Increased Mitochondrial Oxygen Consumption in Adult Survivors of Preterm Birth

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Abstract

Background: Premature birth affects roughly 10% of live births and is associated with long-term increased risk for multiple comorbidities. Though many comorbidities are associated with increased oxidative stress, the potential late impact of extreme premature birth on mitochondrial function has not previously been assessed. We hypothesized that mitochondrial function would be impaired in adult survivors of premature birth.

Methods: Mitochondrial function in peripheral blood mononuclear cells from young adults born moderately to extremely preterm was measured using a Seahorse XF Analyzer at baseline and in response to acute oxidative stress, and compared to age-matched term-born adults. Adult pulmonary function was also obtained.

Results: Young adults born preterm (average gestational age 29 weeks) had increased mitochondrial oxygen consumption at baseline, particularly with respect to basal and non-ATP linked respiration. Maximal and spare capacity were also higher, even in response to acute oxidative stress. Lung function was lower in adults born preterm, and the degree of airflow obstruction correlated only modestly with mitochondrial function.

Conclusion: In conclusion, adults born preterm have higher basal and non-ATP linked mitochondrial respiration. Similar mitochondrial profiles have previously been documented in diabetics, and may support the increased risk for cardiometabolic disease in adults born preterm.

Introduction:

Premature birth, or birth <37 weeks gestation, affects roughly 1 in 10 live births worldwide. As neonatal care practices improve, the vast majority of even extremely preterm infants are now surviving. However, there is a growing recognition that these individuals are at

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increased risk for multiple late comorbidities as they age into adulthood, and the NIH now recommends prematurity be considered a chronic medical condition (1, 2). Adults born preterm are at increased risk for obstructive lung disease, pulmonary and systemic hypertension, cardiovascular disease including heart failure, and metabolic disease including diabetes, obesity and metabolic syndrome (1, 2).

Intriguingly, several of these comorbidities in non-preterm populations have been characterized by increased mitochondrial dysfunction and chronic oxidative stress (3–6), raising the possibility of a neonatal imprinting or priming of critical metabolic machinery following preterm birth. Furthermore, a recent study demonstrated that vascular endothelial cell mitochondrial function at birth may predict death or development of bronchopulmonary dysplasia (BPD) in infants born extremely preterm (7). A second study in a non-preterm cohort demonstrated correlation between maximal mitochondrial respiration in alveolar macrophages and adult lung function including smokers and individuals with chronic obstructive pulmonary disease (COPD) (8).

Therefore, here we sought to characterize mitochondrial function in adults born moderately to extremely preterm, including those with and without a history of neonatal BPD, with a secondary aim to determine whether mitochondrial function associated with adult lung function. Lastly, we wanted to determine if mitochondrial functional differences were based upon biological sex in the preterm participants. Based largely on available data in neonatal populations, we hypothesized that adults born preterm would have higher basal and non-ATP linked respiration, with reduced maximal respiration resulting in reduced reserve respiration compared to adults born term, with increased sensitivity to acute oxidative stressors.

Materials and Methods:

Participants

Adult participants born moderately to extremely premature (n=29) were recruited from the Newborn Lung Project, a cohort of preterm infants born with very low birth weight (< 1,500 g) between 1988 and 1991, or from the general public with confirmation of birth history from neonatal records. All preterm participants were born ≤ 34 weeks. Age-matched term-born adults (n=17) were recruited from the local population. Participants were free of current cardiovascular or respiratory illness and nonsmokers. All participants provided written informed consent, and the protocol was approved by the Institutional Review Board at the University of Wisconsin-Madison.

Peripheral blood mononuclear cell (PBMC) mitochondrial function

After an overnight fast, whole blood was collected in an ACD tube and peripheral blood mononuclear cells (PBMC) isolated by density gradient centrifugation. PBMCs were resuspended in sterile XF assay buffer (DMEM supplemented with 5.5 mM D-glucose, 4 mM L-glutamine, and 1 mM pyruvate, pH 7.4, 37° C) and seeded at 300,000 cells/well on a 96 well poly-d-lysine coated XF microplate. Therefore, the oxygen consumption rates determined during the experiments are a reflection of the amount of oxygen consumed by the 300,000 cells/well for each sample. To determine the susceptibility to secondary

oxidative stressors, a subset of plated cells were exposed to increasing concentrations of the redox cycling agent, DMNQ (doses from 0.5 μM to 20 μM). After a 30 min incubation time at 37°C in a non-CO2 incubator to allow cells to settle, mitochondrial function was assessed in a Seahorse XF Analyzer, using oligomycin 2.5 μM, FCCP 2 μM and antimycin A/rotenone 2 μM, and according to standard protocols (9). All conditions were repeated in a minimum of 8 wells per experimental condition, with values averaged and adjusted for background.

