Supramaximal Testing to Confirm the Achievement of $\dot{V}O_{2max}$ in Acute Hypoxia

by

Benjamin P. Thompson

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Kinesiology and Health Sciences

Waterloo, Ontario, Canada, 2023

AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

ABSTRACT

Incremental exercise tests are often used to quantify an individual's maximal oxygen uptake ($\dot{V}O_{2max}$). To confirm $\dot{V}O_{2max}$, a bout of supramaximal exercise can be performed after maximal exercise and if $\dot{V}O_2$ is within the measurement error (typically ~150 mL) one can presume a true $\dot{V}O_{2max}$ was reached. Upon exposure to acute hypoxia, $\dot{V}O_{2max}$ is known to decrease primarily due to decreases in convective oxygen transport. However, the decrease is variable between individuals, and it is unknown whether a supramaximal can similarly test if $\dot{V}O_{2max}$ is achieved because the power output will still be lower than that of normoxia. Therefore, the purpose of this study was to determine if supramaximal exercise test confirms the achievement of $\dot{V}O_{2max}$ in acute hypoxia. We hypothesized the incremental and supramaximal $\dot{V}O_2$ will be sufficiently similar in acute hypoxia. 20 healthy adults (n = 7 female) completed incremental and supramaximal exercise tests in normoxia and acute hypoxia (FIO_2 = 0.147) on separate days in a randomized order. The incremental exercise tests started at 80W & 60W for males and females in normoxia and 40W & 20W for males and females in hypoxia with a continuous increase of 20W/minute until volitional exhaustion. Following a 20min rest, the supramaximal test increased to 110% of peak power over 20 seconds and continued at constant power until volitional exhaustion. Cardiorespiratory variables were measured using a customized metabolic cart. Supramaximal exercise testing yielded a significantly lower $\dot{V}O_2$ than incremental testing in normoxia (3.87 ± 0.99 vs. 3.76 ± 0.92L/min) and hypoxia (3.27 ± 0.81 vs. 3.16 ± 0.78L/min). HR was significantly lower during the supramaximal test in normoxia (183 ± 7 vs. 176 ± 8bpm) and hypoxia (182 ± 6 vs. 175 ± 6bpm). SpO_2 was significantly higher in the hypoxic supramaximal test than incremental (82.2 ± 4.7 vs. 84.9 ± 4.1%) but not different in normoxia (94.7 ± 2.5 vs. 94.5 ± 4.8%). Equivalence testing indicated that supramaximal $\dot{V}O_2$ was not

sufficiently similar to incremental testing in acute hypoxia. However, the mean difference in incremental and supramaximal $\dot{V}O_2$ in normoxic and acute hypoxic was less than 150mL/min, and physiologically speaking, should verify supramaximal exercise testing in acute hypoxia

ACKNOWLEGMENTS

I would like to thank all the participants that volunteered their time and effort to participate in this study.
I would also like to thank my supervisor, Dr. Paolo Dominelli, for providing me with countless opportunities to learn and grow throughout my degree.
To my committee, Dr. Jason Au, and Dr. Glen Foster, I am grateful for your expertise and support throughout the project.
To my lab mates: Connor Doherty, Leah Mann, Sarah Angus, Jason Chan, Victoria Chang, Allie Pulford-Thorpe, Paige Rynne, and Maddie Wright, thank you for all the help and countless memories over the last couple years.
Finally, I would like to thank my family for their support and encouragement to pursue a master's degree in Canada.

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$AaDO_2$	Alveolar Arterial Oxygen Difference
$AvDO_2$	Arterial to Venous Oxygen Content Difference
C_aO_2	Arterial Oxygen Content
C_vO_2	Venous Oxygen Content
EDV	End Diastolic Volume
EFL	Expiratory Flow Limitation
EF	Ejection Fraction
ESV	End Systolic Volume
FEV	Forced Expiratory Volume
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
FIO_2	Fraction Inspired Oxygen
Hb	Hemoglobin
HH	Hypobaric Hypoxia
HPV	Hypoxic Pulmonary Vasoconstriction
HR	Heart Rate
NH	Normbaric Hypoxia
ODC	Oxy-hemoglobin Dissociation Curve
PCO_2	Partial Pressure Carbon Dioxide
PO_2	Partial Pressure Oxygen
$\dot{Q}$	Cardiac Output
RER	Respiratory Exchange Ratio
SaO_2	Arterial Oxygen Saturation
SpO_2	Oxygen Saturation
SV	Stroke Volume
$V_A/\dot{Q}$	Ventilation Perfusion Mismatch
$\dot{V}_E$	Expiratory Ventilation
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_{2max}$	Maximal Oxygen Uptake
$\dot{V}O_{2peak}$	Peak Oxygen Uptake
W_{max}	Maximal power (watt) output

1. LITERATURE REVIEW

1.1 Physiology of $\dot{V}O_{2max}$

1.1.1 Defining $\dot{V}O_{2max}$

Maximal oxygen uptake ($\dot{V}O_{2max}$), first introduced by Hill and Lupton in 1923, is defined as the maximum oxygen intake during intense exercise in which oxygen uptake can not be increased further, regardless of an increase in effort (1). A $\dot{V}O_{2max}$ test is the gold standard measurement for quantifying the combined integrative pulmonary, cardiovascular, and muscular response to convective oxygen delivery, diffusion into the lungs and tissues, and oxygen transport and utilization by the muscle mitochondria (2). The *Fick* equation defines $\dot{V}O_2$ as the product of cardiac output ($\dot{Q}$) and the arterial to venous oxygen content difference ($C_aO_2 – C_vO_2$) (see Equation 1).

Equation 1. Fick Equation: $\dot{V}O_2 = \dot{Q} * (CaO_2 – CvO_2)$.

