The Effect of Dopamine on Pulmonary Diffusing Capacity and Capillary Blood Volume Responses to Exercise

by

Wade William Michaelchuk

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Faculty of Physical Education and Recreation
University of Alberta

Abstract

Pulmonary diffusing capacity increases during exercise to meet the increasing oxygen (O_2) demand of the body. Expansion of pulmonary capillary blood volume (Vc) and diffusing membrane capacity (Dm) are important contributors to the increased diffusing capacity observed during upright cycle exercise. Recent studies have shown that circulating dopamine, a pulmonary vascular vasodilator, may play an active role in Vc regulation through changes in pulmonary vascular tone. Subsequently, these changes may be responsible for the reduction in exercise tolerance seen during heavy exercise with dopamine receptor blockade. Thus, the purpose of this study was to examine the effect of exogenous dopamine as well as dopamine receptor-2 (D_2-receptor) blockade on pulmonary diffusing capacity (DL_{CO}), Vc, and Dm at baseline and during upright cycle exercise. Additionally, the effect of dopamine and D_2-reeceptor blockade on time-to-exhaustion during heavy cycle exercise at 85% of VO_{2peak} was assessed. Based on previous work, it was hypothesized that dopamine would *increase* DL_{CO}, Vc, Dm, and exercise tolerance, while D_2-receptor blockade would *decrease* DL_{CO}, Vc, Dm, and exercise tolerance. Hemoglobin adjusted DL_{CO}, Vc, and Dm were determined at rest and during exercise in 14 young, healthy, recreationally active, non-smoking subjects (VO_{2peak} 45.8 ± 6.6 mL · kg^{-1} · min^{-1}) using the Roughton and Forster (1957) multiple F_IO_2-DL_{CO} method. Dependent variables were evaluated at baseline, as well as cycling at 60 and 85% of VO_{2peak} under the following randomly assigned conditions: 1) intravenous saline and a placebo pill, 2) intravenous dopamine (2 μg · kg^{-1} · min^{-1}) and a placebo pill, 3) intravenous saline and an oral D_2-receptor antagonist (20 mg metoclopramide). The effect of dopamine on cycle time-to-exhaustion at 85% of VO_{2peak} was also examined in the three conditions. Exogenous dopamine and dopamine blockade had no effect on DL_{CO}, Vc, and Dm at baseline or at any intensity of exercise. Blockade reduced time-to-exhaustion (blockade, 259 ± 120 seconds; placebo, 367 ± 198 seconds; P<0.05), but

intravenous dopamine did not improve time-to-exhaustion. Overall, dopamine does not appear to be important in the regulation of DL_{CO}, Vc, and Dm at rest or during exercise. While endogenous dopamine appears to be important for the maintenance of time-to-exhaustion, providing exogenous dopamine does not appear to enhance exercise tolerance.

Preface

This thesis is an original work by Wade William Michaelchuk. The embedded research project, "The effect of dopamine on pulmonary diffusion, pulmonary capillary blood volume, and exercise tolerance", ID No. Pro00067664 received ethics approval from the University of Alberta Health Research Ethics Board Biomedical Panel November 2016. No part of this thesis has been previously published.

Dedication

This thesis is dedicated to my wife, Rebecca Horne. Rebecca is the driving force that pushed me to reach my academic goals beginning in high school. Her support and belief in me led to my acceptance into the University of Alberta in 2011, helped me to graduate my undergraduate degree with distinction, and motivated me to work hard and complete my Master's degree in two years. Thank you for everything that you do, and here's to a lifetime of pushing each other to succeed both personally and professionally.

"Gravitation cannot be held responsible for people falling in love. How on earth can you explain in terms of chemistry and physics so important a biological phenomenon as first love? Put your hand on a stove for a minute and it seems like an hour. Sit with that special girl for an hour and it seems like a minute. That's relativity." – Albert Einstein

Acknowledgements

To my family – I would like to thank my parents, Margaret and Jeffrey Michaelchuk, and my sister Meghan Michaelchuk. Despite not being entirely certain about what I have been doing at the University of Alberta for the past six years, you have always been trusting and supportive of my education path.

To my research subjects – Thank you all for your effort in my research project without complaint. Although, I'm sure none of you will agree to participate in a project with "exercise tolerance" or "time-to-exhaustion" in the title ever again.

To the Clinical Physiology Lab – Thank you to everyone who has mentored and supported me over the past two and a half years including Desi Fuhr, Vincent Tedjasaputra, Andrew Brotto, Linn Moore, Devin Phillips, Sophie Collins, Salmina Ahmed, Brad Byers, Karishma Kapoor, Ryan St. James, Harley Seville, and Irene Andersson. Everything that this lab touches turns to gold and I am blessed and honored to have been a part of it.

To my research physicians and nurses – My thesis project could not have been completed without you. Thank you to Dr. Tracey Bryan and Dr. Eric Wong for stepping up and providing research supervision times for me amidst their busy schedules. Thank you to Paula Clark, Brittany Rushfeldt, Courtney Blake, and others for being personable and effective research nurses: I received many compliments about our nurses' abilities to place an intravenous line painlessly.

To my supervisory committee – I am truly lucky to have had the opportunity to work with Drs. Michael Stickland and Sean van Diepen. First, I want to thank Dr. Stickland, who is an excellent mentor and a brilliant researcher. Your mentorship style matched me perfectly as you let me

work independently but also provided great feedback and advice when solicited. Your knowledge and passion for cardiopulmonary exercise physiology is very encouraging – although I've told you the Master's degree is the end of my education path, I haven't closed the door on a doctoral degree just yet. You have challenged me in many ways and I have grown significantly as an academic under your leadership. I also want to thank Dr. van Diepen, first and foremost, for taking time out of your schedule to provide me with an abundant amount of physician supervision time. There is no way I would have been able to complete over 56 trials in seven months without you. Additionally, thank you for taking the time to teach me hands-on ultrasound skills. Through these sessions your knowledge and curiosity of the cardiovascular system became clearly evident. Finally, your unique perspective on my thesis project has challenged me and allowed the project to become more diverse overall. This thesis is the culmination of my academic career and it is all thanks to my supervisory committee's leadership and passion.

Table of Contents

List of tables

List of figures

List of abbreviations

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

ANF: Atrial natriuretic factor

COHb: Carboxyhemoglobin

D_1-receptor: Dopamine receptor-1

D_2-receptor: Dopamine receptor-2

DL_{CO}: Diffusing capacity for carbon monoxide

Dm: Diffusing membrane capacity

EIAH: Exercise-induced arterial hypoxemia

ET-1: Endothelin-1

F_IO_2: Fraction of inspired oxygen

Hb: Hemoglobin

[Hb]: Hemoglobin concentration

HPV: Hypoxic pulmonary vasoconstriction

NO: Nitric oxide

PAH: Pulmonary arterial hypertension

PASP: Pulmonary arterial systolic pressure

PGI_2: Prostacyclin

Ppa: Pulmonary artery pressure

PVR: Pulmonary vascular resistance

Pw: Pulmonary wedge pressure

Q: Cardiac output

RV: Residual volume

SaO_2: Arterial oxygen saturation

TLC: Total lung capacity

$\underline{Vc}$: Pulmonary capillary blood volume

$\underline{VO_{2max}}$: Maximal oxygen consumption

$\underline{VO_{2peak}}$: Peak oxygen consumption

$\underline{V/Q}$: Ventilation-perfusion

Chapter I: Introduction

1.1 Background

Pulmonary diffusing capacity increases during exercise to meet the increasing oxygen (O_2) demand of the body (2, 9, 10, 12, 20). This is achieved by expanding pulmonary capillary blood volume (Vc) and diffusing membrane capacity (Dm) through recruitment and distention of the pulmonary capillaries (8, 23), effectively increasing the surface area for diffusion (7). Failure to augment diffusing capacity during exercise would result in early exercise termination, secondary to low arterial O_2 saturation and decreased convective O_2 delivery to the working muscle (4). As cardiac output (Q) and pulmonary artery pressure (Ppa) rise during exercise, recruitment and distention of the pulmonary capillaries increases pulmonary blood flow, resulting in decreased pulmonary vascular resistance (PVR; 14), and limiting the rise in Ppa with exercise. Importantly, if Ppa increases too much during exercise, this could negatively impact exercise performance (1, 22).

Regulation of pulmonary diffusion and vascular pressures during exercise are traditionally thought to occur passively, with increases in both occurring secondary to an increase in central blood volume, leading to the recruitment and distention of the pulmonary capillaries (7). However, there is evidence that the pulmonary circulation is actively influenced by autonomic factors (14, 19) and circulating vasodilators such as dopamine (6, 13). Thus, these mediators may play an important role in Vc and pulmonary diffusion regulation during exercise.

1.2 Purpose

The purpose of this study was to examine the effect of low dose dopamine infusion and a dopamine receptor-2 (D_2-receptor) antagonist on pulmonary diffusion, Vc, Dm, Q, and exercise tolerance. The effects of dopamine and D_2-receptor blockade on the above variables are not well

understood during exercise, therefore this study will advance our fundamental knowledge on the vasoactive regulation of the pulmonary vasculature and pulmonary diffusion during exercise.

1.3 Delimitations

Young, healthy, recreationally active adults were invited to participate in this study. For the exercise diffusion capacity portion, the independent variables were dopamine condition (exogenous dopamine, D_2-receptor blockade, or placebo) and exercise intensity (baseline, 60%, and 85% of VO_{2max}). The dependent variables of this arm include diffusing capacity for carbon monoxide (DL_{CO}) and its two components Vc and Dm (15), systolic pulmonary arterial pressure (PASP), and Q. As a secondary aim, the effect of dopamine on exercise tolerance was also examined. For the exercise tolerance trials, the independent variable was dopamine condition (exogenous dopamine, D_2-receptor blockade, or placebo), and the dependent variables were time-to-exhaustion in seconds, O_2 delivery, Q, and ventilation during exercise. Additional respiratory parameters including volume of O_2 consumption and CO_2 production were also measured. To control for hormonal variations in female participants, menstrual cycle phase was standardized, with all testing days falling within the menstrual cycle phase. For example, if day the preliminary day and day one of testing fell within the follicular phase of the menstrual cycle, then all subsequent testing days were also be performed in the follicular phase. This is important since menstrual cycle phase can affect diffusing capacity, Vc, and exercise tolerance (16). Factors affecting DL_{CO}, including hemoglobin (Hb) concentration, will also be accounted for in measurements.

1.4 Limitations

Roughton and Forster's method for Vc and Dm determination (15) is pragmatic, but it is not without its limitations. First, it is assumed that Θ, the rate of reaction between carbon monoxide

and the red blood cell, does not change with exercise (15). However, Θ is calculated using alveolar partial pressure of O_2 which varies with respiratory exchange ratio—a variable that changes during exercise. Additionally, the calculation of Θ is based on an assumed α, which is a coefficient that assumes a specific body temperature and pH (17). Since both temperature and pH are well known to change during exercise, a value for α was chosen that has been shown to give physiologically reasonable values in young healthy subjects (3) and is consistent with previous work from our research group (2, 20) and other research groups (3). Next, no correction for carboxyhemoglobin (COHb) backpressure was performed. Should COHb backpressure exist, this would affect the measured DL_{CO} (12). This limitation was addressed by recruiting non-smokers, including cigarette, recreational marijuana, and water-pipe users into the experiment. Finally, because of the difficulty of breath-holding during exercise, a six second breath-hold was performed instead of a 10 second breath-hold which is consistent with our previous work (2, 20, 21). In addition, breath-hold time could affect calculated DL_{CO} values. While a shorter breath-hold time would, in theory, affect the measure DL_{CO} value (12), there is evidence that the difference between a six and a 10 second breath hold is minimal (5, 12), thus justifying the use of this technique modification.

Other methodological limitations include the number of testing days required, the potential for testing effects, sampling procedures, and the calculation for PVR. Due to the complexity of the experimental design, individuals committed five different days in the laboratory to complete the study, which increased the difficulty of subject recruitment. Since individuals returned to the laboratory multiple times to perform the same diffusing capacity and exercise tests, testing effects between visits may have arisen. To minimize any testing effects, individuals were provided with a demonstration, familiarization of study procedures on their

preliminary day of testing, and coaching during experimental diffusion trials. In addition, the randomization of drug conditions and workloads helped to control for any potential testing effects. The use of a convenience sample is another limitation of this study, which limits the generalizability of the findings to other populations. Finally, to properly calculate PVR, Ppa and left atrial wedge pressure (Pw) must be measured invasively. Pw was not be measured here because of its invasive nature. Thus, the calculated resistance (*Appendix B*, Eq. 2) is considered total PVR and does not take into account downstream cardiac pressures (i.e., Pw; 10).

1.5 Definitions

Diffusing capacity is the ability of the lungs to transfer O_2 from the alveoli, across the alveolar-capillary membrane, and into the blood stream. Diffusing capacity for O_2 was operationalized using the diffusing capacity for carbon monoxide (DL_{CO}). VO_{2max} refers to the maximum rate of O_2 consumption achievable by the working muscles, signified by a plateau in O_2 consumption with an increase in work rate, while VO_{2peak} is the highest rate of O_2 consumption achieved during a graded exercise test to exhaustion (24). Exercise performance is an umbrella term to describe any type of exercise which quantifies changes before and after an intervention. This may include exercise tolerance tests (e.g., time-to-exhaustion test), exercise capacity, or functional capacity tests such as the 6-minute walk test. Exercise tolerance is the ability to perform work at a pre-determined workload until volitional exhaustion (24), and will be evaluated as time-to-exhaustion during heavy exercise in the current investigation. Steady state exercise is exercise performed below the anaerobic threshold at a constant work rate, with physiological variables such as heart rate and ventilation held within a very narrow range. A Valsalva maneuver occurs when expiration is forced against a closed glottis, creating positive pleural pressure within the thoracic cavity. A Mueller maneuver occurs when inspiration is

forced against a closed glottis, creating negative pleural pressure within the thoracic cavity. Residual volume is the volume of air remaining in the lung after a complete expiration. Total lung capacity is the total volume of air in the lungs after a full inspiration. Vasodilation is the widening of the internal diameter of blood vessels and vasoconstriction is the narrowing of the internal diameter of the blood vessels.

1.6 References

1. **Alkotob ML**, **Soltani P**, **Sheatt MA**, **Katsetos MC**, **Rothfield N**, **Hager WD**, **Foley RJ**, **Silverman DI**. Reduced exercise capacity and stress-induced pulmonary hypertension in patients with scleroderma. *Chest* 130: 176–181, 2006.

2. **Bouwsema MM**, **Tedjasaputra V**, **Stickland MK**. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? *J Appl Physiol* 122: 460–469, 2017.

3. **Ceridon ML**, **Beck KC**, **Olson TP**, **Bilezikian JA**, **Johnson BD**. Calculating alveolar capillary conductance and pulmonary capillary blood volume: comparing the multiple- and single-inspired oxygen tension methods. *J Appl Physiol* 109: 643–53, 2010.

4. **Dempsey JA**, **Wagner PD**. Exercise-induced arterial hypoxemia. *J Appl Physiol* 87: 1997–2006, 1999.

5. **Graham BL**, **Mink JT**, **Cotton DJ**. Effect of breath-hold time on DLCO(SB) in patients with airway obstruction. *J Appl Physiol* 58: 1319–1325, 1985.

6. **Hoshino Y**, **Obara H**, **Iwai S**. Relaxant effect of dopamine on isolated rabbit pulmonary artery. *Life Sci* 39: 2525–2531, 1986.

7. **Hsia C**. Recruitment of lung diffusing capacity - Update of concept and application. *Chest* 122: 1774–1783, 2002.

8. **Johnson RL**, **Spicer WS**, **Bishop JM**, **Forster RE**. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J Appl Physiol* 15: 893–902, 1960.

9. **Krogh M**. The diffusion of gases through the lungs of man. *J Physiol* 49: 271–300, 1915.

10. **Mostyn EM**, **Helle S**, **Gee JBL**, **Bentivoglio LG**, **Bates DV**. Pulmonary diffusing capacity of athletes. *J Appl Physiol* 18: 687–695, 1963.

11. **Naeije R**, **Chesler N**. Pulmonary circulation at exercise. *Compr Physiol* 2: 711–41, 2012.

12. **Ogilvie CM**, **Forster RE**, **Blakemore WS**, **Morton JW**. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 36: 1–17, 1957.

13. **Polak MJ**, **Kennedy LA**, **Drummond WH**. Manipulation of dopamine receptors alters hypoxic pulmonary vasoconstriction in isolated perfused rat lungs. *Life Sci* 51: 1317–1323, 1992.

14. **Reeves JT**, **Taylor AE**. Pulmonary hemodynamics and fluid exchange in the lungs during exercise. *Handb. Physiol. Exerc. Regul. Integr. Mult. Syst.* (1996). doi: 10.1002/cphy.cp120113.

15. **Roughton FJW**, **Forster RE**. Relative importance of diffusion and chemical reaction

rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 11: 290–302, 1957.

16. **Smith JR**, **Brown KR**, **Murphy JD**, **Harms CA**. Does menstrual cycle phase affect lung diffusion capacity during exercise? *Respir Physiol Neurobiol* 205: 99–104, 2015.

17. **Stickland MK**, **Lindinger MI**, **Olfert IM**, **Heigenhauser GJF**, **Hopkins SR**. Pulmonary gas exchange and acid-base balance during exercise. *Compr Physiol* 3: 693–739, 2013.

18. **Stickland MK**, **Welsh RC**, **Haykowsky MJ**, **Petersen SR**, **Anderson WD**, **Taylor DA**, **Bouffard M**, **Jones RL**. Intra-pulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 561: 321–329, 2004.

19. **Suresh K**, **Shimoda LA**. Lung circulation. *Compr Physiol* 6: 897–943, 2016.

20. **Tedjasaputra V**, **Bouwsema MM**, **Stickland MK**. Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity response to exercise. *J. Physiol.* (2016). doi: 10.1017/CBO9781107415324.004.

21. **Tedjasaputra V**, **van Diepen S**, **Collins SE**, **Michaelchuk WW**, **Stickland MK**. Assessment of pulmonary capillary blood volume, membrane diffusing capacity, and intrapulmonary arteriovenous anastomoses during exercise. *J Vis Exp* 120, 2017.

22. **Tolle JJ**, **Waxman AB**, **Van Horn TL**, **Pappagianopoulos PP**, **Systrom DM**. Exercise-induced pulmonary arterial hypertension. *Circulation* 118: 2183–2189, 2008.

23. **Vaughan TR**, **DeMarino EM**, **Staub NC**. Indicator dilution lung water and capillary blood volume in prolonged heavy exercise in normal men. *Am Rev Respir Dis* 113: 757–762, 1976.

24. **Wasserman K**, **Hansen JE**, **Sue DY**, **Casaburi R**, **Whipp BJ**. *Principles of Exericse Testing and Interpretation: Including Pathophysiology and Clinical Applications*. 3rd ed. Balitmore, MD: Lipincott Williams & Wilkins, 1999.

Chapter II. Literature Review

2.1 Gas exchange

2.1.1 Gas exchange during exercise

Exercise represents a significant stress on the pulmonary system, as cardiac output (Q) increases up to six-fold and gas exchange must increase in order to meet the heightened oxygen (O_2) demand of the working muscles (56). If the pulmonary system does not increase gas exchange enough to support the demand of the working muscles during exercise, pulmonary limitations can occur (17, 31, 39, 64). Exercise-induced arterial hypoxemia (EIAH) is evidence of a pulmonary limitation to exercise.

EIAH is defined by a drop in arterial O_2 saturation (SaO_2) ranging in severity from mild to severe and is characterized by the excessive widening of the partial pressure difference between the alveoli and the blood ($A\text{-}aDO_2$; 18). Individuals with the most excessive widening of the $A\text{-}aDO_2$ during maximal exercise experience a significant drop in the partial pressure of arterial O_2 (> 10mmHg) and SaO_2 (> 5-10%) from resting values (31), diminishing their ability to transport O_2 to metabolically active tissues during exercise. Dempsey and Wagner (20) noted that in healthy subjects, $A\text{-}aDO_2$ will progressively increase from near zero at rest to 15-25mmHg during graded exercise to volitional exhaustion and suggest that $A\text{-}aDO_2$ above this value represents a serious gas exchange inefficiency.

2.1.2 Mechanisms for the widening of $A\text{-}aDO_2$

The widening of $A\text{-}aDO_2$ may occur secondary to a gas exchange impairment including diffusion limitation, ventilation-perfusion (V/Q) inequality, or intrapulmonary shunt (20).

Dempsey and colleagues (18) suggested the widening of $A\text{-}aDO_2$ is the result of a diffusion limitation caused by a reduction in transit time of the red blood cells in the pulmonary capillaries. Ayappa et al. (4) challenged the idea that a reduction in pulmonary capillary transit time explains the drop in arterial O_2 saturation by perfusing an isolated animal lung with perfect

V/Q matching and found no change in A-aDO_2 when measured pulmonary transit time was reduced from 0.50-0.62 to 0.14-0.18 seconds. While Ayappa and colleagues' (4) finding may discredit the theory put forward by Dempsey et al. (18), it can alternatively be viewed as evidence that the V/Q inequality seen during heavy exercise at sea level must be present for a diffusion limitation to affect A-aDO_2 (19). In accordance with this theory, Rice et al. (69) suggest the excessive widening of A-aDO_2 at maximal exercise is explained by a combination of V/Q inequality and diffusion limitation.

Diffusion limitation caused by pulmonary edema may also be responsible for the excessive widening of A-aDO_2 during heavy exercise. Hopkins et al. (34) performed broncho-alveolar lavage in elite athletes and found that during intense exercise, these athletes had greater concentrations of red blood cells in the airways than controls. This suggests that the alveolar-capillary membrane has been damaged, leading to interstitial edema, an increased diffusion distance, and therefore a diffusion limitation. However, repeated bouts of maximal exercise improved A-aDO_2, contradicting the theory that structural damage to the alveolar-capillary membrane results in a diffusion limitation which explains the excessive widening of A-aDO_2 in those with EIAH (75).

Intrapulmonary shunts may explain the excessive widening of A-aDO_2 during heavy exercise in EIAH. Intrapulmonary shunting occurs when blood returning to the right side of the heart via the venous circulation passes through the pulmonary circulation, but no gas exchange occurs (77) allowing deoxygenated blood to mix with oxygenated blood in the left ventricle. Importantly, a 2-3% shunt of Q would explain all of the increase in A-aDO_2 during exercise (76). The multiple inert gas elimination technique, currently the gold standard technique for assessing pulmonary gas exchange, does not show significant intrapulmonary shunting at rest or during

exercise in healthy humans (20). In addition, studies utilizing 100% O_2 to quantify shunts during exercise have consistently found insignificant results during exercise (18, 87, 88). However, the existence of large intrapulmonary passageways that bypass the capillary networks have been confirmed in isolated human lungs (50, 83), and saline contrast echocardiography has provided evidence that intrapulmonary shunts open during exercise (22, 49, 77, 78, 82).

Regardless of the precise mechanism for the increase in A-aDO_2 with exercise, the progressive and excessive increase in A-aDO_2 with exercise represents a significant pulmonary limitation to exercise and highlights the importance of gas exchange for exercise performance.

2.1.3 A-aDO_2 and exercise performance

Athletes who experience a widening of A-aDO_2 at sea level have impaired exercise performance, which is evidenced by the decline in performance with artificially induced hypoxemia (45) and their improvement in performance with the administration of slightly hyperoxic gas mixtures (58, 61, 71).

Koskolou and McKenzie (45) artificially induced arterial hypoxemia in well trained male cyclists by carefully reducing the fraction of inspired O_2 (F_IO_2) to achieve mild hypoxemia (SaO_2 of 90%) and moderate hypoxemia (SaO_2 of 87%) during five minute bouts of exhausting cycle ergometer exercise. Total work output during the five minute bouts of exercise was progressively reduced as the level of arterial SaO_2 decreased (45). Additionally, Nielsen and colleagues (58) had trained oarsmen perform exhaustive six minute bouts of exercise on a rowing ergometer with a F_IO_2 of either 21% or 30% in a randomized and blinded fashion. Total work output during the six minute bout of exercise was significantly improved when arterial hypoxemia was prevented using hyperoxia (58). Peltonen and colleagues (61) had national level rowers complete an all-out 2500m rowing ergometer sprint in normoxia, hypoxia (F_IO_2 15.8%), and hyperoxia (F_IO_2

62.2%). During hyperoxia, 500m interval times were significantly reduced and total time to complete the bout of exercise was also reduced (61). Finally, Romer and Dempsey (71) took highly fit endurance trained athletes and found an increase in time-to-exhaustion at ≥ 85% of VO_{2max} on a cycle ergometer when SaO_2 was maintained by increasing the F_IO_2 from 21% to 28%. Taken together, these studies highlight the need for maintaining adequate gas exchange during exercise. Maintaining SaO_2 has positive implications for exercise performance, as athletes who experience an excessive widening of A-aDO_2 and a corresponding drop in SaO_2 will have impaired exercise performance.

