The Effect of Inhaled Nitric Oxide on Maximal Oxygen Consumption in High-fit and Untrained Individuals

by

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Abstract

Previous work suggests that endurance-trained athletes have superior pulmonary vasculature function as compared to untrained individuals, which may contribute to their greater maximal oxygen uptake ($\dot{V}O_{2max}$). Inhaled nitric oxide (iNO) improves pulmonary vascular compliance at rest in healthy individuals, which may translate to greater compliance during exercise and improved $\dot{V}O_{2max}$. The purpose of this study was to examine whether iNO improves $\dot{V}O_{2max}$ in high-fit and untrained individuals. It was hypothesized that iNO will increase $\dot{V}O_{2max}$ in untrained individuals, while no significant change would be observed in high-fit individuals. Sixteen high-fit and sixteen untrained individuals with normal lung function completed the randomized double-blind cross-over study over four sessions. On day 1, participants completed a pulmonary function and standard cardiopulmonary exercise test (CPET). Untrained was defined as a $\dot{V}O_{2max}$ between 30-45 ml · kg^{-1} · min^{-1}, and high-fit was defined as a $\dot{V}O_{2max}$ >55 ml · kg^{-1} · min^{-1} for females and >60 ml · kg^{-1} · min^{-1} for males. A cut-off of 45 ml · kg^{-1} · min^{-1} was used as this represents from the 10th - 50th percentile of fitness in the general population, while a $\dot{V}O_{2max}$ >55 ml · kg^{-1} · min^{-1} for females and >60 ml · kg^{-1} · min^{-1} for males has previously been associated with enhanced cardiac function. On days 2 and 3, participants completed experimental CPETs while breathing either normoxia (placebo, day 2) or 40 ppm of iNO (day 3, order of test day randomized). On day 4, echocardiography was performed during rest and sub-maximal exercise (60 Watts) while participants breathed either iNO or placebo. Expired gas was collected to determine $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and ventilation. Cardiac output was estimated by impedance cardiography and right ventricular systolic pressure (RVSP) was estimated using Doppler Echocardiography. RVSP was significantly reduced with iNO at rest (Placebo: 7.55 ± 0.86 mmHg vs. iNO: 6.35 ± 0.72 mmHg, p< 0.001) and exercise (Placebo: 10.15 ± 1.08 mmHg vs. iNO: 7.29 ± 0.84 mmHg, p= 0.011). $\dot{V}O_{2max}$ was significantly different

between groups (Untrained: 43.3 ± 1.0 ml · kg^{-1} · min^{-1} vs. High-fit: 66.1 ± 1.0 ml · kg^{-1} · min^{-1}, $p < 0.001$), but there was no effect of condition (Placebo: 54.9 ± 1.0 ml · kg^{-1} · min^{-1} vs. iNO: 54.5 ± 1.0 ml · kg^{-1} · min^{-1}, $p = 0.788$) and no group by condition interaction ($p = 0.682$). Peak cardiac output, ventilation and $\dot{V}CO_2$ were also unchanged by iNO in both groups. These data demonstrate that despite reductions in pulmonary vascular resistance, $\dot{V}O_{2max}$ was unaffected by iNO in both high-fit and untrained participants. These results suggest that the pulmonary vasculature does not limit $\dot{V}O_{2max}$ in young healthy individuals, regardless of aerobic fitness level.

Preface

This thesis is an original work by Andrew Brotto. The embedded research project, “The effect of inhaled nitric oxide on maximal oxygen consumption in high-fit and untrained individuals”, received research ethics approval from Health Canada and the University of Alberta Health Research Ethics Board (Biomedical Panel Pro00078715). No part of this thesis has been previously published.

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List of Abbreviations

$A\text{-}aDO_2$: Alveolar-arterial oxygen difference

ANF: Atrial natriuretic factor

CPET: Cardiopulmonary exercise test

DLCO: Diffusing capacity for carbon monoxide

D_M: Diffusing membrane capacity

EDV: End-diastolic volume

EIAH: Exercise induced arterial hypoxemia

ESV: End-systolic volume

F_iO_2: Fraction of inspired oxygen

Hb: Hemoglobin

HPV: Hypoxic pulmonary vasoconstriction

HR: Heart rate

iNO: Inhaled nitric oxide

LAP: Left atrial pressure

LV: Left ventricle

LVSV: Left ventricular stroke volume

LVSW: Left ventricular stroke work

MAP: Mean arterial pressure

Met-Hb: Methemoglobin

NO: Nitric oxide

NO_2: Nitric dioxide

PAP: Pulmonary artery pressure

PAH: Pulmonary arterial hypertension

P_aO_2: Partial pressure of arterial oxygen

PASP: Pulmonary artery systolic pressure

PAWP: Pulmonary artery wedge pressure

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

PVR: Pulmonary vascular resistance

Q: Cardiac output

RAP: Right atrial pressure

RHC: Right heart catheter

RV: Right ventricle

RVEF: Right ventricular ejection fraction

RVSP: Right ventricular systolic pressure

RVSV: Right ventricular stroke volume

RVSW: Right ventricular stroke work

SpO_2: Arterial oxygen saturation

SV: Stroke volume

V_C: Pulmonary capillary blood volume

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

$\dot{V}_E$: Minute ventilation

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

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

$\dot{V}_A/\dot{Q}$: Ventilation-perfusion

Chapter I: Introduction

1.1 Background

During exercise, cardiac output (Q) increases to meet the increasing oxygen demands of the body (Flamm et al., 1990). This is achieved by increasing stroke volume (SV; the amount of blood excreted from the ventricle each heartbeat) and increasing heart rate (HR). Pulmonary blood flow increases proportionately to facilitate oxygen uptake in the lungs and oxygen delivery to the working muscles. With an increase in Q, pulmonary vascular resistance (PVR) is reduced in healthy individuals by recruitment and distention of the pulmonary capillaries (Reeves, 2005). This reduction in PVR limits the rise in pulmonary artery pressure (PAP), as high pulmonary vascular pressures may cause pulmonary edema or damage to the fragile pulmonary capillaries (Wagner et al., 1979). Importantly, large exercise-induced increases in PAP have been shown to negatively impact exercise capacity (Alkotob et al., 2006; Tolle et al., 2008).

Regulation of PVR during exercise has traditionally been thought to occur passively, with increases in blood flow causing recruitment and distention of the pulmonary capillaries, decreasing PVR, and facilitating gas exchange (Hsia, 2002). However, there is evidence that the pulmonary circulation can be influenced by circulating vasodilators (Polak et al., 1992) which could be affected by exercise training. Exercise training has been reported to cause greater left-ventricular systolic and diastolic function and enhanced systemic vascular distensibility to optimize blood flow to the working muscles (Levine et al., 1991; Stickland et al., 2006). Recent work has shown that highly trained individuals demonstrate greater resting and exercising pulmonary capillary blood volume (V_C; Lalande et al., 2012; Tedjasaputra et al., 2016), despite previous work demonstrating that highly trained individuals have similar or even lower pulmonary vascular pressures (Kovacs et al., 2009; Stickland et al., 2006). The increased V_C with similar perfusion pressures would suggest a greater ability for the pulmonary vasculature to distend and/or recruit capillaries in trained individuals. Increased V_C has also been correlated

with greater maximal exercise capacity ($\dot{V}O_{2max}$), suggesting that V_C or the pulmonary circulation may be an important determinant of $\dot{V}O_{2max}$ (La Gerche et al., 2010; Tedjasaputra et al., 2016).

Highly trained individuals demonstrate a lower PAP at a given Q during exercise compared to untrained individuals (Stickland et al., 2006), which would suggest a lower right ventricular (RV) afterload, which may enhance SV. These findings suggest that exercise training may allow for a greater reduction in PVR during exercise which would potentially reduce RV afterload during maximal exercise (Burger et al., 2001; La Gerche et al., 2010; Matsumoto et al., 1997). Previous studies have demonstrated that elevations in PVR and PAP have negative effects on $\dot{V}O_{2max}$ with Q limited by a reduction in right ventricular stroke volume (RVSV; Alkotob et al., 2006; Matsumoto et al., 1997; Tolle et al., 2008). Untrained individuals demonstrate a plateau in SV at approximately 40% of $\dot{V}O_{2max}$ (Gledhill et al., 1994). This plateau in SV could, in part, be due to relative increase in RV afterload secondary to elevated PVR and PAP. Importantly, this may be a limitation unique to untrained individuals as highly trained individuals may have superior pulmonary vascular function and therefore reduced RV afterload.

Inhaled nitric oxide (iNO) is a known selective pulmonary vasodilator (Pepke-Zaba et al., 1991) and has been shown to decrease PVR (Steudal et al., 1999). iNO has been demonstrated to acutely reduce PVR which can attribute to increases in exercise tolerance in heart failure and pulmonary hypertension (Matsumoto et al., 1997; Tolle et al., 2008). Previous work has shown no change in exercise performance with iNO in endurance trained athletes (Durand et al., 1999; Sheel et al., 2001). It has been speculated that this lack of effect is due to the already superior pulmonary vascular function in these athletes and that the pulmonary arterial vasculature has already reached an anatomical limit of dilation during exercise (Dempsey et al., 1999). Untrained healthy individuals, however, have a relatively increased PVR and PAP when compared to

highly trained individuals suggesting that at maximal exercise they could be limited, in part, by the pulmonary vasculature. To-date, no study has investigated the effects of iNO on healthy untrained individuals during exercise.

1.2 Purpose

The purpose of this study was to compare the effect of inhaled nitric oxide on maximal oxygen consumption ($\dot{V}O_{2max}$) in highly trained and untrained young healthy individuals.

1.3 Hypothesis

It was hypothesized that iNO would elicit significant increases in $\dot{V}O_{2max}$ in the untrained group while no significant changes would be observed in the highly endurance trained group. The improvement in $\dot{V}O_{2max}$ would be associated with increased maximal Q, secondary to an iNO-mediated reduction in PVR.

1.4 Delimitations

Due to the potentially learning effect that can be acquired through multiple maximal exercise tests in individuals who have never performed such tests before, the screening maximal exercise test acted as a familiarization day. The screening exercise test was also used to categorize participants into untrained and high-fit groups. In addition, the iNO day and placebo day were randomized to account for any learning or time effect.

Aerobic training influences fitness level which could confound the findings of the study. Since many of these individuals were on strict training regiments, in order to minimize the training effect, experimental trials were no more than 72 hours apart and no less than 24 hours apart. This allowed for a proper recovery between sessions while maintaining relative fitness between the two conditions (Nolan et al., 2014).

Young participants (aged 18-35) were targeted for the study as aging has been shown to impact pulmonary vascular resistance. Further, due to the known negative impacts smoking has on arterial compliance and pulmonary gas exchange, participants were excluded if they reported any smoking history (MacLay et al., 2013).

It has been demonstrated that the phase of the menstrual cycle can have an effect on exercise performance and diffusion capacity (Smekal et al., 2007; Smith et al., 2015). The research design of this study however allowed for each participant to act as their own control. This allowed for all female participants to perform both experimental trials during the same phase of their respective cycle, thus negating any effects the menstrual cycle may have on exercise performance between conditions.

1.5 Limitations

By including both men and women in the study, sample heterogeneity is a possibility since there are known differences between men and women related to vascular function, V_C, and $\dot{V}O_{2max}$ (Harms et al., 2008; Smekal et al., 2007; Smith et al., 2015). In an attempt to control for this limitation, an equal amount of men and women were recruited for both the high-fit and untrained groups.

Due to the process of titrating NO into compressed medical air for the participants to breathe during the trial, the protocol required dry air to be administered through a non-diffusing bag. Breathing dry air could cause discomfort in breathing; however, this was controlled for experimentally by using the same breathing setup for the placebo condition.

The barometric pressure was recorded before each experimental trial. Pneumotach (airflow) and gas analyzers were calibrated before each trial and verified after trials to account for any change in temperature and barometric pressure.

In order to limit participant discomfort, blood samples were not taken during the current study. This limited our ability to correct the diffusing capacity for hemoglobin. This can cause inaccuracies in the calculation of diffusing capacity, especially highly trained females, who may have lower hemoglobin at certain phases of their menstrual cycle (Smith et al., 2015).

