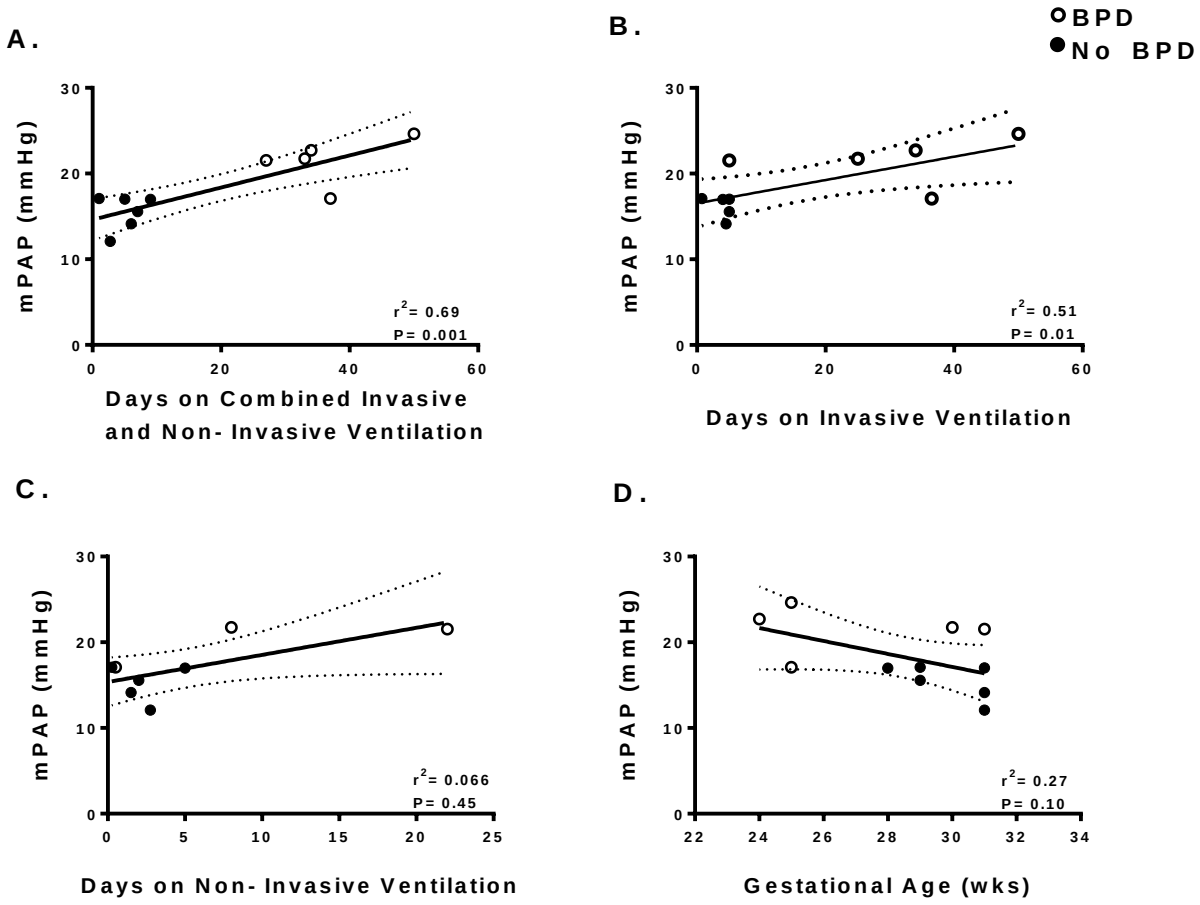

Figure 2: Neonatal predictors of adult pulmonary arterial pressure. Relationship between mean pulmonary arterial pressure (mPAP mmHg) and days on combined invasive and non-invasive ventilation **(A)**, days on invasive ventilation **(B)**, days on non-invasive ventilation **(C)**, and gestational age (weeks) **(D)**. Subjects with a diagnosis of BPD are denoted with open circles. Solid line indicates linear regression and dotted lines indicate borders of 95% confidence interval.

Chapter 2: Altered Right Ventricular Function in Adults Born Preterm

Authors: Arij G Beshish[1], Gregory P Barton[1], Kara N Goss[1,2], Kristin Haraldsdottir [1,4], Ashley M Mulchrone[3], Jacob MacDonald[7], David F Pegelow[1], Mari Palta[5], Luke J Lamers[1], Andrew M Watson[6], Oliver Weiben[7], Christopher Francois[8], Naomi C. Chesler[1,2,3], Marlowe W Eldridge[1,3,4]

Authors Affiliations: Departments of Pediatrics[1], Medicine [2], Biomedical Engineering [3], Kinesiology [4], Population Health Sciences and Biostatistics and Medical Informatics [5], Orthopedic and Rehabilitation Medicine[6] Medical Physics[7], and Radiology[8]. University of Wisconsin, School of Medicine and Public Health, Madison Wisconsin

Introduction:

Advances in perinatal care means that more infants born preterm survive into adulthood. As a result, up to 1 in 10 infants are born preterm (1). At birth, right ventricular (RV) mechanics begin to undergo maturational changes (2). The RV serves as the “systemic ventricle” in utero, as most of the RV output crosses the ductus arteriosus and provides 2/3 of combined ventricular output in the normal fetus. The transitional period in neonates is characterized by peak hemodynamic alterations in the transitional circulation from fetal to postnatal age (closure of fetal shunts, changes in cardiac output, systemic and pulmonary preload, afterload, and resistance) accounting for the progressive decrease in RV wall thickness (2). Physiological development of the RV can be altered in children with bronchopulmonary dysplasia (BPD), persistent patent ductus arteriosus (PDA), and pulmonary hypertension (3).

Alterations in RV function in the first months of age have been seen in infants born preterm (4). At three months of age, infants born preterm had greater RV mass that was independent to the increase in body and cardiac size when compared to infants born at term (5). RV end-systolic area and RV fractional area of change increase in the transitional period in preterm infants, while RV end-diastolic area remains stable(6). Global alterations in myocardial structure and function of both the right and left ventricles were observed in adult survivors of prematurity, including a significant reduction in systolic and diastolic function as well as altered ventricular geometry when compared to age matched controls. (2, 7). Preterm birth is associated with an increased risk of hypertension and cardiovascular mortality (8). Individuals born at gestational ages less than 28 weeks faced a 17-fold increased risk of developing heart

failure (9). However, it is unknown whether the alterations in cardiac function in individuals born preterm is independent or secondary to the increase in afterload observed in individuals born preterm. (cite PV paper when it is out?)

In this study, we investigated right ventricular structure and function in adult survivors of prematurity. We have previously demonstrated that individuals born preterm have increased pulmonary arterial pressures (PAP), stiffer pulmonary arteries (PA) and increase in afterload when compared to controls (**Chapter 1**). We hypothesize that there will be an increase demand in the RV secondary to the increase in afterload leading to decrease in RV function. As a result, preterm subjects will have lesser RV efficiency and decreased right ventricular-pulmonary arterial coupling.

Methods:

Subjects: Preterm subjects (n=11) were recruited from the Newborn Lung Project (10-13) a prospectively followed cohort established at the University of Wisconsin-Madison. Enrolled individuals were born preterm with very low birth weight (≤1500g) between 1988 and 1991 in Wisconsin and Iowa. All of the preterm subjects in this study have been followed serially (10-14). Control subjects (n=11) born at term within the same time period (1988 - 1991) were recruited from the general population in Wisconsin. Term and preterm subjects were free of adult cardiopulmonary disease and were non-smokers, determined by a questionnaire completed at the screening visit. This study was approved by the Institutional Review Board at the University of Wisconsin, School of Medicine and Public Health. Written informed consent was obtained from all subjects.

Screening visit:

Subjects performed a maximal exercise test to determine power output. Please see chapter 1 for details.

