The Effect of Inhaled Nitric Oxide on Maximal Oxygen Consumption During Exercise in Hypoxia.

by

Zahrah Hatimali Rampuri

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ABSTRACT

Background and Rationale. Arterial hypoxemia (decreased arterial oxygen content [CaO_2]) caused by a decrease in inspired partial pressure of oxygen (P_IO_2), can result in a similar reduction in exercise capacity as observed in chronic lung disease. Literature has suggested a discrepancy in the effect of increasing hypoxic severity on peak cardiac output ($\dot{Q}$) and therefore exercise capacity ($\dot{V}O_{2max}$). In moderate hypoxia ($P_IO_2 \geq 85$mmHg), $\dot{V}O_{2max}$ decline has been attributed to arterial desaturation, whereas in severe hypoxia ($P_IO_2 < 85$mmHg), impaired peak $\dot{Q}$, secondary to elevated pulmonary artery pressure (PAP) has been suggested to play a role. Inhaled nitric oxide (iNO) reduces PAP at rest and during exercise in normoxia, and therefore could be used as a tool to evaluate the impact of reducing PAP during hypoxic exercise on $\dot{Q}$ and $\dot{V}O_{2max.}$

Purpose and Hypothesis. The purpose of this study was to better understand the mechanism(s) of exercise intolerance and cardiovascular regulation in hypoxia, by determining if the elevation in PAP in hypoxia is a limiting factor of $\dot{V}O_{2max}$. It was hypothesized that iNO would improve $\dot{V}O_{2max}$ during exercise in both moderate and severe hypoxia by reducing PAP, leading to increased SV and peak $\dot{Q}$. Further, it was hypothesized that the magnitude of improvement in $\dot{V}O_{2max}$ with iNO would be smaller in severe hypoxia due to an underlying diffusion limitation in severe hypoxia which would limit the increase in $\dot{V}O_{2max}$ from augmenting $\dot{Q}$.

Methods. Twelve young, healthy participants with normal lung function were recruited. Participants completed 6 sessions: visit 1) pulmonary function test and normoxic cardiopulmonary exercise test (CPET); visits 2-5) experimental CPETs on separate days breathing the following conditions: A) moderate hypoxia, B) severe hypoxia, C) moderate hypoxia with iNO, and D) severe hypoxia with iNO (order randomized); visit 6) resting and exercise cardiac ultrasound trial while breathing the conditions listed above in a randomized order. $\dot{V}O_2$, ventilation [$\dot{V}_E$], carbon

dioxide production [$\dot{V}CO_2$], ventilatory equivalent to carbon dioxide production [$\dot{V}_E/\dot{V}CO_2$], partial pressure of end-tidal carbon dioxide [$P_{ET}CO_2$]) were measured using a metabolic measurement system. $\dot{Q}$ was estimated using impedance cardiography and right ventricular systolic pressure (RVSP) was estimated via Doppler echocardiography.

Results. $\dot{V}O_{2max}$ was decreased by 0.43±0.07 L/min in moderate hypoxia ($p=0.005$) and 0.78±0.12 L/min in severe hypoxia ($p<0.001$) when compared to normoxia. iNO reduced resting RVSP by 2.53±0.8 mmHg in moderate hypoxia ($p=0.01$) and 1.78±0.2 mmHg in severe hypoxia ($p=0.05$), however, iNO had no effect on $\dot{V}O_{2max}$ in either hypoxic level. Furthermore, peak $\dot{Q}$ was unaffected by hypoxia or iNO.

Discussion and Significance. These findings suggest that the hypoxia-induced increase in PAP does not impair peak $\dot{Q}$ and therefore $\dot{V}O_{2max}$ in healthy participants during hypoxic exercise. This study demonstrated that the pulmonary vasculature and right ventricle likely do not limit hypoxic exercise in healthy individuals suggesting that in hypoxemic patients, the pulmonary vasculature may not directly impair exercise capacity. This work highlights the importance of further understanding mechanism(s) and treatments of exercise limitations in hypoxemic patients, as improving exercise capacity would increase participation in meaningful activities of daily living, and subsequently quality of life. These findings may suggest that the pulmonary hypertension associated with chronic lung disease does not directly impact exercise tolerance.

PREFACE

This thesis is an original piece of work written by Zahrah Hatimali Rampuri. This thesis is part of a larger research project titled “The Effect of Inhaled Nitric Oxide on Maximal Oxygen Consumption During Exercise in Hypoxia”, which has received ethics approval from the University of Alberta Research Ethics Board (Pro00092939).

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1. BMI: Body mass index
2. CaO_2: Arterial oxygen content
3. COPD: Chronic obstructive pulmonary disease
4. COVID-19: Novel coronavirus-19
5. CPET: Cardiopulmonary exercise test
6. DLCO: Diffusing capacity for carbon monoxide
7. DM: Diffusing membrane capacity
8. DO_2: Oxygen delivery
9. ECG: Electrocardiogram
10. EDV: End-diastolic volume
11. EF: Ejection fraction
12. ESV: End-systolic volume
13. f_b: Breathing frequency
14. F_IO_2: Fraction of inspired oxygen
15. Hb: Hemoglobin
16. HPV: Hypoxic pulmonary vasoconstriction
17. HR: Heart rate
18. IC: Inspiratory capacity
19. ICF: International classification of functioning, disability, and health
20. ILD: Interstitial lung disease
21. iNO: Inhaled nitric oxide
22. IVC: Inferior vena cava

23. LAP: Left atrial pressure

24. MAP: Mean arterial pressure

25. mPAP: Mean pulmonary artery pressure

26. Met-Hb: Methemoglobin

27. NO: Nitric oxide

28. NO_2: Nitrogen dioxide

29. PAP: Pulmonary artery pressure

30. PAWP: Pulmonary artery wedge pressure

31. $P_{ET}CO_2$: End-tidal pressure of carbon dioxide

32. P_IO_2: Partial pressure of inspired oxygen

33. PVR: Pulmonary vascular resistance

34. $\dot{Q}$: Cardiac output

35. RAP: Right atrial pressure

36. RHC: Right heart catheter

37. RV: Right ventricle

38. RVSP: Right ventricular systolic pressure

39. RVSW: Right ventricular stroke work

40. RVSWI: Right ventricular stroke work index

41. S_PO_2: Arterial oxygen saturation

42. SV: Stroke volume

43. $\dot{V}CO_2$: Carbon dioxide production

44. $\dot{V}_E$: Minute ventilation

45. $\dot{V}_E/\dot{V}CO_2$: Ventilatory equivalent to carbon dioxide production

46. $\dot{V}O_{2max}$: Oxygen consumption

47. $\dot{V}O_{2max}$: Maximal oxygen consumption

48. $\dot{V}O_{2peak}$: Peak oxygen consumption

49. V_T: Tidal volume

50. V_{TR}: Tricuspid regurgitant velocity

CHAPTER I: Introduction

1.1 Background

Dyspnea, commonly known as breathlessness, is a key contributor to reduced exercise capacity in patients with lung disease(Jensen et al., 2018). Reduced exercise tolerance and increased dyspnea have been associated with decreased quality of life and increased risk of mortality in individuals with lung diseases such as chronic obstructive pulmonary disease or interstitial lung disease(Jensen et al., 2018; Ley et al., 2016; Oga et al., 2003). Patients with advanced lung disease often have damage/destruction of the alveoli resulting in decreased surface area for gas exchange, impaired gas exchange, significant arterial hypoxemia and reduced exercise capacity(Mannino, 2002; West, 2011). Several studies have linked significant exertional arterial hypoxemia to reduced exercise tolerance and increased dyspnea in patients with advanced lung disease(Du Plessis et al., 2018; Hiroyuki et al., 2018; Yannick et al., 2020).

Exposure to hypoxic conditions such as high altitude, where there is a drop in inspired partial pressure of oxygen (P_IO_2), can lead to arterial hypoxemia (a drop in arterial PO_2 or arterial saturation), reduced arterial oxygen content (CaO_2), resulting in a similar reduction in exercise capacity as observed in chronic lung disease(Hackett, 2019; Harvard, 2018). Aerobic exercise capacity is measured by maximal oxygen consumption ($\dot{V}O_{2max}$) and determined by the Fick equation ($\dot{V}O_2=\dot{Q}\ x\ (CaO_2-CvO_2)$). Studies have demonstrated that in hypoxia, the decrease in CaO_2, secondarily to a drop in arterial saturation (S_PO_2), is the primary limiting factor of $\dot{V}O_{2max}$ (Knight et al., 1993; Mollard et al., 2007; Stenberg et al., 1966). However, recent hypoxia research has suggested that cardiac output ($\dot{Q}$) may also limit $\dot{V}O_{2max}$ within a specific range of hypoxia(Calbet et al., 2003). In mild-moderate hypoxia (P_IO_2~97-85 mmHg), peak $\dot{Q}$ remains relatively unchanged in healthy participants while $\dot{V}O_{2max}$ is significantly decreased, suggesting

that $\dot{Q}$ does not play a role in limiting $\dot{V}O_{2max}$ in hypoxia(Reeves et al., 1987b; Stenberg et al., 1966; Wagner, 2017). However, in severe hypoxia (P_IO_2 <85 mmHg), significant reductions in $\dot{Q}$ as well as $\dot{V}O_{2max}$ are observed, suggesting that cardiac function may be limited during exercise(Calbet et al., 2003). Therefore, it remains unclear whether hypoxia affects peak $\dot{Q}$ during exercise, and whether $\dot{Q}$ is a limiting factor of $\dot{V}O_{2max}$ in hypoxia.

Research has suggested that the reduction in peak $\dot{Q}$ with hypoxia is due to hypoxic pulmonary vasoconstriction (HPV)(Naeije, 2011; Stembridge et al., 2019; Swenson, 2013). HPV is a mechanism that, in response to local alveolar hypoxia, diverts blood flow from poorly ventilated lung units to sufficiently ventilated lung units, with the goal of maintaining or improving ventilation/perfusion matching(Swenson, 2013). Although HPV may be a protective mechanism against local alveolar hypoxia in clinical conditions, in the case of global alveolar hypoxia (as may occur in severe hypoxia), it may be maladaptive(Naeije, 2011). Heterogenous HPV can redirect blood flow to small pulmonary arteries that are not actively vasoconstricted, leading to higher perfusion pressures reaching the alveolar-capillary bed in these regions causing capillary hypertension(Naeije, 2011). Due to the fragility of pulmonary capillaries, capillary hypertension can result in pulmonary edema and/or damage to the alveolar-capillary membrane, leading to pulmonary gas exchange impairment(Naeije, 2011; West & Luks, 2016).

Furthermore, HPV would lead to increased pulmonary artery pressure (PAP) and right ventricular (RV) afterload, which may limit $\dot{Q}$ and $\dot{V}O_{2max}$(Naeije, 2011; Stembridge et al., 2019; Swenson, 2013). During maximal intensity exercise in normoxia, distention and recruitment of pulmonary capillaries occurs in response to large increases in $\dot{Q}$ to decrease pulmonary vascular resistance (PVR) and allow for greater increases in $\dot{Q}$(West & Luks, 2016). However, this drop in PVR with increasing $\dot{Q}$ may not occur in hypoxia. Studies have demonstrated that in both

moderate and severe hypoxia, $\dot{V}O_{2max}$ is decreased and PAP and PVR are increased in healthy volunteers when compared with normoxia(Naeije, 2011; Naeije et al., 2010; Wagner et al., 1986a). However, there does not appear to be a relationship between the increase in PAP and the reduction in $\dot{V}O_{2max}$ within hypoxia. Additionally, research in moderate and severe hypoxia suggests that when PVR and PAP are pharmacologically reduced, $\dot{V}O_{2max}$ is improved, likely secondary to improved SV and $\dot{Q}$(Faoro et al., 2009; Naeije et al., 2010). Together, these findings suggest that PVR and PAP are increased in moderate and severe hypoxia, resulting in reductions in $\dot{Q}$ and $\dot{V}O_{2max.}$

Sildenafil (i.e., Viagra^{TM}) is a phosphodiesterase-5 inhibitor that prevents the degradation of cyclic guanosine monophosphate thereby enhancing the effect of endogenous NO (i.e., potentiates vasodilation)(National Center for Biotechnology Information., 2021). Sildenafil has been shown to improve $\dot{V}O_{2max}$ secondary to decreased PAP and PVR and therefore increased $\dot{Q}$ during exercise in severe hypoxia(Fischler et al., 2009; Ghofrani et al., 2004). However, sildenafil is administered orally and affects both the systemic and pulmonary vasculature, therefore it cannot be determined if the improvement in $\dot{V}O_{2max}$ occurred due to reduced HPV or increased oxygen delivery to skeletal muscles through vasodilation of the peripheral vasculature(Ghofrani et al., 2004). Studies using inhaled nitric oxide (iNO), a selective pulmonary vasodilator, have demonstrated reductions in PVR and PAP in acute hypoxic conditions(Frostell et al., 1993; Pepke-Zaba et al., 1991). Frostell et al, studied the effects of iNO on HPV in healthy volunteers at rest(Frostell et al., 1993). As expected, severe hypoxia elicited a significant increase in PAP, PVR and right ventricular stroke work index (RVSWI; measured by right heart catheterization), while the addition of iNO returned PAP, PVR and RSVWI to normoxic levels(Frostell et al., 1993). This suggests that iNO is effective in reversing HPV, thereby normalizing PAP and PVR. However, the

study did not examine the effects of iNO on PAP and PVR during maximal exercise(Frostell et al., 1993). To date, no study has investigated the effects of iNO on $\dot{V}O_{2max}$ and cardiac mechanics during exercise in hypoxia.

1.1.1 Purpose

The primary purpose of this study was to determine the effects of iNO on exercise capacity during moderate and severe hypoxia in young, healthy participants. We sought to better understand the mechanism(s) of exercise intolerance and cardiovascular regulation in hypoxia, by determining if the elevation in PAP secondary to HPV is a limiting factor of $\dot{V}O_{2max}$ in health during moderate and severe hypoxia.

1.1.2 Hypothesis

We expect $\dot{V}O_{2max}$ to be reduced in both moderate and severe hypoxia, with larger reductions in severe hypoxia due to decreased $\dot{Q}$, in addition to arterial desaturation. We hypothesized that iNO would improve $\dot{V}O_{2max}$ during exercise in both moderate and severe hypoxia by reducing PAP, leading to increased SV and peak $\dot{Q}$. However, we hypothesized that the magnitude of improvement in $\dot{V}O_{2max}$ with iNO would be smaller in severe hypoxia due to an underlying diffusion limitation in severe hypoxia which would limit the increase in $\dot{V}O_{2max}$ by augmenting $\dot{Q}$.

1.2 Delimitations

Experimental trials were randomized to limit the potential learning effect of 6 cardiopulmonary exercise tests (CPETs). A minimum of 24 hours was allotted between each CPET to allow for proper recovery and all 6 CPETs were completed within a 3-week period. Furthermore, as the study was a double-blind randomized cross-over design, participants acted as their own control between each experimental condition.

As smoking history is known to affect pulmonary gas exchange(John D & William, 2013) and a BMI>30 is known to affect the pulmonary vasculature(Delorey et al., 2005), all participants were young healthy individuals with minimal smoking history (<10 pack years) and a BMI of 18.5-30.

Lastly, due to the potential effects of the menstrual cycle on $\dot{V}O_{2max}$(Seaton, 1972; Smith et al., 2015), menstrual cycle stage, usage and brand of contraception was reported to allow for tracking of hormone regulation during trials.

1.3 Limitations

1.3.1 Breathing Discomfort.

During the experimental CPETs, compressed hypoxic gas (12.1% oxygen or 13.6% oxygen balanced with nitrogen) was titrated with or without iNO and delivered to participants via a Douglas bag. Since the compressed gas is not humidified prior to inhalation, there was potential for additional breathing discomfort for participants during the CPETs, however, the same delivery system was used during each experimental trial to control for this.

1.3.2 Preset Hypoxic Tanks.

For this study, preset hypoxic tanks (12.1% oxygen and 13.6% oxygen) were used for the 2 hypoxic conditions. When barometric pressure in the laboratory was greater than the normal barometric pressure for Edmonton (~700mmHg), the partial pressure of inspired oxygen (P_IO_2) was elevated as the fraction of inspired oxygen (F_IO_2) from the tanks could not be lowered. This resulted in a slightly elevated P_IO_2 than the target of 79mmHg (severe hypoxia) and 89mmHg (moderate hypoxia) during the placebo trials.

1.3.3 Barometric Pressure and Temperature Fluctuations.

Barometric pressure and temperature in the laboratory varied and were recorded each day. The pneumotach and gas analyzers were calibrated before each trial to account for these fluctuations.

1.3.4 Sample Heterogeneity.

Due to the inclusion of both males and females in the study sample heterogeneity is possible due to known differences in $\dot{V}O_{2max}$ and vascular function(Harms & Rosenkranz, 2008; Smekal et al., 2007; Smith et al., 2015). To mitigate this limitation an equal number of males and females were recruited to participate in the study.