Theoretically, as long as enough reactants are available and the products of oxidative phosphorylation are removed, there should be no limit to one's ability to utilize oxygen. However, the human body's ability to utilize O_2 is limited by enzyme and substrate availability and the delivery of O_2. So, to determine the limitations of $\dot{V}O_2$, we must further investigate each component of the Fick equation.

1.1.2 Cardiac Output

The volume of blood pumped from the heart per unit time, or cardiac output ($\dot{Q}$), is the product of heart rate (HR) and stroke volume (SV). The intrinsic contraction rate of the heart is 100 beats per minute, but parasympathetic stimulation of the heart slows the heart to ~60 beats

per minute at rest in the typical healthy adult. Stroke volume, the volume of blood pumped from the heart each beat, is determined by the difference between end diastolic volume (EDV, i.e., the volume of blood in the ventricle prior to contraction) and end systolic volume (ESV, i.e., the volume of blood in the ventricle post contraction). A typical healthy individual will have an EDV of ~120mL and an ESV of ~50mL for an SV of ~70 mL. Another way to determine the amount of blood leaving the heart each beat is to calculate the ejection fraction (EF). The EF is the percentage of blood pumped from the heart with each beat and is calculated by dividing the volume of blood pumped out of the heart by the volume of blood remaining in the heart post-contraction. A typical healthy heart has an ejection fraction between 50% and 70%. At rest, $\dot{Q}$ is ~5 L/min and increases linearly with $\dot{V}O_2$ to nearly quadruple resting values in healthy young adults during exercise via increased HR and SV (3). Increased HR is achieved via parasympathetic withdrawal and increased sympathetic output to the heart to allow the typical healthy young heart to reach ~200bpm near maximal exercise. Increased SV is achieved via enhanced sympathetic activity to the heart to improve the contractility of the heart. Skeletal and respiratory muscle pumps also increase SV during exercise via increased venous blood returning to the heart. A maximal SV is attained at ~110bpm or ~60% $\dot{V}O_{2max}$ and does not appear to decrease when the HR is ~200 bpm at maximal exercise (3). SV is influenced by preload, afterload, and contractility.

Preload can be defined as the myocardial sarcomere length just before contraction and is approximated using the EDV, since sarcomere length cannot be measured in an intact heart. Changes in preload directly impact SV via a length-tension relationship, causing ventricular output to increase as preload increases according to the Frank Starling Mechanism (4). During exercise, increased venous return via skeletal and respiratory muscle pumps increases the filling

of the left ventricle causing the myocardial sarcomeres to further stretch and thus increase the contraction force leading to an increased SV.

Afterload is the pressure the left ventricle must generate to overcome the aortic pressure to eject blood during systole. Changes in aortic pressure can affect SV, for instance, high arterial pressures result in greater time to generate enough pressure to open the aortic valve causing the aortic valve close sooner and lessen the time to eject blood- increasing the ESV. However, the increased ESV leads to an increase in EDV since the increased ESV is combined with the venous return to increase ventricular filling and contraction force (i.e., contractility). Consequently, preload and SV are increased to offset the previously decreased SV caused by increased afterload.

Contractility, the contraction force of the heart muscle, affects cardiac output through its influence on SV and ESV. The more forceful the contraction is, the greater the SV and less the ESV. For instance, increasing contractility during exercise, increases the velocity of myocardial sarcomere shortening at a given preload and afterload according to the force-velocity relationship. As a result, the ventricle can eject blood more forcefully and quickly, allowing for increased SV and decreased ESV. Increased venous return resulting during exercise combined with increased contractility can also increase SV.

1.1.3 Cardiac Output Limitations

Heart rate is the main modulator on the distribution of $\dot{Q}$ to the active muscles during high intensity exercise in young healthy individuals due to natural limitations on SV (3). Stroke volume increases ~20% to 30% with exercise and may level off ~60% of $\dot{V}O_{2max}$ in healthy young individuals, despite increases in exercise intensity (5), limiting the volume of blood delivered to the active tissue. Further increases in SV could be due to both improved ventricular

filling and ejection but more likely the result of improved ventricular filling via greater ventricular preload from training-induced increases in blood volume and ventricular volume (5, 6).

At rest, the majority of $\dot{Q}$ is directed to vital organs and distributed based on the oxygen uptake of the tissues, however, during exercise, the metabolic demand of the skeletal muscle increases so oxygen delivery to the skeletal muscle must increase proportionately. As exercise intensity increases, blood flow to the heart increases with little to no increase in cerebral blood flow and significant reductions in visceral organs blood flow (7). At maximal exercise, ~80% of $\dot{Q}$ can be delivered to the skeletal muscles in healthy young individuals (8). Systemic vasoconstriction during exercise allows blood to be redistributed to active tissues where local vasodilation occurs to accommodate the increased blood flow. A competition for blood flow exists between skeletal muscle beds, for instance, blood flow is altered when leg exercise was superimposed on arm exercise (9, 10). Leg blood flow was not reduced when arm cranking was superimposed on knee extension, however, the addition of leg cycling to arm exercise resulted in a 5% lower arm vascular conductance (9, 10). A competition for blood flow also exists between locomotor muscle beds and respiratory muscle beds with respiratory muscle blood flow increasing at the expense of skeletal muscle blood flow during high intensity exercise. Respiratory muscles consume 10% to 15% of $\dot{Q}$ during high intensity exercise, and when $\dot{Q}$ is limited, e.g., ~ ≥85% $\dot{V}O_{2max}$, blood flow is redirected to the respiratory muscles via group IV afferent sympathetic medicated redistribution (11). The redistribution of $\dot{Q}$ from the active locomotor muscles to the respiratory muscles increases respiratory muscle blood flow and creates a “competition” for $\dot{Q}$ (12). Subsequently, decreased oxygen delivery to the active limb

muscles causes increased limb muscle fatigue, increased perceived effort and decreased limb muscle performance.