Anthropometric and Pulmonary Function Data Collection

Participants' height and weight was recorded at the beginning of the study visit. Blood pressure was measured at rest using an automatic blood pressure cuff and glucose was measured with a point-of-care glucometer. Subjects also completed spirometry, 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}) (MasterScreen PFT; Jaeger, Hoechberg, Germany) was obtained, and corrected for hemoglobin (Easy life Hb, London, United Kingdom). Predicted values were calculated from the Global Lung Initiative references (10, 11).

Statistical Analysis

Data were initially grouped by birth status (preterm, term), and presented as mean ± standard deviation for continuous variables, and number (percent) for categorical variables. Baseline anthropometric, pulmonary function, and mitochondrial respiration data were compared by t-tests for each continuous variable, or Chi squared for categorical variables. Response to DMNQ dosing was analyzed using a repeated measures mixed effects model. After stratifying the preterm group by a diagnosis of BPD, we used a one-way analysis of variance (ANOVA) to determine overall statistical differences between, term, preterm, and preterm with a history of BPD. Multiple comparisons were made using Tukey's LSD when appropriate. In order to determine main effects of biological sex, birth status, and the interaction, we utilized a two-way ANOVA. Univariate correlations were performed between maximal respiration and spirometric values. Significance level was determined *a priori* at the 0.05 level and all tests were 2-tailed. Statistical analyses were performed in Prism Graphpad (Version 8, GraphPad Software Inc., La Jolla, CA).

Results:

Anthropometric and Pulmonary Function

Preterm subjects had an average gestational age of 29.1 weeks, and were shorter and with higher body mass index than term subjects (Table 1). Vitals signs including blood pressure and oxygen saturation were similar between groups, as was fasting glucose. Although FVC and FEV1 percent-predicted were similar between groups, a greater number of preterm individuals had airflow obstruction based on lower FEV_1/FVC ratio and FEF_{25-75}. DL_{CO} was also lower, with higher hemoglobin, in preterm-born adults.

PBMC Mitochondrial Function

PBMC mitochondrial function assays revealed significantly higher basal respiration in adults born preterm (Table 2; Figure 1). Although maximal and spare capacity were also higher, these findings were not statistically significant at baseline. However, non-ATP coupled respiration was significantly higher in adults born preterm. The preterm group was further stratified into adults with or without a history of BPD in order to assess whether a history of early lung disease would result in a differential PBMC mitochondrial functional response. There were no differences between adults born preterm with and without BPD for all mitochondrial measures of oxygen consumption (spare capacity not shown) (Figure 2). Basal and non-ATP linked respiration was higher in both preterm with and without BPD as compared to term born subjects, respectively. Therefore, preterm subjects with and without BPD were grouped together on further analysis. When exposed to increasing doses of the redox cycling agent DMNQ (2, 3-dimethoxy-1, 4-napthoquinone), basal, maximal, spare, and non-ATP coupled respiration remained significantly higher in PBMCs from preterm-born adults across all doses of DMNQ (Figure 3).

Correlation with Lung Function

Given the aforementioned studies on the ability of mitochondrial function to predict airways disease (BPD and COPD) (8, 12), we assessed for correlation between respiration and lung function. Maximal respiration in preterm participants correlated modestly with FEV1 %-predicted (R^2=0.19, p=0.02) and FVC %-predicted (R^2=0.18, p=0.02), but not in term participants (R^2=0.005, p=0.80 and R^2=0.007, p=0.74 respectively; Figure 4). There was no correlation with DL_{CO}.

Sex Differences in PBMC mitochondrial function

We further stratified groups according to sex to better understand the interaction between birth status and biological sex on PBMC mitochondrial function. Independent of birth status, males exhibited higher non-ATP linked respiration (Figure 5) compared to females. Notably, females born premature exhibit increased maximal, basal, non-ATP linked (uncoupled), and ATP linked (coupled) respiration compared to females born at term (Figure 5), suggesting that the differences in the preterm group at large are driven primarily by female participants. In contrast, males born premature show similar maximal, basal, and ATP-linked respiration compared to term born males. There were no differences in spare respiratory capacity by sex or birth status (data not shown).