2.2 Pulmonary circulation

2.2.1 Introduction

The pulmonary circulation is a low pressure circuit with a mean resting inflow pressure (Ppa) of around 15 mmHg and an outflow pressure (Pw) of around five mmHg (90). Comparatively, the systemic circulation has a mean inflow pressure of approximately 100 mmHg and an outflow pressure of around two mmHg (90). The pulmonary arteries are relatively thinner than systemic arteries as a result of the need for efficient O_2 transport in the pulmonary microcirculation (56, 67, 80). The primary function of the pulmonary circulation is gas exchange (90), although other functions of the pulmonary circulation include filtration and storage of blood, as well as metabolic regulation of vasoactive hormones (80).

2.2.2 Models of flow

Steady-flow hemodynamics is commonly used to understand the pulmonary circulation at rest and during exercise. In this model, a single resistance calculation is made in order to examine the state of the pulmonary circulation using a derivation of Ohm's law for resistance and the Hagen-Poiseuille law for fluid movement through non-distensible circular tubes (56). To calculate pulmonary vascular resistance (PVR), the difference between Ppa and Pw is divided by Q. Since

resistance is determined by vessel diameter, assuming unchanged blood viscosity and total length of the circulation, changes in PVR can infer changes in vascular tone (56, 80).

PVR has been calculated in humans through invasive measurement of Ppa, Pw, and Q at rest and during exercise. The relationship between Ppa and Q has been shown to be approximately linear across the physiological range such that a one L/min increase in Q results in a one mmHg increase in Ppa (56). Resting measurements suggest that PVR increases with age (27, 32), decreases going from the upright to the supine position (46, 47), and shares a parabolic relationship with lung volumes such that PVR is greatest at high and low lung volumes (73).

Steady-flow hemodynamics offers a pragmatic model to better understand the pulmonary circulation at exercise. While the current understanding of the pulmonary circulation supports that changes in Ppa are closely related to changes in Q with the initiation of exercise (56), this model was utilized to better understand the role of dopamine in pulmonary vascular regulation during exercise.

2.2.3 The effect of dynamic exercise

With the initiation of upright exercise, there is a decrease in blood volume in the lower extremities and an increase in blood volume in the thoracic cavity (24). This central shift of blood results in recruitment and distention of the pulmonary capillaries, increasing Vc and the effective surface area for diffusion (36). As Q rises with exercise, recruitment and distention of the pulmonary capillaries decreases PVR (47) allowing increased pulmonary blood flow while limiting the rise in Ppa with exercise (57). Increased Q is associated with an increase in Ppa and Pw (78, 88). At peak exercise, Ppa and Pw can rise as high as 40 mmHg and 20 mmHg respectively (88). Interestingly, Pw explains 80% of the variation in Ppa during exercise, which would suggest that the downstream pressure is an important regulator of upstream pressure in the

pulmonary circulation (67). The idea that Pw is an important regulator of Ppa has been speculated to explain the prominent inter-individual variation in the Ppa response to exercise, as the Pw response to exercise is highly variable (67).

Additionally, Pw and Ppa may be related cardiac function. Using a healthy animal model, Cheng and colleagues (12) demonstrated that the increase in the mitral valve pressure gradient during exercise was secondary to a reduction in early diastolic left-ventricular pressure rather than an increase in left atrial pressure. In an animal model of chronic heart failure, Cheng et al. (13) found that the mitral valve pressure gradient was increased via increases in left atrial pressure, in contrast to their previous work with healthy animals. The former study highlights that the healthy, compliant heart can increase left ventricular filling during exercise by decreasing pressure within that chamber. In contrast, with chronic heart failure, significantly higher resting pressures are present and the limits of myocardial compliance may have been reached. In this model, further increases in left ventricular filling were achieved by heightening left atrial pressure. While pulmonary pressures were not measured in the aforementioned studies (12, 13), the consequence of increasing left atrial pressure would likely be an increase in upstream pressures including Ppa and Pw. Support for this idea comes from Stickland and colleagues' (79) work examining the effect of fitness on the cardiovascular hemodynamic response to exercise. Pulmonary and cardiac pressures and volumes were measured invasively and compared in individuals with high cardiorespiratory fitness (VO_{2max} 60 ± 3 $mL \cdot kg^{-1} \cdot min^{-1}$) and low cardiorespiratory fitness (VO_{2max} 43 ± 6 $mL \cdot kg^{-1} \cdot min^{-1}$) during incremental exercise. The low fitness group had a steeper rise in right atrial pressure, and the high cardiorespiratory fitness group had the same or lower Pw and transmural filling pressure (right atrial pressure – Pw) for a given stroke volume, suggesting enhanced cardiac compliance and pulmonary

distensibility in the high fitness group (79). This work strongly suggests that cardiac function has an effect on upstream pulmonary pressures and that enhanced left ventricular compliance results in lower pulmonary pressures during dynamic, incremental exercise. As Ppa is highly correlated with Pw during dynamic exercise (67), cardiac compliance and cardiac function may be responsible for the variation in both Pw and Ppa observed with exercise (12, 13, 79). Accordingly, pressures downstream from the lungs may have a direct influence on Vc and PVR during exercise.

2.2.4 Invasive evaluation of the pulmonary circulation

Invasive measurements of the pulmonary circulation have been performed in humans to measure Ppa, Pw, Q, and PVR. Holmgren and colleagues (32) performed right heart catheterization in 14 young healthy males and four young healthy females. Subjects performed six minutes of cycle ergometer exercise with varying increments in workload. No change in mean Ppa was reported from rest up to the highest workload of exercise. However, significant inter-individual variation in the Ppa response to exercise was noted. Small increases in Pw occurred with increasing intensity of exercise, the largest individual response being a seven mmHg increase from rest up to the highest workload. PVR was reduced from rest up to the highest workload (32).

Granath, Jonsson, and Strandell (27) performed right heart catheterization at rest and during exercise in both the sitting and the supine positions in 17 healthy older men with a mean age of 71. In both positions, measurements were taken at rest and at two consecutive workloads of increasing intensity. During exercise, mean Ppa and Pw increased from rest up to the highest workload (27). In the supine position, both mean Ppa and Pw were higher at rest and during exercise. Additionally, Q was higher at rest and during exercise in the supine position. In both positions, PVR decreased from rest up to the highest level of exercise (27). This study provided

an early and comprehensive examination of cardiopulmonary physiology at rest and during exercise.

Wagner et al. (88) measured pulmonary pressures invasively in seven young healthy males and one young healthy female. More than half of the subjects were regular cyclists, long distance runners, and mountaineers and three of the subjects were reported to be more sedentary, thus a range of fitness levels were present. Measurements were made at rest and during steady state exercise on a cycle ergometer in increments of 60 W up to 240 W at sea level. Mean Ppa and mean Pw increased with every increase in workload on the cycle ergometer in accordance with the results of Granath et al. (27) and in disparity with the results of Holgren et al. (32). Q increased with every increase in workload, and PVR decreased from rest up to 120W then plateaued with further increases in intensity (88). The quality of Wagner and colleagues' (88) data are strong, as steady state criteria was strictly set within 5% constancy for pedal rate, heart rate, pulmonary pressures, end-tidal carbon dioxide (CO_2) and O_2 partial pressure, respiratory rate, and minute ventilation, whereas the attainment of steady state in each workload was not as convincing in Holgren et al.'s (32) study. Thus, in terms of the pulmonary pressure response to exercise, any differences between studies may be the result of inaccurate confirmation of steady state during cycle ergometer exercise.

Stickland et al. (78) measured pulmonary pressures during exercise in eight healthy, highly fit males with a mean age of 30 years and an average VO_{2max} of 54.7 $mL \cdot kg^{-1} \cdot min^{-1}$. Resting upright and supine data as well as exercise data on a cycle ergometer at 75 W, 150 W, ventilatory threshold, 25 W above ventilatory threshold, and 85% of VO_{2max} was collected. Pulmonary artery pressure (Ppa) and Pw both decreased going from the supine to the upright position, Ppa increased significantly with every increase in workload, and Pw began to increase

after ventilatory threshold. Cardiac output was significantly higher with every increase in workload and PVR was consecutively reduced compared to upright baseline starting at 150 W up to 85% of VO_{2max}. The increase in Ppa going from upright to supine fits Granath et al.'s (27) data, and the increases in both Ppa and Pw during exercise of increasing intensity parallel both Wagner et al.'s (88) and Granath et al.'s (27) results.

Based on the invasive studies of pulmonary hemodynamics reviewed above, it appears that Ppa and Pw are affected by body position, exercise intensity, and cardiac function, while PVR decreases with the initiation of upright exercise (12, 13, 27, 32, 78, 79, 88).

2.2.5 Non-invasive evaluation of the pulmonary circulation

Invasively measuring pressures, flow, and resistance in the pulmonary circulation is the most accurate means of quantifying these variables at rest and during exercise and has been done in a variety of subjects and conditions at rest and during exercise (28, 32, 78, 88). However, Doppler echocardiography appears to be a feasible alternative to invasive measurements of Ppa and Q (3).

To estimate mean Ppa during exercise, the maximum velocity of tricuspid regurgitation is determined using ultrasound and pulmonary artery systolic pressure (PASP) is calculated (91). Once PASP is obtained, mean Ppa can be calculated (11). Argiento and colleagues (3) found their values of mean Ppa to closely match invasively measured values with a mean Ppa around 13 mmHg at rest and 30 mmHg at peak exercise. Further, the mean Ppa/Q relationship in Argiento and colleagues' (3) study was 1.37 $mmHg \cdot min^{-1} \cdot L$, which is in close agreement with invasive studies showing a mean slope 0.94 $mmHg \cdot min^{-1} \cdot L$ in slightly younger subjects (65). In addition, Argiento et al. (3) calculated distensibility noninvasively and found a value of 0.017 ± 0.018 mmHg, which aligns with invasively derived calculations of distensibility in humans (66),

adding confidence to the validity of echocardiographic estimations of pressure and flow in the pulmonary circulation.

Limitations of this technique include the requirement of an experienced ultrasonographer (3) and a semi-supine cycle ergometer when working with young healthy human subjects, as getting adequate tricuspid regurgitation during upright cycling has been shown to be challenging in this group (82). With a semi-supine cycle ergometer, 88% of subjects tested had adequate tricuspid regurgitation at rest and during exercise for the analysis of PASP (3). While non-invasive measurement of the pulmonary circulation seems promising, further research should focus on the validity and reliability of these techniques, directly comparing invasive and non-invasive measures.

2.2.6 Pulmonary arterial pressure and exercise tolerance

In situations when Ppa becomes exceedingly high, exercise tolerance may become reduced. Tolle and colleagues (84) catheterized individuals with resting pulmonary arterial hypertension (PAH; resting mean Ppa > 25 mmHg), exercise-induced pulmonary arterial hypertension (resting mean Ppa < 25 mmHg, exercising mean Ppa > 30 mmHg), and controls during exercise. Mean Ppa was significantly higher in exercise-induced PAH compared to controls and was highest in resting PAH compared to both other groups. Maximum achieved workload and Q during a cardiopulmonary exercise test was significantly lower in both PAH groups (84). The reduction in maximal workload and Q secondary to high Ppa highlights the detrimental effect of high Ppa on exercise performance.

In addition, non-invasive studies have shown that high Ppa may be related to exercise intolerance. Alkotob et al. (1) and Cotrim et al. (16) measured exercise tolerance as time-to-exhaustion during the Bruce treadmill test while performing Doppler echocardiography to

estimate PASP. In both studies, high PASP was associated with a reduction in total time of the Bruce treadmill protocol and in Alkotob and colleagues' (1) study, those in the highest quartile of post-exercise PASP had the shortest exercise time during the Bruce treadmill test. While time-to-exhaustion during the Bruce treadmill test may be a crude measure of exercise tolerance, these non-invasive studies suggest that exercise tolerance is reduced in individuals with high Ppa.

Right ventricular afterload may explain the reduction in Q and exercise performance with an increase in Ppa. Bonderman and colleagues (7) estimated right ventricular afterload by measuring PVR and arterial compliance in pulmonary hypertension and health. They found that in health, PVR was reduced with exercise and arterial compliance increased, whereas PVR was unchanged and arterial compliance decreased with exercise in pulmonary hypertension. The lack of reduction in PVR suggests right heart dysfunction and an inability to appropriately expand Vc and adjust Q, leading to exercise intolerance.

It is evident that high Ppa has a detrimental effect on exercise performance, which is likely due to a reduction in Q (84). The inability to increase Q may be due to increased right heart afterload and a lack of reduction in PVR (7), but other factors such as pulmonary edema might also play a role.

2.2.7 Pulmonary edema, gas exchange, and exercise tolerance

During exercise, pulmonary microvascular pressures increase as a result of the increase in Ppa and Pw, which consequently increase microvascular filtration pressure (67). This increase in microvascular filtration pressure is opposed by an increase in microvascular absorption pressure, preventing fluid accumulation in the pulmonary interstitium (i.e., pulmonary edema) from occurring.

Pulmonary edema may cause an impairment in gas exchange if the fluid accumulating in the interstitium drains into the alveoli causing intra-alveolar edema (67). There is evidence of mild pulmonary edema in humans exercising at sea level (92), but this does not seem to correlate well with indices of gas exchange (i.e., A-aDO_2; 18). This leads to the conclusion that exercise in humans results in pulmonary interstitial edema, but not intra-alveolar pulmonary edema (92). However, sea level natives travelling to high altitudes experience pulmonary edema. In individuals travelling to high altitudes, hypoxic pulmonary vasoconstriction (HPV) results in high Ppa and pulmonary edema (44) and this clearly impacts their exercise tolerance. During high intensity exercise at a simulated altitude of 4090 m, healthy, non-acclimatized subjects developed evidence of pulmonary edema via an ultrasound technique and increased their time to complete a high-intensity exercise protocol compared to at sea level (21).

In summary, a potentiated Ppa response with exercise appears to contribute to exercise intolerance, either through reduced convective O_2 delivery to the muscle secondary to right ventricular afterload, or through pulmonary edema and the resulting impairment in gas exchange. Failure to decrease PVR appropriately with exercise would result in an exaggerated Ppa response to exercise and reduced exercise tolerance.

2.3 Regulators of pulmonary vascular tone

Factors that affect vascular tone in the pulmonary circulation are generally classified as either neural or circulatory and can be further broken down into vasodilators and vasoconstrictors (80).

2.3.1 Pulmonary vasoconstrictors and vasodilators

The most commonly studied pulmonary vasoconstrictors include Endothelin-1 (ET-1), serotonin, and angiotensin II (80). ET-1 is produced by the endothelial cells of pulmonary blood vessels and binds to membrane receptors situated throughout the pulmonary circulation (80). Serotonin

is secreted by pulmonary neuroendocrine cells and can be taken up by the endothelial cells via a transporter protein allowing it to perform vasoactive functions within the pulmonary circulation (80). Angiotensin II is converted from its precursor, angiotensin I, primarily in the pulmonary circulation and causes vasoconstriction of vessels in both intact lung and vessel preparations of many animal species (80).

The most common pulmonary vasodilators studied include nitric oxide (NO), adenosine, atrial natriuretic factor (ANF), prostacyclin (PGI_2), and dopamine (78) The release of NO, a vasodilator primarily produced by the endothelium of the blood vessel, is regulated by the production of NO synthase and performs its role as a vasodilator by promptly diffusing into the smooth muscle layer of the blood vessel, initiating relaxation (80). Adenosine is considered a pulmonary vasodilator, which performs its action by binding to surface receptors on pulmonary blood vessels (80). ANF is released by cardiac myocytes into the pulmonary circulation during periods of increased right atrial stretch and attaches to its receptors on pulmonary blood vessels in order to elicit a vasodilatory response (80). PGI_2 is produced by the endothelium of the vasculature and is thought to cause a vasodilatory response in the underlying smooth muscle layer of both pulmonary and systemic blood vessels (80). Of particular interest is the pulmonary vasodilator dopamine.

2.4 Dopamine

2.4.1 Introduction

Dopamine is a brain neurotransmitter involved in the regulation of locomotion, emotion, and endocrine function (52). Importantly, dopamine also acts peripherally as a catecholamine regulating cardiovascular function and vascular tone (52). There are two predominant classes of dopamine receptors in the human body—dopamine receptor-1 (D_1-receptor) and dopamine

receptor-2 (D_2-receptor)—both of which are found throughout the cardiovascular system (52). When dopamine binds to D_2-receptors on the walls of blood vessels, vasodilation occurs via the inhibition of norepinephrine (6, 52). In addition to dopamine's effect on the blood vessels, higher doses of exogenous dopamine (> 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) can act as an inotrope and increase Q (10, 54). The half-life of dopamine has been reported to be < 10 minutes (9).

2.4.2 Dopamine and the cardiovascular system

As previously noted, dopamine receptors exist in the cardiovascular system, and dopamine plays a vasoactive role within this system. O'Malley et al. (59) used a rat model to show that D_2-receptors are found in great density in the cardiovascular system and are subsequently thought as important mediators of cardiovascular function (59). Additionally, D_2-receptors are thought to be involved in the renal circulation, as administration of a moderate dose of dopamine (5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) results in a pronounced vasodilator effect in the renal blood vessels (25) causing an increase in blood flow and filtration within the kidney (2). The above studies suggest that both D_1 and D_2-receptors exist in the cardiovascular system, and D_2-receptors may be more important in the regulation of vascular tone within the cardiovascular system.

Of interest is dopamine's vasoactive role in the pulmonary vasculature. Hoshino and colleagues (35) infused dopamine into an isolated rabbit pulmonary artery and found that vessel relaxation occurred, suggesting the presence of a dopamine receptor within this vessel. Further, administration of a D_1-receptor antagonist blocked the effect of dopamine, whereas application of a D_2-receptor antagonist had no effect, signifying these receptors are not important in the pulmonary circulation of rabbits (35). Gorman (26) found that in the isolated ferret lung, under conditions of HPV, D_1-receptor agonists caused vasodilation measured as a reduction in Ppa during a period of constant blood flow. D_1-receptor antagonists also blocked the effect of

dopamine, further supporting its role as a pulmonary vasodilator. In this particular model, no evidence for the existence of D_2-receptors in the pulmonary circulation was obtained (26). Polak et al. (63) used an isolated, perfused rat lung model to examine the effect of a D_1-receptor agonist and antagonist on HPV. A hypoxic gas mixture (0% O_2, 74% N_2, 5% CO_2) was ventilated for a brief time in order to initiate HPV, then either an agonist, an antagonist, or both were administered. The dopamine receptor agonist significantly reduced Ppa in comparison to the highest Ppa measured during HPV (63). The study by Polak and colleagues (63) strongly suggests the existence of D_1-receptors in the pulmonary circulation and highlights the effectiveness of dopamine as a pulmonary vasodilator in a state of vasoconstriction. Polak and Drummond (62) performed a similar experiment to the one above (63) in chronically instrumented lambs in order to clarify the role of dopaminergic vasodilation in a normal, resting state. Blockade of the D_1-receptors resulted in pulmonary vasoconstriction, evidenced through the increase in Ppa and PVR compared to control conditions (62). Infusion of fenaldopam, a dopamine agonist, provided an unexpected result as there was a significant increase in Ppa which would be suggestive of vasoconstriction. Since there was no change in PVR, Polak and Drummond (62) proposed that this effect is a result of the inotropic effect of dopamine either caused by direct activation of dopaminergic receptors, or indirectly through sympathetic catecholamines. With a lower dosage of dopamine receptor agonist, it is likely that Ppa and PVR would not have changed (10).

In humans, dopamine has been used to study intrapulmonary arterio-venous anastomoses (IPAVA; (10, 82)) and right-to-left anatomical shunt (68). Bryan and colleagues (10) infused dopamine in young healthy men and women in an effort to elucidate the mechanism of the increase in intrapulmonary shunting with an increase in Q. No claims can be made about PVR, as

Pw was not measured and mean Ppa was not calculated, but low dose dopamine (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) did not change Q or PASP significantly. Tedjasaputra et al. (82) used the D_2-receptor antagonist metoclopramide to study the effect of dopamine on gas exchange and IPAVA recruitment during exercise. Metoclopramide ingestion led to an improvement in gas exchange (i.e. A-aDO_2) during exercise and a decrease in maximal stroke volume and Q (82). While Ppa and PVR were not obtained in this study, the decrease in maximal Q in the metoclopramide condition may be explained by a lack of reduction in PVR, greater increase in Ppa, and heightened right heart afterload (82). Thus, Tedjasaputra and colleagues' (82) study provides indirect evidence for the existence of D_2-receptors in the pulmonary circulation. Kobayashi et al. (43) provide further support for the presence of D_2-receptors in the pulmonary circulation, who localized these receptors in the human pulmonary artery using a radio-ligand binding technique.

Based on both the human and animal studies looking at the effect of dopamine on the pulmonary vasculature, it is clear that D_1-receptors exist within the pulmonary circulation and may be involved in the maintenance of the low resting tone seen in many species (62, 63). Evidence for the existence of D_2-receptors within the pulmonary circulation is not strong in animals, but studies done in humans show support for their existence. Moreover, it appears that endogenous dopamine, through stimulation of a D_2-receptor, may be important for regulation of pulmonary circulation at rest and during exercise. Further elucidation of the effect of dopamine on the pulmonary vasculature at rest and during exercise is needed in humans.

2.4.3 Dopamine and exercise performance

Endogenous dopamine levels increase in a curvilinear fashion with an increase in exercise intensity indicated by the heightened dopamine concentration in the venous blood as a function of the volume of O_2 consumption (33). Dopamine appears to be important during exercise, as

blocking dopamine receptors coincides with an impairment in performance. Tedjasaputra and colleagues (82) blocked D_2-receptors using metoclopramide and found a significant reduction in VO_{2max} and peak power output during graded exercise to exhaustion, as well as reduced time-to-exhaustion at 85% of VO_{2max} compared to control conditions. This decrement in exercise performance occurs even though metoclopramide improved gas exchange and SaO_2 (82). Work in animal models also supports that dopamine blockade reduces exercise tolerance, as Balthazar et al. (5) blocked central D_1 and D_2-receptors in the brains of rats and found that time-to-exhaustion during graded treadmill exercise was significantly reduced in both blocked conditions (5).

While antagonizing dopamine receptors clearly has negative implications for exercise performance, dopamine receptor agonists seem to improve performance under certain conditions. Watson and colleagues (89) administered a dual norepinephrine/dopamine reuptake inhibitor called bupropion to healthy, endurance trained males during exercise in effort to measure its effect on performance in warm (30°C) and cool (18°C) conditions. While bupropion had no effect in cool conditions, the time to complete a set amount of work at 75% of their peak power output was significantly improved in warm conditions (89). Roelands et al. (70) performed a very similar experiment in endurance trained athletes using a dopaminergic reuptake inhibitor called methylphenidate. In accordance with the results of Watson and colleagues (89), time trial performance was significantly improved with methylphenidate in the warm, but not the cool, environmental condition (70). These studies highlight that under conditions of thermal stress, dopamine is important for exercise performance.

To date, studies looking at the effect of dopamine on exercise performance show that dopamine plays an important role in maintaining exercise tolerance as dopamine receptor

blockade results in a clear decrement in performance (5, 82). Studies looking at receptor agonists show no effect of dopamine on performance in environmental conditions near room temperature (70, 89), but the dearth of studies looking at the effect of dopamine on exercise tolerance warrants further investigation into this area.

2.5 Diffusing capacity of the pulmonary system

2.5.1 Introduction

The diffusing capacity for carbon monoxide (DL_{CO}) is used to evaluate the ability of the lungs to diffuse gas from the alveoli into the pulmonary capillaries. When a trace amount of carbon monoxide (CO) is inspired into the healthy human lung, Fick's first law (23) states that it will diffuse into the blood at a rate determined by the partial pressure gradient between the alveoli and the blood, the alveolar membrane thickness, and surface area for diffusion. CO, a diffusion limited gas (90), is measured in place of O_2 because of the difficulty of measuring the diffusing capacity of O_2. With DL_{CO} it is assumed that all of the CO crossing alveolar-capillary membrane will instantaneously combine with hemoglobin (Hb) due to the large affinity of Hb for CO (29). Therefore, since negligible amounts of CO will dissolve into the plasma of the blood, CO back pressure will not develop and the partial pressure gradient will be unaffected by this factor. Given that CO is limited by diffusion and not blood flow (i.e., perfusion; 89), it is an ideal gas for testing the diffusive properties of the lung.

2.5.2 Measuring DL_{CO}: The single-breath method

In order to end a scientific debate regarding the active secretion of O_2 from the alveoli, Dr. Marie Krogh used a version of the single-breath DL_{CO} method that is similar to the one used today (48). Since the method's first successful implementation, there have been a few modifications to the single-breath technique, such as the addition of a tracer gas (e.g., methane) to the gas mixture to

simplify the procedure (38). However, the fundamental methodological procedures remain the same.

The following description is based off of the American Thoracic Society and the European Respiratory Society's statement for the standardized single-breath DL_{CO} measurement procedure (51). Following normal tidal breathing into a respiratory measurement system, the subject is instructed to completely and non-forcefully empty the lungs to residual volume (RV). At this point, the subject is switched from room air to a gas mixture containing O_2, methane, CO_2, nitrogen, and a trace amount of CO. The subject is then instructed to quickly (< 2 seconds) inspire the test gas to total lung capacity (TLC) and hold their breath for 10 seconds. During the breath-hold, the subject is asked to avoid a Valsalva maneuver (positive pleural pressure) or a Mueller maneuver (negative pleural pressure) because these are known to affect DL_{CO} measurements (41, 60). After 10 seconds, the lungs are smoothly and non-forcefully emptied down to RV once again. DL_{CO} is then calculated using Eq. 1 of *Appendix B* (51).

