THE EFFECT OF ALTERED OXYGEN TENSIONS AND EXERCISE ON FLOW-MEDIATED DILATION

by

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ABSTRACT

Breathing hypoxia increases vasodilation during exercise, whereas breathing hyperoxia is known to increase vascular resistance. However, little is known about the effect of altered oxygen tensions on endothelial function, particularly following an acute bout of exercise in trained individuals. The purpose of our study was to assess the effects of constant load cycling and different levels of inspired oxygen (F_IO_2) on endothelial function measured via flow-mediated dilation (FMD) in healthy trained men. It was hypothesized that exercising while breathing normoxic or hypoxic gas would improve FMD, whereas hyperoxic gas would not alter FMD. The study used a randomized crossover design. Thirteen healthy, recreationally active males (22±3 yrs) volunteered to participate. Subjects completed three graded exercise tests breathing either 16% O_2 (HYPO), 21% O_2 (NOX) or 100% O_2 (HYPER) to determine gas-specific maximal workload (W_{max}). Subjects then performed three, 40-minute, constant-load cycling trials at 50% of the gas specific W_{max}. Baseline FMD was measured during rest after 30 minutes of gas exposure and 30 minutes following each exercise trial, while breathing the experimental gas. No differences in baseline diameter, shear rate, time-to-peak dilation or FMD were found at rest or after exercise with any gas. Our data indicates that alterations in F_IO_2 with and without exercise have no affect on vascular measures in young trained males. Exercise could have been more powerful on influencing the vasculature than changes in F_IO_2. Training status and associated vascular remodeling and oxidant production could also account for the lack of change observed with FMD after exercise.

PREFACE

This study was approved by the University of British Columbia Clinical Research Ethics Board (ID: H10-02378).

Chapter 2 is based on data collected and analyzed in the Exercise Physiology Laboratory in the School of Health and Exercise Sciences at the University of British Columbia Okanagan campus by Lisa Wong. Dr. Neil Eves and Lisa Wong were responsible for the study concept and Dr. Philip Ainslie, Kurt Smith, Graeme Koelwyn and Jonathan Smirl additionally contributed to the study design. Drs. Neil Eves and Philip Ainslie were responsible for technical assistance, equipment acquisition, and funding of the study. Lisa Wong and Kurt Smith were responsible for subject recruitment and data collection. Assistance during data collection was provided by Kurt Smith, Graeme Koelwyn, Jonathan Smirl, Thomas Cameron, Rochelle Tonkin and Jordan Cheyne. Lisa Wong was responsible for all data collected, analysis of data and writing of this thesis. This thesis was edited by Dr. Neil Eves.

No portion of this thesis has been published to date.

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LIST OF ABBREVIATIONS

Ach	Acetylcholine
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BH_4	Tetrahydrobiopterin
Ca^{2+}	Calcium
CLT	Constant load exercise trial
cyclic GMP	Cyclic guanosine monophosphate
EDHF	Endothelial derived hyperpolarizing factor
EDRF	Endothelium derived relaxing factor
eNOS	Endothelial nitric oxide synthase
FBF	Forearm blood flow
F_IO_2	Fraction of inspired oxygen
FVR	Forarem vascular resistance
FMD	Flow mediated dilation
GTP	Guanosine triphosphate
GXT	Graded exercise test
Hb	Hemoglobin
HYPER	Hyperoxia (100% oxygen)
HYPO	Hypoxia (16% oxygen)
iNOS	Inducible nitric oxide synthase
IL – 6	Interleukin 6
mRNA	Messenger ribonucleic acid
NMD	Nitroglycerin mediated dilation
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOX	Normoxia (21% oxygen)
PO_2	Partial pressure of oxygen
P2γ receptor	Purinergic 2 gamma
PGI_2	Prostacyclin
R hemoglobin	Relaxed state of hemoglobin
ROI	Region of interest
ROS	Reactive oxygen species

TTP	Time to peak dilation		substances
S_aO_2	Percent saturation of oxygen	*T hemoglobin*	Tense state of hemoglobin
		T_xA_2	Thromboxane
SR_{avg}	Average shear rate	*TNFα*	Tumor necrosis factor alpha
$SRAUC_{ttp}$	Shear rate area under the curve until peak dilation	*W*	Watts
		Wmax	Workrate maximum
TBARS	Thiobarbituric acid reactive		

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CHAPTER 1

INTRODUCTION

The structure and function of the arteries and veins differ depending on their location along the arterial tree. The aorta, pulmonary and carotid arteries, known as conducting arteries, are the most elastic which allow for the changes in blood flow that occur with each ventricular contraction [1]. From the conducting arteries, conduit vessels such as the brachial and femoral arteries branch off, allowing for blood to be directed to specific areas within the body. The resistance arteries branch off from these conduit arteries and perfuse the working tissue with adequate blood. Because resistance arteries are mostly composed of smooth muscle and are primarily sympathetically innervated [2], they can respond quickly to change in sympathetic nerve activity, ensuring that the oxygen delivery is tightly matched to oxygen demand [1].

Although the vasculature can vary greatly depending on its location and organ, every vessel from the conducting arteries to the arterioles share common anatomy. There are three main regions within the vessel wall, each containing varying levels of smooth muscle cells and elastin [3]. The outermost layer is the tunica adventitia, which is primarily composed of fibro-elastic connective tissue. In the middle is the tunica media, which consists of smooth muscle cells and elastin fibers. The tunica intima is the innermost layer and contains fibro-elastic tissue layer beneath a single layer of endothelial cells which line the intima and are directly in contact with the blood [3]. Because the endothelium acts as a ”barrier” between the blood and vessel tissue, interacting with a number of vasoactive factors, it plays an active and vital role in local vascular homeostasis which can impact overall cardiovascular health [1].

1.1 Vascular Endothelium

The vascular endothelium is a semi-permeable single layer of cells that exert significant autocrine, paracrine and endocrine functions [4]. It is the largest of such organs in the body as it lines every vessel of the vascular tree and is estimated to cover 5000m^2, weighing approximately 1% of total body mass [5] and acts as the barrier and facilitator between the circulating blood and vessel wall. To maintain hemostasis, it allows for the passage of cells and nutrients to and from the blood and underlying cells, but it also responds and releases several vasoactive and thromboregulatory substances that influence the health and function of the cardiovascular system[1]. When the endothelium is functioning properly, it is anti-atherogenic, anti-inflammatory and appropriately regulates vascular tone and blood flow [6]. The release of nitric oxide (NO) and subsequent vasodilatation can also interfere with leukocyte adhesion and subsequent plaque formation [6]. NO inhibits the proliferaton of vascular smooth muscle from exposure to platelet-derived growth factors and fibrous plaque formation [7]. Therefore, the ability of the endothelium to react and produce NO and other vasoregulatory substances promotes the structural and functional health of the vasculature.

Sympathetic nerve activity plays a major role in maintaining basal vascular tone but as tissue demands change, local factors can act on the active tissue bed, directing blood to where it is needed. Several local factors that play an important role in vascular function are mechanical (such as from stretch or shear stress that can come from alterations in blood volume), chemical (such as changes in partial pressure of oxygen, pH, adrenaline) or those that are derived from the endothelium (such as NO, prostacyclins, endothelium derived hyperpolarizing factor (EDHF) and ET-1 etc)[8]. For the purpose of this review, only the key elements that are pertinent to this study will be highlighted. However, it should

acknowledged that there are many other factors that play an important role in controlling the dilation and constriction of the vasculature.

Endothelial derived hyperpolarizing factor (EDHF) is an unknown substance which hyperpolarizes smooth muscle tissue via intracellular calcuim (Ca^{2+}) mediated potassium channels and is stimulated by agonists such as bradykinin, acetylcholine and substance P [8, 9]. Although NO and prostacyclin (PGI_2) also hyperpolarize the vascular smooth muscle, when both are blocked, vasodilatation can still occur, suggesting another mechanism, such as EDHF, play a role [10].

The primary mechanism by which the endothelium induces smooth muscle relaxation around the vessels comes from NO. NO was first identified by Furchgott and Zawadzki [11]. It is derived from L-arginine and the reaction is catalyzed by the enzyme nitric oxide synthase (NOS) [1]. NOS can exist in three isoforms; neuronal NOS (nNOS), produces the NO for release at the synaptic cleft as a neurotransmitter, inducible NOS (iNOS), which is expressed in cells exposed to inflammation and endothelial NOS (eNOS) which produces NO endothelial cells of the blood vessel [12]. eNOS is bound to caveolin, a protein found in the caveolae which are invaginations within in the cell membrane [13].

1.1.1 Endothelial Nitric Oxide Synthase

The initial stimulation of eNOS occurs from agonists such as bradykinin, acetylcholine, adenosine triphosphate (ATP), adenosine diphosphate (ADP) or substance P [14], which causes calcium (Ca^{2+}) to be released from the endoplasmic reticulum and a rise in intracellular Ca^{2+}. This increase in intracellular Ca^{2+} activates eNOS and dissociates it from calveolin [15]. As the intracellular Ca^{2+} becomes depleted, extracellular Ca^{2+} is moved

into the cell and attaches to calmodulin. This calmodulin-Ca^{2+} complex then binds to the activated eNOS which converts L-arginine to NO [16]. Once NO has been released, it diffuses across the endothelial layer and into the smooth muscle. There, NO binds with the iron atom of the heme prosthetic group on soluble guanylyl cyclase which catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) [17]. This causes smooth muscle relaxation by reducing Ca^{2+} release in the smooth muscle cell and stimulated Ca^{2+} reuptake by the sarcoplasmic reticulum [18]. When Ca^{2+} stores are depleted, the process reverses itself and eNOS becomes inactivated [19].

Mechanical strain, such as wall tension or shear stress on the endothelial cells and related structures can stimulate NO release [20]. Wall tension or cyclical strain describes wall distension, which is the relation of transmural pressure gradient and the radius of the vessel. The greater the vessel dilates, the greater the tension that is exerted on the cell cytoskeleton and cell matrix adhesion molecules [21]. Shear stress results from the friction of blood flowing against the apical surface of the cell. The amount of tension exerted on the vascular wall is dependent on the rate of flow, viscosity of the blood and physical dimensions of the vessel [21]. Whereas cyclical tension is wall stretch in every direction, shear stress occurs in one direction only. Endothelial cells are primarily stimulated by shear stress, whereas the surrounding smooth muscle cells are deformed via transmural pressure [21].

The exact mechanism by which mechanical strain stimulates eNOS is not fully understood. It is believed that physical forces cause a mechanical-chemical coupling effect, where there structural elements are stimulated that can elicit chemical signals [22]. Specifically, integrins are thought to play a major role as they adhere cells to their substratum and are involved with cell signalling cascades [21]. It is also thought that stretch-sensitive

Ca^{2+} may also be involved. The rapid response of endothelial cells to the onset of shear stress results from the stimulation of these stretch-sensitive Ca^{2+} channels, which cause an increase of Ca^{2+} and stimulate the inwardly rectifying K+ channels [23]. The smooth muscle cell then becomes hyperpolarized and induces vasorelaxation. Longer durations of shear stress can also cause phosphorylation of eNOS via protein kinases [24]. Over time the chronic stimulation of eNOS will cause upregulation by enhancing eNOS gene transcription and stabilizing eNOS mRNA [25], increasing NO production and enhancing the vasodilatory response to shear stress.

1.1.2 Effect of Oxygen Saturation on Vascular Function.

The level of blood oxygenation due to decreased hemoglobin saturation has been shown to be a powerful stimulus for NO induced-changes in vascular diameter [26]. This may occur in situations of limited oxygen availability, prolonged hypoxia or ischemia and can be exacerbated during exercise in these conditions, due to increased metabolic utilization. This is supported by the greater role NO has in mediating vasodilation in working muscles during hypoxia compared to exercise in normoxia [27]. The allosteric change from relaxed (R) hemoglobin (oxygenated hemoglobin) to tense (T) hemoglobin (where there is low oxygen affinity, characterizing deoxygenated hemoglobin) is believed to be the primary sensor that promotes the production of NO during this condition [28].

One vasodilatory regulator is the release of ATP as oxygen is unloaded from hemoglobin [29]. The release of ATP is proportional to the decrease in hemoglobin saturation [30]. When ATP is released, it binds to the $P2_Y$ (purinergic) receptors, which activates vasodilatory substrate production (such as NO) and causes smooth muscle

relaxation [31]. ATP can also be released by mechanical deformation of red blood cells as they are squeezed through narrower vessels, activating G protein and adenylyl cyclase, which is a player in the vasodilatory process [32].

While breathing air, nitrite stored in the erythrocytes, plasma and tissue help produce NO [33]. In hypoxic conditions, the L-arginine/eNOS pathway is perturbed, the plasma nitrite is transported to red blood cells, converted to NO, with deoxygenated hemoglobin acting as the reductase and then released to induce vasodilation [34]. This acts as a functional mechanism that matches blood flow to the degree of hemoglobin deoxygenation [35], and typically begins at an arterial PO_2 of around 30mmHg [36]. Although nitrite can react with both oxygenated and deoxygenated hemoglobin, only deoxygenated hemoglobin will produce NO, due to its lower redox potential [35]. This "push-pull" mechanism allows for NO to be adequately supplied as oxygen levels change [37], using stored nitrites as a readily available source for NO production [35]. With further reductions in arterial partial pressure of oxygen (PO_2), NO can be produced through electron donation from other pathways such as xanthine oxidoreductase [38], cytochrome P450s, and substrates of the electron transport chain [38]. As well, during exercise myoglobin has been shown to act as a reductase similarily to hemoglobin in skeletal and cardiac muscle, however its reaction is much more rapid and could potentially play a more active role during exercise, when oxygen demands increase [39]. The role of myoglobin could be even greater during exercise in hypoxic conditions with increased oxygen demand.

Although the effects of hypoxia on NO production have been studied [27, 34, 40, 41], it is not well known how hyperoxia may affect NO function and arterial response to local stimuli such as shear stress, particularly during exercise. In situations of elevations in P_aO_2, it

has been demonstrated that hyperoxia leads to an increase in vascular resistance and vasoconstriction of arterioles and capillaries [42], likely due to an increase in reactive oxygen species (ROS) [43]. ROS are formed from an oxygen molecule with an unpaired electron, which can react with proteins and lipids and interrupt cellular pathways [44]. When antioxidant defenses are inadequate, this imbalance in oxidant production is known as oxidative stress [44]. It has been proposed that the increase in vascular resistance seen in hyperoxia is from increased oxidant production and oxidative stress. When individuals are exposed to supraphysiological levels of oxygen, forearm vascular resistance increases. However when the subjects are given vitamin C supplementation (a known antioxidant), the vascular constriction was reversed, suggesting that elevated oxidant production was responsible [45], even though only alcohol and caffeine consumption was controlled for. Furthermore, it has been reported that supraphysiological levels of oxygen caused an increase in mitochondrial superoxide production, with a concomitant rise in peroxynitrite formation. Superoxide is highly reactive with NO [46] and creates peroxynitrite, a highly cytotoxic compound. Cellular respiration was supressed, interrupting endothelial cell functioning and diminishing the vasodilatory capacity of the vessel [47, 48]. Thus the elevated oxidant production from hyperoxic exposure is likely responsible for the diminished vasodilatory ability of the vessel.

In low amounts, ROS acts as intracellular messengers and mediate normal cell functioning. However, when large amounts are present, oxidative stress can affect surrounding molecular structures and also impair endothelial function and vascular response. Reaction of ROS with tetrahydrobiopterin (BH_4), a cofactor required for eNOS functioning [49], causes eNOS activity to be reduced, compromising NO production and subsequently

reducing endothelial dependent vasodilation [50]. This can also result in eNOS uncoupling and promote further production of superoxide by eNOS [51], as demonstrated by intracellular supplementation of BH_4. Not only has ROS production been shown to be augmented in hyperoxia [52], but it is also elevated in hypoxic situations [53], including during exercise at high altitude, even in individuals with high aerobic capacity and augmented antioxidant capacity [54]. It is possible that this disruption in the oxidant/anti oxidant balance could alter vascular functioning through NO sequestering and eNOS uncoupling, which may be detectable through flow-mediated dilation, a measure of endothelial function. Acute exercise induces hyperemia [55] and it is possible that any detriments from hyperoxic or hypoxic exposure on vascular function can be negated by the vasodilatory substances released through exercise. However, the effects of exercise in these conditions on endothelial response, as measured by flow-medated dilation, has not been studied.

1.2 Measurement of Vascular Function

Assessment of vascular function is a useful prognostic technique to evaluate the risk of future cardiac events [56]. There are several invasive and non-invasive methods that can be applied to give an overall impression of vascular functioning. Although non-invasive measures are easier to administer, proper technique and analysis must be ensured in order for results to be valid and reliable [57]. However, if done properly, these tools can be very useful in clinical and laboratory settings to provide an index of endothelial function[58]. Several methods have been developed to evaluate arterial stiffness and endothelial function. To assess structural health and arterial elasticity, methods such as pulse wave velocity,

augmentation index or aortic distensibility are used to determine arterial stiffness, with central arterial stiffness correlated to future cardiac events [59, 60].

Where arterial stiffness assesses the structural alterations in the vascular tree, endothelial function can give us an index of vascular functioning before remodelling occurs [61]. The gold standard of endothelial function assessment is done in the coronary arteries using an endothelium dependent stimulus such as acetylcholine and measuring the response via coronary angiography and intravascular ultrasound in order to directly measure coronary diameter and blood flow [62]. Although less invasive methods have been developed, such as assessing the affect of vasoactive substances on change in the brachial artery blood flow [63], the infusion of endothelial dependent substances is still not an ideal diagnostic tool as it does not necessarily assess the responses to normal physiological stimuli.

To assess the endothelial dependent vasodilatory response, a non-invasive method known as flow-mediated dilation (FMD) is commonly used to assess vascular health and predict cardiovascular risk [58]. First published by Celermajer et al (1992) [64], brachial flow-mediated dilation (FMD), is a popular experimental tool which measures the NO mediated response of the endothelium to shear stress on the vascular wall of the brachial artery. It has been correlated to coronary endothelial function [65] and has been shown to be primarily NO mediated [66] as long as certain procedures are followed [57]. It is an attractive technique as it is non-invasive and can assess the effect of a physiological stimulus (shear stress) on the vasculature, without administering an exogenous agonist. Briefly, after a period of zero blood flow initiated by cuff occlusion, the cuff is released and the response of the blood flow and diameter of the brachial artery is recorded [67]. The vessel dilates in response to the shear stress induced by the rapid influx of blood and the release of NO acting upon the

smooth muscle around the artery. Attenuated responses in vessel diameter have been shown to be a predictor of future cardiac events [68], independent of other cardiovascular risk factors, such as smoking [69] and diabetes [70]. This technique is particularly attractive as it has a relatively simple protocol. However, there are many technical aspects that must be considered and high level of skill required in order for valid and reliable measurements to be made [57, 71].