Discussion:

We hypothesized that adults born preterm would have higher basal and non-ATP linked respiration, with reduced maximal respiration resulting in reduced reserve respiration compared to adults born at term. Although higher basal and non-ATP linked respiration was observed, maximal respiration was also higher in adults born preterm leading to preserved spare capacity. Additional analysis demonstrated that much of the increase in basal, maximal and ATP-linked respiration in adults born preterm was driven by increased respiration in females. Further, although we suspected mitochondrial function would worsen under acute oxidative stress with DMNQ, we observed a preservation of mitochondrial function

including higher maximal and spare capacity in preterm participants across a range of doses. Finally, adult maximal mitochondrial respiration correlated, but only modestly, with adult lung function in the preterm population.

Intriguingly, the preterm-born young adults in this study demonstrate a mitochondrial respiration pattern of higher basal, maximal, and uncoupled respiration similar to that previously described in PBMCs from diabetic patients (13), though none of our subjects were diabetic and all had normal fasting glucose despite higher body mass index. Impaired insulin sensitivity has previously been reported in adults born preterm and could further contribute to this phenotype (14), though this was not directly assessed in our study. Collectively, our findings further support the increased cardiometabolic risk that follows preterm birth and may provide insight into mechanisms promoting such risk. Furthermore, mitochondrial function in PBMCs may serve as an important biomarker for diabetes risk, and follow up evaluation for the development of overt diabetes is warranted.

Although previous work demonstrates lower maximal mitochondrial respiration in premature neonates who developed severe BPD (7), we did not uncover significant differences between preterm participants with and without a history of BPD. However, the majority of individuals with BPD in the study had mild BPD by current definitions (i.e. use of low flow oxygen by nasal cannula), and greater differences may be observed if individuals with more severe BPD were included. Further, our sample included relatively few individuals with a history of BPD, and a larger sample size would be helpful in future studies to better stratify the effect of BPD.

We speculate that the higher respiration overall among preterm-born adults may be due to mitohormesis. Specifically, while an acute (neonatal) oxidative stressor causes initial worsening of mitochondrial function, reactive oxygen species serve a critical role of promoting adaptation across the lifespan that may even exceed normal function (15). In support of this hypothesis, we have also seen a similar bimodal cardiac response in an animal model of prematurity using postnatal hyperoxia, where mitochondrial and global cardiac function recover in early adulthood despite initial impairment in infancy (16). Unfortunately, this recovery does not remain protective, as these animals develop heart failure in late adulthood with the effects most pronounced in males (17).

Sexual dimorphism is an important consideration in individuals with a history of premature birth. For example, males are at increased risk of developing BPD compared to females of similar gestational age and birth weight (18–20). Furthermore, BPD is associated with oxidative stress in part due to oxygen treatment postnatally (21, 22), and males exhibit increased markers of oxidative stress and lower antioxidant defense mechanisms compared to females born preterm (23). Biological sex is also an important determinate for prevalence of metabolic syndrome and diabetes in individuals born premature (24, 25). In the general population, type II diabetes has a higher overall prevalence among men (25–27). However, females born preterm have a greater incidence of type II diabetes compared to males (25). Findings from this study show that females born premature demonstrate increased mitochondrial respiration, including higher basal and non-ATP coupled respiration, relative to females born at term. Collectively, these findings may suggest an adaptive mitohormetic

response in females born premature that both protects them from development of BPD but yet increases their risk for later diabetes. Given the similarity of our findings of increased PBMC mitochondrial respiration in adults born preterm with diabetic patients (13), PBMC mitochondrial functional measurements may be a biomarker to track potential risk of cardiometabolic disease in individuals born preterm. Further, these findings underscore the importance of sex-based approaches in determining cardiometabolic risk.

Our results also demonstrate that preterm-born adults consume more oxygen for every given molecule of ATP production. This is in part due to greater non-ATP linked (uncoupled) respiration in preterm-born adults. Uncoupled respiration is primarily due to increased uncoupling protein expression which is thought to be protective against further oxidant stress (28). Future work should determine uncoupling protein expression and oxidative stress generation in adults born preterm, and may provide further pathophysiologic basis for the increased risk of diseases associated with oxidative stress. Preterm-born adults also had higher hemoglobin levels, which have been described in prior studies despite normal erythropoietin levels (29). It is possible that higher global mitochondrial oxygen consumption contributes indirectly to the higher hemoglobin levels, though we did not find evidence of direct correlation between PBMC respiration and hemoglobin. Mitochondrial respiration was only modestly correlated with spirometric measures of lung function among adults born preterm, though to a similar degree as previously reported among smokers and patients with COPD (8). In order to improve our understanding of the relationship between mitochondrial respiration and lung function in adults born preterm, future works incorporating a wider range of lung function to include adults born preterm with clinically relevant obstructive lung disease, including those who are active smokers may be necessary to assessing the role of PBMC mitochondrial function as a candidate biomarker as a predictor of future pulmonary outcomes or responses to treatment. Additionally, lung specific cell types would be helpful to further establish this relationship. Finally, whether the correlation with lung function would be stronger earlier in life is unknown and including children and adolescents into the study design may shed more light on the postulation of mitohormesis in prematurity.