1.1.4 Ventilation

To better understand the limitations of the arterial to venous oxygen content difference on $\dot{V}O_{2max,}$ we must first discuss the influence of ventilation and oxygenation.

In response to exercise, tidal volume increases first and levels off at ~40 to 60% of vital capacity and is ~60% of peak exercise capacity (13). Increases in tidal volume are achieved by increases in inspiratory and expiratory reserve volumes. Expiration during exercise becomes active and internal intercostal and abdominal muscles are recruited to aid in expiration, utilizing more expiratory reserve volume as tidal volume increases. Mechanical work is minimized while alveolar ventilation is optimized because dead space volume is fixed, so, increases in tidal volume with exercise limit the dead space to tidal volume ratio before greater increases in breathing frequency are needed to increase ventilation. A minute ventilation of 90 to 100L/min is not uncommon to see during exercise with more fit individuals being in excess of 150 to 200 L/min during high intensity exercise. Upon the initiation of exercise, ventilation and heart rate are rapidly increased via spontaneous excitation of neural circuits controlling the cardiorespiratory and locomotor system (14, 15). This central command response is initiated because waiting for peripheral chemosensors to sense changes in pH and CO_2 could result in dangerous levels of pH and CO_2. At very high ventilation, inspiratory time is reduced from ~1 second to ~0.5 seconds and expiration becomes active and expiratory time is reduced. It's not unusual to observe ~60 breaths per minute and tidal volumes of 2.5 to 3.5L during high intensity exercise and is achieved by a feed forward mechanism from the peripheral chemosensors and type III and IV sensory afferents in the active respiratory and skeletal muscles.

1.1.5 Ventilation Limitations

Expiratory flow can become limited during high intensity exercise when ventilation and breathing frequency is high. Large tidal volumes during high intensity exercise may match the maximal flow volume loop and signify expiratory flow limitations (EFL) have occurred. Expiratory flow limitations are seen in ~50% of healthy males and females during exercise and the occurrence of EFL appears to be similar between sexes (16). Airway anatomy and elastic recoil of the lung contribute to EFL. So, any attempt to increase expiratory flow is not matched by a higher expiratory flow at a given lung volume and the attempt to increase expiratory flow may result in an increased work of breathing, greater oxygen demand of the respiratory muscles and a greater redistribution of blood away from active tissue and to the respiratory muscles (17, 18). Expiratory flow limitations may also lead to relative hypoventilation, reduced alveolar ventilation and arterial hypoxemia and ultimately performance decrements relative to non-expiratory flow limited individuals (19, 20).

1.1.6 Oxygenation

The alveolar and arterial oxygen pressure difference ($AaDO_2$) measures the difference between the alveolar and arterial oxygen pressure and is important for determining lung gas exchange impairment. The increased AaO_2 difference observed during exercise can be offset in most individuals via increased alveolar ventilation and thus alveolar partial pressure to maintain arterial oxygen pressures near resting levels. A varying degree of gas exchange impairment can be seen in all exercising individuals and is the result of a ventilation perfusion inequality ($\dot{V}_A/\dot{Q}$) and diffusion limitation (21). A $\dot{V}_A/\dot{Q}$ is the mismatched of ventilation and blood flow in various regions of the lungs that impair O_2, and CO_2 transfer. A diffusion limitation is an increasingly

important factor limiting pulmonary gas exchange as a subject's $\dot{V}O_2$ increases and/or the inspired PO_2 decreases (22–24).

Traditionally, the limiting factor for determining $\dot{V}O_{2max}$ in healthy individuals at sea level has not been oxygenation since SaO_2 remains high, 95% SaO_2, during intense exercise (25). Oxygen saturation remains high during exercise despite decreases in PO_2 due to the shape of the oxyhemoglobin dissociation curve (ODC). The ODC is nearly flat between a PO_2 of 90 mmHg and 100mmHg and acts as a buffer for SaO_2 in case of a drop in PO_2. For instance, a 40 mmHg drop in PO_2, from 100 mmHg to 60 mmHg, only results in a 10% reduction in SaO_2 and SaO_2 would still be 50% despite PO_2 dropping to 26.6 mmHg. As a result, the maintenance of arterial oxygen content allows the C_aO_2 portion of Equation. 1, to remain high and maintains the gradient for the diffusion of oxygen from the capillary to the cell.

1.1.7 Oxygenation Limitations

It is estimated that exercise-induced arterial hypoxemia (EIAH) occurs in about 50% of endurance elite athletes, despite significant reductions in PaO_2 during high intensity exercise at sea-level being uncommon in most individuals (25, 26). Exercise-induced arterial hypoxemia may develop during intense exercise because of diffusion limitations and short red blood cell transit times in the pulmonary capillaries (26). However, EIAH is not typically seen in untrained men and women, with the exception of EFL women (20), so EIAH is not thought of a limitation to oxygenation in the untrained individuals.

1.1.8 RBC/Hemoglobin

Oxygen is predominantly transported throughout the body via hemoglobin (Hb) molecule within red blood cells. Normal Hb levels are ~14-15 g/dL for healthy men and slightly lower for women (8). Each hemoglobin molecule contains 2 alpha and 2 beta subunits, and each subunit

can bind one molecule of oxygen for a total of 4 bound oxygen per Hb molecule. At rest, hemoglobin molecules are nearly fully saturated with oxygen. Oxygen can also be dissolved in the blood and transported throughout the body, however, oxygen solubility is very low and only accounts for 0.03mL O_2/dL blood, provided normal alveolar PO_2. Since a normal individual has ~5L of blood, only ~15mL of dissolved oxygen is transported in their blood, ~2% of total arterial oxygen. Total blood volume also influences oxygen delivery, for example, endurance training can increase total blood volume and RBC mass which can influence filling pressures in the heart and thus $\dot{Q}$ (27).