The single-breath method for DL_{CO} has pragmatic advantages over the direct measurement of the diffusing capacity for O_2, including no requirement for arterial blood sampling and the availability of machines designed to automate the process (37). For these reasons, the single-breath method for DL_{CO} is a commonly used physiological and clinical test of pulmonary diffusing capacity.

2.5.3 Factors affecting DL_{CO}

In the following section, relevant factors affecting measured DL_{CO} values will be considered. Importantly, the length of breath-holding during the maneuver affects measured DL_{CO} such that breath-hold times less than 10 seconds will reduce DL_{CO} and breath-hold times greater than 10 seconds will yield higher values for DL_{CO} (60). In addition, changes in intrathoracic pressure

caused by performing a Valsalva maneuver lowers measured DL_{CO} values (41, 60). It is speculated that this may be due to the reduction in venous return during such a maneuver, leading to decreased Q (60). Ogilvie and colleagues (60) found that alveolar volume only had a small influence on measured DL_{CO} and for that reason, they did not standardize DL_{CO} for alveolar volume. More recent work has established that changes in alveolar volume affect DL_{CO} significantly, which highlights the importance of accounting for alveolar volume in the calculation (40). Body position also affects DL_{CO} measurements: the highest values are seen in the supine position and the lowest are seen in the standing position (60). Partial pressure of alveolar O_2 also affects the measured DL_{CO} values. For every mmHg drop in alveolar partial pressure of O_2, a 0.31% to 0.35% increase in DL_{CO} is to be expected (51).

Multiple studies have found that DL_{CO} decreases with an increased venous carboxyhaemoglobin (COHb; 49, 51, 58, 84) because less Hb binding sites are available and the partial pressure gradient for CO between the alveoli and the pulmonary capillary is reduced. While this may be accurate, correction for COHb is only recommended if baseline values are greater than 2% since prediction equations account for COHb levels up to 2% (51). In addition to COHb, DL_{CO} varies with Hb concentration, and measurements should be standardized for this variable (15). When performing multiple measurements of DL_{CO} on one occasion, the time between breath-holds must be long enough to allow all of the tracer gas and CO to wash out of the cardiorespiratory system. It is recommended that four minutes between single-breath DL_{CO} maneuvers be given at rest (51) and two minutes between maneuvers during exercise (81). With a host of factors affecting the measured DL_{CO} value using the single-breath method, it is apparent that careful consideration must be put into standardizing the testing procedure.

2.5.4 Pulmonary capillary blood volume and diffusing membrane capacity

DL_{CO} is thought to be comprised of two components: Vc and Dm (72). Dm represents the conductance of CO through the alveolar-capillary membrane, plasma, and interior of the red cell, while Vc represents the amount of blood in the pulmonary capillaries (90). The reciprocal of this relationship ($1/DL_{CO}$) represents the resistance to CO transport through intervening tissues from the alveoli to the red cell (36). Eq. 2 (*Appendix B*) shows the formula Roughton and Forster (72) derived to non-invasively partition out Vc and Dm using the single-breath DL_{CO} method at multiple O_2 tensions.

Roughton and Forster (72) recognized that the combination of CO with Hb is not instantaneous as initially thought in previous investigations (48). Therefore, they included Θ in their equation, which describes the rate of reaction between CO and Hb per mL of blood. Θ is inversely proportional to partial pressure of O_2 (38, 72), and when $1/\Theta$ is plotted against $1/DL_{CO}$, Vc and Dm can be determined (72). Performing multiple single-breath DL_{CO} maneuvers at varying O_2 tensions (i.e., 21%, 40%, 60%) creates a linear relationship where the slope of the regression line is equal to 1/Vc and the y-intercept represents 1/Dm (72). Taking the reciprocal of these values then produces the values for Vc and Dm. Figure 4 (*Appendix A*) shows the graphical relationship between $1/\Theta$ and $1/DL_{CO}$ at various O_2 tensions and the corresponding values for 1/Vc and 1/Dm.

Since Roughton and Forster (72) developed the multiple F_IO_2 DL_{CO} method for Vc and Dm determination, this technique has been used in a variety of settings including exercise (8, 14, 81). Research in this area has consistently shown that DL_{CO} increases from rest up to peak exercise secondary to increases in both Vc and Dm (8, 14, 81). In addition, it appears that DL_{CO} increases as a function exercise intensity regardless of cardiorespiratory fitness and age (14, 81).

However, there are important differences in the pulmonary diffusion response to exercise between highly fit individuals and those with lower fitness as well as young and old individuals (14, 81). In highly fit individuals, DL_{CO} is greater than in individuals with lower fitness secondary to greater Dm, while no difference in Vc was observed (81). When comparing young and old individuals, younger individuals exhibit a greater DL_{CO} response to exercise than their older counterparts which is due to a greater Vc and Dm response to exercise (14). Regardless of differences in the pulmonary diffusion response with aging and cardiorespiratory fitness, the increase in DL_{CO} with exercise is adequate to meet the heightened metabolic demand of the working muscle during exercise.

2.5.5 Sex Differences in DL_{CO}

There is a lack of consensus in the literature about whether differences in DL_{CO} exist between the sexes. DL_{CO} is lower in females compared to males after accounting for height, body surface area, and body mass index (42). However, Bouwsema, Tedjasaputra, and Stickland (8) suggest there is no difference between sexes when DL_{CO} is standardized to alveolar volume.

Research that examines how the menstrual cycle phases effect DL_{CO} contributes to the literature that argues sex differences do exist. For instance, Harms and Rosenkranz (30) found that resting DL_{CO} is reduced in the early follicular stage, and Smith et al. (74) found DL_{CO} is elevated during the mid-luteal phase secondary to an increase in Vc during heavy exercise. However, only two O_2 tensions were utilized in the latter study, bringing into question the accuracy of the measurements. The contested body of knowledge regarding sex differences in DL_{CO} warrants further investigation and suggests the standardization of menstrual cycle should be done routinely when studying female participants.

2.6 Summary

DL_{CO}, and its components Vc and Dm, must increase during exercise in order to meet the increase in the O_2 demand of the working muscle (48, 55, 60, 85). Failure to effectively increase diffusion during exercise would result in a gas exchange impairment, negatively affecting exercise performance secondary to decreased convective O_2 delivery to the muscle (45). While the currently accepted, passive model of pulmonary blood flow does not account for smooth muscle regulation of the pulmonary vasculature (56), recent evidence suggests that dopamine, a naturally occurring substrate, may play an active role in pulmonary vascular and Vc regulation during exercise (82). Thus, the purpose of this study is to investigate the role of dopamine in regulating diffusing capacity, Ppa, Vc and Dm during exercise, as well as exercise tolerance. It is hypothesized that dopamine will increase Vc and Dm, leading to a reduction in PVR and a corresponding decrease in Ppa. This response will allow an increase in DL_{CO}, Q, and exercise tolerance relative to control. Conversely, metoclopramide (D_2-receptor antagonist) will attenuate the increase in Vc and Dm and the reduction in PVR, leading to an increase in Ppa. In this condition, DL_{CO}, Q, and exercise tolerance will be reduced.

2.7 References

1. **Alkotob ML**, **Soltani P**, **Sheatt MA**, **Katsetos MC**, **Rothfield N**, **Hager WD**, **Foley RJ**, **Silverman DI**. Reduced exercise capacity and stress-induced pulmonary hypertension in patients with scleroderma. *Chest* 130: 176–181, 2006.

2. **Aperia A**. Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism. *Annu Rev Physiol* 62: 621–647, 2000.

3. **Argiento P**, **Chesler N**, **Mulè M**, **D'Alto M**, **Bossone E**, **Unger P**, **Naeije R**. Exercise stress echocardiography for the study of the pulmonary circulation. *Eur Respir J* 35: 1273–8, 2010.

4. **Ayappa I**, **Brown L V**, **Wang PM**, **Katzman N**, **Houtz P**, **Bruce EN**, **Lai-Fook SJ**. Effect of blood flow on capillary transit time and oxygenation in excised rabbit lung. *Respir Physiol* 105: 203–216, 1996.

5. **Balthazar CH**, **Leite LHR**, **Ribeiro RMM**, **Soares DD**, **Coimbra CC**. Effects of blockade of central dopamine D1 and D2 receptors on thermoregulation, metabolic rate and running performance. *Pharmacol Reports* 62: 54–61, 2010.

6. **Beaulieu J-M**, **Gainetdinov RR**. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63: 182–217, 2011.

7. **Bonderman D**, **Martischnig AM**, **Vonbank K**, **Nikfardjam M**, **Meyer B**, **Heinz G**, **Klepetko W**, **Naeije R**, **Lang IM**. Right ventricular load at exercise is a cause of persistent exercise limitation in patients with normal resting pulmonary vascular resistance after pulmonary endarterectomy. *Chest* 139: 122–127, 2011.

8. **Bouwsema MM**, **Tedjasaputra V**, **Stickland MK**. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? *J Appl Physiol* 122: 460–469, 2017.

9. **Brunton L**, **Lazo J**, **Parker K**. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 11th ed. New York: McGraw-Hill, 2005.

10. **Bryan TL**, **van Diepen S**, **Bhutani M**, **Shanks M**, **Welsh RC**, **Stickland MK**. The effects of dobutamine and dopamine on intrapulmonary shunt and gas exchange in healthy humans. *J Appl Physiol* 113: 541–8, 2012.

11. **Chemla D**, **Castelain V**, **Humbert M**, **Hébert J-L**, **Simonneau G**, **Lecarpentier Y**, **Hervé P**. New formula for predicting mean pulmonary artery pressure using systolic pulmonary artery pressure. *Chest* 126: 1313–1317, 2004.

12. **Cheng CP**, **Igarashi Y**, **Little WC**. Mechanism of augmented rate of left ventricular filling during exercise. *Circ Res* 70: 9–19, 1992.

13. **Cheng CP**, **Noda T**, **Nozawa T**, **Little WC**. Effect of heart failure on the mechanism of exercise-induced augmentation of mitral valve flow. *Circ Res* 72: 795–806, 1993.

14. **Coffman KE**, **Carlson AR**, **Miller AD**, **Johnson BD**, **Taylor BJ**. The effect of aging and cardiorespiratory fitness on the lung diffusing capacity response to exercise in healthy humans. *J. Appl. Physiol.* (2017). doi: 10.1152/japplphysiol.00694.2016.

15. **Cotes J**. Iron-deficiency anaemia: Its effect on transfer factor for the lung (diffusing capacity) and ventilation and cardiac frequency during sub-maximal exercise. 1972.

16. **Cotrim C**, **Simões O**, **Loureiro MJ**, **Cordeiro P**, **Lopes L**, **Almeida S**, **Ialá M**, **Miranda R**, **Carrageta M**. Stress echocardiography in the evaluation of exercise physiology in patients with severe arterial pulmonary hypertension. New methodology. *Rev Port Cardiol* 24: 1451–1460, 2005.

17. **Dempsey JA**. Is the lung built for exercise? *Med Sci Sports Exerc* 18: 143–155, 1986.

18. **Dempsey JA**, **Hanson PG**, **Henderson KS**. Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. *J Physiol* 355: 161–175, 1984.

19. **Dempsey JA**, **Sheel AW**, **Haverkamp HC**, **Babcock MA**, **Harms CA**. The John Sutton Lecture: CSEP, 2002]. Pulmonary system limitations to exercise in health. *Can J Appl Physiol* 28 Suppl: S2-24, 2003.

20. **Dempsey JA**, **Wagner PD**. Exercise-induced arterial hypoxemia. *J Appl Physiol* 87: 1997–2006, 1999.

21. **Edsell ME**, **Wimalasena YH**, **Malein WL**, **Ashdown KM**, **Gallagher CA**, **Imray CH**, **Wright AD**, **Myers SD**. High-intensity intermittent exercise increases pulmonary interstitial edema at altitude but not at simulated altitude. *Wilderness Environ. Med.*: 2014.

22. **Eldridge MW**, **Dempsey JA**, **Haverkamp HC**, **Lovering AT**, **Hokanson JS**. Exercise-induced intrapulmonary arteriovenous shunting in healthy humans. *J Appl Physiol* 97: 797–805, 2004.

23. **Fick A**. Ficks first law of diffusion. *Ann Phys* 170: 59, 1855.

24. **Flamm SD**, **Taki J**, **Moore R**, **Lewis SF**, **Keech F**, **Maltais F**, **Ahmad M**, **Callahan R**, **Dragotakes S**, **Alpert N**. Redistribution of regional and organ blood volume and effect on cardiac function in relation to upright exercise intensity in healthy human subjects. *Circulation* 81: 1550–1559, 1990.

25. **Goldberg L**. Cardiovascular and renal actions of dopamine: Potential clinical applications. *Pharmacol Rev* 24: 1–29, 1972.

26. **Gorman P**. Vasodilator response to dopamine in the ferret pulmonary circulation. *Br J Pharmacol* 94: 212–218, 1988.

27. **Granath A**, **Jonsson B**, **Strandell T**. Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. *Acta Physiol Scand* 176: 425–446, 1964.

28. **Granath A**, **Strandell T**. Relationships between cardiac output, stroke volume and

intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men. *Acta Med Scand* 176: 447–466, 1964.

29. **Haldane J**. The Action of Carbonic Oxide on Man. *J Physiol* 18: 430–462, 1895.

30. **Harms CA.**, **Rosenkranz S**. Sex differences in pulmonary function during exercise. *Med Sci Sports Exerc* 40: 664–668, 2008.

31. **Harms CA**, **McClaran SR**, **Nickele GA**, **Pegelow DF**, **Nelson WB**, **Dempsey JA**. Exercise-induced arterial hypoxaemia in healthy young women. *J Physiol* 507: 619–628, 1998.

32. **Holmgren A**, **Jonsson B**, **Sjostrand T**. Circulatory data in normal subjects at rest and during exercise in the recumbent position, with special reference to the stroke volume a different work intensities. *Acta Physiol Scand* 49: 343–363, 1960.

33. **Hopkins SR**, **Bogaard HJ**, **Niizeki K**, **Yamaya Y**, **Ziegler MG**, **Wagner PD**. Beta-adrenergic or parasympathetic inhibition, heart rate and cardiac output during normoxic and acute hypoxic exercise in humans. *J Physiol* 550: 605–616, 2003.

34. **Hopkins SR**, **Schoene RB**, **Henderson WR**, **Spragg RG**, **Martin TR**, **West JB**. Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090–1094, 1997.

35. **Hoshino Y**, **Obara H**, **Iwai S**. Relaxant effect of dopamine on isolated rabbit pulmonary artery. *Life Sci* 39: 2525–2531, 1986.

36. **Hsia C**. Recruitment of lung diffusing capacity - Update of concept and application. *Chest* 122: 1774–1783, 2002.

37. **Hughes JMB**. The single breath transfer factor (Tl,co) and the transfer coefficient (Kco): a window onto the pulmonary microcirculation. *Clin Physiol Funct Imaging* 23: 63–71, 2003.

38. **Hughes JMB**, **Borland CDR**. The centenary (2015) of the transfer factor for carbon monoxide (T(LCO)): Marie Krogh's legacy. *Thorax* 70: 391–394, 2015.

39. **Johnson BD**, **Saupe KW**, **Dempsey JA**. Mechanical constraints on exercise hyperpnea in endurance athletes. *J Appl Physiol* 73: 874–86, 1992.

40. **Johnson DC**. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. *Respir Med* 94: 28–37, 2000.

41. **Johnson RL**, **Spicer WS**, **Bishop JM**, **Forster RE**. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J Appl Physiol* 15: 893–902, 1960.

42. **Kendrick A**, **Laszlo G**. CO transfer factor on exercise: age and sex differences. *Eur Respir J* 3: 2340891, 1990.

43. **Kobayashi Y**, **Cavallotti D**, **Ricci A**, **Amenta F**. Localisation of dopamine D2-like

receptors in pulmonary artery of the human and rabbit but not of the rat. *Eur J Pharmacol* 261: 229–236, 1994.

44. **Korzeniewski K**, **Nitsch-Osuch A**, **Guzek A**, **Juszczak D**. High altitude pulmonary edema in mountain climbers. *Respir Physiol Neurobiol* 209: 33–38, 2015.

45. **Koskolou MD**, **McKenzie DC**. Arterial Hypoxemia and Performance During Intense Exercise. *Eur J Appl Physiol Occup Physiol* 68: 80–86, 1994.

46. **Kovacs G**, **Berghold A**, **Scheidl S**, **Olschewski H**. Pulmonary arterial pressure during rest and exercise in healthy subjects: A systematic review. *Eur Respir J* 34: 888–894, 2009.

47. **Kovacs G**, **Olschewski A**, **Berghold A**, **Olschewski H**. Pulmonary vascular resistances during exercise in normal subjects: a systematic review. *Eur Respir J* 39: 319–28, 2012.

48. **Krogh M**. The diffusion of gases through the lungs of man. *J Physiol* 49: 271–300, 1915.

49. **Lovering AT**, **Stickland MK**, **Eldridge MW**. Intrapulmonary shunt during normoxic and hypoxic exercise in healthy humans. In: *Advances in Experimental Medicine and Biology*. 2008, p. 31–45.

50. **Lovering AT**, **Stickland MK**, **Kelso AJ**, **Eldridge MW**. Direct demonstration of 25- and 50-microm arteriovenous pathways in healthy human and baboon lungs. *Am J Physiol Heart Circ Physiol* 292: H1777–H1781, 2007.

51. **MacIntyre N**, **Crapo RO**, **Viegi G**, **Johnson DC**, **van der Grinten CPM**, **Brusasco V**, **Burgos F**, **Casaburi R**, **Coates A**, **Enright P**, **Gustafsson P**, **Hankinson J**, **Jensen R**, **McKay R**, **Miller MR**, **Navajas D**, **Pedersen OF**, **Pellegrino R**, **Wanger J**. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 26: 720–735, 2005.

52. **Missale C**, **Nash SR**, **Robinson SW**, **Jaber M**, **Caron MG**. Dopamine receptors: from structure to function. *Physiol Rev* 78: 189–225, 1998.

53. **Mohsenifar Z**, **Tashkin DP**. Effect of carboxyhemoglobin on the single breath diffusing capacity: derivation of an empirical correction factor. *Respiration* 37: 185–191, 1979.

54. **Molloy DW**, **Ducas J**, **Dobson K**, **Girling L**, **Prewitt RM**. Hemodynamic management in clinical acute hypoxemic respiratory failure. Dopamine vs dobutamine. *Chest* 89: 636–640, 1986.

55. **Mostyn EM**, **Helle S**, **Gee JBL**, **Bentivoglio LG**, **Bates DV**. Pulmonary diffusing capacity of athletes. *J Appl Physiol* 18: 687–695, 1963.

56. **Naeije R**, **Chesler N**. Pulmonary circulation at exercise. *Compr Physiol* 2: 711–41, 2012.

57. **Naeije R**, **Vanderpool R**, **Dhakal BP**, **Saggar R**, **Saggar R**, **Vachiery JL**, **Lewis GD**. Exercise-induced pulmonary hypertension: Physiological basis and methodological concerns. *Am. J. Respir. Crit. Care Med.* 187: 576–583, 2013.

58. **Nielsen HB**, **Boushel R**, **Madsen P**, **Secher NH**. Cerebral desaturation during exercise reversed by O2 supplementation. *Am J Physiol* 277: H1045-52, 1999.

59. **O'Malley KL**, **Harmon S**, **Tang L**, **Todd RD**. The rat dopamine D4 receptor: sequence, gene structure, and demonstration of expression in the cardiovascular system. *New Biol* 4: 137–146, 1992.

60. **Ogilvie CM**, **Forster RE**, **Blakemore WS**, **Morton JW**. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 36: 1–17, 1957.

61. **Peltonen JE**, **Rantamäki J**, **Niittymäki SP**, **Sweins K**, **Viitasalo JT**, **Rusko HK**. Effects of oxygen fraction in inspired air on rowing performance. *Med Sci Sport Exerc* 27: 573–579, 1995.

62. **Polak MJ**, **Drummond WH**. Systemic and pulmonary vascular effects of selective dopamine receptor blockade and stimulation in lambs. *Pediatr Res* 33: 181–184, 1993.

63. **Polak MJ**, **Kennedy LA**, **Drummond WH**. Manipulation of dopamine receptors alters hypoxic pulmonary vasoconstriction in isolated perfused rat lungs. *Life Sci* 51: 1317–1323, 1992.

64. **Powers SK**, **Lawler J**, **Dempsey JA**, **Dodd S**, **Landry G**. Effects of incomplete pulmonary gas exchange on VO2max. *J Appl Physiol* 66: 2491–2495, 1989.

65. **Reeves JT**, **Dempsey JA**, **Grover RF**. Pulmonary circulation during exercise. In: *Pulmonary Vascular Physiology and Physiopathology*, edited by Weir E, Reeves J. New York: Marcel Dekker, 1989, p. 107–133.

66. **Reeves JT**, **Linehan JH**, **Stenmark KR**. Distensibility of the normal human lung circulation during exercise. *Am J Physiol Lung Cell Mol Physiol* 288: L419–L425, 2005.

67. **Reeves JT**, **Taylor AE**. Pulmonary hemodynamics and fluid exchange in the lungs during exercise. *Handb. Physiol. Exerc. Regul. Integr. Mult. Syst.* (1996). doi: 10.1002/cphy.cp120113.

68. **Rennotte MT**, **Reynaert M**, **Clerbaux T**, **Willems E**, **Roeseleer J**, **Verlter C**, **Rodenstein D**, **Frans A**. Effects of two inotropic drugs, dopamine and dobutamine, on pulmonary gas exchange in artificially ventilated patients. *Intensive Care Med* 15: 160–165, 1989.

69. **Rice AJ**, **Thornton AT**, **Gore CJ**, **Scroop GC**, **Greville HW**, **Wagner H**, **Wagner PD**, **Hopkins SR**. Pulmonary gas exchange during exercise in highly trained cyclists with arterial hypoxemia. *J Appl Physiol* 87: 1802–1812, 1999.

70. **Roelands B**, **Hasegawa H**, **Watson P**, **Piacentini MF**, **Buyse L**, **De Schutter G**, **Meeusen RR**. The effects of acute dopamine reuptake inhibition on performance. *Med Sci Sports Exerc* 40: 879–885, 2008.

71. **Romer LM**, **Dempsey JA**. Effects of exercise-induced arterial hypoxemia on limb muscle

fatigue and performance. *Proc Aust Physiol Soc* 36: 71–75, 2005.

72. **Roughton FJW**, **Forster RE**. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 11: 290–302, 1957.

73. **Simmons D**, **Linde L**, **Miller J**, **O'Reilly R**. Relation between lung volume and pulmonary vascular resistance. *Circ Res* 4: 465–471, 1961.

74. **Smith JR**, **Brown KR**, **Murphy JD**, **Harms CA**. Does menstrual cycle phase affect lung diffusion capacity during exercise? *Respir Physiol Neurobiol* 205: 99–104, 2015.

75. **St Croix CM**, **Harms CA**, **McClaran SR**, **Nickele GA**, **Pegelow DF**, **Nelson WB**, **Dempsey JA**. Effects of prior exercise on exercise-induced arterial hypoxemia in young women. *J Appl Physiol* 85: 1556–1563, 1998.

76. **Stickland MK**, **Lindinger MI**, **Olfert IM**, **Heigenhauser GJF**, **Hopkins SR**. Pulmonary gas exchange and acid-base balance during exercise. *Compr Physiol* 3: 693–739, 2013.

77. **Stickland MK**, **Lovering AT**. Exercise-induced intrapulmonary arteriovenous shunting and pulmonary gas exchange. *Exerc. Sport Sci. Rev.* 34: 99–106, 2006.

78. **Stickland MK**, **Welsh RC**, **Haykowsky MJ**, **Petersen SR**, **Anderson WD**, **Taylor DA**, **Bouffard M**, **Jones RL**. Intra-pulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 561: 321–329, 2004.

79. **Stickland MK**, **Welsh RC**, **Petersen SR**, **Tyberg J V**, **Anderson WD**, **Jones RL**, **Taylor DA**, **Bouffard M**, **Haykowsky MJ**. Does fitness level modulate the cardiovascular hemodynamic response to exercise? *J Appl Physiol* 100: 1895–1901, 2006.

80. **Suresh K**, **Shimoda LA**. Lung circulation. *Compr Physiol* 6: 897–943, 2016.

81. **Tedjasaputra V**, **Bouwsema MM**, **Stickland MK**. Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity response to exercise. *J. Physiol.* (2016). doi: 10.1017/CBO9781107415324.004.

82. **Tedjasaputra V**, **Bryan TL**, **van Diepen S**, **Moore LE**, **Bouwsema MM**, **Welsh RC**, **Petersen SR**, **Stickland MK**. Dopamine receptor blockade improves pulmonary gas exchange but decreases exercise performance in healthy humans. *J Physiol* 593: 3147–57, 2015.

83. **Tobin CE**. Arteriovenous shunts in the peripheral pulmonary circulation in the human lung. *Thorax* 21: 197–204, 1966.