MRI visit:

MRI was used to characterize right ventricular (RV) volumes at rest. All imaging was performed on a clinical 3.0 T system (Discovery MR750, GE Healthcare) with an 8-channel cardiac coil. To characterize RV volumes at rest, a standard clinical, multi-slice, balanced steady-state free precession (bSSFP) sequence (FIESTA) (15) was acquired covering the heart in a short axis orientation with the following notable scan parameters: field of view (FOV) = 350 x 350 mm, spatial resolution = 1.3672 x 1.3672 mm, slice thickness = 7 mm, number of slices = 17, reconstructed cardiac phases = 20. This

acquisition was performed across a 13 s breath hold with prospective ECG gating. RV segmentation was performed with the software package Segment (16).To produce estimates of end-systolic and end-diastolic RV volumes (ESV, EDV), RV stroke volumes (SV) and ejection fraction (EF), the RV endocardial walls for all slices at these time points were manually traced out to generate a region-of-interest (ROI) covering the ventricle. The ROI area for each slice was multiplied by the slice thickness, and the volumes from all slices in the same time frame were added together to produce a ventricular volume estimate. Right ventricular – pulmonary artery coupling (Nvv) was calculated as ESV/SV, as previously described (17-19). End arterial ekastance (Ea) was calculated from right heart catheter hemodynamics as stroke volume (SV) divided by pulmonary arterial pulse pressure. End systolic elastance (Ees) was back calculated as Nvv multiplied by end arterial elastance (Ea). Cardiac index (CI) was calculated as stroke volume index (SVI) multiplied by heart rate (HR). Proximal pulmonary artery relative area of change (RAC) was calculated as the ratio of the change of PA area during a single cardiac cycle and the PA area at diastole (20). Pulse wave velocity (PWV) was calculated by dividing the distance between two known points on the PA (path length) by the time taken to move between them (21).

RV strain analysis: Three slices from the RV (apex, mid and base) tagged short-axis cine MR images were used to analyze circumferential strain (mean peak strain, systolic and diastolic strain rate) using Segment strain analysis module (22). RV efficiency was calculated by dividing SVI by mean peak circumferential strain

Statistical Analysis: Data were grouped by birth status (preterm, term) Baseline anthropometric data and cardiac MRI data were compared across birth status using unpaired Wilcoxon Rank Sum test. All statistical analyses were performed in R (Foundation for Statistical Computing, Vienna, Austria) (23). Significance was determined at $P < 0.05$

Results:

Subject characteristics:

Preterm subjects had an average gestational age of 28 ± 2 weeks, and birth weight of 1071 ± 310 grams. Adult anthropometric data for preterm and term subjects are shown in **Table 1**. Subjects were well matched with respect to anthropometric data.

RV function:

To determine RV function, RV end-systolic and end-diastolic volumes were measured. Although ESVI was similar, EDVI and therefore SVI were lower in preterm subjects when compared to controls. Preterm subjects had EF that was %5 lower than those of controls (**Figure1**). In order to determine a mechanical explanation for the reduced EF, peak RV circumferential strain as well as RV systolic and diastolic strain rate were measured. RV circumferential stain and RV systolic strain were significantly higher in preterm subjects when compared to controls (**Figure 2).**

RV afterload:

PA stiffness was determined by measuring RAC and PWV. Preterm subjects had higher PWV when compared to controls however there were no differences in RAC **(Figure 3)**.

RV-PV coupling:

RV-PV coupling, defined as Ees/Ea was measured to determine how well the RV and PA interact with one another. RV-PV coupling was lower in preterm subjects when compared to controls. (**Figure 4**).

Discussion:

We have previously demonstrated that preterm subjects have increased mean pulmonary arterial pressure (mPAP), and afterload as well as decreased arterial vascular compliance (Cp) when compared to controls (Chapter 1). In this study, we hypothesized that adults born preterm with no history of pulmonary or cardiovascular disease will have a decreased RV function when compared to controls, that is secondary to the increase in PAP and afterload. Here we demonstrate that preterm subjects have a altered RV function compared to terms evident by decreased EDVI, SVI and EF. Despite having lower SVI, preterm subjects had higher RV strain to controls. Preterm subjects also had lower coupling compared to controls suggesting that they have a less efficient RV.

In early stages of RV adaptation to increased afterload, there is concentric hypertrophy of the ventricle (24). Preterm subjects exhibited a lower EDVI and SVI when compared to controls. Our findings are consistent with that seen by Lewandowski and colleagues (2). It is important to note that preterm subjects participating in our study differ from those in the Lewandowski study in that they are part of a prospectivly-studied cohort in the United States followed since birth who were born at earlier gestational ages. Preterm subjects also had lower EF compared to controls. A reduction in preload, and an increased afterload explain the reduction in SVI. However the reduced EF suggest preterm term subjects have an lowered systolic RV function when compared to controls.

Myocardial strain is a measure of ventricular displacement or relative length change during the cardiac cycle (26). RV strain is increased in pulmonary hypertension (27). RV

circumferential strain at peak systole as well as RV circumferential systolic strain rate were significantly higher in preterm subjects as compared to controls. These findings suggest preterm subjects have a hyperdynamic RV under resting conditions. Interestingly, hyperdyanmic contractile properties as measured by increased twitch kinetics and greater Ca^{2+}- sensitivity of force development has been observed in models of familial hypertrophic cardiomyopathy (HCM) (28). Our results of increased RV systolic strain and strain rate suggests that the twitch properties in the RV of preterm subjects may display a similar phenotype as compared to HCM. We normalized SVI to RV mean peak circumferential strain as a way to assess how much volume is ejected relative to a surrogate of RV work (i.e. strain). We found that preterm subjects demonstrated lower efficiency as measured by SVI/RV strain, suggesting that more work may be required to eject a given volume of blood. Additionally, preterm subjects spent greater percentage of the cardiac cycle in systole compared to controls, suggesting that they have a reduced filling time. Also, control subjects spent approximately 30% of the cardiac cycle with a RV strain value of 0%, while the preterm subjects had a greater than 0% value until the very end of the cardiac cycle. These findings suggest that there may be some alteration in diastolic relaxation in the RV of preterm subjects as compared to controls which may also contributed to the lower EDVI. Taken together, our findings indicate that preterm subjects are showing early signs of altered RV function that may continue to deteriorate over time.

We have previously demonstrated that individuals born preterm have a higher resting mPAP, elevated afterload and stiffer PA when compared to controls (chapter 1). PWV, a noninvasive measurement of pulmonary artery stiffness was found to be

significantly higher in preterm subject when compared to controls indicating a stiffer PA in preterm subjects. This correlates with our right heart catheter measurement of stiffness, (Cp) which was lower in preterm subjects. There were no differences in RAC between both groups.

The interplay between RV function and the pulmonary vasculature, which in turn affects ventricular performance, is defined as ventricular-arterial coupling and is an expression of global RV efficiency (29). This relation is expressed as the ratio between (E_{ES}) and (E_A). When E_{es}/E_a ratio is higher than one, , the RV and arterial system are optimally coupled to produce stroke work, a measure of the efficiency of RV work (30). When E_{es}/E_a ratio is <1, the RV becomes progressively less efficient. Coupling of RV systolic function to afterload is used as predictor of survival in patients with chronic heart failure and PAH (31-34). Preterm subjects had lower coupling compared to controls.

It is important to note that preterm individuals participating in this study were health active young adults which may result in some degree of selection bias. However, given that we have demonstrated reduced RV function in this relatively healthy group, these data may underestimate the degree of PA and RV pathology in preterm individuals as a whole. We did not measure RV mass, however, future analysis of RV mass is required to delineate if the increased mPAP observed in preterm subjects results in RV hypertrophy.