The oxyhemoglobin dissociation curve is characterized by a sigmoid-shaped curve and depicted by plotting the partial pressure of oxygen and the percentage of oxygen saturated hemoglobin. The ODC is influenced by temperature, pH, and CO_2, and 2,3-Diphosphoglycerate (2,3-DPG), an organic phosphate that binds to Hb and alters the affinity for oxygen. A right shift of the ODC is the result of increased temperature, increased CO_2, or decreased pH and favors unloading of oxygen at the active tissue. A right shift of the ODC causes increased unloading of oxygen at the active tissues and results in lower mixed venous oxygen content and allows for increased pressure gradient to drive oxygen from the pulmonary to arterial capillaries. The P_{50} value, half saturation tension of oxygen, is lower at the arterial side of the capillary relative to the venous side (28) because of the continuous change in blood compositions from the mixing of metabolites as blood enters the capillary. As a result, there is a large rightward shift of the ODC within the capillary that increases the unloading of O_2 from Hb (29). A left shift of the ODC is the result of decreased temperature, decreased CO_2, or increased pH and favors the loading of oxygen to Hb. In the lung, the effects of metabolites on oxyhemoglobin affinity are reduced, relative to active tissues, due to lower temperatures, decreased alveolar CO_2 via alveolar gas

exchange and the mixing of venous blood from in/active tissues and other organs causing relatively lower [H^+] and CO_2 in the venous blood and attenuating the effects of the rightward shift seen in the active tissues (30).

1.1.9 RBC/Hemoglobin Limitations

The rightward shift of the ODC during intense exercise may impair the loading of oxygen to Hb in the lung, however, the magnitude of the ODC right shift during exercise is contingent on exercise intensity and active muscle mass (30). The P_{50} value can increase from ~27 mmHg at rest to ~34 mmHg in arterial blood during intense exercise and result in impaired oxyhemoglobin affinity and hinder oxygen loading in the pulmonary capillaries and decrease SaO_2 from ~97.5% to 95% during high intensity exercise (31). This decrease in SaO_2 would result in a slight lower arterial to venous oxygen content difference because of a decreased CaO_2. Increased 2-3, DPG in trained individuals may further decrease SaO_2 due to decreased oxyhemoglobin affinity (32). Also, SaO_2 can be further decreased due to the diffusion limitation from reduced transit time of RBC in the pulmonary capillaries when $\dot{Q}$ is high (33, 34).

1.1.10 In Summary: Limitations of $\dot{V}O_{2max}$ in Healthy Adults

Since large muscle mass exercise is mainly limited by blood flow and oxygen delivery (35), the increase in SV of only 20-30% with exercise coupled with a plateau near 60% $\dot{V}O_{2max}$ results in SV limiting the ability of oxygen delivery to active tissues during intense exercise, limiting $\dot{V}O_{2max}$ (5). The prevalence of expiratory flow limitations in about ~50% of healthy males and females may results in relative hypoventilation, reduced alveolar ventilation and arterial hypoxemia with the attempt to increase expiratory flow resulting in an increased work of breathing and a subsequent greater redistribution of blood to the respiratory muscles during high intensity exercise (17–20). Since SaO_2 remains high, ~95% during intense exercise, oxygenation

has not been thought of as a limiting factor for determining $\dot{V}O_{2max}$ in healthy untrained individuals at sea level (25), with the exception women who are EFL (20).

1.2. Response to exercise in Acute Hypoxia

1.2.1 What is Hypoxia?

Hypoxia is a state in which oxygen is not adequately available in the tissues to maintain homeostasis. Low tissue oxygen levels may occur due to low atmospheric oxygen pressure or inadequate oxygen delivery to the tissues. At altitude, the main effect of hypoxia is the reduction in atmospheric pressure which in turn reduces the partial pressure of inspired oxygen (P_IO_2). Exposure to hypoxia is accompanied by a series of physiological changes, initiated by a drop in the partial pressure of oxygen, to help maintain adequate oxygen delivery to the tissues.

1.2.2 Ventilatory Response

Central chemosensors are located in the medulla are responsible for changes in $\dot{V}_E$ via arterial partial pressure of carbon dioxide (PCO_2) causing pH changes in the cerebral spinal fluid, albeit a much slower ventilatory response than the peripheral chemosensors (36). The peripheral chemosensors, located in the carotid arteries and aortic arch, are responsible for sensing and rapidly responding to changes in arterial PO_2, PCO_2, pH and K^+ (37). A decreased arterial PO_2 at altitude is sensed by glomus cells and neurotransmitters are released to stimulate the central nervous system to increase $\dot{V}_E$ and subsequently PO_2 (38). The hypoxic ventilatory response (HVR) is responsible for increasing ventilation at rest and during exercise at altitude. The HVR increases alveolar partial pressure of oxygen (PAO_2) and thus arterial partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SaO_2) and ultimately arterial oxygen content (CaO_2) to meet the increased demand for oxygen at altitude despite the $AaDO_2$ being increased during exercise (39). Hyperventilation during exercise in hypoxia removes more CO_2 and increases

PAO_2 and by increasing pH, can also shift the ODC leftward and increase SaO_2 by six to twelve percentage units (39).