There are some strengths of this study to consider. This was the first study to determine PBMC mitochondrial function in a cohort of young adults with a history of premature birth. Secondly, this study begins to shed light on sexually dimorphic adaptations that could be an important differentiation for cardiometabolic disease risk in the preterm population. Limitations of our study include a relatively small sample size as well as utilization of a circulating cell type. Although readily available, changes in PBMC mitochondrial function may not fully reflect mitochondrial function at the organ level, and functional correlations may be stronger with a tissue-specific cell type. Given the established protocols (9) that were used to measure PBMC mitochondrial function, OCR was greater in many of the samples after administration of rotenone/Antimycin A as compared to the OCR after oligomycin administration. This did not permit delineating the relative contributions of proton leak and non-mitochondrial OCR from non-ATP linked OCR. This limitation highlights the heterogeneity of response in PBMC mitochondrial function and using a higher concentration of rotenone/Antimycin A in future experiments may be necessary to ensure adequate complex III inhibition in order to better determine mitochondrial mechanistic

differences (i.e. proton leak and non-mitochondrial OCR from the non-ATP linked OCR) between biological sex and preterm and term born adults, respectively. Further, although we assessed tolerance of acute oxidative stressors, we did not measure the consequences of such tolerance, such as greater production of reactive oxygen species that could be deleterious over time. Chronic airway oxidative stress was previously demonstrated to be elevated in adolescents born preterm (30), and may further serve as a driver of mitochondrial adaptation over the lifetime.

In conclusion, we identified increased oxygen consumption in PBMCs from adults born preterm, similar to a profile previously documented in diabetes. Though the association with lung function was modest at best, the findings may support the increased risk for cardiometabolic disease in adults born preterm, which may be more pronounced for females. Future studies should address additional cell types, measurement of reactive oxygen species, and direct evaluation of the electron transport chain complexes. Given the potential as a longitudinal biomarker, there is further need to understand how intrauterine and postnatal factors alter and may prime mitochondria for the lifetime.

Acknowledgements:

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Impact:

- Adults born preterm have higher maximal but also higher basal and non-ATP linked mitochondrial respiration. Similar mitochondrial profiles have previously been documented in diabetics, and may support the increased risk for cardiometabolic disease in adults born preterm.

- Prior studies demonstrate a link between perinatal mitochondrial function and risk for development of bronchopulmonary dysplasia. Here, maximal mitochondrial respiration correlates modestly with adult lung function.

- Peripheral blood mononuclear cell mitochondrial function may be a biomarker of both early lung function and late cardiometabolic risk after preterm birth.
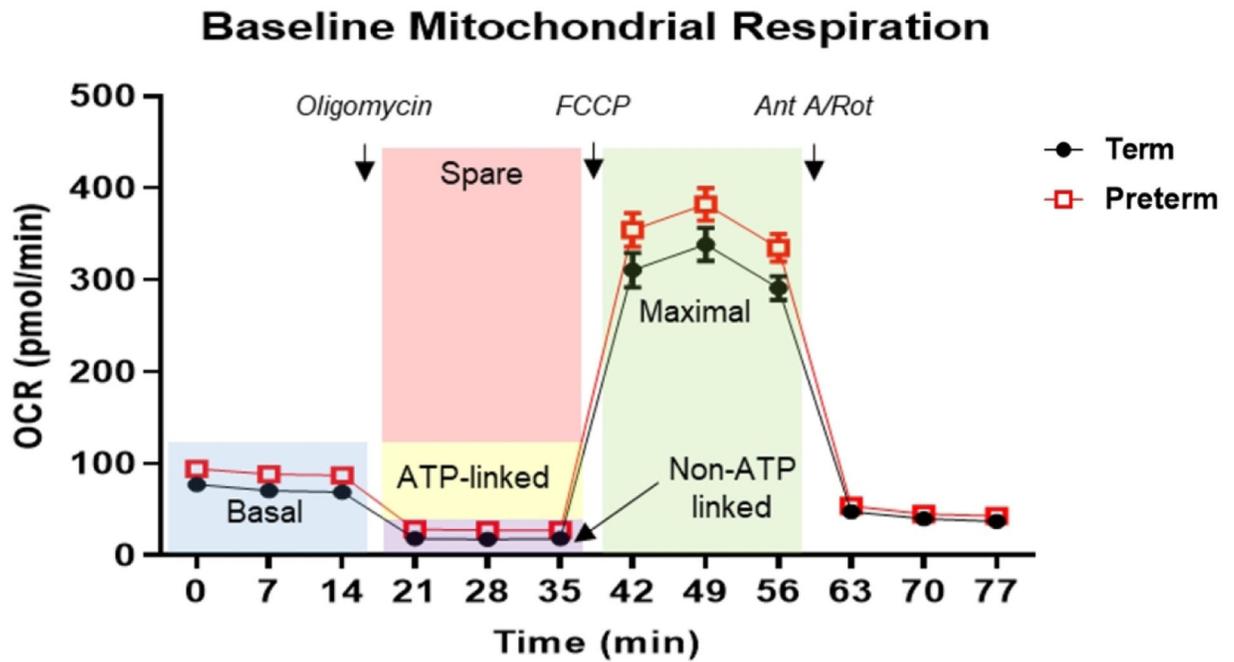
Baseline Mitochondrial Respiration

