

Title: Early Pulmonary Vascular Disease in Young Adults Born Preterm

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At a glance:

Scientific knowledge: Premature birth, defined as less than 37 weeks gestation, affects 10% of all live births in the United States. Advances in neonatal care have resulted in the increased survival of infants born at extremely low gestational ages and the vast majority now reach adulthood. Although prematurity is a known risk factor for neonatal and childhood pulmonary vascular disease, little is known about the impact of prematurity on the pulmonary vasculature and right ventricle into adulthood.

What this study adds: Among young adults born premature, with an average gestational age of 29 weeks and no history of adult cardiovascular or respiratory disease, 45% (5 of 11) have elevated resting pulmonary vascular pressures meeting criteria for borderline or overt pulmonary hypertension. In addition to a stiffer, less recruitable vascular bed, otherwise healthy adults born preterm demonstrate a blunted cardiac response to exercise suggesting early cardiac dysfunction. These findings support the concept that premature birth is associated with an increased risk for the development of adult pulmonary vascular disease, specifically pulmonary hypertension, as well as right ventricular dysfunction. Further studies are needed to determine the etiology and rate of progression of pulmonary vascular and right ventricular dysfunction in this at-risk population.

Abstract:

Rationale: Premature birth affects 10% of live births in the United States, and is associated with alveolar simplification and altered pulmonary microvascular development. However, little is known about the long-term impact prematurity has on the pulmonary vasculature.

Objective: Determine the long-term effects of prematurity on right ventricular and pulmonary vascular hemodynamics.

Methods: Preterm subjects (n=11) were recruited from the Newborn Lung Project, a prospectively followed cohort at the University of Wisconsin-Madison, born preterm with very low birth weight (≤ 1500 g; average gestational age 28 weeks) between 1988 and 1991. Control subjects (n=10) from the same birth years were recruited from the general population. All subjects had no known adult cardiopulmonary disease. Right heart catheterization was performed to assess right ventricular and pulmonary vascular hemodynamics at rest and during hypoxic and exercise stress.

Results: Preterm subjects had higher mean pulmonary arterial pressures (mPAP), with 27% (3/11) meeting criteria for borderline pulmonary hypertension (mPAP 19-24 mmHg) and 18% (2/11) meeting criteria for overt pulmonary hypertension (mPAP ≥ 25 mmHg). Pulmonary vascular resistance and elastance were higher at rest and during exercise, suggesting a stiffer vascular bed. Preterm subjects were significantly less able to augment cardiac index or right ventricular stroke work during exercise. Among neonatal characteristics, total ventilatory support days was the strongest predictor of adult pulmonary pressure.

Conclusion: Young adults born preterm demonstrate early pulmonary vascular disease, characterized by elevated pulmonary pressures, a stiffer pulmonary vascular bed, and right ventricular dysfunction, consistent with an increased risk of developing pulmonary hypertension.

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Introduction:

Premature birth, defined as less than 37 weeks of completed gestation, affects 10% of all live births in the United States (1). Preterm birth is associated with alveolar simplification and altered pulmonary microvascular development, and is a known risk factor for neonatal and childhood pulmonary vascular disease (2-4). Premature infants with pulmonary vascular disease in the first week of life are more likely to develop bronchopulmonary dysplasia (BPD) and pulmonary hypertension (PH) at 36 weeks postmenstrual age (5). In adolescence, those born premature have higher estimated pulmonary artery pressures (PAP) by echocardiography than aged-matched term-born controls, with the highest values in preterm subjects with a history of BPD (6). In addition, registry studies have identified a 3 to 5-fold increased risk for development of PH in adolescents and young adults born premature, even after adjustment for congenital heart defects and pulmonary diseases (7, 8).

Still, how prematurity affects the pulmonary vasculature into adulthood is unclear. A cardiac magnetic resonance imaging study in young adults born preterm demonstrated greater right ventricular (RV) mass and lower RV ejection fraction compared to age-matched term-born controls (9). Whether or not pulmonary vascular disease was present in this study remains unknown. Finally, a non-invasive limited echocardiography-based exercise study in adults born premature demonstrated an exaggerated increase in estimated PAP. Paradoxically, this effect was seen primarily in preterm-born adults without a history of BPD (10).

We hypothesized that adults born moderately to extremely preterm would have (1) elevated resting PAP, (2) an abnormal pulmonary vascular response to exercise consistent with decreased recruitable pulmonary vascular surface area and a stiffer pulmonary vascular bed, and (3) an exaggerated hypoxic vasoconstrictor response. To test our hypotheses, we measured invasive pulmonary vascular and RV hemodynamics at rest and in response to physiologic stressors, including exercise, hypoxia, and hypoxic exercise, in young adults born preterm as compared to that of controls born at term within the same time period. Some of these results were previously reported in the form of abstracts (11, 12).

Methods:

Subjects:

Preterm subjects (n=11) were recruited from the Newborn Lung Project, a cohort of infants born preterm with very low birth weight (≤ 1500 g) between 1988 and 1991 in Wisconsin and Iowa and prospectively followed at the University of Wisconsin-Madison (13-16). Comprehensive neonatal medical records were available for all subjects. Control subjects (n=10) born at term within the same 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. 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 (17). Questions assess physical activity at work, in transport and leisure time, and how much time is spent sedentary, with scores used to determine the metabolic equivalent of task minutes per week as a measure of general activity level.