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CHAPTER II: The Effects of Inhaled Nitric Oxide on Maximal Oxygen Consumption During Exercise in Hypoxia

2.1 Introduction

2.1.1 Hypoxic Exposure and Cardiac Output

Hypoxic exposure, such as that experienced at high altitude can lead to symptoms such as headaches, dizziness, confusion, nausea, fatigue, shortness of breath and exercise intolerance (Hackett, 2019; Harvard, 2018). Aerobic exercise capacity, measured by maximal oxygen consumption ($\dot{V}O_{2max}$), decreases by approximately 2-4% with every 300-meter gain in altitude (Burtscher et al., 2018). $\dot{V}O_{2max}$ is the product of cardiac output ($\dot{Q}$) and the arterial venous oxygen difference (CaO_2-CvO_2). Interestingly, despite $\dot{V}O_{2max}$ being significantly decreased in healthy participants under mild-moderate hypoxic conditions (partial pressure of inspired oxygen [P_IO_2] ~97mmHg to 85mmHg), peak $\dot{Q}$ remains relatively unaffected (Reeves et al., 1987b; Stenberg et al., 1966; Wagner, 2017). Conversely, $\dot{Q}$ as well as $\dot{V}O_{2max}$ have been shown to be reduced in severe hypoxic (P_IO_2 <85 mmHg) conditions(Calbet et al., 2003). The discrepancies within current research regarding the $\dot{Q}$ response to hypoxic exercise may be due to an apparent 'hypoxic threshold' whereby once surpassed, $\dot{Q}$ may play a major role in limiting $\dot{V}O_{2max}$. In other words, in mild to moderate hypoxia ($P_IO_2 \sim 97$ mmHg to 85mmHg), the diffusive component (i.e., lung and muscle diffusing capacity) of the oxygen transport system may be the main limiting factor of $\dot{V}O_{2max}$. Conversely, in severe hypoxia ($P_IO_2 \leq 80$mmHg) there may be a shift where, in addition to the diffusive component, the convective component of the oxygen transport system (i.e., $\dot{Q}$) also contributes to impaired $\dot{V}O_{2max}$. However, the point where this impairment in $\dot{Q}$ occurs remains unclear. Additionally, the mechanism(s) behind this impairment in cardiac function is also unclear.

2.1.2 Hypoxic Pulmonary Vasoconstriction

Previous work suggests that the reduction in peak $\dot{Q}$ with hypoxia is due to heterogenous hypoxic pulmonary vasoconstriction (HPV)(Naeije, 2011; Stembridge et al., 2019; Swenson,

2013). In response to local alveolar hypoxia, HPV diverts blood flow from poorly ventilated lung units to sufficiently ventilated lung units, in order to maintain or improve ventilation/perfusion matching(Swenson, 2013). However, in response to global alveolar hypoxia (as may occur in severe hypoxia), HPV may be maladaptive(Naeije, 2011). HPV leads to increased pulmonary artery pressure (PAP) and right ventricular (RV) afterload, which may limit $\dot{Q}$ and $\dot{V}O_{2max}$(Naeije, 2011; Stembridge et al., 2019; Swenson, 2013). During maximal intensity exercise in normoxia, as $\dot{Q}$ increases, distention and recruitment of pulmonary capillaries occurs to decrease pulmonary vascular resistance (PVR) and allow for greater increases in $\dot{Q}$(West & Luks, 2016). However, this drop in PVR with increasing $\dot{Q}$ during exercise may not occur in hypoxia. In both moderate and severe hypoxic exercise, $\dot{V}O_{2max}$ is decreased, while PVR and PAP are greater as compared to normoxia in healthy volunteers (Naeije, 2011; Naeije et al., 2010; Wagner et al., 1986a). Together, these findings suggest that PVR and PAP are increased in moderate and severe hypoxia, resulting in reductions in $\dot{Q}$ and ultimately $\dot{V}O_{2max.}$

2.1.3 Inhaled Nitric Oxide

Sildenafil (phosphodiesterase-5 inhibitor), commonly known as Viagra, has been shown to decrease PVR and PAP, increase $\dot{Q}$ and therefore improve $\dot{V}O_{2max}$ in severe hypoxia(Fischler et al., 2009; Ghofrani et al., 2004). However, sildenafil is administered orally and therefore affects both the systemic and pulmonary vasculature. As such, it is unclear if the improvement in $\dot{V}O_{2max}$ is secondary to reduced HPV, increased oxygen delivery (DO_2) to skeletal muscles through vasodilation of the peripheral vasculature, or a combination of the two (Ghofrani et al., 2004). Studies using iNO, a selective pulmonary vasodilator, have demonstrated reductions in PVR and PAP in acute severe hypoxic conditions(Frostell et al., 1993; Pepke-Zaba et al., 1991). Frostell et al., studied the effects of 40ppm iNO on HPV in healthy volunteers at rest(Frostell et al., 1993)

and found that iNO returned PAP, PVR and right ventricular stroke work index (RVSWI; measured by right heart catheterization) to normoxic levels. This suggests that iNO is effective in reducing HPV, and consequently PVR and PAP. To date, no study has investigated the effects of iNO on $\dot{V}O_{2max}$ and cardiac mechanics during exercise in hypoxia.

2.1.4 Wagner Model

Traditionally, $\dot{Q}$ has been considered the key determinant of $\dot{V}O_{2max}$ in healthy individuals, however Dr. Peter Wagner developed a model which takes a multisystem approach to evaluating limitations to $\dot{V}O_{2max}$(Wagner, 2017). Specifically, Wagner's model suggests that all variables in the oxygen transport system may affect $\dot{V}O_{2max}$ at sea level and can be divided into two components; 1) the convective component (i.e. $\dot{Q}$ and arterial oxygen content [CaO_2]), and 2) the diffusive component (i.e. diffusive membrane in the muscle [D_M]])(Wagner, 2017). However, at altitude, since diffusion limitation can occur within the lungs as well as in the muscles, both the convective and diffusive components may be reduced, resulting in a lower $\dot{V}O_{2max}$. Wagner conducted a theoretical analysis and demonstrated that $\dot{V}O_{2peak}$ increases by 25% in severe hypoxia ($P_IO_2 \leq$ 80mmHg) if lung and muscle diffusing capacities are increased by 50% and 40% respectively. However, $\dot{V}O_{2peak}$ was only increased by a further 1.7% when $\dot{Q}$ was increased by 25%. Together, these findings suggest that the diffusive components of the oxygen transport system may play a larger role in determining $\dot{V}O_{2max}$ as compared to the convective components in severe hypoxia(Wagner, 2017). Evaluating $\dot{V}O_{2peak}$ with and without iNO using Wagner's model in both moderate and severe hypoxia may provide important information about determinants of $\dot{V}O_{2peak}$ in hypoxia.

2.1.5 Purpose and Hypothesis

The purpose of the current study was to evaluate the mechanism(s) of exercise intolerance and cardiovascular regulation in hypoxia. We sought to determine if the elevation in PAP secondary to HPV is a limiting factor of $\dot{V}O_{2max}$ in health during moderate and severe hypoxia.

As previously shown, we expect $\dot{V}O_{2max}$ to be reduced in both moderate and severe hypoxia, with larger reductions in severe hypoxia due to reductions in arterial saturation (S_PO_2), SV, and $\dot{Q}$. We hypothesized that iNO would improve $\dot{V}O_{2max}$ during exercise in both moderate and severe hypoxia secondary to reduced PAP, leading to increased SV and peak $\dot{Q}$. However, the magnitude of improvement in $\dot{V}O_{2max}$ with iNO would be smaller in severe hypoxia due to an underlying diffusion limitation in severe hypoxia which would limit the increase in $\dot{V}O_{2max}$ by augmenting $\dot{Q}$.

2.1.6 Significance

Body structure and function are a key component in understanding disease as outlined by the International Classification of Functioning, Disability and Health(Organization, 2002). The decreased $\dot{V}O_{2max}$ observed in healthy individuals breathing hypoxia (due to decreased P_IO_2 and the resulting arterial hypoxemia [decreased CaO_2]) may offer insight into the pathophysiological responses to exercise in patients with chronic lung disease whereby alveolar hypoxia may occur (e.g. chronic obstructive pulmonary disease [COPD] and interstitial lung disease [ILD])(Mannino, 2002; West, 2011). These patients often have a physiological impairment in which damage/destruction of the alveoli results in decreased surface area for gas exchange, causing impaired gas exchange, and significant exertional arterial hypoxemia (Mannino, 2002; West, 2011). Arterial hypoxemia is a known contributor to exertional dyspnea and exercise intolerance (activity limitation) in these patients, limiting their ability to participate in meaningful activities of daily living(Du Plessis et al., 2018; Hiroyuki et al., 2018; Jensen et al., 2018; Yannick et al., 2020).

Ultimately, the prolonged inability to participate in meaningful activities secondary to exertional dyspnea and exercise intolerance has been shown to contribute to reduced quality of life (Ley et al., 2016; Oga et al., 2003).

Therefore, understanding the body structure(s) and functions that limit hypoxic exercise (i.e., activity) in healthy individuals will allow us to better understand the exercise limitations observed in clinical populations that demonstrate significant hypoxemia on exertion. If impaired $\dot{Q}$ secondary to elevated PAP is found to play a role in limiting $\dot{V}O_{2max}$ in hypoxia, next steps would be to evaluate potential therapies (e.g., pharmacological interventions) that would improve exercise capacity in clinical conditions and therefore increase participation in meaningful activities of daily living, and subsequently quality of life.

2.2 Methods

2.2.1 Ethical Approval and Participant Description

The study was approved by the University of Alberta Health Research Ethics Board (Pro00092939) and Health Canada (HC6-24-c231426), and all participants provided written informed consent. A total of 12 young, healthy males and females (18-40 years old, 5 females) with no history of cardiovascular, metabolic, pulmonary, neurological, or musculoskeletal disease were included in this study. All participants had <5-year smoking history and a BMI between 18.5-30. Participants on phosphodiesterase type 5 (PDE-5) inhibitors were excluded. For safety reasons, pregnant females were also excluded from the study.

2.2.2 Study Design

The study used a randomized placebo-controlled double-blind cross-over design. Six sessions were completed over a 3-week period, in the following order (see Appendix B for schematic): **Day 1)** Participant enrollment, medical history, standard pulmonary function test

(PFT) and an incremental cardiopulmonary exercise test (CPET) breathing normoxia (21% inspired oxygen) to determine exercise capacity and peak work rate. **Days 2-5)** Four experimental CPETs on separate days while breathing either **A)** moderate hypoxia, **B)** severe hypoxia, **C)** moderate hypoxia with iNO, and **D)** severe hypoxia with iNO (randomized). **Day 6)** Resting and exercise cardiac ultrasound trials while breathing normoxia, moderate and severe hypoxia, and moderate and severe hypoxia with iNO (order randomized), to determine RVSP. All experimental trials were randomized using a random number generator in Excel. Participants breathed through an identical apparatus during all experimental CPETs and were asked to refrain from caffeine, alcohol, and vigorous exercise for a minimum of 12 hours before testing. Blinding was preserved by ensuring both participant and metabolic cart operator were unaware of the intervention being administered. In addition, white noise was played during all trials to avoid any auditory cues that may have indicated which intervention was being delivered. Female menstrual cycle phase was recorded for each trial, however menstrual cycle phase was not standardized, as literature suggests that $\dot{V}O_{2max}$ is not affected by menstrual cycle phase (Jurkowski et al., 1981; Smekal et al., 2007). Due to the current novel coronavirus (COVID-19) pandemic, participants were required to screen for symptoms using the Alberta Health Services COVID-19 Self-Assessment and to measure body temperature each day upon arrival to the laboratory. If participants presented with any COVID-19 related symptoms, their test was rescheduled following Alberta Health Services recommendations. Participants were excluded from the study if they tested positive for COVID-19 prior to, or while enrolled in the study.

2.2.3 Intervention

Hypoxia

Using a simple randomization technique participants completed the following 4 conditions during the experimental CPETs: A) moderate hypoxia (13.6% oxygen; P_IO_2 ~ 89 mmHg), B) severe hypoxia (12.1% oxygen; P_IO_2 ~ 79 mmHg), C) moderate hypoxia with 40ppm iNO, and D) severe hypoxia with 40ppm iNO. Hypoxia was delivered via an air-blender system using medical air (21% oxygen) and 100% nitrogen. During each trial participants' arterial oxygen saturation was monitored using forehead pulse oximetry and was continuously recorded within the LabChart software. Two trials were terminated as S_PO_2 surpassed the predefined safety cut-off ($S_PO_2 \leq 65\%$).

Inhaled Nitric Oxide (iNO)

A dose of 40ppm iNO was delivered using the SoKINOX^{TM} device (VitalAire^{TM}) which is Health Canada approved. iNO was delivered via a non-rebreathe circuit connected to a flow sensor, NO sample line, and inspired oxygen sample line. During the trials, iNO was delivered with moderate or severe hypoxia and additional oxygen was titrated to maintain the targeted P_IO_2 (moderate hypoxia: 89mmHg and severe hypoxia: 79mmHg) throughout the trials. During the placebo trials, the SoKINOX was set at 0ppm NO. Nitrogen dioxide and methemoglobin were monitored using the SoKINOX^{TM} device and co-oximetry. The trials were immediately terminated if nitrogen dioxide or methemoglobin levels reached ≥2ppm and ≥5%, respectively. One trial was terminated as methemoglobin surpassed the safety cut-off.

2.2.4 Pulmonary Function Test (PFT)

A full pulmonary test (PFT) was completed as per current guidelines, which included spirometry, single breath diffusing capacity for carbon dioxide (DLCO), and lung volume measures including plethysmography(Graham et al., 2019). All pulmonary function measures

were obtained using a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA).

2.2.5 Cardiopulmonary Exercise Tests (CPET)

All incremental CPETs (screening and incremental) were completed on an electronically braked cycle ergometer (Ergoselect II 1200; Ergoline, Blitz, Germany). Cardiorespiratory data, S_PO_2, heart rate (HR) and $\dot{Q}$ were collected continuously during all CPETs. Cardiorespiratory data ($\dot{V}O_2$, ventilation [$\dot{V}_E$], carbon dioxide production [$\dot{V}CO_2$], ventilatory equivalent to carbon dioxide production [$\dot{V}_E/\dot{V}CO_2$], partial pressure of end-tidal carbon dioxide [$P_{ET}CO_2$] etc.) were measured using a metabolic measurement system, S_PO_2 was recorded using forehead pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA), and HR was estimated via a 12-lead electrocardiogram (ECG) (CardioSoft, GG Medical Systems, Milwaukee, WI, USA). Impedance cardiography (Physioflow® device, Manatec® Biomedical) was used to estimate $\dot{Q}$ and has been strongly correlated with the direct Fick method during exercise ($r=0.94$, $p<0.01$) in healthy participants (Richard et al., 2001). Arterial blood pressure was determined using manual auscultation during the last 30 seconds of the first minute of each stage. All continuous ventilatory and cardiovascular data were reported during the first 30 seconds of the last minute of each stage. Inspiratory capacity (IC) maneuvers were used to calculate operating lung volumes and were collected during the last 30 seconds of each stage and at the end of exercise, as recommended (Guenette et al., 2013). Ratings of perceived exertion and dyspnea were collected using the modified Borg scale (0-10 points) prior to the IC maneuver (Guenette et al., 2013) and at end-exercise. The following 4 criteria were required to achieve $\dot{V}O_{2max}$: 1) volitional exhaustion, 2) a plateau in $\dot{V}O_2$, 3) respiratory exchange ratio >1.1, and 4) achievement of predicted maximum heart rate(Stickland et al., 2012).

Screening CPET: A screening CPET was completed to determine baseline $\dot{V}O_{2max.}$ The screening CPET began with a 2-minute baseline period with participants seated on the cycle ergometer, followed by a 2-minute warmup period at 0-watts. The workload was increased by 25 watts every 2 minutes until anaerobic threshold, and then every minute until volitional exhaustion.

Experimental CPETs: The experimental CPETs began at a workload of 0 watts, with workload being increased by 25 watts every 2 minutes until volitional exhaustion. The experimental CPETs were conducted in a random order while participants breathed 1 of the 4 hypoxic conditions stated above (*2.2.3 Intervention: Hypoxia*). Upon completion of the first 8 participants, the protocol was revised to start at 50 watts for 2-minutes and was increased by 25 watts every minute until volitional exhaustion. This was done to maintain the ideal 8-12-minute test time to obtain an accurate $\dot{V}O_{2max}$ without overburdening participants(Datta et al., 2015). All trials that were terminated due to surpassing S_PO_2 or methemoglobin cut-offs were repeated.

2.2.6 Resting and Exercise Cardiac Ultrasound

Resting and exercise cardiac ultrasounds were completed on participants to examine cardiac structure and function in hypoxia with and without iNO, using the Vivid Q ultrasound machine (GE Healthcare, Fairfield, CT, USA). Following a 10-minute baseline supine resting period, resting cardiac ultrasound was completed in the following conditions: A) normoxia; B) moderate hypoxia with and without iNO; and C) severe hypoxia with and without iNO (hypoxic level randomized). Participants then proceeded to perform exercise at 50W on a supine cycle ergometer while breathing the same conditions described above in a randomized order (see Appendix B for protocol schematic). A single-lead ECG was used to determine HR, $\dot{Q}$ was estimated using impedance cardiography, S_PO_2 and methemoglobin were monitored continuously as using forehead pulse-oximetry and finger co-oximetry. All echocardiograms were performed

by one experienced sonographer and a minimum of 3 clear cardiac cycles were recorded for each window.

Ultrasound analysis:

Ultrasound analysis was completed on the EchoPAC software (GE Healthcare, Fairfield, CT, USA) by an expert who was blinded to the order of experimental conditions.