Figure 1: Mitochondrial respiration in PBMCs from adults born preterm (red squares) is elevated compared to adults born at term (black circles).
Basal, maximal, and non-ATP linked respiration are higher in adults born preterm at baseline. Oxygen consumption rate values in these experiments reflect the consumption of oxygen of 300,000 cells/well. Data points represented as Mean ± SEM. PBMC: peripheral blood mononuclear cells. OCR: oxygen consumption rate.
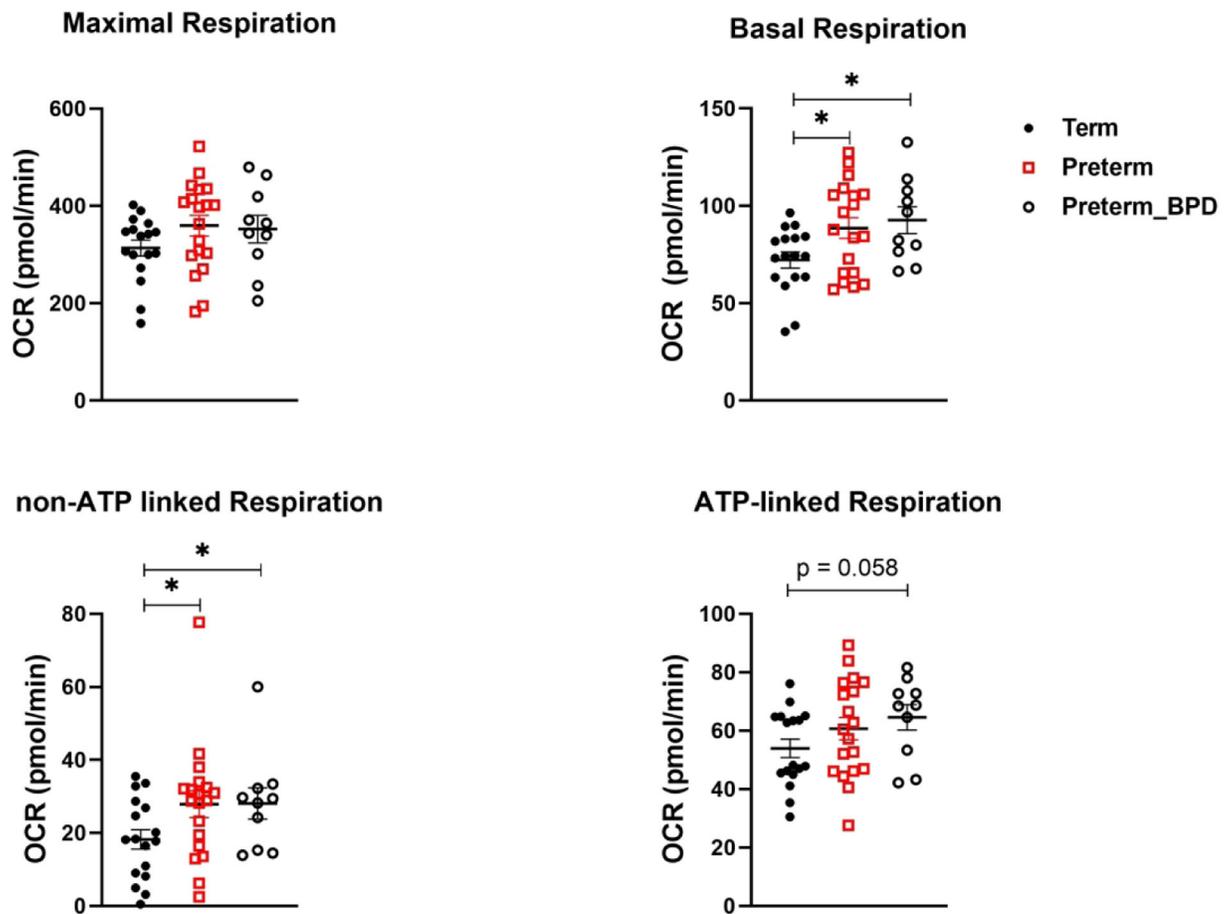
Figure 2: Mitochondrial respiration in PBMCs from adults born at term, preterm, and preterm with a history of bronchopulmonary dysplasia (BPD).