84. **Tolle JJ**, **Waxman AB**, **Van Horn TL**, **Pappagianopoulos PP**, **Systrom DM**. Exercise-induced pulmonary arterial hypertension. *Circulation* 118: 2183–2189, 2008.

85. **Turino GM**, **Bergofsky EH**, **Goldring RM**. Effect of exercise on pulmonary diffusing capacity. *J Appl Physiol* 18: 447–456, 1963.

86. **Viegi G**, **Paoletti P**, **Carrozzi L**, **Baldacci S**, **Modena P**, **Pedreschi M**, **Di Pede F**, **Mammini U**, **Giuntini C**. CO diffusing capacity in a general population sample: relationships with cigarette smoking and airflow obstruction. *Respiration* 60: 155–161, 1993.

87. **Vogiatzis I**, **Zakynthinos S**, **Boushel R**, **Athanasopoulos D**, **Guenette JA**, **Wagner H**, **Roussos C**, **Wagner PD**. The contribution of intrapulmonary shunts to the alveolar to arterial oxygen difference during exercise is very small. *J Physiol* (2008). doi: 10.1113/jphysiol.2007.150128.

88. **Wagner PD**, **Gale GE**, **Moon RE**, **Torre-Bueno JR**, **Stolp BW**, **Saltzman HA**. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260–270, 1986.

89. **Watson P**, **Hasegawa H**, **Roelands B**, **Piacentini MF**, **Looverie R**, **Meeusen R**. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *J Physiol* 565: 873–83, 2005.

90. **West JB**. *Respiratory physiology : The essentials.* 9th ed. Baltimore, MD: Philadelphia : Wolters Kluwer Health/Lippincott Williams & Wilkins, c2012., 2012.

91. **Yock PG**, **Popp RL**. Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. *Circulation* 70: 657–662, 1984.

92. **Zavorsky GS**. Evidence of pulmonary oedema triggered by exercise in healthy humans and detected with various imaging techniques. *Acta Physiol.* 189: 305–317, 2007.

Chapter III: The Effect of Dopamine on Pulmonary Diffusing Capacity and Capillary Blood Volume Responses to Exercise

3.1 Introduction

Pulmonary diffusing capacity increases during exercise to meet the increasing oxygen (O_2) demand of the body (16, 21, 23, 39). Expansion of pulmonary capillary blood volume (Vc) and diffusing membrane capacity (Dm) are important contributors to the increased diffusing capacity observed during upright cycle exercise (34). Increases in Vc and Dm are traditionally thought to occur passively during upright exercise, secondary to a central shift in blood volume into the thoracic cavity (10). This central shift leads to increases in pulmonary vascular pressures and results in recruitment and distention of the pulmonary capillaries, increasing Vc and Dm (14, 27, 41).

Pharmacological studies have shown that a variety of mediators—including nitric oxide, adenosine, and dopamine—may modulate pulmonary vascular tone (32) and thus may be important in Vc regulation. In particular, dopamine has been found to be a pulmonary vascular vasodilator (13, 26), and recent work suggests that dopamine may play an important role in pulmonary vascular regulation during exercise (35). Specifically, Tedjasaputra et al. (35) found that dopamine receptor-2 (D_2-receptor) blockade reduced time-to-exhaustion during near maximal cycle exercise secondary to a reduced maximal cardiac output (Q) and stroke volume. While pulmonary hemodynamic measurements were not available, dopamine blockade resulted in an exaggerated ventilatory response to exercise, which some have suggested occurs in the presence of elevated pulmonary vascular pressures (22, 37). These findings are also consistent with work showing that exercise tolerance is reduced in clinical conditions of high pulmonary arterial pressure such as primary pulmonary hypertension (1, 38).

Should endogenous dopamine improve pulmonary vascular compliance, dopamine receptor blockade would result in reduced Vc and increased pulmonary vascular pressures for a

given Q. Similarly, providing exogenous dopamine would cause pulmonary vascular vasodilation, increased Vc, and a reduction in pulmonary vascular pressures for a given Q. Accordingly, the primary aim of this study was to examine the effect of exogenous dopamine and dopamine receptor blockade on carbon monoxide diffusing capacity (DL_{CO}), Vc, Dm, pulmonary artery systolic pressure (PASP), and the resultant effect on time-to-exhaustion.

3.2 Methods

3.2.1 Subjects

Fourteen young, healthy women and men were recruited for this study. While there was no direct method to determine the effect size of dopamine modulation on DL_{CO}, Vc or Dm, a sample size calculation was performed based on data from previous work with D_2-receptor blockade (35). Tedjasaputra et al. (35) demonstrated a mean difference in time-to-exhaustion between blockade and placebo of 193 ± 145.7 seconds. With the desired power level of 0.80, α=0.05, and three comparison groups, it was calculated that 13 subjects would be sufficient to detect a significant difference in time-to-exhaustion between conditions. All subjects provided their written informed consent to participate in the study, which was approved by the Human Research Ethics Board at the University of Alberta (Pro00067664). Subjects were physically active non-smokers and all had normal lung function. For female participants, while data collection was not standardized to a specific phase of the menstrual cycle across female participants, all data were collected within the same phase of the menstrual cycle for a specific participant. As an example, if the preliminary and first experimental days were conducted during the follicular phase, then experimental days two and three were also collected during the follicular phase. Subject characteristics and pulmonary function data are listed in Table 1.

3.2.2 Study design overview

Participants first underwent preliminary testing which included a full lung function test and a graded exercise test until volitional exhaustion. No less than 48 hours later, participants conducted exercise DL_{CO} trials across three different days. Each trial was double-blinded and randomized to one of the following conditions: 1) dopamine (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ intravenous) and a placebo pill, 2) dopamine blockade with metoclopramide (20 mg oral) and intravenous saline, or 3) intravenous saline and a placebo pill. These trials, which were separated by a minimum of 24 hours, utilized the Roughton and Forster (29) multiple F_IO_2 DL_{CO} method to estimate Vc and Dm at rest, 60%, and 85% of VO_{2peak}. Following the exercise DL_{CO} maneuvers and a 15 minute seated rest period, participants subsequently performed a time-to-exhaustion test at 85% of VO_{2peak}. See Figure 3 (*Appendix A*) for research design schematic.

3.2.3 Preliminary testing

Upon arrival to the laboratory, participants were cleared for exercise using the physical activity readiness questionnaire plus (PAR-Q+). Additionally, participants were screened for any cardiopulmonary and mental health medications. After screening, participants performed a full lung function test including spirometry, lung volumes, and resting 10 second DL_{CO} using a calibrated Vmax metabolic system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA). To familiarize the subjects with the multiple F_IO_2 DL_{CO} method (29), resting six second breath-holds were also performed at three different F_IO_2 values (F_IO_2 = 0.21, 0.40, 0.60).

Lastly, a graded exercise test until volitional exhaustion was performed on an electronically braked cycle ergometer (Ergoselect II 1200 Ergoline, Blitz, Germany) to characterize VO_{2peak}. Consistent with previous work (5, 34), the initial workload was set to 50 W and increased by 25 W every two minutes until ventilatory threshold, at which point the workload was increased by 25 W every minute until exhaustion. Attainment of VO_{2peak} was

based on meeting three out of the following criteria: 1) volitional exhaustion; 2) an increase in oxygen consumption < 100mL/min with an increase in power output; 3) respiratory exchange ratio (RER) > 1.1; 4) attainment of age-predicted maximum heart rate. Expired gases were collected into the mixing chamber of the metabolic measurement system listed above and mean 30 second data were reported. Heart rate was determined using 3-lead electrocardiography (CardioSoft, GG Medial Systems, Milwaukee, WI, USA), O_2 saturation was determined using finger pulse oximetry (Radical-7; Masimo Corporation, Irvine, CA, USA), and Q was determined using transthoracic impedance cardiography (Physioflow, Manatec, Paris, France).

3.2.4 Exercise DL_{CO} trials

Subject preparation. One hour prior to testing, either a placebo pill or 20 mg metoclopramide were ingested. Upon arrival to the laboratory, subjects were instrumented with an intravenous catheter (ProtectIV Plus, Smiths Medical, Southington, CT, USA) into a large peripheral vein in the arm and attached to an infusion pump (Alaris PC-8015, CardinalHealth, San Diego, CA, USA) via an extension kit (Alaris SmartSite, CareFusion, San Diego, CA, USA). Dopamine was infused at a rate of 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and saline was infused at a rate to obtain a similar infusion volume to dopamine. On any given testing day, no more than 30mL of fluid was infused into a subject. Lastly, electrodes were placed on the subject to determine Q via transthoracic impedance cardiography (Physioflow, Manatec, Paris, France) according to the manufacturer's specifications.

Dopamine blockade. Metoclopramide was used as a dopamine receptor-2 (D_2-receptor) antagonist. Consistent with previous work (35), 20 mg was consumed orally one hour prior testing to allow for peak blood concentration of the drug to occur while testing (30, 33).

Exogenous dopamine. Low dose intravenous dopamine was used to stimulate dopamine receptors. To avoid any inotropic effects of the drug, the rate of 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was chosen as this rate is not associated with any increases in heart rate, stroke volume, or Q in humans (6).

Both the participant and the researcher collecting the data were blinded to the condition. Metoclopramide and placebo pills were packaged and coded by a researcher external to the study. Similarly, the saline and dopamine infusions were set up by the research nurse and hidden from the data collection researchers using a blinding bag. Following data collection and data analysis, the study conditions were revealed to the primary researcher by the external researcher and entered into an un-blinded master file for statistical analysis.

Experimental protocol. Following instrumentation and 10 minutes of infusion wash-in time, DL_{CO} was determined at rest and during exercise at 60% and 85% of previously determined VO_{2peak}. The exact power output was determined by linear regression of the VO_2 and power output data obtained during the graded exercise test. A discontinuous exercise protocol was used at both 60% and 85% of VO_{2peak}. Exercise DL_{CO} breath-holds occurred after a minimum of two minutes at the particular power output, once heart rate was within 5 heart beats of the expected heart rate for the workload. To minimize fatigue, active recovery (< 100 W) was performed between breath-holds and workloads. Immediately prior to the second breath-hold for a given workload, ratings of perceived exertion (RPE) for both breathing and leg discomfort were collected. The order of exercise workloads was randomized with the exception of rest, which was always performed first.

Diffusing capacity for carbon monoxide (DL_{CO}) was determined through a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA) using a

modified six second breath-holding technique (5, 34, 36). Consistent with the American Thoracic Society and the European Respiratory Society's statement for the standardized single-breath DL_{CO} measurement procedure (19), subjects performed normal tidal breathing, then completely and non-forcefully emptied the lungs to residual volume (RV). At this point, subjects were switched to the DL_{CO} gas mixture. Subjects were then instructed to inspire the test gas to total lung capacity in under two seconds and hold their breath. During the breath-hold, subjects were asked to avoid a Valsalva maneuver (positive pleural pressure) or a Mueller maneuver (negative pleural pressure) because these are known to affect DL_{CO} measurements (15, 23). After the breath-holding period, subjects were asked to smoothly and non-forcefully empty their lungs down to RV once again.

Hemoglobin concentration ([Hb]) was measured at rest and immediately following the second breath-hold within a given exercise workload (HemoCue 201+, HemoCue AB, Angelholm, Sweden). DL_{CO} was corrected for [Hb] using the equation by Marrades and colleagues (20): $DL_{COadj} = DL_{CO} * ((10.22+[Hb])/(1.7+[Hb]))$. For each workload, DL_{CO} breath-holds were performed at three F_IO_2 values (0.21, 0.40, 0.60) with a minimum of two minutes wash out time between. For breath-holds with an F_IO_2 of 0.40 or 0.60, subjects pre-breathed five breaths from a Douglas bag (Hans Rudolph, Shawnee, KS, USA) containing the equivalent F_IO_2 to ensure a stable alveolar O_2 partial pressure. The order of F_IO_2 was randomized for each workload. Carbon monoxide concentration was 0.3% in each tank, and methane (0.3%) was used to determine alveolar volume (V_A) and confirm adequate gas equilibration in the lung for each breath-hold. Importantly, DL_{CO} breath-holds were repeated if V_A values varied by greater than 10% from previous V_A values for a given workload.

DL_{CO} was calculated using the equation (19): $DL_{CO} = (V_A/((^{BHT}/_{60})*(P_b - P_{H2O}))*\ln(FA_{TR}*FI_{CO})/(FI_{TR}*FA_{CO})$, where BHT is the breath-hold time in seconds, P_b is the barometric pressure, P_{H2O} is water vapor pressure, ln is natural logarithm, FA_{TR} is fraction of alveolar tracer gas, FI_{CO} is fraction of inspired carbon monoxide, FI_{TR} is fraction of inspired tracer gas, and FA_{CO} is fraction of alveolar carbon monoxide (19). Vc and Dm were determined with the equation: $1/DL_{CO} = 1/Dm + 1/(\Theta_{CO}*Vc)$, where theta ($\Theta_{CO}$) is the reaction rate between carbon monoxide and hemoglobin and is calculated using the equation $1/\Theta_{CO} = 0.0058*P_AO_2 + 0.73$ (29). Alveolar partial pressure of O_2 was calculated at every workload using the alveolar air equation (41): $P_AO_2 = F_IO_2 (P_{Bar} - P_{H2O}) - P_ACO_2 \times (1 - F_IO_2)/RER$, where P_AO_2 is partial pressure of alveolar O_2, F_IO_2 is the fraction of inspired oxygen, P_{Bar} is barometric pressure, P_{H2O} is water vapor pressure, P_ACO_2 is partial pressure of alveolar carbon dioxide (CO_2), and RER is respiratory exchange ratio.

For young healthy subjects free of lung disease, P_ACO_2 is assumed to be equivalent to the partial pressure of arterial CO_2 (P_aCO_2; 37), and P_aCO_2 was estimated from the partial pressure of end-tidal CO_2 values. For each workload, $1/DL_{CO}$ versus $1/\Theta_{CO}$ was plotted for all F_IO_2 values and a regression line was calculated. The minimum acceptable coefficient of determination (r^2) value was set to 0.95 and DL_{CO} maneuvers were repeated when the r^2 fell below this threshold. Values for 1/Vc (slope of the regression line) and 1/Dm (y-intercept of the regression line) were then determined (29). We have previously used this approach to evaluate Vc and Dm in healthy subjects and observed excellent reliability, as well as values which are consistent with previous work (5, 34).

Time-to-exhaustion. To examine exercise tolerance and the cardiovascular effects of exogenous dopamine and D_2-receptor blockade during exercise, high intensity time-to-exhaustion trials

were performed at the end of each experimental trial. Following a 15-minute period of seated rest, participants exercised on the cycle ergometer at 85% of VO_{2peak} until exhaustion. Time-to-exhaustion in seconds was recorded using a stopwatch. Cardiac output was determined using transthoracic impedance cardiography (Physioflow, Manatec, Paris, France), heart rate by ECG, and O_2 saturation by pulse oximetry (Radical-7; Masimo Corporation, Irvine, CA, USA). Expired gases were collected during the time-to-exhaustion trial via a metabolic measurement system (Encore229 Vmax, SensorMedics, Yorba Linda, CA, USA).

3.2.5 Exercise PASP trials

At least one week after the exercise DL_{CO} trials, participants returned to perform baseline and exercise echocardiography at 60% of VO_{2peak} in all three drug conditions. To enhance tricuspid regurgitant envelopes, exercise was performed semi-supine with 15 degrees of downward tilt on a tilt table (InnovaProducts, Mount Pleasant, WI, USA) attached to a modified cycle ergometer (828E, Monark Exercise, Vansbro, SE). Subjects were instrumented in an identical manner to the exercise DL_{CO} trials. All experimental drugs were administered within a single testing session and the first two drug conditions randomized were dopamine and saline. Due to metoclopramide's half-life of three to six hours (30), blockade was always the final experimental condition. As a result of this design, the participant remained blind to all experimental conditions, however the researcher became un-blinded to the final experimental condition. Between conditions, subjects were disconnected from the infusion pump and given 30 minutes of seated rest before beginning echocardiography in the next experimental condition. All echocardiograms were performed by an experienced sonographer (Vivid Q, GE Healthcare, Fairfield, CT, USA).

The pulmonary arterial pressure gradient was estimated from the peak tricuspid regurgitant jet velocity measured in the four chamber view (42). The inferior vena cava diameter was measured from subcostal longitudinal images, and a collapsibility index was calculated as percent difference between the minimal and maximal diameter of the inferior vena cava during a sniff maneuver. Right atrial pressure (RAP) was estimated using the inferior vena cava collapsibility index (25) and PASP was calculated using the following equation (42): PASP = $4(V_{TR})^2$ + RAP, where V_{TR} is peak tricuspid regurgitant jet velocity in meters per second and RAP is right atrial pressure measured in mmHg.

PASP measurements at rest and 60% of VO_{2peak} were attempted in the first seven subjects, but none of the subjects displayed sufficient tricuspid regurgitation envelopes at rest or during exercise for valid PASP determination. As a result of this, no further PASP measurements were attempted and this phase of the study was abandoned.

3.2.6 Statistical analysis

For all inferential analyses, the probability of type I error was set at 0.05. Mean differences in DL_{CO}, Vc, Dm, [Hb], Q, stroke volume, and heart rate were compared at rest, 60%, and 85% of VO_{2peak} in all dopamine conditions using a 3x3 repeated measures ANOVA (SigmaPlot, v.13, Systat Software, San Jose, CA, USA). Exercise tolerance, cardiovascular, and metabolic variables were compared at rest, 120 seconds, and exhaustion in all conditions using a 3x3 repeated measures ANOVA. Ratings of perceived exertion (RPE) were compared in all conditions during exercise at 60% and 85% of VO_{2peak} using a 2x3 repeated measures ANOVA. Where main effects were established, the Holm-Sidak post hoc test was used to determine where the differences occurred. To evaluate associations between pulmonary diffusion variables and time-to-exhaustion, variables were plotted on a scatterplot to determine the line of best fit, the

Pearson correlation coefficient, the coefficient of determination, and the *P*-value for relationships (SigmaPlot, v.13, Systat Software, San Jose, CA, USA).

3.3 Results

All subjects tolerated the study procedures well. Subjects were young, healthy, recreationally active men and women, had a mean VO_{2peak} of 45.8 ± 6.5 $mL \cdot kg^{-1} \cdot min^{-1}$ and normal lung function. Descriptive characteristics are listed in Table 1. Information regarding reliability of DL_{CO}, Vc, and Dm measurements are presented in Table 2.

3.3.1 Diffusing capacity, pulmonary capillary blood volume, and diffusing membrane capacity

A main effect of intensity on DL_{CO} was observed in the current investigation ($P<0.001$). DL_{CO} increased from baseline up to 85% of VO_{2peak} in all conditions (baseline vs. 60%, $P<0.001$; baseline vs. 85%, $P<0.001$; 60% vs. 85%, $P<0.001$). There was no main effect of condition ($P=0.938$) and no interaction effect ($P=0.932$) for DL_{CO} (Fig. 1.A).

A main effect of intensity on Vc was observed ($P<0.001$), as Vc increased from baseline up to 85% of VO_{2peak} in all conditions (baseline vs. 60%, $P<0.001$; baseline vs. 85%, $P<0.001$; 60% vs. 85%, $P=0.011$). There was no main effect of condition ($P=0.125$) and no interaction effect ($P=0.759$) for Vc (Fig. 1.B).

A main effect of intensity on Dm was observed ($P<0.001$), as Dm increased from rest up to 85% of VO_{2peak} in all conditions (baseline vs. 60%, $P<0.001$; baseline vs. 85%, $P<0.001$; 60% vs. 85%, $P<0.001$). There was no main effect of condition ($P=0.318$) and no interaction effect ($P=0.984$) for Dm (Fig. 1.C).

A main effect of intensity was observed for [Hb] ($P<0.001$). [Hb] increased from baseline to 60% of VO_{2peak}, but there was no further increase from 60% to 85% VO_{2peak} (baseline vs.

60%, *P*=0.009; baseline vs. 85%, *P*=0.001; 60% vs. 85%, *P*=0.340). There was no main effect of condition (*P*=0.388) and no interaction effect (*P*=0.785) for [Hb] (Table 3).

The cardiovascular responses to exercise at 60% and 85% of VO_{2peak} are listed in Table 3. No differences were observed between conditions at 60% of VO_{2peak} for breathing RPE (saline, 2.4 ± 0.9; dopamine, 2.5 ± 0.8; blockade, 2.4 ± 1.2; *P*=0.875) and leg RPE (saline, 3.4 ± 1.1; dopamine, 3.5 ± 0.8; blockade, 3.3 ± 0.7; *P*=0.770), and similarly, no differences were seen at 85% of VO_{2peak} for breathing RPE (saline, 4.4 ± 1.7; dopamine, 4.1 ± 1.3; blockade, 4.6 ± 1.3; *P*=0.875) and leg RPE (saline, 5.8 ± 1.9; dopamine, 5.7 ± 1.2; blockade, 5.4 ± 1.3; *P*=0.774).

3.3.2 Time-to-exhaustion during heavy exercise

Respiratory and cardiovascular responses to exhaustive exercise at 85% of VO_{2peak} are listed in Table 4 and Table 5. All comparisons were made against the placebo condition unless otherwise stated.

For time-to-exhaustion, a main effect of condition was observed (*P*=0.040). While, time-to-exhaustion was not different with dopamine (*P*=0.700), it was significantly reduced with blockade as compared to control (*P*<0.001).

There was no main effect of condition for O_2 consumption (*P*=0.961) and no interaction effect was observed (*P*=0.390). While ventilation did not have a main effect of condition (*P*=0.404), an interaction effect was observed (*P*=0.018). Ventilation with dopamine was reduced at 120 seconds compared to placebo (*P*=0.038). For respiratory exchange ratio, there was no effect of condition (*P*=0.244) and no interaction effect (*P*=0.537). No main effect of condition or interaction effect were observed for O_2 saturation (*P*=0.087 and *P*=0.848 respectively).

A main effect of time for Q was observed (P<0.001), as Q increased from baseline up to exhaustion in all conditions (baseline vs. 120 seconds, P<0.001; baseline vs. exhaustion, P<0.001; 120 seconds vs. exhaustion, P=0.018). There was no main effect of condition (P=0.243) and no interaction effect (P=0.181) for Q.

For stroke volume, a main effect of time was observed (P<0.001) as stroke volume increased from baseline up to 120 seconds. No further increase in stroke volume from 120 seconds to exhaustion occurred (baseline vs. 120 seconds, P<0.001; baseline vs. exhaustion, P<0.001; 120 seconds vs. exhaustion, P=0.182). There was no main effect of condition (P=0.145) and no interaction effect (P=0.538) for stroke volume.

A main effect of time and condition for heart rate was observed (P<0.001 and P<0.001 respectively). Heart rate increased from baseline up to exhaustion (baseline vs. 120 seconds, P<0.001; baseline vs. exhaustion, P<0.001; 120 seconds vs. exhaustion, P<0.001). Heart rate was significantly lower in the blockade condition compared to the placebo condition at baseline (P=0.005) and exhaustion (P=0.027).There was no interaction effect (P=0.594) for heart rate.

A main effect of time was observed (P<0.001) for O_2 saturation. While it was unchanged from baseline to 120 seconds, O_2 saturation was reduced at exhaustion (baseline vs. 120 seconds, P=0.716; baseline vs. exhaustion, P=0.023; 120 seconds vs. exhaustion, P=0.036). There was no main effect of condition (P=0.087) and no interaction effect (P=0.848) for O_2 saturation.

A main effect of time and condition for O_2 delivery was observed (P<0.001 and P=0.010) respectively). O_2 delivery increased from baseline up to 120 seconds, but was not different at exhaustion (baseline vs. 120 seconds, P<0.001; baseline vs. exhaustion, P<0.001; 120 seconds vs. exhaustion, P=0.073). O_2 delivery was significantly lower in the blockade condition and the

dopamine condition compared to the placebo condition at 120 seconds only (P=0.026 and P=0.036 respectively). There was no interaction effect (P=0.844) for O_2 delivery.

For mean arterial pressure, a main effect of time and condition was observed (P<0.001 and P=0.025 respectively). Mean arterial pressure increased from baseline up to 120 seconds. No further increase in mean arterial pressure from 120 seconds to exhaustion occurred (baseline vs. 120 seconds, P=0.002; baseline vs. exhaustion, P=0.001; 120 seconds vs. exhaustion, P=0.836). Mean arterial pressure was significantly lower in the dopamine condition compared to the placebo condition at baseline only (P=0.043).There was no interaction effect for mean arterial pressure (P=0.488).

A main effect of time was observed (P<0.001) for total conductance, as it increased from baseline up to 120 seconds. No further increase in conductance from 120 seconds to exhaustion occurred (baseline vs. 120 seconds, P<0.001; baseline vs. exhaustion, P<0.001; 120 seconds vs. exhaustion, P=0.086). There was no main effect of condition (P=0.422) and no interaction effect (P=0.877) for conductance.

3.3.3 Determinants of time-to-exhaustion

Correlations were used to examine the determinants of time-to-exhaustion at 85% of VO_{2peak} and are listed in Table 6. Within each condition (i.e. placebo, dopamine or D_2-receptor blockade), DL_{CO}, Vc, and Dm at rest and during exercise were correlated to time-to-exhaustion within the respective condition.