1.2.3 Oxygenation Response

Hypoxic Pulmonary Vasoconstriction (HPV), an intrinsic mechanism of the lung for matching ventilation and perfusion to optimize oxygen delivery, occurs in response to alveolar hypoxia and works by vasoconstricting arteries in the lungs to divert blood flow to better ventilated portions of the lungs, causing the whole lung to vasoconstrict and raise pulmonary artery pressure in environmental hypoxia (40). In hypoxia, PAO_2, mixed venous PO_2 and likely pulmonary capillary PO_2 are reduced and the increased tissue oxygen extraction during exercise causes lower mixed venous oxygen saturation (41) which increases the time needed for blood to equilibrate with oxygen in the pulmonary capillaries, further challenging the diffusion capacity of the lungs during exercise (42). The increase in $\dot{Q}$ during exercise decreases the transit time of the RBC through the pulmonary capillaries to an insufficient level to reach equilibrium between PAO_2 and capillary PO_2 causing a reduction in SaO_2. The result is an increased widening of the $AaDO_2$ and reduced SaO_2 at altitude and could explain the reduction in $\dot{V}O_{2max}$ seen at altitude. The reduction of oxygen delivery to the active muscle is the main cause of reduced $\dot{V}O_2$ in acute hypoxia (35).

1.2.4 RBC/Hemoglobin Response

In exercising tissue, increases in PCO_2 , $[H^+]$, and temperature shifts the ODC to the right to increase the unloading oxygen in the active tissue. Increased 2-3, DPG will also shift the ODC to the right to reduce oxyhemoglobin affinity and increase the unloading of oxygen to the active tissues (43). However, the rightward shift is somewhat blunted by the hypoxia-induced hyperventilation which causes increased pH and increased oxyhemoglobin affinity. For instance,

excess exhalation of CO_2 causes a left shift of the ODC and increases the oxyhemoglobin affinity and may impair the release of oxygen in the peripheral tissues (39).

1.2.5 $\dot{V}O_{2max}$ Response

It is widely known that $\dot{V}O_{2max}$ will be impaired for an individual at altitude, but the degree of impairment varies between individuals, $\dot{V}O_{2max}$ and altitude (44). Performance decrements at altitude have been seen at low as 580m (45) with performance decrements linearly decreasing to 2,800m (44). Significant correlations (r=0.54-0.94) exist between normoxic $\dot{V}O_{2max}$ and percent decline of $\dot{V}O_{2max}$ at altitude (44, 46, 47). For instance, untrained individuals do not have as great of a percent reduction in $\dot{V}O_{2max}$ at altitude, relative to sea level $\dot{V}O_{2max}$, as trained individuals, therefore, the greater the subject's normoxic $\dot{V}O_{2max}$, the greater the percent reduction of $\dot{V}O_{2max}$ in hypoxia (44, 48). The larger reduction in $\dot{V}O_{2max}$ seen in trained participants compared to untrained participants appears to be due to a greater level of arterial desaturation within trained participants (45, 49, 50). The reduction of oxygen delivery to the active muscle is the main cause of reduced $\dot{V}O_2$ in acute hypoxia (35). Large muscle mass exercise, such as biking, is mainly limited by oxygen delivery which in turn depends on blood flow and oxygen content of the arterial blood (35). Given the reduced oxygen content in acute hypoxia and reduction of blood flow to the locomotor muscles at high intensity exercise, via respiratory muscle metaboreflex, it is fair to attribute the reduced oxygen content and blood flow to the active tissues at high intensity exercise to the reduction in VO_2 in acute hypoxia (51).

1.3 Technical aspects of maximal exercise testing

1.3.1 $\dot{V}O_{2max}$ Testing

A $\dot{V}O_{2max}$ test may be conducted on a treadmill or cycle ergometer and follow a protocol that increases speed or work rate progressively, about 8 to 12 minutes, until the participant can

no longer sustain the work rate (2). Oxygen uptake should increase linearly with work rate and may plateau near end exercise (52). The peak oxygen uptake ($\dot{V}O_{2peak}$) is the highest $\dot{V}O_2$ achieved in a given test regardless of effort and used to classify a $\dot{V}O_{2max}$ test that did not meet the primary criteria (53) The primary criterion for determining a $\dot{V}O_{2max}$ is observing a small to no increase in oxygen consumption despite an increase in work rate (54). The primary criterion was initially defined as an oxygen uptake increase < 150 ml/min despite an increase in workload (55) but other studies have found 0 - 100 ml/min (54, 56, 57), and 250 ml/min (58) was sufficient for defining a $\dot{V}O_{2max}$. Secondary criterion are used to determine $\dot{V}O_{2peak}$ if a plateau in $\dot{V}O_2$ is not observed. The most common secondary criteria are an $RER_{max} > 1.10$ and HR_{max} within 10 bpm of the subject's age predicted max while other criterion, such as blood lactate and RPE have been used to determine $\dot{V}O_{2max}$ (54, 59). No consensus in the values used to define secondary criteria was found in a review of studies published in 5 journals across 3 time periods (59) The review found 7 different values (i.e. ≤ 2.1 ml/kg/min , ≤ 200 ml/min, ≤ 150 ml/min, etc.) were used to determine $\dot{V}O_2$ plateau, 2 different values (i.e. ≥ 10 mmol/L and ≥ 8 mmol/L) used for the concentration of blood lactate, 8 different values (i.e. ≥ 1.20, ≥ 1.15, ≥ 1.13, etc.) used for RER, 10 different values (i.e. ± 5, ± 10, ± 15 beats/min of age predicted HR_{max}, etc.) for HR, and 3 different values (i.e. ≥ 19, ≥ 18, and ≥17) for RPE were used as secondary criteria. The absence of a standardized criteria to determine $\dot{V}O_{2max}$ makes accessing the test potentially susceptible to misinterpretation.