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Chapter II: The effect of inhaled nitric oxide on maximal oxygen consumption in high-fit and untrained individuals

2.1 Introduction

At the initiation of exercise, there is an increased oxygen demand in the working muscles which is addressed by an increase in cardiac output (Q). Translocation of blood volume centrally towards the heart helps to increase both left atrium pressure (LAP) and right atrium pressure (RAP) facilitating filling of the respective ventricles, increasing stroke volume (SV) and therefore Q (Flamm et al., 1990; Gledhill et al., 1994). Mean pulmonary artery pressure (PAP) is also increased with exercise which helps facilitate blood flow through the pulmonary vasculature. With the rise in PAP, there is recruitment and distension of the pulmonary capillaries, resulting in a reduction in pulmonary vascular resistance (PVR; Reeves, 2005). A rise in PAP as a result of increasing Q can have negative effects on gas exchange as the pulmonary capillaries are susceptible to hydrostatic pulmonary edema (West, 2008). Furthermore, a large increase in LAP has also been linked to the development of pulmonary edema (Drake et al., 2002). Therefore, with increasing exercise intensity, it is imperative that the increase in LAP and PAP not be excessive in order to protect the pulmonary vasculature. LAP is typically determined from pulmonary arterial wedge pressure (PAWP), and therefore the relationship between PAP, PAWP and Q is defined by the PVR equation.

$$PVR = (PAP - PAWP) / Q$$

As previously mentioned, PVR in healthy individuals decreases with exercise, which is secondary to pulmonary vascular recruitment and distensibility (Kovacs et al., 2009; Naeije et al., 2012). However, Stickland et al. further demonstrated that the recruitment of anatomical intrapulmonary shunts may also decrease PVR during exercise (Stickland et al., 2004), though the full extent of these shunts are not known. As such, large increases in PAP during exercise are

prevented through the combination of pulmonary capillary recruitment and distension, as well as potentially the recruitment of intrapulmonary shunts.

In a untrained health adult individual, SV reaches a plateau at submaximal exercise (approximately 40% of maximal oxygen uptake; $\dot{V}O_{2max}$), and further increases in Q are attained almost exclusively through increases in heart rate (Gledhill et al., 1994). However, highly trained individuals demonstrate a continuous rise in SV up to maximal intensity (Gledhill et al., 1994; Stickland et al., 2006), which is attributed to superior diastolic function (i.e. greater preload/end-diastolic volume; EDV) as well as superior systolic function and lower afterload, which results in lower end-systolic volume (ESV; Pluim et al., 2000).

Though the left side of the heart has been the primary focus of exercise physiology research, recent work has demonstrated that pulmonary circulation and right ventricular (RV) function may be important determinants of SV during high intensity exercise. Recent work suggests that highly trained individuals demonstrate enhanced pulmonary capillary blood volume (V_C) at rest and during exercise (Lalande et al., 2012; Tedjasaputra et al., 2016). This increased V_C is present despite lower PAP at a given Q as compared to untrained individuals (Kovacs et al., 2009; Stickland et al., 2006). Greater V_C despite similar or lower PAP and PVR (D'Andrea et al., 2011; La Gerche et al., 2010; Stickland et al., 2006) would suggest greater compliance in the pulmonary vasculature in endurance trained participants compared to untrained. Previous work has demonstrated elevations in PAP and PVR have negative impacts on $\dot{V}O_{2max}$, secondary to limitations on SV and consequently Q (Alkotob et al., 2006; Matsumoto et al., 1997; Tolle et al., 2008). Elevated PAP leads to elevated RV afterload, increased right ventricular stroke work (RVSW) and a reduction in SV (Burger et al., 2001). In clinical populations, such as pulmonary hypertension, chronically elevated RV afterload results in impaired RV function and ultimately

RV failure (Hasuda et al., 2000). The difference in PAP and PVR in high-fit and untrained individuals challenges previous assumptions that exercise training strictly affects the systemic vasculature and that no pulmonary vascular adaptations are seen following training (Hagberg et al., 1988).

Inhaled nitric oxide (iNO) is a selective pulmonary vasodilator which has been shown to reduce PVR and PAP (Koizumi et al., 1994; Pepke-Zaba et al., 1991). Due to iNO's ability to selectively vasodilate the pulmonary artery, iNO has been shown to be an effective method of increasing exercise capacity in patients with pulmonary hypertension (Pepke-Zaba et al., 1991) and heart failure (Matsumoto et al., 1997). These clinical groups already have increased PVR at rest which limits the patients' ability to perform maximal exercise. However, very limited studies have been performed with iNO in health during exercise. Durand et al. performed a maximal graded exercise test on nine healthy highly trained male athletes ($\dot{V}O_{2max}$ > 60 ml · kg^{-1} · min^{-1}) and found no difference in $\dot{V}O_{2max}$ between placebo and iNO (65.0 ± 0.9 ml · kg^{-1} · min^{-1} vs 66.2 ± 1.6 ml · kg^{-1} · min^{-1}, respectively). It has been suggested the lack of change in $\dot{V}O_{2max}$ in this study was due to the high fitness level of the participants. Durrand et al. recruited highly trained individuals, which as discussed, would already have high V_C (Lalande et al., 2012; Tedjasaputra et al., 2016) and likely lower PVR (D'Andrea et al., 2011). Chronic exercise training may allow these athletes to maximally dilate the pulmonary artery without the aid of exogenous NO and thus no improvement in $\dot{V}O_{2max}$ would be observed with iNO (Dempsey, 1986). In contrast, untrained individuals with lower $\dot{V}O_{2max}$ may have higher exercise PVR due to lower NO bioavailability and as a result, may be responsive to iNO. Therefore, the purpose of the current study was to determine the effect of iNO on $\dot{V}O_{2max}$ in high-fit and untrained individuals. Higher PVR seen in untrained individuals may be indicative of lower NO bioavailability. Therefore, it

was hypothesized that iNO would increase $\dot{V}O_{2max}$ in untrained individuals, whereas, in high-fit individuals, no change would be expected with iNO. Should $\dot{V}O_{2max}$ increase with iNO in untrained individuals, this would demonstrate that the pulmonary circulation could, in part, be a limiting factor of exercise capacity in health.

2.2 Methods

2.2.1 Participants

Sixteen untrained individuals ($\dot{V}O_{2max}$: 39.8 ± 3.7 ml · kg^{-1} · min^{-1}) and sixteen high-fit individuals ($\dot{V}O_{2max}$: 63.2 ± 7.3 ml · kg^{-1} · min^{-1}) were recruited to complete the study. All participants were under 40 years of age, were non-smokers and had no known history of pulmonary, metabolic, or cardiovascular disease. Untrained individuals were categorized by a $\dot{V}O_{2max}$ between 30-45 ml · kg^{-1} · min^{-1} and high-fit individuals were categorized by a $\dot{V}O_{2max}$ greater than 55 ml · kg^{-1} · min^{-1} for females and 60 ml · kg^{-1} · min^{-1} for males. A $\dot{V}O_{2max}$ of 30-45 ml · kg^{-1} · min^{-1} is considered average for healthy inactive males and females, achieving a maximal Q of approximately 25 L · min^{-1} (Rowell et al., 1993). Further, individuals with a $\dot{V}O_{2max}$ between 30-45 are between the 10th- 50th percentile of the population according to ACSM guidelines (Kaminsky et al., 2015). Individuals with a $\dot{V}O_{2max}$ greater than 55 ml · kg^{-1} · min^{-1} for females and 60 ml · kg^{-1} · min^{-1} for males demonstrate enhanced systolic and diastolic function, and Q approximately 35 L · min^{-1} (Levine et al., 1991). The sample size was calculated from previous work in patients with chronic heart failure which demonstrated a 10 ± 1% improvement in $\dot{V}O_{2max}$ with iNO (Matsumoto et al., 1997). We assumed an effect size of approximately half of that observed in heart failure and an a priori sample size calculation determined sixteen non-endurance trained individuals would be sufficient in detecting an effect of iNO on $\dot{V}O_{2max}$ (α=0.05, β=0.8). Sixteen endurance trained individuals were used for comparison (N=32).

2.2.2 Study Design

The study was a randomized, double-blind, placebo-controlled, crossover design and received ethical approval from Health Canada and the University of Alberta Health Research Ethics Board (Biomedical Panel Pro00078715). After providing written informed consent and filling out a PAR-Q+, all participants completed four visits. Day 1 included medical history screening, pulmonary function testing, and an incremental cardiopulmonary cycle exercise test (CPET) to volitional exhaustion in order to determine $\dot{V}O_{2max}$. A supramaximal verification test was performed afterward to confirm $\dot{V}O_{2max}$ (Scharhag-Rosenberger et al., 2011). On days 2 and 3, participants completed a CPET while breathing either normoxia (placebo, day 2) or 40 ppm iNO (day 3). The order of days 2 and 3 was randomized. Participants were asked to abstain from caffeine and alcohol for at least six hours prior to testing, and any exercise 12 hours prior to testing. On Day 4 echocardiograms were conducted at rest and during sub-maximal exercise do evaluate cardiac function and right ventricular systolic pressure (RVSP) with and without iNO.

2.2.3 Cardiopulmonary Testing

The initial screening CPET consisted of 2-minute steady state resting period, followed by 2-minutes of pedaling at 50 watts with a 25-watt stepwise increase in work rate every 2-minutes until volitional exhaustion. Peak work rate was defined as the highest work rate that the subject was able to maintain for greater than 30-seconds. Following the CPET, participants were given an active cool down for 2-minutes and then rested for a further 8-minutes before completing the supramaximal verification test (Scharhag-Rosenberger et al., 2011). Participants then cycled for 1-minute at 60% of the maximum workload they had achieved on the incremental CPET. The workload was then increased 25 W above the CPET peak workload and further increased every minute until volitional exhaustion.

Experimental CPET's consisted of 2-minutes of steady-state rest, followed by 2-minutes of exercise at 60 watts. Exercise intensity was then increased to 60% of maximal peak wattage obtained during the screening CPET and increased in increments between 10-25 watts every minute until volitional exhaustion. The modified experimental CPET protocol was designed such that high-fit and untrained participants would exercise for similar durations (Guenette et al., 2007). Peak work rate was defined as the highest work rate that the subject maintained for greater than 30-seconds. Using a modified Borg scale, participants rated their perceived breathing and leg discomfort every 2-minutes and at the end of the exercise, followed by an inspiratory capacity (IC) maneuver. All standard ventilatory and cardiovascular measurements were continuously recorded and averaged in 30-second intervals. The highest 30-second average for oxygen consumption was accepted as $\dot{V}O_{2max}$ (i.e. the primary outcome of the study). Immediately after test termination, participants were asked to report their primary reason for stopping (breathing, legs, both, or other) and to select qualitative phrases that best described how their breathing felt at peak exercise (Simon et al., 1990).

All exercise tests were performed on an electronically braked cycle ergometer (Ergoselect II 1200 Ergoline, Blitz, Germany) using a cardiorespiratory metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA). Arterial oxygen saturation (SpO_2) and methemoglobin (MET-Hb) was estimated using finger pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA). METHb percentage is a measure of hemoglobin that is unable to bind oxygen and can be a by-product of iNO (Wessel et al., 1994). Heart rate was measured using 3-lead electrocardiography (CardioSoft, GE Medical Systems, Milwaukee, WI, USA), and blood pressure was taken manually while auscultating the brachial artery. Q was continuously monitored beat-by-beat and recorded in 30-second averages with impedance

cardiography (PhysioFlow, Manatec, Paris, France). Impedance cardiography is a non-invasive estimated measure of Q which has been validated with right heart catheterization (r= 0.87) and magnetic resonance imaging (Panagiotou et al., 2017).

2.2.4 Intervention

iNO has been used in many clinical populations and shown to be a selective pulmonary vasodilator (Matsumoto et al., 1997; Pepke-Zaba et al., 1991). A 40 ppm dose of iNO was given using a customized NO delivery system (SoKINOX, Vitalaire, Ontario, Canada). Briefly, the device consisted of a non-rebreathe circuit connected to a flow sensor and NO was delivered with medical grade normoxic air (~21% O_2 and balance N_2). Oxygen (O_2) was titrated in to maintain 21% inspired oxygen throughout the test. The placebo condition consisted of participants breathing medical grade normoxic gas which was delivered by the same non-rebreathing system (soKINOX) as the NO condition. Concentrations of inspired O_2, NO, and nitrogen dioxide (NO_2) gas were continuously monitored from a sample line positioned within the breathing tube immediately before the mouthpiece. Both NO and placebo tanks were set up in tandem, all identifying information was removed and both cylinder tanks appeared identical. A 5-minute wash-in was completed prior to all experimental exercise trials, regardless of condition. The lead researcher and participant were completely blinded to the condition of the trial. Only the research assistant and supervising physician were aware of the condition (placebo or iNO) on each day.