In conclusion, we have identified that young adults born preterm with no apparent underlying illness have altered RV function (decreased EDVI. SVI, RV EF and ventricular-vascular coupling) that is secondary to the increase in afterload. Preterm

subjects also demonstrated lower RV efficiency when compared to controls. Taken together, these findings suggest that individuals with history of preterm birth may be at risk of developing RV dysfunction that if left untreated, may lead to RV failure. Given the increase in mortality risk associated with decreased coupling (31, 34), further studies are needed to determine mechanisms of pulmonary vascular disease and RV dysfunction in this population.

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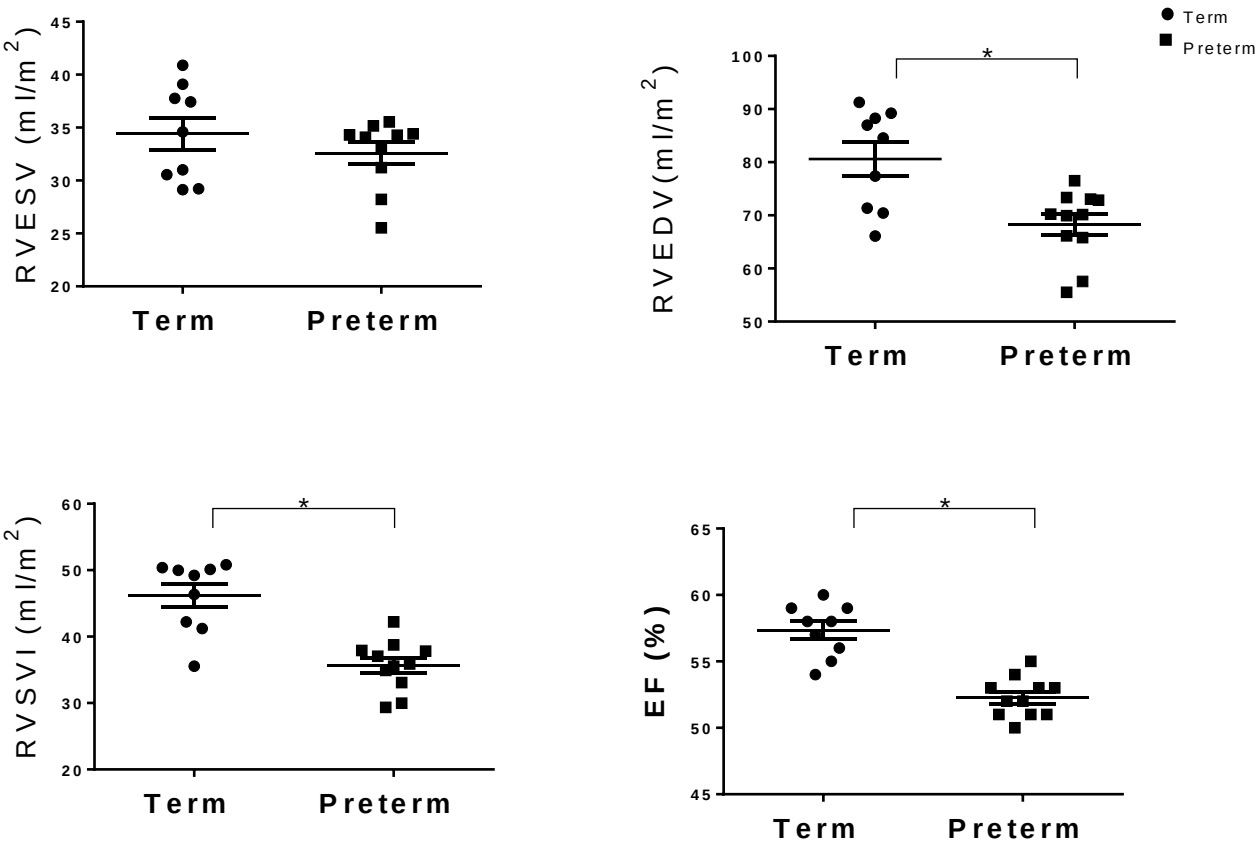
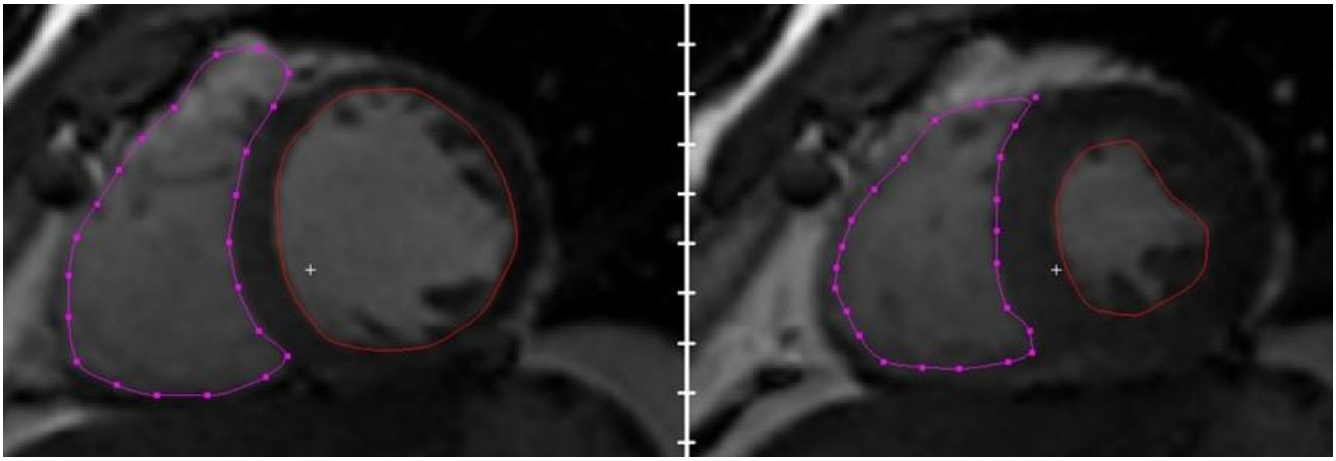
Table 1. Anthropometric data for term and preterm subjects

	Term	**Preterm**
N (males)	11(7)	11(5)
Age (years)	26.7 ± 0.8	26.9 ± 1.1
Weight (kg)	70.9 ± 11.1	69.9 ± 10.1
Height (m)	1.71 ± 0.10	1.72 ± 0.11
BMI (kg/m^2)	23.5 ± 1.3	22.9 ± 3.0

Values are reported as mean ± SD. BMI: body mass index. Each parameter was compared between both groups using unpaired Wilcoxon Rank Sum test.





LV and the RV at end diastole LV and the RV at end systole

Figure 1: Above: Right ventricular hemodynamics in term and preterm subjects. Values are reported as mean ± SD. ESVI: end-systolic volume index, EDVI: end-diastolic volume index, SVI: stroke volume index EF: ejection fraction. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test. * indicates a P value ≤ 0.05
Below: Short axis cine MR images of the right and left ventricle at end-systolic and end-diastolic volumes