VO_{2peak} was associated with time-to-exhaustion in saline (r=0.60, P=0.03) and dopamine (r=0.66, P=0.01) conditions, but not with blockade (r=0.46, P=0.10).

Baseline DL_{CO} was associated with time-to-exhaustion in the dopamine condition

(r=0.47, *P*=0.01) and placebo conditions (r=0.21, *P*=0.49) but not with blockade (r=0.01, *P*=0.990). Baseline Vc and Dm were not correlated with time-to-exhaustion in any condition.

At 60% of VO_{2peak}, DL_{CO} was related to time-to-exhaustion in the dopamine condition (r=0.70, *P*=0.01), but not in blockade (r=0.26, *P*=0.38) or placebo (r=0.12, *P*=0.71) conditions. Additionally, Vc at 60% of VO_{2peak} showed a positive correlation with time-to-exhaustion in the dopamine condition (r=0.68, *P*=0.01), but not in the other conditions. Dm at 60% of VO_{2peak} was not correlated to time-to-exhaustion in any condition.

Diffusing capacity at 85% of VO_{2peak} was related to time-to-exhaustion in the dopamine condition (r=0.63, *P*=0.02), but not in blockade (r=0.04, *P*=0.88) or placebo (r=0.31, *P*=0.30) conditions. Pulmonary capillary blood volume at 85% of VO_{2peak} showed a positive correlation with time-to-exhaustion in both the placebo (r=0.70, *P*=0.01) and dopamine (r=0.69, *P*=0.02) conditions but not with blockade (r=0.39, *P*=0.17). Consistent with the previous workload, Dm at 85% of VO_{2peak} was not correlated to time-to-exhaustion in any condition.

The change in Vc from baseline to 85% of VO_{2peak}, an index of pulmonary capillary network expansion, was associated with time-to-exhaustion in the placebo condition (r=0.71, *P*=0.001), trending toward significance in the dopamine condition (r=0.54, *P*=0.09), and was not related to time-to-exhaustion in the blockade condition (r=0.28, *P*=0.34). The change in Dm from baseline to 85% of VO_{2peak} was not related to time-to-exhaustion in any condition (Table 6).

Correlations were also performed to examine if changes in Vc and Dm between conditions were associated with changes in time-to-exhaustion between conditions. No relationship exists between the change in Vc at 85% and the change in time-to-exhaustion for any condition (dopamine – saline, r=0.21; blockade – saline, r=0.29; Fig. 2.A). Additionally, no

relationship was observed between the change in Dm at 85% and the change in time-to-exhaustion for any condition (dopamine – saline, r=0.15; blockade – saline, r=0.19; Fig. 2.B).

3.4 Discussion

The present study examined the effect of exogenous dopamine and D_2-receptor blockade on DL_{CO}, its components Vc and Dm, and the resultant effect on time-to-exhaustion during heavy cycle exercise. In the current investigation, dopamine had no effect on DL_{CO}, Vc or Dm. While D_2-receptor blockade reduced time-to-exhaustion at 85% of VO_{2peak}, providing exogenous dopamine did not improve exercise tolerance. These results suggest that dopamine does not appear to be important in the regulation of DL_{CO}, Vc, and Dm at rest or during exercise in the current sample. Additionally, while endogenous dopamine appears to be important for the maintenance of time-to-exhaustion, providing exogenous dopamine does not further enhance exercise tolerance in young, healthy, active subjects.

3.4.1 The effect of dopamine on pulmonary diffusing capacity during exercise

Consistent with previous work, DL_{CO} increased with exercise through increases in Vc and Dm (5, 14, 34). Animal studies have shown direct evidence that dopamine is a pulmonary vasodilator (13, 26), and human studies have provided evidence that dopamine plays a role in pulmonary vascular regulation as well (6, 35). As a result, we hypothesized that stimulating pulmonary D_2-receptors would decrease pulmonary vascular resistance, allowing an increase in Vc and DL_{CO}. However, contrary to the initial hypothesis, neither dopamine nor dopamine blockade had any effect on DL_{CO}, Vc, or Dm at any intensity of exercise tested. The current investigation suggests that dopamine is not important in pulmonary vascular regulation during exercise in health as no differences in DL_{CO}, Vc, or Dm were found with either exogenous dopamine or dopamine blockade.

In addition, neither exogenous dopamine nor dopamine blockade seem to be important for the cardiovascular response to exercise at 60% and 85% of VO_{2peak}, as no differences were seen in Q, stroke volume, or heart rate. This response was expected at baseline, as prior work found no change in Q, stroke volume, or heart rate with infusion of dopamine at the same rate as the current investigation in young healthy men and women (6). The exercise heart rate response was consistent with results from Lundby and colleagues (18) who found blocking D_2-receptors had no effect on exercise heart rate at sea level. However, in contrast to the current results, Stickland and colleagues (31) found heart rate to be significantly increased during leg extension exercise with low dose dopamine infusion. It is possible that the divergent result comes from differences in exercise modality. Overall, dopamine does not appear to play an important role in the regulation of pulmonary diffusing capacity or the cardiovascular response during submaximal upright cycle exercise.

3.4.2 The effect of dopamine on time-to-exhaustion

The current investigation provides no evidence of improvements in time-to-exhaustion with exogenous dopamine in normoxia at room temperature, which is aligned with previous work examining the effect of dopamine receptor agonists on exercise tolerance (28, 40). Previous studies have found that dopamine receptor agonists improve exercise tolerance under conditions of significant thermal stress only (28, 40). Consistent with work in humans (35) and animals (2), dopamine receptor blockade reduced exercise tolerance. In the investigation by Tedjasaputra and colleagues (35), time-to-exhaustion with dopamine blockade was reduced, and was partly explained by a reduction in Q and stroke volume. The current data do not support a cardiovascular impairment during the time-to-exhaustion trials with dopamine blockade because Q, stroke volume, and O_2 delivery were not different in any condition at exhaustion. While heart rate was significantly lower with blockade at exhaustion, the small reduction did not result in a

change in Q and cannot explain the 47% reduction in time-to-exhaustion seen in this investigation. Differences in fitness may contribute to the differential findings between studies, as Tedjasaputra and colleagues (35) worked with highly fit subjects while the present study recruited recreationally active subjects. In the dopamine and blockade conditions, O_2 delivery was reduced at the two-minute point in the time-to-exhaustion trials. This finding suggests that there may be a time course effect on O_2 delivery with both drugs, since these differences disappear at exhaustion. The difference in O_2 delivery at two minutes appears to be driven by a reduced, although not statistically significant, Q for both dopamine and blockade conditions. Based on the current study, a cardiovascular limitation to exhaustive exercise with blockade at 85% of VO_{2peak} is not evident.

During exercise, mean arterial pressure was not different at any time-point during the time-to-exhaustion trial. However, this study found mean arterial pressure to be reduced at baseline with low dose dopamine infusion. This finding contrasts previous investigations using the same dose of dopamine, which demonstrate that resting mean arterial pressure was unchanged during dopamine infusion (6, 9, 31). Differences in the type of baseline assessment may explain the differences in these results. Specifically, the current study examined pre-exercise baseline whereas other investigations examined quiet rest. Thus, this study provides new insight into the effect of dopamine on mean arterial pressure during pre-exercise baseline.

Generally, the respiratory response to exhaustive exercise at 85% of VO_{2peak} revealed that no difference exists between conditions. Consistent with the baseline values reported in this study, Edgell and colleagues (9) found that resting ventilation was not different in healthy controls with low dose dopamine infusion. However, the present study found that at the two minute time-point of the time-to-exhaustion trials, ventilation was significantly reduced in the

dopamine condition. Boetger et al. (4) revealed that dopamine slows the transient ventilatory response to an increase in workload, thus it is not surprising that ventilation is lower with dopamine at two minutes in the current study. At exhaustion, there was no difference in ventilation between conditions. In support of this, Henson and colleagues (12) also showed no difference in ventilation at peak exercise with dopamine infusion in young healthy subjects.

Low dose dopamine infusion inhibits the carotid chemoreceptor (31), which is the primary O_2 sensing organ in the human body and is an important regulator of ventilation (24). Stickland et al. (31) provide evidence that low dose dopamine infusion effectively inhibits the carotid chemoreceptor and reduces the ventilatory response to handgrip exercise during hypoxia. Thus, it might be expected that D_2-receptor blockade would sensitize the carotid chemoreceptor and enhance the ventilatory response to exercise consistent with Tedjasaputra and colleagues' (35) work showing an increase in peak exercise ventilation with D_2-receptor blockade during incremental exercise to exhaustion. However, in the current investigation, D_2-receptor blockade had no effect on peak exercise ventilation during exhaustive exercise at 85% of VO_{2peak}. It is possible that the incremental nature of exercise in Tedjasaputra and colleagues' study (35) is important for the differential findings and further investigation is warranted.

The current investigation confirms that, in young healthy subjects, exogenous dopamine does not result in an increase in time-to-exhaustion, but exogenous dopamine may slow the ventilatory response to exercise.

3.4.3 Determinants of time-to-exhaustion

Previous work in healthy subjects with a range of cardiorespiratory fitness levels has demonstrated a relationship between resting Vc and VO_{2peak} (17, 34), suggesting that athletes may exhibit greater pulmonary vascular distensibility than non-athletes. In the present study,

VO_{2peak} was significantly associated with time-to-exhaustion in the placebo and dopamine conditions, highlighting that cardiorespiratory fitness may play a significant role in determining exercise tolerance. To examine if indices of pulmonary vascular distensibility are important determinants of exercise tolerance, correlations of time-to-exhaustion with DL_{CO}, Vc, and Dm were performed.

At rest, DL_{CO} was associated with time-to-exhaustion in the placebo and dopamine conditions, but Vc and Dm were not correlated to time-to-exhaustion in any condition. In the investigation by Lalande and colleagues (17), VO_{2max} was significantly associated with resting Vc highlighting that the greater pulmonary distensibility in athletes may be responsible for their increased cardiorespiratory fitness. Surprisingly, even with the significant association between VO_{2peak} and time-to-exhaustion seen in the current study, it appears that baseline Vc was not an important determinant of time-to-exhaustion during heavy cycle exercise.

While resting Vc does not appear to be associated with exercise tolerance, Vc at 85% of VO_{2peak} as well as the change in Vc from baseline to 85% of VO_{2peak} are significantly correlated with time-to-exhaustion in the placebo and dopamine conditions (Table 6). Since the change in Vc from rest to maximal exercise is thought to indicate the degree of pulmonary capillary network expansion (3, 8), these results suggest that the ability to expand the pulmonary capillary network during exercise may be an important determinant of exercise tolerance in healthy subjects.

To examine if dopamine contributes to pulmonary capillary network expansion and changes in exercise tolerance, we investigated correlations between the change in Vc and Dm at 85% of VO_{2peak} between conditions (e.g., change in Vc at 85% of VO_{2peak} with dopamine

subtracted from placebo) and the change in time-to-exhaustion between experimental conditions (i.e., time-to-exhaustion with dopamine subtracted from placebo). This analysis revealed that when comparing the blockade condition to placebo, changes in Vc (Fig. 2.A) and Dm (Fig. 2.B) were not related to changes in time-to-exhaustion. The same result was obtained when comparing the dopamine condition to placebo (Fig. 2). These results suggest that, while the Vc response to exercise may play a role in determining VO_{2max} (34) and time-to-exhaustion, the change in Vc with dopamine or dopamine blockade does not explain any changes in time-to-exhaustion across conditions.

3.4.4 Study limitations

Currently, there is no consensus on the correct value for θ_{CO} when calculating Vc and Dm. Consistent with our recent publications (5, 34), the values for α and β were set at 0.0058 and 0.73 respectively (29), as these values provide reasonable estimations of Vc and Dm at rest and during exercise. These values for α and β assume a pH of 8.0 and moderate red cell permeability (7). While this assumption is a limitation of the current technique, these values for α and β provide physiologically reliable values and have been used previously (5, 7, 34).

The standard method for DL_{CO} breath-holding recommends 10 second breath-holds (19), however the current investigation used six second breath-holds which may decrease the accuracy of the DL_{CO} measurement. Despite this limitation, this six second breath-holding period is consistent with our previous work (5, 34), and there is evidence that shortening breath-hold time in this manner does not change DL_{CO} significantly (11, 23). Using a strict methodology for DL_{CO} measurement, including ensuring that V_A values are within 10% of each other and an $r^2 > 0.95$ for the relationship between $1/DL_{CO}$ and $1/\Theta$, we obtained reproducible values for Vc and Dm which further enhances the quality of the present results (Table 2).

In agreement with our previous publications using DL_{CO} (5, 34), no correction was made for carboxyhemoglobin (COHb) backpressure. To minimize the potential effect of increasing COHb with breath-holding, at least four minutes were given between breath-holds at rest and two minutes between breath-holds during exercise. Additionally, no more than 12 breath-holds were performed in a single testing session. The following study design features were also utilized to help control for COHb: 1) young, healthy, non-smoking subjects were recruited; 2) the order of F_IO_2-DL_{CO} maneuvers were randomized; 3) the order of exercise workloads were randomized; and 4) drug interventions were randomized to allow valid between-group comparisons.

No previous work has assessed the effect of low dose dopamine infusion and D_2-receptor blockade on pulmonary diffusing capacity, Vc, and Dm during heavy exercise. Thus, a sample of 14 subjects were recruited and tested based off of an a priori power calculation from previous work examining the effect D_2-receptor blockade on time-to-exhaustion (35). By basing the sample size estimation on the exercise tolerance data instead of pulmonary diffusion data, there was the chance that the sample size recruited may have been underpowered to detect a difference in our primary outcomes of the study. However, a post-hoc sample size calculation was performed using Vc data at 85% of VO_{2peak} and revealed that, with a mean difference of 11 ± 51 mL, more than 300 subjects would be required to detect a statistically significant difference in Vc between dopamine and placebo conditions. Based on previous work which highlighted that a 10 mL difference in peak exercise Vc was not statistically different between groups (34), we would suggest that an 11 mL change in Vc at peak exercise is not likely to be physiologically important. As such, the inability to detect a difference in Vc between conditions is likely not the result of being statistically underpowered.

3.4.5 Conclusion

This study examined the effect of exogenous dopamine and D_2-receptor blockade on pulmonary diffusing capacity (DL_{CO}), Vc, Dm, and the resultant effect on time-to-exhaustion during heavy upright cycle exercise. Exogenous dopamine does not appear important in the regulation of DL_{CO}, Vc, and Dm at rest or during exercise in healthy subjects. Additionally, D_2-receptor blockade reduced time-to-exhaustion at 85% of VO_{2peak}, but did not affect DL_{CO}, Vc, Dm, or Q. While dopaminergic receptors appear important for the maintenance of exercise tolerance, dopamine does not contribute in the regulation of DL_{CO}, Vc, and Dm at rest or during exercise in young healthy subjects.

Table 1. Subject characteristics and pulmonary function

	Mean			% Predicted		
N	14					
Male/Female	8/6					
Age, years	27.4	±	5.0			
Height, m	1.76	±	0.07			
Mass, kg	75.0	±	12.6			
Body Mass Index, $kg \cdot m^2$	24.2	±	2.6			
VO_{2peak}, $L \cdot min^{-1}$	3.5	±	0.9	123.8	±	27.9
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	45.8	±	6.6	111.5	±	21.8
TLC, L	6.82	±	0.95	98.9	±	7.9
FEV_1, L	4.49	±	0.79	106.9	±	8.4
FVC, L	5.46	±	0.88	107.4	±	8.4
FEV_1/FVC, %	82.2	±	6.2	99.2	±	7.5
Resting DL_{CO}, $mL \cdot min^{-1} \cdot mmHg^{-1}$	32.5	±	6.5	93.1	±	10.8

Values are expressed as mean ± SD. VO_{2peak} = peak O_2 consumption; FEV_1 = forced expired volume in 1 s; FVC = forced vital capacity; DL_{CO} = pulmonary diffusing capacity for carbon monoxide.

Table 2. Reliability of diffusing capacity components during exercise

	Rest	60%	85%
	Coefficient Of Variation (%)	Coefficient Of Variation (%)	Coefficient Of Variation (%)
BHT	2.81	2.27	1.50
IVC	2.96	3.04	3.85
FE_{CH4}	1.68	2.18	2.86
V_A	2.62	2.73	4.30
HR	2.48	1.38	1.48

BHT = breath-hold time; IVC = inspired vital capacity; FE_{CH4} = fraction of expired methane; V_A = alveolar volume; HR = heart rate.

Table 3. Cardiovascular and hemoglobin responses at baseline and during exercise

		Baseline			60% of VO_{2peak}			85% of VO_{2peak}		
Q,	PLA	5.6	±	1.1	16.0	±	3.9	19.3	±	5.2
$L \cdot min^{-1}$	DA	5.8	±	0.7	15.2	±	2.5	17.5	±	2.8
	BK	5.7	±	1.0	16.4	±	3.3	18.6	±	4.2
SV,	PLA	82.4	±	23.1	106.3	±	27.4	113.2	±	29.4
$mL \cdot beat^{-1}$	DA	79.9	±	14.1	98.9	±	17.3	107.2	±	17.7
	BK	84.9	±	20.4	109.1	±	20.9	113.2	±	25.0
HR,	PLA	70	±	11	149	±	13	164	±	12
$beat \cdot min^{-1}$	DA	73	±	11	154	±	13	166	±	12
	BK	69	±	10	149	±	11	160	±	10
Hb Concentration,	PLA	14.3	±	0.9	14.8	±	1.2	15.1	±	1.7
$g \cdot dL^{-1}$	DA	14.0	±	1.5	14.8	±	1.2	15.0	±	1.6
	BK	14.1	±	1.0	14.6	±	1.1	14.7	±	1.6

Values are expressed as mean ± SD. Q = cardiac output; SV = stroke volume; HR = heart rate; Hb = hemoglobin.

Table 4. Cardiovascular responses during exhaustive exercise at 85% of VO_{2peak}

		Baseline	2 Minutes	Exhaustion	*P* time	*P* condition	*P* interaction
Time, seconds	PLA	0 ± 0	120 ± 0	367 ± 198	n/a	**0.040**	n/a
	DA	0 ± 0	120 ± 0	378 ± 194			
	BK	0 ± 0	120 ± 0	259 ± 120*			
Q, $L \cdot min^{-1}$	PLA	7.5 ± 1.6	19.1 ± 5.1	21.3 ± 6.4	**<0.001**	0.243	0.181
	DA	7.7 ± 1.1	17.5 ± 4.1	20.0 ± 4.6			
	BK	7.1 ± 2.1	17.8 ± 5.3	19.9 ± 5.1			
SV, $mL \cdot beat^{-1}$	PLA	81.6 ± 23.1	116.5 ± 36.0	119.3 ± 38.9	**<0.001**	0.145	0.538
	DA	77.6 ± 16.6	105.3 ± 27.1	111.5 ± 26.2			
	BK	81.5 ± 22.8	109.8 ± 34.1	116.7 ± 31.5			
HR, $beat \cdot min^{-1}$	PLA	102 ± 25	167 ± 10	179 ± 9	**<0.001**	**<0.001**	0.594
	DA	101 ± 14	167 ± 10	180 ± 10			
	BK	92 ± 12*	163 ± 10	171 ± 13*			
SpO_2, %	PLA	96.4 ± 2.7	96.6 ± 3.1	94.8 ± 3.1	**0.015**	0.087	0.848
	DA	95.1 ± 2.5	94.9 ± 3.8	94.1 ± 2.5			
	BK	96.5 ± 2.2	96.1 ± 4.1	95.2 ± 3.1			
O_2 Delivery, $mLO_2 \cdot min^{-1}$	PLA	1621 ± 687	3891 ± 1244	4174 ± 1344	**<0.001**	**0.010**	0.844
	DA	1421 ± 297	3435 ± 926*	3837 ± 961			
	BK	1297 ± 371	3438 ± 1049*	3814 ± 1121			
MAP, mmHg	PLA	90.1 ± 13.1	98.1 ± 16.4	100.1 ± 13.7	**<0.001**	**0.025**	0.448
	DA	81.9 ± 9.1*	93.1 ± 10.9	95.6 ± 14.8			
	BK	85.6 ± 8.7	101.5 ± 14.9	98.3 ± 13.2			
Q/MAP, $mL \cdot min^{-1} \cdot mmHg^{-1}$	PLA	83.4 ± 17.6	192.5 ± 44.2	206.9 ± 38.8	**<0.001**	0.422	0.877
	DA	94.7 ± 14.0	186.3 ± 51.6	211.4 ± 42.4			
	BK	83.5 ± 23.3	178.3 ± 48.5	202.4 ± 61.0			

Values are expressed as mean ± SD. PLA = placebo; DA = dopamine; BK = blockade; Q = cardiac output; SV = stroke volume; HR = heart rate; SpO_2 = arterial O_2 saturation; MAP = mean arterial pressure. * indicates statistically significant difference compared to the placebo condition ($P<0.05$).

Table 5. Respiratory responses during exhaustive exercise at 85% of VO_{2peak}

		Baseline	2 Minutes	Exhaustion	*P* time	*P* condition	*P* interaction
VO_2, $L \cdot min^{-1}$	PLA	0.51 ± 0.35	2.81 ± 0.74	3.24 ± 0.96	**<0.001**	0.961	0.390
	DA	0.50 ± 0.16	2.84 ± 0.73	3.30 ± 0.85			
	BK	0.61 ± 0.37	2.78 ± 0.67	3.21 ± 0.86			
VE, $L \cdot min^{-1}$	PLA	16.0 ± 3.3	97.1 ± 21.0	132.0 ± 28.6	**<0.001**	0.404	**0.018**
	DA	17.7 ± 6.2	85.9 ± 24.9*	132.7 ± 32.4			
	BK	17.5 ± 6.6	94.6 ± 20.3	124.8 ± 32.3			
RER	PLA	0.81 ± 0.08	1.09 ± 0.07	1.15 ± 0.12	**<0.001**	0.244	0.537
	DA	0.82 ± 0.07	1.05 ± 0.11	1.14 ± 0.09			
	BK	0.80 ± 0.08	1.07 ± 0.13	1.12 ± 0.09			

Values are expressed as mean ± SD. PLA = placebo; DA = dopamine; BK = blockade; VO_2 = volume of O_2 consumption; VE = ventilation; RER = respiratory exchange ratio. * indicates statistically significant difference compared to the placebo condition ($P<0.05$).

Table 6. Determinants of time-to-exhaustion

	Baseline			60% of VO_{2peak}			85% of VO_{2peak}		
	r	r^2	*P* value	r	r^2	*P* Value	r	r^2	*P* Value
Placebo									
DL_{CO}	0.21	0.04	0.49	0.12	0.01	0.71	0.31	0.10	0.30
Vc	0.16	0.03	0.59	0.15	0.02	0.63	**0.70**	**0.49**	**0.01**
Δ Vc							**0.71**	**0.50**	**0.001**
Dm	0.04	0.00	0.90	-0.56	0.31	0.86	-0.46	0.21	0.12
Δ Dm							-0.43	0.19	0.14
Dopamine									
DL_{CO}	**0.69**	**0.47**	**0.01**	**0.70**	**0.48**	**0.01**	**0.63**	**0.39**	**0.02**
Vc	0.44	0.19	0.13	**0.68**	**0.46**	**0.01**	**0.69**	**0.47**	**0.02**
Δ Vc							0.54	0.29	0.09
Dm	0.04	0.00	0.90	-0.56	0.31	0.86	-0.46	0.21	0.12
Δ Dm							-0.04	0.00	0.90
Blockade									
DL_{CO}	0.01	0.00	0.99	0.26	0.07	0.38	0.04	0.00	0.88
Vc	0.19	0.03	0.53	0.16	0.03	0.59	0.39	0.15	0.17
Δ Vc							0.28	0.08	0.34
Dm	-0.17	0.03	0.55	0.32	0.10	0.26	-0.13	0.016	0.67
Δ Dm							-0.04	0.00	0.90

r = Pearson correlation coefficient; r^2 = coefficient of determination; VO_{2peak} = peak O_2 consumption; DL_{CO} = diffusing capacity for carbon monoxide measured in $mL \cdot min^{-1} \cdot mmHg^{-1}$; Vc = pulmonary capillary blood volume measured in mL; Dm = diffusing membrane capacity measured in $mL \cdot min^{-1} \cdot mmHg^{-1}$; Δ Vc = change in Vc from baseline to 85% of VO_{2peak}; Δ Dm = change in Dm from baseline to 85% of VO_{2peak}.