1.3 Exercise Hyperemia

The mechanisms responsible for increased blood flow at the onset of exercise and the maintenance of increased flow during sustained exercise are still not fully understood. Many neural, metabolic, and mechanical mechanisms [72] have been proposed. Evidence indicates that it is the coordinated actions of these neural, metabolic, and mechanical pathways that are responsible for the increased flow [73]. Attempts at trying to isolate the primary cause of hyperemia have given negative results, likely due to the number of redundant pathways involved in this critical physiological process. ACh spill-over [74] from active nerve endings, NO, oxygen levels in the blood and adenosine among others have been suggested as potential stimulators of vasodilatation during exercise [55] however it is only when multiple pathways are blocked can a reduction in blood flow be seen [75].

Adenosine and ATP are considered vasodilators during hypoxic exposure and exercise because of their involvement with muscle contraction. Adenosine concentrations have been strongly correlated (r=.98) to leg blood flow [76] and increase with exercise [77] although it does not seem to be obligatory in exercise hyperemia [55]. ATP has been well recognized as a potent endothelium-dependent vasodilator, exerting its action by activating

P2γ purinergic receptors on the endothelium and stimulating the release of endothelial vasodilators such as NO [31]. ATP sources during exercise could originate from working skeletal muscle, endothelial cells, sympathetic nerve terminals or mechanical deformation of red blood cells [55, 78]. ATP is also released from hemoglobin during deoxygenation [79], its release from endothelial cells stimulated during hypoxia [80]. This release of ATP is augmented during exercise in hypoxic conditions; however, during hyperoxia, or when carbon monoxide is present and hemoglobin is unable to go through a conformational change, ATP release is attenuated [81]. After acute hyperoxic exercise, it is possible that ATP release will continue to be attenuated, as hemoglobin is still unable to make a conformational change, blunting the release of other vasodilatory agonists and diminishing the capacity of the vessel to dilate. It is also possible that the exercise stimulus can negate the blunting effects of hyperoxia by increasing shear stress and stimulating NO release through another mechanism, improving the vascular response during exercise recovery.

Prostanoids such as prostaglandin (PGI_2) and thromboxane (T_xA_2) work synergistically to control vasodilation. They are formed from arachadonic acid and are stimulated similarly to NO through increases in Ca^{2+} concentration and shear stress. It appears that prostanoids have a synergistic effect with NO in the hyperemic response to exercise, further supporting redundancy in mechanisms of vascular control. When prostaglandins are locally blocked, there is a transient reduction in local blood flow during exercise [82] whereas when NOS and prostaglandins are both inhibited, blood flow is greatly reduced [83]. During hypoxia, where there is an augmented blood flow response to exercise compared to normoxia, a similar relationship exists; NO is the primary vasodilator and prostanoids play an important, but not essential role in the vasodilatation seen in this

condition [84]. However, it is not known whether the increase in blood flow during hypoxic exercise affects vascular reactivity and whether the vessel remains more sensitive to a shear stress stimulus following the exercise.

Although NO has shown to be more dominant in blood flow control during rest and recovery [85, 86], it does play a role in the hyperemic effect of exercise, particularly in propagating vasodilation to upstream vessels [87]. The release of NO during activity can come from mechanical stimulation and shear stress from increased blood flow, as mentioned above, as well as increases in intracellular Ca^{2+} resulting from muscle contractions [88]. The binding of agonists such as bradykinin, acetylcholine and ATP, which are released during muscle contractions also act on eNOS to produce NO [88]; however, whether NO release would be attenuated or augmented with exercise in hypoxia or hyperoxia is still not yet known.

Although the aforementioned substances such as NO and prostaglandins are all involved with the hyperemic response to exercise, many of their acute post exercise effects on endothelial function have not been studied. Because altered P_aO_2 can change the vasodilatory response during exercise, it is not known whether the post-exercise effects on the endothelium will be altered as well. This is particularly relevant in athletic and clinical situations where hypoxia is often administered to elicit a training effect. For example, when athletes are exposed to hypoxia to improve aerobic fitness by improving the capacity to delivery oxygen. Conversely, hyperoxia is delivered in clinical situations when patients experience hypoxemia and require supplemental oxygen to meet metabolic needs.

1.4 Exercise and the Endothelium

Physical activity accounts for approximately 40% of the risk reduction for cardiovascular disease, independent of other traditional risk factors [89]. Specifically, exercise training has been shown to improve endothelial function before improvements in other major risk factors (e.g., plasma lipids, blood pressure, blood glucose, waist–to–hip ratio and body mass index) [61]. The endothelium and its effects on vascular functioning, remodelling and health may be the primary site where exercise exerts its effects on cardiovascular fitness. It has been shown that even an acute bout of exercise can improve plasma leves of NO release in healthy individuals [90]. However, in highly trained individuals, the response may be altered due to the high training status and vascular adaptations to repetitive shear stress [91]. Over time as one continues to train, these shear stimuli can induce vascular changes primarily through NO bioavailability, activity and ultimately vascular remodelling and adaptation [91-93]. However, in conditions where the vascular milieu is altered (such as high or low oxygen states), it is unclear whether an acute bout of exercise will alter the responsiveness of the vessel.

Intensity of exercise has also been shown to affect the NO response to acute exercise. In a study by Goto et al (2007), 8 young healthy males performed three randomized cycling exercise bouts at 25%, 50% and 75% of maximal oxygen uptake (VO_{2max}) and had measurements of forearm blood flow (FBF), 8-isopostane (a measure of oxidative stress) and plasma norepinephrine taken [94]. Elevated forearm blood flow after moderate (50% VO_{2max}) intensity cycling exercise was determined to be primarily NO-mediated, as assessed from forearm blood flow changes after inhibition of NOS via N^G-monomethyl-L-arginin. Moreover, since there was no concomitant rise in 8-isoprostane or norepinephrine, the

elevated blood flow response could have been from attenuated NO degradation or increased NO production or potentially other chemical or hormonal vasodilatory stimuli that are released during exercise. Conversely, high-intensity (75% $\dot{V}O_{2max}$) cycling exercise increased 8-isoprostane and norepinephrine, unfortunately, no measurements of FBF were completed post-exercise in this condition. It is likely that such increases in oxidative stress, as gauged by changes in 8-isoprostane, would decrease the FBF unless there was a large increase in antioxidant status to counteract the oxidant production. However, 8-isoprostane may not be the best marker of ROS and other factors such as increased sympathetic activity or the release of other inflammatory or oxidants could account for the lack of change observed. The mild–intensity (25% $\dot{V}O_{2max}$) exercise bout did not result in any changes in FBF, forearm vascular resistance (FVR), 8-isoprostane and norepinephrine. This was likely due to the insufficient shear stimuli elicited by such a low intensity of exercise.

The greatest change observed in FMD after moderate exercise observed in the previous study was also supported by a more recent study by Johnson et al [95] where healthy trained men ($\dot{V}O_{2peak}$ 64.0mL kg^{-1} min^{-1}) performed running exercise at a high (80% $\dot{V}O_{2peak}$) or moderate (50% $\dot{V}O_{2peak}$) intensity, for either a short (30 minutes) or long (60 minutes) duration, respectively in order to match work output. Vascular function was assessed using FMD, while oxidative stress was measured via thiobarbituric acid reactive substances (TBARS). High intensity medium duration exercise resulted in the highest TBARS concentration (1.6 µmol/L increase from baseline) and lowest FMD response (~ 5%) immediately post exercise. However, there was an elevated response of FMD (~7%) one hour post exercise that was not seen in the other exercise trials. This period when FMD is decreased immediately after exercise has been demonstrated in other studies that have

examined the influence of high intensity exercise on FMD in trained individuals [96, 97]. It is possible that the lack of FMD response during this period was due to increased oxidative stress induced by the high intensity exercise, diminishing the NO availability for vasodilation. It is also possible that vasoconstricting factors such as endothelin-1 or incresaed sympathetic activity from high intensity exercise could have been more dominate during this time, overriding any vasodilatory affects of exercise and diminishing the FMD response.

In highly trained individuals [97] ($\dot{V}O_{2max}$ 75.9±0.8 ml^{-1} $kg\text{-}^{1}$ min^{-1}), this detriment in FMD as reported elsewhere [95] was also demonstrated after high intensity interval exercise. Both sedentary ($\dot{V}O_{2max}$ 47.7±1.7 ml^{-1} $kg\text{-}^{1}$ min^{-1}) and highly trained individuals completed a high intensity exercise bout of 5x5 minutes at 90% of HR_{max} [97]. The highly trained group had 38% lower FMD after exercise despite an increase in NO activity, measured via assay by measuring the conversion of nitrate to nitrite via nitrate reductase to give total plasma nitrite concentration. It is interesting to note that the elevations in NO remained above 80% from baseline, even after 48 hrs from exercise, where as the sedentary control group levels of NO decreased to baseline levels after 24 hrs. The continual increase in NO may be from upregulation of eNOS protein from exercise [98] and continual phosphorylation of eNOS. The latter may have been enhanced in these highly active individuals, who had already undergone vascular remodelling. The authors suggested that the apparent "detriment" in FMD after exercise may not necessarily have been due to sequestering of NO but rather that increases in vessel diameter after exercise reduced the shear stress stimulus acted upon the endothelium, compared to vessels of smaller diameter. It should be noted, however, that this study used upper arm occlusion, which can evoke different FMD responses compared to the more NO mediated forearm occlusion [99].

It is possible that limb specific effects of exercise could account for the observations in FMD of the brachial artery. A study by Dawson et al (2008) assessed marathon runners before and after a marathon race [96]. Although this study assessed vascular effects of high intensity exercise, it demonstrates how blood flow patterns in the upper and lower limb could cause different effects. The subjects demonstrated no change in upper limb FMD after racing whereas femoral artery FMD declined by nearly 3%. Previous literature has documented shear rate patterns in the upper limb vs lower limb [100] with upper limbs experience retrograde flow during cycle ergometry. However, whether these patterns will remain under altered P_aO_2 conditions is not yet known.

In contrast to individuals with normal endothelial function, acute exercise is a powerful stimulus to improve vascular function in those with endothelial dysfunction [101]. In healthy, post-menopausal women with blunted FMD (5.3% ± 0.5), acute moderate exercise (45 minutes at 60% $\dot{V}O_{2max}$) restored FMD (9.9% ± 0.5) to levels similar to those of pre-menopausal women (12.1% ± 1.5). However, exercise in the pre-menopausal group had no significant effect (12.1% ± 1.5 to 14.4% ± 1.2 after exercise). It should be noted that, because of the related age difference, the pre-menopausal women had greater aerobic capacity than the post-menopausal group ($p<0.001$). The authors suggested that pre-menopausal women already have nearly maximal NO bioavailability such that any other stimulation, such as exercise, would not cause any further increaes in endothelial function, as assessed using FMD. The lack of response observed from the acute bout of exercise was because of a functional "ceiling-effect".

Another study of interest by Harris et al [102] assessed the FMD response to three intensities of acute treadmill exercise at 25%, 50% and 75% of $\dot{V}O_{2peak}$ in active and inactive

overweight men (BMI≥25kg/m^2). Inactive individuals had lower FMD response compared to the active group after acute exercise in all conditions, and change in FMD was not associated with changes in systemic inflammatory markers tumor necrosis factor alpha (TNFα) and interleukin-six (IL-6). The authors postulated that "waiting room climate" could have accounted for the vasoconstriction observed in the inactive group. No further explanation was provided regarding the vasoconstrictive response or what the authors meant by "waiting room climate". It is possible that they meant anxiety of being tested (ie: "white-coat" syndrome), causing elevations in sympathetic activity and vasoconstriction or perhaps temperature of the environment. It should be noted that the inactive group had greater FMD at baseline than the active group, but it did not appear to be significantly different. The FMD results of this study need to be interpreted with caution as the total hyperemic stimulus was not measured [103], thus the shear stimulus could have differed between groups. This could help describe the disparity in results between both groups. As well, defining the groups as "active" and "inactive" may not have been entirely valid as subjects self-reported their activity status and despite their "activity level" being significantly different (0.7±0.3hrs vs 4.4±0.5hrs respectively), their $\dot{V}O_2$peak was not statistically different (30.9±1.2 ml/kg/min vs 34.2±1.7 ml/kg/min respectively). Furthermore, assessment of oxidant and antioxidant status could have shed more light on the mechanistic response to exercise in these individuals. Inactive individuals may have elevated ROS production from exercise and without an established antioxidant system to help prevent oxidative stress from happening. Active individuals have improved antioxidant capacity due to increased levels of physical activity, thus being able to tolerate the elevated oxidant production better.

It is possible that individuals fall along a potential inverted U-shaped curve of FMD response (Figure 1.1); thus, individuals with low physical activity level and low antioxidant capacity having the lowest FMD response due to elevated oxidative stress. Such changes may explain the findings from the inactive and overweight individuals in [102]. Next along the continuum towards the center would be individuals with existing endothelial function but are able to increase their NO production enough to counteract ROS production after exercise and thereby increasing their FMD response. On the right of the continuum would be highly trained individuals, with remodelled vasculature adapted to repetitive shear stress, already utilizing their maximum NO capacity. Although this model is only conceptual and has not been tested, changing the vascular milieu and altering baseline FMD response due to hypoxic or hyperoxic exposure may shift highly trained individuals into the middle category. This would make them more sensitive to the vascular affects of acute exercise as changes in P_aO_2 could alter the sensitivity of the vasculature to the hyperemic stimuli from exercise. However, whether exercise will be strong enough of a stimulus or whether the gas exposure will alter FMD enough is unknown.

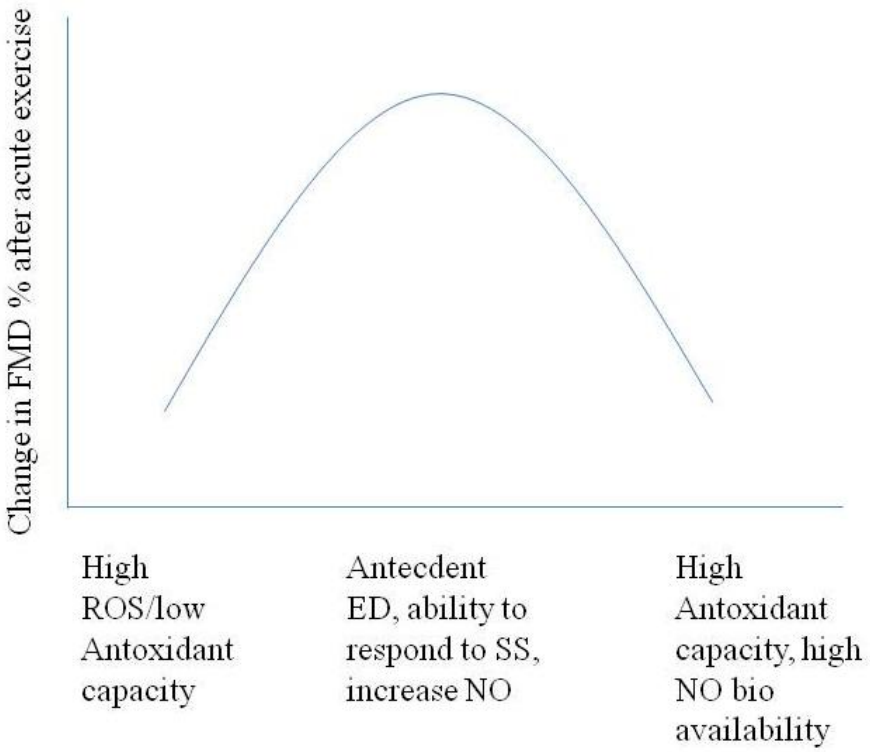

Figure 1.1 Conceptual model to predict change in FMD after acute exercise.

ROS = Reactive oxygen species, ED = Endothelial dysfunction, SS = Shear stress, NO = Nitric Oxide

1.4.2 Effect of Altered P_aO_2 on Flow-mediated Dilation

To our knowledge, only one study has assessed the affects of P_aO_2 on FMD [104]. There did not appear to be any difference in hypoxic and normoxic response to reactive hyperemia. Fifteen minutes of hypoxia (12.5% O_2) did not alter the FMD response compared to normoxic exposure. On contrast, hyperoxia (100% O2) induced vasoconstriction at baseline (-4%) and greater relative change in FMD (+4.6%). However, limitations in the methodology and design of the study should be noted. For example, gas exposure was not randomly administered and serial measurements of FMD were made, possibly confounding the FMD results by repeatedly inducing a shear stimulus on the vasculature. In addition,

dilatory response was reported as a ratio of FMD to nitroglycerin mediated dilation (NMD), whereas changes in absolute or relative dilatory response were not reported. The values reported were diameters at 60s post release and may not illustrate the true peak diameter as time to peak can differ between individuals [105]. This study assessed individuals with increased risk of cardiovascular disease thus does not fully illustrate the normal physiological effects such gases might have on a healthy endothelium. It is known that exposure to hypoxia can cause increases in peripheral vascular tone [106], oxidative stress [107] and attenuated dilatory response to shear stress [104]; whether exercise in these conditions can help mediate the vascular responses needs to be investigated, and forms the focus of this thesis.

1.5 Relevance

The effects of P_aO_2 on vascular health at rest and after exercise are not well-understood. In disease conditions, detriments in arterial oxygen saturation are associated with cardiovascular disease risk, such as in chronic obstructive pulmonary disease, chronic heart failure and obstructive sleep apnea [108]. Transient and chronic hypoxemia is associated with increased inflammation and oxidative stress, which disrupts the vascular and endothelial milieu promoting atherosclerosis and impairing cardiovascular health. Many of these patients receive oxygen therapy and are often exposed to hyperoxia to improve their functional capacity. However, whether these improvements in functional capacity are negated by cardiovascular effects from increased oxidant production is not known. Similarly, training in hypoxia [109] or hyperoxia [110] is commonly performed by athletes to increase physiological stress to try and gain greater physiological adaptation. The vascular affects of such training has not been well-studied, although it is known that such training can cause

increased oxidative stress and can possibly have negative vascular consequences. It is also not known how a healthy endothelium and vasculature will react to alterations in oxygen tension and whether exercise will help mediate these effects.