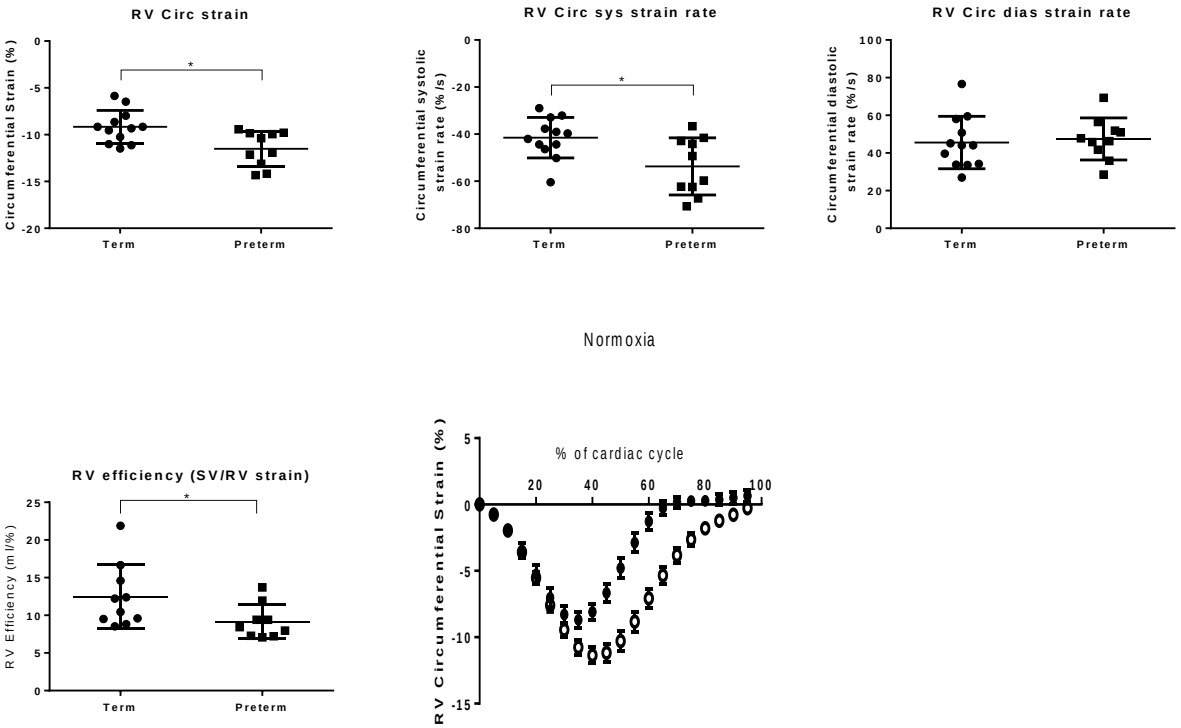


Figure 2: Above: Right ventricular strain and RV Circumferential strain as a function of the cardiac cycle in term and preterm subjects. Values are reported as mean ± SEM. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test. * indicates a P value ≤ 0.05

Below: Representative image of the determination of right ventricular circumferential strain.

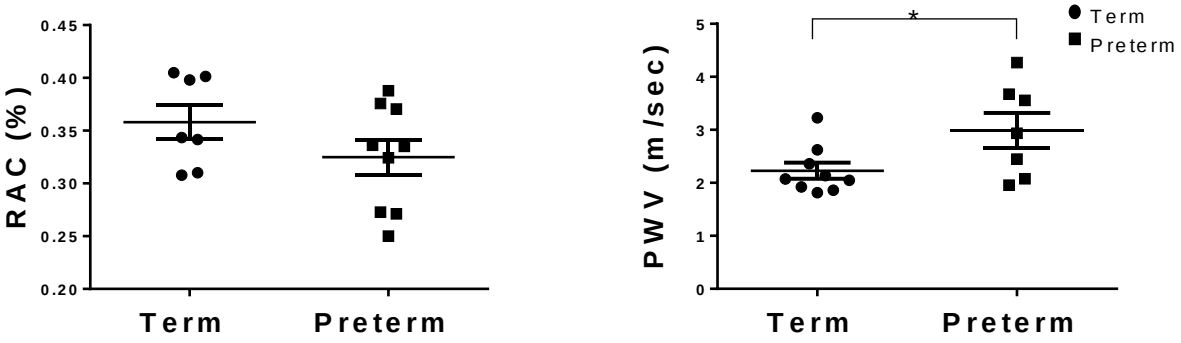


Figure 3: Pulmonary artery relative area of change (RAC) and pulse wave velocity (PWV) in term and preterm subjects. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test. * indicates a P value ≤ 0.05

Chapter 3: Effects of Hypoxia on Pulmonary Vascular Reactivity in Young Adults Born Preterm

Authors: Arij G Beshish[1], Kara N Goss[1,2], Gregory P Barton[1], Kristin Haraldsdottir [1,4], Ashley M Mulchrone[3], David F Pegelow[1], Mari Palta[5], Luke J Lamers[1], Andrew M Watson[6], Naomi C. Chesler[1,2,3], Marlowe W Eldridge[1,3,4]

Authors Affiliations: Departments of Pediatrics[1], Medicine [2], Biomedical Engineering [3], Kinesiology [4], Population Health Sciences and Biostatistics and Medical Informatics [5], Orthopedic and Rehabilitation Medicine[6]. University of Wisconsin, School of Medicine and Public Health, Madison Wisconsin

Introduction:

High altitude pulmonary edema (HAPE) is a non-cardiogenic pulmonary edema which typically occurs in lowlanders who ascend rapidly to altitudes greater than 2500-3000 meters (1). At sea level, HAPE susceptible individuals have normal resting pulmonary function (2) and similar gas exchange to HAPE resistant individuals at rest and submaximal exercise in normoxia and hypoxia (3, 4). However, HAPE susceptible individuals have an augmented flow dependent pulmonary vascular reactivity (2). A recent case report demonstrated an excessive gas exchange impairment during normoxic and hypoxic exercise in a young HAPE susceptible adult with a history of preterm birth (5). These findings suggest that individuals born preterm have an increase susceptibility for developing HAPE, although the underlying mechanisms remain to be determined.

Hypoxia exposure results in pulmonary vasoconstriction (HPV) that causes an increase in pulmonary arterial pressure and vascular resistance (6). In pre-clinical models of prematurity, exposure to hypoxia during the first days of life induces a transient increase of pulmonary artery pressure, and predisposes to exaggerated pulmonary vasoconstrictor responses that persists into adulthood (7). At altitude young healthy adults who had suffered from transient hypoxia during the first few days of life experienced an increase in pulmonary artery pressure that was more than 50 % larger than in control subjects (8). Previous reports have also determined altered ventilatory responses to hypoxia in individuals born preterm (9, 10).

In this study, we examined the effects of hypoxia and exercise on the pulmonary vascular and right ventricular function in young healthy adults born preterm with no

history of adult cardiopulmonary disease. We hypothesized that when exposed to hypoxia, adults born preterm would exhibit an increase in pulmonary vascular reactivity evident by an exaggerated increase in pulmonary artery pressure and pulmonary vascular resistance when compared to age matched term-born controls. To test our hypothesis, we measured pulmonary and right ventricular hemodynamics at rest and compared it to the response in hypoxia in term and preterm born subjects. We also performed hypoxic exercise to determine if the addition of exercise to hypoxia would further unmask abnormal pulmonary hemodynamic and right ventricular functional responses in preterm subjects.

Methods:

Subjects:

Preterm subjects (n=11) were recruited from the Newborn Lung Project,(11-14) a prospectively followed cohort established at the University of Wisconsin-Madison. Enrolled individuals were born preterm with very low birth weight (≤1500g) between 1988 and 1991 in Wisconsin and Iowa. Comprehensive medical records for these individuals are available and included maternal, prenatal, birth, and newborn intensive care data. Furthermore, all of the preterm subjects in this study have been followed serially.(11-15) Control subjects (n=10) born at term within the same time period (1988 - 1991) were recruited from the general population. Term and preterm subjects were free of adult cardiopulmonary disease and were non-smokers. This study was approved by Institutional Review Board at the University of Wisconsin, School of Medicine and Public Health. Written informed consent was obtained from all subjects.