Basal and non-ATP linked respiration were higher in adults born preterm with and without a history of BPD as compared to those born at term. There were no differences in mitochondrial respiration between the adults born preterm with and without a history of BPD. Data represented as Mean ± SEM. * $p < 0.05$.
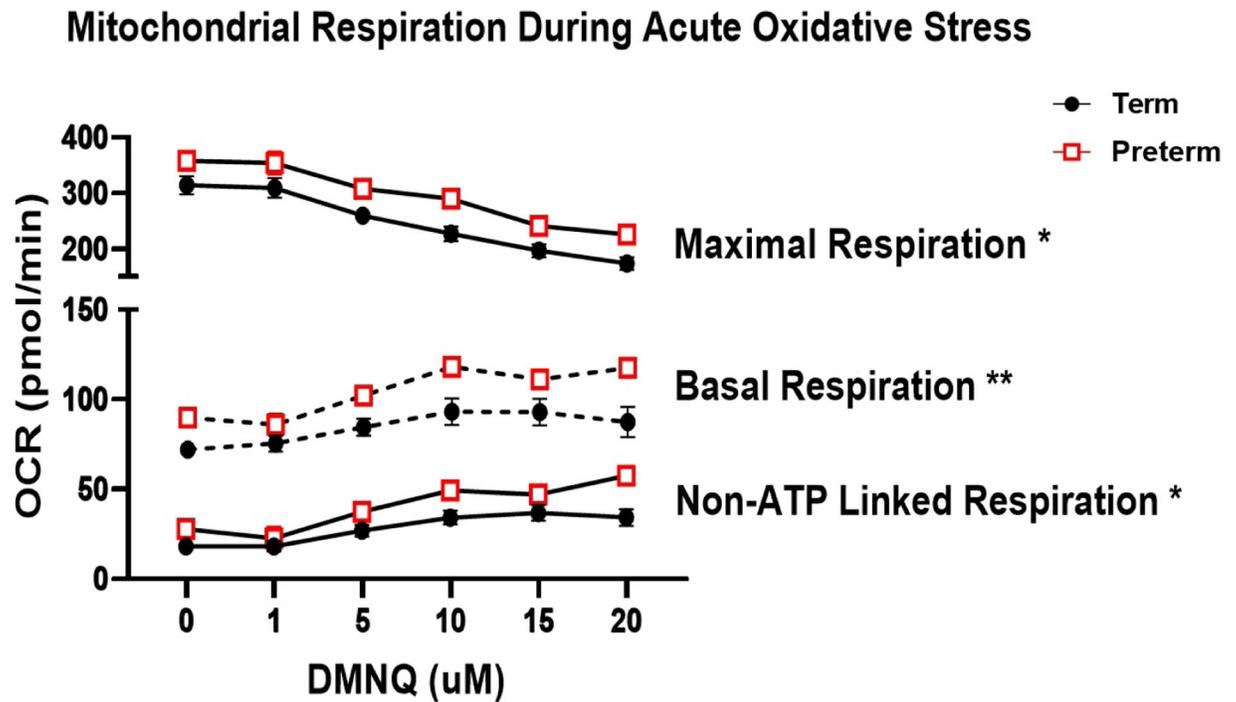
Figure 3: Mitochondrial respiration in PBMCs from adults born preterm remain higher compared to adults born at term across a range of increasing doses of DMNQ, an oxidant stressor (repeated measures mixed effects analysis, *p<0.05, **p<0.01 for birth effect). Data points represented as Mean ± SEM. PBMC: peripheral blood mononuclear cells. OCR: oxygen consumption rate. DMNQ: 2, 3-dimethoxy-1, 4-napthoquinone.
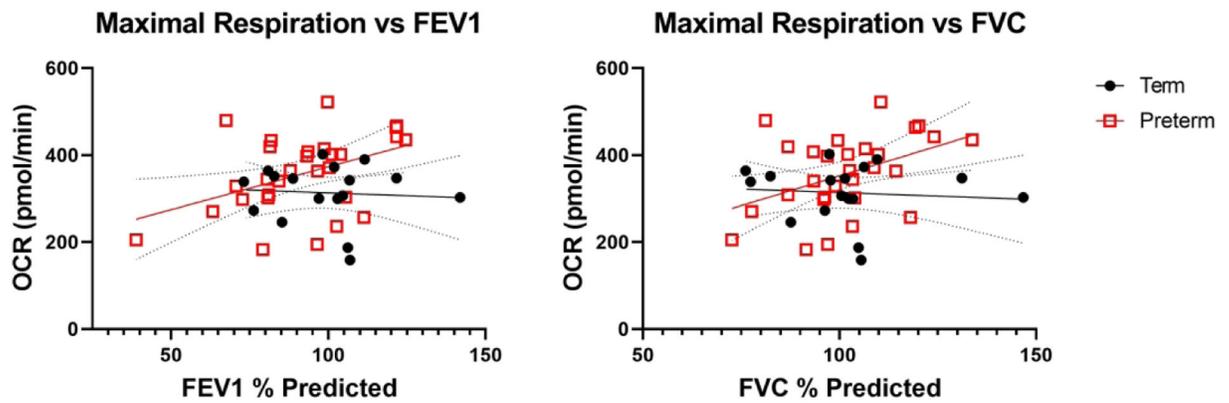
Figure 4: Mitochondrial respiration correlated modestly with lung function in adults born preterm, but not in adults born at term.