1.6 Objective

To assess the effects of constant workload exercise while breathing hypoxic, normoxic and hyperoxic gas on FMD.

1.7 Hypothesis

Exercising while breathing a normoxic or hypoxic gas will improve FMD, whereas breathing hyperoxic gas will not alter the FMD response.

CHAPTER 2

EXERCISE IN HYPOXIA AND HYPEROXIA DO NOT ALTER THE FLOW-MEDIATED DILATORY RESPONSE IN HEALTHY YOUNG MEN

2.1 Background

Acute exercise has shown to be a powerful stimulus to improve the vascular response in individuals who have pre-existing endothelial dysfunction [101, 102]. Flow-mediated dilation is a non-invasive method of assessing NO-mediated endothelial function [64] and its application in the acute exercise model is gaining attention to understand the acute affects of exercise on vascular function [111]. The effects of altered oxygen status on FMD is not well known, particularly in conjunction with exercise.

Exercise in hypoxic conditions can increase the release of vasodilatory substances which can induce an augmented vasodilatory responses [27]. It is possible that such affects could remain elevated during the recovery period [86], contributing to the vascular adaptations seen with exercise over time [92]. However, whether this will impact the effects of FMD measured during this recovery period after exercise is not known. The endothelial effects of hyperoxia (>21%O_2) are even less well known. Supraphysiological levels of oxygen (e.g., 100%) is associated with increased vascular tone and resistance [42] as well as increased oxidative stress [52] which can negate any vasodilatory effects from exercise. However, whether exercise can mitigate these effects such that endothelial response will not be affected has not been studied.

Only one previous study has assessed the effects of altered oxygen tension at rest on FMD [104]. Hypoxic exposure did not alter the FMD response whereas hyperoxia increased FMD, compared to normoxia. However, these measurements were completed within the

same day, in the same gas order so it is possible that these methodological issues could have affected the results. Exercise could also potentially alter the hypoxic or hyperoxic effects on FMD by increasing NO availability [98] and increasing antioxidant capacity [112], thereby decreasing oxidative stress. However, the vascular affects of such stimulus has not been studied and it is uncertain how a healthy endothelium and vasculature might react to alterations in oxygen tension and whether exercise will help mediate any effects that can be seen after the exercise bout. The purpose of this study is to assess the effects of constant workload exercise while breathing hypoxic (16% O_2), normoxic (21% O_2) and hyperoxic (100% O_2) gas on FMD in healthy active males. We hypothesized that exercising while breathing a normoxic or hypoxic gas will improve FMD, whereas breathing hyperoxic gas during exercise will not alter the FMD response.

2.2 Methodology

2.2.1 Subjects

Seventeen healthy, recreationally active males (age: 21 ± 1yr) volunteered to participate in the study. Males were selected in order to eliminate the potential effects of estrogen on vascular function in female subjects [113]. Subjects were recruited from the University of British Columbia Okanagan campus and the surrounding community. The risks, benefits and procedures of the protocol were explained to the subjects and written informed consent was provided. All documents and procedures of the study were approved by the Clinical Research Ethics Board at the University of British Columbia.

Subjects were non-smokers who were normal weight to heigh ratio (Body mass index <30 kg/m^2), normotensive (blood pressure <140/90 mmHg at rest) and were free of any illness, history of diabetes, cardiovascular disease, and musculoskeletal injuries that could limit their ability to exercise. Subjects were not taking any prescription medications or vitamin supplementation. One subject who reported asthmatic symptoms was instructed to take his bronchodilator immediately prior to each experimental session. Before each experimental day, subjects were asked to refrain from caffeine, alcohol, smoke inhalation and exposure, and strenuous exercise for at least 24 hrs prior to testing. Subjects were permitted to eat a light, low fat, low sugar meal 2 hours prior to testing and were requested to eat the same meal prior to each testing session. Water was permitted ad libitum throughout exercise.

2.2.2 Study Design

This study was performed using a single blind, randomized crossover design. Participants came to the Exercise Physiology Laboratory in the School of Health and Exercise Sciences on six occasions, with each testing session separated by at least 48 hrs. Subjects completed three graded exercise tests (GXT) during the first three visits to determine the gas specific workload for the experimental constant load exercise trials (CLT) that were performed in visits 4 – 6. The intensity for each CLT was set at a relative intensity of 50% of the maximum wattage achieved during the GXT on each gas. Special care was taken to ensure that testing sessions were scheduled around periods of activity that may have caused alterations in vascular response such as increased stress (i.e. exams) or changes in sleep and dietary habits (i.e. holidays). As well, CLT sessions were performed at the same time of day for each test to control for circadian variation in vascular function [114].

All exercise sessions were performed on a Velotron cycle ergometer (Dynafit Pro, RacerMate, Seattle, WA) with the same equipment set up (Figure 2.1). Expired gas analysis (Vista-MX, VacuMed, Ventura, CA) during GXTs was used for ventilation only due to the well documented difficulties of measuring oxygen consumption in hypoxia and 100% oxygen [115], as varied inspired oxygen fractions (F_IO_2) were utilized and metabolic gas analysis was not required. Subjects breathed gas mixtures of compressed air (21% O_2, 79% N_2), hypoxic gas (16% O_2, 84% N_2) or oxygen (100% O_2) from a large K-sized cylinder, connected to a large (150L) non-diffusion bag (8900 series; Hans Rudolph; Kansas City, MO) through a low-resistance non-rebreathing valve attached to a face mask (Hans rudolph 2700 series, Hans Rudolph, Kansas City, MO). The order of the gases for both the graded exercise tests and the experimental trials were randomized. Sixteen percent oxygen was selected in order to simulate 3,000m in altitude, the top end of a hypoxic stimulus used in training. Pure oxygen was utilized in order to maximize the effects of hyperoxic delivery.

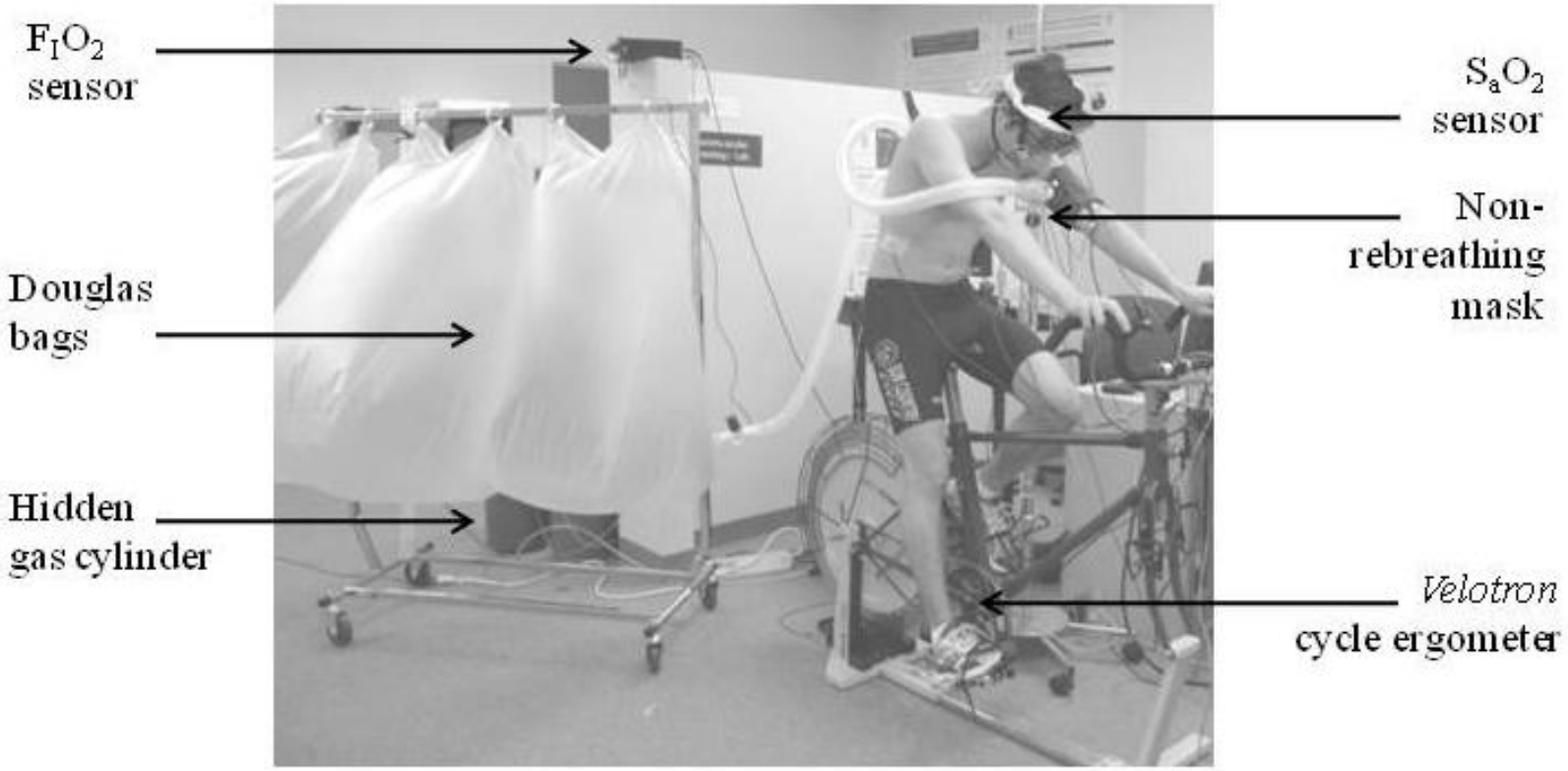

Figure 2.1 Exercise testing experimental set-up.

2.2.3 Measurements and Procedures

2.2.3.1 Graded exercise test

Subjects sat quietly on the cycle ergometer for 5 minutes while breathing the experimental gas mixture. Subjects then completed a standardized 5 minute warm-up at 100W before the incremental test began (Figure 2.2). Exercise intensity was increased by 30W every 2 minutes from the starting wattage of 150W until the second ventilatory threshold (VT2) determined as the point where there was a large non-linear increase in ventilation as per the method of Wasserman (1987) by which $\dot{V}_E$ rises disproportionately to $\dot{V}CO_2 / \dot{V}O_2$ [116]. After VT2 was achieved, workload was increased 30W every minute until volitional exhaustion. Gas specific peak wattage, which was the last workload the subject fully completed, was recorded to determine work rate for CLT. Heart rate and oxyhemoglobin saturation were measured continuously throughout the test using a three lead electrocardiogram (ECG; Tango+, SunTech Medicals, Morrisville, NC) and a forehead pulse oximeter (N3000, Covidien-Nellcor, Boulder, CO) respectively.

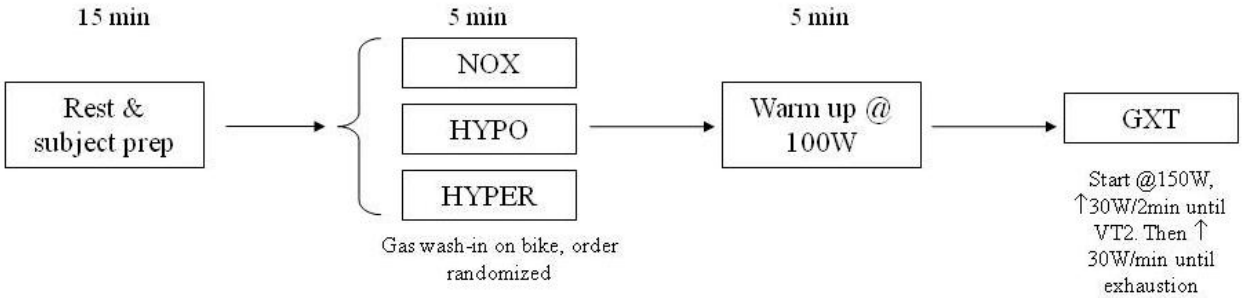

Figure 2.2 Graded exercise test (GXT) testing protocol.

NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia, W = watts, GXT = graded exercise test

2.2.3.2 Experimental constant load trials

The three experimental constant load trials were separated by at least 48 hours and performed at the same time of day (Figure 2.3). On arrival at the lab, subjects rested on a bed for thirty minutes and were administered the experimental gas. Following this wash-in period, baseline flow-mediated dilation (FMD) was measured. Subjects were then moved to the bike while still breathing the experimental gas and completed a 5-minute standardized warm-up of 5 minutes at 25% of the maximal wattage achieved during their gas specific incremental exercise test. Subjects then completed 40 minutes of exercise at 50% Wmax immediately followed by a 5 minute cool-down of 25% Wmax. The mean wattage in each trial was 50±3%, 50±2% and 50±4% for NOX, HYPER and HYPO respectively. Heart rate and oxyhemoglobin saturation were recorded every 10 minutes using a forehead pulse oximeter. After exercise, subjects lay supine and continued to breathe the experimental gas mixture until the completion of the post exercise FMD measurement, which occurred after 30 minutes of recovery.

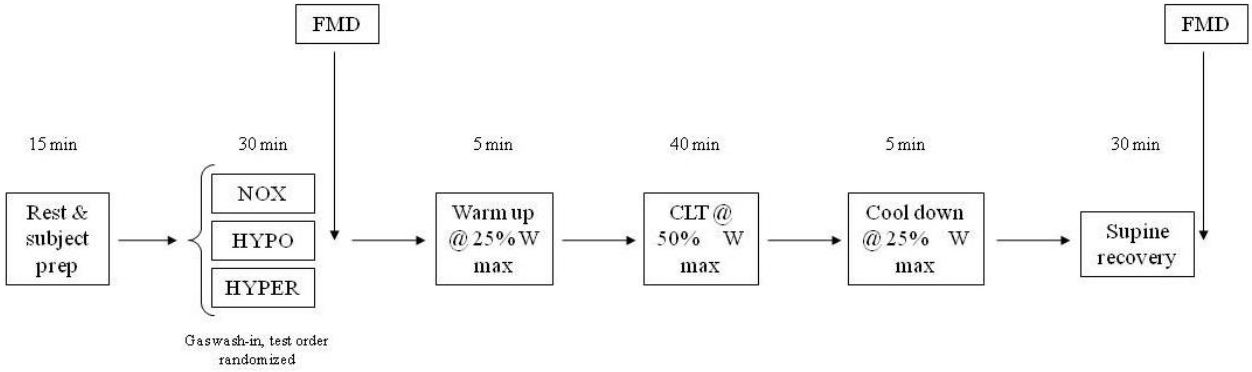

Figure 2.3 Experimental exercise session protocol.

NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia, W = watts, CLT = Constant load trial

2.2.3.3 Flow-mediated dilation

Assessment of brachial FMD followed the protocol and guidelines of Corretti et al [67] and Thijssen et al [57]. During the measurement, the room was dimly lit, quiet and temperature controlled. Subjects rested supine for 30 minutes while breathing the appropriate gas mixture before measurements began. The right arm was extended at approximately 80º from the body and was fully supported. To induce reactive hyperemia, a cuff was placed on the forearm and inflated with a rapid cuff inflator (D.E. Hokanson, Bellevue, WA) to 220mmHg for five minutes. Image acquisition of the brachial artery and placement of ultrasound transducer was above the antecubital fossa in the longitudinal plane. Measurements were obtained with a Terason ultrasound (T3000, Terason Ultrasound, Burlington, MA) equipped with vascular software for 2-D imaging, colour and spectral Doppler, and a 12mHz multifrequency linear array probe. Once the brachial artery was located in the distal third of the upper arm, the 2-D image of the intima-wall interface was optimized. Both the 2-D image and continuous Doppler velocity assessment of brachial artery blood flow was obtained simultaneously at an insonation angle of 60º. Baseline 2-D images of the brachial artery and Doppler velocity assessment were recorded for two minutes prior to cuff inflation and then from 30s prior to and 5 minutes post cuff deflation. Camtasia screen capture software (TechSmith, Okemos, MI) was used to make the video recording of the ultrasound images at a rate of 30Hz.

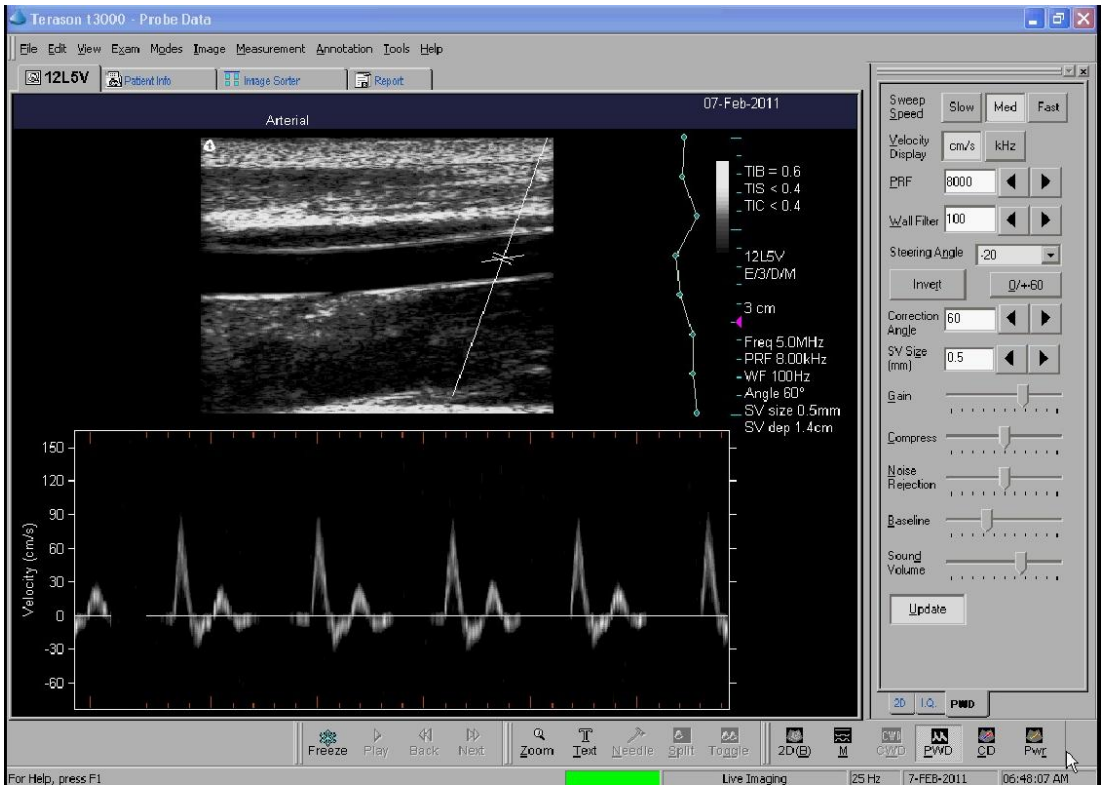

Figure 2.4 Ultrasound interface. The top half of the screen represents the B-mode 2-dimensional image of the brachial artery. Below, is the Doppler trace of the blood flow velocity moving through the brachial artery.