Screening Visit:

During the initial screening visit, general anthropometric data as well as Global Physical Activity Questionnaire (GPAQ) was collected (Chapter 1). A maximal exercise test was performed on a cycle ergometer (Velotron; RacerMate, Seattle, WA) in normoxia (FiO_2 0.21) and hypoxia (FiO2 0.12) to determine maximal oxygen consumption (VO_{2max}), maximal carbon dioxide production, maximal minute ventilation, as well as maximal power output (P_{max}) in both conditions. Exercise started at 65 W and resistance was increased by 15 W every minute. Subjects were asked to maintain a cadence of 60-70 revolutions per minute (rpm). The exercise test was concluded when subjects were no longer able to maintain 55 rpm for more than five seconds. Venous lactate was

measured at each stage of exercise both in normoxia and hypoxia. Subjects were given a 30-minute rest period between exercises for heart rate to go back to baseline prior to hypoxic exercise bout.

Right Heart Catheter Visit:

This has been previously described in detail in chapter 1. Hemodynamic measurements were obtained at rest and during exercise in both normoxia and hypoxia.

Measurements of Right Ventricular and Pulmonary Vascular Hemodynamics:

Resting and exercise hemodynamics including right ventricular pressure (RVP) and pulmonary arterial pressure (PAP) were continuously recorded using a Powerlab data acquisition system (Chart 5 for Windows; AD Instruments, Sydney, Australia). Cardiac output (CO) at rest and during exercise was calculated using the direct Fick method (VO_2 divided by arterial-venous oxygen content difference). Stroke volume (SV) was calculated as CO divided by HR. CO and SV were indexed for body surface area. Mean pulmonary arterial pressure (mPAP) was averaged over a period of 30 seconds at steady state exercise to be sure that the mPAP was measured over multiple respiratory cycles. Total pulmonary vascular resistance (TPVR) was calculated as mPAP divided by CO. Pulmonary arterial compliance (Cp) was calculated as SV divided by PP and arterial elastance (Ea) was calculated as mPAP divided by SV.(16)

Statistical analysis:

Data were initially grouped by birth status (preterm, control) and condition (rest, hypoxia) and rest, hypoxic exercise). Baseline anthropometric data, and resting, hypoxia and hypoxic exercise hemodynamic data were compared across birth status using 2-way ANOVA. Percent Changes from rest to hypoxia as well as from rest to hypoxic exercise were compared across birth status using unpaired Wilcoxon Rank Sum Tests. Adjustment was made for multiple pairwise comparisons using the method initially suggested by Holm.(17) Significance was determined at $P < 0.05$.

Results:

Exercise data is shown in **Table 1**. Preterm subjects had lower $V_{E\ max}$, P_{max} and $VO_{2\ max}$ when compared to controls both during normoxic and hypoxic exercise. Preterm subjects also achieved lactate threshold earlier than controls in normoxic and hypoxic exercise **(Figure1)**.

Hemodynamics:

Normoxic Rest and Exercise: (previously described in Chapter 1)

Hypoxia rest:

When exposed to hypoxia, preterm subjects had higher mPAP (20.9 ± 4.7 mmHg vs. 16.7 ± 5.0 mmHg) and sRVP (31.8 ± 2.7 mmHg vs. 27.6 ± 3.6 mmHg) pressures when compared to controls. Preterm subjects also had higher SWI, Ea, CI and HR when compared to controls ($p < 0.05$). There were no differences in TPVR and SVI between both groups. **(Table 2)**. Preterm subjects had a steeper mPAP/CO relationship in both normoxia and hypoxia when compared to controls **(Figure 2).** When looking at the realtion between CO and mPAP, Preterm subjects When looking at the percent change from normoxia to hypoxia, preterm subjects showed a significantly reduced sRVP change when compared to controls (15.5 ± 13.5 mmHg vs. 25.8 ± 14.2) Changes were similar in mPAP, TPVR, Ea, Cp, CI, SVI and HR in both groups. **(Figure 3).**

Hypoxic exercise: 2 out of the 11 preterm subjects were unable to tolerate hypoxic exercise, bring down the number of subjects to 9. Preterm and term subjects demonstrated a similar increase in mPAP and sRVP, with hypoxic exercise **(Table 2).**

During hypoxic exercise, Cp remained significantly lower in preterm subjects. At steady-state hypoxic exercise, SWI, TPVR, Ea, HR, SVI, and CI were similar between groups (Table 2). When determining the percent change from normoxia to hypoxic exercise, preterm subjects demonstrated a lesser augmentation in mPAP, sRVP, SWI, CI, SVI and HR when compared to controls born at term **(Figure 4).** Percent changes in TPVR, Ea and Cp from rest to hypoxic exercise were similar between both groups.

Discussion:

We hypothesized that when exposed to hypoxia, adults born preterm would have an increase in pulmonary vascular reactivity evident by an exaggerated increase in PA pressure and pulmonary vascular resistance when compared to term-born controls. To test our hypothesis, we measured the pulmonary vascular response to hypoxia in healthy young adults born preterm and compared it to those of term-born controls. We have identified that at baseline (normoxic rest), preterm subjects had higher mPAP when compared to controls that was mainly driven by an increase in afterload and stiffness of the pulmonary artery. TPVR was also higher in preterm subjects although the differences were not significant. However, when exposed to acute hypoxia, preterm subjects did not display a difference in hypoxic pulmonary vascular response when compared to controls as evident by percent change from baseline in mPAP and TPVR. Interestingly, preterm subjects exhibited a lower percent change in sRVP from rest to hypoxia when compared to controls despite a similar increase in mPAP compared to control subjects. We also measured right heart and pulmonary vascular responses to hypoxic exercise. Notably, adults born preterm demonstrated a diminished mPAP, sRVP, CI, SVI, and HR response from rest to hypoxic exercise when compared to age matched controls. The reduced mPAP response to hypoxic exercise in preterm subjects was not due to altered pulmonary vascular reactivity since the percent change in TPVR was similar between groups from rest to hypoxic exercise. Taken together, the reduced RV systolic pressure response to hypoxia and hypoxic exercise in combination with reduced CI and SVI response from normoxic rest to hypoxic exercise suggests

abnormal RV function in our preterm subjects despite apparently similar pulmonary vascular reactivity.

We have previously determined in chapter 1 that at rest, preterm subjects had higher resting mPAP when compared to controls that was mainly driven by an increase in afterload (Ea) and stiffer pulmonary artery (decreased Cp). Although not significant, preterm subjects had higher resting TPVR when compared to controls. Acute exposure to hypoxia induces pulmonary vasoconstriction, increases pulmonary arterial pressure and a rapid increase in afterload (18). When exposed to hypoxia, preterm subjects had a higher mPAP and TPVR when compared to controls. However, pulmonary vascular response as measured by the change from normoxia rest to hypoxia rest in preterm subjects was similar to controls suggesting preterm subjects do not have increased hypoxic pulmonary vascular reactivity. Preterm subjects had a steeper mPAP/CO relationship in both normoxia and hypoxia when compared to controls. These findings are similar to what is seen in individuals susceptible to HAPE, who have an augmented flow-dependent pulmonary vasoconstriction (2).

During submaximal exercise, preterm subjects demonstrated an increased pulmonary vascular stiffness and failure to fully decrease TPVR. (Chapter 1). The combined effect of exercise and hypoxia has an additive effect on PAP (16) which was observed in both preterm and term-born subjects. Preterm subjects continued to have increased mPAP, Ea and pulmonary vascular stiffness in hypoxic exercise compared to controls. The findings in hypoxic exercise in both groups mimic the response in normoxic exercise suggesting these changes are an effect of exercise rather than hypoxia.

At high altitude, hypoxia affects right ventricular function indirectly through a pressure overload due, in turn to hypoxic pulmonary vasoconstriction (19). Preterm subjects had higher sRVP when exposed to hypoxia when compared to controls. There were no differences in SWI and SVI between the two groups in hypoxia. However, preterm subjects showed a decrease in percent change of sRVP from normoxia to hypoxia that was independent of the afterload, suggesting that there is some intrinsic pathology occurring in the RV independent to the pulmonary artery.