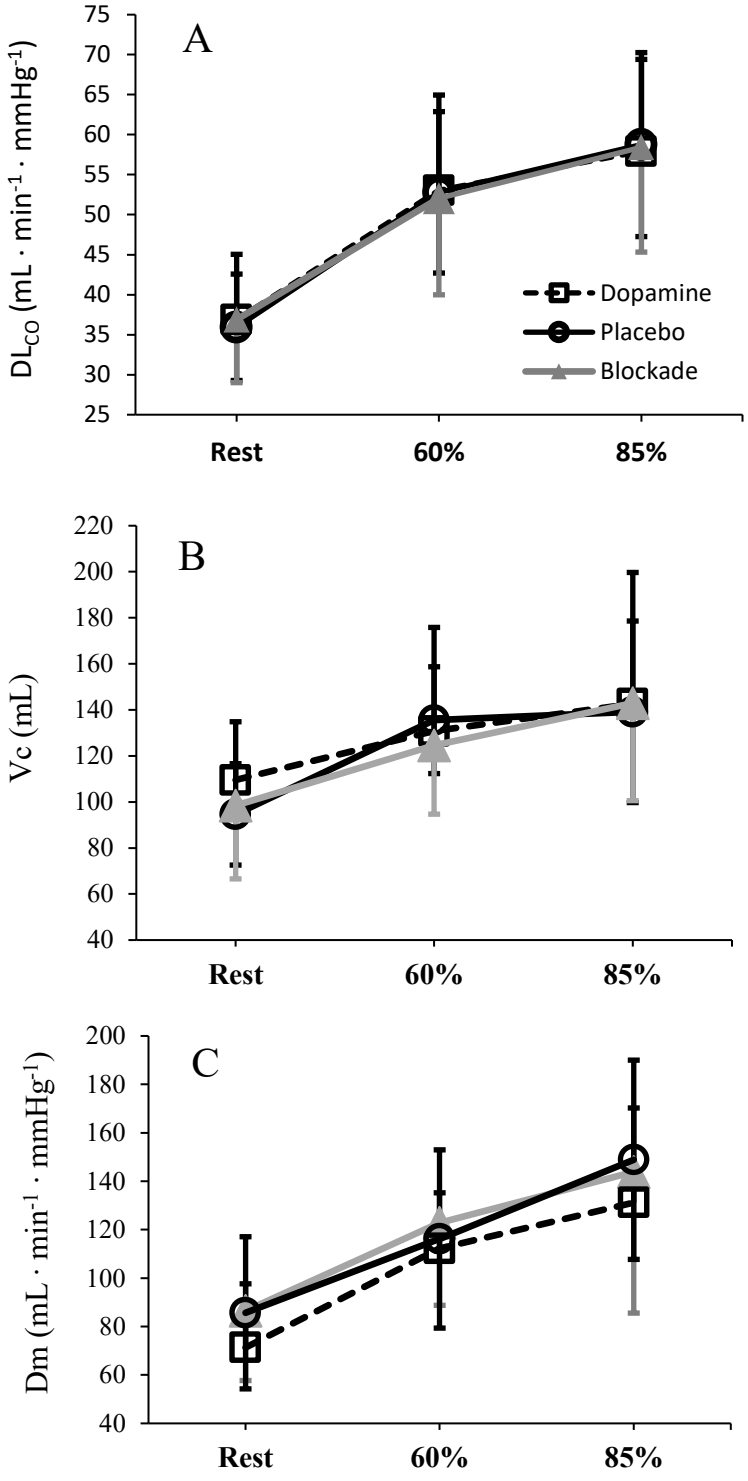

Figure 1. A. Diffusing capacity response at baseline and to exercise at 60% and 85% of VO_{2peak}. B. Pulmonary Vc response at baseline and to exercise at 60% and 85% of VO_{2peak}. C. Membrane diffusing capacity response at baseline and to exercise at 60% and 85% of VO_{2peak}. Values are expressed as mean ± SD.

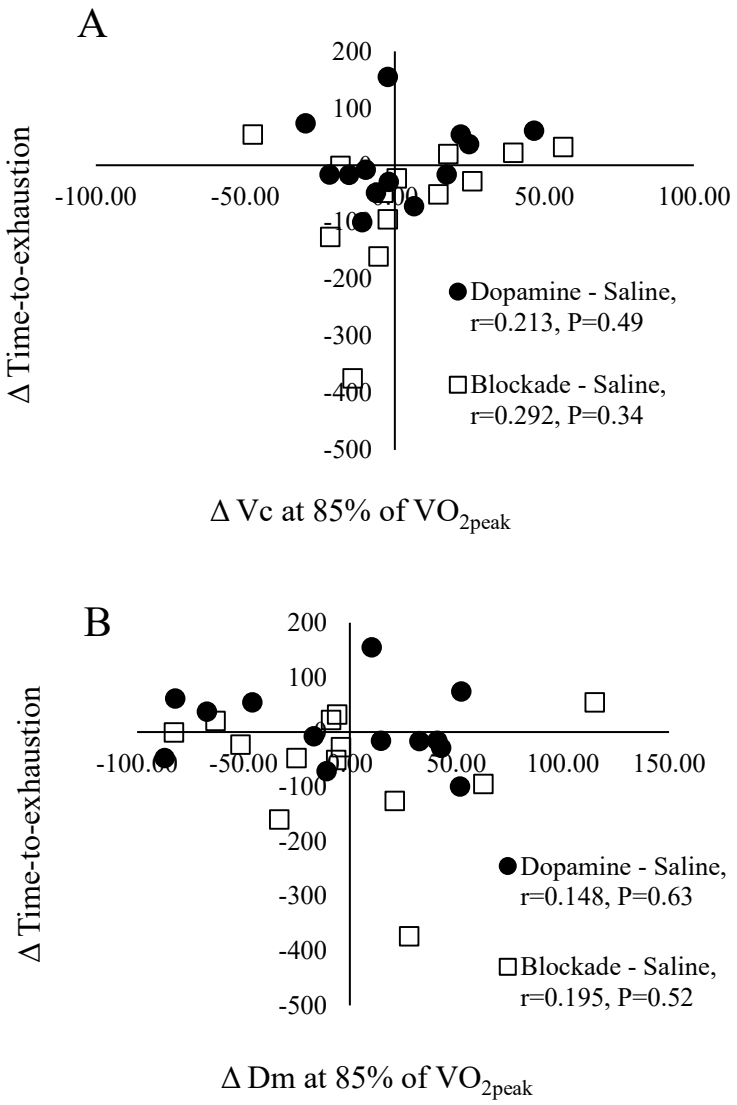

Figure 2. A. Correlation between the change in Vc between drug conditions at 85% of VO_{2peak} and the change in time-to-exhaustion between drug conditions. B. Correlation between the change in Dm between drug conditions at 85% of VO_{2peak} and the change in time-to-exhaustion between drug conditions.

3.5 References

1. **Alkotob ML**, **Soltani P**, **Sheatt MA**, **Katsetos MC**, **Rothfield N**, **Hager WD**, **Foley RJ**, **Silverman DI**. Reduced exercise capacity and stress-induced pulmonary hypertension in patients with scleroderma. *Chest* 130: 176–181, 2006.

2. **Balthazar CH**, **Leite LHR**, **Ribeiro RMM**, **Soares DD**, **Coimbra CC**. Effects of blockade of central dopamine D1 and D2 receptors on thermoregulation, metabolic rate and running performance. *Pharmacol Reports* 62: 54–61, 2010.

3. **Bates ML**, **Hotter JA**, **Zhang H**, **Farrell ET**. Overbuilt for exercise or not: how do lung structure and distensibility impact exercise capacity? *J Physiol* 591: 605–606, 2013.

4. **Boetger CL**, **Ward DS**. Effect of dopamine on transient ventilatory response to exercise. *J Appl Physiol* 61: 2102–2107, 1986.

5. **Bouwsema MM**, **Tedjasaputra V**, **Stickland MK**. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? *J Appl Physiol* 122: 460–469, 2017.

6. **Bryan TL**, **van Diepen S**, **Bhutani M**, **Shanks M**, **Welsh RC**, **Stickland MK**. The effects of dobutamine and dopamine on intrapulmonary shunt and gas exchange in healthy humans. *J Appl Physiol* 113: 541–8, 2012.

7. **Ceridon ML**, **Beck KC**, **Olson TP**, **Bilezikian JA**, **Johnson BD**. Calculating alveolar capillary conductance and pulmonary capillary blood volume: comparing the multiple- and single-inspired oxygen tension methods. *J Appl Physiol* 109: 643–53, 2010.

8. **Coffman KE**, **Carlson AR**, **Miller AD**, **Johnson BD**, **Taylor BJ**. The effect of aging and cardiorespiratory fitness on the lung diffusing capacity response to exercise in healthy humans. *J. Appl. Physiol.* (2017). doi: 10.1152/japplphysiol.00694.2016.

9. **Edgell H**, **McMurtry MS**, **Haykowsky MJ**, **Paterson I**, **Ezekowitz JA**, **Dyck JRB**, **Stickland MK**. Peripheral chemoreceptor control of cardiovascular function at rest and during exercise in heart failure patients. *J Appl Physiol* 118, 2015.

10. **Flamm SD**, **Taki J**, **Moore R**, **Lewis SF**, **Keech F**, **Maltais F**, **Ahmad M**, **Callahan R**, **Dragotakes S**, **Alpert N**. Redistribution of regional and organ blood volume and effect on cardiac function in relation to upright exercise intensity in healthy human subjects. *Circulation* 81: 1550–1559, 1990.

11. **Graham BL**, **Mink JT**, **Cotton DJ**. Effect of breath-hold time on DLCO(SB) in patients with airway obstruction. *J Appl Physiol* 58: 1319–1325, 1985.

12. **Henson LC**, **Ward DS**, **Whipp BJ**. Effect of dopamine on ventilatory response to incremental exercise in man. *Respir Physiol* 89: 209–224, 1992.

13. **Hoshino Y**, **Obara H**, **Iwai S**. Relaxant effect of dopamine on isolated rabbit pulmonary artery. *Life Sci* 39: 2525–2531, 1986.

14. **Hsia C**. Recruitment of lung diffusing capacity - Update of concept and application. *Chest* 122: 1774–1783, 2002.

15. **Johnson RL**, **Spicer WS**, **Bishop JM**, **Forster RE**. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J Appl Physiol* 15: 893–902, 1960.

16. **Krogh M**. The diffusion of gases through the lungs of man. *J Physiol* 49: 271–300, 1915.

17. **Lalande S**, **Yerly P**, **Faoro V**, **Naeije R**. Pulmonary vascular distensibility predicts aerobic capacity in healthy individuals. *J Physiol* 590: 4279–88, 2012.

18. **Lundby C**, **Møller P**, **Kanstrup IL**, **Olsen NV**. Heart rate response to hypoxic exercise: role of dopamine D2-receptors and effect of oxygen supplementation. *Clin Sci (Lond)* 101: 377–383, 2001.

19. **MacIntyre N**, **Crapo RO**, **Viegi G**, **Johnson DC**, **van der Grinten CPM**, **Brusasco V**, **Burgos F**, **Casaburi R**, **Coates A**, **Enright P**, **Gustafsson P**, **Hankinson J**, **Jensen R**, **McKay R**, **Miller MR**, **Navajas D**, **Pedersen OF**, **Pellegrino R**, **Wanger J**. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 26: 720–735, 2005.

20. **Marrades RM**, **Diaz O**, **Roca J**, **Campistol JM**, **Torregrosa J V**, **Barberà JA**, **Cobos A**, **Félez MA**, **Rodriguez-Roisin R**. Adjustment of DL(CO) for hemoglobin concentration. *Am J Respir Crit Care Med* 155: 236–241, 1997.

21. **Mostyn EM**, **Helle S**, **Gee JBL**, **Bentivoglio LG**, **Bates DV**. Pulmonary diffusing capacity of athletes. *J Appl Physiol* 18: 687–695, 1963.

22. **Myers J**, **Froelicher VF**. Hemodynamic determinants of exercise capacity in chronic heart failure. *Ann Intern Med* 115: 377–386, 1991.

23. **Ogilvie CM**, **Forster RE**, **Blakemore WS**, **Morton JW**. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 36: 1–17, 1957.

24. **Olsen EBJ**, **Vidruk EH**, **Dempsey JA**. Carotid body excision significantly changes ventilatory control in awake rats. *J Appl Physiol* 64: 666=671, 1988.

25. **Otto C**. *Textbook of Clinical Echocardiography*. 3rd ed. Philadelphia, PA: Elsevier Saunders, 2004.

26. **Polak MJ**, **Kennedy LA**, **Drummond WH**. Manipulation of dopamine receptors alters hypoxic pulmonary vasoconstriction in isolated perfused rat lungs. *Life Sci* 51: 1317–1323, 1992.

27. **Reeves JT**, **Taylor AE**. Pulmonary hemodynamics and fluid exchange in the lungs during exercise. *Handb. Physiol. Exerc. Regul. Integr. Mult. Syst.* (1996). doi: 10.1002/cphy.cp120113.

28. **Roelands B**, **Hasegawa H**, **Watson P**, **Piacentini MF**, **Buyse L**, **De Schutter G**,

Meeusen RR. The effects of acute dopamine reuptake inhibition on performance. *Med Sci Sports Exerc* 40: 879–885, 2008.

29. **Roughton FJW**, **Forster RE**. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 11: 290–302, 1957.

30. **Schuppan D**, **Schmidt L**, **Heller M**. Preliminary pharmacokinetics of metoclopramide in humans. Plasma levels following a single oral and intravenous dose. *Arzneimittelforschung* 29: 151–154, 1979.

31. **Stickland MK**, **Fuhr DP**, **Haykowsky MJ**, **Jones KE**, **Paterson DI**, **Ezekowitz JA**, **McMurtry MS**. Carotid chemoreceptor modulation of blood flow during exercise in healthy humans. *J Physiol* 589: 6219–30, 2011.

32. **Suresh K**, **Shimoda LA**. Lung circulation. *Compr Physiol* 6: 897–943, 2016.

33. **Taylor W**, **Bateman D**. Oral bioavailability of high-dose metoclopramide. *Eur J Clin Pharmacol* 61: 41–44, 1986.

34. **Tedjasaputra V**, **Bouwsema MM**, **Stickland MK**. Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity response to exercise. *J. Physiol.* (2016). doi: 10.1017/CBO9781107415324.004.

35. **Tedjasaputra V**, **Bryan TL**, **van Diepen S**, **Moore LE**, **Bouwsema MM**, **Welsh RC**, **Petersen SR**, **Stickland MK**. Dopamine receptor blockade improves pulmonary gas exchange but decreases exercise performance in healthy humans. *J Physiol* 593: 3147–57, 2015.

36. **Tedjasaputra V**, **van Diepen S**, **Collins SE**, **Michaelchuk WW**, **Stickland MK**. Assessment of pulmonary capillary blood volume, membrane diffusing capacity, and intrapulmonary arteriovenous anastomoses during exercise. *J Vis Exp* 120, 2017.

37. **Theodore J**, **Robin ED**, **Morris AJ**, **Burke CM**, **Jamieson SW**, **Van Kessel A**, **Stinson EB**, **Shumway NE**. Augmented ventilatory response to exercise in pulmonary hypertension. *Chest* 89: 39–44, 1986.

38. **Tolle JJ**, **Waxman AB**, **Van Horn TL**, **Pappagianopoulos PP**, **Systrom DM**. Exercise-induced pulmonary arterial hypertension. *Circulation* 118: 2183–2189, 2008.

39. **Turino GM**, **Bergofsky EH**, **Goldring RM**. Effect of exercise on pulmonary diffusing capacity. *J Appl Physiol* 18: 447–456, 1963.

40. **Watson P**, **Hasegawa H**, **Roelands B**, **Piacentini MF**, **Looverie R**, **Meeusen R**. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *J Physiol* 565: 873–83, 2005.

41. **West JB**. *Respiratory physiology : The essentials.* 9th ed. Baltimore, MD: Philadelphia : Wolters Kluwer Health/Lippincott Williams & Wilkins, c2012., 2012.

42. **Yock PG**, **Popp RL**. Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. *Circulation* 70: 657–662, 1984.

Chapter IV: General Discussion

4.1 Interactions among pulmonary diffusing capacity, cardiovascular fitness, aging, and dopamine

This study was designed to assess the effect of exogenous dopamine and dopamine receptor-2 (D_2-receptor) blockade on DL_{CO}, its components Vc and Dm, and exercise tolerance during heavy exercise. In the current investigation, it appeared that dopamine and D_2-receptor blockade did not alter the response of DL_{CO}, Vc, and Dm at rest and during exercise up to 85% of peak O_2 consumption (VO_{2peak}). During exercise, it is well known that DL_{CO}, Vc, and Dm rise with increasing exercise intensity to meet the increasing O_2 demand of the body (5, 7, 23), and the current study provides no exception to this finding. In accordance with studies which tested the effect of dopamine receptor agonists on exercise tolerance (20, 26), dopamine did not significantly improve time-to-exhaustion at 85% of VO_{2peak} in healthy humans. Additionally, this study aligns with work that shows dopamine blockade reduces exercise tolerance in both humans (24) and animals (1). Overall, this study highlights that pulmonary vascular dopaminergic receptors do not appear important in the regulation of pulmonary diffusion during exercise in health.

4.1.1 Cardiorespiratory fitness

Recent work has shown that those with greater cardiorespiratory fitness exhibit higher DL_{CO} during exercise, secondary to a greater Dm response (23). When the current sample is split into groups of low cardiorespiratory fitness (Lo-Fit) and high cardiorespiratory fitness (Hi-Fit), the Hi-Fit group (Table 9, *Appendix C*) exhibits a considerably higher DL_{CO} during exercise than the Lo-Fit group (Table 8, *Appendix C*). In the placebo condition, this appears to be driven by a 21.8% increase in Dm at 60% of VO_{2peak} and a 32.7% increase in Vc at 85% of VO_{2peak} (*Appendix C*). Commentary on the effect of cardiorespiratory fitness in this section will utilize

trends in the current data rather than formal statistics to provide comparisons, as the current sample is underpowered to detect differences between fitness groups.

In addition to differences in pulmonary diffusion between Hi-Fit and Lo-Fit individuals, it is also known that those with greater cardiorespiratory fitness exhibit lower pulmonary pressures for a given O_2 consumption (22), which signifies greater pulmonary vascular distensibility during exercise. However, further elucidation of the mechanism for the enhanced pulmonary vascular distensibility in those with greater cardiorespiratory fitness is needed.

A possible mechanism for the greater pulmonary vascular distensibility seen in Hi-Fit individuals may be heightened dopamine receptor sensitivity. Hopkins and colleagues (10) showed that endogenous dopamine levels increase during exercise up to 100% of VO_{2max}. Additionally, at a low dose, dopamine binds to pulmonary dopaminergic receptors to cause vasodilation (11, 17). Thus, Hi-Fit individuals may have a greater pulmonary vascular response to the increase in dopamine with exercise than Lo-Fit individuals, resulting in a state of relative vasodilation and improved pulmonary vascular distensibility. If accurate, dopamine receptor sensitivity may partially explain differences in the components of pulmonary diffusion (i.e., greater Vc or Dm) as a function of cardiorespiratory fitness seen in previous work (12, 23).

Since pulmonary pressure measurements were not obtained in the current investigation, the mean Ppa-cardiac output (Q) relationship could not be used to assess pulmonary vascular distensibility as has been done previously (12). However, the change in Vc from rest to maximal exercise represents pulmonary capillary network expansion (2, 7), meaning that a larger Vc response to exercise would suggest greater pulmonary vascular distensibility. To support the theory that heightened pulmonary vascular distensibility in Hi-Fit individuals is related to

dopamine receptor sensitivity during exercise, the change in Vc from the placebo condition to the dopamine condition would be expected to be greatest in Hi-Fit individuals.

Indeed, when comparing the dopamine condition to placebo in the Lo-Fit group, Vc at 60% of VO_{2peak} is reduced by 8.9% and Vc at 85% of VO_{2peak} is decreased by 2% (Table 8, *Appendix C*). In contrast, the Hi-Fit exhibited a 19.1% increase in Vc at 60% of VO_{2peak} and a 13.7% increase in the Vc at 85% of VO_{2peak} in the dopamine condition compared to placebo (Table 9; *Appendix C*). The current data indicate a divergent dopamine response based on relative fitness level. Further, the dopamine data suggest that Hi-Fit individuals do in fact have greater pulmonary vascular distensibility than Lo-Fit individuals secondary to enhanced pulmonary dopamine receptor sensitivity. When considering the dopamine blockade data, Hi-Fit individuals had a 3% reduction in the Vc response at 85% of VO_{2peak} compared to the placebo condition, while Lo-Fit individuals had a 7.9% increase in Vc at 85% of VO_{2peak} with blockade. Thus, while the pulmonary vasculature of Lo-Fit individuals in the current investigation does not appear to be sensitive to changes in dopamine, the heightened Vc response with exogenous dopamine and the attenuated Vc response seen with D_2-receptor blockade in the Hi-Fit group highlights that dopamine may exhibit some control over the pulmonary vasculature of Hi-Fit individuals. Further, this potentially increased pulmonary dopamine receptor sensitivity may partly explain the greater pulmonary vascular distensibility seen in Hi-Fit individuals.

Further, if dopamine receptor sensitivity is important for greater pulmonary vascular distensibility in Hi-Fit individuals, then it would be expected that the change in Vc at 60% and 85% of VO_{2peak} from the placebo to the dopamine condition would be related to cardiorespiratory fitness. To assess this, a correlation was conducted between the above variables (Fig. 5, *Appendix C*). At 60% of VO_{2peak}, a moderately strong positive correlation ($r=0.47$, $P=0.09$) was

shown between the change in Vc from the placebo to the dopamine condition and relative VO_{2peak} (Fig. 5.A, *Appendix C*). Interestingly, this relationship was not maintained at 85% of VO_{2peak} (r=0.12, *P*=0.71). In addition, the change in Dm from the placebo to the dopamine condition was not related to VO_{2peak} at either workload (Fig. 5.B, *Appendix C*). This suggests that the effectiveness of the dopamine receptors in Hi-Fit individuals is more important during submaximal rather than heavy exercise.

Clear differences in the pulmonary systems of Hi-Fit and Lo-Fit individuals have been identified in previous work (7, 12, 22, 23). Based on the current data, it may be possible that those with greater cardiorespiratory fitness may have enhanced D_2-receptor sensitivity which results in a state of relative pulmonary vascular vasodilation, helping to explain the heightened pulmonary vascular distensibility seen in Hi-Fit individuals (12, 22). Future work should further investigate the role of cardiorespiratory fitness in modulating pulmonary D_2-receptor sensitivity and pulmonary vascular distensibility during exercise.

4.1.2 Aging

The aging process is associated with decreases in pulmonary distensibility (18) and increases in pulmonary artery systolic pressure (PASP) in healthy individuals (13), which may place older adults in a category of individuals who would benefit from a pulmonary vasodilator such as dopamine. One example of work showing changes in the pulmonary systems of older adults comes from Lam et al. (13), who measured PASP in a large sample of individuals aged 45 and older. They found that the median PASP was 26 mmHg in their sample and that age was positively correlated to PASP. The study by Lam and colleagues (13) can be compared to the work by D'Andrea et al. (8), who studied control subjects with a mean age of 27.5 years and found an average resting PASP of 17.6 mmHg. Based on these large echocardiographic studies,

it is evident that there is an increase in pulmonary pressures with increasing age (8, 13). Thus, those of increasing age may be in a physiological state where dopamine is more effective in altering pulmonary pressures, pulmonary diffusion, and Vc during exercise through greater expansion of the pulmonary capillary network.

Interesting work by Coffman and colleagues (7) examined DL_{CO} and its components Vc and Dm in young healthy adults compared to older healthy adults. This work highlights that expansion of the pulmonary capillary network is sufficient to increase DL_{CO}, Vc, and Dm during exercise in healthy individuals regardless of age (7). To make this conclusion, Coffman et al. (7) examined the Vc-Q relationship during exercise up to 90% of VO_{2peak}. Their analysis showed that the slopes between young and old are approximately the same from rest to peak exercise intensity, which led to the conclusion that expansion of the pulmonary capillary network is adequate in healthy adults irrespective of age (7). While this may be accurate, there is a physiologically significant difference in the Vc-Q response between young and old healthy adults, as the slope of this response is reduced by 34% in older adults (young healthy Vc-Q slope = 1.87; old healthy Vc-Q slope = 1.23) in Coffman and colleagues' study (7). It is possible that in older adults, the pulmonary D_2-receptors become partially deficient leading to a decrease in the slope of the Vc-Q response to exercise. Stimulation of pulmonary vascular dopamine receptors through exogenous administration of dopamine may help to improve the Vc-Q response in this group of individuals.

Findings from the current study may offer a role for dopamine in the recruitment and distention process in older adults. Expansion of the pulmonary capillary network (i.e., change in Vc from rest to peak exercise) was positively correlated with time-to-exhaustion during heavy exercise in both the dopamine and placebo conditions, but not the blockade condition (Fig. 2). It

is possible that in older adults, the pulmonary D_2-receptors become partially deficient leading to a decrease in the slope of the Vc-Q response to exercise. Stimulation of pulmonary vascular dopamine receptors through exogenous administration of the drug may help to improve the Vc-Q response in this group of individuals. To better understand dopamine's role in regulating pulmonary diffusion, Vc, Dm, Q, and exercise tolerance, it would be beneficial to replicate the present study in healthy older adults.

4.2 Dopamine's role in exercise tolerance: Central fatigue

In contrast to previous work in humans (24), the current study cannot explain the reduction in time-to-exhaustion during heavy cycle exercise with D_2-receptor blockade via a cardiovascular limitation in Q, stroke volume, or heart rate. The reduction in exercise tolerance is also not explained by changes in DL_{CO}, Vc, or Dm during heavy exercise.