Pulmonary Function Testing: Subjects underwent baseline pulmonary function testing, including forced expiratory volume at 1 sec (FEV_1), forced vital capacity (FVC), FEV_1/FVC , and forced expiratory flow at 25-75% (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 (Easy life Hb, London, United Kingdom). Predicted values and lower limits of normal for pulmonary function tests were calculated as previously described by the Global Lung Initiative (18, 19).

Graded Maximal Exercise Testing: Each subject performed a 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}) in normoxia (F_{IO_2} 0.21) and hypoxia (F_{IO_2} 0.12). P_{max} was determined as the wattage at the highest completed stage for the given oxygen condition. These values were used to establish the workload (70% of each individual's P_{max}) for the right heart catheterization study visit, ensuring that each subject was exercising at or near their anaerobic threshold. VO_{2max} was determined from a rolling 30-second average of VO_2 . A test was considered a valid VO_{2max} using the following criteria: (1) a plateau in VO_2 , (2) respiratory exchange ratio

≥ 1.1 , and (3) maximal heart rate (HR) higher than 90% predicted max ($220 - \text{age}$). Stage completion required the subjects to finish at least 30 sec of a given stage. Exercise started at 65 W and workload was increased by 15 W every minute. Subjects were asked to maintain a cadence of 60-70 revolutions per minute, and testing concluded when subjects were no longer able to maintain 55 revolutions for more than five seconds despite verbal encouragement. Subjects were given a 45-minute rest period between exercise bouts to ensure that the HR returned to baseline.

Right Heart Catheter Visit:

The right internal jugular vein was accessed under sterile conditions with local anesthesia, and a 40 cm long 8F J-shaped Flexor sheath positioned in the RV. Two 3.5F high-fidelity solid-state pressure sensor catheters (Mikro-Cath; Millar, Houston, TX) were placed via the sheath, one in the RV and the other in the pulmonary artery (PA), with position confirmation through transducing appropriate waveforms. A 4F fluid-filled catheter was placed in the PA for venous blood sampling. All right heart catheter insertions were performed by the same interventional cardiologist (LJL). A 3F arterial catheter was placed in a radial artery.

Invasive Cardiopulmonary Exercise Testing: After securing the catheters, subjects were transported from the catheterization lab to the exercise physiology lab. They 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 determined by the continuously adjustable resistance and the step frequency. After acquiring resting data, subjects exercised for 5 minutes at

70% P_{\max} to achieve steady state HR under normoxic and hypoxic conditions. Exercise data were acquired once steady state was achieved. Mixed venous and arterial blood samples were collected from the PA and radial 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 in PowerLab (Lab chart version 8 for Windows; ADInstruments; Colorado Springs, CO). VO_2 at rest and during exercise was determined offline by a blinded reviewer (KH).

Right Ventricular and Pulmonary Vascular Hemodynamic Measurements:

Resting and exercise hemodynamics including RV pressure (RVP) and PAP were continuously recorded using Powerlab. RV and PA pressure waveforms were analyzed offline by two blinded reviewers (KNG and AGB) to determine catheter locations, and discrepancies were adjudicated by a third blinded reviewer (MWE). RV waveforms were identified by characteristic up-sloping end-diastolic pressure tracing signifying ventricular filling followed by a sharp upstroke to a peaked tracing. PAP tracings were identified by a sharp upstroke followed by a dicrotic notch on the backside of the waveform, with continued downslope until the next cardiac cycle. For subjects with flattened RV waveforms where the measured systolic RVP was lower than the systolic PAP, likely secondary to the Millar catheter catching between trabeculae during contraction, the systolic PAP was used as the best estimate of systolic RVP. Waveforms that did not meet these criteria were excluded. All subjects had analyzable RV and PA waveforms at rest. Pressure tracings were averaged over a period of 30

seconds at steady state rest and exercise to ensure measurements over multiple respiratory cycles, and measurements between the two reviewers averaged for analysis.

Cardiac output (CO) for each condition was calculated using the direct Fick method. Stroke volume (SV) was calculated as CO divided by HR. Stroke work (SW) was calculated as systolic RVP multiplied by SV. CO, SV and SW were indexed for body surface area (CI, SVI, and SWI, respectively), based on the formula of Mosteller (20). Total pulmonary vascular resistance (TPVR) was calculated as mean PAP (mPAP) divided by CO. PA pulse pressure (PP) was calculated as the difference between systolic and diastolic PAP. Pulmonary vascular compliance (Cpa) was calculated as SV divided by PP, and arterial elastance (Ea) was calculated as mPAP divided by SV, as previously described (21).