In populations such as heart failure, the greatest risk of iNO usage is that it can increase LAP resulting in increased pulmonary capillary pressure. Greater than normal pulmonary capillary pressure forces excess fluid through the pulmonary walls causing edema within the lung (Drake et al., 2002). This risk can be avoided by screening for cardiopulmonary disease

during the initial screening grade exercise test. Further, formation of NO_2 from NO and O_2 reaction can potentially lead to pulmonary edema, however, it is extremely uncommon while breathing NO at doses of ≤80 ppm (Pepke-Zaba et al., 1991; Steudal et al., 1999). As previously mentioned, throughout the trial, inspired O_2, NO and NO_2 were closely monitored with gas analyzers (soKINOX, Vitalaire, Ontario, Canada). This ensured that 40 ppm of NO was always being delivered and NO_2 never reached greater than 2 ppm. Further, NO competes with O_2 to bind with hemoglobin, creating methemoglobin (MET-Hb). An elevation in MET-Hb not only reduced oxyhemoglobin saturation, but it can also influence oxygen unloading at the muscle, by shifting the oxyhemoglobin dissociation curve to the left, promoting the retention of O_2 to hemoglobin. Thus, MET-Hb was continuously monitored using carbon-monoxide oximetry (Radical 7, Masimo, Irvine, CA, USA), and MET-Hb did not rise above 5% during any trials.

2.2.5 Pulmonary Function

Spirometry, lung volume including plethysmography and diffusing capacity for carbon monoxide (DLCO) measurements were completed as per current guidelines (Graham et al., 2017) using a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorda Linga, CA, USA). Measurements were expressed as absolute values and percent of predicted normal values (Quanjer et al., 2012).

2.2.6 Echocardiography

Echocardiographic images were collected to assess cardiac structure and function in accordance with current guidelines (Lang et al., 2015). Images were collected using a commercially available ultrasound (Vivid Q, GE Healthcare, Fairfield, CT, USA). During testing, the participant started in an upright position at rest and then proceeded to perform exercise at 60 watts on a cycle ergometer. A single-lead electrocardiograph was used during both rest and exercise. Participants rested quietly for 10 minutes prior to obtaining baseline data such

as heart rate, estimated Q (cardiac impedance), systemic arterial blood pressure (manual auscultation of brachial artery), and SpO_2 (pulse oximetry). Following baseline collection, echocardiography data were collected while participants breathed room air. Once room air data were obtained, iNO was administered and additional echocardiography data were obtained. Data were then obtained during submaximal exercise (60 watts). All echocardiograms were performed by a single experienced sonographer and were acquired within a range of 70-90 frames per second. Five consecutive cardiac cycles were recorded, and measurements were made in triplicate for each condition. These were averaged by the same sonographer who remained blinded to the experimental condition for all analysis (EchoPAC, GE Healthcare, Fairfield, CT, USA).

Left ventricular stroke volume (LVSV) was determined by multiplying the cross-sectional area of the LV outflow tract and the velocity-time integral of aortic outflow (Lang et al., 2015). Using two-dimensional echocardiography (apical 4- and 2-chamber images), left (Simpson's biplane) and right (single plane) ventricular volumes were assessed by calculating EDV and ESV. Both left and right ventricular ejection fraction was calculated using the following formula:

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

RVSP (also referred to in literature as pulmonary artery systolic pressure; PASP) was calculated using tricuspid regurgitant peak velocity measured, using continuous wave Doppler, and estimated RAP, in the following formula (Lang et al., 2015; Rudski et al., 2010):

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

RAP was estimated through imaging of the inferior vena cava (IVC) from a subcostal view at rest and during an inspiratory sniff. A value of 3 mmHg was given for RAP if the diameter of the IVC was < 2.1 cm and collapsed >50% with a sniff test. If IVC diameter was > 2.1cm and collapsed <50% with a sniff test, RAP was estimated as 15 mmHg. If either IVC diameter was >2.1 or the collapsibility was less than 50%, RAP was estimated to be 8 mmHg (Lang et al., 2015).

2.2.7 Statistical Analysis

Data are presented as mean ± standard error (SE) unless otherwise stated. Statistical significance was set a priori at $p < 0.05$. Unpaired student T-test was used to evaluate subject characteristics and pulmonary function between groups. A two-way repeated-measures analysis of the variance (ANOVA) was used to analyze the effect of placebo versus iNO on $\dot{V}O_{2max}$ in high-fit and untrained individuals (primary outcome). Secondary outcomes on submaximal measures were compared using a multifactorial repeated-measures ANOVA. A two-way ANOVA will be used for further analysis of the differences in $\dot{V}O_2$ and secondary outcomes at maximal intensity between conditions. A Bonferroni post-hoc was completed to locate differences in an ANOVA when a significant interaction was found. All statistical analysis was performed using IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY).

2.3 Results

2.3.1 Participants

Descriptive characteristics for the low- and high-fit individuals are displayed in Table 1. There were no between-group differences in age, height, and pulmonary function data. The high-fit individuals had a significantly lower BMI than the untrained individuals (Untrained: 24.0 ± 2.3 kg · m^{-2} vs. High-fit: 21.6 ± 1.4 kg · m^{-2}; $p<0.001$). Reflecting the study criteria, the high-fit group had significantly higher baseline exercise capacity (Untrained: 39.8 ± 3.7 ml · kg^{-1} · min^{-1}

vs. High-fit: 63.2 ± 7.3 ml · kg^{-1} · min^{-1}; p<0.001) and peak power output (Untrained: 231 ± 49W vs. High-fit: 328 ± 64W; p<0.01) as compared to the untrained group.

2.3.2 Primary Outcome

$\dot{V}O_{2max}$ was greater in the high-fit group (Untrained: 43.3 ± 1.0 ml · kg^{-1} · min^{-1} vs. High-fit: 66.1 ± 1.0 ml · kg^{-1} · min^{-1}, p< 0.001), but there was no effect for condition (Placebo: 54.9 ± 1.0 ml · kg^{-1} · min^{-1} vs. iNO: 54.5 ± 1.0 ml · kg^{-1} · min^{-1}, p= 0.788) and no group by condition interaction (p= 0.682).

2.3.3 Hemodynamic responses

All peak hemodynamic data are displayed in Table 2. Peak Q was higher in the high-fit (Untrained: 16.6 ± 1.0 L · min^{-1} vs. High-fit: 19.9 ± 1.0 L · min^{-1}, p= 0.018), however, no differences across treatment conditions (Placebo: 17.1 ± 1.0 L · min^{-1} vs. iNO: 19.4 ± 1.0 L · min^{-1}, p= 0.108) were observed. Further, no interaction between fitness level and iNO was observed in peak Q (p=0.674). Submaximal analysis indicated no significant difference in Q between placebo and iNO at baseline, 60 watts, or 6-min iso-time (p=0.846). Peak heart rate and mean systemic arterial pressure (MAP) were not different between groups (p= 0.08, p=0.565, respectively), and unaffected by iNO (Heart rate: condition p= 0.949, condition by group interaction p= 0.615; MAP condition p= 0.470, condition by group interaction p= 0.802).

2.3.4 Gas-exchange responses

All gas-exchange outcomes are reported in Table 2. Ventilation ($\dot{V}_E$) was significantly lower in the untrained group (Untrained: 106.6 ± 5.1 L · min^{-1} vs. High-fit: 137.4 ± 5.1 L · min^{-1}, p< 0.01), however no effect for condition (Placebo: 123.0 ± 5.1 L · min^{-1} vs. iNO: 121.0 ± 5.1 L · min^{-1}, p= 0.782) or interaction (p= 0.872) was observed. Ventilatory efficiency ($\dot{V}_E/\dot{V}CO_2$) was different between the two groups (Untrained: 28.7 ± 1.0 vs. High-fit: 26.9 ± 1.0, p= 0.027) with no significant difference between conditions (Placebo: 28.0 ± 1.0 vs. iNO: 27.6 ± 1.0, p= 0.538)

or group-by-condition interaction (p= 0.837). No submaximal differences were detected in $\dot{V}_E/\dot{V}CO_2$ between conditions in either group (p=0.681). End-tidal pressure of CO_2 ($P_{ET}CO_2$) was not different between iNO and placebo in either high- or low fit group at submaximal workloads (p=0.298), as well as at peak exercise (p=0.932). Lastly, breathing frequency had a main effect for group (Untrained: 44 ± 1.0 breaths · min^{-1} vs. High-fit: 52 ± 1.0 breaths · min^{-1}, p< 0.001) with no difference between condition (p= 0.632) or group by condition interaction (p= 0.945).

SpO_2 was not different between groups (Untrained: 95 ± 1% vs. High-fit: 94 ± 1%, p=0.277) and no difference was observed between condition (Placebo: 95 ± 1% vs. iNO: 94 ± 1%, p= 0.185). Further, no group by condition interaction was observed (p= 0.777). Lastly. at submaximal workloads, there was no difference in SpO_2 between placebo and iNO in either high-fit or untrained group (p=0.613).

2.3.5 Echocardiography

All echocardiography results are listed in Table 3. Echocardiography data was collected on a subset of participants (n=13) in which clear tricuspid regurgitation signal could be obtained. Pooled data of all participants demonstrated that resting right ventricular systolic pressure (RVSP) was significantly lower with iNO at rest (Placebo: 7.55 ± 0.86 mmHg vs. iNO: 6.35 ± 0.72 mmHg, p< 0.001) and submaximal exercise (Placebo: 10.15 ± 1.08 mmHg vs. iNO: 7.29 ± 0.84 mmHg, p= 0.011). There was however no significant effect of condition on right ventricle stroke volume (RVSV; p= 0.936) or right ventricle ejection fraction (RVEF; p= 0.542). Left ventricular function was not different between conditions. Due to the small sample size, no statistics were run comparing high-fit versus untrained groups.

2.3.6 Sex-Differences

Table 4 report data separated by sex to examine potential sex differences. $\dot{V}O_{2max}$ was significantly different between men and women (Male: 2.39 ± 0.06 vs. Female: 1.80 ± 0.06 L · min^{-1}; p<0.001), however there was no sex by condition interaction (p=0.551). Further, no difference in Q estimated using impedance cardiography was determined between men and women (Male: 13.4 ± 0.7 vs. Female: 11.3 ± 0.6 L · min^{-1}; p=0.054) nor was a sex by condition interaction observed (p=0.659). These data indicate that the responses to iNO are similar between men and women.

2.4 Discussion

The current study aimed to evaluate the effects of pulmonary vasodilation using iNO on $\dot{V}O_{2max}$ in healthy high-fit and untrained individuals, and three key findings emerge. First, despite reducing RVSP, iNO had no effect on $\dot{V}O_{2max}$ in either the untrained or high-fit participants. Secondly, no changes in Q were observed in either group when iNO was compared to placebo. Lastly, neither $\dot{V}_E/\dot{V}CO_2$ nor SpO_2 was affected with iNO in either group, suggesting no significant changes in ventilation-perfusion ($\dot{V}_A/\dot{Q}$) matching with iNO. Collectively, these data suggest that regardless of fitness level, $\dot{V}O_{2max}$ in healthy individuals is not limited by the pulmonary circulation or the right ventricle.

2.4.1 Effect of iNO on hemodynamic function during exercise

It has been well documented that endurance trained athletes have superior cardiovascular function due to adaptations from chronic exercise training (Gledhill et al., 1994; Levine et al., 1991; Stickland et al., 2006). Previous work has suggested that the pulmonary circulation is actively influenced by circulating vasodilators (Polak et al., 1992), which could be affected by exercise training. In the current study, iNO was confirmed to be an effective pulmonary vasodilator, as RVSP was reduced both at rest and during submaximal exercise with iNO. This

reduction in RVSP, with no change in Q, would reflect a reduction in PVR, and in turn a likely decrease in RV afterload. By reducing afterload, a decrease in RVESV and an increase in RVSV would be expected, however, this was not observed. These results indicate that the increase in afterload demonstrated during exercise on the RV does not seem to be a limiting to Q, and subsequently $\dot{V}O_{2max}$.

Contrary to our hypothesis, the reduction in RVSP with iNO did not translate into enhanced SV, Q and greater $\dot{V}O_{2max}$ in untrained individuals. The lack of change in $\dot{V}O_{2max}$ seen in previous studies with iNO in high-fit individuals (Durand et al., 1999; Sheel et al., 2001) was thought to be due to these individuals achieving maximal anatomical dilation of the pulmonary artery through exercise (Dempsey et al., 1999). However, in the current study, it was hypothesized that untrained individuals do not normally experience this maximal dilation, and that a reduction in PVR with iNO would facilitate greater Q, and thus an increased $\dot{V}O_{2max}$. While iNO reduced RVSP, this did not translate into a change in RV hemodynamic function (i.e. Q, SV) which indicates that regardless of fitness level, the RV does not seem to limit exercise capacity in healthy subjects.

2.4.2 Effect of iNO on ventilation during exercise

During incremental exercise, exercise-induced arterial hypoxemia (EIAH; decrease in arterial oxygen saturation greater than 5%) can result in a reduction in $\dot{V}O_{2max}$ (Dempsey et al., 1999). Previous work has demonstrated that $\dot{V}_A/\dot{Q}$ mismatch accounts for most of the gas exchange impairment commonly seen in high endurance trained individuals (Hopkins et al., 1994; Rice et al., 1999). Though the exact mechanism for $\dot{V}_A/\dot{Q}$ mismatch is not known, previous work has suggested high pulmonary vascular pressure, leading to pulmonary edema could be the cause for the increased alveolar-arterial difference for oxygen (A-aDO_2; Wagner et al., 1986).