When looking at the percent change from rest to hypoxic exercise, preterm subjects had a diminished increase in mPAP when compared to controls. Additionally, there were no differences in the change in TPVR between groups from rest to hypoxic exercise. Therefore, this attenuated mPAP response to hypoxic exercise was likely due to a decreased augmentation of CI. The blunted CI response to hypoxic exercise can be explained by the reduced change in HR and SVI. SVI is a determinant of RV function which was blunted in preterm subjects when compared to controls, although not statistically significant, the finding of a reduced SVI response from rest to hypoxic exercise in preterm adults appears to be a physiologically relevant finding. A possible link between a reduced SVI, CI and mPAP change from rest to hypoxic exercise could be explained by the attenuated increase in sRVP in preterm subjects compared to controls. Together, these findings suggest that intrinsic factors may be responsible for the altered systolic RV function in preterm subjects. Cardiac MRI was performed to evaluate RV function in these subjects (tern and preterm) determined that preterm subjects had lower ESVI, SVI and EF when compared to controls. Furthermore, preterm subjects demonstrated lower RV efficiency and a decrease in RV-PV coupling when

compared to controls (chapter 2). These findings further supports data obtained by RHC in that preterm subjects have reduced RV function when compared to controls born at term.

Preterm subjects had reduced power output and VO_2 at maximal exercise in both normoxia and hypoxia. Adults born preterm have a lower anaerobic threshold when compared to control subjects and tend to have lower work efficiency (in review). The lower anaerobic threshold points to early lactate production as demonstrated by preterm subjects having a lower lactate threshold when compared to controls. The lower exercise capacity both in normoxia and hypoxia in preterm subjects can be explained by the altered RV function in these individuals causing a decrease in stroke volume and cardiac output response to exercise which may lead to hypoperfusion of the active skeletal muscle.

One of the limitations, but also an interesting observation was that out of the 11 preterm subjects, 9 were able to complete a maximal exercise test in normoxia and hypoxia while two preterm subjects were unable to tolerate hypoxic exercise (one experienced a pre-syncopal event and the other experienced syncope during the initial screening test) reducing the number of subjects that participated in hypoxic exercise to nine. The demands of this study may have resulted in a selection bias, with the healthiest of the adults born preterm selecting to participate in this study while those who may have a more severe PA and RV phenotype did not participate.

In conclusion, preterm subjects have higher resting PA pressure and vascular resistance when compared to controls at baseline resting conditions. However, contrary to our hypothesis, acute hypoxic exposure did not result in an exaggerated hypoxic

pulmonary vascular response in preterm subjects. The utilization of two physiological stressors (hypoxia and hypoxic exercise) unmasked altered RV function in preterm subjects which may explain the decrease in exercise tolerance in this group and the inability of two preterm subjects to complete hypoxic exercise. Taken together, these results suggest that adults born with a history of prematurity must take caution when exercising at altitude.

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Table 1: Metabolic parameters at maximal exercise in normoxia and hypoxia.

	NORMOXIA		HYPOXIA	
	Term	Preterm	Term	Preterm
N (males)	10(7)	11(5)	10(7)	9(5)
$V_{E\ max}$ (L/min)	121.0 ± 24.1	100.5 ± 18.7*	118.5 ± 29.4	98.6 ± 15.2*
$VO_{2\ max}$ (L/min/kg)	4.0 ± 1.6	2.6 ± 0.4*	2.9 ± 0.8	1.9 ± 0.4*
P_{max} (watt)	255 ± 38	189 ± 41*	212 ± 37	164 ± 19

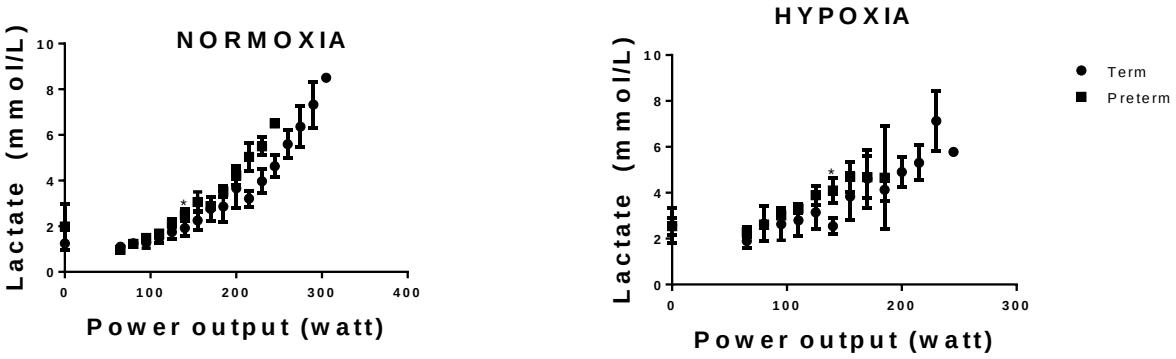
Values are reported as mean ± SD. $V_{E\ max:}$ Maximal minute ventilation, $VO_{2\ max:}$ maximal oxygen consumption, , $P_{max:}$ maximal power output. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test. * indicates a P value ≤ 0.05

Table 2: Resting normoxic, hypoxic and exercise hypoxic right ventricular and pulmonary vascular hemodynamics in preterm and term subjects.

	NORMOXIA		HYPOXIA		HYPOXIC EXERCISE	
	Term	**Preterm**	**Term**	**Preterm**	**Term**	**Preterm**
sPAP (mmHg)	21.8 ± 2.3	27.0 ± 5.1*	27.3 ± 4.56	30.6 ± 5.9*	39.3 ± 6.3	41.7 ± 8.6*
dPAP (mmHg)	11.0 ± 2.2	13.4 ± 3.3	11.2 ± 5.2	15.3 ± 4.3	13.1 ± 6.4	16.0 ± 6.7
mPAP (mmHg)	14.6 ± 1.9	18.2 ± 3.9*	16.7 ± 5.0	20.9 ± 4.7*	22.0 ± 6.4	23.4 ± 7.7
sRVP (mmHg)	22.5 ± 3.6	28.7 ± 3.7*	27.6 ± 3.6	31.8 ± 2.7*	40.8 ± 7.6	39.9 ± 6.4
HR (beats/min)	77 ± 12	84 ± 12*	84 ± 16	93 ± 15*	132.6 ± 17.3	129.6 ± 7.6
CI (L/min/m^2)	4.1 ± 1.2	4.5 ± 6	4.3 ± 0.8	4.6 ± 0.6	11.8 ± 3.4	9.5 ± 2.9
TPVR (mmHg/L/min)	1.8 ± 0.4	2.0 ± 0.4	1.8 ± 0.6	2.1 ± 0.8	1.1 ± 0.4	1.3 ± 0.2
Ea (mmHg/ml)	0.1 ± 0.0	0.2 ± 0.0*	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.0*
Cp (ml/mmHg)	11.0 ± 2.8	8.0 ± 1.3*	7.5 ± 2.6	7.4 ± 1.8	7.0 ± 1.2	5.2 ± 1.2*

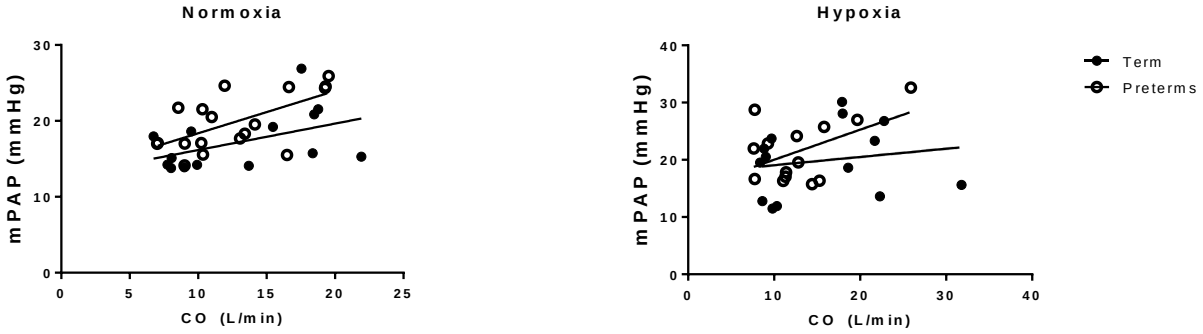
Values are reported as mean ± SD. sPAP: systolic pulmonary arterial pressure, mPAP: mean pulmonary arterial pressure, sRVP: systolic right ventricular pressure, HR: heart rate, CI: cardiac index, TPVR: total pulmonary vascular resistance, Ea: arterial elastance, Cp: Pulmonary arterial compliance. Each parameter was compared between both groups (term and preterm) using 2-WAY ANOVA.* indicates a P value ≤ 0.05 comparing term to preterm for a given condition

Figure 1: Blood lactate response to exercise at different power outputs in term and preterm subjects.