1.3.2 Supramaximal Verification Testing

Alternatively, having subjects perform a $\dot{V}O_2$ verification test after completing a $\dot{V}O_{2max}$ test was recommended to avoid relying on primary and secondary criteria to determine $\dot{V}O_{2max}$ or $\dot{V}O_{2peak}$ (60). A supramaximal test is a commonly used as a verification test (61–64) and would

confirm the $\dot{V}O_{2max}$ result if the verification test $\dot{V}O_2$ was similar to, within measurement error, the $\dot{V}O_2$ from the max test since cycling at a supramaximal workload should increase the oxygen demand of the participant beyond that of the max test. However, if the $\dot{V}O_2$ during the supramaximal test increased beyond that of the gas analyzer measurement error the subject did not reach $\dot{V}O_{2max}$ and instead a VO_{2peak}.

Conducting the supramaximal and maximal test in the same visit may provide increased likelihood of verifying $\dot{V}O_{2max}$ since a priming bout of exercise has shown to improve the ability to identify a $\dot{V}O_2$ plateau (65). A 20-minute rest period following the max test is recommended when using supramaximal testing to confirm $\dot{V}O_{2max}$ in normally active populations (66) but no difference was found in the ability to confirm $\dot{V}O_{2max}$ using a rest period of 10 minutes (67), 20 minutes or 60 minutes (66) between maximal and supramaximal exercise bouts. A supramaximal work rate about 10% higher than peak power output from the $\dot{V}O_{2max}$ test has proven effective in verifying $\dot{V}O_{2max}$ (59, 61). However, supramaximal intensities have ranged from 105 to 130% of $\dot{V}O_2$ peak power output with no significant difference between maximal and supramaximal $\dot{V}O_2$ (61–64). Sustaining a supramaximal power is accomplished by recruiting anaerobic energy pathways to meet the increased metabolic demand of a supramaximal effort. Aerobic energy production may plateau, denoting $\dot{V}O_{2max}$, so increased anaerobic contributions are needed to meet the demand of the supramaximal workload. The supramaximal test should be maintained for a duration long enough for oxygen kinetics to allow achievement of the same or greater $\dot{V}O_2$ if possible (2). A supramaximal test is typically tolerated for three to six minutes (2), but findings suggest a supramaximal test of 2 minutes is adequate to produce a $\dot{V}O_2$ like the max test $\dot{V}O_2$ (61).

Supramaximal testing to confirm the achievement of $\dot{V}O_{2max}$ has been successfully validated in children (68), sedentary (61), athletes (69), obese populations (70, 71) and cystic fibrosis patients (72). Supramaximal testing has also confirmed the achievement of $\dot{V}O_{2max}$ in acclimatized runners residing at 2,350m (69). However, supramaximal testing has not been verified in lowlanders exposed to acute hypoxia. Therefore, the purpose of this study is to determine if supramaximal exercise testing confirms the achievement of $\dot{V}O_{2max}$ in acute hypoxia. We seek to determine if a difference lies in the ability of an individual to produce a maximal $\dot{V}O_2$ in acute hypoxia using the maximal and supramaximal exercise tests and determine if cardiopulmonary measures differ between the two exercise bouts in acute hypoxia and normoxia.

2. STUDY RATIONALE

The purpose of this study was to determine if supramaximal exercise testing confirms the achievement of $\dot{V}O_{2max}$ in acute hypoxia. We sought to determine if a difference lies in the ability of an individual to produce a maximal $\dot{V}O_2$ in acute hypoxia using incremental and supramaximal exercise tests and determine if cardiopulmonary measures differ between the two exercise bouts in acute hypoxia and normoxia. Therefore, our research provided quantitative data regarding the use of supramaximal testing to confirm the achievement of $\dot{V}O_{2max}$ in acute hypoxia.

3. RESEARCH QUESTION AND HYPOTHESIS

Research Questions

1. Is $\dot{V}O_2$ during incremental and supramax exercise tests similar, within 1 mL/kg/min, in acute hypoxia ($F_iO_2 = 0.147$)?

Hypothesis

$H_{\theta a}$: The difference in maximal and supramaximal $\dot{V}O_2$ is no different or even smaller than an oxygen uptake of -1 mL/kg/min (lower boundary: - δ)

$H_{\theta b}$: The difference in maximal and supramaximal $\dot{V}O_2$ is no different or even bigger than an oxygen uptake of 1 mL/kg/min (upper boundary: + δ)

Alternative hypothesis

The difference in means is bigger than the lower boundary and smaller than the upper boundary

4. METHODS

4.1 Ethics

This study has been reviewed and received ethics clearance through a University of Waterloo Research Ethics Committee (ORE #41941). The research methods and protocols adhere to the recommendations outlined by the Declaration of Helsinki concerned with the use of human participants, except for registration in a database.

4.2 Participants

The proposed sample size was 20 participants, and we attempted to recruit equal numbers of men and women for the study, but the study will not investigate sex differences. The sample size was determined by reviewing the sample size from 12 supramaximal exercise tests from 11 studies to determine the average sample size. The average sample size was 20 subjects. Therefore, the sample size for our study was 20 participants. We determined the sample size using the above methods because determining the sample size for a two one sided t-tests (TOST) required writing code and conducting thousands of equivalence test to determine at what sample size the equivalence occurs 80% of the time. Currently, software does not exist to conduct power calculations for TOST, and we do not have the expertise to run said simulations quickly and effectively, so we estimated our sample size using previous literature. An issue with estimating the sample size using previous literature instead of calculating a sample size is that we have no basis for effect, rather we are estimating our basis for effect. Calculating the sample size using a power calculation provides an accurate basis for effect to determine the sample size needed to see an effect.

Healthy, non-obese (BMI < 30 kg/m^2) males and females between the ages of 18 and 40 years were recruited to participate in this study. Table 1 outlined the specific participant inclusion and exclusion criteria.