However, few studies have examined the effects of iNO during exercise in healthy humans. Neither Durand et al. (1999) nor Sheel et al. (2001) reported any significant change in A-aDO_2 in highly trained individuals with iNO during exercise. The current study supports these findings, as no changes in SpO_2 were seen in either the highly trained or untrained individuals with iNO. Had exercise-induced pulmonary edema been significant, we would have expected that SpO_2 would have improved with the iNO intervention, as iNO would have reduced PAP and thus edema (Wagner et al., 1986).

Additionally, $\dot{V}_E/\dot{V}CO_2$ has been shown to be an indicator of $\dot{V}_A/\dot{Q}$ mismatch (Lewis et al., 2008; Neder et al., 2016). A disproportionate increase in $\dot{V}_E$ with respect to carbon dioxide production ($\dot{V}CO_2$) suggests greater dead space (areas of ventilation with little to no perfusion). With increased greater perfusion of the lung, dead space should have been reduced and therefore we would have expected $\dot{V}_E/\dot{V}CO_2$ to be reduced with iNO. However, shunt (i.e. areas of perfusion with little or no alveolar ventilation) may have occurred with iNO which would have increased $\dot{V}_A/\dot{Q}$ mismatch. In the current study, no difference was seen in $\dot{V}_E/\dot{V}CO_2$ between iNO and placebo in either high-fit or untrained groups suggesting no effect of iNO on $\dot{V}_A/\dot{Q}$ matching in health. Our findings of a reduction in RVSP during exercise with iNO, combined with previous work demonstrating no effect of iNO on A-aDO_2 at peak exercise (Durand et al., 1999; Sheel et al., 2001) question the significance of exercise-induced pulmonary edema as a major contributor to gas exchange impairment in health.

2.4.3 Limitations

Impedance cardiography was used as a non-invasive technique to determine Q during the exercise trials. Impedance cardiography is an indirect estimation of Q, using changes in electrical conductivity of the thorax to estimate blood flow through the heart to calculate Q (Charloux et

al., 2000). Though not as robust as the direct Fick method which is considered the gold standard for measuring Q, impedance cardiography has been strongly correlated with direct Fick method in healthy populations at rest (r=0.87; Panagiotou et al., 2017) and during exercise (r=0.94; Charloux et al., 2000; Richard et al., 2001). Using a reliable, non-invasive method for estimating Q avoided unnecessary discomfort for the participants partaking in the study without compromising the validity of the primary outcome (i.e. $\dot{V}O_{2max}$).

SpO_2 monitored using pulse oximetry was similar between iNO and placebo in both groups suggesting partial pressure of oxygen (P_aO_2) was not likely impacted by iNO. However, SpO_2 has limited sensitivity to detect minor changes in oxygenation (Mardirossian et al., 1992). The absence of blood gas analysis and direct measure of A-aDO_2 limits the scope of this study in determining if gas exchange was affected by iNO in either group. Further, due to the competitive binding of NO and CO on hemoglobin, it is not possible to use the multiple-F_iO_2 technique to calculate changes in capillary blood volume (V_C) while breathing iNO. As such, we were unable to directly confirm that iNO increased V_C at rest or during exercise.

Power calculations were calculated *a priori* using previous iNO exercise data in heart failure patients (Matsumoto et al., 1997), and assuming the iNO intervention would be half as effective in healthy individuals. These calculations indicated a sample size of sixteen untrained individuals would be sufficient to detect an effect of iNO on $\dot{V}O_{2max}$ (α=0.05, β=0.8). Sixteen high-fit individuals were then recruited for comparison (N=32). A *post-hoc* power calculation was completed after data collection and with a mean difference of 0.00 ± 0.05 L · min^{-1} in absolute $\dot{V}O_{2max}$ in the untrained group. The sample size determined from the power calculation indicated that more than 200 participants would have to be recruited to find a significant difference between iNO and placebo in the untrained group. From this *post-hoc* analysis, we can

conclude that the lack of change in $\dot{V}O_{2max}$ demonstrated with iNO in untrained individuals is not likely due to being statistically underpowered.

Consistent with previous iNO work (Durand et al., 1999; Matsumoto et al., 1997; Pepke-Zaba et al., 1991; Sheel et al., 2001; Tolle et al., 2008), 40 ppm of iNO was administered during exercise trials. The echocardiography data demonstrated that 40 ppm of iNO was successful at reducing RVSP by 3.0 mmHg during exercise, while NO_2 and MET-Hb were maintained well below safety levels. It is possible that a higher level of iNO may have resulted in a greater reduction in RVSP and potentially an improvement in $\dot{V}O_{2max}$. Though the literature has shown that an upwards of 80 ppm of iNO can be administered before the risk of pulmonary edema due to increased NO_2 formation (Steudal et al., 1999), doses up to 80 ppm have not been shown to be any more effective at reducing PAP as compared to 40 ppm (Steudal et al., 1999). It is possible that higher levels of iNO may have resulted in greater reductions in RVSP and translated into changes in $\dot{V}O_{2max}$. Further, due to the challenge in obtaining echocardiographic images of tricuspid regurgitation in young healthy participants, only 13 individuals had sufficient tricuspid regurgitation for RVSP analysis with iNO. Due to the limited number of data between group comparisons (i.e. trained vs. untrained) of the iNO response was not possible.

The menstrual cycle has been shown to affect $\dot{V}O_{2max}$ and diffusion capacity in females (Smith et al., 2015). In the current study, the menstrual cycle was not standardized between participants for the exercise trials. However, since the study was a randomized cross over design, each participant acted as their own control. For all females, each experimental trial was completed within the same phase (i.e. within 2-4 days) for a given participant so as to limit menstrual cycle variance on $\dot{V}O_{2max}$. Contraceptive usage was also tracked as an indication of hormone regulation during experimental trials.

2.5 Conclusion

This study examined the effect of iNO on $\dot{V}O_{2max}$ in untrained and highly trained individuals during incremental exercise. While iNO decreased RVSP at rest and during submaximal exercise, iNO did not significantly affect $\dot{V}O_{2max}$ in either the highly trained or untrained groups. These findings suggest that regardless of fitness level, $\dot{V}O_{2max}$ is not limited by the pulmonary circulation or the right ventricle.

Table 1. Subject characteristics and pulmonary function in high- and untrained participants

	Untrained		High-Fit		
	Mean	%Predicted	Mean	%Predicted	P-value
N (male, female)	8,8		8,8		
Age (years)	25.6 ± 4.0		27.0 ± 6.2		0.446
Height (m)	1.71 ± 0.1		1.72 ± 0.1		0.651
BMI (kg/m^2)	24.0 ± 2.3		21.6 ± 1.4		<0.01*
FVC (L)	4.94 ± 1.2	102.8 ± 11.4	4.99 ± 1.1	103.8 ± 10.6	0.925
FEV_1 (L)	3.98 ± 0.8	99.3 ± 10.4	4.00 ± 0.9	99.8 ± 10.6	0.967
FEV_1/FVC (%)	81.3 ± 6.4	96.4 ± 6.6	80.3 ± 7.5	95.9 ± 8.6	0.688
DLCO ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	28.7 ± 7.3	86.3 ± 11.9	32.6 ± 10.1	96.3 ± 16.1	0.225
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.81 ± 0.62	101.8 ± 11.8	4.06 ± 0.98	147.9 ± 18.6	<0.01*
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	39.8 ± 3.7	95.2 ± 6.6	63.2 ± 7.3	152.7 ± 14.6	<0.01*
Power Output (Watts)	231 ± 49	118 ± 17	328 ± 64	174 ± 36	<0.01*
RER	1.24 ± 0.1		1.17 ± 0.1		<0.01*

Values are expressed as the mean ± SD. 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. *Significantly different than Untrained group ($p<0.05$)

Table 2. Peak physiological and performance responses with placebo and iNO in high-fit and untrained participants

	Low		High		P-value		
Variable	Placebo	iNO	Placebo	iNO	Group	Condition	Interaction
Power Output (W)	220 ± 14.3	222 ± 14.6	329 ± 18.3	328 ± 18.4	<0.001*	0.992	0.917
$\dot{V}O_{2max}$ (L·min^{-1})	3.06 ± 0.18	3.06 ± 0.17	4.32 ± 0.24	4.26 ± 0.23	<0.001*	0.886	0.882
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	43.2 ± 1.2	43.4 ± 1.4	66.6 ± 1.7	65.6 ± 1.5	<0.001*	0.788	0.682
$\dot{V}CO_2$ (L·min^{-1})	3.76 ± 0.25	3.77 ± 0.23	5.09 ± 0.28	5.06 ± 0.25	<0.001*	0.977	0.940
RER	1.23 ± 0.01	1.25 ± 0.02	1.19 ± 0.01	1.20 ± 0.01	0.003*	0.476	0.751
$\dot{V}E$ (L·min^{-1})	107.0 ± 6.39	106.2 ± 5.78	140.0 ± 8.23	135.8 ± 8.10	<0.001*	0.782	0.872
Vt (L)	2.59 ± 0.18	2.69 ± 0.18	2.91 ± 0.17	2.88 ± 0.16	0.143	0.844	0.691
fb	44.5 ± 1.7	43.8 ± 2.0	52.4 ± 1.8	51.4 ± 1.7	<0.001*	0.632	0.945
$\dot{V}E/\dot{V}CO_2$	28.8 ± 0.93	28.5 ± 0.89	27.3 ± 0.53	26.6 ± 0.59	0.027*	0.538	0.837
$\dot{V}E/\dot{V}CO_{2Nadir}$	25.0 ± 0.75	24.9 ± 0.71	24.1 ± 0.39	23.9 ± 0.47	0.123	0.836	1.000
$PETCO_2$ (mmHg)	36.2 ± 1.15	36.8 ± 1.09	37.9 ± 0.77	38.7 ± 0.70	0.063	0.487	0.932
SpO_2 (%)	95.3 ± 1.03	94.3 ± 0.85	94.5 ± 0.74	93.1 ± 0.98	0.277	0.185	0.777
Q (L·min^{-1})	15.7 ± 1.12	17.4 ± 1.34	18.5 ± 1.04	21.3 ± 1.73	0.018*	0.108	0.674
HR (beats·min^{-1})	183 ± 2	184 ± 3	180 ± 2	179 ± 3	0.077	0.949	0.615
MAP (mmHg)	105 ± 2	102 ± 3	103 ± 2	102 ± 2	0.565	0.470	0.802
Dyspnea	7.3 ± 0.45	6.6 ± 0.49	8.6 ± 0.36	8.6 ± 0.41	<0.001*	0.472	0.388
Leg Discomfort	9.1 ± 0.20	9.1 ± 0.32	8.9 ± 0.31	9.0 ± 0.39	0.690	1.000	0.842

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; $PETCO_2$: Pressure of end-tidal carbon dioxide; fb: Breathing frequency; Vt: Tidal volume; HR: Heart rate; Q: Cardiac output; SpO_2: Percent arterial oxygen saturation; MAP: Mean arterial pressure. *Signifies p<0.05.

Table 3. Echocardiography during rest and submaximal exercise with placebo and iNO in participants (n=13)

	Rest			Exercise at 60W		
	Placebo	iNO	P-value	Placebo	iNO	P-value
Right Ventricle						
RVSP (mmHg)	7.55 ± 0.86	6.35 ± 0.72	<0.001*	10.15 ± 3.73	7.29 ± 0.84	0.011*
Q ($L \cdot min^{-1}$)	1.13 ± 0.1	1.15 ± 0.1	0.921	1.36 ± 0.1	1.58 ± 0.1	0.119
ESV (mL)	16.3 ± 1.5	14.7 ± 1.7	0.212	12.2 ± 1.5	10.1 ± 0.8	0.488
EDV (mL)	35.3 ± 2.5	33.5 ± 3.7	0.520	27.5 ± 2.5	27.3 ± 2.5	0.700
SV (mL)	19.0 ± 1.3	18.8 ± 2.3	0.936	15.3 ± 1.1	17.2 ± 1.9	0.178
EF (%)	54 ± 2	52 ± 3	0.542	55 ± 2	61.6 ± 2.7	0.213
TAPSE (cm)	2.3 ± 0.1	2.3 ± 0.1	0.864	2.4 ± 0.1	2.4 ± 0.1	0.317
Left Ventricle						
ESV (mL)	28.9 ± 2.5	27.2 ± 2.0	0.288	24.1 ± 1.4	23.7 ± 1.6	0.776
EDV (mL)	84.3 ± 6.1	85.2 ± 5.6	0.730	89.7 ± 4.4	90.5 ± 4.5	0.816
SV (mL)	55.4 ± 4.1	58.0 ± 4.0	0.178	65.5 ± 3.5	66.8 ± 3.2	0.700
EF (%)	66 ± 1	68 ± 1	0.146	73 ± 1	74 ± 1	0.464

Values are expressed as the mean ± SE. RVSP: Right ventricular systolic pressure; ESV: End-systolic volume; EDV: End-diastolic volume; SV: Stroke volume; EF: Ejection fraction; TAPSE: Tricuspid annular plane systolic excursion. *Signifies $p<0.05$.