RVSP was calculated using the following equation:

$$(1)\ RVSP = 4(V_{TR})^2 + RAP$$

where V_{TR} is the estimated maximum tricuspid regurgitant jet velocity and RAP is the estimated right atrial pressure(Rudski et al., 2010). RAP was estimated from the diameter and collapsibility of the inferior vena cava (IVC)(Lang et al., 2015; Rudski et al., 2010). If the diameter and collapsibility of the IVC were: 1) <2.1cm and >50% then RAP was estimated to be 3 mmHg, 2) >2.1cm and <50% then RAP was estimated to be 15 mmHg, and 3)>2.1cm or <50% then RAP was estimated to be 8 mmHg(Lang et al., 2015). Mean pulmonary arterial pressure (mPAP) was estimated using the following equation(Chemla et al., 2004):

$$(2)\ mPAP = (0.61 \times RVSP) + 2.$$

Right ventricular stroke work (RVSW) was calculated using the following equation(Chemla et al., 2013):

$$(3)\ RVSW = (1.25 \times mPAP - RAP) \times SV$$

where SV was estimated using impedance cardiography due to difficulty of estimating right ventricular SV through echocardiography during exercise. Left and right ventricular volumes were estimated by calculating end-diastolic volume (EDV) and end-systolic volume (ESV) from apical 4-chamber images(Lang et al., 2015). Left and right ventricular ejection fraction were calculated using the following equation:

(4) EF = (EDV-ESV)/EDV

2.2.7 Statistical Analyses

Our laboratory previously examined the effects of iNO (40 ppm) on exercise capacity ($\dot{V}O_{2max}$) in patients with mild COPD and healthy controls(Phillips et al., 2021). In COPD, $\dot{V}O_{2max}$ was increased by 0.23±0.04 L/min with iNO ($\dot{V}O_{2max}$ =1.8±0.1 L·min^{-1}) versus placebo ($\dot{V}O_{2max}$ =1.53±0.10 L·min^{-1}) in patients with mild COPD (calculated effect size: 0.997)(Phillips et al., 2021). We assumed a similar effect size in healthy participants during hypoxic maximal exercise, and an *a priori* sample size calculation determined that 10 participants should be sufficient to detect a significant effect of iNO on exercise capacity in hypoxia (effect size= 0.997, α=0.05, power=0.8) using the G-Power 3.1 statistical software. Therefore, we recruited 12 participants (5 females) to account for a 20% drop-out rate.

All statistical analyses were completed using IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY). A factorial repeated measures analysis of variance (ANOVA) was used to evaluate the effects of iNO versus placebo on $\dot{V}O_{2max}$ (primary outcome) in moderate and severe hypoxia Furthermore, a factorial repeated measures ANOVA was used to evaluate the effects of iNO versus placebo on secondary outcome measures (ex. $\dot{Q}$) at baseline, 50 watts, 75 watts, 100 watts, and $\dot{V}O_{2max}$ in moderate and severe hypoxia and when compared to normoxia. Lastly, a factorial repeated measures ANOVA was used to evaluate the effects of iNO versus placebo on RVSP at rest and 50 watts in moderate and severe hypoxia and when compared to normoxia. Statistical significance was set *a priori* to $p<0.05$. When main effects or interactions were found, a Bonferroni post-hoc test was completed to locate differences. Shapiro Wilk normality test was used to determine normal distribution and Brown-Forsythe was used to test for equal variance. When statistical test assumptions were violated a Friedman non-parametric test was used.

2.3 Results

2.3.1 Participants

Descriptive statistics for all participants are displayed in Table 1. A total of 12 participants (5 females) aged 22±1 years with a normal BMI of 22.3±0.4 kg/m^2 were included in the study. Participants had normal lung function and DLCO values. Mean absolute $\dot{V}O_{2max}$ was 45.3±3.5 mL/kg/min.

2.3.2 Primary Outcome: $\dot{V}O_2$

Individual and mean $\dot{V}O_{2max}$ responses to iNO in moderate and severe hypoxia are depicted in Figure 1. The main effect of P_IO_2 on $\dot{V}O_{2max}$ ($p<0.001$). $\dot{V}O_{2max}$ was significantly lower in moderate ($p=0.005$) and severe hypoxia ($p<0.001$) when compared to normoxia. $\dot{V}O_{2max}$ was decreased in severe hypoxia relative to moderate hypoxia ($p=0.02$). There was no difference in $\dot{V}O_{2max}$ with iNO (main effect $p=0.35$), and no P_IO_2 x intervention interaction ($p=0.42$). Mean submaximal (baseline, 50-watts, 75-watts, and 100-watts) and peak $\dot{V}O_2$ data are reported in Figure 1. $\dot{V}O_2$ during submaximal exercise was not different in either moderate or severe hypoxic conditions (placebo and iNO) when compared to normoxia.

2.3.3 Hemodynamic Response

All peak hemodynamic data are displayed in Table 2. Submaximal and peak $\dot{Q}$, HR, and SV are reported in Figure 2. There were no significant differences in submaximal or peak $\dot{Q}$ and SV in either moderate or severe hypoxia when compared to normoxia. There were no significant differences in peak or submaximal HR in moderate hypoxia when compared to normoxia. In severe hypoxia, peak HR was significantly increased from normoxia ($p=0.002$), however no differences were observed during submaximal exercise. iNO did not affect submaximal or peak $\dot{Q}$, HR, and SV when compared to placebo. Importantly, mean arterial pressure (MAP) was not significantly

different between placebo and iNO conditions at submaximal or peak exercise, demonstrating that there were no systemic vascular effects of iNO during hypoxic exercise.

2.3.4 Oxygen Delivery Response

Submaximal and peak DO_2, S_PO_2, and venous partial pressure of oxygen (P_VO_2) data are reported in Figure 3. As expected, submaximal and peak S_PO_2 were gradually decreased with increasing hypoxic severity. Furthermore, peak DO_2 was significantly decreased in all hypoxic conditions when compared to normoxia ($p<0.001$) with no differences at submaximal workloads. Additionally, peak and submaximal P_VO_2 were not significantly different in either hypoxic level when compared to normoxia. iNO did not affect submaximal or peak S_PO_2, DO_2, and P_VO_2 when compared to placebo.

2.3.5 Ventilatory Responses

All gas-exchange data are displayed in Table 2 and submaximal and peak $\dot{V}_E$, $\dot{V}_E/\dot{V}CO_2$, and $P_{ET}CO_2$ data are reported in Figure 4. $\dot{V}_E$ was significantly increased at peak in severe hypoxia when compared to normoxia ($p=0.02$), but there were no differences between moderate hypoxia and normoxia at peak. There were no differences in submaximal $\dot{V}_E$ between either moderate or severe hypoxia and normoxia. $\dot{V}_E/\dot{V}CO_2$ ratio at each submaximal workload and peak was not significantly different in all hypoxic conditions when compared to normoxia. $\dot{V}_E/\dot{V}CO_2$ nadir was significantly elevated in all hypoxic conditions when compared to normoxia ($p\leq0.001$). $\dot{V}_E/\dot{V}CO_2$ slope was not different in either hypoxic level when compared to normoxia. $P_{ET}CO_2$ was significantly decreased at peak exercise ($p<0.001$) in all hypoxic conditions when compared to normoxia. $P_{ET}CO_2$ was significantly decreased at all submaximal workloads in severe hypoxia ($p\leq0.001$) when compared to normoxia. There were no differences in dyspnea or leg discomfort between hypoxia and normoxia. Lastly, iNO did not have a significant effect on submaximal or

peak $\dot{V}_E$, $\dot{V}CO_2$, $\dot{V}_E/\dot{V}CO_2$ (ratio, nadir, or slope), $P_{ET}CO_2$, tidal volume (V_T), breathing frequency (f_b), dyspnea, or leg discomfort.

2.3.6 Echocardiography

Echocardiography data were obtained on a subset of participants at rest (n=10) and during exercise (n=4) where a clear and analyzable V_{TR} was captured. While RVSP was attempted in 10 participants, only 6 participants had clear and analyzable V_{TR} in all conditions at rest (Figure 5). Resting P_IO_2, HR, $\dot{Q}$, S_PO_2 and RVSP data (n=6) for normoxia, moderate hypoxia, and severe hypoxia are displayed in Table 3. At rest, in a sample of 6, there was a main effect of P_IO_2 (p=0.03) and intervention (p=0.007) on RVSP. There was no interaction between P_IO_2 and intervention (p=0.57). At rest, iNO significantly decreased RVSP in moderate hypoxia by 2.53±0.8 mmHg (p=0.01) and severe hypoxia by 1.78±0.2 mmHg (p=0.05). Although only 6 participants had clear V_{TR} in *all conditions*, within moderate and severe hypoxia 7 and 8 participants had a clear V_{TR}, respectively. As a result, a second analysis was conducted using paired t-tests to examine the effects of iNO versus placebo *within* each hypoxia level (Figure 6). iNO significantly decreased RVSP in moderate hypoxia by 2.41±0.4 mmHg (n=8, p=0.008) and in severe hypoxia by 1.76±0.2 mmHg (n=7, p=0.01). Additionally, S_PO_2 was gradually decreased with increasing hypoxic severity when compared to normoxia ($p<0.05$), while HR and $\dot{Q}$ were not affected by hypoxia or iNO. During exercise, in a sample of 4, there was no main effect of P_IO_2 (p=0.176) or intervention (p=0.474) and no P_IO_2 x intervention interaction (p=0.172). Moderate hypoxia appeared to increase exercising RVSP by 2.5±0.9 mmHg when compared to normoxia with no effect of iNO. In severe hypoxia, exercise RVSP appeared to increase by 6.3±0.9 mmHg when compared to normoxia and decrease by 3.2±0.8 mmHg following iNO inhalation.

2.4 Discussion

To date, this is the first study to evaluate the effects of iNO on $\dot{V}O_{2max}$ in healthy individuals during exercise in moderate and severe hypoxia. We found that 1) as anticipated, $\dot{V}O_{2max}$ was decreased in moderate hypoxia with further reductions in severe hypoxia; and 2) iNO reduced resting RVSP in moderate and severe hypoxia, however, $\dot{V}O_{2max}$ and peak $\dot{Q}$ were unaffected by iNO. Together, our findings suggest that the hypoxia-induced increase in pulmonary artery pressure does not impair peak $\dot{Q}$ and therefore $\dot{V}O_{2max}$ in healthy participants during acute hypoxic exercise.

2.4.1 Effect of iNO on Cardiovascular Function in Hypoxia

Studies in moderate hypoxia have suggested peak $\dot{Q}$ is unaffected when compared to normoxia, while other studies have suggested peak $\dot{Q}$ may be decreased in severe hypoxia(Calbet et al., 2003; Mollard et al., 2007; Naeije, 2010; Naeije, 2011; Peltonen et al., 2001; J. P. Richalet et al., 1988; Savard et al., 1995; Stenberg et al., 1966). We hypothesized that there may be a hypoxic threshold where there is a larger increase in PAP in severe hypoxic conditions. This elevated PAP would lead to increased RV afterload, therefore decreasing SV, peak $\dot{Q}$ and ultimately $\dot{V}O_{2max}$. In the current study, RVSP was decreased with the addition of iNO in both levels of hypoxia. This decrease in RVSP with iNO suggests a reduction in RV afterload which would be expected to increase SV, $\dot{Q}$, and ultimately $\dot{V}O_{2max}$. However, peak $\dot{Q}$ remained unchanged with iNO in either level of hypoxia, suggesting that HPV may not limit peak $\dot{Q}$ during exercise in health.

At peak exercise S_PO_2 and DO_2 were significantly decreased in moderate hypoxia with further decreases in severe hypoxia. It is plausible that the observed decrease in $\dot{V}O_{2max}$ in the current study was secondary to a decrease in DO_2 to working skeletal muscles, resulting in earlier

transition to anaerobic metabolism, quicker muscular fatigue, and earlier cessation of exercise. Taken together, our study findings demonstrate that in moderate and severe hypoxia, $\dot{V}O_{2max}$ is likely decreased due to the direct impact of hypoxia on CaO_2 and DO_2, and that elevations in RVSP (i.e., RV afterload) with hypoxia are unlikely to impact peak $\dot{Q}$ and $\dot{V}O_{2max}$ in either moderate or severe hypoxia.

Studies have shown that in moderate (P_IO_2~92mmHg) and severe hypoxia (P_IO_2=70mmHg), 50 mg sildenafil improves peak workload and $\dot{V}O_{2max}$, secondary to increased S_PO_2 and peak $\dot{Q}$(Ghofrani et al., 2004; Hsu et al., 2006). Furthermore, in severe hypoxia (P_IO_2=70mmHg), 50 mg sildenafil was effective in reducing RVSP both at rest and during exercise, suggesting a reduction in RV afterload(Ghofrani et al., 2004). Conversely, Kjaergaard et al., demonstrated that 100mg of sildenafil did not improve $\dot{V}O_{2max}$ and had no effect on S_PO_2 or peak $\dot{Q}$ at maximal exercise in moderate hypoxia (P_IO_2~89mmHg)(Kjaergaard et al., 2007). However, Kjaergaard et al, found that 100mg sildenafil was only effective in reducing RVSP at rest and not during exercise(Kjaergaard et al., 2007). Taken together, it remains unclear if sildenafil is effective in improving peak $\dot{Q}$ and $\dot{V}O_{2max}$ secondary to reducing RVSP and RV afterload in healthy participants during hypoxic exercise. The advantage of iNO is that it only targets the pulmonary vasculature and does not cause systemic vasodilation. Our study demonstrated that while 40ppm iNO is effective in reducing RVSP at rest in moderate and severe hypoxia, peak $\dot{Q}$ and $\dot{V}O_{2max}$ are unaffected in both hypoxic conditions. Due to our limited RVSP data during exercise, it is difficult to determine if iNO was effective in reducing RVSP during hypoxic exercise. Interestingly, when sildenafil improved peak $\dot{Q}$ and peak workload or $\dot{V}O_{2max}$, the sample population studied were trained cyclists/triathletes ($\dot{V}O_{2max}$=59.7±9.5 mL/min/kg)(Hsu et al., 2006) and healthy mountaineers(Ghofrani et al., 2004). Conversely, when sildenafil or iNO had

no effect on peak $\dot{Q}$ or $\dot{V}O_{2max}$, the sample populations were young healthy individuals with average fitness [$\dot{V}O_{2max}$=36.7±6.0 mL/min/kg(Kjaergaard et al., 2007) and 39.8±3.7 mL/min/kg(current study)]. Based on these findings, the varying effect of sildenafil or iNO on peak $\dot{Q}$ and $\dot{V}O_{2max}$ in moderate and severe hypoxia may be due to fitness level, however further research is needed to examine this.

2.4.2 Effect of iNO and Hypoxia on Respiratory Function

$\dot{V}_E/\dot{V}CO_2$ nadir was increased in moderate and severe hypoxia when compared to normoxia, suggesting decreased ventilatory efficiency. This elevated $\dot{V}_E/\dot{V}CO_2$ nadir in both hypoxic conditions was driven by elevated $\dot{V}_E$, as $\dot{V}CO_2$ remained unchanged. The increased $\dot{V}_E$ was secondary to V_T expansion, since f_b remained unaffected. The increased ventilatory response to hypoxic exercise is likely due peripheral chemoreceptor stimulation(Prabhakar & Peng, 2004). Peripheral chemoreceptors respond to decreases in arterial PO_2 and stimulate medullary respiratory centers to increase $\dot{V}_E$(Prabhakar & Peng, 2004), and therefore decreased $P_{ET}CO_2$, as observed in the current study. Earlier work suggests that increased mean PAP may increase $\dot{V}_E$ and result in reduced $P_{ET}CO_2$(Yasunobu et al., 2005). If $\dot{V}_E$ was driven by elevated PAP, then decreasing PAP with iNO would result in reduced $\dot{V}_E$ and subsequently $\dot{V}_E/\dot{V}CO_2$. However, despite reducing RVSP during both moderate and severe hypoxia, iNO had no effect on $\dot{V}_E$, $\dot{V}_E/\dot{V}CO_2$, or $P_{ET}CO_2$. Our findings suggest that the increased ventilatory response to hypoxic exercise is not the result of increased PAP, but rather secondary to peripheral chemoreflex stimulation.

2.4.3 Wagner Model

Wagner's model suggests that both the convection (i.e., $\dot{Q}$ and CaO_2) and diffusion (i.e., D_M) components of oxygen transport are impaired in hypoxia; thus decreasing $\dot{V}O_{2max}$(Wagner, 2017). However, Wagner suggests that in severe hypoxia, improving $\dot{Q}$ would cause minimal

increases in $\dot{V}O_{2max}$ secondary to a pulmonary and/or peripheral diffusion limitation(Wagner, 2017). As such, we hypothesized that if iNO decreased RV afterload secondary to RVSP, the increase in $\dot{Q}$ and therefore $\dot{V}O_{2max}$, would be greater in moderate hypoxia when compared to severe hypoxia. In the current study, the convective component of oxygen transport was gradually decreased as hypoxic severity increased (Figure 7). As iNO had no effect on $\dot{Q}$ in either moderate or severe hypoxia (Figure 7), it was not possible to experimentally evaluate the contribution of altered convective oxygen delivery on $\dot{V}O_{2max}$ in hypoxia. The decrease in the convective component with hypoxia was likely due to a pulmonary diffusion limitation (i.e., reduced CaO_2), since submaximal and peak $\dot{Q}$ were unaffected by hypoxia or iNO. Furthermore, if a peripheral diffusion limitation had been present, we would have expected to observe an increase in P_VO_2 secondary to reduced oxygen extraction. Although not statistically significant, submaximal and peak P_VO_2 appeared to be decreased in both moderate (Figure 3, graph E) and severe hypoxia (Figure 3, graph F) when compared to normoxia, suggesting greater oxygen extraction and therefore no peripheral diffusion limitation. Taken together, our findings suggest that in moderate and severe hypoxia, the decrease in $\dot{V}O_{2max}$ is likely due to an impairment in the convection component of oxygen transport secondary to a pulmonary diffusion limitation (i.e., decreased CaO_2).