Statistical Analysis:

Data were initially grouped by birth status (preterm, term) and condition (rest, exercise, hypoxic rest, hypoxic 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 preterm subjects at rest versus exercise under normoxic conditions) were made using paired Wilcoxon Rank Sum tests. The difference in the response to normoxic exercise between term and preterm subjects was evaluated through separate linear mixed effects regression models to predict the dependent variables (i.e. CI, HR, and SVI), with the interaction between birth status and

condition (rest, exercise) as a fixed effect and individual as a random effect. The relationships between mPAP and CO as well as RVP and SV were compared between term and preterm groups using separate linear mixed effects regression models to predict the y variables (mPAP and RVP, respectively). The interaction between birth status and the x variables (CO and SV) were included as fixed effects and individual as a random effect to account for the inclusion of both rest and exercise values from the same individuals. Among the preterm participants, separate univariable regressions were developed to evaluate the relationship between mPAP and gestational age and ventilator days. Significance level was determined *a priori* at the 0.05 level and all tests were 2-tailed. All data is presented as mean \pm SD, unless otherwise noted. All statistical analyses were performed in R (Foundation for Statistical Computing, Vienna, Austria)(22), and graphs generated using Prism Graphpad (Version 7, GraphPad Software Inc., La Jolla, CA).

Results:

Subject characteristics:

Preterm subjects had an average gestational age of 28.6 ± 2.7 weeks (range 24-31), and birth weight of 1087 ± 297 grams (range 675 – 1497). 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 FEV₁ 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. As a group, the preterm subjects were above the lower limit of normal for spirometry and exercise capacity, consistent with selection of a healthy relatively fit preterm population, though DL_{CO} and DL_{CO}/V_A fell at the lower limit of normal. Additional neonatal characteristics of the preterm subjects are shown in **Supplemental Table 1**.

Hemodynamics:

Rest: Preterm subjects had 41% higher resting PAP, with mPAP 19.7 ± 4.6 vs 14.0 ± 3.4 mmHg in term subjects (**Table 1**). Resting CO and CI were similar among preterm and term subjects, but TPVR and Ea were significantly higher, suggesting a stiffer pulmonary vasculature in preterm subjects. Distribution of key hemodynamic variables in reference to accepted clinical thresholds are shown in **Figure 1** (upper limit for TPVR defined by Kovacs et al (23)). Although systolic RVP was 19% higher among preterm subjects, the resting RV SWI was not significantly different between groups.

Hemodynamic Response to Exercise: At 70% of P_{max} , preterm and term subjects exercised at a similar VO_2 (1.2 ± 0.3 vs 1.5 ± 0.5 L/min, respectively, $p=0.13$). Mean PAP remained higher in preterm subjects but was not statistically different from term subjects, likely due to the small sample size (**Table 1**; $p=0.11$). However, TPVR and Ea remained significantly higher consistent with a less distensible, less recruitable pulmonary vascular surface area. Although RV hemodynamics were not different at submaximal exercise between groups, the RV response to exercise was significantly

different between groups. Specifically, CI increased by 96% in term but only 49% in preterm subjects ($p=0.01$), SVI increased by 23% in term but decreased by 3% in preterm subjects ($p=0.07$), and SWI increased 69% in term but only 15% in preterm subjects ($p=0.04$) (**Figure 2**). Together, these results suggest a significant cardiac limitation to exercise in young adults born preterm.

To better understand the relationships between PAP and flow from rest to exercise and to compare mPAP and TPVR at iso-flow states, we plotted CO versus mPAP for term and preterm subjects (**Figure 3**). Given that the slope of each line represents the TPVR, preterm subjects have a non-significantly higher vascular resistance for any given flow ($p=0.07$). In addition, to better understand the relationship between RV work and CO, we plotted systolic RVP vs SV. Preterm subjects demonstrated a trend toward lower volume ejected for any RVP ($p=0.06$), consistent with early RV dysfunction during exercise.

Hemodynamic Response to Hypoxia and Hypoxic Exercise: During hypoxic rest, there were no significant differences between groups (**Table 2**). Although we hypothesized there would be increased hypoxic pulmonary vasoconstriction in preterm subjects, comparison of the response to hypoxia among groups showed no differences ($P>0.16$ for all variables). Two preterm subjects did not complete hypoxic exercise due to presyncopal symptoms during the screening visit hypoxic VO_{2max} test. Only TPVR remained significantly higher among preterms during hypoxic exercise, likely due to the smaller sample size. The cardiac response to hypoxic exercise was comparable to normoxic exercise, with an 83% increase in SWI in term subjects compared to 27% in preterm subjects ($p=0.002$).

Correlation with Neonatal Clinical Data: In an effort to identify neonatal characteristics that might predict 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 (continuous positive airway pressure), 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 better predictor than gestational age (**Figure 4**). Notably, those infants requiring prolonged ventilatory support were also diagnosed with BPD, defined clinically by use of supplemental oxygen at 36 weeks postmenstrual age.

Discussion:

We hypothesized that moderate to extreme premature birth persistently impairs pulmonary vascular function, such that adults born preterm have elevated resting PAP, an abnormal pulmonary vascular response to exercise due to a stiffer less recruitable vascular bed, and an exaggerated hypoxic vasoconstrictor response. To test our hypothesis, we measured pulmonary vascular and RV hemodynamics at rest and in response to physiologic stressors, including exercise, hypoxia, and hypoxic exercise using high-fidelity pressure catheters. Here we have identified that young adults born premature, with an average gestational age of 28.6 weeks and no known history of adult cardiopulmonary disease, have elevated resting pulmonary vascular pressures, increased resistance, and were significantly less able to augment cardiac index or RV

stroke work in response to exercise. Contrary to our initial hypothesis, the hypoxic pulmonary vasoconstrictor response appears intact in these individuals, which was also recently reported by Laurie et al (10).