Table 1: Participant Inclusion and Exclusion Requirements

Inclusion Criteria	Exclusion Criteria
• Age: 18-40 years old • Healthy, non-obese (BMI < 30 kg/m^2) individuals • Experience with high intensity exercise (previously completed a $\dot{V}O_{2max}$ test) • Regular physical activity, (2+ days/week totaling 150 minutes of moderate-to-vigorous physical activity)	• Chronic health condition(s) i.e., metabolic (i.e., Type 1 or 2 diabetes) cardiovascular (i.e., hypertension), respiratory (i.e., COPD) digestive (i.e., ulcerative colitis) disorders. • Pregnant, suspect you may be pregnant, or nursing • Hormone replacement therapy • Arthritis • Smoker

4.3 Experimental Overview

Participants underwent pulmonary function testing followed by a maximal exercise test and a supramaximal exercise test to confirm the achievement of $\dot{V}O_{2max}$ in normoxia and acute hypoxia (FIO_2 =0.15). Participants completed two randomized testing visits separated by at least 48 hours, see *Figure 1***:** Outline of Testing Protocol and *Figure 2*: Sample Outline of Maximal and Supramaximal Testing Procedures for more detailed testing protocol. The visit order was

determined using a random integer generator with equal distribution of ‘normoxic’ and ‘hypoxic’ visits. The participant was not informed of the gas mixture prior to, during, or after the visit. The Douglas bag was filled prior to the subject’s arrival into the lab and was refilled during both visits, as needed. The participant was not able to see the gas tank valves from the cycle ergometer and was blinded to which gas tank was used during the refilling process. The participants could only see their cadence during exercise on the cycling ergometer. The power output on the cycle ergometer was covered to prevent speculation of a reduced power output and subsequent guess to which gas the participant was breathing due to the potentially increased perception of fatigue at an earlier power output. The order of events was identical during both visits, see Figure 1. This allowed for consistent order for both visits. Participants breathed from gas tanks during both visits to maintain consistency of dry gas.

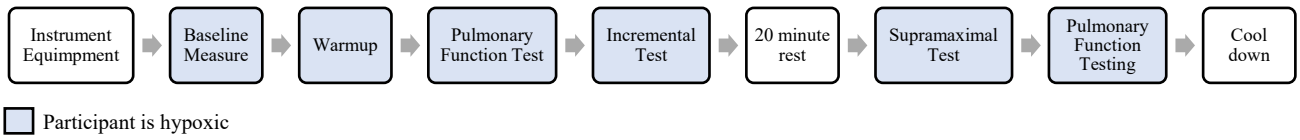

Figure 1: Outline of Testing Protocols

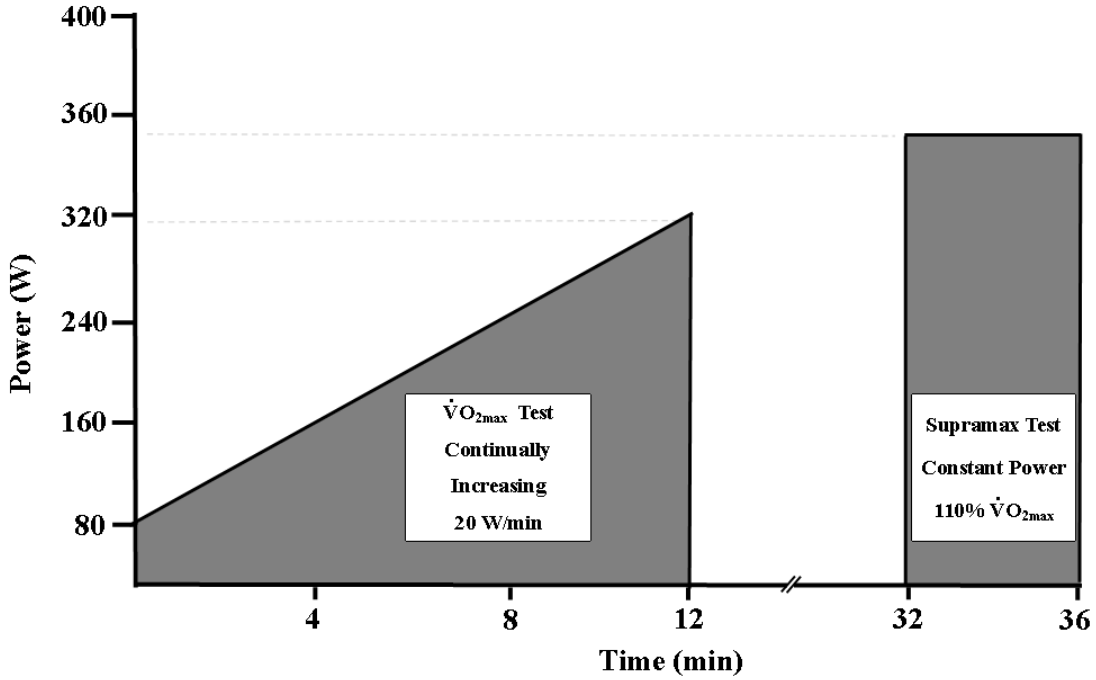

Figure 2: Maximal and Supramaximal Testing Protocol Sample

4.4 Pulmonary Function Testing

Forced Vital Capacity (FVC) and Forced Expired Volume in one second (FEV_1) maneuvers will be performed according to ATS/ERS standards. Participants will be specifically instructed to, "breath in as deeply as possible" (to reach Total Lung Capacity) and "breath out as hard, fast, and completely as possible" (to reach Residual Volume). Testing will be completed at varying intensities (i.e., 100%, 90%, 80%, 70%) to allow for determination of a maximal flow for a given volume without the effects of excessive gas compression (73). Pulmonary function testing will be conducted to determine eligibility for the study. If FEV_1 is less than 80% predicted, the participant does not have normal pulmonary function and will be excluded from the study. The post-exercise testing was conducted to account for thoracic gas compression and bronchodilation during exercise (73).