2.2.3.4 Image analysis

Images were analyzed offline using custom-designed edge detection and wall tracking software independent of investigator bias [117]. Analyses of the images were performed with an icon-based graphical programming language and toolkit (LabView 6.02, National Instruments, Austin, TX)[105]. Firstly, two regions of interest (ROIs) were defined and calibrated, to ensure the pixel size in the computer corresponded to the correct actual values. To assess vessel diameter and blood flow velocity, a region of interest was selected around the optimal area of the B-mode image and the Doppler strip respectively. For vessel diameter, a pixel-density algorithm automatically detected the angle-corrected near and far

wall lines for each pixel column in the ROI. The edges of the vessel wall were determined where the pixel intensity changed most rapidly (Figure 2.5).

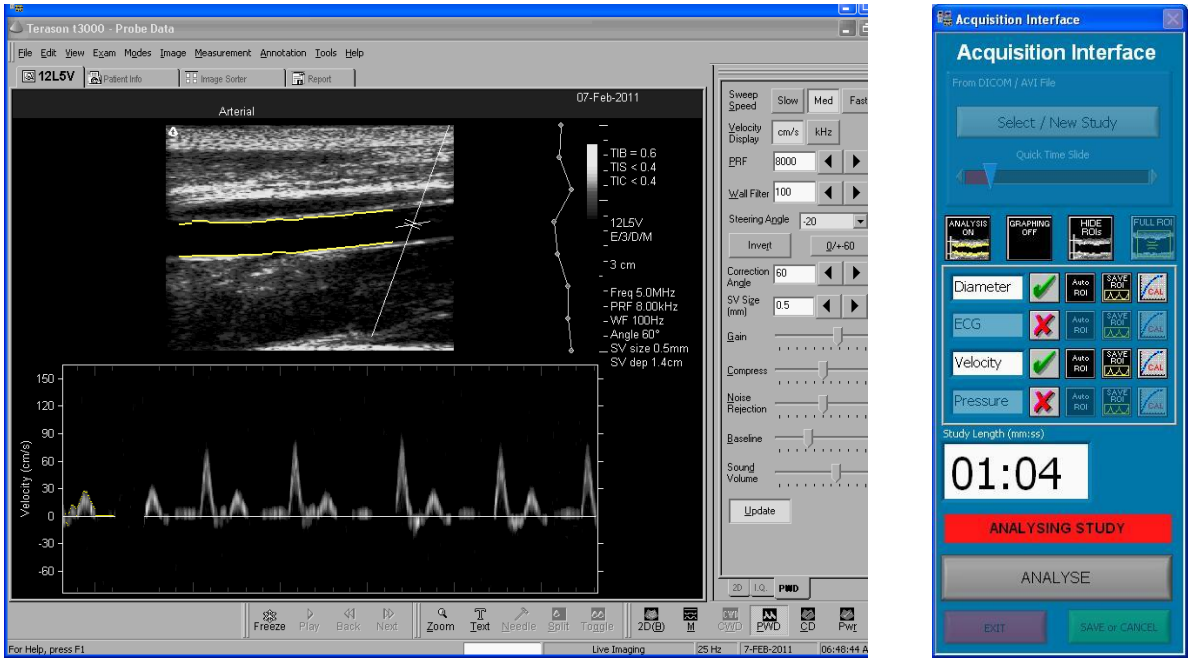

Figure 2.5 Video analysis interface. The right portion displays the acquisition interface where the user can select the region of interest of the 2D image and the Doppler trace. The top half of the screen highlights the region of interest selected for the software to detect the wall of the vessel. Below is the Doppler trace of the vessel where blood flow velocity is shown.

The ROI diameter can contain from 200-400 pixel columns per frame, with the software analyzing the images at 30 frames a second. Similarly, Doppler velocity was automatically assessed with the ROI around the waveform envelope. Using the simultaneous Doppler velocity and vessel diameter data, blood flow (vessel cross sectional area x Doppler velocity) and shear rate (velocity x 4, divided by vessel diameter). Once the video was analyzed, the software plotted diameter data against time. In the analysis window, event cursors that represent the beginning and end of baseline and FMD were placed at the correct time points of the study (Figure 2.6). Any erroneous data points that were falsely identified as peak diameter by the software were manually removed from the data set. Changes were

represented as a change from baseline. This method has been shown to be significantly better than manual methods previously used [117].

Additional analyses to better understand the FMD response were conducted. FMD responses were normalized for $SRAUC_{ttp}$ and SR as this has been shown to better describe the FMD response [103, 118, 119].

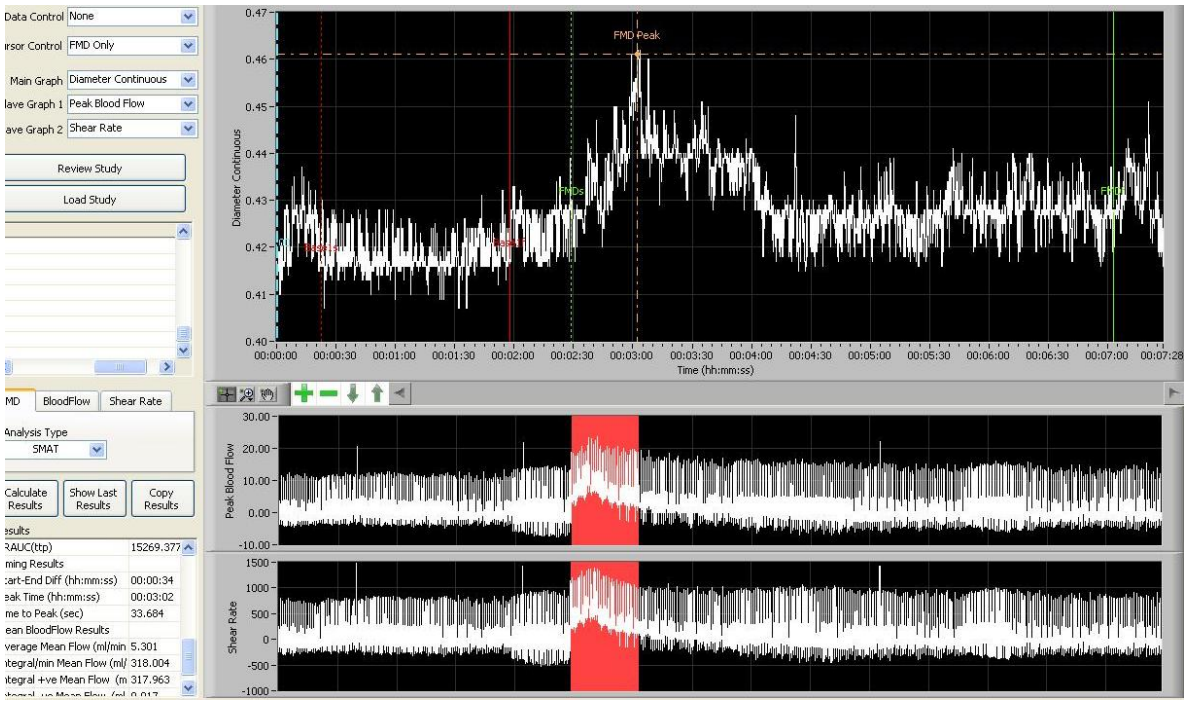

Figure 2.6 Data analysis interface. The top window displays the diameter trace of the vessel during the FMD procedure, with cursors placed to indicate the beginning and end of baseline and FMD during the recording. Below, peak blood flow and shear rate are displayed with the highlighted portion indicating the time to peak dilation. The bottom left panel displays calculated values.

2.2.4 Power Calculation and Statistical Analysis

Previous to this study, no investigation has examined the vascular response to altered P_aO_2 and exercise. One previous study [104] has addressed the effect of altered inspired oxygen levels on brachial artery FMD in older patients with increased risk of cardiovascular

disease at rest. It was impossible from this study to determine the potential effect size that altering inspired gas may have on FMD following exercise. However, we assumed a similar standard deviation of the change score as reported in this study of ~2%, with the 13 subjects in this study we should be able to detect a true difference in the mean change score of ±2.1% with a power of 0.8 and an alpha level of 0.018 adjusted for multiple comparisons.

To determine the change in all dependent variables across both the constant load trials and graded exercise tests, a one-way ANOVA was used. Additionally, a 2 (time) x3 (gas) repeated measures ANOVA was performed and a Tukey post hoc test was performed when the ANOVA determined a significant effect. An alpha level of 0.05 was used for all analysis. Additional analysis were completed with relative FMD normalized to shear rate area under the curve, until time to peak dilation ($SRAUC_{ttp}$), which was completed by dividing the FMD results by $SRAUC_{ttp.}$ Group analyses were completed to assess affects of time of day of testing (ie: before or after 12:00) as circadian rhythm has shown to have an affect on FMD [120] and exercise responses [114], as well as fitness level. Linear regression analyses were completed to determine if there was any relation between FMD response and level of oxygen desaturation during exercise, shear rate, FMD, baseline diameter and S_pO_2.

2.3 Results

Seventeen healthy, recreationally active males volunteered to participate in this study. Thirteen completed the whole study protocol. Of the 4 that did not complete the study protocol, two withdrew due to illness, one withdrew due to scheduling conflicts and one was dropped from the study due to technical difficulties obtaining appropriate ultrasound images. Specifically, there was difficulty obtaining a clear 2-D image of the brachial artery with

clearly defined vessel walls suitable for analysis. On average, subjects were 22 ± 3 yrs old with a mean body mass of 76 ± 9kg, a height of 179 ± 6 cm and fitness level of 4.7 ± 0.75 W_{max}/kg. Competetive elite cyclists are commonly >6.0 W_{max}/kg [121]. Complete individual data can be found in Appendix C.

2.3.1 Exercise Trials

2.3.1.1 Graded exercise test

Peak power output achieved in the graded exercise test were 358 ± 71W, 341 ± 42W and 390 ± 71W for NOX, HYPO and HYPER trials, respectively. Only the difference between the HYPO and HYPER trial was significant for peak power outputs ($p=0.04$). Average S_pO_2 at the end of the exercise test during the NOX, HYPO and HYPER GXT was 94.2 ± 3.4%, 86.6 ± 4.9%, 98.4 ± 0.5% respectively. As expected, S_pO_2 in hypoxia was lower than NOX and HYPER ($p<0.05$). These data are presented in **Error! Reference source not found.**1.

2.3.1.2 Constant load trial

Compared to NOX (97.3 ± 1.9%) and HYPER (99.4 ± 0.5%), S_pO_2 during CLT was significantly lower in HYPO (89.6 ± 2.9%) ($p<0.001$). Heart rate achieved in the NOX, HYPO and HYPER GXT tests averaged 192 ± 11bpm, 191 ± 13bpm, and 193 ± 12bpm, respectively. During the CLT, heart rate reached 162 ± 11bpm, 164 ± 9bpm, and 158 ± 13bpm respectively. Heart rates in the GXT and CLT trials did not differ between gas conditions ($p>0.05$). These data are presented in Table 2.1. Generally, subjects were unable to ascertain what gas they were breathing until the completion of the trial.

Table 2.1 Exercise data. S_pO_2 = percentage of oxyhemoglobin saturation, HR = heart rate, W_{max} = workrate max, W = watts. * p<0.05 HYPER, **p<0.05 NOX and HYPER

	NOX	**HYPO**	**HYPER**
Rest			
S_pO_2 (%)	98.4 ± 1.1	97.8 ± 1.9	98.9 ± 1
HR (bpm)	70 ± 5	71 ± 5	68 ± 5
Graded exercise test			
W_{max} (W)	358 ± 71	341 ± 42*	390 ± 71
S_pO_2 (%)	94.2 ± 3.4	86.6 ± 4.9**	98.4 ± 0.5
HR_{max} (bpm)	192 ± 11	191 ± 13	193 ± 12
Constant load trial			
Work rate (W)	179 ± 23	168 ± 24	194 ± 28
S_pO_2 (%)	97.3 ± 1.9	89.6 ± 2.9**	99.4 ± 0.5
HR (bpm)	162 ± 11	164 ± 9	158 ± 13

2.3.2 Flow-mediated Dilation

The vascular responses to exercise in the three gas conditions are depicted in Table 2.2. Forty minutes of moderate intensity exercise did not induce a change in brachial FMD response in any of the gas conditions when analyzed as an absolute or relative change (Figure 2.8). Baseline arterial diameter (Figure 2.7), SR_{avg} (Figure 2.10), $SRAUC_{ttp}$ (Figure 2.9) and TTP (Figure 2.11) also did not significantly differ between any of gas conditions before or after exercise. Subgroup analysis categorizing subjects according to the time of day of testing and aerobic fitness levels found no discernable relationships between exercise or the three different gas conditions (p.0.05). As well, further analysis of FMD after normalizing for

SRAUC did not reveal any further differences (p.0.05). There was no change in FMD response, SRAUC, or time to peak dilation after exercise in any gas condition. Because we felt that alterations in S_pO_2 could alter shear rate, and therefore impact the FMD stimulus, we performed regression analyses to assess whether there was any relationship between S_pO_2 and shear rate (average overall shear rate as well as area under the curve until peak dilation shear rate). Regression analysis revealed no relationship between changes in S_pO_2 and FMD (relative and absolute) before or after exercise (p=0.05). Although S_pO_2 and average SR were moderately related (r^2=0.38, P=0.03), there was no correlation between $SRAUC_{ttp}$ and S_pO_2.

Table 2.2 Flow-mediated dilation data. NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia, FMD = flow-mediated dilation, SR_{avg} = average shear rate, $SRAUC_{ttp}$ = shear rate area under the curve until peak dilation, TTP = time to peak dilation, Δ = change

	Resting diameter (mm)	**FMD peak (mm)**	**FMD peak (%)**	**SR_{avg} (1/s)**	**$SRAUC_{ttp}$ (10^3)**	**TTP (s)**
NOX						
Baseline	39.7 ± 4.3	42.0 ± 4.1	6.0 ± 2.4	391 ± 112	18.0 ± 7.3	47.0 ± 18.8
30min post	39.3 ± 4.0	42.1 ± 2.2	7.1 ± 4.1	410 ± 115	25.6 ± 12.9	63.9 ± 29.9
Δ from baseline	-0.4 ± 4.0	0.1 ± 2.2	1.2 ± 4.5	19 ± 133	7.6 ± 9.0	16.9 ± 31.2
HYPO						
Baseline	39.6 ± 4.6	42.1 ± 5.0	6.5 ± 3.7	396 ± 141	20.1 ± 10.2	56.4 ± 23.0
30min post	39.3 ± 4.7	41.5 ± 4.6	5.5 ± 3.0	362 ± 153	20.2 ± 12.2	55.9 ± 24.0
Δ from baseline	-0.2 ± 2.3	-0.7 ± 1.2	-1.0 ± 4.6	-34 ± 138	20.2 ± 17.5	-0.52 ± 51.0
HYPER						
Baseline	38.1 ± 3.5	40.4 ± 4.1	5.9 ± 2.6	391 ± 119	14.6 ± 5.3	42.5 ± 24.7
30min post	38.1 ± 3.7	40.7 ± 3.9	7.0 ± 3.1	417 ± 117	20.9 ± 8.4	50.1 ± 13.1
Δ from baseline	0.1±1.2	0.3 ± 1.3	1.1 ± 3.3	26 ± 120	6.4±8.4	7.6 ± 24.7

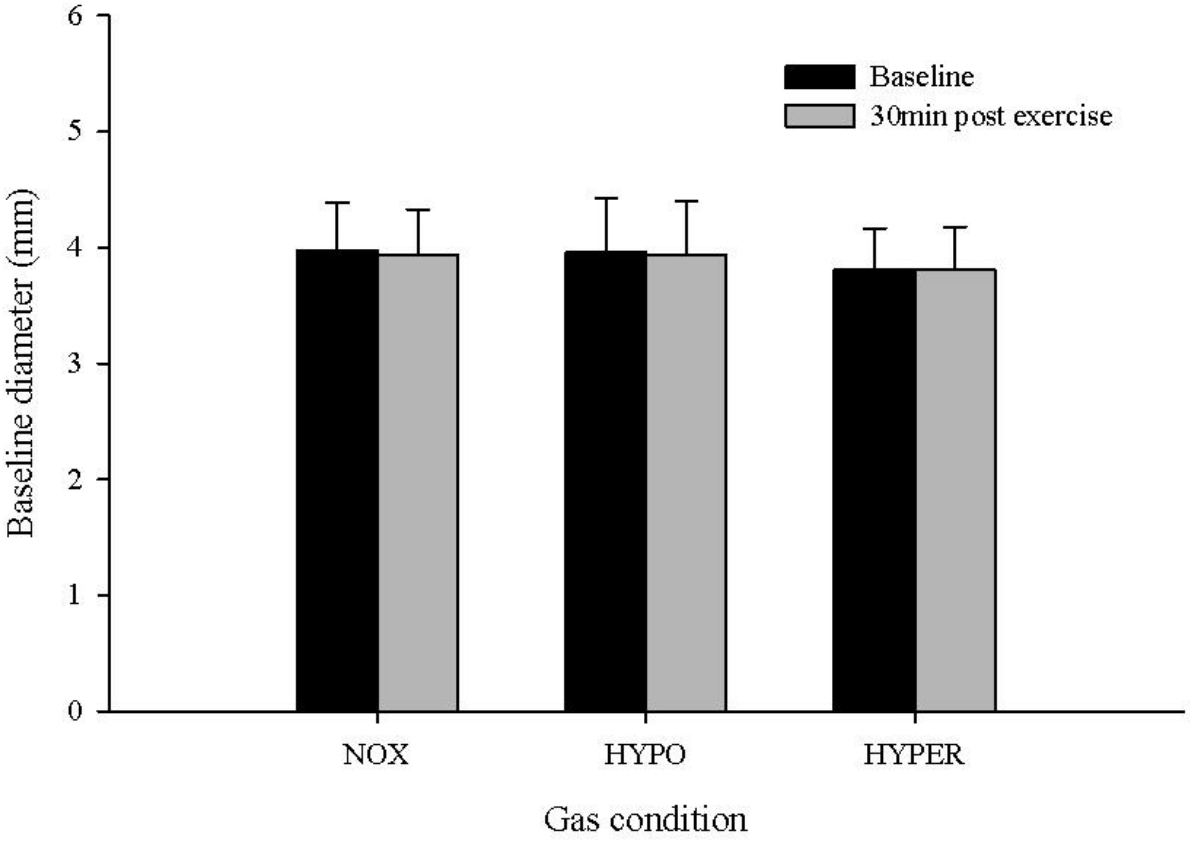

Figure 2.7 Baseline brachial artery diameter before and 30 min after exercise. NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia. Values are mean ± SD