The average mPAP for our preterm population was 19.7 mmHg, with 2 subjects (18%) presenting with resting mPAP ≥ 25 mmHg, consistent with overt PH, and 3 of 11 subjects (27%) presenting with resting mPAP 19-24 mmHg, consistent with borderline PH. Importantly, a growing body of work supports a significant increase in mortality risk even for mild elevations in PAP. Specifically, recent large studies have identified an adjusted mortality hazard ratio of 1.23-1.31 when borderline PH was defined as mPAP 19-24 mmHg (24, 25), 2.37 when defined as mPAP 17-26 mmHg (26), and 4.03 when defined as mPAP 21-24 mmHg (27). Furthermore, in a serial catheterization study, 61% of subjects with borderline PH developed overt PH on repeat catheterization, suggesting potential to progress and worsening overall prognosis (25).

The finding of increased resting PAP and TPVR in this otherwise healthy preterm-born population is notable. Specifically, as a group the preterm subjects had pulmonary function above the lower limit of normal, with the only between-group differences being in diffusion capacity. We do not believe these subjects are outliers in the literature. Spirometric and DLCO values in other studies of adults born premature, particularly when recruiting participants willing to undergo exercise testing, frequently fall above the lower limit of normal yet may be statistically lower than controls (28, 29). The significantly lower DL_{CO} and DL_{CO}/V_A in preterm subjects, falling just above the lower limit of normal, suggests a decreased microvascular surface area among adults

born premature, and likely contributes to the exaggerated increase in PAP with exercise.

The degree of RV dysfunction unmasked by exercise in these preterm-born young adults is surprising. RV dysfunction in PH has previously been related to the degree of pulmonary vascular stiffness (30), but typically occurs in the setting of significantly higher pressures. However, the effects of mild chronic elevations in PAP over the course of a lifetime, beginning in a developmentally immature host, are not well described. Based on the developmental origins of health and disease hypothesis, a complex interplay between adaptive and maladaptive responses to environmental cues in a developmentally plastic host would be expected (31, 32). Intriguingly, using a rodent model of BPD, we have previously identified persistently elevated PAP throughout the lifespan. During adolescence, DLCO is maintained due to an adaptive increase in CO, but RV dysfunction develops in mid-adulthood (33-35). In humans born premature, cardiac imaging studies demonstrate biventricular hypertrophy beginning during early postnatal development with RV dysfunction and reduced ejection fraction apparent in early adulthood (36, 37).

Our study is now the second to report a cardiac limitation to exercise in seemingly healthy young adults born premature. Using exercise stress echocardiograms, Huckstep et al demonstrated a decreased cardiac reserve with less augmentation of left ventricular ejection fraction during exercise (38). The relative contributions of left versus right ventricular dysfunction during exercise remain to be elucidated in adults born preterm. However, prior exercise comparisons of left versus right heart failure suggest less SV reserve but larger HR responses to exercise in RV

failure (39), and the RV may again be the more affected ventricle in adults born premature. Our small sample size and the unavailability of cardiac response to exercise data for all subjects limits our ability to identify neonatal predictors of RV dysfunction. However, based on our findings, one hypothesis for the paradoxical lack of exaggerated increase in echocardiographically-estimated PAP among preterm-born adults with a history of BPD, as identified by Laurie et al, is more exaggerated RV dysfunction in this population (10).

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. Among our subjects, the strongest predictor of elevated mPAP was the number of 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. 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.

One of the primary limitations of our study is the small sample size. However, the use of direct invasive measures, physiologic stressors, and availability of extensive neonatal records allowed us to conclude that otherwise healthy young adults born preterm, particularly those requiring extended neonatal respiratory support, have increased pulmonary pressures, increased pulmonary vascular resistance, and an impaired cardiac response to exercise. 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 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 RV 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. Finally, we were unable to obtain pulmonary vascular wedge pressures given the use of high-fidelity solid-state pressure catheters, prompting our use of TPVR as a surrogate for pulmonary vascular resistance. We did however measure compliance, which contributes to the pulsatile RV afterload, and E_a , which computes composite RV afterload. Together, these represent a robust measure of RV afterload and were impaired in preterm subjects.

In conclusion, young adults born premature with no history of adult cardiopulmonary disease have elevated resting pulmonary vascular pressures, with 45% meeting clinical cut-points for abnormal, as well as evidence of increased pulmonary vascular stiffness and early RV dysfunction. Given the findings of early RV dysfunction at relatively low-level elevations in PAP, future studies should evaluate the role of earlier treatment of pulmonary vascular disease in order to maintain RV function in this high-risk population.

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Figure Legends:

Figure 1: Distribution of resting hemodynamic data in term and preterm subjects in reference to clinical thresholds. For mean pulmonary artery pressure (mPAP) and cardiac index (CI), solid red line represents accepted clinical thresholds for pulmonary hypertension and cardiac dysfunction, respectively. Dashed red line represents cut-point for borderline pulmonary hypertension. For total pulmonary vascular resistance (TPVR) and elastance (Ea), dotted black line represents 1 standard deviation and solid black line represents 2 standard deviations above published and internal normative values, respectively. Error bars are standard deviation.