Preterm forced expiratory volume at 1 sec (FEV1) %-predicted R^2=0.19, p=0.02 and forced vital capacity (FVC) %-predicted R^2=0.18, p=0.02; term FEV1 %-predicted R^2=0.005, p=0.80 and FVC %-predicted R^2=0.007, p=0.74.
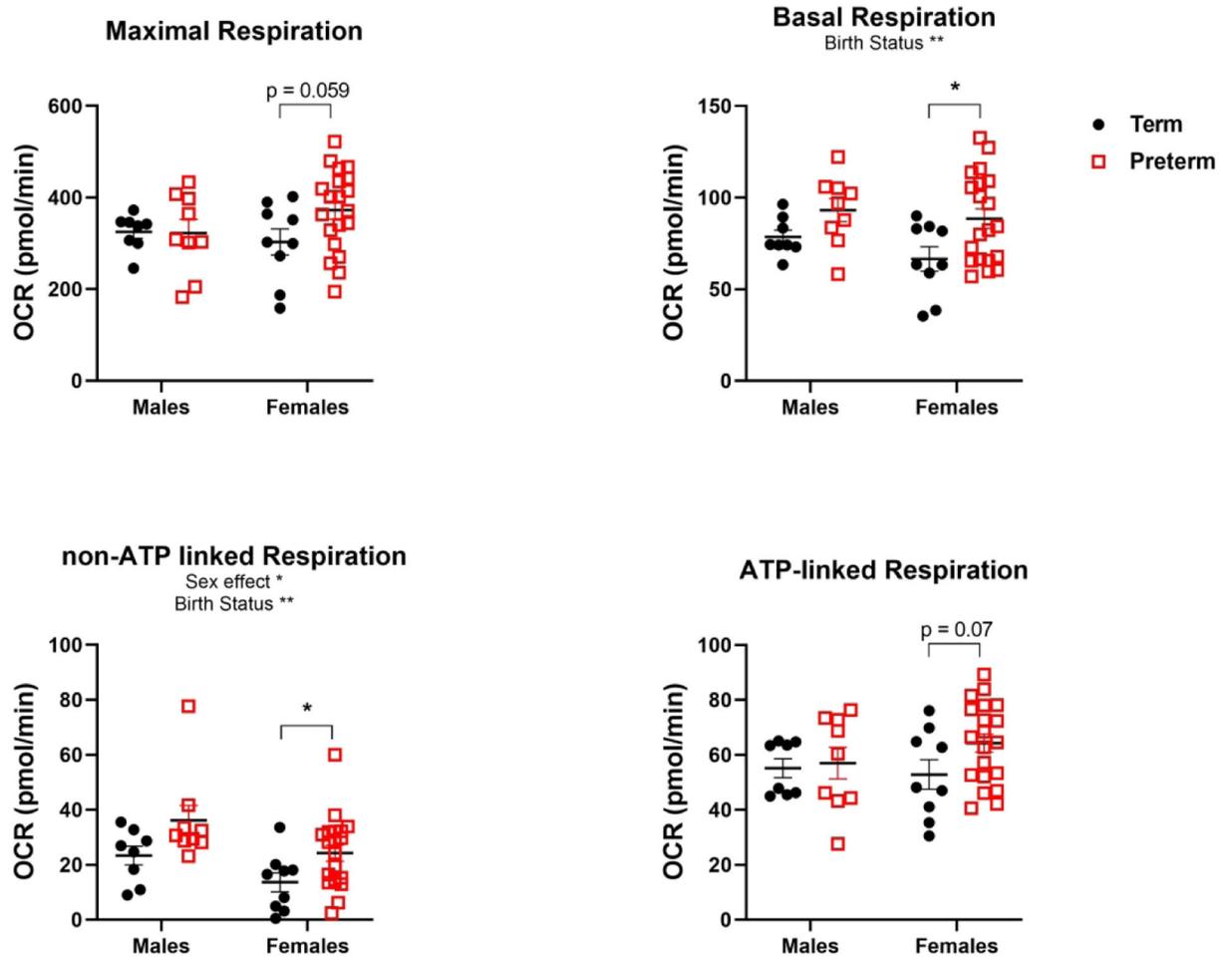
Figure 5: Biological sex dependence on PBMC mitochondrial function in adults born preterm. There is an overall effect of biological sex as males exhibit greater non-ATP linked respiration compared to females, independent of birth status. Maximal, basal, non-ATP linked, and ATP-linked respiration is higher in females born premature compared to females born at term. Data represented as Mean ± SEM. * $p < 0.05$, ** $p < 0.01$.