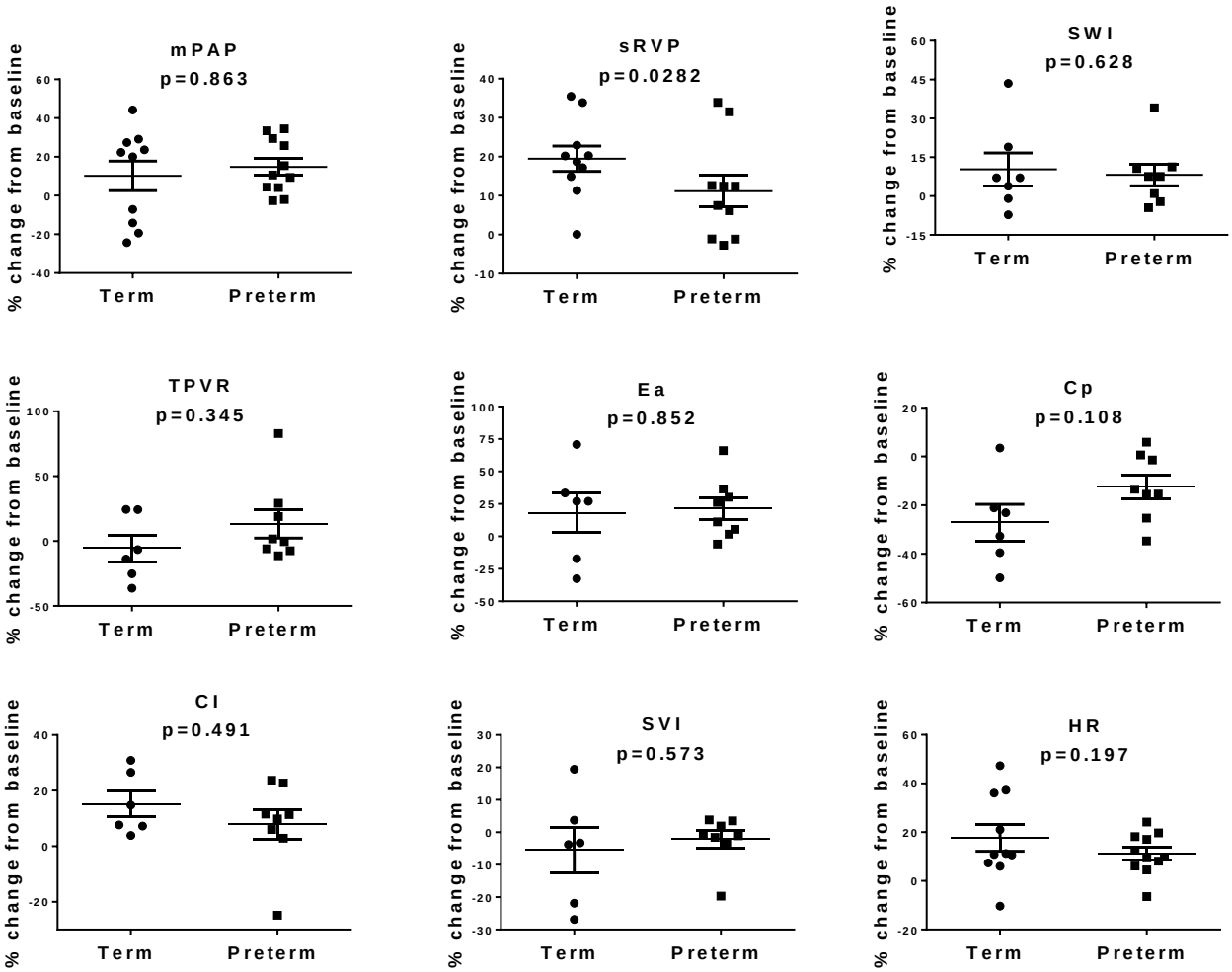
A. Normoxic exercise. B. Hypoxic exercise. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test. .* indicates a P value ≤ 0.05

.**Figure 2: Relationship between cardiac output and mPAP in terms and preterm subjects**



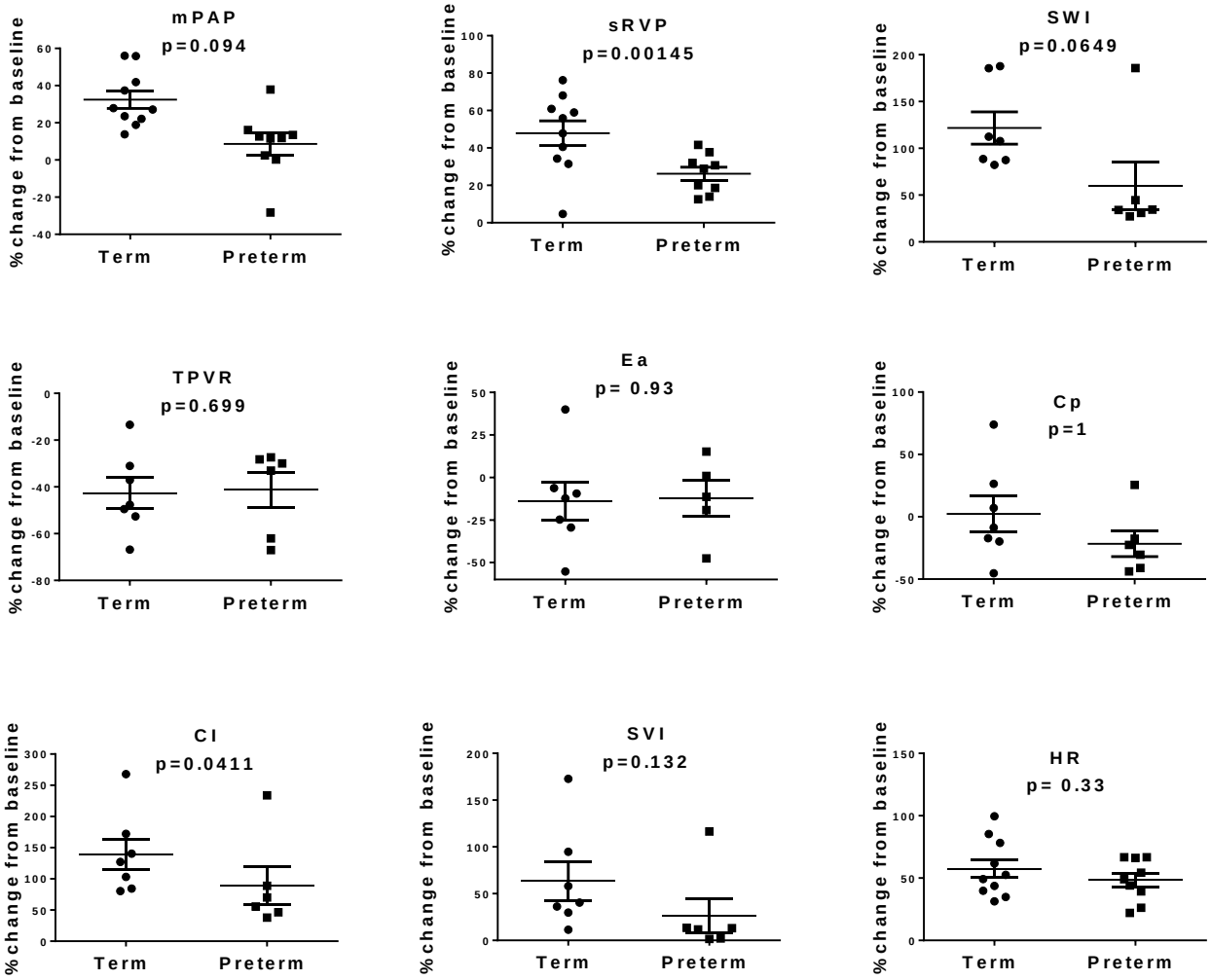
A. Normoxia. B. Hypoxia. Solid line indicates linear regression