2.4.4 Limitations and Considerations

2.4.4.1 Power

Sample size was calculated *a priori* assuming iNO would have a similar effect on $\dot{V}O_{2max}$ in healthy participants during maximal hypoxic exercise as observed previously in our laboratory in patients with mild chronic obstructive pulmonary disease(Phillips et al., 2021). Following data collection, a *post-hoc* power calculation was completed based on our observed a mean difference

of 0.00±0.28 L/min and 0.090±0.24 L/min in absolute $\dot{V}O_{2max}$ in moderate and severe hypoxia, respectively with iNO. The power calculation revealed that a sample size of 21,464 would be needed to detect a significant difference between iNO and placebo in moderate hypoxia, while 186 participants would be needed to detect a significant difference in severe hypoxia. Importantly, the potential absolute change in $\dot{V}O_{2max}$ with iNO in either hypoxic condition was well below physiological significance. From this analysis, it can be concluded that the unchanged $\dot{V}O_{2max}$ with iNO in both hypoxic conditions is not likely a result of being statistically underpowered.

2.4.4.2 Echocardiography

The echocardiography data in this study were collected during one session which could have allowed for iNO spillover during placebo testing of the subsequent hypoxic level. However, this is unlikely due to the 2-6 second half-life of iNO(Bernasconi & Beghetti, 2002) and its rapid inactivation by hemoglobin. In addition, to mitigate any potential confounding effect of iNO spillover, the level of hypoxia was randomized for each participant. RVSP data between hypoxic levels were not collected, resulting in the inability to verify that RVSP had returned to baseline; however, researchers ensured that participants' vitals (i.e., HR and S_PO_2) returned to baseline prior to starting the next hypoxic level.

The RVSP response at $\dot{V}O_{2max}$ in normoxia and hypoxia was not evaluated in this study. Due to the difficulty of obtaining images at maximal exercise, RVSP data were collected at a rest and a submaximal workload of 50 watts. This allowed for greater image quality; however, this assumes that that the effects of iNO on RVSP in hypoxia would be similar at rest, submaximal, and maximal exercise. Moderate and severe hypoxia increased RVSP during submaximal exercise (50 watts). However, in comparison to moderate hypoxia, there was a trend towards less of a reduction in RVSP with iNO in severe hypoxia. Of note, the submaximal exercise RVSP sample

size was very small (n=4) due to difficulty obtaining analyzable tricuspid regurgitation images during exercise with hypoxia. As a result, inferences were made using resting RVSP data. Previous work from our lab has shown that iNO reduces RVSP at rest and during exercise in normoxia(Brotto, In Review), and it is likely that the iNO response that was observed at rest would be present during submaximal and maximal exercise. In hypoxia, mean PAP measured via right heart catherization is gradually increased from rest to maximal exercise (Reeves et al., 1987a). Furthermore, the magnitude of PAP increase in hypoxia is greater as exercise intensity increases(Reeves et al., 1987a). Additionally, sildenafil has been shown to gradually decrease RVSP at rest and maximal exercise in hypoxia(Ghofrani et al., 2004). Together, this may suggest that the magnitude of RVSP decline that occurs with iNO during maximal exercise may be larger than that demonstrated at rest.

2.4.4.3 Impedance Cardiography

The direct Fick method is considered to be the gold standard for measuring cardiac output; however, this was impractical at this stage due to the invasiveness of the technique. Impedance cardiography, although not as precise as the Fick method, is a non-invasive technique used to estimate $\dot{Q}$ by measuring the electrical changes within the thoracic cavity to estimate the flow of blood through the heart(Charloux et al., 2000). Impedance cardiography has been shown to slightly overestimate $\dot{Q}$ during exercise when compared to the Fick method in patients with cardiovascular disease such as hypertension and congestive heart failure (Kemps et al., 2008; Scherhag et al., 2005). However, impedance cardiography has been strongly correlated with the direct Fick method during exercise ($r=0.94$, $p<0.01$) in healthy participants and has good between test variability within participants (Richard et al., 2001). Therefore, we are confident that we were able to reliably estimate $\dot{Q}$ via impedance cardiography, while avoiding unnecessary participant discomfort.

2.4.4.4 Additional Limitations

Diet was not standardized and circulating nitrates and nitrites were not measured, and therefore it is possible that some participants had elevated endogenous NO resulting in a greater vasodilatory response during iNO administration. However, due to a within-subject design each participant served as their own control and therefore the level of endogenous NO would likely have been similar within participants.

It is possible that alleviating HPV with iNO could have negatively impacted ventilation-perfusion matching and therefore impaired pulmonary gas exchange. However, arterial saturation as estimated by forehead pulse oximetry was not different between placebo and iNO trials, suggesting that iNO did not significantly impair ventilation-perfusion matching.

2.5 Conclusion

This study examined the effects of iNO on $\dot{V}O_{2max}$ in healthy participants during hypoxic exercise. iNO did not significantly affect $\dot{V}O_{2max}$ in either moderate or severe hypoxia. Furthermore, peak $\dot{Q}$ remained unchanged despite decreased resting RVSP with iNO in both moderate and severe hypoxia. Together, the study findings suggest that the elevation in RVSP with hypoxia is unlikely to play a role in limiting peak $\dot{Q}$ and therefore $\dot{V}O_{2max}$ during hypoxic exercise in healthy participants. Future studies could evaluate the effects of longer hypoxic exposure (≥1 hour) on exercise capacity in young healthy participants as longer exposure to hypoxia may elicit a greater HPV response, thereby leading to impaired $\dot{Q}$ and further $\dot{V}O_{2max}$ decline.

Table 1. Participant Characteristics

	Mean	%Predicted
Demographics		
N (female, male)	12 (5,7)	
Age (years)	22.0± 1.0	
Height (m)	1.76 ± 0.04	
BMI (kg/m^2)	22.3 ± 0.4	
Pulmonary Function		
FVC (L)	5.19 ± 0.5	110.0 ± 6.9
FEV_1 (L)	4.27 ± 0.4	105.0 ± 5.9
FEV_1/FVC (%)	83.0 ± 2.0	95.3 ± 2.3
DLCO (ml/min/mmHg)	32.6 ± 2.9	118.6 ± 10.0
Cardiopulmonary Exercise Test		
$\dot{V}O_{2max}$ (L/min)	3.32 ± 0.4	139.1 ± 9.3
$\dot{V}O_{2max}$ (ml/kg/min)	39.8 ± 3.7	
Peak Power Output (Watts)	283 ± 29	145.7 ± 10.3
RER	1.16 ± 0.02	

Values are expressed as the mean ± SEM. BMI: Body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; DLCO: diffusing capacity of the lung for carbon monoxide; $\dot{V}O_2$: oxygen uptake; RER: respiratory exchange ratio.

Table 2. Peak Physiological Responses with Placebo and iNO in Moderate and Severe Hypoxia

	Moderate Hypoxia			Severe Hypoxia			P_IO_2 effect	Interaction effect
Variable	**Placebo**	**iNO**	**p-value**	**Placebo**	**iNO**	**p-value**	**p-value**	**p-value**
P_IO_2 (mmHg)	90.3 ± 0.5	88.6 ± 0.2	0.389	81.0 ± 0.4	79.1 ± 0.1	0.922	<0.001	0.343
Power Output (W)	225 ± 24	220 ± 24	0.922	196 ± 19	204 ± 22	0.989	0.042	-
$\dot{V}O_{2max}$ (L/min)	2.89± 0.3	2.90 ± 0.3	0.922	2.54 ± 0.2	2.63 ± 0.2	0.224	0.021	0.499
$\dot{V}CO_2$ (L/min)	3.48 ± 0.3	3.56 ± 0.4	0.999	3.08 ± 0.3	3.2 ± 0.3	0.999	0.065	-
RER	1.30 ± 0.02	1.35 ± 0.04	0.141	1.30 ± 0.03	1.38 ± 0.05	0.141	0.819	0.693
$\dot{V}_E$ (L/min)	103.6 ± 8.8	106.2 ± 5.8	0.061	96.9 ± 9.3	102.1 ± 9.1	0.123	0.068	0.801
V_t (L)	2.77 ± 0.3	2.92 ± 0.3	0.413	2.81 ± 0.3	2.79 ± 0.3	0.413	0.432	0.236
f_b (breaths/min)	39.9 ± 2.1	41.7 ± 2.2	0.225	37.4 ± 2.4	39.8 ± 1.6	0.111	0.135	0.792
$\dot{V}_E/\dot{V}CO_2$	30.3 ± 0.8	31.2 ± 0.9	0.778	31.8 ± 0.9	32.7 ± 0.8	0.229	0.485	-
$\dot{V}_E/\dot{V}CO_{2\ Nadir}$	28.2 ± 0.8	28.7 ± 0.8	0.306	29.6 ± 0.6	30.0 ± 0.7	0.392	0.004	0.894
$P_{ET}CO_2$ (mmHg)	36.6 ± 0.9	36.2 ± 0.8	0.545	34.6 ± 0.6	34.8 ± 0.9	0.761	0.003	0.595
SpO_2 (%)	77.6 ± 1.4	76.2 ± 1.3	0.821	69.9 ± 0.8	69.4 ± 1.0	0.946	<0.001	-
Q (L/min)	17.8 ± 1.2	17.4 ± 1.5	0.496	18.8 ± 1.4	17.9 ± 1.3	0.496	0.469	-
HR (beats/min)	178 ± 2	177 ± 2	0.316	173 ± 3	176 ± 3	0.316	0.062	0.053
MAP (mmHg)	99 ± 3.2	102 ± 3	0.564	103 ± 2	94 ± 2	0.564	0.886	-
Dyspnea	9.3 ± 0.3	8.9 ± 0.3	0.235	9.3 ± 0.3	9.8 ± 0.1	0.081	0.044	0.064
Leg Discomfort	9.8 ± 0.1	9.4 ± 0.3	0.288	9.3 ± 0.3	9.5 ± 0.2	0.288	0.288	-

Values are expressed as mean ± SE. $\dot{V}O_{2max}$: Maximal oxygen consumption; $\dot{V}_E$: Ventilation; RER: Respiratory exchange ratio; $\dot{V}CO_2$: Carbon dioxide production; $P_{ET}CO_2$: Pressure of end-tidal carbon dioxide; f_b: Breathing frequency; V_t: Tidal volume; HR: Heart rate; Q: Cardiac output; SpO_2: Percent arterial oxygen saturation; MAP: Mean arterial pressure. P-value represents main effect of intervention (iNO), P_IO_2 effect p-value denotes main effect of hypoxia, and interaction p-value denotes interaction between hypoxia and iNO. Note, when non-parametric statistics were used no p-value is available.

Table 3. Echocardiography-derived Measurements at Rest in Normoxia, Moderate and Severe Hypoxia

Variable	Normoxia	Moderate Hypoxia	Moderate Hypoxia + iNO	Severe Hypoxia	Severe Hypoxia + iNO
P_IO_2 (mmHg)	137.7 ± 1.0	89.5 ± 0.4*	89.3 ± 0.2*	79.7± 0.3*$^{\#}$	79.2± 0.2*$^{\#}$
HR (beats/min)	68.0 ± 7.0	67.0 ± 6.0	72.0 ± 8.0	71.0 ± 7.0	71.0 ± 7.0
Q (L/min)	6.0 ± 0.6	6.0 ± 0.7	6.5 ± 0.8	5.9 ± 0.7	6.5 ± 1.0
SpO_2 (%)	98.0 ± 0.3	88.0 ± 1.1*	85.0 ± 1.8*	82.0 ± 1.0*$^{\#}$	79.5 ± 1.7*$^{\#}$
RVSP (mmHg)	21.8 ± 2.1	23.0 ± 1.7	20.5 ± 0.9$^{\$}$	24.1 ± 1.0	22.3 ± 1.3$^{\$}$

Values are expressed as mean ± SE (n=6). P_IO_2: Partial pressure of inspired oxygen; HR: Heart rate; Q: Cardiac output; SpO_2: Percent arterial oxygen saturation; RVSP: Right ventricular systolic pressure. *statistically different from normoxia ($p<0.05$), $^{\#}$statistically different from moderate hypoxia ($p<0.05$), $^{\$}$statistically different from placebo.

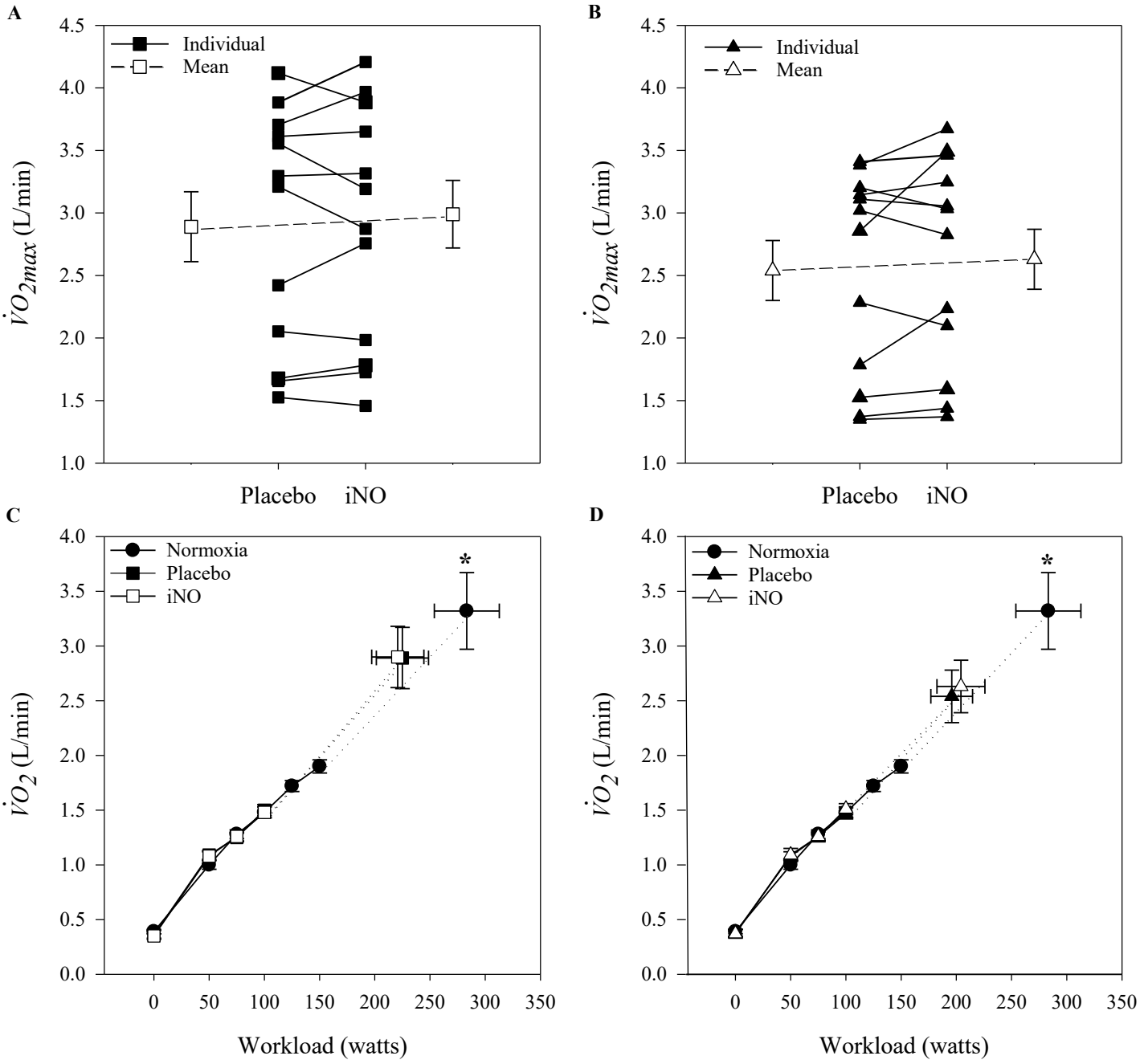

Figure 1. Individual (closed symbols) and mean ± SEM (open symbols) $\dot{V}O_{2max}$ responses to iNO in moderate hypoxia (A) and severe hypoxia (B). $\dot{V}O_2$ response to placebo (closed symbols) and iNO (open symbols) at baseline and during exercise at 50 watts, 75 watts, 100 watts and $\dot{V}O_{2max}$ in moderate hypoxia (C) and severe hypoxia (D). *statistically different from all hypoxic conditions ($p \leq 0.005$).