Table 4. Peak physiological and performance responses with placebo and iNO in male and female participants

	Male		Female		P-value	
Variable	Placebo	iNO	Placebo	iNO	Sex	Interaction
Power Output (W)	328 ± 17.2	329 ± 16.9	222 ± 16.2	221 ± 15.6	<0.001*	0.583
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	4.32 ± 0.24	4.29 ± 0.21	3.05 ± 0.17	3.03 ± 0.18	<0.001*	0.551
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	57.9 ± 3.5	57.5 ± 3.2	51.9 ± 3.1	51.5 ± 3.1	0.191	0.964
$\dot{V}E$ ($L \cdot min^{-1}$)	145.1 ± 7.78	142.9 ± 6.80	100.9 ± 4.11	99.1 ± 4.23	<0.001*	0.893
$\dot{V}E/\dot{V}CO_2$	27.2 ± 0.38	27.1 ± 0.56	28.9 ± 0.99	28.1 ± 0.96	0.210	0.232
$\dot{V}E/\dot{V}CO_{2Nadir}$	23.1 ± 0.31	23.1 ± 0.47	26.0 ± 0.61	25.7 ± 0.55	<0.001*	0.390
SpO_2 (%)	94.7 ± 0.68	93.8 ± 0.96	95.1 ± 1.07	93.7 ± 0.88	0.687	0.900
Q ($L \cdot min^{-1}$)	19.2 ± 1.20	23.1 ± 1.87	15.5 ± 0.81	16.3 ± 0.83	0.002*	0.051
HR ($beats \cdot min^{-1}$)	184 ± 2	184 ± 3	179 ± 2	180 ± 3	0.185	0.738
Dyspnea	8.1 ± 0.48	7.8 ± 0.51	7.7 ± 0.39	7.4 ± 0.53	0.493	1.000
Leg Discomfort	9.3 ± 0.15	9.5 ± 0.16	8.8 ± 0.32	8.6 ± 0.45	0.069	0.168

Values are expressed as mean ± SE. $\dot{V}O_{2max}$: Maximal oxygen consumption; $\dot{V}E$: Ventilation; HR: Heart rate; Q: Cardiac output; SpO_2: Percent arterial oxygen saturation. *Signifies $p<0.05$.

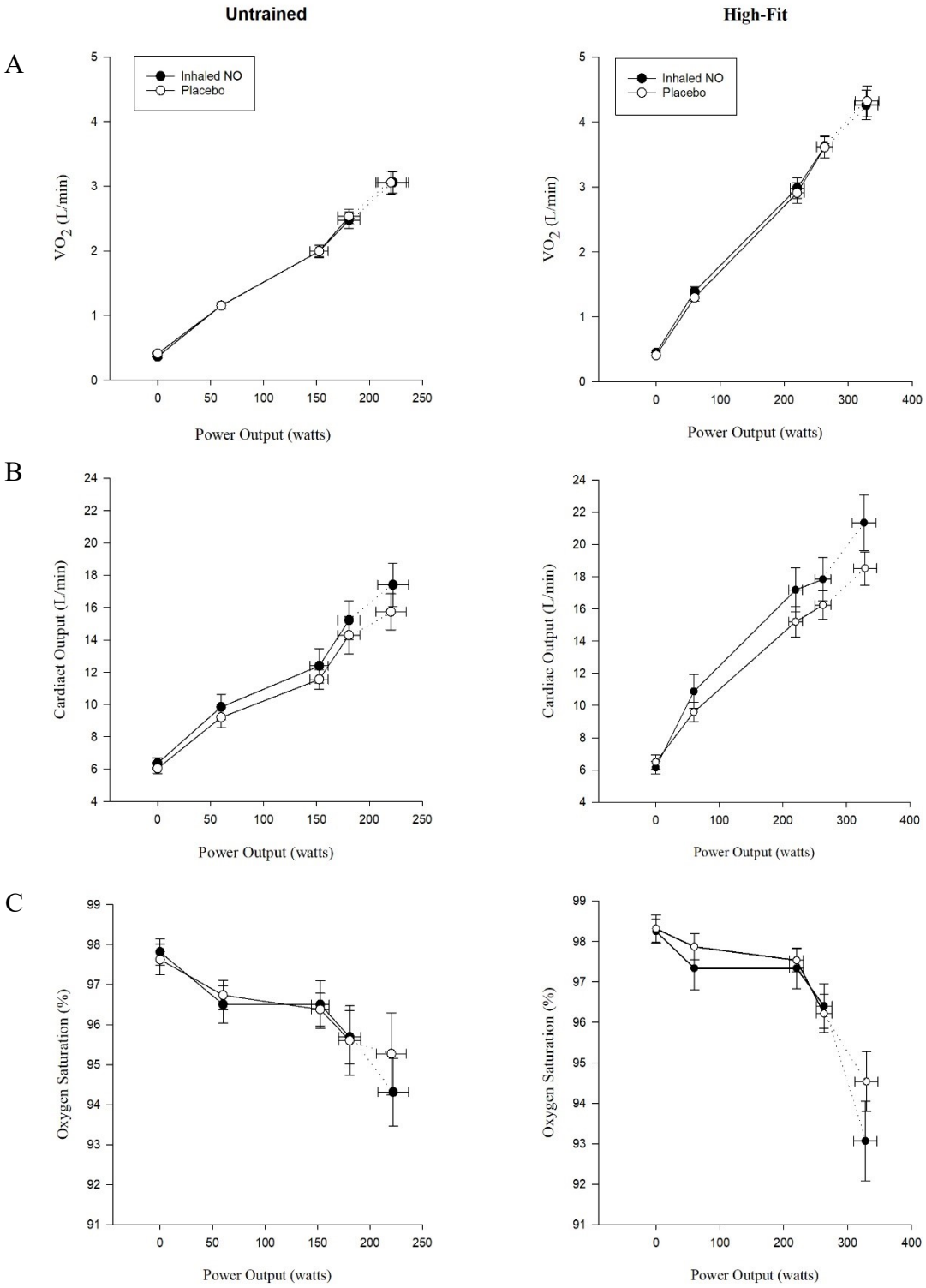

Figure 1A. $\dot{V}O_2$ response to placebo and iNO at baseline and during exercise at 60 watts, 4-minute iso-time, 6-minute iso-time, and $\dot{V}O_{2max}$ in high- and untrained individuals. B. Cardiac output response to placebo and iNO at baseline and during exercise at 60 watts, 4-minute iso-time, 6-minute iso-time, and $\dot{V}O_{2max}$ in high- and untrained individuals. C. Arterial oxygen saturation response to placebo and iNO at baseline and during exercise at 60 watts, 4-minute iso-time, 6-minute iso-time, and $\dot{V}O_{2max}$ in high- and untrained individuals. Values are expressed as mean ± SE.

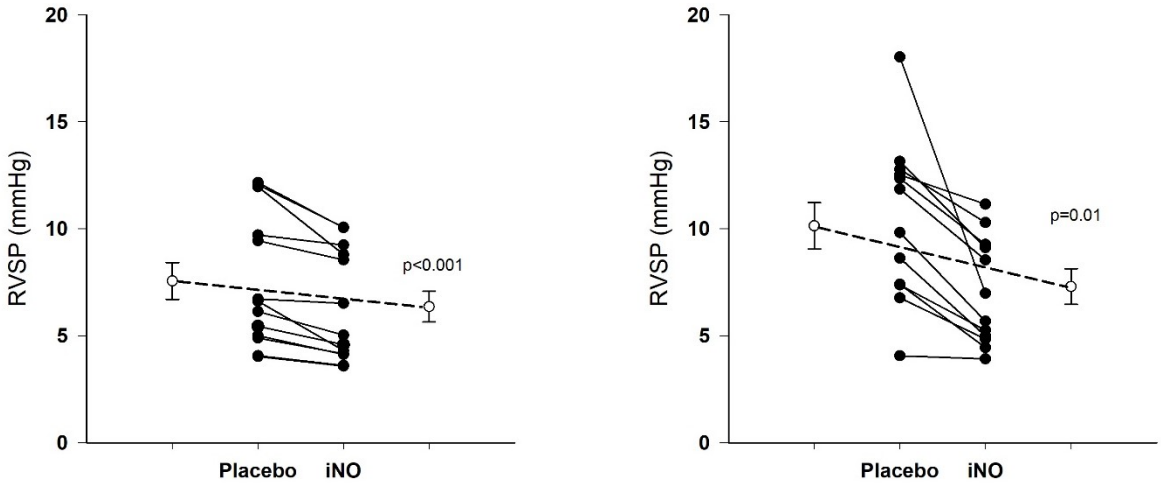

Figure 2. Effect of iNO and placebo on pulmonary artery systolic pressure at rest and during exercise at 60 watts. * signifies overall $p<0.05$

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Chapter III: General Discussion

3.1 Effect of Inhaled Nitric Oxide on Maximal Oxygen Consumption in High- and Untrained Individuals

The purpose of this study was to evaluate the effects of inhaled nitric oxide (iNO) on maximal oxygen consumption ($\dot{V}O_{2max}$) in untrained individuals as compared to high-fit individuals. We found that despite iNO decreasing right ventricular systolic pressure (RVSP) at rest and during exercise, iNO did not have an effect on $\dot{V}O_{2max}$ in either group. This was in contrast to our hypothesis in which we predicted that $\dot{V}O_{2max}$ would improve with iNO in the untrained group. However, these findings were congruent with previous research that showed no change in $\dot{V}O_{2max}$ in highly trained individuals (Durand et al., 1999).

3.1.1 Effect of Inhaled Nitric Oxide on the Right Ventricle

To our knowledge, our study is the first to examine the effect of iNO on right ventricle (RV) function during exercise in health. The current study aimed to examine the potential limitation that the RV may have on exercise capacity in healthy individuals. Previous literature demonstrates that individuals with chronically elevated pulmonary artery pressure (PAP) due to various chronic disease (i.e chronic obstructive pulmonary disease, heart failure, pulmonary hypertension) demonstrate an inverse relationship between PAP and right ventricular ejection fraction (RVEF) due to elevations in afterload (Burger et al., 1997; Nagel et al., 1996). A chronic increase in afterload leads to reduced stroke volume (SV), limiting cardiac output (Q) and decreasing exercise capacity (Matsumoto et al., 1997; Tolle et al., 2008). The pathophysiologic elevations in PAP measured in disease are not typically observed in healthy individuals, however differences are shown in healthy individuals with varying fitness levels (D'Andrea et al., 2011; Kovacs et al., 2009). During exercise, increased PAP would cause increased afterload on the RV. This increased afterload would result in increased in right ventricle stroke work (RVSW) and decreased RVEF (Burger et al., 2001). RVSW is defined as the amount of work required to

produce a given SV (Ibe et al., 2018). RVSW relative to PAP and right atrial pressure (RAP) is described in the equation below (Chemla et al., 2013):

$$RVSW = (PAP\text{-}RAP) \times SV$$

As depicted in the equation, the greater the difference between PAP and RAP, the greater the RVSW. At rest, RVSW is lower than left ventricular stroke work (LVSW; La Gerche et al., 2011). However, during exercise, RVSW increases at a disproportionate rate compared to LVSW (La Gerche et al., 2013). Further, endurance athletes have been shown to demonstrate higher RV end-systolic wall stress than non-trained participants (La Gerche et al., 2011). Greater RV wall stress has been shown to cause greater RV remodeling in endurance trained individuals, leading to greater RV mass (La Gerche et al., 2011). Importantly however, at matched power output, endurance trained individuals demonstrate similar RV function to untrained individuals (La Gerche et al., 2011; Stickland et al., 2006). The disproportionate increase in RVSW would suggest that the RV may limit exercise capacity, however this was not demonstrated in the current study.

An estimation of RVSW (Chemla et al., 2013) calculated using RVSP data from the current study determined a significant reduction in RVSW with iNO at rest (Placebo: 801.0 ± 122.4 mmHg · mL vs. iNO: 641.7 ± 84.2 mmHg · mL; p=0.01) and during submaximal exercise (Placebo: 1037.9 ± 118.1 mmHg · mL vs. iNO: 771.1 ± 108.3 mmHg · mL; p=0.02). If the disproportionate increase in RVSW was a limiting component of exercise capacity, the 26% reduction in RVSW with iNO would have elicited an increase in $\dot{V}O_{2max}$ due a decreased afterload and corresponding increase in SV. Assuming the reduction in RVSW was maintained during maximal exercise with iNO, the lack of change seen in $\dot{V}O_{2max}$ indicates that regardless of

the high RVSW demonstrated during maximal exercise, the afterload experienced by the RV does not significantly impact exercise capacity in healthy individuals.