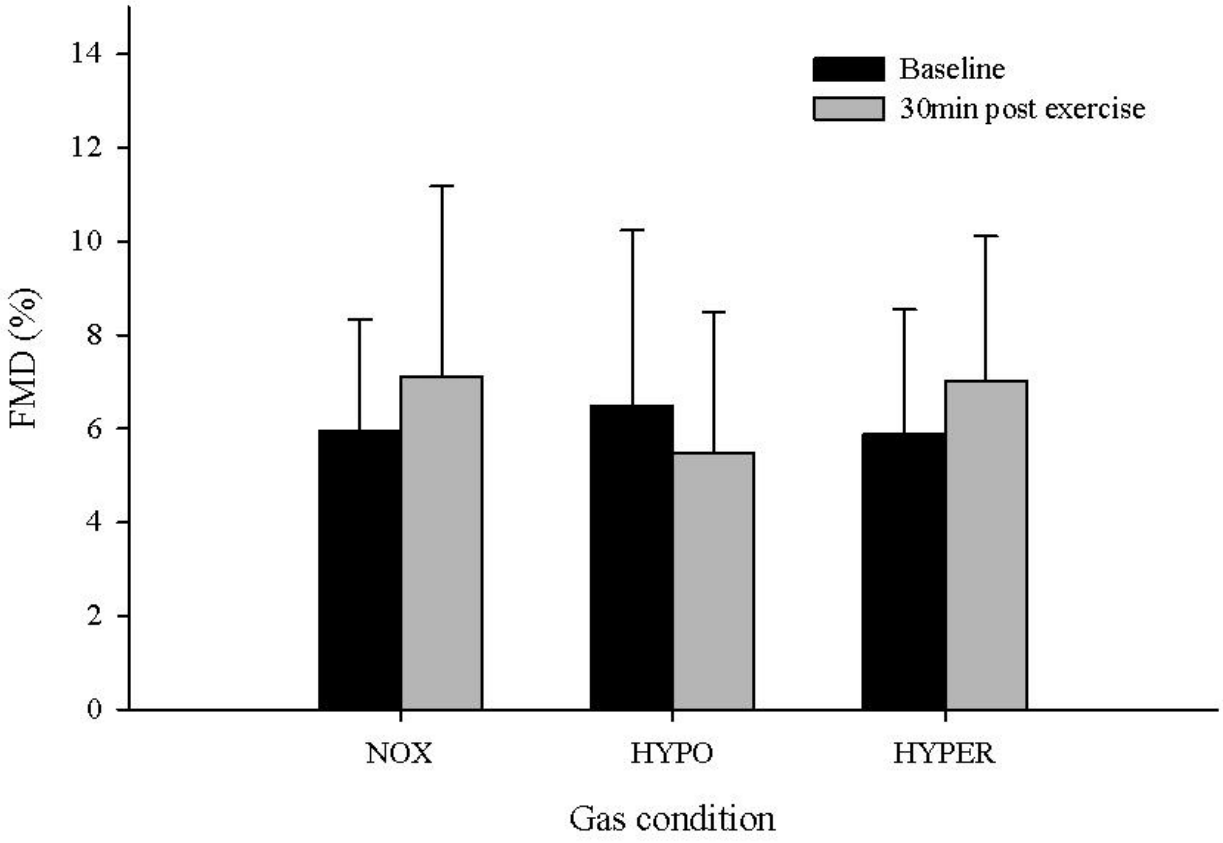

Figure 2.8 Relative change in flow-mediated dilation (FMD) before and after exercise. NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia. Values are mean ± SD

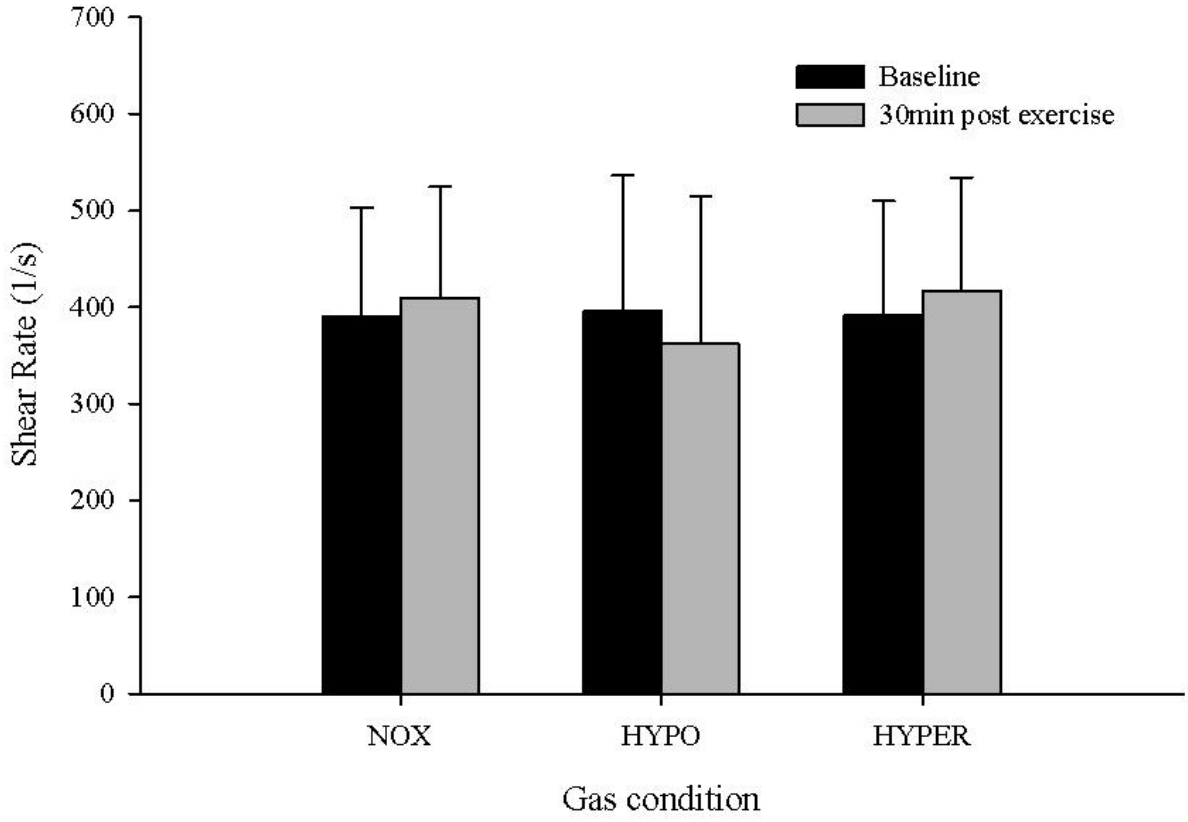

Figure 2.9 Shear rate area under the curve (SRAUC), until peak dilation.
NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia. Values are mean ± SD

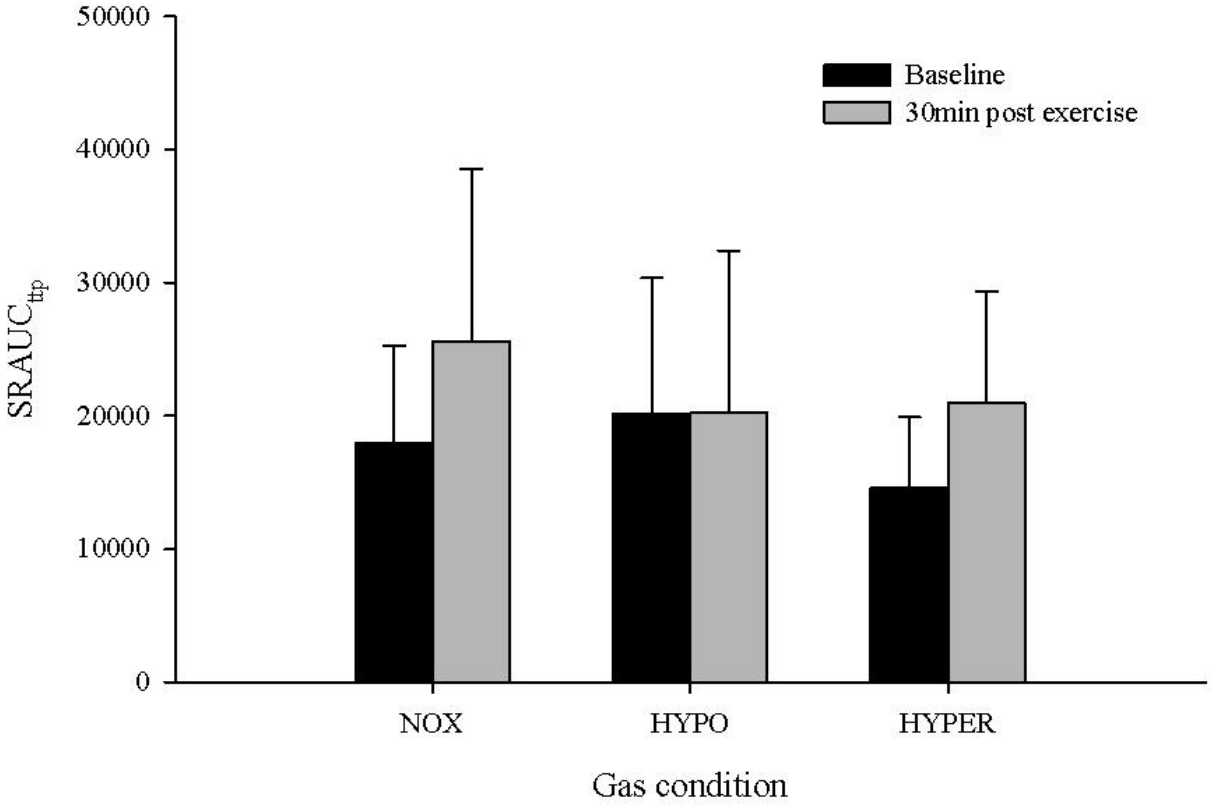

Figure 2.10 Average shear rate until peak dilation during FMD before and after exercise. NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia. Values are mean ± SD

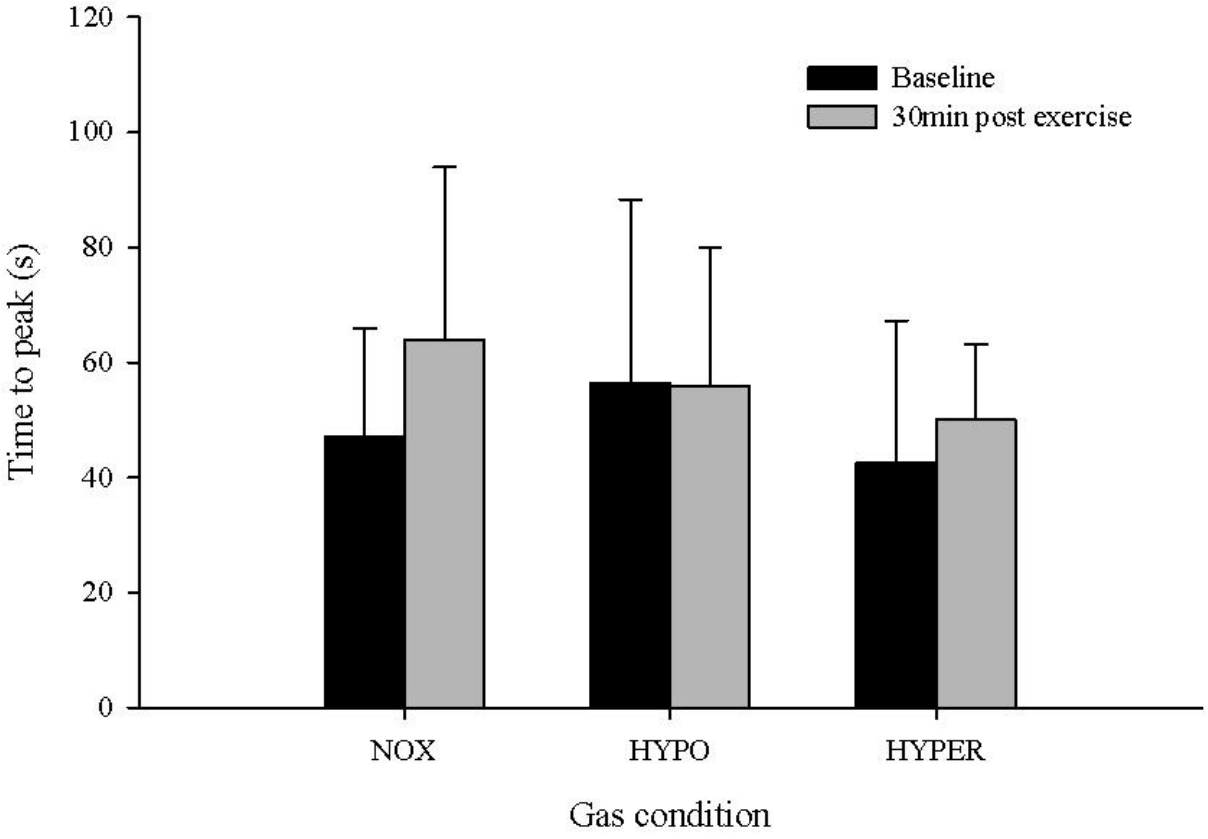

Figure 2.11 Time to peak dilation during FMD before and after exercise.
NOX = normoxia, HYPO = hypoxia, HYPER = hyperoxia. Values are mean ± SD

2.5 Discussion

This is the first study to investigate the effects of an acute bout of exercise on FMD under varying levels of arterial oxygen concentration. We hypothesized that FMD would be augmented after exercise in both normoxia and hypoxia due to the known increases in shear stress [27]. In contrast, we reasoned that exercise during hyperoxia would have no effect on FMD due to elevations in ROS production [45] and subsequent sequestering of NO, negating the shear stress induced vasodilatory response [48, 122]. The lack of change in FMD due to 40 minutes of exercise in the hyperoxic condition supported our hypothesis; however, the lack of any effect of exercise on absolute or relative FMD in the NOX and HYPO conditions is in contrast to our hypothesis. These findings did not change when the data was normalized for shear rate ($p = 0.309$), which takes into consideration the known differences in flow rates associated with different P_aO_2 tensions [122].

2.5.1 Baseline

The finding that a large changes in oxygen tension do not affect FMD values at rest is interesting due to the known affects on vessel diameter [104]. Hyperoxia has been documented to cause vasoconstriction whereas exercise in hypoxia induces vasodilation. The lack of change during hyperoxic and hypoxic gas exposure at rest was also surprising and in contrast to the only other study that has investigated the effects of changes in oxygen tension on FMD during rest [104]. Frobert et al assessed the effects of acute normoxic, hypoxic (12.5% O_2) and hyperoxic (100% O_2) exposure at rest on individuals with cardiovascular risk and healthy age matched controls. Hyperoxic exposure at rest caused vasoconstriction (-4%), whereas hypoxic exposure did not alter baseline diameter measures from normoxia. As well,

the FMD/NMD (an index of endothelial dependent dilation independent of brachial artery diameter, was significantly less in hypoxia, compared to the other conditions; a finding described as a decreased sensitivity to reactive hyperemia. The method of using FMD/NMD [123] is based on the assumption that baseline arterial measures are similar between conditions (ie: gas exposure) in order to be able to use the FMD/NMD for comparison. However, this assumption was violated as baseline diameters were not the same across all conditions. Upon our recalculation of their data, when the diameter measurements are calculated out to provide FMD in relative terms (ie: %), normoxic, hypoxic and hyperoxic FMD responses were 4.58%, 3.13% and 5.63%. Several differences between the this study and ours could account for the disparate results. Our measurements were completed on separate days, with the gas order randomized whereas the Frobert et al study assessed the gas affects in the same order, only 15 minutes apart, on the same day. The authors also only assessed the diameter change at 60s post release of the cuff whereas we were able to use video analysis software that analyzed the true peak diameter change, irrespective of time. Only taking a discrete time point can potentially underestimate the true peak diameter, particularly when comparing groups [105]. Depending on the condition or subject cohort, the peak dilation does not always occur at a specific time point, thus the ability to measure the true peak diameter is a more valid measurement of the FMD response. Finally, our subjects were also younger and were considered recreationally trained, whereas the control subjects in the Frobert et al study were older and no mention was made of their activity or training level. Because accurate comparisons between studies is not possible, the differences in the results may not necessarily “contradict” each other but perhaps give us an indication of the acute vascular affects of varying oxygen exposure in different populations.