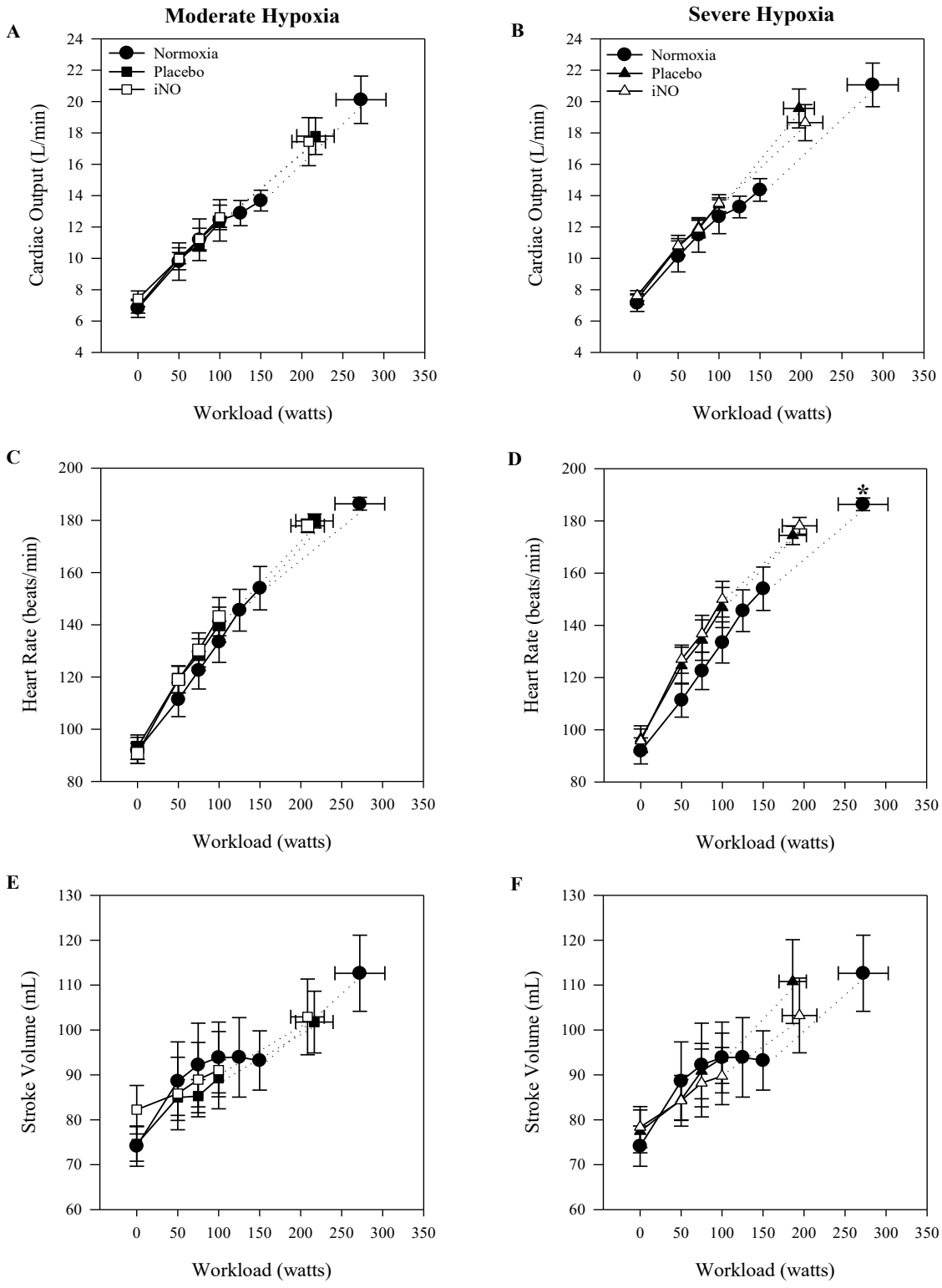

Figure 2. Cardiac output (A and B), heart rate (C and D) and stroke volume (E and F) responses to placebo (closed symbols) and iNO (open symbols) at baseline and during exercise at 50 watts, 75 watts, 100 watts and $\dot{V}O_{2max}$ in moderate hypoxia (left column) and severe hypoxia (right column). Values are expressed as mean ± SEM. *statistically different from hypoxia ($p<0.005$).

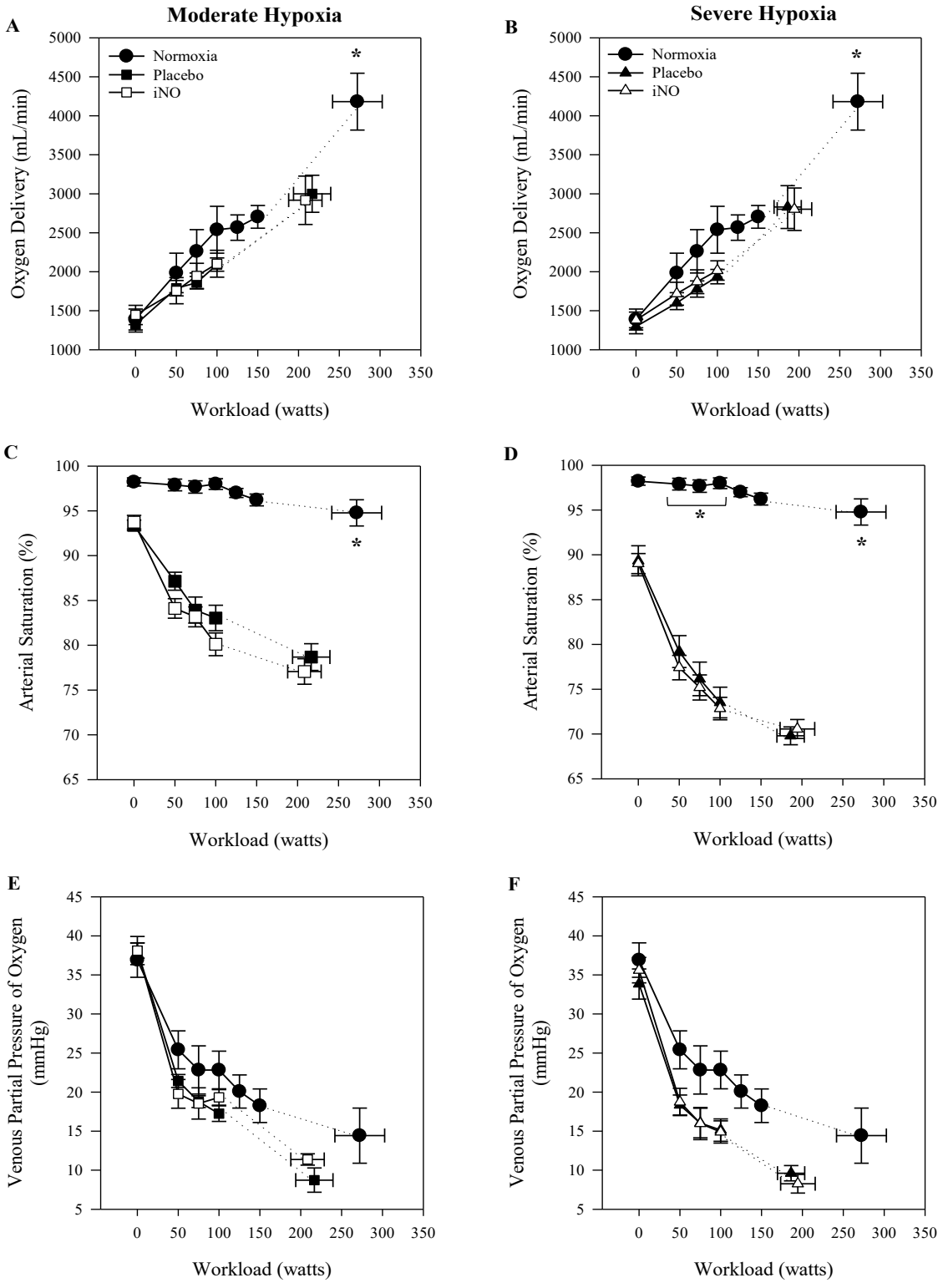

Figure 3. Oxygen delivery (A and B), arterial saturation (C and D) and venous partial pressure of oxygen (E and F) responses to placebo (closed symbols) and iNO (open symbols) at baseline and during exercise at 50 watts, 75 watts, 100 watts and $\dot{V}O_{2max}$ in moderate hypoxia (left column) and severe hypoxia (right column). Values are expressed as mean ± SEM.* statistically different from hypoxia ($p<0.001$).

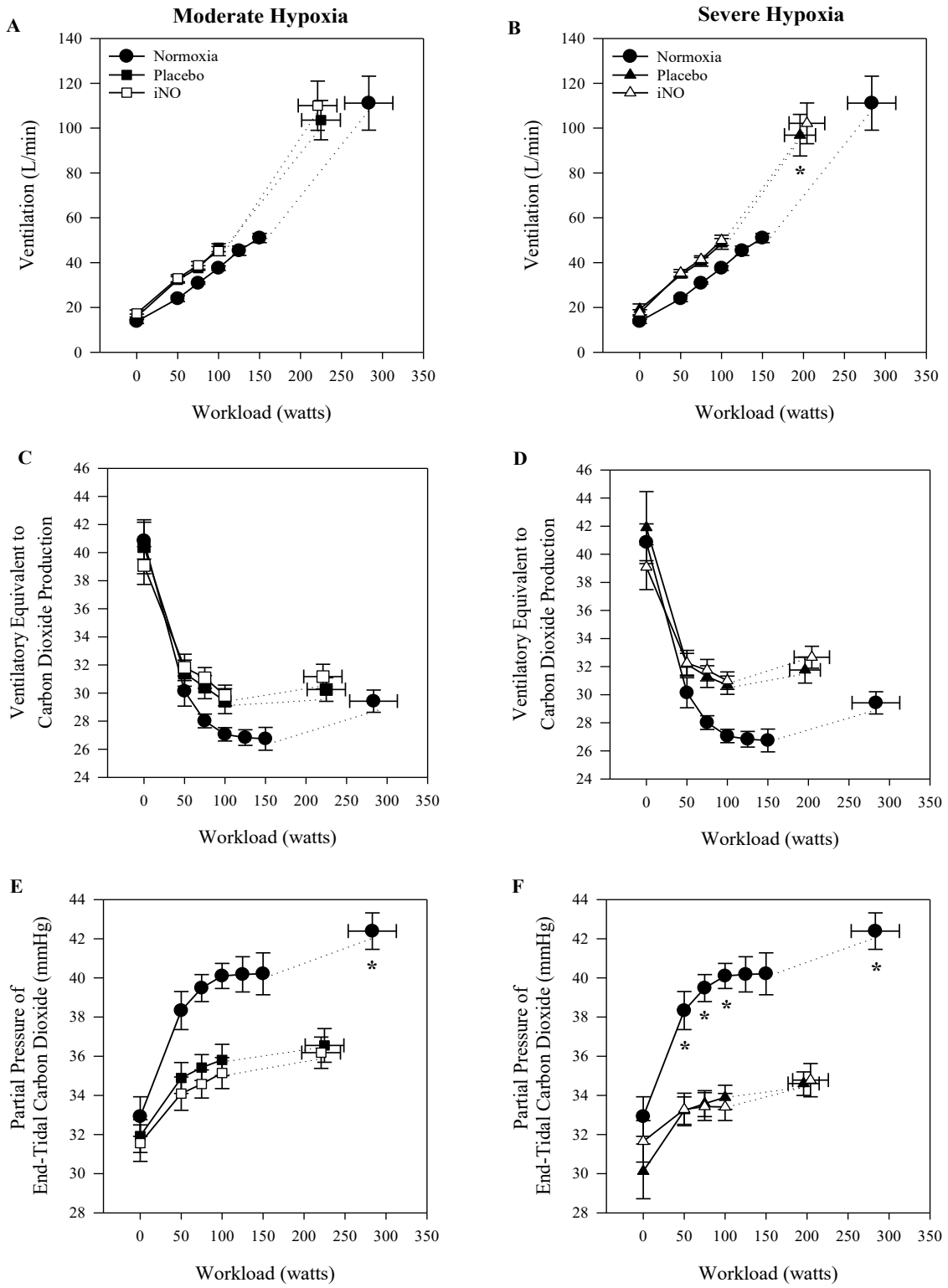

Figure 4. Ventilation (A and B), ventilatory equivalent to carbon dioxide production (C and D), and partial pressure of end-tidal carbon dioxide (E and F) responses to placebo (closed symbols) and iNO (open symbols) at baseline and during exercise at 50 watts, 75 watts, 100 watts and $\dot{V}O_{2max}$ in moderate hypoxia (left column) and severe hypoxia (right column). Values are expressed as mean ± SEM. *statistically different from hypoxia ($p<0.05$).

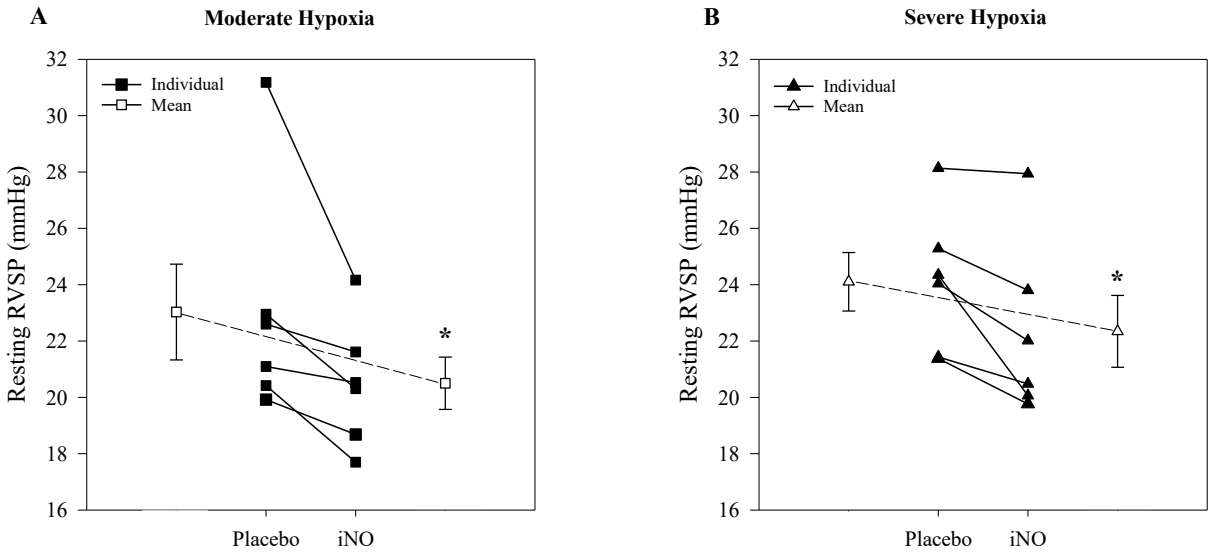

Figure 5. Individual (closed symbols) and mean ± SEM (open symbols) resting right ventricular systolic pressure (RVSP) responses to iNO in moderate hypoxia (A, n=6) and severe hypoxia (B, n=6). *statistically different from placebo ($p \leq 0.05$).

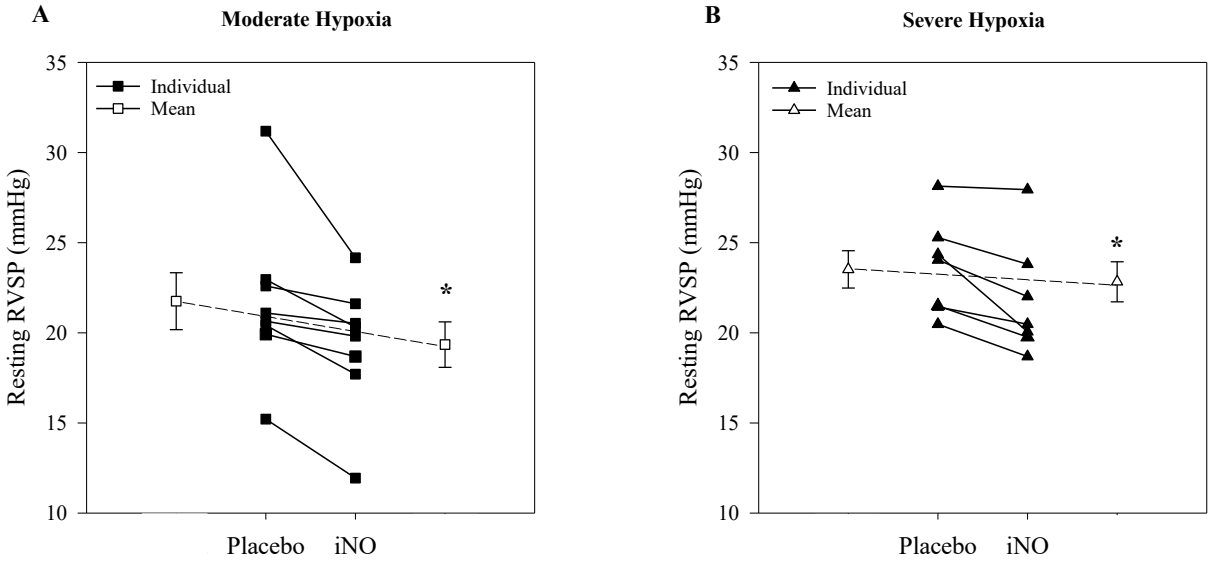

Figure 6. Individual (closed symbols) and mean ± SEM (open symbols) resting right ventricular systolic pressure (RVSP) responses to iNO in moderate hypoxia (A, n=8) and severe hypoxia (B, n=7). *statistically different from placebo ($p \leq 0.01$).