An exploratory analysis comparing the change in RVSP with iNO in the untrained and high-fit groups suggests a difference, though not significant, between the two groups (Untrained: 1.76 ± 0.46 mmHg vs. High-fit: 0.85 ± 0.23 mmHg; p=0.08). This trend would suggest that untrained individuals may be more responsive to exogenous NO. RAP was estimated using imaging of the inferior vena cava (IVC) at rest and during an inspiratory sniff. No difference between untrained and high-fit was seen in the estimation of RAP at rest in the current study. Invasive measure of RAP has shown no significant difference between fitness levels during incremental exercise (Stickland et al., 2006). The trend seen in a greater RVSP response in untrained individuals compared to high-fit however did not translate into any changes in $\dot{V}O_{2max}$ secondary to increased Q. Unfortunately, due to the technical difficulties in acquiring tricuspid regurgitation through echocardiography in healthy individuals, the sample size in the current study was not large enough to detect these differences in RVSP between untrained individuals (n=5) as compared to high-fit individuals (n=8). As such the difference between high- and untrained groups and the effects of iNO on pulmonary hemodynamics remains speculation.

3.1.2 Vasoactive Regulation of Pulmonary Vasculature

The influence of exercise training and fitness on pulmonary vascular regulation is not fully understood. Alpha dopamine receptors have been shown to effect regulation of vascular tone, specifically in the pulmonary artery in vitro during isolated lung manipulations in rats (Polak et al., 1992). Further experiments in humans at resting conditions demonstrated that during L-NMMA (a NO synthase inhibitor), significantly increased pulmonary vascular resistance (PVR), while decreasing Q secondary to a decrease in SV (Stamler et al., 1994). These

studies demonstrate the importance of various mediators on pulmonary hemodynamics. This is contrary to original work which suggested pulmonary vasculature regulation was a passive process, and as such exogenous mediators (such as iNO) would not have significant effects (Wagner et al., 1979, West, 2008).

Though contrary to our hypothesis, the lack of change in $\dot{V}O_{2max}$ with iNO seen in the current study is supported by previous studies investigating the hemodynamic effects of other pulmonary vasodilators during exercise in health. Hsu et al. (2006) administered sildenafil (phosphodiesterace-5 inhibitor) in trained individuals during submaximal exercise. Hsu et al. (2006) determined that no change in cardiovascular or performance outcomes was observed during normoxic conditions. However, sildenafil increased SV, Q, and arterial oxygen saturation (SpO_2) during submaximal exercise at a constant work rate when the participants were exposed to hypoxic conditions (F_iO_2= 0.128). Participants also achieved a significant increase in exercise performance during a 6 km bicycling time trial with sildenafil at the same simulated altitude (Hsu et al., 2006). During simulated altitude (i.e. hypoxia), hypoxic pulmonary vasoconstriction (HPV) has been shown to occur (Wagner et al., 1986). The increased regulation on pulmonary vascular tone is thought to optimize blood flow to areas of greatest ventilation in order to optimize gas exchange and reduce hypoxemia (Groves et al., 1987; Wagner et al., 1986). Though HPV is thought to be a protective mechanism to maximize ventilation-perfusion ($\dot{V}_A/\dot{Q}$) matching, the increase in vascular tone also increases PVR, and as such afterload on the RV. Similar to clinical populations, an increase in PVR reduces Q secondary to reduced SV and decreases exercise capacity. The increase in exercise capacity demonstrated during hypoxia after the administration of sildenafil suggests a release of HPV, allowing or an increase in SV and Q (Hsu et al., 2006).

Further, recent work in our lab has demonstrated that exogenous low-dose dopamine during exercise did not influence exercising pulmonary capillary blood volume (V_C) or exercise tolerance in recreationally active individuals (Michealchuk, under review). During dopamine blockade, exercise tolerance was reduced, suggesting dopamine has a regulatory effect on the pulmonary vasculature. When combined with the current study it has been demonstrated that healthy individuals are not limited by the RV, however, increases to PVR outside of normal physiological limits due to hypoxia (Hsu et al., 2006) or reduced vasodilatory regulators (Michaelchcuk, under review) may reduce exercise capacity.

3.1.3 iNO Dosage during Exercise

Previous work using iNO as a pulmonary vasodilator in clinical populations have demonstrated increases in exercise capacity with 40 ppm (Alkotob et al., 2006; Matsumoto et al., 1997; Pepke-Zaba et al., 1991; Tolle et al., 2008). Due to the reaction of NO with oxygen species, concentrations above 80 ppm have shown to drastically increase NO_2 production, which is known to cause pulmonary edema (Steudal et al., 1999). iNO administered in lambs showed no significant difference in PVR when concentrations ranged from 40-80 ppm (Koizumi et al., 1994). Previous research in healthy humans at rest during hypoxic exposure however demonstrated PAP decreased from 28 mmHg to 20 mmHg when breathing 40 ppm but saw a further decrease to 18 mmHg when exposed to 80 ppm (Frostell et al., 1993). Demonstrated by these findings, increasing the dosage of iNO in the current study from 40 ppm to 80 ppm may have elicited marginally greater reduction in RVSP in the participants, however this would have been at the cost of elevated NO_2 species which may have increased participant risk.

In the current study, RVSP was decreased from 7.55 mmHg to 6.35 mmHg at rest and 10.15 mmHg to 7.29 mmHg during submaximal exercise. Though significant, these changes in RVSP may not have been substantial to unload the RV and impact SV. With respect to Frostell et al. (1993) findings, a greater dosage of 80 ppm may have demonstrated greater impacts on RVSP, however with the complexity of exercise, increased NO_2 and methemoglobin (MET-Hb) most likely would have occurred.

3.1.4 Sex differences with iNO

Women have smaller lungs and airways than men even when matched for height (Bouwsema et al., 2017). Numerous studies have examined the implications of this anatomical difference on pulmonary gas exchange and ventilatory mechanics during exercise. Exercise-induced arterial hypoxemia (EIAH) has been shown to be much more prevalent in females, specifically at much lower fitness levels then matched males (Hopkins et al., 2004). Proposed mechanisms of EIAH include relative alveolar hypoventilation, $\dot{V}_A/\dot{Q}$ inequality, and diffusion limitations during exercise (Guenette et al., 2007). To summarize, women have a higher work of breathing at the same minute ventilation ($\dot{V}_E$) and are more prone to EIAH than males (Dominelli et al., 2015; Guenette et al., 2007). Despite a large body of work investigating sex differences in respiratory mechanics and mechanisms of EIAH, there is minimal work investigating sex differences in pulmonary vascular limitations to exercise. To our knowledge, the current study is the first to include women as participants when examining the effect of iNO during exercise. We conducted an exploratory analysis to determine if there were any sex differences in the iNO response during exercise (see Table 4). As expected, men had greater absolute $\dot{V}O_{2max}$ than women, however there was no between condition effect (p=0.952), and no group by condition interaction (p=0.879). Though two highly trained women experienced EIAH (a drop in SpO_2 greater than 5%; Dempsey et al., 1999), this was unaffected by iNO. Combined, these findings

suggest that the bioavailability of NO during exercise and the iNO response to exercise is not different between men and women.

3.2 Limitations and Considerations

The power calculation for the current study was based on the expected difference of iNO on $\dot{V}O_{2max}$. As a result, we were not appropriately powered to detect a change in iNO between high-fit and untrained groups in respect to RVSP. A *post-hoc* power calculation completed after data collection, with a mean difference of 1.20 ± 0.91 mmHg (p=0.07) RVSP between untrained and high-fit group in response to iNO determined that 25 participants in each group would have had to be recruited to find a significant difference in iNO response. These data suggest that there may be a difference in the RVSP response to iNO between groups; however, this needs to be confirmed with a larger sample.

In healthy individuals, technical challenges occur when trying to estimate tricuspid regurgitation. Previous work has shown that semi-supine is the most echogenic position for healthy individuals, however this becomes problematic during exercise (Chemla et al., 2004). Further, with increased $\dot{V}_E$ that occurs during exercise, imaging can be obscured by the inflation of the lungs, further increasing challenges to imaging (Lang et al., 2015). Using an experienced echocardiographer, the current study screened all participants for being echogenic to imaging tricuspid regurgitation, and only those participants in which clear images were used in analysis.

One of the major limitations to previous impedance cardiography devices is the reliance on baseline impedance for calibration and estimation of SV (RV). Traditionally this makes the device susceptible to problematic variability based on hydration status (thoracic blood volume) and resistivity of the blood (Hsu et al., 2006). This is important in the current study, as iNO is expected to change V_C which would greatly change baseline impedance. Physioflow however,

does not use baseline impedance for either calibration or estimation of values, but rather relies solely on a change in impedance (Charloux et al., 2000). Assuming proper baseline calibration is completed, changes to thoracic blood flow should not interfere with data collection (Hsu et al., 2006). However, the authors recognize that the accuracy of the Physioflow has not been confirmed while breathing iNO, and as such could have contributed to error in stroke volume determination.

3.3 Future Directions

To our knowledge, the use of iNO in healthy untrained individuals has not been previously studied. In the current study, the effect of iNO on RVSP was quite small, though consistent throughout the participants. As mentioned above, a greater dosage of iNO may produce greater changes in RVSP and could be worth exploring. This small decrease in RVSP may not have been enough to unload the RV and improve $\dot{V}O_{2max}$. It has been well established that hypoxia causes HPV in healthy individuals, increasing afterload on the RV secondary to increased PVR. Hypoxia has been associated with reduced exercise capacity, specifically due to the increase in PVR due to HPV. In future studies, a double-blind randomized control trial much like the current study should be performed, however, within each condition (iNO and placebo), different levels of hypoxia should be administered. Further, by incorporating both a high-fit and untrained group, the different responses seen in each group from iNO would indicate the role exercise training has on pulmonary NO bioavailability and its ability to release HPV.

3.4 Summary

The effect of iNO on $\dot{V}O_{2max}$ was examined in young healthy, low- and high-fit individuals. It was determined that despite a decrease in RVSP, $\dot{V}O_{2max}$ was unaffected by iNO in either the low- or high-fit individuals. The lack of change determined in $\dot{V}O_{2max}$ suggests that regardless of

fitness level, neither the pulmonary circulation nor the right ventricle are limiting factors of exercise capacity in health.

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Appendices

Appendix A: Literature Review

A.1 Pulmonary Circulation during Exercise

Maximal oxygen consumption ($\dot{V}O_{2max}$) is a measure of the capacity for aerobic exercise (West, 2008). $\dot{V}O_2$ is derived by using the Fick equation:

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

where Q is the cardiac output (the product of heart rate and stroke volume) and CaO_2 and CvO_2 are the oxygen contents of the arterial and venous blood, respectively (Stickland et al., 2012). In healthy subjects, it is commonly accepted that the cardiovascular system (i.e. Q) is the main limitation to $\dot{V}O_{2max}$, (Wagner, 1996).

The primary function of the pulmonary circulation is to complete gas exchange, however, it also contributes to filtration of unwanted materials in the circulation, blood storage, and some metabolic regulation (Suresh et al., 2016; West, 2008). In relation to the systemic circulatory system, the pulmonary vasculature functions under much lower perfusion pressures. Mean resting pulmonary arterial (i.e. inflow) pressure is around 15 mmHg and pulmonary venous (i.e. outflow) pressure is around 5 mmHg (Kovacs et al., 2009; Naeije et al., 2012; West, 2008). This allows the walls of the pulmonary vessels to have significantly less smooth muscle than vessels in the systemic system, facilitating more efficient exchange of oxygen through the pulmonary microcirculation (Reeves, 2005).

A.1.1 Pulmonary Artery Pressure

As demonstrated in the Fick equation, as metabolic demand increases during exercise, Q must increase to meet the oxygen demand. Cardiac output (i.e. flow) is related to differences in pressure over resistance, and represented by the equation:

$$Q = \frac{Pressure}{Resistance}$$

Even though the pulmonary vessels are very compliant allowing distention in response to an increase in Q and reducing resistance (West, 2008), pulmonary artery pressure (PAP) increases during exercise. This is demonstrated in a study by Wagner et al. (1986) in which PAP was measured using right heart catheterization (RHC) during incremental cycle exercise. Measurements were taken at rest and during steady-state exercise starting at 60 watts and increasing resistance by 60 watt increments up to 240 watts at sea level. Mean PAP and Q increased with every workload on the cycle ergometer, with the greatest values reaching 37.2 ± 6.1 mmHg and 23.9 ± 1.2 L · min^{-1} at 240 watts respectively (Wagner et al., 1986).