2.5.2 Post-exercise Effects

The lack of a change in FMD following the exercise bout in each of the gas conditions is again surprising and in contrast to what was expected. One explanation for this finding may be that in healthy, well trained individuals as used in this study, moderate aerobic activity was not a great enough stimulus to increase the release of endothelium-dependent dilators such as ACh, adenosine or NO to see a change 30 minutes after exercise. Conversely, it is possible that our exercise stimulus caused an increase in vasoconstrictors, balancing any vasodilatory influences from exercise. We deemed our subjects to be trained (4.7W/kg) but not elite, as competitive elite cyclists have a documented fitness level > 6.0W/kg [121]. It is possible that these individuals who are accustomed to increases in shear stress were able to re-establish homeostasis quickly after exercise, or that this level of exercise did not disrupt homeostasis enough to induce a supercompensatory effect that could be detected with FMD. One major consideration we made was to ensure an equal relative exercise intensity stimulus was created in each condition, which could account for the lack of difference in FMD between each condition. The idea of using a the same relative intensity across exercise session is to simulate the same metabolic demand and therefore the same shear stimulus across exercise sessions.

One unique aspect of this study was that we took great care to ensure that each workload created essentially the same relative exercise intensity stimulus regardless of the gas mixtures used. This matching of intensity could have resulted in the same shear stimulus on the vasculature between each condition, thus accounting for the lack of difference between each gas. Conversely, it is possible that cardiac output differed in each gas condition in order to maintain consistent oxygen delivery to working muscles, which could

have altered the overall blood flow and shear stimulus in each condition (i.e. increased in hypoxia and redued in hyperoxia). However, heart rate was similar between each gas condition and no change was detected in blood flow or shear stress at rest in each condition. Without direct brachial artery blood flow measurements during exercise, we cannot confirm which mechanism occurred during exercise. Another possibility was that the amount of shear induced was not enough to disrupt homeostasis above habitual levels to induce a supercompensatory affect. It is possible that these healthy, trained individuals likely have adequate antioxidant capacity to counteract any oxidative stress induced by gas exposure and moderate exercise and negate any effects they may have on vascular functioning. Tinken et al [124] demonstrated that training can cause an initial improvement in FMD. However, the FMD response returns to baseline values after 8 weeks of training, indicating that structural adaptations occur with training in order to accommodate the repetitive shear stress.

Another possible reason for the lack of change observed in the brachial artery is the differential blood flow patterns that can occur with lower limb exercise [100]. Dawson et al [96] found that brachial artery FMD was unchanged after prolonged exercise, whereas femoral FMD decreased by 2.5% from baseline. It was suggested that oxidative stress after prolonged exercise could be isolated to the active muscle bed, accounting for the impairment of FMD after exercise. Additionally, the authors suggested NO synthase substrate or cofactor depletion, could impair vasodilator function. Although we did not measure femoral FMD, it is possible that the NO and ROS/inflammatory effects of lower limb exercise could have been enhanced or diminished in the hyperoxic or hypoxic conditions. The lack of change found in the brachial artery reported after marathon exercise could be due to the different flow and shear rate patterns of the upper limbs after lower limb exercise. Increased

retrograde flow has been documented during cycling [125] and can acutely impair FMD [125]. This supports our findings of no change in brachial FMD after lower limb exercising in a healthy trained cohort.

Johnson et al [95] manipulated exercise intensity and duration to study the individual effects on change in FMD after an acute bout of exercise. Exercise equivalent to moderate duration activity with moderate intensity (ie: 80% $\dot{V}O_{2peak}$ until equivalent work output of 50% $\dot{V}O_{2peak}$ for 30 minutes was achieved), induced the greatest change in FMD (~5% to ~7%) immediately after exercise. Based on these results, one would have expected our protocol to induce similar results in positive change in FMD after exercise, as our exercise intensity (50% workrate max) and duration (40 minutes) were comparable. However, our results are difficult to compare again due to methodological differences. In their study, FMD responses were either elevated or diminished significantly immediately after exercise and returned to levels close to baseline one hour after exercise, depending on whether exercise intensity or duration was manipulated. Our measurements were performed 30minutes post exercise which falls during this one hour post exercise period, which may explain the lack of significant change in FMD observed. Thirty minutes was chosen to ensure enough time for heart rate to return to resting levels before FMD measures were made. As well, Johnson et al completed two FMD measurements (one immediately after exercise and another one hour later). It is unknown how multiple measurements after exercise could affect the FMD response and whether the results are due to the effects of exercise hyperemia or repetitive FMD measurements. Furthermore, no apparent considerations were made for post exercise measurements such as ventilation, temperature etc which may also confound their initial post FMD measurements.

2.5.3 Baseline Diameter and Shear Stress Considerations

The FMD values in our cohort at baseline and after exercise, are consistent with others that are reported [95, 97, 101, 126]. It is possible that in healthy trained individuals, lower shear stress is inflicted on the endothelium due to a larger resting diameter as a result of vascular remodelling. To support this contention, our subjects had a mean resting diameter of 4.0 ± 0.4 mm which is similar to studies using trained young males (brachial artery diameters of 4.1 ± 0.1 mm and 3.9 ± 0.1 [97, 127]). In individuals with existing vascular dysfunction, such as inactive postmenopausal women [101] the smaller vessel diameter (3.2 ± 0.2mm) is likely more sensitive to the shear stress. Celermajer and colleagues [64], demonstrated that the greater the vessel diameter at baseline, the lower the FMD response as measured at rest ($r=-0.81$, $p<0.0001$). This larger diameter could be indicative of the exercise-induced remodelling due to repetitive exposure to increased blood flow and normalization to accommodate continual shear stress [91, 92]. In contrast, however, Jazuli and Pyke [128] recently found that there was little correlation with baseline brachial artery diameter and FMD response; rather, there was a strong correlation between FMD and the magnitude of the shear stimulus. However, this finding by Jazuli and Pyke may not apply to our study as we were unable to directly manipulate the amount of shear inflicted on the vessel wall thus alterations in baseline, and subsequently shear stress, could have affected peak dilatory capacity measured in FMD.

Although we were unable to directly control the magnitude of the shear stimulus, we were able to normalize our data to the shear rate area under the curve until peak dilation (i.e. total shear stimulus until dilation). In doing this, we found no relation between shear rate and absolute or relative FMD. This suggests that the amount of shear stimulus was not correlated

to the dilatory response of our study. This observation supports previous work in which shear rate area under the curve only explains 14% of the FMD response and that other vasoactive substances may play more of a role in these populations [129]. Other factors we were unable to measure or control for (i.e. oxidative stress, NO availability, sympathetic activity, deoxygenated hemoglobin) may have played a greater role in the dilatory response. It is possible that for the individuals in this study, the exercise conditions, as well as varying gas conditions altered certain pathways upstream or downstream from the shear stress stimulus that could impacted the vasodilatory response. For example, the decreased NO (or other vasodilatory agonists) bioavailability or signalling could have been altered during the hypoxic or hyperoxic gas condition, thus changing the stimulus on the vascular smooth muscle, regardless of the amount of shear stress induced.

2.5.4 Nitric Oxide and Hypoxia

NO is elevated after acute exercise [94] and contributes to the post exercise vasodilation, even in non-exercising beds [130, 131]. Elevated NO production has been shown during hypoxic exercise and contributes to the increased vasodilatory response; however, whether it remains elevated during hypoxic recovery is not known [27]. It should be noted that such measurements of NO may not accurately affect NO function in the body as a diet high in nitrates or mechanisms up or downstream from NO could also be affected. Furthermore, it is possible that these effects of the gas and exercise are detectable in the working limb (ie: femoral FMD) as upper and lower limb FMD responses can differ after lower limb exercise [96]. Since hypoxic exercise has shown to increase oxidant production, even in trained individuals with high antioxidant status [54], it is possible that increased

oxidant production impairs the FMD response via NO sequestering during recovery. However, without any blood measures to assess the oxidant and antioxidant balance, it is difficult to make any definitive statements about the response of the endothelium after exercise in these conditions. Another possibility for the lack of change in brachial FMD with exercise could have been our conservative hypoxic stimulus (16% O_2) compared to levels used in other studies or that exercise has a greater influence on FMD, regardless of F_IO_2 [122, 132-134]. The lack of change in baseline diameter could indicate a diminished vasodilatory response from the hypoxic stimulus which supports the conservative stimulus. During CLT, average S_PO_2 during HYPO trials dropped to 89.6 ± 2.9%, which is less of a reduction compared to other studies using hypoxia [135] and would be remain above the 40mmHg threshold in which nitrite red blood cell-dependent vasodilation occurs [36]. However, the addition of exercise should have induced an additional stimulus so likely another factor, such as differential limb effects of exercise [96], contributed to the lack of change in brachial FMD observed.

From our S_aO_2, data we were able to determine that many of our subjects experienced exercise induced arterial hypoxemia. Five demonstrated mild desaturation (S_aO_2 93-95%), 1 was moderate (S_aO_2 88–93%) and 2 were severe (S_aO_2 <88%) [136]. Due to the desaturation with heavy exercise in these individuals in air, it is possible that they may have been physiologically accustomed to lower blood oxygen levels during exercise. More, specifically their endothelium function and vascular structure may have already adapted to regular exposures to low arterial oxygen tension. This could help explain why there was no difference in FMD observed after the HYPO condition, despite the moderate exercise induced arterial hypoxemia that occurred (86.6 ± 4.9%).

2.5.5 Hyperoxia and ROS

It is also possible that increased oxidative stress from elevated ROS production in HYPER supports our rationale and our hypothesis that increased oxidants diminished the bioavailability of free NO thereby reducing the FMD response [48]. Hyperoxic administration, by reducing the forearm blood flow response to ACh administration [45], has been shown to impair forearm endothelial dependent vasodilation. This observation in hyperoxia is reversed when subjects are administered an antioxidant, specifically vitamin C, demonstrating that the diminished vasodilatory response due to hyperoxic exposure is primarily ROS mediated [45]. It is also possible that an increase in sympathetic nerve activity during exercise diminished the vasodilatory capacity of the vessel. Hyperoxia has shown to decrease muscle sympathetic nerve activity during rest but increase it during exercise [137]. Thus, if this effect persists, it may act to diminish the FMD response after exercise.

2.5.6 Methodological Considerations

Change in time to peak dilation is an important characteristic that should be considered when assessing FMD responses [138]. Any differences in the time it takes to reach peak dilation implies that some conditions require longer to reach maximal dilation than others, possibly due to increased stiffening, changes in enzyme kinetics, or availability of substrates [105]. However, because there were no differences in time to peak dilation between any condition, it is difficult to apply this concept to our study. The elevated time to peak dilation was related to the elevated shear stress in all conditions except in the post exercise HYPO and pre exercise HYPER condition but there was no significant difference between gas conditions ($p>0.05$). A potential reason for the lack of relationship in the pre

HYPER shear rate area under the curve (SRAUC) and time to peak dilation is the increased oxidative stress production from hyperoxic exposure at rest [45, 48].

Another possibility for the variance and lack of change with FMD response could be due to the circadian effect on exercise and FMD measurement. In designing the study, we believed that as long as the subjects were tested at the same time of day for each test, we would reduce variance in our individual FMD measurement. However, to fit with subject schedules and lab availability we had to schedule subjects throughout the day. This could have negated the post exercise response as FMD measurements have recently been shown to be change depending on time of day that exercise is performed [114]. Most of our subjects (8 out of 13 subjects) were tested before 10:00hr. A study by Jones et al [114] found that not only was FMD blunted in the morning (800h), but that the FMD response after acute cycling exercise was also attenuated, compared to exercise completed in the afternoon (1600h). The afternoon group in the study by Jones et al., had a higher FMD before exercise (10.8%) , however, the post exercise FMD was 4% lower; this lead to no difference in FMD after exercise between the morning and afternoon group. No differences were observed in baseline diameter. Potential suggestions for this observation were diurnal rhythms in sympathetic nerve activity [139] or alterations in oxidative stress and inflammation [140, 141] throughout the day. It should be noted that the individuals in the aforementioned Jones et al study [114] were considered healthy but untrained and it is unknown how the circadian response would be in trained individuals. A subgroup analysis of our data separating subjects according to time of day testing revealed no significant differences between baseline diameter, FMD response (absolute and relative) as well as SRAUC were found ($p>0.05$); therefore, we are confident that time of testing did not affect our results.

2.5.7 Limitations

One limitation in our measurement of FMD was that our cuff placement was not optimal to induce maximal shear stress. During pilot work, cuff placement in the antecubital fossa of the forearm caused the brachial artery to "snap" out of view during cuff release. The image of the brachial artery could no longer be obtained so in order to ensure that we could maintain imaging of the artery, cuff placement was slightly below the antecubital fossa. It is possible that this cuff placement altered the shear stimulus required for optimal FMD response [99]. Because cuff placement was consistent across all trials, any differences in results should have been due to gas and/or exercise effect However, it is possible that this did not induce adequate shear stress thereby masking any subtle changes that could have altered the NO stimulus.

Despite our best abilities to control for any variance, it is also possible that changes in exercise and gas exposure affected FMD within our margin of error for FMD thus accounting for the variance we observed. Coefficient of variance in FMD has been reported as high as 13.9% [142]; our values for FMD were well within this range. It is possible that the angle of insonation for both the Doppler blood flow velocity as well as 2-D imaging was not optimal thus potentially over or under estimating the values we observed. Doppler is best measured parallel to the blood flow, whereas 2-D image acquisition is best measured perpendicular to the area of interest, therefore measuring at a $\leq 60^{\circ}$ insonation angle gives us the best "compensation" for both measures [57]. However, all measurements were performed consistently and we took great care to adhere to the same standards and techniques for image acquisition; thus, any changes we observed would have been due to the effects of the gas and exercise.

The software, though robust in its automated edge-detection abilities and calculation of blood velocity and vessel diameter [117] also presents some limitations. It is possible that any movement of the ultrasonographer or the subject could alter the image quality obtained by the ultrasound. As such, the area of analysis could move out of the ROI as defined on the user by the computer system. This effect will alter what pixels the software will detect as the edge of the lumen and therefore diameter measurements. However, it should be noted that such effects should be minimal as the software analyzes the image file at 30Hz and any momentary discrepancies in edge detection would be minimized over the course of the video. As well, if a "false" peak diameter was detected at any point, or if there were any discrepancies in diameter measurements, the user is able to remove specific sections of the video so that it would not be included in the analysis.

2.5.8 Summary

In conclusion, this is the first study to investigate the acute effects of changes in P_aO_2 at rest and during exercise in young, healthy individuals. Our findings demonstrated that there is no alteration in FMD after exercise when exercising at the same relative intensity in hypoxic or hyperoxic conditions. This is an important finding as alterations in oxygen content via O_2 administration and altitude are used as a training tool to help increase aerobic capacity. An important implication of this work may also be the clinical application of this where hypoxemia is associated with cardiovascular risk. As we gain a better understanding of the impact in healthy individuals, the findings need to be further investigated in situations where patients experience chronic or repetitive bouts of hypoxemia, many of whom are given supplemental oxygen to improve daily functional capacity or during exercise rehabilitation.

More work is needed to understand these effects in other populations (women, sedentary, aged etc) and the endothelial response to exercise as well as how changes in P_aO_2 interact with the endothelium. Much work is needed before we can begin to identify the mechanisms that impact the endothelium during these exposures to altered oxygen tension. This pilot work has demonstrated no effect of gas exposure and relative exercise in this cohort on FMD. In this context, we can begin to explore and expand upon this data in order to gain a better understanding of these stimuli and the application of FMD in the acute exercise model.

CHAPTER 3

THESIS DISCUSSION

The vascular endothelium plays a key role in maintaining vascular homeostasis and overall cardiovascular health. Changes in the vascular milieu in health and exercise can alter the state and functioning of the endothelium, eventually resulting in vascular remodelling [92]. Acute moderate intensity exercise has shown to be a powerful stimulus to improve FMD response in individuals who have pre-existing endothelial dysfunction [101, 102]; however, the impact on healthy individuals is less clear [95, 97, 101], with variations in study populations, methodology and timing potentially impacting the divergent results. The effects of hypoxic or hyperoxic gas exposure are also not well studied in healthy individuals. It is known that hypoxic exposure during exercise can increase vasodilatory response, from augmented activity of substrates such as NO [27], which could potentially increase the response to reactive hyperemia after exercise. With increased blood flow it is possible that the endothelium is more sensitive to shear stress, increasing NO production, which could play a role in the aerobic adaptations to hypoxic exercise. Whereas hypoxic exposure can increase blood flow, hyperoxic exposure during exercise has been shown to decrease blood flow [122]. This could possiby be due to increased oxidative stress from elevated P_aO_2 [45] could alter the prooxidant/antioxidant balance and cause upstream disruptions in vascular functioning.

Based on these observations, we hypothesized that exercise in hyperoxic conditions would cause no change in FMD response, whereas an increase would occur after hypoxic exercise. However, our data indicates that exercise does not alter relative or absolute FMD, regardless of the gas exposure in young, healthy, trained males. Furthermore, baseline

diameter, shear rate area under the curve, time to peak dilation and total shear rate did not differ between gas conditions nor was there any difference before or following exercise. Although this is somewhat surprising for this type of exercise (ie moderate intensity vs marathon running [96]), there are several mechanistic and methodological reasons why P_aO_2 and exercise have no effect on vascular function in this specific subset of individuals.

The exact mechanisms of exercise hyperemia are not fully understood. Adenosine, ATP as well as prostaglandins and NO have all been considered players in the vasodilatory response of exercise [55]. Mechanical deformation causing release of intracellular calcium, as well as the stimulation from agonists such as bradykinin can induce other substances to be released [88]. It is this repetitive stress from regular exercise training that causes the vasculature to remodel itself to accommodate increases in blood flow such that in trained individuals, acute exercise may have less of an affect than in an individual who is less active. In trained individuals, it is not necessarily less shear stress but the ability to accommodate elevated exercise hyperemia better due to vascular remodelling, such that the FMD response may not be as great.

In low oxygen situations, NO can also be released as oxygen hemoglobin saturations change causing ATP to be released [79], activating downstream vasodilatory mechanisms [31]. Offloading of oxygen from hemoglobin can also produce NO as nitrite reacts with the deoxygenated hemoglobin, using it as the reductase [35]. During hypoxic exercise, increased NO is produced which contributes to increased vasodilation see during this condition [41]. However, during rest when the FMD measurements are taken there was little to no change in arterial saturation with hypoxia. Therefore the deoxygenated hemoglobin signal that increases the bioavailability of NO seen in hypoxic situations is likely reduced. Additionally,

despite vasodilation and increased blood flow, hypoxic exposure is associated with increased sympathetic activity [143] and increased oxidant production, even in individuals with robust antioxidant status [144]. It is possible that exercise could have exacerbated the ROS production through pathways such as the xanthine oxidase [145]. This could further attenuate the FMD response by sequestering any available NO or other active vasodilators that could be activated, negating the supervasodilatory affects of hypoxic exercise and diminishing the potential effects of exercise on FMD.