Figure 3: Percent change from normoxia rest to hypoxia rest of right ventricular and pulmonary vascular hemodynamics in term and preterm subjects.



Values are reported as mean ± SEM. mPAP: mean pulmonary arterial pressure, sRVP: systolic right ventricular pressure, SWI: stroke work index, TPVR: total pulmonary vaascular resistance, Ea: arterial elastance, Cp: pulmonary vascular compliance, CI: cardiac index, SVI: stroke volume index, HR: heart rate. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test.

Figure 4: Percent change from normoxia rest to hypoxic exercise at rest of right ventricular and pulmonary vascular hemodynamics in term and preterm subjects.



Values are reported as mean ± SEM. mPAP: mean pulmonary arterial pressure, sRVP: systolic right ventricular pressure, SWI: stroke work index, TPVR: total pulmonary vaascular resistance, Ea: arterial elastance, Cp: pulmonary vascular compliance, CI: cardiac index, SVI: stroke volume index, HR: heart rate. Each parameter was compared between both groups (term and preterm) using unpaired Wilcoxon Rank Sum test.

Summery and conclusion:

In this study we determined that preterm subjects with no history of pulmonary or cardiovascular disease had higher mean pulmonary arterial pressures (mPAP) when compared to term born controls, with 36% meeting criteria for borderline pulmonary hypertension (resting mPAP 19-24 mmHg). This of important clinical significance since an increase in all-cause mortality beginning at an mPAP of 19 mmHg, with an adjusted mortality hazard ratio of 1.23 when borderline PH was defined as mPAP 19-24 mmHg (1). It has also been reported that Small increases in mPAP, even at values currently considered normal, are independently associated with increased mortality (2). Right ventricular systolic pressure and afterload were higher and pulmonary vascular compliance lower at rest among preterm subjects. One possible mechanism to our findings could be a consequence of the disruption in vascular growth seen in infants born preterm resulting in decreased vessel density throughout the pulmonary microvascular network (3) and decreased cross-sectional area for blood flow leading to an increased pulmonary vascular resistance (PVR) which alters vasoreactivity and causes structural remodeling (3). Weather subclinical pulmonary vascular disease persists versus re-emerges in humans born premature is yet to be determined.

The elevated mPAP in adults born preterm was driven by a mild elevation in afterload (Ea). Increase in resistance and decrease in compliance, both of which were found in the preterm subjects attributed to the increase in Ea. The significantly lower vascular compliance observed in preterm subjects was consistent with a stiffer pulmonary vascular bed. Both the increased TPVR and decreased compliance in

preterm subjects maybe explained by a decrease in microvascular surface area, which is consistent with the lower DL_{CO} and DL_{CO}/V_A found in these adults born preterm. In addition to a higher TPVR and decreased compliance when compared to controls during exercise, the decrease in TPVR from rest to exercise (ΔTPVR) was significantly blunted in preterm subjects, providing further evidence of an inability of the pulmonary vasculature to distend and recruit with exercise in preterm subjects. These findings may also suggest that adults born preterm are using a greater percentage of their total vascular surface area at rest and have lesser vasculature to recruit when exercising.

During exercise, preterm subjects had higher total pulmonary vascular resistance and lower compliance, and demonstrated less ability to augment stroke volume. Limitations in SV reserve with exercise have also been identified in adult patients with overt pulmonary hypertension (4, 5). Since HR is the main mechanism for increasing cardiac output in PH, these patients compensate for the inability to augment SV during exercise by increasing their HR, most likely as a result of increased sympathetic activation (4). Taken together, these finding suggest that the increase in afterload is causing alteration in RV function.

Univariate linear regression analysis identified the strongest predictor of elevated mPAP was the number of total days on combined invasive and non-invasive ventilatory support. Subjects with the longest durations of invasive ventilation had a neonatal diagnosed of BPD suggesting a diagnosis of BPD may be the primary modifier of long-term pulmonary vascular risk.

Utilization of two physiological stressors (hypoxia and hypoxic exercise) unmasked altered RV function in preterm subjects. When exposed to acute hypoxia, although

having higher PA pressure and resistance, preterm subjects did not have a difference in hypoxic pulmonary vascular response when compared to controls as evident by percent change from baseline in mPAP and TPVR. Preterm subjects had higher sRVP when exposed to hypoxia when compared to controls with no differences observed in SWI and SVI between the two groups. However, preterm subjects showed a decrease in percent change of sRVP from normoxia to hypoxia that was independent of the afterload, suggesting some intrinsic pathology occurring in the RV independent to the pulmonary artery. This may also explain the cause of hypoxia intolerance in two of our preterm subjects.

When looking at the percent change from rest to hypoxic exercise, preterm subjects had a reduced increase in mPAP when compared to controls with no differences observed in the change in TPVR between both groups. Therefore, this attenuated mPAP response to hypoxic exercise was likely due to a decreased augmentation of CI which can be explained by the reduced change in HR and SVI. SVI, a determinant of RV function was found to be altered in preterm subjects.

Right ventricular end-diastolic volume, stroke volume index and ejection fraction were significantly lower in preterm subjects when compared to controls. Preterm subjects had higher global right ventricular strain and systolic strain rate compared to controls. However, right ventricular-pulmonary vascular coupling was lower in preterm subjects compared to controls suggesting they have a less efficient right ventricle.

The degree of altered RV function in preterm subjects seems to be disproportionate to the increase in afterload. Preterm subjects showed limitations in SV reserve with exercise similar to what is seen in adult patients with overt pulmonary hypertension (4,

5). HR is the main mechanism for increasing cardiac output in PH, they compensate for the inability to augment SV during exercise by increasing their HR. However, alterations in RV function in patients with pulmonary hypertension usually occur later in the course of the disease with mPAP >40 mmHg. Our findings suggest that there may be an intrinsic RV dysfunction independent of increase in afterload. A pre-clinical model of prematurity found mitochondrial dysregulation that persists into adulthood with eventual RV dysfunction (6). Whether this is the case humans is yet to be determined.

Taken together, Young adults born preterm demonstrate early pulmonary vascular disease, and altered right ventricular function suggesting an increased risk to develop overt pulmonary hypertension and right heart failure. Given the increased mortality risk associated with both borderline pulmonary hypertension and decreased pulmonary vascular compliance, and their association with altered right heart function, further studies will be needed to determine mechanisms and rate of progression of pulmonary vascular disease in this population. Future studies should also address the possibility of intrinsic RV dysfunction independent of the increase in afterload as well as addressing screening methods and treatment options.

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