Table 1:

Baseline characteristics in adults born term and preterm.

	TERM (n=17)	PRETERM (n=29)	p-value
Baseline Adult Characteristics			
Current Age, years	25.3±4.0	26.6±4.0	0.30
Female sex	9 (53%)	20 (69%)	0.44
Height (inches)	68.1±3.4	65.6±2.8	0.02
Weight (kg)	69.9±9.6	76.7±24.6	0.21
Body Mass Index (kg/m^2)	23.3±1.9	27.4±8.5	0.02
Fasting Glucose	83±8	84±9	0.69
Systolic Blood Pressure (mmHg)	116±12	115±10	0.65
Diastolic Blood Pressure (mmHg)	74±7	71±4	0.18
Resting Oxygen Saturation (%)	97.8±0.9	97.8±1.1	0.93
Neonatal Characteristics			
Gestational Age (weeks)	39.8±1.0	29.1±2.5	<0.01
Birth Weight (g)	3388±571	1183±373	<0.01
Pulmonary Function Testing			
FVC (% predicted)	101.6±17.0	101.7±13.8	0.98
FEV1 (% predicted)	99.2±16.8	91.9±19.2	0.19
FEV1/FVC	0.83±0.06	0.76±0.10	0.01
FEF$_{25-75}$ (% predicted)	93.8±23.8	73.2±29.7	0.02
DLCOc (% predicted)	97.7±18.3	82.7±11.3	0.01
Hemoglobin (g/dL)	14.2±1.7	15.8±2.3	0.01

Numbers represent mean ± standard deviation for continuous variables, and number (percent) for categorical variables. Comparisons by t-tests for each continuous variable, or Chi squared for categorical variables. Abbreviations: FVC: forced vital capacity; FEV1: forced expiratory volume in 1 second; FEF$_{25-75}$: mean forced expiratory flows from 25–75% of FVC; DLCOc: diffusion capacity of the lung for carbon monoxide, corrected for hemoglobin.

Table 2:

Baseline mitochondrial function in adults born term and preterm

	TERM (n=17)	PRETERM (n=29)	p-value
Baseline Mitochondrial Function			
Basal Respiration (pmol/min)	72.2±16.4	90.0±22.0	0.004
Maximal Respiration (pmol/min)	313.5±64.9	357.2±88.3	0.07
Spare Capacity (pmol/min)	241.3±53.6	267.1±74.0	0.19
ATP-linked Respiration (pmol/min)	54.0±12.7	62.1±15.4	0.07
Non-ATP linked Respiration (pmol/min)	18.2±10.7	28.0±14.7	0.02