The use of FMD in the acute model is still relatively new [111] and optimal timing of the post FMD measurement is still not known. Some studies take the measurement immediately post exercise [95], whereas others take the measurements 30 minutes [114] (such as in our study), 45 minutes [101] and one hour [97] post exercise. These studies show disparate results, potentially from the differences in protocol and subject population, or the use of a discrete time to take the post exercise measurements. Perhaps to "normalize" between individuals and studies, such that results between studies can be more comparable to isolate specific mechanisms, the timing of FMD after exercise should be based on a physiological measure for example, return to baseline blood velocity in the vessel of interest. The timing of velocity returning to baseline can also be an indicator of vascular function as the body returns to homeostasis and in itself can be an interesting finding in the acute exercise response.

The continued hypoxic exposure during recovery could have also negated the typical drop in sympathetic tone seen after activity, blunting the response to reactive hyperaemia after exercise [146], without changing baseline diameter values. Typically, muscle sympathetic nerve activity and vascular resistance is diminished by approximately 30% after

exercise [147]. However, as our subjects continued to breathe the hypoxic gas during recovery, this decrease in sympathetic activity may not have occurred, and may have acted to diminish the FMD response [146]. However, whether this was a sufficient stimulus is not known, as varying methods of stimulating sympathetic nerve activity can alter the effect on FMD [148]. In contrast to this argment, increases in sympathetic stimulation have been shown to have no affect on femoral FMD in young individuals after exercise [149]. It can also be argued that exercise in the NOX condition, that FMD should increase due to elevated NO production from exercise [150], and decreased vascular tone. It would be interesting to see whether exercise in hypoxia blunts the FMD response overall, or whether it changes the time course in which FMD returns to baseline. As mentioned before, the time course of FMD measurements after exercise is an area that still needs to be explored. Perhaps this scenario is one in which relative physiological measures (i.e. taking post exercise measurements upon return to baseline values) would provide more insight into the post exercise response to alterations in vascular milieu and the mechanisms behind this response.

Supraphysiological levels of oxygen are associated with increased oxidative stress [52] which can impair vascular function through increased vascular tone and resistance [42]. It is possible that the elevations in oxidative stress sequester available NO [46], converting it to the highly cytotoxic reactive oxygen species perioxynitrate, diminishing the vasodilatory capacity of the smooth muscle. Perioxynitrate, and other oxidants can decrease NO production by impairing eNOS functioning by reacting with tetrahydrobiopterin, a cofactor required for eNOS to produce NO [49]. Reducing substrates required for eNOS functioning as well as eNOS uncoupling compromises NO production [151] and subsequently impairs endothelial function [50]. Conversely, it is also possible that our subjects who are healthy,

well-trained, and express no cardiovascular risk, have a high antioxidant capacity and are able to withstand large elevations in oxidant production [152]. Trained individuals have an elevated antioxidant reponse 30 minutes after exercise [153]. Perhaps the increased oxidant production that could have occurred with hyperoxic exercise caused a supercompensatory response in which elevated antioxidants were released to counteract such ROS activity. However, further work which would include blood measures would be required to confirm this.

Another potential mechanism explaining the lack of exercise effect on FMD could be the hyperoxic exposure during recovery that the subjects received. Exposure to 100% oxygen has also shown to increase sympathetic nerve activity and increase vascular tone and resistance [154], which could alter the dilatory response in FMD [146], overcoming the hyperemic effects of exercise. However, blood pressure is normally not altered with hyperoxia, thereby maintaining homeostasis. Additionally, hyperoxia alters the vasodilatory signals from hemoglobin due to the occupation of oxygen binding sites on the hemoglobin molecule [81] so it is possible that the vasodilatory stimulus from hemoglobin was diminished in this condition. As such, it could be possible that any supercompensatory effects that exercise could have had on NO production from the unloading of oxygen from hemoglobin was not negated by oxidative stress but rather the lack of the agonist release from oxygenated hemoglobin. The lack of effect of hyperoxia on NO function is supported by a previous study which reported that breathing hyperoxia did not alter the NO mediated vasodilatory response or stimulated NO bioavailability [155]. This demonstrates that it is not the hyperoxic effects on NO directly, but potentially it is the effect of hyperoxia on the signalling processes responsible for NO production.

The mode of exercise coud have also affected the FMD response. It is possible that because we used cycling, a primarily lower leg exercise as our exercise stimulus, and measured brachial artery FMD, that our stimulus was not optimal for seeing a brachial artery response, as there are limb specific adaptations to exercise [96, 156]. However, cycling exercise has been demonstrated to elicit the shear stress in the brachial artery required for vascular remodelling, even though it is a non-exercising limb [131, 157]. Increases in cardiac output and blood flow, even to non-exercising limbs should elicit an exercise–induced hyperemic response. Typically, blood flow is restricted to inactive muscle beds due to increases in sympathetic nerve activity [131], but as exercise continues, this vasoconstriction is reversed. This functional sympatholysis occurs as the initial sympathetic nerve activity is overridden by local vasodilatory mediators causing limb vasodilation [158]. Not only does increased cardiac output and therefore elevated shear stress and circulating vasodilatory factors induce vasodilation, but also a thermoregulatory affect occurs, with inactive muscle beds dilating in order to help thermoregulate the body [131, 159]. As vasodilation occurs, sensitivity to shear stress may increase, further releasing other vasodilatory factors and perpetuating the vasodilatory process [57]. However, because we had the subjects recover for 30 minutes, it is possible that the vasodilatory effects in the brachial artery had returned to baseline by the time the measurements were conducted, resulted in no change from baseline.

Not only does the nature of exercise affect the post exercise response but the subject population also can alter the magnitude of FMD response. It is possible that the training background of our subjects were too varied to see a consistent trend with a cycling protocol. Some of our subjects were recreationally active (n=4), others were aerobically trained (n=5), whereas others were strength trained (n=4). As well, because of their training status, it is

possible that our protocol or perhaps our cuff placement did not induce enough shear stress as many are endurance trained. The intensity of exercise has shown to impact endothelial response, with moderate intensity being the optimal stimulus [95]. As average heart rates ranged around 150–160 bpm, our stimulus was likely under anaerobic threshold for individuals in this age group and of moderate intensity. As these individuals appear to have remodelled vessel structure, with large resting diameters of 4.0±0.4 mm, it is possible that their vasculature is accustomed and normalized to repetitive shear stress or that the shear stress was not high enough to elicit a response (see Chapter 2) [91, 92].

3.1 Methodological Limitations

There are several physiological reasons why there was no detectable change in FMD before and after exercise in each gas condition, whether it was cohort specific (i.e. characteristics of trained individuals), or gas specific (i.e. continual exposure during recovery) or limb specific (i.e. brachial vs femoral artery). The application of FMD in the acute exercise model has been increasing in recent years [111] and it is still relatively new. There are no consistent results in the literature regarding acute exercise. Differences in timing, exercise duration, intensity, subject population and methodology could account for the disparity. Several guidelines have been published, attempting to update and standardize methodological considerations [57, 67, 71]; however, there are still differences in analysis methods [104, 117] and cuff placement [97, 124], both of which are known to alter FMD results [99, 105]. As well, depending on the age [160], training status [97, 102] and level of cardiovascular risk [149], the magnitude of the FMD response can differ. However, as a non-invasive method, FMD is attractive to use after either whole body [161] or local [162]

exercise to help provide more insight into endothelial responses regarding different modes of exercise might [163, 164]. Although there may be discrepancies between studies, which could account for varied results in the literature, we took great care to ensure that each exercise session was normalized such that only the influence of gas and exercise would be assessed. Exercise intensity was normalized between gas conditions in order to elicit the same relative stimulus and other subject factors such as activity, dietary intake and circadian affects were tightly controlled. As proposed in chapter one, this FMD response to acute exercise could be part of a U-shaped relationship where the FMD of healthy individuals do not respond to acute exercise because of their already robust vascular and endothelial structure. This lack of response an interesting finding and adds to the growing body of literature on the acute affects of exercise on FMD. This is the first study assessing altered oxygen tension as well as exercise; therefore, we have formed a basis to pose future questions to attempt to better understand the mechanistic alterations the vasculature undergoes when exposed to these conditions in certain populations.

3.2 Future Studies

Although the findings in this study indicated no affect of gas exposure and exercise, there are still many more questions that arise from this pilot work. The subject population were young, male and recreationally active. Studying the sex-differences with the same background could give different insight into the estrogen affects on vascular response in these conditions. Females tend to have smaller vessel diameter so it could respond very differently to males in response to shear stress. As well, it is known that estrogen can improve endothelial function, similar to that of shear stress [113]. Heat shock proteins,

which are stimulated by shear stress and estrogen can phosphorylate eNOS, release NO [165]. Studying menstrual cycle effects on FMD can allow us to further understand the sensitivity of the endothelium to changes in estrogen and progesterone. Additionally, we can better understand whether hormone supplementation, such as oral contraceptives have any impact on endothelial function and cardiovascular health. Even though exercise has already been documented to be as beneficial as hormone therapy [113], it is not yet know whether changes in oxygen tension will impact these positive effects. Studying sex effects is important as it is known that cardiovascular disease affects women and men differently.

Studying the training effects on the vasculature (ie: with same level of fitness or training specificity) could provide a better understanding about the cardioprotective effects of exercise and how we can apply them to other populations. For example, for a range of exercise intensities, understanding how low fitness individuals experience vascular benefit at a lower or higher exercise intensity compared to high fitness individuals would be important in designing rehabilitation programs. As well, would the nature of exercise impact the response as effectively, for example, intervals vs constant load trials? It is known that high intensity interval training has the same FMD effects as long slow endurance training in young healthy populations [166], however, whether this is applicable in other populations, is not known. Aging has also been shown to affect FMD response, particularly to exercise [160]; therefore, it would be interesting to assess the increases in oxidative stress and arterial stiffness associated with age and how it might alter the vascular response to acute and chronic exercise.

Future studies could also be performed to assess the relationship between chronic and acute effects of exercise and P_aO_2. In clinical patients, who experience chronic hypoxemia at

rest, FMD is diminished [167]. Acute exercise is known to increase inflammation in this group as well [168]. Whether correcting this chronic hypoxemia in an acute bout of exercise can improve oxidant and inflammatory status, potentially improving FMD is not yet known. In essence, this design would be the opposite of the present study, where we induced hypoxemia in healthy normoxic individuals. Our individuals had a presumably robust antioxidant, anti-inflammatory status, and it would be interesting to understand the exercise cardioprotective mechanisms that affect cardiovascular and endothelial functioning from an oxidative stress and inflammatory standpoint in a clinical group. This is particularly relevant in situations where individuals may receive domiciliary oxygen (e.g., patients with COPD) and may also participate regularly in pulmonary rehabilitation.

As discussed above, circadian rhythm is understood to have a role in the post exercise hypotension response [114, 127]. It would be relevent to assess if P_aO_2 affects may be altered on the time of day, and whether there would be a greater affect on FMD with and without exercise (i.e. are the post hypotensive differences to exercise in the morning and afternoon more sensitive to alterations in oxygen tension?). Again, this could have major implications for pulmonary rehabilitation in order to gain the maximal benefit from such therapy. If the P_aO_2 affects are more powerful at a certain time of day, it would be ideal to have patients exercise at a time that is suitable for their disease status in order to gain the maximal benefit.

A future study could also assess the effects of a more severe hypoxic exposure, than used in the present study on FMD. The nitrite red blood cell-dependent vasodilation is initiated at approximately 40mmHg [36] thus to see the link between this mechanism and possible effects on FMD, a lower P_aO_2 will need to be assessed than the one used in the present study. The rationale for the selection of our stimulus was that it was translatable since

it is commonly used in athletic training scenarios. However, using a more severe stimulus could give us more insight into the vascular impact on other sports, such as mountaineering, particularly because it requires physical exertion in addition to the strong hypoxic exposure. Blood measures could also help elucidate the $P_aO_2$affects on the vasculature, particularly in combination with exercise. Examples could be plasma and red blood cell nitrite content to ascertain level of NO activity, oxidant/antioxidant measures, oxidative stress as well as sympathetic nerve activity. However, the timing of these measurements in conjunction with other vascular measurements such as FMD after exercise needs to be better understood. Using FMD in the acute exercise model is still relatively new and lacks confirmation with other studies and other biological markers [111]. This also may differ in other populations such as those who are sedentary or have a clinical condition as their response to acute exercise may differ.

3.3 Conclusion

Our data indicate that one bout of moderate exercise breathing a hypoxic, normoxic or hyperoxic gas does not alter FMD 30 minutes after exercise in young, trained males. Whether these findings are applicable in other populations is unknown. Although it is difficult to begin comparing results between studies due to differences in methodology and subject population, this study adds to the growing body of literature regarding endothelial responses to acute exercise. We are only now beginning to better understand the mechanisms behind the cardiovascular effects of exercise and the importance of physical activity in maintaining the health and homeostasis of the body.

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APPENDICES

Appendix A – Informed Consent

INFORMED CONSENT FORM

Title of Project: The effect of altered oxygen tensions and exercise on blood flow regulation
Investigators: Neil Eves, PhD (Principle investigator)
Philip Ainslie, PhD
Lisa Wong, MSc candidate
Kurt Smith, PhD candidate
Institution: School of Human Kinetics
University of British Columbia
Contact Person: Lisa Wong
Email: lewong@interchange.ubc.ca

INTRODUCTION: You are being invited to participate in this study because you are a healthy male/female between the ages of 18 and 40. If you have a documented history of diabetes, cardiovascular disease or any injuries limiting your ability to perform exercise, you are not eligible for this study. Also, if you smoke, are overweight (body mass index ≥30 kg/m^2), or have a resting blood pressure over 140/90mmHg, then you are not eligible to participate. Pregnancy or not being able to comfortably communicate in English will also exclude you from participation.

YOUR PARTICIPATION IS VOLUNTARY: Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision. All results will be kept confidential.
Please take time to read the following information carefully and to discuss it with your family, friends, and/or doctor before you decide.

BACKGROUND: The effects of breathing different amounts of oxygen during exercise and how it might affect your blood vessels is not understood. Understanding how breathing different levels of oxygen affects your blood vessels is important to allow us to understand how going up mountains and diseases where patients have low oxygen levels in the blood may affect the way our blood vessels work. Exercise has been shown to help keep your blood vessels healthy but how exercise and different amounts of oxygen affect how your blood vessels work is not known.
PURPOSE: The purpose of this project is to investigate how the blood vessels in your arms and brain respond to exercise while breathing either a low, normal or high amount of oxygen. We will investigate exercise that starts easy and gets harder and exercise which stays at the same load for a 40-minutes to see if different types of exercise have a different effect on how your blood vessels respond.
WHO CAN PARTICIPATE IN THIS STUDY: Healthy males/females between the ages of 18 and 40 with no history of diabetes, cardiovascular disease or any injuries limiting

your ability to perform exercise. Also, if you smoke, are overweight (body mass index ≥30 kg/m^2), or have a resting blood pressure over 140/90mmHg, then you are not eligible to participate. Pregnancy or not being able to comfortably communicate in English will also exclude you from participation.

WHAT DOES THIS STUDY INVOLVE?: This pilot study will need you to come to the exercise physiology laboratory at the University of British Columbia Okanagan on six separate occasions. 12 volunteer subjects will be enrolled for the study. The first three visits will consist of exercise which starts easy and gets harder until you feel tired and have to stop. During these three tests, you will breathe either low oxygen, high oxygen or room air. We will do our blood vessel measurements of your brain and arm before and after exercise to see how the exercise in each condition has affected you. We will also monitor your blood flow and oxygen levels throughout the test. The order of these visits will be random so you will not know which gas you are breathing. The next three visits will consist of three exercise tests that will be at a constant intensity for 40min (which is the equivalent of a fairly moderate workout). Just like the first three visits, you will breathe a different gas each test (ie: low oxygen, high oxygen and room air), and again the order will be random. We will do our vascular measurements before and after to again see how the exercise may have affected your blood vessels. Prior to each visit, we will ask you to eat a light, low fat meal, however this must be at least 4 hours before you come in as you cannot eat for least 4 hours prior to the testing session. You will also be asked to stop all vitamin and nutritional supplements for a week before testing and to not have any caffeinated products, alcohol, participate in exercise, or smoke (including secondary exposure to smoke) for at least 24 hours prior to testing.

PROCEDURES: If you decide to participate, you will be asked to come into the laboratory on six occasions.
Visits 1 through 3 will be randomized so you do not know which gas you are receiving. Tests will be separated at least 48 hours apart.

Incremental Exercise Test, Brain Blood Flow and Brachial Blood Flow Measurements (Time commitment = 1.5 hours / visit)

Prior to the exercise test, we will have you lie on a bed and breathe the selected gas mixture for 15minutes. At the end of this breathing period, we will complete our first set of arm and brain blood vessel measurements, prior to exercise. During the exercise test, we will monitor your oxygen saturation levels and take measurements of your blood flow through your arm and brain using non-invasive ultrasound. After the exercise test, and a standard cool-down, we will have you continue breathing the gas while lying down and will repeat these vascular measurements after 30 minutes.

(1) Incremental Exercise Test

- **What are these?** This exercise test starts easy and slowly gets harder until you become tired and cannot exercise any more. This test will be done on a stationary bicycle and you will breathe through a mouthpiece while wearing a

nose clip to collect your expired air. You will breathe either room air (21% oxygen), a low oxygen mixture (16% oxygen) or a high oxygen mixture (100% oxygen). Although we will require approximately 45 minutes preparing and performing this test, you will only exercise for approximately 8-12 minutes.

(2) Blood flow

- **What is this?** This measurement is done by using an ultrasound to send a sound wave into your arm to tell us how fast blood is moving through your blood vessel and also takes a picture of your vessel so we can measure its diameter. This is a safe, non-invasive technique and will be done periodically during exercise and recovery.