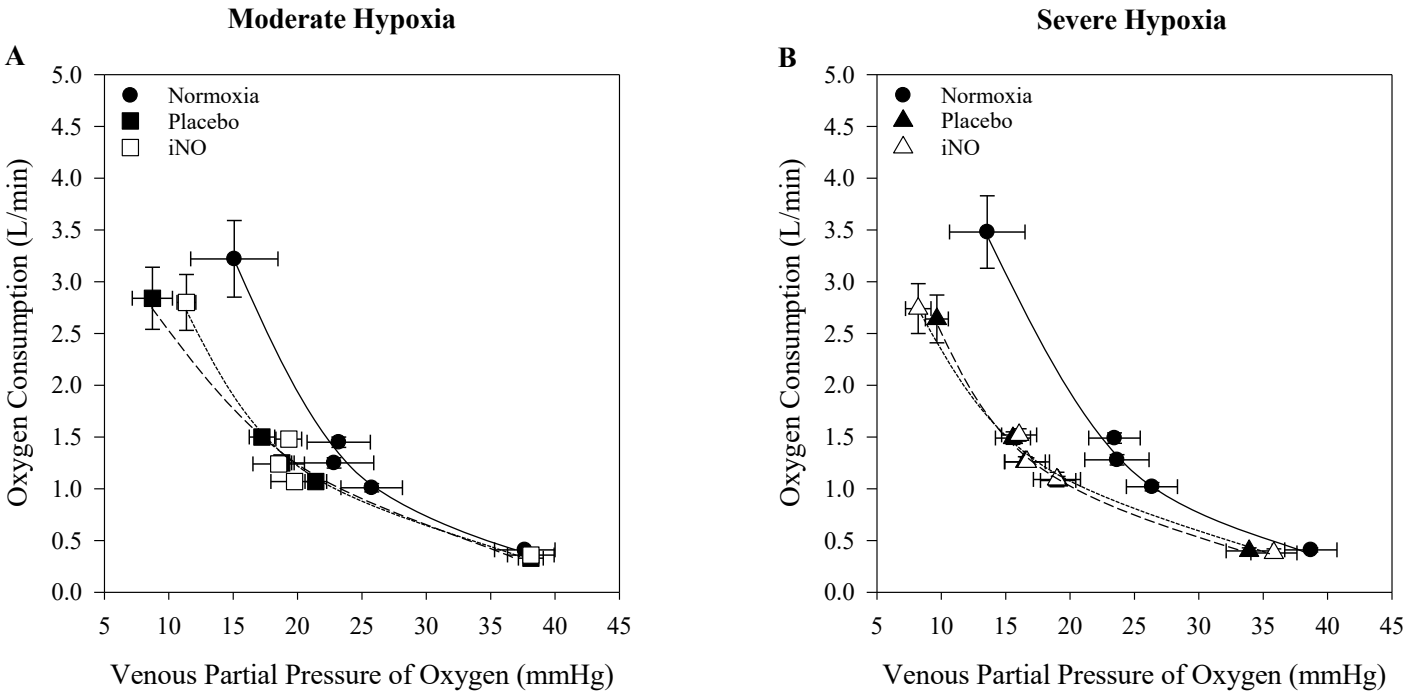

Figure 7. Wagner model for placebo (closed symbols with dashed line) and iNO (open symbols with dotted line) in moderate hypoxia (A) and severe hypoxia (B). Values are expressed as mean ± SEM at baseline, 50-watts, 75-watts, 100-watts, and peak exercise.

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CHAPTER III: General Discussion

3.1 The Effect of Inhaled Nitric Oxide on Maximal Oxygen Consumption During Exercise in Hypoxia

The purpose of this study was to evaluate the effects of iNO on $\dot{V}O_{2max}$ in healthy individuals during exercise in moderate and severe hypoxia. As we expected, both hypoxic levels reduced $\dot{V}O_{2max}$, however, iNO had no effect. This differed from our hypothesis that iNO would improve $\dot{V}O_{2max}$ during exercise in both moderate and severe hypoxia by reducing PAP, leading to increased SV and peak $\dot{Q}$.

3.1.1 The Effect of iNO on Right Ventricular Work

Right ventricular stroke work (RVSW) is the amount of work required by the right ventricle to obtain a certain SV and can be defined by equation 3 (refer to *Chapter II: Ultrasound Analysis*)(Chemla et al., 2013). As depicted in the equation, the larger the difference between mean PAP and RAP, the greater the RVSW. During exercise, HPV will increase PAP resulting in elevated RVSW secondary to increased RV afterload. RVSW was estimated using resting RVSP and SV as there was insufficient submaximal RVSP data. iNO decreased RVSW in moderate hypoxia by 10% (mean change: 218.5±114.1 mmHg/mL) and 3% (mean change: 72.1±11.7 mmHg/mL) in severe hypoxia. Despite this reduction in RVSW in moderate hypoxia, peak $\dot{Q}$ and $\dot{V}O_{2max}$ remained unaffected suggesting that the pulmonary vasculature may not play a significant role in limiting peak $\dot{Q}$ during exercise. However, in severe hypoxia it is likely that the decrease in RVSW was not sufficient to affect $\dot{V}O_{2max}$.

3.1.2 Apparent Hypoxic Threshold

A recent study in our laboratory found that iNO decreases RVSP by 2.95±0.28 mmHg in normoxia (Brotto, In Review). The current study showed that in moderate hypoxia and severe hypoxia, iNO decreased RVSP at rest by 2.53±0.77 mmHg and 1.78±0.24 mmHg, respectively.

iNO did not have as large of an effect on RVSP in moderate hypoxia when compared to normoxia. Furthermore, when compared to normoxia and moderate hypoxia, the effect of iNO in severe hypoxia was minimal. It is possible that the magnitude of HPV with increasing hypoxia becomes too large to be overcome by 40ppm of iNO, suggesting a possible "hypoxic threshold" at which point iNO is no longer effective in reversing HPV.

3.2 Limitations and Considerations

3.2.1 Bias in Partial Pressure of Oxygen

Inspired partial pressure of oxygen (P_IO_2) can be determined by the following equation:

$$(5)\ P_IO_2 = F_IO_2 \times (P_b - 47mmHg)$$

where F_IO_2 is the fraction of inspired oxygen, P_b is the barometric pressure and 47mmHg is the partial pressure of water vapor. As seen in the equation above, P_IO_2 can increase or decrease with proportional changes in F_IO_2 and P_b. This study initially used tanks pre-set with concentrations of moderate (13.6% balanced with nitrogen) and severe hypoxia (12.1% balanced with nitrogen) to deliver each hypoxic condition. When the Pb in the laboratory exceeded the normal Pb in Edmonton (~700mmHg) the P_IO_2 for placebo trials was higher than the targeted 89mmHg (moderate hypoxia) and 79mmHg (severe hypoxia), due to the inability to undershoot the F_IO_2 from the pre-set tanks. Because of this limitation, hypoxia delivery was modified by titrating medical air (21% oxygen) with 100% nitrogen to reach the desired P_IO_2 targets for each condition. This modification made delivery of the target P_IO_2 more accurate, despite this, the group mean placebo P_IO_2 remained over the target values. This did not occur with the iNO trials as the addition of NO lowered the F_IO_2, allowing for P_IO_2 reduction. Despite the limitation of pre-set tanks, the P_IO_2 for all trials (placebo and iNO) were still within the target ranges of 87-91mmHg (moderate hypoxia) and 77-81mmHg (severe hypoxia).

3.2.2 Inhaled Nitric Oxide Dosage

Previous work has demonstrated that 40ppm iNO effectively dilates the pulmonary vasculature and improves exercise capacity in clinical populations such as pulmonary hypertension and mild COPD(Mollard et al., 2007; Naeije, 2010; Naeije, 2011; Peltonen et al., 2001; Pepke-Zaba et al., 1991; Phillips et al., 2021; J. P. Richalet et al., 1988; Savard et al., 1995; Stenberg et al., 1966). Previous work in our laboratory has demonstrated that 40ppm iNO is effective in reducing RVSP in normoxia as rest (25.2±1.3 mmHg to 22.3±1.0 mmHg) and during exercise (33.8±2.6 mmHg to 31.8±3.3mmHg) in healthy participants(Brotto, In Review). Furthermore, Frostell et al, demonstrated that 40ppm iNO was effective in normalizing PAP (mean change: 5.3±0.2 mmHg, $p<0.01$) to baseline values in healthy participants during moderate hypoxia (P_IO_2~86mmHg) at rest(Frostell et al., 1993). Of note, Frostell et al, examined 9 firefighters, which may not be reflective of the general population due to potential vascular impairment/damage secondary to occupational smoke inhalation(Burgess et al., 1999). The potential vascular impairment (e.g., inadequate NO synthase function) may explain why 40ppm iNO was twice as effective in reducing PAP at rest in moderate hypoxia when compared the current study. It is possible that the magnitude of RVSP decline observed in our study was not sufficient to reduce RV afterload and thus improve peak $\dot{Q}$ and $\dot{V}O_{2max}$ in moderate hypoxia. Similarly, there may have not been a sufficient vasodilatory stimulus to overcome the level of HPV in severe hypoxia, thus explaining the lack of change in RVSP with iNO. The study by Frostell and colleagues, demonstrated further decreases in PAP when iNO dosage was increased to 80ppm(Frostell et al., 1993). Taken together, a larger dose of iNO (i.e., 80ppm) may have had a larger impact on RVSP in moderate and severe hypoxia.

3.2.3 Estimation of RVSP using Echocardiography

Invasive techniques (e.g., right heart catherization) are the most accurate methods of directly measuring PAP and PVR during rest and exercise(Champion et al., 2009). However, invasive methods also present safety risks as well as increased participant discomfort and therefore noninvasive measures such as echocardiography can be used to as an appropriate alternative to estimate RVSP(Argiento et al., 2010; Ghofrani et al., 2004; Reichenberger et al., 2007; Zhao et al., 2001). Despite increased participant comfort, echocardiography does have its limitations. To obtain an adequate image of tricuspid regurgitation velocity jet, exercise must be performed in a semi-supine position and an experienced sonographer is required(Argiento et al., 2010). Furthermore, as V_E increases with exercise, we are faced with greater difficulty with image acquisition, reduced image quality and increased artifact (Lang et al., 2015).

Previous work suggests that contrast enhanced echocardiography provides more accurate estimations of RVSP with increased quality image acquisition at rest (93%), submaximal (80%) and maximal exercise (69%)(Claessen et al., 2016). Furthermore, RVSP derived from contrast enhanced echocardiography was strongly correlated to directly measured PAP at rest (r=0.92) and peak exercise (r=0.91) (Claessen et al., 2016). Taken together, future studies on hypoxic exercise could use enhanced echocardiography to increase the image quality and acquisition.

3.2.4 Effects of Menstrual Cycle on $\dot{V}O2_{max}$

There is much debate in research about controlling for menstrual cycle phase; however, current literature suggests that $\dot{V}O_{2max}$ is not affected by menstrual cycle phase(Jurkowski et al., 1981; Smekal et al., 2007). Smekal et al, examined the effects of menstrual cycle (follicular and luteal phase) on exercise variables in 19 healthy eumenorrheic females(Smekal et al., 2007). Participants performed 2 incremental exercise tests on a cycle ergometer, 1 during the follicular

phase and 1 during the luteal phase of their cycle(Smekal et al., 2007). Diet, time of day, and oral contraceptive intake were controlled between exercise tests(Smekal et al., 2007). The authors found no significant differences between follicular and luteal phase in $\dot{V}O_{2max}$ or power output(Smekal et al., 2007). Furthermore, Jurkowski et al, demonstrated no differences in $\dot{V}O_{2max}$, power output or $\dot{Q}$ during incremental exercise in 9 healthy females across menstrual cycle phase, despite increased $\dot{V}_E$ during the luteal phase(Jurkowski et al., 1981). Therefore, it is unlikely that $\dot{V}O_{2max}$ would have been affected by menstrual cycle phase in the current study.

3.3 Current Study in the Context of Rehabilitation Science

This study may provide insight into our understanding of the pathophysiology of chronic lung diseases such as COPD and ILD within the context of rehabilitation sciences. The International Classification of Functioning (ICF), Disability and Health attempts to utilize a standard framework to describe health and physical function in disease (Organization, 2002). The ICF acknowledges that disability and disease are complex phenomena that incorporate body structure and function, individual factors in the forms of participation and activity, and environmental and personal contextual factors(Organization, 2002). In the context of research, utilizing the ICF when developing research questions and study designs may allow researchers to better understand all components of disability and disease, and identify relevant therapeutic targets (Organization, 2002).

The ICF defines *body functions* as "physiological functions of body systems (including psychological functions)" and *body structures* as "anatomical parts of the body such as organs, limbs and their components" (Organization, 2002). Patients with chronic lung disease often have a body structure impairment at the level of the gas exchanging units (alveoli) and the pulmonary vasculature. The damage and/or destruction of the alveoli can result in reduced surface area for

gas exchange, causing impaired gas exchange, and significant exertional arterial hypoxemia; which represents a body function impairment(Mannino, 2002; West, 2011). Arterial hypoxemia is a known contributor to exertional dyspnea and exercise intolerance in these patients, limiting their ability to participate in meaningful activities of daily living (Du Plessis et al., 2018; Hiroyuki et al., 2018; Jensen et al., 2018; Yannick et al., 2020). Ultimately, this prolonged limitation to participate in meaningful activities of daily living contributes to reducing quality of life (Ley et al., 2016; Oga et al., 2003).

The current study focused on manipulating body structure (pulmonary vasculature and right ventricle) and function (physiological exercise response to hypoxia and iNO) in health. $\dot{V}O_{2max}$ was incrementally decreased, while RVSP was incrementally increased with increasing hypoxia, respectively. However, the increase in RVSP was not accompanied by a decrease in peak $\dot{Q}$, which would suggest that right ventricular SV was unaffected by hypoxia (cardiac function). Together, these findings suggest that the pulmonary vasculature and right ventricle may not play a role in limiting exercise capacity in healthy individuals, regardless of hypoxic severity.

Patients with COPD and ILD have been shown to have reduced exercise capacity (activity limitation) when compared to healthy age-matched controls(Du Plessis et al., 2018; Phillips et al., 2021). In the current study, we simulated advanced lung disease conditions via acute hypoxia in healthy participants and demonstrated gradual $\dot{V}O_{2max}$ decline with worsening hypoxia. The observed S_PO_2 reduction in moderate hypoxia was comparable to that observed in patients with moderate to severe ILD (Du Plessis et al., 2018). Our laboratory has previously demonstrated an improvement in $\dot{V}O_{2peak}$, secondary to decreased $\dot{V}_E/\dot{V}CO_2$ and dyspnea with iNO in patients with mild COPD (Phillips et al., 2021). Despite no change in peak $\dot{Q}$, iNO was shown to reduce resting RVSP in mild COPD by 3.7±1.4 mmHg (Phillips et al., 2021), suggesting a decrease in RV

afterload. In the current study, $\dot{V}O_{2max}$ and peak $\dot{Q}$ were unaffected by iNO despite reducing resting RVSP in both hypoxic conditions. Together, these findings suggest that in patients with mild COPD and moderate to severe ILD, the pulmonary vasculature may not play a significant role in limiting peak $\dot{Q}$ but still impairs exercise capacity and thus participation in activities of daily living. Future research is needed to determine if increased RV afterload, secondary to elevated PAP in patients with moderate to severe COPD and ILD limits peak $\dot{Q}$ thereby limiting exercise capacity. The improvement in $\dot{V}O_{2\text{max}}$ with iNO in mild COPD is promising but needs to be evaluated in more severe COPD and in ILD. Following this, next steps would include evaluating more practical pharmacological therapies (e.g., sildenafil) that would improve exercise capacity in these clinical conditions and therefore increase participation in meaningful activities of daily living, and subsequently quality of life.

3.4 Future Directions

Examining body structure and function in chronic lung disease is difficult due to patients often having multiple co-morbidities which may confound results. Due to such co-morbidities, understanding which conditions may or may not affect specific body structures/functions is difficult. Therefore, the current study examined the effects of hypoxic exercise on $\dot{V}O_{2max}$ in young healthy participants with the aim of simulating the exercise intolerance observed in chronic lung disease. Longer hypoxic exposure (1 hour or more) may have elicited a greater HPV response, potentially leading to impaired $\dot{Q}$ and further $\dot{V}O_{2max}$ decline. Future studies could evaluate the effects of longer hypoxic exposure on exercise capacity in young healthy participants, as patients with advanced lung disease often experience *chronic* alveolar hypoxia which may increase right ventricular afterload, decreasing SV, $\dot{Q}$ and therefore $\dot{V}O_{2max}$. Additionally, future research should examine the effect of iNO or sildenafil in moderate and severe

hypoxia in high fit and low fit participants, as literature would suggest that when given sildenafil, individuals with higher fitness have improvements in peak $\dot{Q}$ and $\dot{V}O_{2max}$ during hypoxic exercise when compared to low fit individuals.

3.5 Summary

The effect of iNO on $\dot{V}O_{2max}$ during hypoxic exercise was examined in young healthy participants. iNO had no effect on $\dot{V}O_{2max}$ in either moderate or severe hypoxia, despite decreasing resting hypoxic RVSP. Furthermore, peak $\dot{Q}$ remained unaffected by either hypoxic level or iNO, suggesting that regardless of hypoxia severity, the pulmonary vasculature and right ventricle may not play a role in limiting peak $\dot{Q}$ and consequently exercise capacity in young healthy participants.

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APPENDIX A: Literature Review

Every year, thousands of tourists make trips to high altitude destinations and expose themselves to hypoxic conditions (Burtscher et al., 2018). Hypoxia is commonly defined as a reduction in the partial pressure of oxygen in inspired air (P_IO_2) and can occur through changes in barometric pressure (i.e., high altitude ascent) or changes in the fraction of inspired oxygen (F_IO_2). Exposure to hypoxia can cause symptoms such as headaches, dizziness, confusion, nausea, fatigue, shortness of breath and exercise intolerance (Hackett, 2019; Harvard, 2018). Exposure to extreme hypoxia can lead to high altitude pulmonary edema (Hackett, 2019). Furthermore, hypoxia can lead to arterial hypoxemia which is defined by decreased oxygen content within the arterial blood and can result in reduced exercise capacity.