Stickland et al. (2004) further supported these results by reporting that PAP and Q increased significantly with exercise in high-fit individuals. Eight healthy, physically active males ($\dot{V}O_{2max}$: 54.7 ± 9.0 ml · kg^{-1} · min^{-1}) were tested during exercise on a cycle ergometer. Measurements were taken while supine resting, upright resting, 75 watts, 150 watts, at the ventilatory threshold (VT), 25 watts above VT, and 90% of $\dot{V}O_{2max}$. During exercise, Q increased at each workload with PAP and pulmonary artery wedge pressure (PAWP) at peak exercise (Q_{max}: 29.7 L · min^{-1}) being approximately 27 mmHg and 15 mmHg respectively. These data are consistent with Wagner et al. (1986), and both illustrate an increase in PAP in the face of increased Q.

A.1.2 Pulmonary Vascular Resistance

Though it has been well established that PAP increases with exercise, large inter-individual variation has been reported. During exercise 80% of PAP variation can be explained by PAWP, suggesting that downstream pressure is an important regulator of PAP (Reeves,

2005). PAWP is used as a surrogate for left atrial pressure (LAP), with the difference between PAP and PAWP corresponding to the pressure of flow through the pulmonary circulation. Pulmonary vascular resistance (PVR) is calculated using the following equation:

$$PVR = \frac{Ppa - Pw}{Q}$$

At the onset of upright exercise, there is a decrease in blood volume in the lower extremities and an increase in blood to the thoracic cavity (Flamm et al., 1990). The shift of blood and an increase in Q increases PAP and results in the recruitment and distention of pulmonary capillaries (Hsia, 2002). Since the radius of a vessel is such a prominent factor in determining resistance, by recruitment and distention of the pulmonary capillaries, PVR decreases. Normal resting PVR has been reported to be 1.25 mmHg · L^{-1} · min^{-1} in the upright position (Kovacs et al., 2012). It is important to note that using non-invasive measurements to calculate PVR does not consider PAWP and so is denoted total PVR (TPVR; Naeije et al., 2012).

As previously established, PAP increases with exercise. Stickland et al. (2004) reported decreased PVR with increasing exercise intensities up to 90% $\dot{V}O_{2max}$. Further, PVR remained unchanged compared to 25 watts above VT. This potentially suggests that at high-intensity exercise, PVR may plateau. Importantly, without a reduction in PVR, PAP can increase substantially with exercise and negatively impact exercise tolerance (Tolle et al., 2008). Tolle et al. (2008) found that elevated PAP and PVR negatively impacted $\dot{V}O_{2max}$ in patients with exercised-induced pulmonary arterial hypertension (PAH). One-hundred and nine patients underwent graded exercise testing (GXT) with RHC to measure PAP and PAWP. Compared to controls, individuals with exercise-induced PAH had a much greater increase in PAP during the GXT from rest to maximal exercise (18.6 ± 3.2 mmHg to 36.6 ± 5.7 mmHg compared to 13.9 ±

2.9 mmHg to 27.4 ± 3.7 mmHg). Further, PVR at maximal exertion was much greater in the exercise-induced PAH patients as compared to controls (2.01 ± 0.75 mmHg · L^{-1} · min^{-1} and 0.78 ± 0.25 mmHg · L^{-1} · min^{-1}, respectively). The increased PAP was suggested to play a significant role in the reduced $\dot{V}O_{2max}$ seen in the exercise-induced PAH patients ($\dot{V}O_{2max}$: 66.5 ± 16.3 % predicted) compared to controls ($\dot{V}O_{2max}$: 91.7 ± 13.7 % predicted; relative $\dot{V}O_{2max}$ not reported). Since PVR is inversely related to Q, an increase in PVR would potentially cause a decrease in Q which would reduce $\dot{V}O_{2max}$.

Stickland et al. (2004) reported a decrease in PVR during increasing exercise intensities with a potential plateau in PVR near maximal exercise. A plateau in PVR has also been shown to occur at simulated extreme altitude and was related to decreased exercise performance (Groves et al., 1987). Further, work in exercise-induced PAH also has demonstrated that increased PAP and PVR were related to lower exercise performance (Kovacs et al., 2012; Tolle et al.,2008). These findings indicate that a greater PAP response to exercise may reduce $\dot{V}O_{2max}$, presumably through an increase in afterload of the right ventricle (RV).

A.1.3 Right Ventricle during Exercise

Earlier work has focused on the left ventricle and its relation to exercise however it is not until recently that research has shifted to focus on the RV and its impact on exercise performance. Burger et al. (2001) found that during exercise, afterload became the predominant determent of right ventricle ejection fraction (RVEF; Burger et al., 2001). Sixteen male patients with coronary artery disease performed submaximal supine exercise (median = 100 watts; workload dependent on previous exercise test performed; mean not reported) for five minutes. Using right heart catheterization PAP and right atrial pressure (RAP) were measured and right ventricular stroke work (RVSW) and RV end-systolic volume was calculated. A strong

correlation between RVSW and RV end-systolic volume (r = -0.89) was seen at the end of each exercise session suggesting that RVSW was not predominantly influenced by preload as was seen at rest. These data suggested that due to increased PAP, greater stroke work was needed to maintain RV end-systolic volume, and as such SV. (Burger et al., 2001).

Burger et al. (2001) findings supported that of Francios et al. (1985) which reported that exercising RVEF was correlated with exercise capacity during symptom limited exercise tests. Forty-one patients with chronic left ventricular failure performed symptom limited bicycle exercise tests ($\dot{V}O_{2max}$: 12.8 ± 5.2 ml · kg^{-1} · min^{-1}) while PAP and PAWP were measured using RHC. PVR remained unchanged during exercise (2.0 ± 1.5 mmHg · L^{-1}), and PAP increased significantly (31.8 ± 8.2 mmHg - 50.4 ± 12.9 mmHg). PVR was negatively correlated to exercise capacity (r = -0.55), suggesting that reduced RVEF secondary to increased afterload on the RV was an important determinant of exercise capacity in heart failure patients. Reduced RVEF, and as a result decreased SV would decrease maximal Q, which would reduce $\dot{V}O_{2max}$ in accordance with the Fick equation.

Both Burger et al. (2001) and Franciosa et al. (1985) found RV limitation to exercise performance due to increased afterload in clinical populations, however, this has been recently suggested to occur in healthy individuals as well. La Gerche et al. (2012) found that healthy individuals with lower TPVR had greater Q and lower pulmonary artery systolic pressure (PASP), a non-invasive measure used to estimate PAP, suggesting less afterload on the RV during maximal exercise. Right ventricular systolic pressure (RVSP) is a synonymous term with PASP in literature and will be used for the purposes of this paper. Fifty-five subjects completed maximal bike tests in which agitated saline contrast echocardiography was used. The authors suggested that a positive agitated saline contrast echo was not evidence of an intra-pulmonary

shunt (Stickland et al., 2004), but rather reflected greater pulmonary vascular distensibility. Subjects that demonstrated greater pulmonary transit of agitated contrast (PTAC) achieved significantly lower RVSP at maximal exercise (52.3 ± 9.8 mmHg) than those that had lower PTAC (62.6 ± 13.7 mmHg) while achieving significantly higher Q (16.1 ± 3.4 L · min^{-1} vs 13.9 ± 2.9 L · min^{-1} respectively). TPVR was also greater in low PTAC subjects as compared to the high PTAC subjects ($p < 0.0001$; absolute numbers not reported). The significance of RVSP and TPVR on exercise performance suggests that in health, afterload on the RV may be a limitation to exercise (La Gerche et al., 2010).

A.1.4 Effect of Exercise Training on Pulmonary Circulation

Endurance trained athletes exhibit enhanced cardiovascular function when compared to lower fit, non-athletes (Stickland et al., 2006), however, it has been generally accepted that aerobic training does not affect lung structure or function (Hagberg et al.,1988). Work however by Lalande et al. (2012) challenges this previously accepted concept. Lalande et al. (2012) found that individuals with the highest aerobic capacity have greater resting capillary blood volume (V_C) and lower TPVR at maximal exercise. Twenty-four healthy individuals performed a maximal exercise test ($\dot{V}O_{2max}$: 41.8 ± 7.1 ml · kg^{-1} · min^{-1}) to characterize fitness levels. On a separate day, the participants completed a second maximal exercise test in combination with echocardiography, PTAC, and lung diffusing capacity measurements. Diffusion capacity was estimated using diffusion capacity for carbon monoxide (DLCO) technique. Diffusion capacity can be separated into the V_C component and membrane diffusing capacity (D_M) component, estimated using DLNO/DLCO technique, and calculated using the Roughton-Forster relationship (Roughton & Forster, 1957). Higher $\dot{V}O_{2max}$ was correlated with elevated resting V_C ($r = 0.60$) suggesting greater resting distention and recruitment of the capillaries (Lalande et al., 2012). Further, higher $\dot{V}O_{2max}$ was negatively correlated with TPVR ($r = -0.54$). Importantly, TPVR was

calculated using RVSP estimations from echocardiography and therefore pulmonary venous pressure data was not used for the calculation of PVR.

Additionally, Tedjasaputra et al. (2016) demonstrated greater resting V_C in endurance trained athletes as compared to less fit controls, further suggesting a pulmonary adaptation to chronic aerobic training. Stickland et al. (2006) has previously demonstrated a lower resting PAP and PAWP in trained subjects. A higher resting V_C while maintaining lower PAP and PAWP suggests greater distensibility and recruitment of the pulmonary vasculature which may be important for decreasing PVR and limiting excess RV afterload during exercise (La Gerche et al., 2010; Lalande et al., 2012; Reeves, 2005; Tedjasaputra et al., 2016).

A.2 Modulators of Pulmonary Vascular Tone

A.2.1 Pulmonary Vasodilators

Although the pulmonary vasculature has much less smooth muscle tone than that of the systemic circulation, it is still modulated by exogenous and endogenous factors. The most common of these factors studied are pulmonary vasodilators such as nitric oxide, adenosine, atrial natriuretic factor (ANP), prostacyclin (PGI_2), and dopamine (Suresh et al., 2016). Adenosine causes vasodilation of the pulmonary arteries by binding to surface receptors coupled with a G-protein adenylyl cyclase (Suresh et al., 2016). Adenosine has been shown to increase pulmonary blood flow in healthy humans (Heinonen et al., 2013), however, it is still not known whether it contributes to reducing pulmonary vascular basal tone (Suresh et al., 2016). ANP has been shown to reduce PVR and PAP in pigs (Cigarini et al., 1989) and rats (Jin et al., 1988) as well as individuals diagnosed with chronic obstructive pulmonary disease (Adnot et al., 1989). PGI_2 is produced by the endothelium and is thought to cause a vasodilatory response in the smooth muscle in both pulmonary and systemic vasculature, however, studies examining the

exact role of PGI_2 have produced contradicting results in its contribution to pulmonary vasomotor tone (Suresh et al., 2016).

Dopamine is most commonly associated with its role as a neurotransmitter, however, it is also a catecholamine that is extremely important in regulating vascular tone, both in the pulmonary and systemic system (Missale et al., 1998). At low doses, dopamine binds to D_2-like receptors which are found on the walls of the pulmonary blood vessels, causing vasodilation via inhibition of norepinephrine (Missale et al., 1998). At higher doses, however, (>10 $\mu g \cdot kg^{-1} \cdot min^{-1}$) it can increase stroke volume and Q (Bryan et al., 2012).

A.3 Nitric Oxide

A.3.1 Introduction

Nitric oxide (NO) is a commonly studied molecule due to its influence on the vascular tone and resistance specifically in the pulmonary system (Suresh et al., 2016). NO is synthesized by one of three different nitric oxide synthases (NOS) which are characterized by the location of synthesis (Carvajal et al., 2000). The three different isoforms of NOS are neural NOS (nNOS), endothelial NOS (eNOS), and calcium independent NOS (iNOS) (Carvajal et al., 2000). Though it is important to recognize the different forms of NO synthesis, the focus of this overview will be on eNOS as it is predominant in the pulmonary vasculature (Suresh et al., 2016).

NO synthesis is initiated by the sheer stress on the vessel walls during increased blood flow. This stress releases calcium which activates eNOS to produce NO. (Carvajal et al., 2000). NO diffuses from the endothelium to bind with soluble guanylate cyclase (s-GC) on the smooth muscle surrounding the blood vessels and increases cyclic guanosine monophosphate (cGMP) production. Increased cGMP relaxes the smooth muscle and results in vasodilation (Carvajal et al., 2000). This process of smooth muscle relaxation and blood vessel dilation has been shown in

multiple vascular sections within the body, including the pulmonary vasculature (Landgraf et al., 1986; Perrella et al., 1991; Rees et al., 1989).