Figure 2: Cardiac response to exercise in term and preterm subjects. Preterm subjects demonstrate a significantly blunted cardiac response to exercise, demonstrated by lower augmentation of cardiac index (CI). This difference is driven primarily by stroke volume. Solid lines represent group response, while lighter dashed lines represent individual responses.

Figure 3: Correlation of hemodynamic parameters at rest and during exercise in term and preterm subjects. Left: Cardiac output (CO) versus mean pulmonary artery pressure (mPAP) demonstrates a greater pulmonary vascular resistance (slope) for a given volume of pulmonary flow among preterm subjects. Right: Systolic right ventricular pressure (sRVP) versus stroke volume (SV) demonstrates a lower volume

ejected for any RV pressure among preterm subjects. Individual data points missing for 4 term and 2 preterm subjects due to failure to meet pre-specified quality measures.

Figure 4: Correlation of neonatal characteristics with mean pulmonary artery pressure (mPAP). Subjects diagnosed with bronchopulmonary dysplasia (BPD), defined by use of supplemental oxygen at 36 weeks postmenstrual age, are denoted by open circles (males) or open triangles (females).

	Term N=10	Preterm N=11	P value
Anthropometric Data			
Age (years)	26.3±0.9	27.3±0.8	0.02
Sex (M/F)	7/3	5/6	0.26
Weight (kg)	72.8±9.8	69.3±13.3	0.45
Height (cm)	176.6±7.8	170.0±11.3	0.23
BMI (kg/m ²)	23.3±1.7	23.9±3.0	0.66
Pulmonary Function Testing			
FVC (L)	5.4±1.1	4.7±0.7	0.16
– % predicted	103.8±13.2	103.8±16.6	0.73
– GLI LLN (L)	4.2±0.7	3.7±0.9	0.16
FEV1 (L)	4.5±1.0	3.8±0.7	0.11
– % predicted	101.9±15.1	99.1±20.1	0.37
– GLI LLN (L)	3.5±0.5	3.1±0.7	0.16
FEF ₂₅₋₇₅ (L/s)	4.5±1.6	3.6±1.2	0.24
– % predicted	98.3±30.3	87.6±32.6	0.45
– GLI LLN (L/s)	2.9±0.3	2.7±0.4	0.08
FEV1/FVC	0.82±0.06	0.80±0.06	0.73
DLCO (ml/min/Torr)	32.4±6.5	24.4±4.3	0.004
– % predicted	97.8±9.1	81.5±6.4	<0.001
– GLI LLN (ml/min/Torr)	24.4±4.2	21.6±5.2	0.19
DLCO/VA (ml/min/Torr/L)	5.6±0.8	4.7±0.4	0.002
– % predicted	113.3±18.0	96.3±8.9	0.01
– GLI LLN (ml/min/Torr)	4.7±0.2	4.6±0.2	0.29
VA (L)	6.0±1.4	5.3±0.8	0.07
– % predicted	93.9±14.5	94.3±10.9	0.86
– GLI LLN (L)	5.2±0.7	4.7±1.0	0.26
Exercise Testing			
VO _{2max} (L/min)	3.5±0.7	2.6±0.6	0.005
– % predicted	135.5±31.7	112.4±37.0	0.17
VO _{2max} (ml/kg/min)	50.0±10.4	38.1±8.6	0.01
P _{max} (watts)	236.5±48.9	187.7±36.0	0.02
P _{max} at 70% VO _{2max} (watts)	172.8±31.5	130.5±24.6	0.005
GPAQ (MET/week)	3368±2550	3420±2006	0.79

Table 1: Baseline characteristics of term and preterm subjects. Continuous variables are presented as mean ± standard deviation. BMI: body mass index. FVC: forced vital capacity. FEV1: forced expiratory volume at 1 second. FEF₂₅₋₇₅: forced expiratory flow at 25-75% of pulmonary volume. DLCO: diffusion capacity for carbon monoxide. VA: alveolar volume. GLI LLN: Global Lung Initiative lower limits of normal, GLI also used for percent predicted. VO_{2max}: maximal oxygen consumption. Wasserman

used for percent predicted, which accounts for both weight and sex. P_{\max} : maximal power output. GPAQ: Global Physical Activity Questionnaire. MET: metabolic equivalent of task. P values are unadjusted Wilcoxon Rank Sum tests (Chi-square for sex).