A.1 Hypoxemia in Lung Disease

Dyspnea, commonly known as breathlessness, is a key contributor to reduced exercise capacity in patients with lung disease (Jensen et al., 2018). Reduced exercise tolerance and increased dyspnea have been associated with decreased quality of life and increased risk of mortality in individuals with lung diseases such as chronic obstructive pulmonary disease (COPD) or interstitial lung disease (ILD) (Jensen et al., 2018; Ley et al., 2016; Oga et al., 2003). Patients with advanced lung disease often have damage or destruction of the gas exchanging units of the lungs (i.e. alveoli) (Mannino, 2002; West, 2011). This damage/destruction of the alveoli results in decreased surface area for gas exchange, ultimately resulting impaired gas exchange, significant arterial hypoxemia and a reduction in exercise capacity (West, 2011). The arterial hypoxemia that occurs in patients with chronic lung disease can result in a similar reduction in $\dot{V}O_{2max}$ as observed in hypoxia. Thus, studying the exercise response under hypoxic conditions may provide

pathophysiological insights into our understanding of exercise limitation(s) in clinical conditions that demonstrate significant hypoxemia on exertion.

A.2 Determinants of $\dot{V}O_{2max}$ in hypoxia

Aerobic exercise capacity, measured by maximal oxygen consumption ($\dot{V}O_{2max}$), decreases by approximately 2-4% with every 300-meter gain in altitude (Burtscher et al., 2018; Stickland et al., 2012). A higher maximal oxygen uptake ($\dot{V}O_{2max}$) indicates a greater ability to transport and utilize oxygen during exercise (Bassett & Howley, 2000; Stickland et al., 2012). $\dot{V}O_{2max}$ is determined by the Fick equation:

$$(1)\ \dot{V}O_2 = \dot{Q}\ \times (CaO_2 - CvO_2)$$

Where $\dot{Q}$ is cardiac output,, and CaO_2 and CvO_2 are arterial and venous oxygen content, respectively (Stickland et al., 2012). $\dot{Q}$ is the product of stroke volume (SV) and heart rate (HR) as demonstrated by the following equation (Kemp & Conte, 2012):

$$(2)\ \dot{Q} = HR \times SV$$

The partial pressure of oxygen in the alveoli (P_AO_2) is determined by the abbreviated alveolar air equation (West & Luks, 2016):

$$(3)\ P_AO_2 = P_IO_2 - \left[\frac{P_ACO_2}{RER}\right]$$

where P_ACO_2 and RER are the partial pressure of carbon dioxide and the respiratory exchange ratio, respectively. As demonstrated by the above equation, reductions in P_IO_2 would result in decreased P_AO_2 and assuming normal gas exchange, would result in a proportional decline in the arterial partial pressure of oxygen (PaO_2) (West & Luks, 2016). Manipulating P_IO_2 leads to reductions in arterial oxygen saturation (SaO_2) and CaO_2 due to decreased PaO_2 in hypoxia when

compared to sea level and can be demonstrated by the following equation (Knight et al., 1993; Stenberg et al., 1966; Stickland et al., 2012):

$$(4)\ C_aO_2 = (1.34\ x\ H_b\ x\ S_aO_2) + (0.0031\ x\ P_aO_2)$$

where H_b is the hemoglobin concentration in the blood. Furthermore, this decline in CaO_2 would consequently reduce $\dot{V}O_{2max}$ (see equation 1). In two separate papers, Stenberg et al, as well as and Knight et al. examined the hemodynamic response to maximal cycle exercise in healthy participants at simulated altitude (P_IO_2=87 mmHg) when compared to sea level (P_IO_2=147mmHg) (Knight et al., 1993; Stenberg et al., 1966). Both studies found that decreased $\dot{V}O_{2max}$ was associated with reductions in arterial oxygen saturation (SaO_2) and CaO_2 in hypoxia when compared to sea level and was related to decreased $\dot{V}O_{2max}$ (Knight et al., 1993; Stenberg et al., 1966). The hypoxic breathing conditions in the above experiments would decrease both P_AO_2 and PaO_2, which would result in a reduction in SaO_2 and CaO_2 as indicated by equation 4 (West & Luks, 2016). Using the Fick equation as a guide, $\dot{V}O_{2max}$ is reduced in hypoxia because of the proportional decrease in P_IO_2, PaO_2, SaO_2, and thus CaO_2.

A.3 Wagner's Theoretical Model on Determinants of $\dot{V}O_{2max}$

Traditionally, $\dot{Q}$ has been considered the key determinant of $\dot{V}O_{2max}$ in healthy individuals, however Dr. Peter Wagner developed a model which takes a multisystem approach to evaluating limitations to $\dot{V}O_{2max}$. Specifically, the Wagner model incorporates each factor involved in the oxygen transport cascade (P_IO_2, ventilation, lung diffusing capacity, $\dot{Q}$, hemoglobin concentration and muscle diffusing capacity) (Wagner, 2017). In 1996, Wagner developed this theoretical model that evaluated the *steps* of oxygen transport as a *system* divided into two components; 1) the convective component of oxygen transport, and 2) the diffusive component of oxygen transport (Wagner, 2017). The convective component includes all steps of oxygen transport from the

environment to the muscle microvessels and includes the following components of the Fick equation; $\dot{Q}$ and CaO_2 (equation 1) (Wagner, 2017). The diffusive component includes the last step of oxygen transport, from the muscle microvessels to the mitochondria and can be demonstrated by the Law of Diffusion (Wagner, 2017):

$$(5)\ \dot{V}O_2 = D_M \times (P_{cap}O_2 - P_{mito}O_2)$$

where D_M is the diffusion membrane in the muscle, $P_{cap}O_2$ and $P_{mito}O_2$ are partial pressure of oxygen in the capillary and the mitochondrial, respectively (Wagner, 2017). Assuming that $P_{cap}O_2$ is proportional to end-capillary partial pressure of oxygen (i.e. venous partial pressure of oxygen; P_VO_2) and that $P_{mito}O_2$ is very low (< 3 mmHg) and therefore negligible, equation 5 can be simplified to the following equation (Wagner, 2017):

$$(6)\ \dot{V}O_2 = D_M \times k \times PvO_2$$

where, k is a constant that represents the ratio between mean $P_{cap}O_2$ and P_VO_2 (typically k = ~2) (Wagner, 2017). Wagner's model suggested that when both equations were plotted on a graph with P_VO_2 on the x-axis and $\dot{V}O_2$ on the y-axis, the point at which both lines intersect represents a person's $\dot{V}O_{2max}$ (Wagner, 2017) (see Figure 1 below). Therefore, Wagner's model suggests that *all variables* in the oxygen transport *system* (P_IO_2, ventilation, lung diffusing capacity, $\dot{Q}$, hemoglobin concentration and muscle diffusing capacity) may affect $\dot{V}O_{2max}$ at sea level (Wagner, 2017).

According to Wagner's model, since diffusion limitation can occur within the lungs as well as within muscles, in a human at altitude, the convective curve would be compressed downward and toward the left (due to diffusion limitation of the lung), while the slope of the diffusive curve would shift downwards about the origin (where x=0 and y=0, due to diffusion limitation within

the muscle), resulting in a new lower set point for $\dot{V}O_{2max}$ as can be seen in the figure 1 (Wagner, 2017).

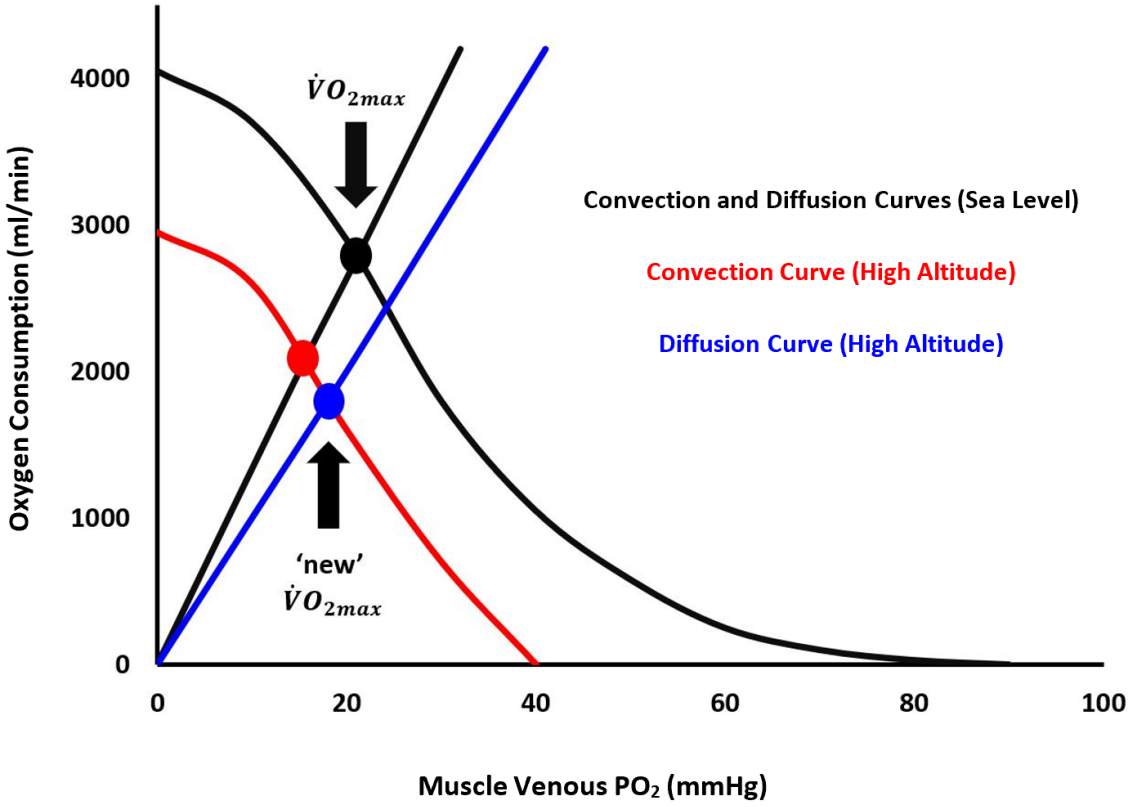

Figure 1: Theoretical representation of Wagner's model representing the change in the convection and diffusion slopes at altitude (reduced PO_2) adapted from *Wagner* (Wagner, 2017). The black circle represents $\dot{V}O_{2max}$ at sea level. At high altitude, the red circle represents the decrease in $\dot{V}O_{2max}$ following the downward decompression of the convection curve due to the pulmonary diffusion limitation (and thus hypoxemia). Similarly, the blue circle represents the further decrease in $\dot{V}O_{2max}$ following the downward shift about the origin of the diffusion curve due to a peripheral (i.e., muscle) diffusion limitation.

Furthermore, a theoretical analysis conducted by Wagner using data from the study conducted by Oelz et al. in 1986, demonstrated that at extreme high altitude (i.e. severe hypoxia; P_IO_2 <85mmHg), the magnitude that each variable within the oxygen transport system influencing

$\dot{V}O_{2max}$ is different than observed at sea level (Wagner, 2017). Specifically, Wagner demonstrated that by causing a 50% improvement in lung diffusing capacity and a 40% improvement in muscle diffusing capacity, $\dot{V}O_2$ is increased by only 25% in severe hypoxia. However, when $\dot{Q}$ is improved by 25% in addition to increasing both lung and muscle diffusing capacities, $\dot{V}O_2$ is only increased by a further 1.7%. This suggests that changes in the diffusive components (i.e. lung and muscle diffusing capacity) of the oxygen transport system may play a larger role in affecting $\dot{V}O_{2max}$ when compared to the convective components (i.e. $\dot{Q}$) in severe hypoxia (Wagner, 2017). Interestingly, research has shown that in mild-moderate hypoxia (P_IO_2 ~ 97-85 mmHg), peak $\dot{Q}$ remains relatively unchanged in healthy participants while $\dot{V}O_{2max}$ is significantly decreased, suggesting that cardiac output does not play a role in limiting $\dot{V}O_{2max}$ in hypoxia, as theorized by Wagner (Reeves et al., 1987b; Stenberg et al., 1966; Wagner, 2017). However, in severe hypoxia (P_IO_2 <85mmHg,), significant reductions in $\dot{Q}$ as well as $\dot{V}O_{2max}$ are observed, suggesting that cardiac function may be limited during exercise; which contrasts with Wagner's theoretical model(Calbet et al., 2003). Therefore, based on the current research it remains unclear whether hypoxia affects peak $\dot{Q}$ during exercise, and whether $\dot{Q}$ is a limiting factor of $\dot{V}O_{2max}$ in hypoxia.

The discrepancies within current research regarding the $\dot{Q}$ response during exercise in hypoxia may be due to an apparent 'hypoxic threshold'. In mild to moderate hypoxia (P_IO_2 ~ 97 mmHg to 85mmHg), the diffusive component of the oxygen transport system may be the main limiting factor of $\dot{V}O_{2max}$. However, in severe hypoxia (P_IO_2 <85mmHg) there may be a shift, where the convective component of the oxygen transport system also limits $\dot{V}O_{2max}$. In short, $\dot{Q}$ may become impaired in severe hypoxia and further contribute to the decrease in $\dot{V}O_{2max}$. However, the hypoxic threshold where this change in $\dot{Q}$ occurs remains unclear, and may vary between individuals, and disease severity. For example, patients with severe hypoxemia may exhibit $\dot{Q}$

limitations earlier when compared to those with less severe hypoxemia during exercise. Additionally, the mechanism(s) behind this change in cardiac function is also unclear.

A.4 Cardiac Output in Moderate Hypoxia

Studies in mild to moderate acute hypoxia have demonstrated minimal decreases in maximal HR and SV, while still demonstrating significant reductions in $\dot{V}O_{2max}$ when compared to normoxia, suggesting that $\dot{Q}$ is not the main contributor to exercise intolerance in mild to moderate hypoxia (Mollard et al., 2007; Naeije, 2010; Peltonen et al., 2001; Stenberg et al., 1966). Specifically, Mollard et al, assessed the effects of acute hypoxia of increasing severity (P_IO_2= 132 to 81 mmHg) on 8 healthy untrained males during maximal exercise (Mollard et al., 2007). $\dot{V}O_{2max}$ and maximal HR were progressively reduced with each P_IO_2 decrement, suggesting that the lower $\dot{V}O_{2max}$ may be driven by lower maximal HR (Mollard et al., 2007). However, in the same study, arterial oxygen saturation (S_PO_2) was also progressively decreased with worsening hypoxia. Multivariate analysis revealed that S_PO_2 was a stronger predictor of $\dot{V}O_{2max}$ decline when compared to maximal HR in acute mild to moderate hypoxia (Mollard et al., 2007). Additionally, studies that have shown reductions in maximal HR, SV and therefore $\dot{Q}$ have only been observed in *chronic moderate hypoxia*, whereas $\dot{Q}$ remains relatively unchanged in *acute moderate hypoxia* (P_IO_2 ~85 mmHg) (Benoit et al., 2003; J. Richalet et al., 1988; Savard et al., 1995). Chronic hypoxia results in acclimatization, at which point various organ systems adapt to the decreased P_IO_2 making it difficult to determine the factor(s) contributing to the reduction in SV (Kennedy, 2006; Stembridge et al., 2019). In chronic hypoxia, the body undergoes dehydration resulting in hypovolemia (reduced blood volume) (Kennedy, 2006). Hypovolemia causes reduced venous return (i.e. preload) and increased hematocrit (increased density of red blood cells) resulting in increased blood viscosity (Kennedy, 2006). Reduced venous return leads to decreases in SV and therefore $\dot{Q}$

(Kennedy, 2006). Therefore, arterial desaturation, and not $\dot{Q}$, is likely the main contributor to reduced $\dot{V}O_{2max}$ in conditions of acute mild to moderate hypoxia in health.

A.5 Cardiac Output in Severe Hypoxia

In acute severe hypoxia (P_IO_2 <85mmHg,) there is a larger reduction in $\dot{V}O_{2max}$ that cannot be explained by arterial desaturation and impaired pulmonary gas exchange alone (Calbet et al., 2003; Jeffries et al., 2019). Calbet et al, studied the effects of acute severe hypoxia (F_IO_2= 0.105, P_IO_2= 74 mmHg) on maximal exercise capacity in 9 healthy male volunteers(Calbet et al., 2003). They found that $\dot{V}O_{2max}$ was decreased by approximately 50% in severe hypoxia when compared to normoxia(Calbet et al., 2003). The authors demonstrated that two thirds of this reduction in $\dot{V}O_{2max}$ could be accounted for by the reduction in SpO_2 and pulmonary gas exchange impairment, while one third was due to decreased maximal $\dot{Q}$(Calbet et al., 2003). Furthermore, studies have demonstrated that maximal HR is minimally changed during exercise in severe acute hypoxia, therefore, SV may account for the observed decline in $\dot{Q}$ (Benoit et al., 2003; Calbet et al., 2003; J. Richalet et al., 1988; Savard et al., 1995). The mechanism(s) associated with this decline in SV and $\dot{Q}$ in acute severe hypoxia remains unclear, however, heterogenous hypoxic pulmonary vasoconstriction has been suggested to play a role (Calbet et al., 2003; Naeije, 2011).