(3) Flow-mediated dilation (FMD)

- **What is this?** This measurement is done by using an ultrasound to send a sound wave into your arm that tells us about how your blood vessels respond following occlusion of blood flow in your arm. This will be done with you lying down and having a pumped up blood pressure cuff on your arm for 5 minutes. We will then do an ultrasound of your brachial artery as it responds to the increased blood flow following the release of the blood pressure cuff. We will do two measurements:
    - First at rest, breathing the gas mixture for ten minutes
    - Thirty minutes after the exercise test while still breathing the gas mixture.

(4) Brain Blood Flow measurements

- **What is this?** This measurement is used to monitor the speed of blood flow to the blood vessels feeding your brain. A probe will be attached, via a comfortable fitting headband, to your cheekbone on your head to monitor blood flow velocity. This probe uses an Doppler ultrasound system, which is a device that measures how fast your blood is moving by sending tiny sound waves out and seeing how they reflect back. It is a safe and non-invasive technique.

Visits 4 – 6 will be separated by at least 48 hours apart.

Exercise Trial and FMD (Time commitment = 3.0 hours/visit)

This session will be used to measure the blood flow response to 40 min exercise session at a set intensity, breathing room air, low oxygen or high oxygen. You will not be told what gas that you are breathing. Prior to the test, we will have you rest while breathing the gas mixture for 15 minutes while we do our baseline set of brain and arm blood vessel measurements. We will then have you exercise, where we will closely monitor your oxygen levels. After the exercise trial you will do a standard 5 minute recovery and then rest for 30min. At the end of this recovery period, we will do our blood vessel measurements on you again. As well, just like the graded exercise tests, we will measure blood flow throughout the test and during your recovery.

(1) Constant load Cycling Time Trial

- **What is this?** This session will be performed on a cycle ergometer and will require you to complete 40 minutes of exercise at moderate intensity. During the test we will collect the gases you breathe out by getting you to breathe through a mouthpiece while wearing a nose clip. We will also deliver the gas to you through this valve as you exercise. During the test we will measure your heart rate by placing a strap around your chest and we will measure your blood oxygen levels by a small probe placed on your forehead.

RISKS:

- Exercise: The exercise that you will be performing is regarded as safe. Graded exercise test data from other investigations, suggest that the likelihood of dying from sudden cardiac death is 2 per 100,000 tests. This usually only occurs in people who already have some form of heart disease. Following all of the exercise sessions you may experience muscle soreness, which will disappear within a few days.

- Hypoxia: There are no risks associated with mild exposures to high altitude, a condition that will be simulated in this experiment. The level of low oxygen (hypoxia) that you will be exposed to is equal to approximately 3500 m. This is approximately equal to being at the summit of Catskill Mountain, New York. At this level of simulated high altitude you will breathe more quickly and more deeply. These sensations will go away very quickly when the mouthpiece is removed and you breathe room air. Your responses to the exposures to low levels of oxygen will be monitored during the test, and the test will be terminated if abnormal responses are observed (not anticipated). There is no risk of developing altitude sickness over the short exposure (mild symptoms sometimes develop >6 hrs). The low oxygen level we are using is also higher than conditions used in previous studies.

- Ultrasound: Ultrasound is non-invasive, painless technique used for measuring blood flow in this experiment. It poses no risk.

BENEFITS: You will not benefit directly from participation in this study however you will be provided with the fitness results of your exercise tests which could assist you to improve your exercise training. Your participation in this experiment will contribute an understanding of the effects different oxygen levels on blood vessel response and how different exercise conditions might play a role. This knowledge can be used to gain a better understanding of how blood vessels are affected by different exercise and oxygen levels and can provide us with a better understanding

of the cardiovascular responses to exercise. This is particularly relevant for individuals with chronic diseases and poor arterial health.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE? Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected.

The study doctor(s)/investigators may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interests.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law, this data cannot be destroyed.

COMPENSATION AND INJURY: Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION? A trained research assistant will be available on every occasion to explain the procedure and answer any questions. If you have any other questions or desire further information about this study before or during participation, you can contact Dr. Neil Eves at (250) 807 - 9676.

WHAT WILL THE STUDY COST ME? There will be no reimbursement for participating in this study. You will, however, receive your fitness results.

CONFIDENTIALITY: Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada or the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY? Please note that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services' at 604-822-8598 or by email at RSIL@ors.ubc.ca.

CONSENT: In signing this form you are consenting to participate in this research project. Furthermore, signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

- *I have read and understood the subject information and consent form.*
- *I have had sufficient time to consider the information provided and to ask for advice if necessary.*
- *I have had the opportunity to ask questions and have had satisfactory responses to my questions.*
- *I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*
- *I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*
- *I understand that I am not waiving any of my legal rights as a result of signing this consent form.*
- *I understand that there is no guarantee that this study will provide any benefits to me.*
- *I have read this form and I freely consent to participate in this study.*
- *I have been told that I will receive a dated and signed copy of this form.*

_____	_____	_____
Signature of Subject	Printed name	Date
_____	_____	_____
Signature of Witness	Printed name	Date
_____	_____	_____
Principle Investigator or/ designated representative	Printed name	Date

Appendix B – Raw Data

Table A.1 Raw subject data

Subject #	Time of Day of Test	Age	Weight (kg)	Height (cm)	Body Mass Index	Fitness (W/kg)
1	800	20	81	182	24.5	4.07
2	1530	22	85	187	24.3	4.94
3	800	20	63	172	21.3	4.29
4	830	23	69	174	22.8	4.35
5	1230	21	72	180	22.2	5.00
6	1600	24	70	180	21.6	3.43
7	800	31	95	182	28.7	4.11
8	700	25	81	171	27.7	4.07
9	530	20	77	174	25.4	5.06
10	715	20	68	177	21.7	4.85
11	845	20	81	187	23.2	6.30
12	1100	18	65	171	22.2	5.54
13	1700	19	82	185	24.0	5.12

Table A.2 Raw exercise data. NOX = 21%O_2, HYPO = 16%O_2, HYPER = 100%O_2, HR = Heart rate, bpm = beats per minute, GXT = Graded exercise test, W = Watts, S_pO_2 = Percentage of oxyhemoglobin saturation, CLT = Constant load trial, WMax = Work rate max

Subject #	Gas	Resting HR (bpm)	GXT REST S_PO_2	GXT MAX S_PO_2	GXT Max (W)	GXT MAX HR	CLT S_PO_2	CLT HR (bpm)	CLT (W)	%W Max
1	NOX	66	99	95	330	175	95	172	180	55
2	NOX	65	99	95	420	190		173	210	50
3	NOX	75	98	96	270	199	100	153	135	50
4	NOX	67	99	96	300	194	96	150	135	45
5	NOX			87	360			156	180	50
6	NOX	73	96	97	240	185	98	164	180	75
7	NOX	66	100	96	390	195	96	178	195	50
8	NOX	73	99	95	330	173	98	140	165	50
9	NOX	65	99	98	390	190	98.9	153	185	47
10	NOX	82	97	91	330	200		159	180	55
11	NOX	69	99	95	510	190	99	168	195	38
12	NOX	64	98	88	360	207	95	161	180	50
13	NOX	72	98	95	420	210		175	210	50
1	HYPO	62	98	82	300	180	88	165	165	55
2	HYPO	74	99	83	420	198	85	179	210	50
3	HYPO	64	97	93	270	204	92	170	135	50
4	HYPO	72	97	77	310	200	89	159	125	40
5	HYPO			88	360	165	86	163	180	50
6	HYPO		99	90	330	190	95	166	165	50
7	HYPO		98	84	360	198	90	174	180	50
8	HYPO	73	100	90	300	178	87	149	150	50
9	HYPO	73	100	90	360	184	94	152	180	50
10	HYPO		93	79	300	183	89	158	150	50
11	HYPO	74	98	89	360	190	89	164	180	50
12	HYPO	74	97	81	330	206	91	160	165	50
13	HYPO		98	87	390	210	90	177	195	50
1	HYPER	70	96	100	330	174	100	161	180	55
2	HYPER	61	99	100	480	198	100	173	240	50
3	HYPER	74	100	99	300	205	99	170	150	50
4	HYPER		100	97	300	194		142	150	50
5	HYPER	72	100	96	360			157	210	58
6	HYPER		99	98	360	188	100	156	180	50
7	HYPER		99	99	420	199	99	165	210	50
8	HYPER	63	100	99	330	171	99	127	165	50
9	HYPER	67	98	100	420	190	99	149	210	50
10	HYPER		97	97	390	200		165	195	50
11	HYPER	69	98	99	540	190	100	163	210	50
12	HYPER		100	98	390	202	99	155	195	50
13	HYPER		100	97	450	210		175	225	50

Table A.3 Raw flow-mediated dilation (FMD) data for normoxia (NOX)
Pre = Before exercise, Post = 30 minutes after exercise, BL = baseline diameter, Δ = difference between before and after exercise, Avg = average, SR = Shear rate, SRAUC = Shear rate area under the curve until peak dilation, TTP = time to peak dilation

Subject #	BL pre (cm)	BL post (cm)	BL Δ	FMD Peak pre (cm)	FMD Peak post (cm)	FMD cm diff	FMD Peak pre (%)	FMD Peak post (%)	FMD % Δ	Avg SR pre (1/s)	Avg SR post (1/s)	Avg SR Δ	SRAUC pre (ttp)	SRAUC post (ttp)	SRAUC Δ	TTPpre (sec)	TTP post (sec)	TTP diff
1	0.375	0.385	0 01	0.391	0.4 6	0.015	4.27	5.45	1.18	382.93	542.84	159.92	36550.65	58010.93	21460.28	95.46	106.84	11.379
2	0.445	0.425	-0.02	0.474	0.448	-0.026	6.52	5.41	-1.11	289.92	378.58	88.66	16476.92	23372.47	6895.55	56.79	61.73	4.946
3	0.337	0.35	0.013	0.372	0.373	0.001	10.39	6.57	-3.82	580.18	358.38	-221.81	17516.22	29637.01	12120.79	30.20	82.73	52.526
4	0.393	0.402	0.009	0.417	0.415	-0.002	6.11	3.23	-2.88	376.18	401.95	25.78	13980.01	19649.61	5669.60	37.17	48.89	11.721
5	0.375	0.345	-0.03	0.403	0.367	-0.036	7.47	6.38	-1.09	481.48	458.66	-22.82	19726.38	40446.11	20719.73	40.98	88.18	47.203
6	0.373	0.369	-0.004	0.397	0.4	0.003	6.43	8.4	1.97	486.79	450.52	-36.27	19848.51	16926.48	-2922.03	40.78	37.57	-3.203
7	0.448	0.445	-0.003	0.468	0.526	0.058	4.46	18.2	13.74	322.95	454.08	131.13	14266.38	23154.20	8887.83	44.19	51.01	6.822
8	0.438	0.417	-0.021	0.462	0.461	-0.001	5.48	10.55	5.07	328.91	453.65	124.74	24579.83	15269.38	-9310.45	74.77	33.68	-41.09
9	0.462	0.462	0	0.47	0.477	0.007	1.73	3.25	1.52	280.23	252.53	-27.70	10171.82	6959.19	-3212.63	36.30	27.58	-8.718
10	0.357	0.352	-0.005	0.383	0.386	0.003	7.28	9.66	2.38	547.37	652.12	104.76	18775.98	30270.78	11494.81	34.34	46.44	12.1
11	0.34	0.341	0.001	0.365	0.358	-0.007	7.35	4.99	-2.36	394.72	275.94	-118.78	19383.69	23652.09	4268.40	49.09	85.74	36.648
12	0.389	0.394	0.005	0.397	0.407	0.01	2.06	3.3	1.24	190.14	399.32	209.18	6086.44	15809.72	9723.28	31.98	39.60	7.625
13	0.426	0.423	-0.003	0.459	0.453	-0.006	7.75	7.09	-0.66	419.13	246.27	-172.86	16329.11	29832.45	13503.35	38.97	121.23	82.266

Table A.4 Raw flow-mediated dilation (FMD) data for hypoxia (HYPO)
Pre = Before exercise, Post = 30 minutes after exercise, BL = baseline diameter, Δ = difference between before and after exercise, Avg = average, SR = Shear rate, SRAUC = Shear rate area under the curve until peak dilation, TTP = time to peak dilation

Subject #	BL pre (cm)	BL post (cm)	BL Δ	FMD Peak pre (cm)	FMD Peak post (cm)	FMD cm diff	FMD Peak pre (%)	FMD Peak post (%)	FMD % Δ	Avg SR pre (1/s)	Avg SR post (1/s)	Avg SR Δ	SRAUC pre (ttp)	SRAUC post (ttp)	SRAUC Δ	TTP pre (sec)	TTP post (sec)	TTP diff
1	0.366	0.37	0.004	0.384	0.392	0.008	4.92	5.95	1.03	650.07	633.85	-16.22	24768.98	52022.43	27253.46	38.11	82.11	43.99
2	0.438	0.415	-0.023	0.456	0.447	-0.009	4.11	7.71	3.60	189.09	339.53	150.44	17671.26	15989.79	-1681.48	93.35	47.08	-46.27
3	0.35	0.331	-0.019	0.373	0.374	0.001	6.57	12.99	6.42	406.25	355.00	-51.25	18325.04	15604.28	-2720.75	45.09	43.98	-1.11
4	0.375	0.391	0.016	0.409	0.402	-0.007	9.07	2.81	-6.26	358.58	343.65	-14.93	10260.82	16884.75	6623.94	28.62	49.16	20.54
5	0.376	0.368	-0.008	0.388	0.377	-0.011	3.19	2.45	-0.74	551.53	396.31	-155.22	21909.42	29633.47	7724.06	39.75	74.78	35.03
6	0.376	0.379	0.003	0.397	0.394	-0.003	5.59	3.96	-1.63	277.14	354.67	77.53	17929.77	17775.54	-154.23	64.68	50.15	-14.52
7	0.409	0.468	0.059	0.48	0.491	0.011	17.36	4.91	-12.45	488.93	454.32	-34.61	20849.81	26222.24	5372.43	42.66	57.73	15.07
8	0.512	0.488	-0.024	0.538	0.508	-0.03	5.08	4.1	-0.98	340.75	157.78	-182.97	12779.31	13259.86	480.55	37.51	84.09	46.58
9	0.451	0.425	-0.026	0.469	0.45	-0.019	3.99	5.88	1.89	364.34	164.87	-199.47	51665.72	470.83	-51194.89	141.81	2.85	-138.96
10	0.358	0.357	-0.001	0.387	0.384	-0.003	8.1	7.56	-0.54	458.89	591.72	132.83	17012.50	20673.27	3660.77	37.09	34.96	-2.14
11	0.355	0.335	-0.02	0.379	0.356	-0.023	6.76	6.27	-0.49	555.28	403.27	-152.01	16313.83	27268.14	10954.31	29.44	67.60	38.17
12	0.393	0.389	-0.004	0.405	0.393	-0.012	3.05	1.03	-2.02	195.05	389.97	194.92	14708.38	16175.01	1466.63	75.42	41.50	-33.92
13	0.388	0.398	0.01	0.414	0.421	0.007	6.7	5.78	-0.92	307.88	119.61	-188.27	18332.58	10819.62	-7512.96	59.56	90.34	30.78

Table A.5 Raw flow-mediated dilation (FMD) data for hyperoxia (HYPER)
Pre = Before exercise, Post = 30 minutes after exercise, BL = baseline diameter, Δ = difference between before and after exercise, Avg = average, SR = Shear rate, SRAUC = Shear rate area under the curve until peak dilation, TTP = time to peak dilation

Subject #	BL pre (cm)	BL post (cm)	BL Δ	FMD Peak pre (cm)	FMD Peak post (cm)	FMD cm diff	FMD Peak pre (%)	FMD Peak post (%)	FMD % Δ	Avg SR pre (1/s)	Avg SR post (1/s)	Avg SR Δ	SRAUC pre (ttp)	SRAUC post (ttp)	SRAUC Δ	TTP pre (sec)	TTP post (sec)	TTP diff
1	0.361	0.37	0.009	0.383	0.398	0.015	6.09	7.57	1.48	358.10	527.42	169.33	12175.96	20978.78	8802.82	34.01	39.73	5.72
2	0.393	0.408	0.015	0.429	0.426	-0.003	9.16	4.41	-4.75	462.45	315.37	-147.08	8075.19	13720.39	5645.20	17.47	43.53	26.07
3	0.324	0.33	0.006	0.345	0.351	0.006	6.48	6.36	-0.12	576.79	560.01	-16.78	23362.74	22530.32	-832.42	40.51	40.22	-0.29
4	0.408	0.396	-0.012	0.438	0.421	-0.017	7.35	6.31	-1.04	478.08	338.52	-139.57	12727.75	11965.33	-762.41	26.64	35.33	8.70
5	0.356	0.352	-0.004	0.369	0.373	0.004	3.65	5.97	2.32	485.00	462.23	-22.76	14348.20	22771.37	8423.17	29.61	49.27	19.66
6	0.374	0.376	0.002	0.393	0.391	-0.002	5.08	3.99	-1.09	405.32	334.35	-70.97	3872.92	22680.82	18807.90	9.58	68.02	58.44
7	0.42	0.441	0.021	0.458	0.468	0.01	9.05	6.12	-2.93	376.17	578.32	202.16	19364.26	33563.84	14199.57	51.48	58.06	6.58
8	0.451	0.44	-0.011	0.474	0.463	-0.011	5.1	5.23	0.13	361.14	364.78	3.64	11729.35	9976.02	-1753.33	32.49	27.38	-5.10
9	0.417	0.408	-0.009	0.448	0.449	0.001	7.43	10.05	2.62	134.00	176.77	42.77	13472.71	9759.23	-3713.49	100.47	55.24	-45.23
10	0.359	0.354	-0.005	0.392	0.398	0.006	9.19	12.43	3.24	372.52	516.52	144.00	19357.00	37935.16	18578.16	51.96	73.46	21.50
11	0.345	0.323	-0.022	0.358	0.349	-0.009	3.77	8.05	4.28	499.00	488.06	-10.94	20235.96	22707.45	2471.50	40.57	46.54	5.97
12	0.381	0.377	-0.004	0.381	0.386	0.005	0	2.39	2.39	213.39	417.60	204.21	17190.63	24129.12	6938.49	80.55	57.81	-22.75
13	0.37	0.375	0.005	0.386	0.422	0.036	4.32	12.53	8.21	366.05	345.98	-20.06	13545.17	19504.07	5958.90	37.03	56.38	19.35