D_2-receptors are found in abundance in the cardiovascular system, as well as centrally in the brain and central nervous system (3, 15). Although the pulmonary D_2-receptors were targeted in the current investigation, there is no way to assess if the central dopamine receptors were also blocked through our intervention. It is possible that blocking central dopamine receptors led to a reduction in central motor output to the working muscle during exercise, resulting in central rather than peripheral fatigue during the time-to-exhaustion trials. While this is outside of the scope of the current investigation, future work should examine if indices of central motor output (e.g., supramaximal quadriceps twitch force) are reduced after heavy exercise with D_2-receptor blockade.

4.3 Additional considerations

This investigation studied the effects of dopamine and D_2-receptor blockade in young healthy subjects at rest and during exercise up to 85% of VO_{2peak}. By means of the narrow frame of

subjects tested, these results cannot be generalized beyond young, healthy, recreationally active adults. Other limitations of the current study include the use of echocardiography in attempt to obtain pulmonary artery pressures and the use of a non-invasive Q measurement.

4.3.1 Inadequate tricuspid regurgitation

Due to insufficient tricuspid regurgitation envelopes of the young healthy subjects in this study, estimation of PASP was not possible. In a previous publication from our group (24), measurement of PASP was attempted in young healthy subjects during upright cycling with little success. In the current study, we attempted to improve the quality of the signal by having young healthy subjects perform semi-supine exercise. This endeavor did not provide adequate tricuspid regurgitation in any of the healthy subjects tested. Future work should utilize saline contrast echocardiography to enhance the tricuspid regurgitation envelope as recommended in the literature (14, 16), or directly measure pulmonary pressures invasively.

4.3.2 Non-invasive cardiac output estimation

This study utilized transthoracic impedance cardiography (Physioflow, Manatec, Paris, France) to non-invasively estimate Q at rest and during exercise. While the most accurate way to assess Q is invasively sampling arterial blood gases then using the Fick equation to calculate Q directly (21, 25). A non-invasive method to determine Q was chosen to improve subject recruitment. Importantly, the transthoracic impedance cardiography device chosen for this study has been validated against the direct Fick method for Q in both healthy adults (19) and clinical samples (6) and has shown to be an acceptable measure of Q at rest and during exercise in both samples. Additionally, this device has shown to have good reliability in trials involving repeated measures (19) and was always properly adjusted to baseline Q before every trial in the current study. As a result of this, a clean signal was obtained in 98% of trials at rest, 95% of trials at 60% of VO_{2peak}, and 90% of trials at 85% of VO_{2peak}.

The slope of the relationship between Q and O_2 consumption during exercise provides further insight into the validity of the non-invasive Q measurement used in this investigation. During the maximal exercise test to exhaustion on the preliminary day of testing, the average slope of the Q-O_2 consumption plot was 4.6 with successful measurement in 12 out of 14 subjects. For two of the maximal exercise tests, there was technical difficulty and no Q data was obtained for these subjects. The Q-O_2 consumption slope obtained in this study is similar to other studies that have invasively measured Q up to maximal exercise, including one study that found an average slope of 4.9 (4) and another study that found an average slope of 6.0 (9). While this study's use of a non-invasive Q measurement may not be the gold standard procedure in the field, it likely streamlined the study recruitment process and aligns well with other research that measured Q in other validated ways.

4.4 Summary

The effect of dopamine and D_2-receptor blockade on pulmonary diffusion, pulmonary capillary blood volume, and diffusing membrane capacity was examined in young, healthy, recreationally active adults. It was found that dopamine was not an important regulator of pulmonary diffusion or any of its components at rest and during exercise up to 85% of VO_{2peak}. D_2-receptor blockade reduced time-to-exhaustion during heavy exercise, but exogenous dopamine administration did not improve exercise tolerance any further. Further sub-analysis suggests that the ability to expand the pulmonary capillary network may be an important determinant of exercise tolerance, and that dopamine receptor sensitivity may be important for differences in the pulmonary vasculature seen with cardiorespiratory fitness and aging. Future work should examine the effect of dopamine on the pulmonary vasculature and diffusion responses in older adults, and high versus low cardiorespiratory fitness, as well as further elucidate the mechanism for reduced

exercise tolerance seen during D_2-receptor blockade (i.e., central fatigue during exhaustive exercise).

4.5 References

1. **Balthazar CH**, **Leite LHR**, **Ribeiro RMM**, **Soares DD**, **Coimbra CC**. Effects of blockade of central dopamine D1 and D2 receptors on thermoregulation, metabolic rate and running performance. *Pharmacol Reports* 62: 54–61, 2010.

2. **Bates ML**, **Hotter JA**, **Zhang H**, **Farrell ET**. Overbuilt for exercise or not: how do lung structure and distensibility impact exercise capacity? *J Physiol* 591: 605–606, 2013.

3. **Beaulieu J-M**, **Gainetdinov RR**. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63: 182–217, 2011.

4. **Beck K**, **Randolph L**, **Bailey K**. Relationship between cardiac output and oxygen consumption during upright cycle exercise in healthy humans. *J Appl Physiol* 101: 1474–1480, 2006.

5. **Bouwsema MM**, **Tedjasaputra V**, **Stickland MK**. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? *J Appl Physiol* 122: 460–469, 2017.

6. **Charloux A**, **Lonsdorfer-Wolf E**, **Richard R**, **Lampert E**, **Oswald-Mammosser M**, **Mettauer B**, **Geny B**, **Lonsdorfer J**. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: Comparison with the direct Fick method. *Eur J Appl Physiol* 82: 313–320, 2000.

7. **Coffman KE**, **Carlson AR**, **Miller AD**, **Johnson BD**, **Taylor BJ**. The effect of aging and cardiorespiratory fitness on the lung diffusing capacity response to exercise in healthy humans. *J. Appl. Physiol.* (2017). doi: 10.1152/japplphysiol.00694.2016.

8. **D'Andrea A**, **Naeije R**, **D'Alto M**, **Argiento P**, **Golia E**, **Cocchia R**, **Riegler L**, **Scarafile R**, **Limongelli G**, **Di Salvo G**, **Citro R**, **Caso P**, **Russo MG**, **Calabrò R**, **Bossone E**. Range in pulmonary artery systolic pressure among highly trained athletes. *Chest* 139: 788–792, 2011.

9. **Granath A**, **Jonsson B**, **Strandell T**. Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. *Acta Physiol Scand* 176: 425–446, 1964.

10. **Hopkins SR**, **Bogaard HJ**, **Niizeki K**, **Yamaya Y**, **Ziegler MG**, **Wagner PD**. Beta-adrenergic or parasympathetic inhibition, heart rate and cardiac output during normoxic and acute hypoxic exercise in humans. *J Physiol* 550: 605–616, 2003.

11. **Hoshino Y**, **Obara H**, **Iwai S**. Relaxant effect of dopamine on isolated rabbit pulmonary artery. *Life Sci* 39: 2525–2531, 1986.

12. **Lalande S**, **Yerly P**, **Faoro V**, **Naeije R**. Pulmonary vascular distensibility predicts aerobic capacity in healthy individuals. *J Physiol* 590: 4279–88, 2012.

13. **Lam CSP**, **Borlaug BA**, **Kane GC**, **Enders FT**, **Rodeheffer RJ**, **Redfield MM**. Age-

associated increases in pulmonary artery systolic pressure in the general population. *Circulation* 119: 2663–2670, 2009.

14. **Lopes LR**, **Loureiro MJ**, **Miranda R**, **Almeida S**, **Almeida AR**, **Cordeiro A**, **Cotrim C**, **Carrageta M**. The usefulness of contrast during exercise echocardiography for the assessment of systolic pulmonary pressure. *Cardiovasc Ultrasound* 6: 51, 2008.

15. **Missale C**, **Nash SR**, **Robinson SW**, **Jaber M**, **Caron MG**. Dopamine receptors: from structure to function. *Physiol Rev* 78: 189–225, 1998.

16. **Parasuraman S**, **Walker S**, **Loudon B**, **Gollop N**, **Wilson A**, **Lowery C**, **Frenneaux M**. Assessment of pulmonary artery pressure by echocardiography—A comprehensive review. *Int J Cardiol Hear Vasc* 12: 45–51, 2016.

17. **Polak MJ**, **Kennedy LA**, **Drummond WH**. Manipulation of dopamine receptors alters hypoxic pulmonary vasoconstriction in isolated perfused rat lungs. *Life Sci* 51: 1317–1323, 1992.

18. **Reeves JT**, **Linehan JH**, **Stenmark KR**. Distensibility of the normal human lung circulation during exercise. *Am J Physiol Lung Cell Mol Physiol* 288: L419–L425, 2005.

19. **Richard R**, **Lonsdorfer-Wolf E**, **Charloux A**, **Doutreleau S**, **Buchheit M**, **Oswald-Mammosser M**, **Lampert E**, **Mettauer B**, **Geny B**, **Lonsdorfer J**. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur J Appl Physiol* 85: 202–207, 2001.

20. **Roelands B**, **Hasegawa H**, **Watson P**, **Piacentini MF**, **Buyse L**, **De Schutter G**, **Meeusen RR**. The effects of acute dopamine reuptake inhibition on performance. *Med Sci Sports Exerc* 40: 879–885, 2008.

21. **Stickland MK**, **Welsh RC**, **Haykowsky MJ**, **Petersen SR**, **Anderson WD**, **Taylor DA**, **Bouffard M**, **Jones RL**. Intra-pulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 561: 321–329, 2004.

22. **Stickland MK**, **Welsh RC**, **Petersen SR**, **Tyberg J V**, **Anderson WD**, **Jones RL**, **Taylor DA**, **Bouffard M**, **Haykowsky MJ**. Does fitness level modulate the cardiovascular hemodynamic response to exercise? *J Appl Physiol* 100: 1895–1901, 2006.

23. **Tedjasaputra V**, **Bouwsema MM**, **Stickland MK**. Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity response to exercise. *J. Physiol.* (2016). doi: 10.1017/CBO9781107415324.004.

24. **Tedjasaputra V**, **Bryan TL**, **van Diepen S**, **Moore LE**, **Bouwsema MM**, **Welsh RC**, **Petersen SR**, **Stickland MK**. Dopamine receptor blockade improves pulmonary gas exchange but decreases exercise performance in healthy humans. *J Physiol* 593: 3147–57, 2015.

25. **Wagner PD**, **Gale GE**, **Moon RE**, **Torre-Bueno JR**, **Stolp BW**, **Saltzman HA**. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260–270, 1986.

26. **Watson P**, **Hasegawa H**, **Roelands B**, **Piacentini MF**, **Looverie R**, **Meeusen R**. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *J Physiol* 565: 873–83, 2005.

Bibliography

Alkotob ML, **Soltani P**, **Sheatt MA**, **Katsetos MC**, **Rothfield N**, **Hager WD**, **Foley RJ**, **Silverman DI**. Reduced exercise capacity and stress-induced pulmonary hypertension in patients with scleroderma. *Chest* 130: 176–181, 2006.

Aperia A. Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism. *Annu Rev Physiol* 62: 621–647, 2000.

Argiento P, **Chesler N**, **Mulè M**, **D'Alto M**, **Bossone E**, **Unger P**, **Naeije R**. Exercise stress echocardiography for the study of the pulmonary circulation. *Eur Respir J* 35: 1273–8, 2010.

Ayappa I, **Brown L V**, **Wang PM**, **Katzman N**, **Houtz P**, **Bruce EN**, **Lai-Fook SJ**. Effect of blood flow on capillary transit time and oxygenation in excised rabbit lung. *Respir Physiol* 105: 203–216, 1996.

Balthazar CH, **Leite LHR**, **Ribeiro RMM**, **Soares DD**, **Coimbra CC**. Effects of blockade of central dopamine D1 and D2 receptors on thermoregulation, metabolic rate and running performance. *Pharmacol Reports* 62: 54–61, 2010.

Bates ML, **Hotter JA**, **Zhang H**, **Farrell ET**. Overbuilt for exercise or not: how do lung structure and distensibility impact exercise capacity? *J Physiol* 591: 605–606, 2013.

Beaulieu J-M, **Gainetdinov RR**. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63: 182–217, 2011.

Beck K, **Randolph L**, **Bailey K**. Relationship between cardiac output and oxygen consumption

during upright cycle exercise in healthy humans. *J Appl Physiol* 101: 1474–1480, 2006.

Boetger CL, **Ward DS**. Effect of dopamine on transient ventilatory response to exercise. *J Appl Physiol* 61: 2102–2107, 1986.

Bonderman D, **Martischnig AM**, **Vonbank K**, **Nikfardjam M**, **Meyer B**, **Heinz G**, **Klepetko W**, **Naeije R**, **Lang IM**. Right ventricular load at exercise is a cause of persistent exercise limitation in patients with normal resting pulmonary vascular resistance after pulmonary endarterectomy. *Chest* 139: 122–127, 2011.

Bouwsema MM, **Tedjasaputra V**, **Stickland MK**. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? *J Appl Physiol* 122: 460–469, 2017.

Brunton L, **Lazo J**, **Parker K**. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 11th ed. New York: McGraw-Hill, 2005.

Bryan TL, **van Diepen S**, **Bhutani M**, **Shanks M**, **Welsh RC**, **Stickland MK**. The effects of dobutamine and dopamine on intrapulmonary shunt and gas exchange in healthy humans. *J Appl Physiol* 113: 541–8, 2012.

Ceridon ML, **Beck KC**, **Olson TP**, **Bilezikian JA**, **Johnson BD**. Calculating alveolar capillary conductance and pulmonary capillary blood volume: comparing the multiple- and single-inspired oxygen tension methods. *J Appl Physiol* 109: 643–53, 2010.

Charloux A, **Lonsdorfer-Wolf E**, **Richard R**, **Lampert E**, **Oswald-Mammosser M**, **Mettauer B**, **Geny B**, **Lonsdorfer J**. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: Comparison with the direct Fick

method. *Eur J Appl Physiol* 82: 313–320, 2000.

Chemla D, **Castelain V**, **Humbert M**, **Hébert J-L**, **Simonneau G**, **Lecarpentier Y**, **Hervé P**. New formula for predicting mean pulmonary artery pressure using systolic pulmonary artery pressure. *Chest* 126: 1313–1317, 2004.

Cheng CP, **Igarashi Y**, **Little WC**. Mechanism of augmented rate of left ventricular filling during exercise. *Circ Res* 70: 9–19, 1992.

Cheng CP, **Noda T**, **Nozawa T**, **Little WC**. Effect of heart failure on the mechanism of exercise-induced augmentation of mitral valve flow. *Circ Res* 72: 795–806, 1993.

Coffman KE, **Carlson AR**, **Miller AD**, **Johnson BD**, **Taylor BJ**. The effect of aging and cardiorespiratory fitness on the lung diffusing capacity response to exercise in healthy humans. *J. Appl. Physiol.* (2017). doi: 10.1152/japplphysiol.00694.2016.

Cotes J. Iron-deficiency anaemia: Its effect on transfer factor for the lung (diffusing capacity) and ventilation and cardiac frequency during sub-maximal exercise. 1972.

Cotrim C, **Simões O**, **Loureiro MJ**, **Cordeiro P**, **Lopes L**, **Almeida S**, **Ialá M**, **Miranda R**, **Carrageta M**. Stress echocardiography in the evaluation of exercise physiology in patients with severe arterial pulmonary hypertension. New methodology. *Rev Port Cardiol* 24: 1451–1460, 2005.

D'Andrea A, **Naeije R**, **D'Alto M**, **Argiento P**, **Golia E**, **Cocchia R**, **Riegler L**, **Scarafile R**, **Limongelli G**, **Di Salvo G**, **Citro R**, **Caso P**, **Russo MG**, **Calabrò R**, **Bossone E**. Range in pulmonary artery systolic pressure among highly trained athletes. *Chest* 139: 788–792, 2011.

Dempsey JA. Is the lung built for exercise? *Med Sci Sports Exerc* 18: 143–155, 1986.

Dempsey JA, **Hanson PG**, **Henderson KS**. Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. *J Physiol* 355: 161–175, 1984.

Dempsey JA, **Sheel AW**, **Haverkamp HC**, **Babcock MA**, **Harms CA**. The John Sutton Lecture: CSEP, 2002]. Pulmonary system limitations to exercise in health. *Can J Appl Physiol* 28 Suppl: S2-24, 2003.

Dempsey JA, **Wagner PD**. Exercise-induced arterial hypoxemia. *J Appl Physiol* 87: 1997–2006, 1999.

Edgell H, **McMurtry MS**, **Haykowsky MJ**, **Paterson I**, **Ezekowitz JA**, **Dyck JRB**, **Stickland MK**. Peripheral chemoreceptor control of cardiovascular function at rest and during exercise in heart failure patients. *J Appl Physiol* 118, 2015.

Edsell ME, **Wimalasena YH**, **Malein WL**, **Ashdown KM**, **Gallagher CA**, **Imray CH**, **Wright AD**, **Myers SD**. High-intensity intermittent exercise increases pulmonary interstitial edema at altitude but not at simulated altitude. *Wilderness Environ. Med.*: 2014.

Eldridge MW, **Dempsey JA**, **Haverkamp HC**, **Lovering AT**, **Hokanson JS**. Exercise-induced intrapulmonary arteriovenous shunting in healthy humans. *J Appl Physiol* 97: 797–805, 2004.

Fick A. Ficks first law of diffusion. *Ann Phys* 170: 59, 1855.

Flamm SD, **Taki J**, **Moore R**, **Lewis SF**, **Keech F**, **Maltais F**, **Ahmad M**, **Callahan R**, **Dragotakes S**, **Alpert N**. Redistribution of regional and organ blood volume and effect on cardiac function in relation to upright exercise intensity in healthy human subjects.

Circulation 81: 1550–1559, 1990.

Goldberg L. Cardiovascular and renal actions of dopamine: Potential clinical applications. *Pharmacol Rev* 24: 1–29, 1972.

Gorman P. Vasodilator response to dopamine in the ferret pulmonary circulation. *Br J Pharmacol* 94: 212–218, 1988.

Graham BL, **Mink JT**, **Cotton DJ**. Effect of breath-hold time on DLCO(SB) in patients with airway obstruction. *J Appl Physiol* 58: 1319–1325, 1985.

Granath A, **Jonsson B**, **Strandell T**. Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. *Acta Physiol Scand* 176: 425–446, 1964.

Granath A, **Strandell T**. Relationships between cardiac output, stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men. *Acta Med Scand* 176: 447–466, 1964.

Haldane J. The Action of Carbonic Oxide on Man. *J Physiol* 18: 430–462, 1895.

Harms CA, Rosenkranz S. Sex differences in pulmonary function during exercise. *Med Sci Sports Exerc* 40: 664–668, 2008.

Harms CA, **McClaran SR**, **Nickele GA**, **Pegelow DF**, **Nelson WB**, **Dempsey JA**. Exercise-induced arterial hypoxaemia in healthy young women. *J Physiol* 507: 619–628, 1998.

Henson LC, **Ward DS**, **Whipp BJ**. Effect of dopamine on ventilatory response to incremental exercise in man. *Respir Physiol* 89: 209–224, 1992.

Holmgren A, **Jonsson B**, **Sjostrand T**. Circulatory data in normal subjects at rest and during exercise in the recumbent position, with special reference to the stroke volume a different work intensities. *Acta Physiol Scand* 49: 343–363, 1960.

Hopkins SR, **Bogaard HJ**, **Niizeki K**, **Yamaya Y**, **Ziegler MG**, **Wagner PD**. Beta-adrenergic or parasympathetic inhibition, heart rate and cardiac output during normoxic and acute hypoxic exercise in humans. *J Physiol* 550: 605–616, 2003.

Hopkins SR, **Schoene RB**, **Henderson WR**, **Spragg RG**, **Martin TR**, **West JB**. Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090–1094, 1997.

Hoshino Y, **Obara H**, **Iwai S**. Relaxant effect of dopamine on isolated rabbit pulmonary artery. *Life Sci* 39: 2525–2531, 1986.

Hsia C. Recruitment of lung diffusing capacity - Update of concept and application. *Chest* 122: 1774–1783, 2002.

Hughes JMB. The single breath transfer factor (Tl,co) and the transfer coefficient (Kco): a window onto the pulmonary microcirculation. *Clin Physiol Funct Imaging* 23: 63–71, 2003.

Hughes JMB, **Borland CDR**. The centenary (2015) of the transfer factor for carbon monoxide (T(LCO)): Marie Krogh's legacy. *Thorax* 70: 391–394, 2015.

Johnson BD, **Saupe KW**, **Dempsey JA**. Mechanical constraints on exercise hyperpnea in endurance athletes. *J Appl Physiol* 73: 874–86, 1992.

Johnson DC. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon

monoxide transfer coefficient (KCO) for alveolar volume. *Respir Med* 94: 28–37, 2000.

Johnson RL, **Spicer WS**, **Bishop JM**, **Forster RE**. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. *J Appl Physiol* 15: 893–902, 1960.

Kendrick A, **Laszlo G**. CO transfer factor on exercise: age and sex differences. *Eur Respir J* 3: 2340891, 1990.

Kobayashi Y, **Cavallotti D**, **Ricci A**, **Amenta F**. Localisation of dopamine D2-like receptors in pulmonary artery of the human and rabbit but not of the rat. *Eur J Pharmacol* 261: 229–236, 1994.

Korzeniewski K, **Nitsch-Osuch A**, **Guzek A**, **Juszczak D**. High altitude pulmonary edema in mountain climbers. *Respir Physiol Neurobiol* 209: 33–38, 2015.

Koskolou MD, **McKenzie DC**. Arterial Hypoxemia and Performance During Intense Exercise. *Eur J Appl Physiol Occup Physiol* 68: 80–86, 1994.

Kovacs G, **Berghold A**, **Scheidl S**, **Olschewski H**. Pulmonary arterial pressure during rest and exercise in healthy subjects: A systematic review. *Eur Respir J* 34: 888–894, 2009.

Kovacs G, **Olschewski A**, **Berghold A**, **Olschewski H**. Pulmonary vascular resistances during exercise in normal subjects: a systematic review. *Eur Respir J* 39: 319–28, 2012.

Krogh M. The diffusion of gases through the lungs of man. *J Physiol* 49: 271–300, 1915.

Lalande S, **Yerly P**, **Faoro V**, **Naeije R**. Pulmonary vascular distensibility predicts aerobic capacity in healthy individuals. *J Physiol* 590: 4279–88, 2012.

Lam CSP, **Borlaug BA**, **Kane GC**, **Enders FT**, **Rodeheffer RJ**, **Redfield MM**. Age-associated

increases in pulmonary artery systolic pressure in the general population. *Circulation* 119: 2663–2670, 2009.

Lopes LR, **Loureiro MJ**, **Miranda R**, **Almeida S**, **Almeida AR**, **Cordeiro A**, **Cotrim C**, **Carrageta M**. The usefulness of contrast during exercise echocardiography for the assessment of systolic pulmonary pressure. *Cardiovasc Ultrasound* 6: 51, 2008.

Lovering AT, **Stickland MK**, **Eldridge MW**. Intrapulmonary shunt during normoxic and hypoxic exercise in healthy humans. In: *Advances in Experimental Medicine and Biology*. 2008, p. 31–45.

Lovering AT, **Stickland MK**, **Kelso AJ**, **Eldridge MW**. Direct demonstration of 25- and 50-microm arteriovenous pathways in healthy human and baboon lungs. *Am J Physiol Heart Circ Physiol* 292: H1777–H1781, 2007.

Lundby C, **Møller P**, **Kanstrup IL**, **Olsen NV**. Heart rate response to hypoxic exercise: role of dopamine D2-receptors and effect of oxygen supplementation. *Clin Sci (Lond)* 101: 377–383, 2001.

MacIntyre N, **Crapo RO**, **Viegi G**, **Johnson DC**, **van der Grinten CPM**, **Brusasco V**, **Burgos F**, **Casaburi R**, **Coates A**, **Enright P**, **Gustafsson P**, **Hankinson J**, **Jensen R**, **McKay R**, **Miller MR**, **Navajas D**, **Pedersen OF**, **Pellegrino R**, **Wanger J**. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 26: 720–735, 2005.

Marrades RM, **Diaz O**, **Roca J**, **Campistol JM**, **Torregrosa J V**, **Barberà JA**, **Cobos A**, **Félez MA**, **Rodriguez-Roisin R**. Adjustment of DL(CO) for hemoglobin concentration.

Am J Respir Crit Care Med 155: 236–241, 1997.

Missale C, **Nash SR**, **Robinson SW**, **Jaber M**, **Caron MG**. Dopamine receptors: from structure to function. *Physiol Rev* 78: 189–225, 1998.

Mohsenifar Z, **Tashkin DP**. Effect of carboxyhemoglobin on the single breath diffusing capacity: derivation of an empirical correction factor. *Respiration* 37: 185–191, 1979.

Molloy DW, **Ducas J**, **Dobson K**, **Girling L**, **Prewitt RM**. Hemodynamic management in clinical acute hypoxemic respiratory failure. Dopamine vs dobutamine. *Chest* 89: 636–640, 1986.

Mostyn EM, **Helle S**, **Gee JBL**, **Bentivoglio LG**, **Bates DV**. Pulmonary diffusing capacity of athletes. *J Appl Physiol* 18: 687–695, 1963.

Myers J, **Froelicher VF**. Hemodynamic determinants of exercise capacity in chronic heart failure. *Ann Intern Med* 115: 377–386, 1991.

Naeije R, **Chesler N**. Pulmonary circulation at exercise. *Compr Physiol* 2: 711–41, 2012.