A.6 Hypoxic Pulmonary Vasoconstriction

Hypoxic pulmonary vasoconstriction (HPV) is a mechanism that, in response to local alveolar hypoxia, diverts blood flow from poorly ventilated lung units to sufficiently ventilated lung units, with the goal of maintaining or improving ventilation/perfusion ($\dot{V}_A/\dot{Q}$) matching (Swenson, 2013). Hambraeus-Jonzon et al. demonstrated the stimulus-response relationship of HPV and P_IO_2 (Hambraeus-Jonzon et al., 1997). Seventeen healthy participants were given intravenous anesthesia while their lungs were separately and synchronously ventilated. Perfusion

distributions were measured using the multiple inert gas elimination technique (MIGET) (Hambraeus-Jonzon et al., 1997). The right lung was continuously ventilated with 100% oxygen, while the left lung was ventilated with stepwise reductions in F_IO_2 (100%, 12%, 8%, and 5% oxygen balanced with nitrogen) for 25 minutes at each F_IO_2. During bilateral ventilation with hyperoxia, 52% and 48% of cardiac output perfused the left and right lung, respectively. During unilateral ventilation with 12%, 8%, and 5% oxygen, left lung perfusion decreased to 47%, 40% and 30% of cardiac output, respectively. Additionally, when the left lung was immediately taken from 100% to 5% oxygen there was an observed 20% reduction in left lung perfusion. The findings of this study suggest that the magnitude of HPV is directly related to the severity of hypoxia. Additionally, this study demonstrated that in response to local alveolar hypoxia, HPV diverts blood flow away from poorly oxygenated lung regions (i.e., hypoxic left lung) towards sufficiently ventilation lung regions. Melot et al. evaluated $\dot{V}_A/\dot{Q}$ matching in hypoxia (F_IO_2= 0.125; P_IO_2 ~ 90 mmHg) and normoxia (F_IO_2=0.21; P_IO_2 ~151 mmHg) using MIGET in 7 healthy volunteers. They found that acute severe hypoxia leads to reductions in blood flow to hypoxic lung regions (i.e. poorly ventilated lung regions) thereby improving overall $\dot{V}_A/\dot{Q}$ matching and proposed this was due to HPV (Mélot et al., 1987). The researchers then evaluated the effects of nifedipine, a vasodilator that has been shown to decrease HPV and found worsened $\dot{V}_A/\dot{Q}$ mismatch (Mélot et al., 1987). Taken together, the findings of this study suggest that HPV occurs in an attempt to maintain $\dot{V}_A/\dot{Q}$ matching (Mélot et al., 1987).

Although HPV may be a protective mechanism against local alveolar hypoxia, in the case of global alveolar hypoxia (as may occur in severe hypoxia), it may be maladaptive (Naeije, 2011). Global alveolar hypoxia leads to heterogenous HPV which can cause pulmonary hypertension and pulmonary edema (Naeije, 2011; Swenson, 2013). Heterogenous HPV can redirect blood flow to

small pulmonary arteries that are not actively vasoconstricting, leading to higher perfusion pressures reaching the alveolar-capillary bed in these regions and causing capillary hypertension (Naeije, 2011). Due to the fragility of pulmonary capillaries, capillary hypertension can result in pulmonary edema and/or cause damage to the alveolar-capillary membrane, leading to an impairment in pulmonary gas exchange (Naeije, 2011; West & Luks, 2016).

A.7 Increased Right Heart Work

Effects of Pulmonary Artery Pressure and Pulmonary Vascular Resistance on Stroke Volume

Research has suggested that heterogenous HPV may lead to larger increases in pulmonary artery pressure (PAP) and (Calbet, 2003; Faoro et al., 2009; Naeije, 2010; Naeije, 2011; Peltonen et al., 2001; Stembridge et al., 2019; Swenson, 2013; Wagner et al., 1986b)increased right ventricular afterload which may limit $\dot{Q}$ and $\dot{V}O_{2max}$ (Naeije, 2011; Stembridge et al., 2019; Swenson, 2013). Afterload is defined by all of the factors that contribute to the total myocardial wall stress during systole, including end-systolic pressure (ESP), end-systolic volume (ESV), and ventricular wall thickness (Norton, 2001). Stroke volume is determined by the following equation (Kemp & Conte, 2012; Klabunde, 2012):

$$(7)\ SV = EDV - ESV$$

where EDV is end-diastolic volume (volume of blood in the ventricle at the end of diastolic filling) and ESV (volume of blood in the ventricle after ejection) (Klabunde, 2012). When afterload is increased (i.e., larger hydraulic resistance) the ventricle must contract more forcefully to adequately eject blood during systole. If the ventricle is unable to contract with such force, a lower blood volume is ejected from the ventricle, increasing ESV and reducing SV (see equation 7).

Increases in PVR would increase the pressure the right ventricle must work against to eject blood from the ventricle resulting in increased ESV and therefore reduced SV (equation 7). PVR is determined by the following equation:

$$(8)\ PVR = \frac{PAP - PAWP}{\dot{Q}}$$

where, PAWP is pulmonary artery wedge pressure (PAWP). The difference between PAP and PAWP is used to determine the driving pressure throughout the pulmonary circulation as PAWP is used as an estimate of left atrial pressure. During maximal intensity exercise at normoxia, distention and recruitment of pulmonary capillaries occurs in response to large increases in $\dot{Q}$ (West & Luks, 2016). The resistance within a blood vessel is determined by the following equation (West & Luks, 2016):

$$(9)\ PVR = \frac{8\ x\ n\ x\ L}{\pi\ x\ r^4}$$

Where *n* is blood viscosity, *L* is blood vessel length, and *r* is the radius of the blood vessel. As seen from equation 9, increases in the radius (*r*) of a blood vessel would result in an exponential decrease in resistance (PVR). Recruitment of capillaries refers to an increase in the number of perfused capillaries while distention refers to an increase in capillary diameter due to increased blood flow to exercising muscle (West & Luks, 2016). Therefore, recruitment and distention of pulmonary capillaries would effectively increase *r* and therefore reduce PVR (West & Luks, 2016). This reduction in PVR, allows for greater increases in $\dot{Q}$ since PVR and $\dot{Q}$ are inversely related (equation 8). However, this drop in PVR with increasing $\dot{Q}$ may not occur in hypoxia.

Studies have demonstrated an increase in PVR during exercise in severe hypoxia (Calbet et al., 2003; Faoro et al., 2009; Peltonen et al., 2001; Swenson, 2013). Stembridge et al, demonstrated significant increases in PVR secondary to HPV in 12 healthy males at rest following

acclimatization in chronic moderate hypoxia (Stembridge et al., 2019). Furthermore, Naeije et al, reported decreased $\dot{V}O_{2max}$ and increased PAP and PVR in healthy volunteers during 1 hour of hypoxic breathing (F_IO_2= 0.12; P_IO_2 ~ 86 mmHg) when compared with normoxic breathing (F_IO_2=0.21; PIO2 ~151 mmHg) (Naeije et al., 2010). Additionally, when volunteers were given 100 mg of sitaxsentan, a drug that prevents vasoconstriction by blocking endothelin A receptors), $\dot{V}O_{2max}$ was improved secondary to decreased PAP and PVR in hypoxia (Naeije et al., 2010). Overall, research suggests that in moderate and severe hypoxia, PVR is increased, resulting in reductions in $\dot{Q}$ (equation 3) and $\dot{V}O_{2max.}$ However, when PVR is pharmacologically decreased, $\dot{V}O_{2max}$ has been shown to improve, likely secondary to improved SV and therefore $\dot{Q}$. Lastly, studies have demonstrated elevations in PAP in severe hypoxia when compared to normoxia (Faoro et al., 2009; Wagner et al., 1986b). A study using right heart catheterization measured mean PAP in 8 healthy individuals during incremental exercise (60 watt increments up to 240 watts) at sea level and at simulated altitudes of 3,048m (P_IO_2=99.9 mmHg) and 4,572m (P_IO_2=80.2 mmHg) in a hypobaric chamber (Wagner et al., 1986b). PAP and $\dot{Q}$ were increased while $\dot{V}O_{2max}$ was decreased at each altitude when compared to sea level, however there were larger increases in PAP when compared to $\dot{Q}$ (Wagner et al., 1986b). The findings of this study suggest that with increasing severity of hypoxia, PAP rises (with no change in PAWP) more than $\dot{Q}$, which would increase PVR (equation 8). The elevated PVR would increase right ventricular afterload causing a reduction in $\dot{Q}$ and therefore $\dot{V}O_{2max.}$ Therefore, HPV-mediated increases in PVR and PAP could potentially increase right ventricular afterload, reducing SV, and therefore $\dot{Q}$ and as a result, negatively affecting $\dot{V}O_{2max}$ (Stickland et al., 2012). One way to reduce PVR and PAP is with selective pulmonary vasodilators such as nitric oxide (Ichinose & Zapol, 2017; Pepke-Zaba et al., 1991; Steudel et al., 1999).

A.8 Nitric Oxide

Nitric oxide (NO) is an important signaling molecule within the body and has become an area of interest in biomedical research for its potent localized vasodilatory effects (Ichinose & Zapol, 2017). Endogenous NO is synthesized by three forms of nitric oxide synthases (NOS) and are distinguished by their location within the body (Ichinose & Zapol, 2017). The three NOS forms are neuronal NOS (nNOS) found primarily in nervous tissue, inducible NOS (iNOS) expressed in various inflammatory cells, and endothelial NOS (eNOS) found in endothelial cells (Ichinose & Zapol, 2017). In 1998, NO was determined to modulate the vascular tone of both the systemic and pulmonary circulations by inducing smooth muscle relaxation (Ichinose & Zapol, 2017).

Endogenous NO, produced by eNOS, diffuses from the endothelium into vascular smooth muscle where it activates soluble guanylate cyclase (SGC) to increase the production of cyclic guanosine monophosphate (cGMP) (Ichinose & Zapol, 2017). Increased cGMP results in vasodilation, via relaxation of vascular smooth muscle secondary to activation of cGMP-dependent protein kinase (Ichinose & Zapol, 2017). When not acting on vascular smooth muscle, NO quickly binds to hemoglobin and is converted to its inactive forms (nitrite and nitrate) (Ichinose & Zapol, 2017). Exogenous inhaled nitric oxide (iNO) can therefore selectively target the pulmonary vasculature while having little to no effect on the systemic vasculature (Pepke-Zaba et al., 1991). Pepke-Zaba et al., showed that iNO can effectively treat pulmonary artery hypertension, while having no effect on system vascular resistance (Pepke-Zaba et al., 1991). Furthermore, phosphodiesterase-5 inhibitors such as sildenafil (i.e. Viagra) inhibit the degradation of cGMP and therefore prolong the vasodilatory effects of NO (Ichinose & Zapol, 2017).

Inhaled nitric oxide has been shown to reduce PVR and PAP in acute severe hypoxic conditions (Frostell et al., 1993; Pepke-Zaba et al., 1991). Frostell et al, studied the effects of

40ppm iNO on HPV in 9 healthy volunteers (Frostell et al., 1993). Participants breathed the following gas mixtures for 10 minutes each: 21% oxygen (control), 12% oxygen (hypoxia), and 12% oxygen with 40ppm iNO in a supine position (Frostell et al., 1993). PAP was measured using right heart catherization and PVR and right ventricular stroke work index (RVSWI) were calculated (Frostell et al., 1993). As expected, breathing 12% oxygen elicited a significant increase in PAP, PVR and RVSWI, however when breathing 12% oxygen with 40ppm iNO, PAP, PVR and RSVWI all returned to normoxic values (Frostell et al., 1993). This suggests that iNO is effective in reversing HPV, thereby maintaining normal PAP and PVR values. However, the study did not examine the effects of iNO on PAP and PVR during maximal exercise (Frostell et al., 1993). Lastly, studies using sildenafil, a PDE-5 inhibitor (inhibits vasoconstriction), have demonstrated improved $\dot{V}O_{2max}$ secondary to decreased PAP and therefore PVR and increased $\dot{Q}$ during exercise in severe hypoxia (Fischler et al., 2009; Ghofrani et al., 2004). However, sildenafil is administered orally and affects both systemic and pulmonary vasculature, therefore it cannot be determined if $\dot{V}O_{2max}$ improvement occurred due to alleviated HPV or increased oxygen delivery to skeletal muscles through vasodilation of the systemic vasculature (Ghofrani et al., 2004).

A.9 References

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APPENDIX B: Supplemental Figures

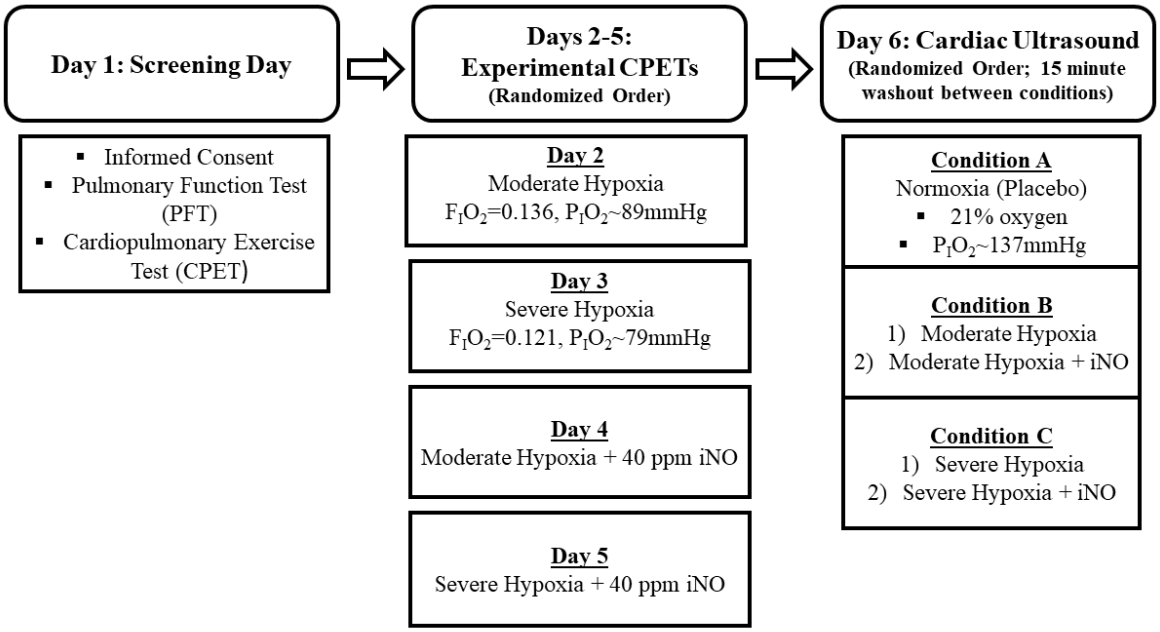

Figure 1. Study Design Schematic.

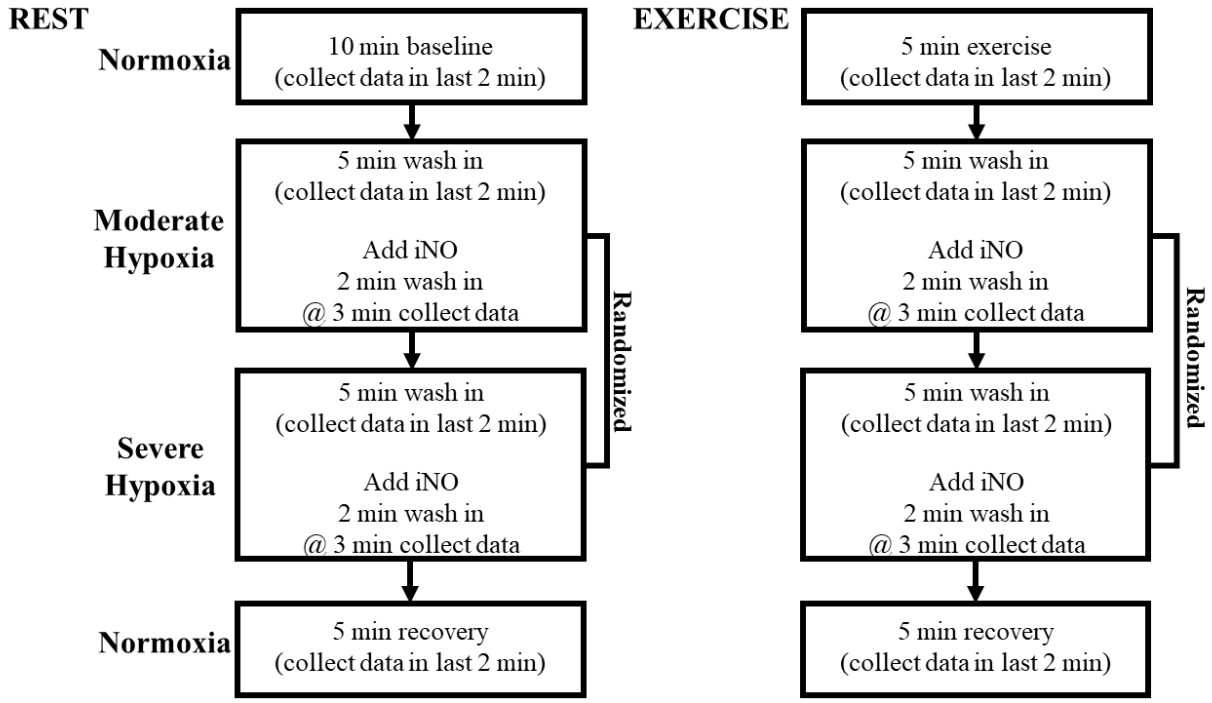

Figure 2. Echocardiography Protocol Schematic

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