A.3.2 Inhaled Nitric Oxide

Inhaled NO (iNO) has been shown to cause vasodilation selectively in the pulmonary artery (Pepke-Zaba et al., 1991). Interestingly, the exact mechanism as to how the iNO reaches the pulmonary arterial smooth muscle is still uncertain. A possible explanation could be that during inhalation, NO diffuses from the airway into the pulmonary artery before reaching the alveoli through precapillary gas exchange (Jameson, 1964). NO has a strong affinity for heme and will bind to hemoglobin if not directly acting on the vascular smooth muscle. Binding of NO to hemoglobin subsequently converts NO to inactive nitrite and nitrate (Krasuski et al., 2000). The attraction of NO to heme and dissociation of NO inhibits any effects it may have on the systemic system, localizing the vasodilation to the pulmonary vasculature.

The importance of basal NO to the regulation of pulmonary vascular tone was demonstrated in a study by Stamler et al (1994). The NO synthesis inhibitor, L-NMMA, was administered in eleven healthy participants, and RHC evaluated hemodynamic responses such as mean RAP, PAP, and PAWP, while a second catheter was inserted into the radial or brachial artery to measure systemic systolic and diastolic pressures. L-NMMA was then infused in increasing dosages. Systemic vascular resistance increased by 63.4% and mean blood pressure increased by 15.5% at the highest dosage of L-NMMA (Stamler et al., 1994). Stamler et al. (1994) reported no significant change in PAP, however, L-NMMA increased PVR by 39.8% and reduced Q. This increase in PVR demonstrates the importance of endogenous NO on resting pulmonary artery tone.

Frostell et al (1993) found that iNO decrease PAP and PVR at rest while breathing hypoxia, a known pulmonary vasoconstricting condition. Nine healthy adults were given room air, hypoxia ($FiO_2 = 0.12$), hypoxia ($FiO_2 = 0.12$) with 40ppm NO, and room air with 40ppm NO. RHC and radial artery catheters were inserted to measure pulmonary and systemic pressure respectively. Breathing iNO with room air had no significant change on PAP or Q. As expected, breathing hypoxia caused an increase in PAP, PVR, and Q, however when iNO was inspired, PAP and PVR decreased significantly. These findings demonstrated that iNO can help reverse the vasoconstrictive characteristics of hypoxia at rest.

Stamler et al. and Frostell et al. both substantiated the importance of NO on the resting pulmonary vascular tone, however, these studies were conducted at rest with relatively low Q and PAP, and therefore the impact of iNO during exercise is less clear.

A.3.3 Effect of Inhaled Nitric Oxide on Exercise

It is well known that exercise induced arterial hypoxemia (EIAH) may occur in highly trained athletes during high intensity exercise (Dempsey et al., 1984; Wagner et al., 1986). The decreased partial pressure of oxygenation (P_aO_2) demonstrated in EIAH is the result of an increased alveolar-arterial oxygen pressure differences ($A\text{-}aDO_2$). During exercise, athletes exhibit high PAP (Reeves, 2005), which is theorized to potentially disrupt the capillary endothelial resulting in interstitial edema (West, 2008). Edema may result in ventilation-perfusion inequality and potentially diffusion limitation, both of which are known to influence EIAH in aerobically trained athletes at maximal exercise (West, 2008, Rice et al., 1999).

Due to iNO's vasodilatory effects on the resting PAP, Durand et al (1999) studied the effects of NO inhalation during incremental exercise to see if iNO would limit EIAH. A group of nine highly trained male athletes ($\dot{V}O_{2max}$: 65 ml · kg^{-1} · min^{-1}) was tested, all of whom exhibited

EIAH prior to being enrolled in the study. The athletes performed two maximal exercise tests in a single day while arterial blood gas was measured using brachial catheterization. Participants were given a 90-minute break in between to allow for A-aDO_2 to return to resting levels. One exercise test was performed with 15 ppm of NO and the other on room air (randomization not stated). There was no significant difference in $\dot{V}O_{2max}$ or peak workload between the placebo and iNO exercise tests. However, the authors reported that during room air inhalation, P_aO_2 decreased between 75-100% of $\dot{V}O_{2max}$, but during iNO, P_aO_2 did not decrease over the same intensity (Durand et al., 1999). Lower A-aDO_2 could suggest a potential decrease in PAP during the iNO condition, resulting in less pulmonary edema, which could, in turn, improve ventilation-perfusion matching and reduce diffusion limitation. However, it is very important to note that none of the arterial gas measurements were corrected for body temperature, preventing accurate determination of A-aDO_2 during exercise.

Sheel et al. (2001) investigated iNO during high intensity exercise and found no effect on A-aDO_2. Eight highly fit male participants performed a screening incremental bike test to exhaustion to determine $\dot{V}O_{2max}$ ($\dot{V}O_{2max}$: 65.3 ml · kg^{-1} · min^{-1}). On a separate day, participants warmed up on the bike for 10-15 minutes before pedaling at 100% of $\dot{V}O_{2max}$ for five minutes. This was done four times, each under differing conditions (room air, hypoxia (F_iO_2 = 0.14), 20ppm of NO, and 20ppm of NO and hypoxia (F_iO_2 = 0.14). Heart rate, metabolic data, as well as temperature-corrected arterial blood gases, were monitored throughout exercise. Inspired NO did not affect P_aO_2 or A-aDO_2 at rest or during exercise in room air or hypoxia (Sheel et al., 2001). Differences observed by Durand et al. (1999) when compared to Sheel et al. (2001) may be explained by the lack of arterial blood gas temperature correction by Durand et al.

It has been suggested that high aerobically trained individuals already achieve maximal pulmonary dilation (Dempsey, 1986) which could explain the negative effects of iNO found by Sheel et al. (2001). Maximal dilation within the pulmonary artery in highly trained individuals was also supported by Tedjasaputra et al. (2016), who reported that individuals with high aerobic fitness exhibited significantly increased V_C when compared to low aerobically fit individuals. This would suggest that aerobically fit individuals exhibit maximal dilation, while less fit individuals may require pharmacological support (i.e. iNO) to fully dilate the pulmonary vasculature.

A.4 Sex Differences in Maximal Oxygen Consumption

A.4.1 Introduction

Women have been shown to have smaller lungs compared with age- and height- matched men (Mclaran et al., 1998). This has caused speculation that women may inherently be more susceptible to EIAH than men due to a diffusion capacity plateau during maximal exercise, limiting $\dot{V}O_{2max}$ (Smith et al., 2015). Harms et al. (1999) reported EIAH in healthy active females (within 15% of predicted $\dot{V}O_{2max}$), while healthy men have not been reported to experience EIAH within 15% of predicted $\dot{V}O_{2max}$ values. Bouwsema et al. (2017) however reported that when expressed relative to alveolar volume, there was no difference between men and women in diffusion capacity or V_C.

A.4.2 Effect of Menstrual Cycle on $\dot{V}O_{2max}$

There is considerable debate within the literature as to the effects of menstrual cycle on exercise performance. Jurkowski et al. (1981) studied nine healthy females ($\dot{V}O_{2max}$: 41.8 ml · kg^{-1} · min^{-1}) who reported regular menstrual cycles and examined exercise response in the luteal and follicular phase. Within each phase, the participant completed a standard incremental exercise test on one day, and on a second the participant's rode at 40%, 70%, and 90% of $\dot{V}O_{2max}$ till

exhaustion. Lactate, Q, and $\dot{V}O_2$ were collected at each workload. Progesterone was elevated during the luteal phase, and since progesterone is known to stimulate ventilation ($\dot{V}_E$), unsurprisingly $\dot{V}_E$ was increased in the luteal phase when compared to the follicular phase. There was, however, no corresponding difference in $\dot{V}O_{2max}$, workload, and Q between phases, indicating that cardiopulmonary responses to exercise were not affected by the menstrual cycle (Jurkowski et al.,1981). A follow-up study by Smekal et al. (2007) had 19 healthy women perform maximal incremental exercise tests on a cycle ergometer in both the luteal and follicular phase. Similarly, the authors reported no significant difference in $\dot{V}O_{2max}$, heart rate, or power output across the menstrual cycle (Smekal et al., 2007).

Despite evidence that exercise performance is not affected by menstrual phase, there are data demonstrating relevant changes to pulmonary circulation and diffusion across the menstrual cycle. Work by Seaton et al. (1972) reported greater resting DLCO and V_C during the luteal phase as compared to the follicular phase. Smith et al. (2015) hypothesized that this greater V_C during the luteal phase would also be observed during exercise. Eight women ($\dot{V}O_{2max}$ range: 35-53 ml · kg^{-1} · min^{-1}; mean $\dot{V}O_{2max}$ not reported) performed a pulmonary function test and an incremental treadmill test. Each woman conducted multiple F_iO_2 DLCO measures at six different workloads (rest, 40, 60, 80, 90, and 100% of $\dot{V}O_{2max}$) during early-follicular, late-follicular, and mid-luteal stages of their respective menstrual cycle. Exercising DLCO was higher during the mid-luteal stage of the menstrual cycle at 90% and 100% $\dot{V}O_{2max}$ (Smith et al., 2015). This increase in DLCO was secondary to greater V_C, while no differences in D_M were observed. While DLCO varied across the menstrual cycle, no difference in $\dot{V}O_{2max}$ was seen across phases. These findings suggest that any change in DLCO was not sufficient to alter oxygen saturation or impact $\dot{V}O_{2max}$.

Evidence suggests that menstrual cycle phase influences physiological parameters in females during exercise. It was reported that females have higher V_C during the luteal phase at rest (Seaton, 1972) and during exercise (Smith et al., 2015). Consistently, however, all previously mentioned studies found no difference in $\dot{V}O_{2max}$ or Q between different menstrual phases. This suggests that the changes that occur due to the menstrual cycle have no effect on cardiorespiratory outcomes when achieving maximal intensities (Jurkowski et al., 1981; Smekal et al., 2007; Smith et al., 2015).

A.5 Non-Invasive Evaluation of Pulmonary Circulation

Invasive measurements of pulmonary circulation are the most accurate means of attaining pressure and pulmonary resistance data both at rest and during exercise (Champion et al., 2009). However, invasive measurements have a high safety risk associated with them, and therefore RVSP obtained by Doppler echocardiography is frequently used as an alternative method for obtaining PAP estimates (Argiento et al., 2010; Forton et al., 2016; Kovacs et al., 2009).

Yock et al. (1984) first determined that patients with elevated RV pressures have tricuspid regurgitation signals that can be detected by ultrasound. The maximum velocity of tricuspid regurgitation measured by Doppler ultrasound can be used to calculate RVSP (Yock, 1984), and a mean PAP can further be estimated. Estimated RVSP via Doppler echocardiography has been correlated with RHC studies using healthy controls ($r = 0.98$; Chemla et al., 2004). A study by Argiento et al. (2010) used ultrasound to evaluate RVSP and PAP in 25 healthy volunteers (12 females and 13 males) at rest and during an incremental exercise test using a semi-recumbent cycle ergometer. The authors reported resting mean PAP around 13 mmHg at rest and around 30 mmHg at peak exercise which is closely matched with invasive measures (Argiento et al., 2010). No correlation calculations were conducted as different

samples were tested, however, the mean PAP/Q relationship in Argiento et al. (2010) study was 1.37 mmHg · min^{-1} · L^{-1} which agrees with invasive studies showing a slope of 0.94 ± 9.4 mmHg · min^{-1} · L^{-1} (Naeije et al., 2012).

More recently, Forton et al. (2016) compared the effect of body position on exercise capacity and pulmonary vascular pressure using non-invasive Doppler echocardiography. Thirty volunteers (15 men and 15 women) were recruited to perform incremental cycle tests in an upright, semi-recumbent, and supine position. Upright cycling mean $\dot{V}O_{2max}$ in 26 volunteers was 43 ml kg^{-1} min^{-1}. The authors achieved images in 87% (26/30) of all participants in all three body positions. Resting mean PAP was not significantly different between body position at rest (approximately 15 mmHg) or at maximal exercise (approximately 34 mmHg; exact values not reported), however increased with exercise intensity as was consistent with other non-invasive (Argiento et al., 2010; Kovacs et al., 2009) and invasive literature (Stickland et al., 2004; Wagner et al., 1986).

Limitations to non-invasive determination of RVSP includes the need for an experienced echocardiographer (Argiento et al., 2010), as well as the requirement to perform exercise in a semi-supine position in young healthy subjects in order to achieve an adequate image of tricuspid regurgitant flow (Tedjasaputra et al., 2016). Importantly, while widely used with exercise, at present there are no data that have directly compared non-invasive RVSP estimates with measured PAP in exercising humans, and as such the validity of this technique remains unclear (Forton et al., 2016). Though RVSP is a promising method for estimating PAP, because it is not a direct measurement and proper validation studies have not been performed, other measurable variables are better suited as a primary outcome.

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