	<u>Normoxic Rest</u>			<u>Normoxic Exercise</u>			<u>Hypoxic Rest</u>			<u>Hypoxic Exercise</u>		
	Term (n=10)	Preterm (n=11)	P value	Term (n=10)	Preterm (n=11)	P value	Term (n=10)	Preterm (n=11)	P value	Term (n=10)	Preterm (n=9)	P value
Pulmonary Hemodynamics												
sPAP (mmHg)	21.0±3.5	25.6±5.6	0.06	29.6±5.8	32.5±5.8	0.34	25.4±5.3	29.8±6.3	0.25	36.8±7.2	39.3±9.0	0.73
dPAP (mmHg)	8.7±3.6	14.5±4.1	0.005	11.6±5.5	16.9±4.6	0.06	11.2±5.0	16.3±4.8	0.15	14.7±6.8	19.7±6.6	0.36
mPAP (mmHg)	14.0±3.4	19.7±4.6	0.006	19.6±5.8	23.9±4.9	0.11	17.8±5.4	22.4±5.1	0.15	25.2±6.7	28.4±7.3	0.69
CI (L/min/m ²)	5.1±1.2	5.7±2.1	0.77	10.0±2.3	8.5±2.2	0.22**	5.3±1.6	5.2±1.9	0.94	10.2±1.8	8.1±2.7	0.12
HR (bpm)	74±12	82±12	0.07	121±19	126±16	0.59	84±17	92±15	0.21	132±18	132±9	0.99
SVI (ml/beat/m ²)	69.8±15.9	69.7±26.7	0.94	86.0±18.0	67.3±16.5	0.07	64.2±19.4	57.1±19.5	0.66	79.7±11.1	61.3±19.3	0.06
TPVR (mmHg/L/min)	1.5±0.4	2.1±0.8	0.03	1.0±0.4	1.6±0.3	0.02	1.9±0.7	2.8±1.2	0.09	1.3±0.4	2.0±0.5	0.02
PP (mmHg)	12.4±2.9	11.1±2.6	0.28	17.9±3.8	15.6±2.8	0.39	14.2±2.8	13.6±3.1	0.52	22.2±2.8	19.5±4.2	0.18
Cpa (ml/mmHg)	10.7±2.5	11.2±3.1	0.88	9.6±1.8	7.6±1.9	0.10	8.5±3.7	7.5±2.4	0.89	6.8±1.1	5.8±1.8	0.21
Ea (mmHg/ml)	0.11±0.02	0.18±0.07	0.007	0.11±0.05	0.20±0.04	0.004	0.16±0.07	0.25±0.13	0.07	0.18±0.06	0.26±0.07	0.12
Right Ventricular Hemodynamics												
sRVP (mmHg)	23.4±3.2	27.8±5.5	0.05	32.0±6.6	34.2±6.2	0.41	27.3±5.1	29.2±6.1	0.54	39.0±7.5	35.6±6.6	0.40*
edRVP (mmHg)	7.1±3.1	8.3±3.7	0.86	5.4±4.0	5.5±4.0	0.86	7.1±4.7	5.0±5.1	0.5	6.5±5.2	3.7±3.6	0.35
SWI (mmHg*ml/m ²)	1651±480	1986±919	0.33	2788±1196	2280±801	0.44*	1709±575	1677±881	0.99	3119±854	2138±953	0.07**

Table 2: Hemodynamic assessment at rest, exercise, hypoxic rest, and hypoxic exercise in term and preterm subjects. Values presented as mean ± standard deviation. PAP: pulmonary artery pressure, (s) systolic, (d) diastolic, (m) mean. CI: cardiac index. HR: heart rate. SVI: stroke volume index. TPVR: total pulmonary vascular resistance (mPAP/CI). PP: pulse pressure (sPAP-dPAP). Cpa: pulmonary artery compliance (SV/PP). Ea: elastance (mPAP/SV). RVP: right ventricular pressure, (s) systolic, (ed) end-diastolic. SWI: stroke work index (sRVP*SVI). Comparisons between term and preterm subjects during each condition are made using unpaired Wilcoxon Rank Sum test. *p<0.05, **p<0.01 for differential response to exercise (interaction) between term and preterm subjects.