Naeije R, **Vanderpool R**, **Dhakal BP**, **Saggar R**, **Saggar R**, **Vachiery JL**, **Lewis GD**. Exercise-induced pulmonary hypertension: Physiological basis and methodological concerns. *Am. J. Respir. Crit. Care Med.* 187: 576–583, 2013.

Nielsen HB, **Boushel R**, **Madsen P**, **Secher NH**. Cerebral desaturation during exercise reversed by O2 supplementation. *Am J Physiol* 277: H1045-52, 1999.

O'Malley KL, **Harmon S**, **Tang L**, **Todd RD**. The rat dopamine D4 receptor: sequence, gene structure, and demonstration of expression in the cardiovascular system. *New Biol* 4: 137–

146, 1992.

Ogilvie CM, **Forster RE**, **Blakemore WS**, **Morton JW**. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 36: 1–17, 1957.

Olsen EBJ, **Vidruk EH**, **Dempsey JA**. Carotid body excision significantly changes ventilatory control in awake rats. *J Appl Physiol* 64: 666=671, 1988.

Otto C. *Textbook of Clinical Echocardiography*. 3rd ed. Philadelphia, PA: Elsevier Saunders, 2004.

Parasuraman S, **Walker S**, **Loudon B**, **Gollop N**, **Wilson A**, **Lowery C**, **Frenneaux M**. Assessment of pulmonary artery pressure by echocardiography—A comprehensive review. *Int J Cardiol Hear Vasc* 12: 45–51, 2016.

Peltonen JE, **Rantamäki J**, **Niittymäki SP**, **Sweins K**, **Viitasalo JT**, **Rusko HK**. Effects of oxygen fraction in inspired air on rowing performance. *Med Sci Sport Exerc* 27: 573–579, 1995.

Polak MJ, **Drummond WH**. Systemic and pulmonary vascular effects of selective dopamine receptor blockade and stimulation in lambs. *Pediatr Res* 33: 181–184, 1993.

Polak MJ, **Kennedy LA**, **Drummond WH**. Manipulation of dopamine receptors alters hypoxic pulmonary vasoconstriction in isolated perfused rat lungs. *Life Sci* 51: 1317–1323, 1992.

Powers SK, **Lawler J**, **Dempsey JA**, **Dodd S**, **Landry G**. Effects of incomplete pulmonary gas exchange on VO2max. *J Appl Physiol* 66: 2491–2495, 1989.

Reeves JT, **Dempsey JA**, **Grover RF**. Pulmonary circulation during exercise. In: *Pulmonary Vascular Physiology and Physiopathology*, edited by Weir E, Reeves J. New York: Marcel Dekker, 1989, p. 107–133.

Reeves JT, **Linehan JH**, **Stenmark KR**. Distensibility of the normal human lung circulation during exercise. *Am J Physiol Lung Cell Mol Physiol* 288: L419–L425, 2005.

Reeves JT, **Taylor AE**. Pulmonary hemodynamics and fluid exchange in the lungs during exercise. *Handb. Physiol. Exerc. Regul. Integr. Mult. Syst.* (1996). doi: 10.1002/cphy.cp120113.

Rennotte MT, **Reynaert M**, **Clerbaux T**, **Willems E**, **Roeseleer J**, **Verlter C**, **Rodenstein D**, **Frans A**. Effects of two inotropic drugs, dopamine and dobutamine, on pulmonary gas exchange in artificially ventilated patients. *Intensive Care Med* 15: 160–165, 1989.

Rice AJ, **Thornton AT**, **Gore CJ**, **Scroop GC**, **Greville HW**, **Wagner H**, **Wagner PD**, **Hopkins SR**. Pulmonary gas exchange during exercise in highly trained cyclists with arterial hypoxemia. *J Appl Physiol* 87: 1802–1812, 1999.

Richard R, **Lonsdorfer-Wolf E**, **Charloux A**, **Doutreleau S**, **Buchheit M**, **Oswald-Mammosser M**, **Lampert E**, **Mettauer B**, **Geny B**, **Lonsdorfer J**. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur J Appl Physiol* 85: 202–207, 2001.

Roelands B, **Hasegawa H**, **Watson P**, **Piacentini MF**, **Buyse L**, **De Schutter G**, **Meeusen RR**. The effects of acute dopamine reuptake inhibition on performance. *Med Sci Sports Exerc* 40: 879–885, 2008.

Romer LM, **Dempsey JA**. Effects of exercise-induced arterial hypoxemia on limb muscle fatigue and performance. *Proc Aust Physiol Soc* 36: 71–75, 2005.

Roughton FJW, **Forster RE**. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 11: 290–302, 1957.

Schuppan D, **Schmidt L**, **Heller M**. Preliminary pharmacokinetics of metoclopramide in humans. Plasma levels following a single oral and intravenous dose. *Arzneimittelforschung* 29: 151–154, 1979.

Simmons D, **Linde L**, **Miller J**, **O'Reilly R**. Relation between lung volume and pulmonary vascular resistance. *Circ Res* 4: 465–471, 1961.

Smith JR, **Brown KR**, **Murphy JD**, **Harms CA**. Does menstrual cycle phase affect lung diffusion capacity during exercise? *Respir Physiol Neurobiol* 205: 99–104, 2015.

St Croix CM, **Harms CA**, **McClaran SR**, **Nickele GA**, **Pegelow DF**, **Nelson WB**, **Dempsey JA**. Effects of prior exercise on exercise-induced arterial hypoxemia in young women. *J Appl Physiol* 85: 1556–1563, 1998.

Stickland MK, **Fuhr DP**, **Haykowsky MJ**, **Jones KE**, **Paterson DI**, **Ezekowitz JA**, **McMurtry MS**. Carotid chemoreceptor modulation of blood flow during exercise in healthy humans. *J Physiol* 589: 6219–30, 2011.

Stickland MK, **Lindinger MI**, **Olfert IM**, **Heigenhauser GJF**, **Hopkins SR**. Pulmonary gas exchange and acid-base balance during exercise. *Compr Physiol* 3: 693–739, 2013.

Stickland MK, **Lovering AT**. Exercise-induced intrapulmonary arteriovenous shunting and pulmonary gas exchange. *Exerc. Sport Sci. Rev.* 34: 99–106, 2006.

Stickland MK, **Welsh RC**, **Haykowsky MJ**, **Petersen SR**, **Anderson WD**, **Taylor DA**, **Bouffard M**, **Jones RL**. Intra-pulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 561: 321–329, 2004.

Stickland MK, **Welsh RC**, **Petersen SR**, **Tyberg J V**, **Anderson WD**, **Jones RL**, **Taylor DA**, **Bouffard M**, **Haykowsky MJ**. Does fitness level modulate the cardiovascular hemodynamic response to exercise? *J Appl Physiol* 100: 1895–1901, 2006.

Suresh K, **Shimoda LA**. Lung circulation. *Compr Physiol* 6: 897–943, 2016.

Taylor W, **Bateman D**. Oral bioavailability of high-dose metoclopramide. *Eur J Clin Pharmacol* 61: 41–44, 1986.

Tedjasaputra V, **Bouwsema MM**, **Stickland MK**. Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity response to exercise. *J. Physiol.* (2016). doi: 10.1017/CBO9781107415324.004.

Tedjasaputra V, **Bryan TL**, **van Diepen S**, **Moore LE**, **Bouwsema MM**, **Welsh RC**, **Petersen SR**, **Stickland MK**. Dopamine receptor blockade improves pulmonary gas exchange but decreases exercise performance in healthy humans. *J Physiol* 593: 3147–57, 2015.

Tedjasaputra V, **van Diepen S**, **Collins SE**, **Michaelchuk WW**, **Stickland MK**. Assessment of pulmonary capillary blood volume, membrane diffusing capacity, and intrapulmonary arteriovenous anastomoses during exercise. *J Vis Exp* 120, 2017.

Theodore J, **Robin ED**, **Morris AJ**, **Burke CM**, **Jamieson SW**, **Van Kessel A**, **Stinson EB**,

Shumway NE. Augmented ventilatory response to exercise in pulmonary hypertension. *Chest* 89: 39–44, 1986.

Tobin CE. Arteriovenous shunts in the peripheral pulmonary circulation in the human lung. *Thorax* 21: 197–204, 1966.

Tolle JJ, **Waxman AB**, **Van Horn TL**, **Pappagianopoulos PP**, **Systrom DM**. Exercise-induced pulmonary arterial hypertension. *Circulation* 118: 2183–2189, 2008.

Turino GM, **Bergofsky EH**, **Goldring RM**. Effect of exercise on pulmonary diffusing capacity. *J Appl Physiol* 18: 447–456, 1963.

Vaughan TR, **DeMarino EM**, **Staub NC**. Indicator dilution lung water and capillary blood volume in prolonged heavy exercise in normal men. *Am Rev Respir Dis* 113: 757–762, 1976.

Viegi G, **Paoletti P**, **Carrozzi L**, **Baldacci S**, **Modena P**, **Pedreschi M**, **Di Pede F**, **Mammini U**, **Giuntini C**. CO diffusing capacity in a general population sample: relationships with cigarette smoking and airflow obstruction. *Respiration* 60: 155–161, 1993.

Vogiatzis I, **Zakynthinos S**, **Boushel R**, **Athanasopoulos D**, **Guenette JA**, **Wagner H**, **Roussos C**, **Wagner PD**. The contribution of intrapulmonary shunts to the alveolar to arterial oxygen difference during exercise is very small. *J Physiol* (2008). doi: 10.1113/jphysiol.2007.150128.

Wagner PD, **Gale GE**, **Moon RE**, **Torre-Bueno JR**, **Stolp BW**, **Saltzman HA**. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260–270, 1986.

Wasserman K, **Hansen JE**, **Sue DY**, **Casaburi R**, **Whipp BJ**. *Principles of Exericse Testing and Interpretation: Including Pathophysiology and Clinical Applications*. 3rd ed. Balitmore, MD: Lipincott Williams & Wilkins, 1999.

Watson P, **Hasegawa H**, **Roelands B**, **Piacentini MF**, **Looverie R**, **Meeusen R**. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *J Physiol* 565: 873–83, 2005.

West JB. *Respiratory physiology : The essentials.* 9th ed. Baltimore, MD: Philadelphia : Wolters Kluwer Health/Lippincott Williams & Wilkins, c2012., 2012.

Yock PG, **Popp RL**. Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. *Circulation* 70: 657–662, 1984.

Zavorsky GS. Evidence of pulmonary oedema triggered by exercise in healthy humans and detected with various imaging techniques. *Acta Physiol.* 189: 305–317, 2007.

Appendix A: Supplemental Figures

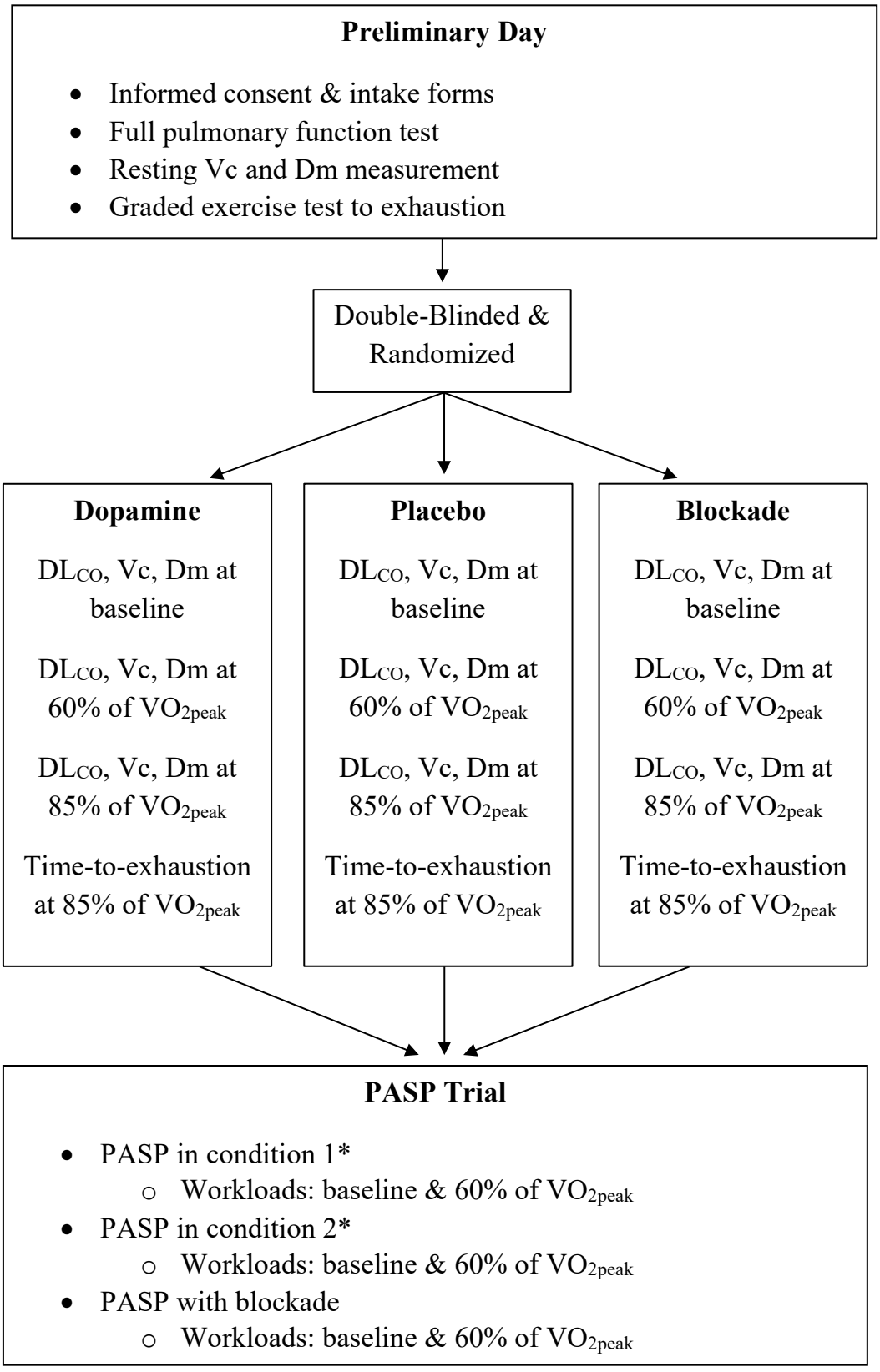

Figure 3. Research design schematic

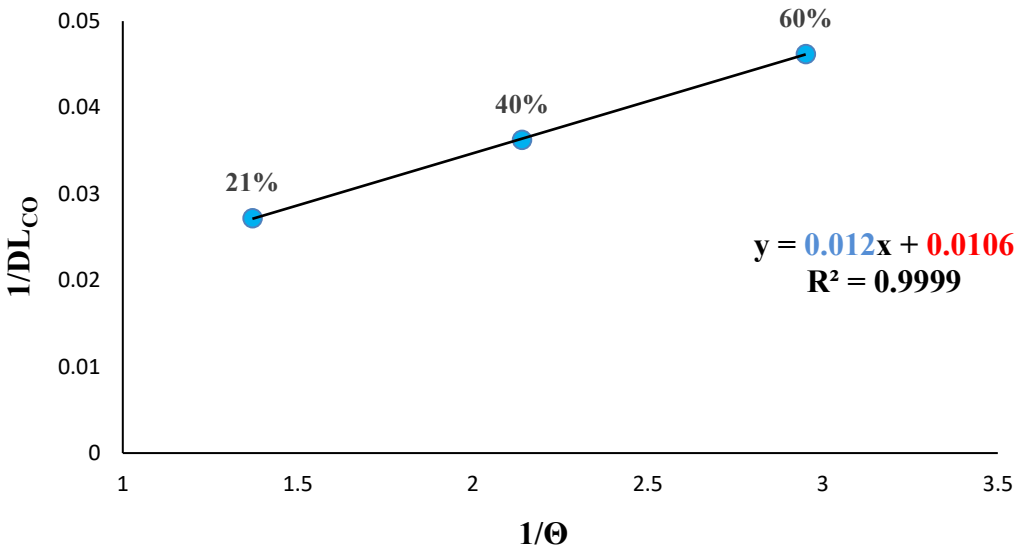

Figure 4. Graphical representation of 1/Θ versus $1/DL_{CO}$ at various O_2 tensions. The slope of the line (highlighted in blue) represents 1/Vc, whereas the y-intercept (highlighted in red) represents 1/Dm. The inverse of these values will give Vc and Dm. Note: The minimum acceptable r^2 value is 0.95.

Appendix B: Calculations

(1) $$DLCO = (VA/(\frac{t}{60} x\ (Pb - PH_2O))xln(\frac{FA_{TR}x\ FI_{CO}}{FI_{TR}xFA_{CO}})$$

Where DL_{CO} is the diffusing capacity for carbon monoxide, VA is alveolar volume in STPD, t is time in seconds, Pb is barometric pressure, PH_2O is water vapor pressure, ln is natural logarithm, FA_{TR} is fraction of alveolar tracer gas, FI_{CO} is fraction of inspired carbon monoxide, FI_{TR} is fraction of inspired tracer gas, and FA_{CO} is fraction of alveolar carbon monoxide. From MacIntyre et al. (2005).

(2) $$\frac{1}{DLCO} = \frac{1}{Dm} + \frac{1}{\theta Vc}$$

DL_{CO} is the diffusing capacity for carbon monoxide, Dm is the membrane diffusing capacity, Vc is the volume of blood in the pulmonary capillaries, and Θ is the rate of reaction between carbon monoxide and hemoglobin. From Roughton & Forster (1957).

(3) $$PVR = \frac{Ppa}{Q}$$

Where PVR is the resistance within the pulmonary vasculature, Ppa is the mean pulmonary arterial pressure, and Q is pulmonary blood flow. From Naeije & Chesler (2012).

(4) $$\frac{1}{\theta} = 0.0055 * PAO2 + 0.73$$

Where Θ is the rate of reaction between carbon monoxide and hemoglobin and P_AO_2 is the partial pressure of oxygen in the alveoli. From Roughton & Forster (1957).

(5) $$DLCO_{adj} = DLCO\ x\ \frac{10.22+[Hb]}{1.7+[Hb]}$$

Where DLCO is the diffusing capacity for carbon monoxide and [Hb] is the concentration of hemoglobin in the venous blood in g/deciliter of blood. Θ is the rate of reaction between carbon monoxide and hemoglobin and P_AO_2 is the partial pressure of oxygen in the alveoli. From Marrades et al. (1997).

Appendix C: Cardiorespiratory Fitness Data

Table 7. Hi-fit versus lo-fit subject characteristics

	Lo-Fit			Hi-Fit		
N	8			6		
Male/Female	4/4			4/2		
Age, years	26.5	±	3.2	28.7	±	6.9
Height, m	1.74	±	0.07	1.78	±	0.06
Mass, kg	72.1	±	11.2	78.7	±	14.5
Body Mass Index, $kg \cdot m^2$	23.7	±	2.3	24.7	±	3.0
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	41.5	±	3.2	51.7	±	5.0

Values are expressed as mean ± SD. Hi-Fit = high cardiorespiratory fitness; Lo-Fit = low cardiorespiratory fitness; VO_{2peak} = peak O_2 consumption.

Table 8. Lo-fit pulmonary diffusion response to exercise

		Baseline			60% of VO_{2peak}			85% of VO_{2peak}		
DL_{CO},	PLA	33.4	±	3.7	49.6	±	5.5	54.4	±	6.7
$mL \cdot min^{-1}$	DA	33.2	±	4.7	47.9	±	6.3	53.0	±	4.9
$\cdot mmHg^{-1}$	BK	33.6	±	4.5	47.3	±	7.4	54.0	±	8.0
Vc,	PLA	90.7	±	24.7	128.6	±	22.0	123.4	±	29.4
mL	DA	102.4	±	22.7	117.1	±	22.1	126.4	±	17.7
	BK	84.3	±	19.4	110.8	±	18.7	133.2	±	25.0
Dm,	PLA	79.4	±	31.4	108.3	±	24.8	147.6	±	41.8
$mL \cdot min^{-1}$	DA	60.9	±	10.8	108.0	±	26.4	138.4	±	40.3
$\cdot mmHg^{-1}$	BK	84.9	±	31.0	117.4	±	29.8	127.8	±	25.5

Values are expressed as mean ± SD. DL_{CO} = diffusing capacity for carbon monoxide; Vc = pulmonary capillary blood volume; Dm = diffusing membrane capacity; Lo-Fit = low cardiorespiratory fitness; VO_{2peak} = peak O_2 consumption.

Table 9. Hi-fit pulmonary diffusion response to exercise

		Baseline			60% of VO_{2peak}			85% of VO_{2peak}		
DL_{CO},	PLA	39.4	±	8.4	57.3	±	13.4	63.8	±	14.8
$mL \cdot min^{-1}$	DA	42.4	±	9.2	59.7	±	14.7	65.3	±	15.0
$\cdot mmHg^{-1}$	BK	40.5	±	10.0	58.4	±	14.7	64.2	±	16.8
Vc,	PLA	93.0	±	20.1	138.5	±	25.6	163.8	±	48.5
mL	DA	120.1	±	27.0	164.9	±	55.0	186.3	±	72.4
	BK	123.1	±	32.8	146.1	±	31.3	158.4	±	54.5
Dm,	PLA	100.7	±	29.6	131.9	±	47.8	143.6	±	44.0
$mL \cdot min^{-1}$	DA	86.7	±	34.3	115.8	±	18.9	129.5	±	40.5
$\cdot mmHg^{-1}$	BK	80.5	±	28.1	126.6	±	41.3	164.3	±	83.8

Values are expressed as mean ± SD. DL_{CO} = diffusing capacity for carbon monoxide; Vc = pulmonary capillary blood volume; Dm = diffusing membrane capacity. Hi-Fit = high cardiorespiratory fitness; VO_{2peak} = peak O_2 consumption.

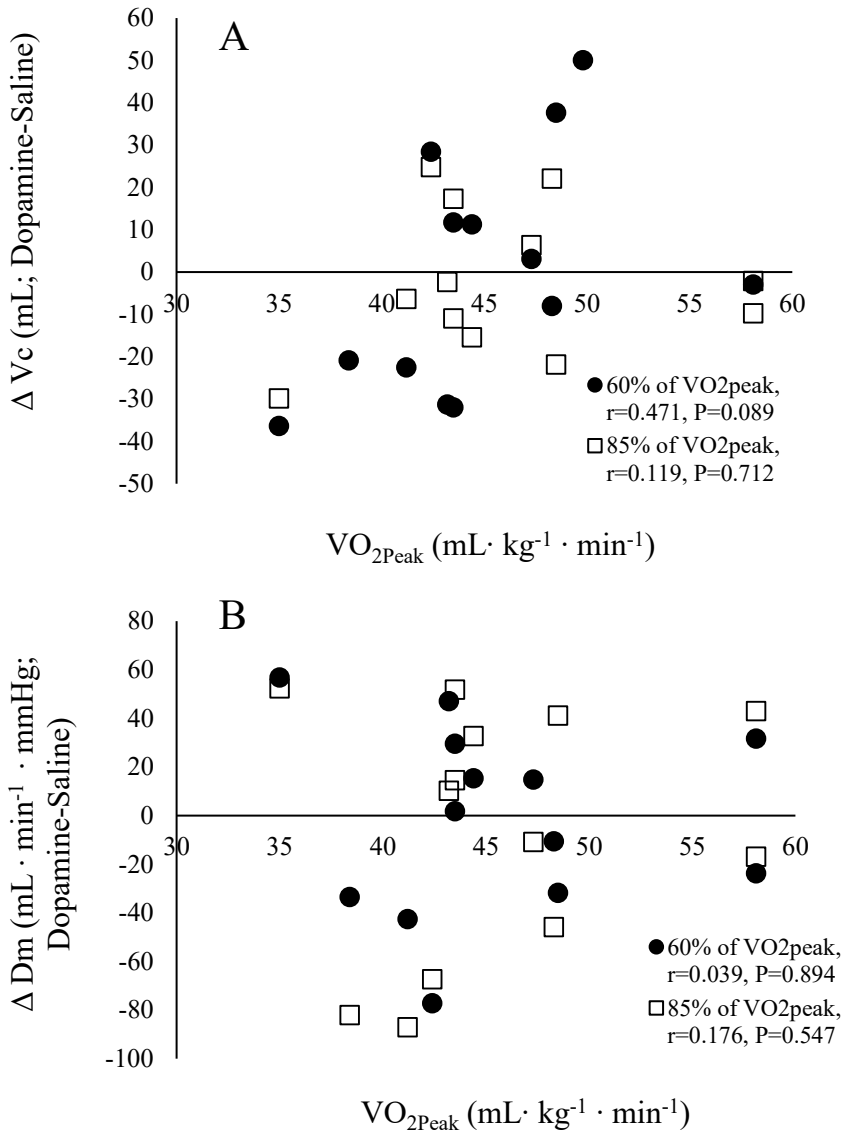

Figure 5. A. Correlation between cardiorespiratory fitness and the change in Vc from the placebo to the dopamine condition during exercise at 60% and 85% of VO_{2peak}. B. Correlation between cardiorespiratory fitness and the change in Dm from the placebo to the dopamine condition during exercise at 60% and 85% of VO_{2peak}.