Figure 1

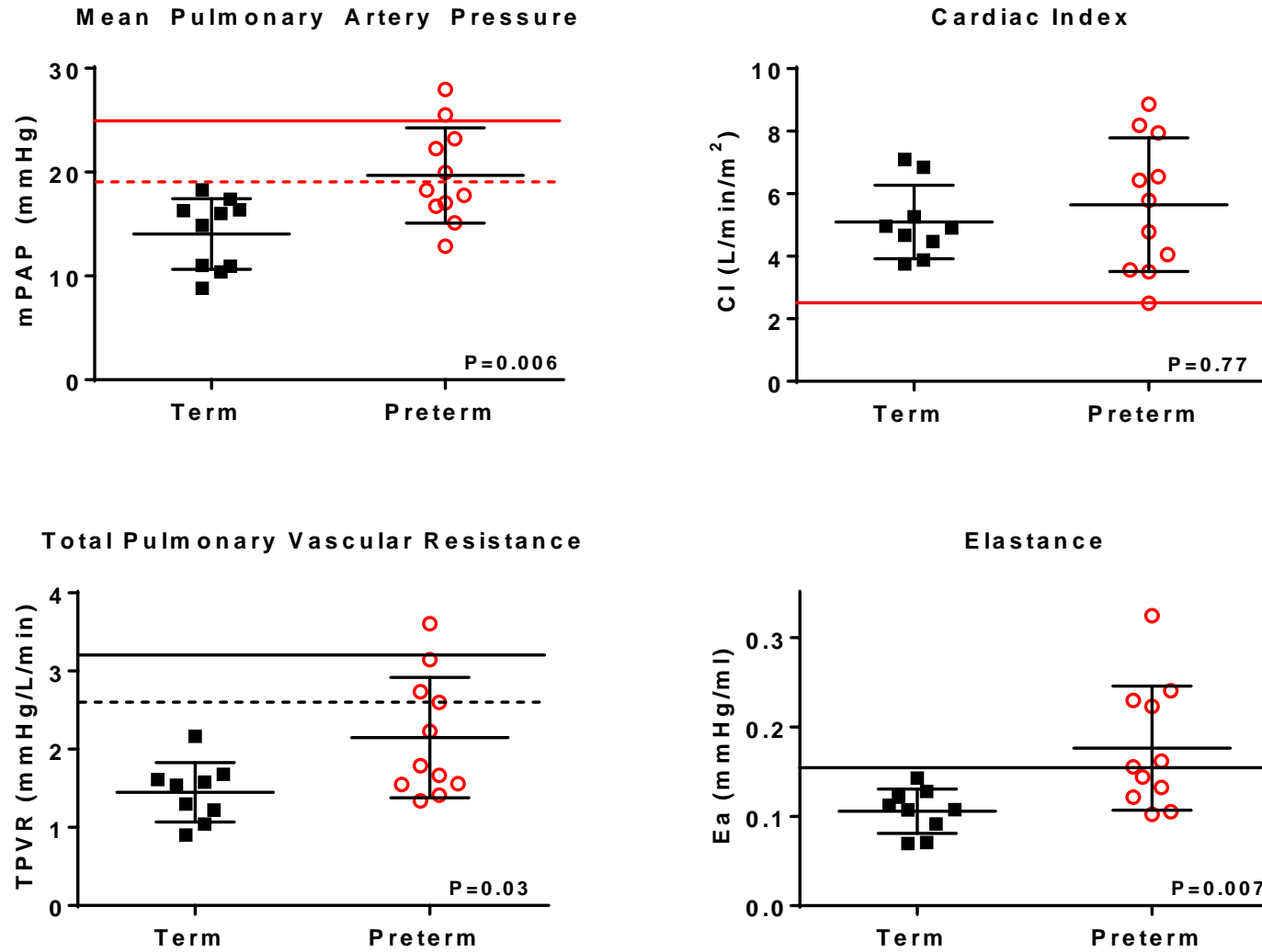


Figure 2

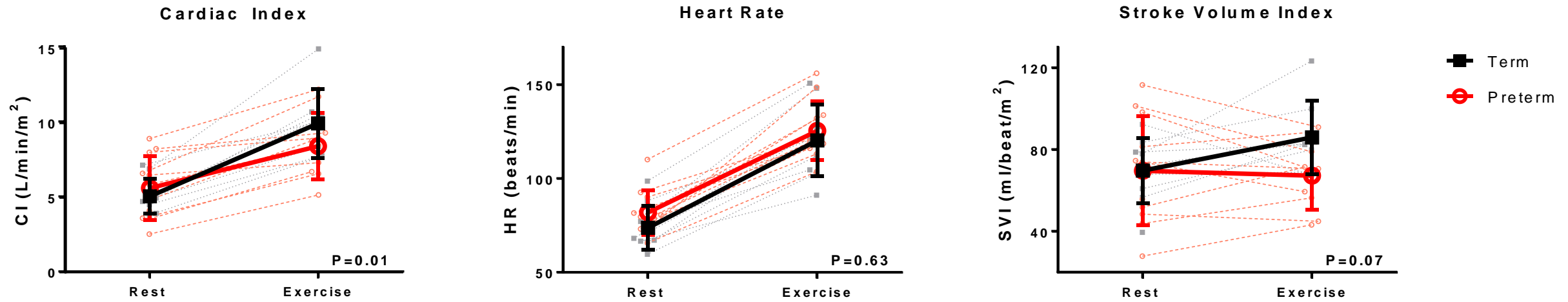


Figure 3

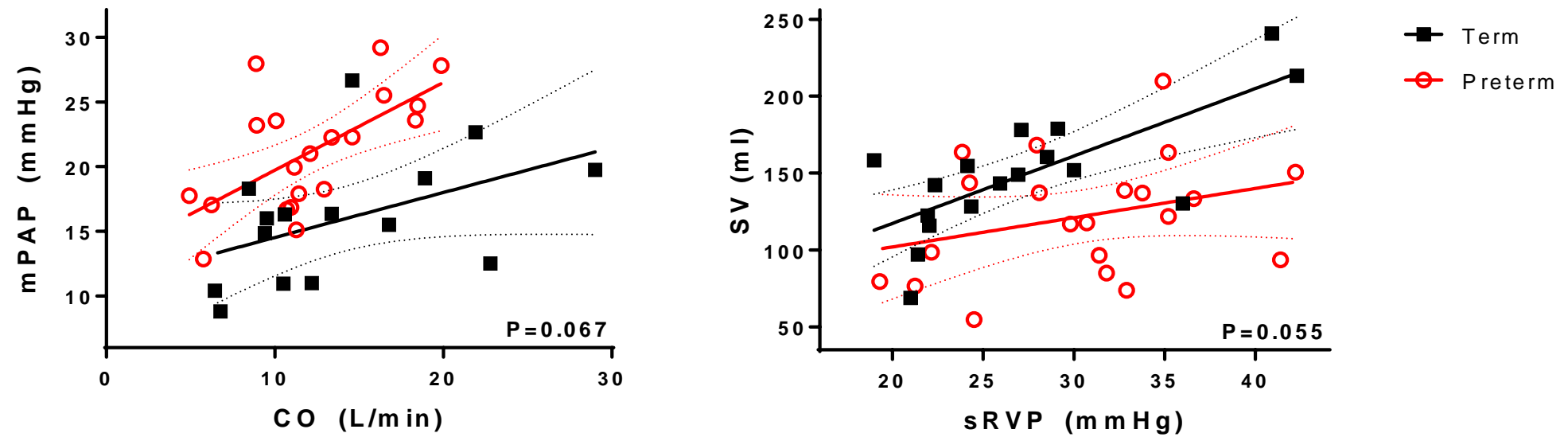
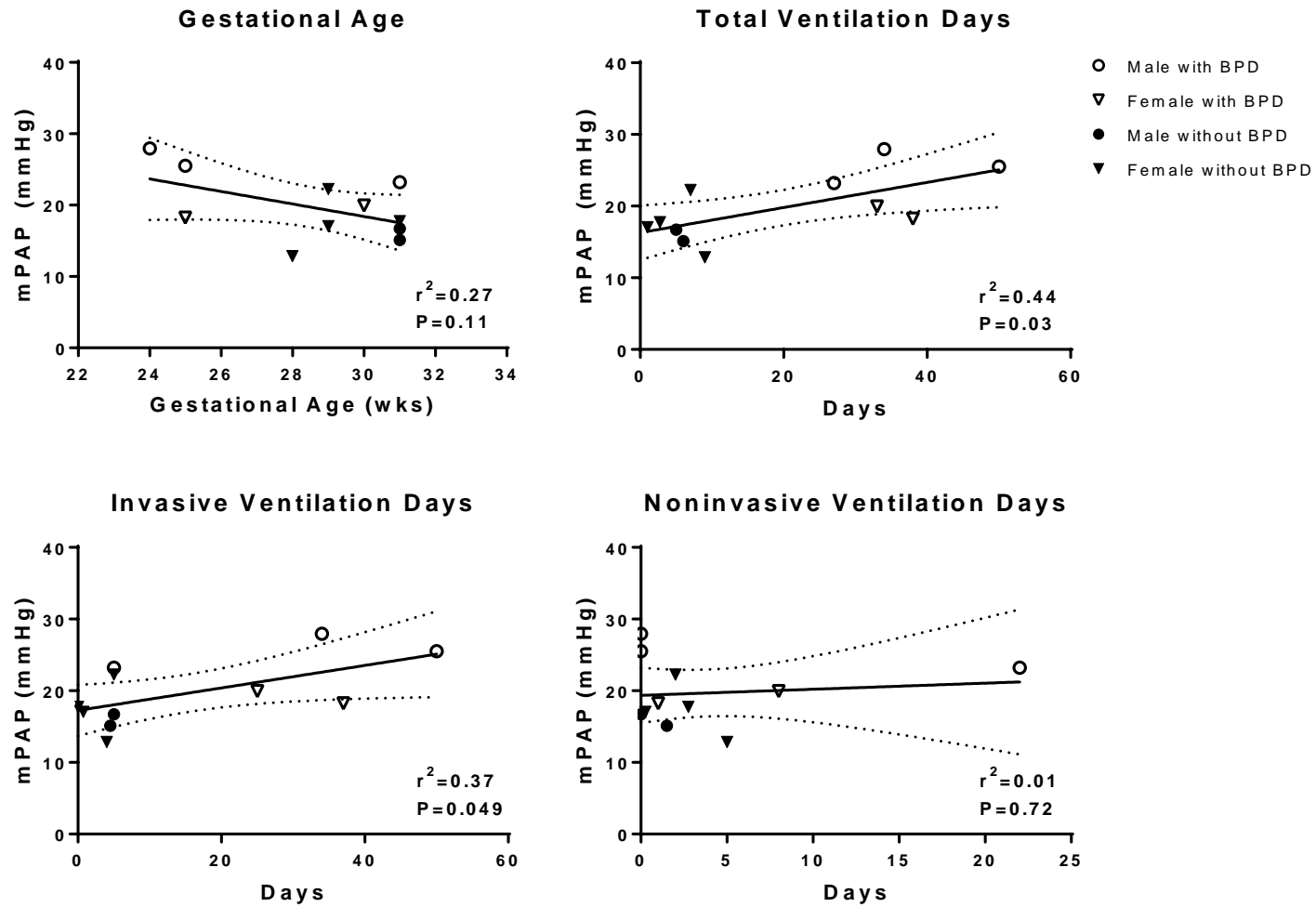


Figure 4



N (males)	11(5)
Gestational age (weeks)	28.6 ± 2.7 (24 – 31)
Birth weight (grams)	1087 ± 297 (675 – 1497)
Apgar score at 1 minute	3 ± 2
Apgar score at 5 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)	2/9
Days of invasive ventilation	15.5 ± 17.7 (0 – 50)
Days of noninvasive ventilation	3.9 ± 6.5 (0 – 22)
Days on oxygen	93 ± 150 (1 – 514)
Days in the NICU	64 ± 30 (33 – 107)
BPD diagnosis at discharge (yes/no)	5/6
Persistent PDA (yes/no)	7/4
Diagnosis of PH by echo in the NICU	0

Supplemental Table 1. Birth and neonatal clinical data for preterm subjects. Values are indicated in mean ± SD with range in parentheses. NICU: neonatal intensive care unit, BPD: bronchopulmonary dysplasia, PDA: patent ductus arteriosus, PH: pulmonary hypertension.