4.5 Maximal Exercise Test

Maximal exercise testing was conducted on an electronically braked cycle ergometer (LC7TT, Monark Exercise AB, Vansbro, Sweden) and followed a continuous protocol. In the normoxic visit, males and females started at 80 watts and 60 watts, respectively, with the power increased 20 watts per minute until volitional fatigue. Different starting power outputs were chosen in attempt to match test duration for men and women. In the hypoxic visit, males and females started at 40 watts and 20 watts, respectively, with the power increased 20 watts per minute until volitional fatigue. Pilot testing was used to determine a starting power output for the incremental test in which test duration may be matched between condition to ensure test duration was within the recommended 8 -12-minute duration (74). Continuous measures of mixed expired gases were collected using a sample line connected into the mixing chamber. During the hypoxic visit, the inspired oxygen was continuously sampled, using a second oxygen analyzer, from a sample port located just before the pneumotach, instead of sampling at the mouth of the Douglas Bag, to ensure a more accurate representation of the inspired gas.

4.6 Supramaximal Exercise Test

Supramaximal exercise testing was conducted on the same electronically braked cycle ergometer. Participants cycled at 110% of their peak power output, from the $\dot{V}O_{2max}$ test, until volitional fatigue. The supramaximal test started at the same power output as the $\dot{V}O_{2max}$ test for 5 seconds before power increased to 110% power output over 15 seconds. Then, the supramaximal test continued at 110% power output for the duration of the test. The ramp in power was implemented to allow the participant to progressively build to 110% peak power and limit the oxygen deficit experienced at the start exercise. For example, the male's normoxic max test started at 80W, so, the male's normoxic supramaximal test began at 80W for 5 seconds before power increased to 110% $\dot{V}O_{2max}$ power output over the next 15 seconds and continued at

110% $\dot{V}O_{2max}$ power output until volitional fatigue. A 2-minute sample of the supramaximal test protocol is found in Figure 3.

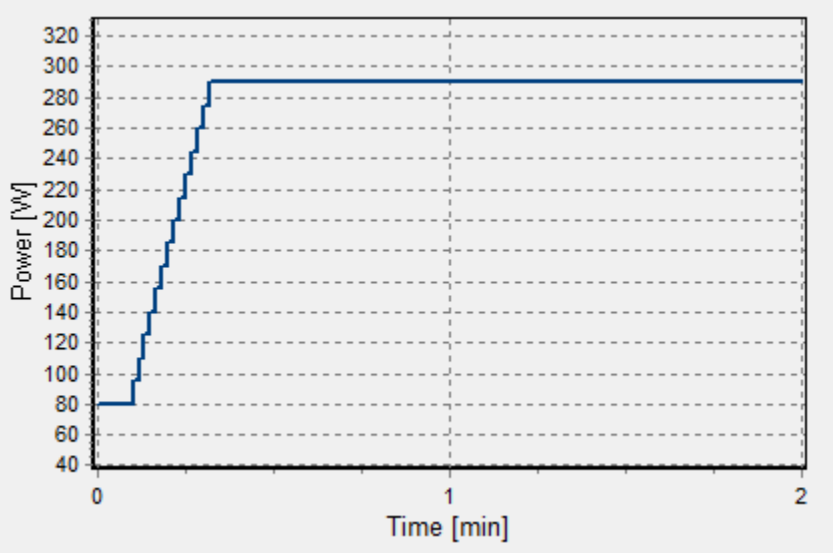

Figure 3: Supramaximal Testing Protocol Sample

The participant should theoretically increase their $\dot{V}O_2$ when cycling at a supramaximal workload, but if the subject reached $\dot{V}O_{2max}$, the oxygen consumption should plateau, validating $\dot{V}O_{2max}$. If the participant's $\dot{V}O_2$ increased beyond that of the gas analyzer measurement error, the participant did not reach $\dot{V}O_{2max}$. Therefore, the supramaximal test was used to verify the achievement of $\dot{V}O_{2max}$.

4.7 Hypoxic Gas Mixture

Medical grade gas tanks contained ~15% O_2 and balanced nitrogen as well as compressed room air and was provided by Linde Canada Inc. Gas traveled from the Douglas Bag through large bore tubing and into a 3-way valve and to the participant. A 3-way valve was used to switch between room air and the compressed gas mixture. The valve did not audibly alert the participant when switched and the participant was not aware of the valve's switching. The participant was only informed of the gas mixture after completing the entire study.

4.8 Data Collection

Raw data was collected at 200 Hz using a 16-channel analog-to-digital data acquisition system (PowerLab/16SP model ML 795; ADInstruments, Colorado Springs, CO). Exercise was completed on an electronically braked cycle ergometer (LC7TT, Monark Exercise AB, Vansbro, Sweden). Heart rate was continuously measured using a non-coded chest transmitter (Model T34; Polar Electro Oy, Kempele, Finland). Oxygen-saturated hemoglobin was continuously measured using a pulse oximeter placed on the finger (Nonin 7500; Nonin Medical Inc., Plymouth, Minn., USA). A second finger pulse oximeter was used as a secondary measurement of SpO_2 (Model M170; Shenzhen Fitfaith Technology Co. Ltd., Shenzhen, China). Inspired and expired flow was measured using two calibrated pneumotachometers (Model 3813; Hans Rudolph). Both pneumotachometers were calibrated using a 3L syringe. The expired pneumotachometer was heated to 37ºC while the inspired pneumotachometer was at room temperature. Expired gases were collected in a mixing chamber before entering nafion tubing routed through a sealed glass jar that contained drierite to ensure the gases are dried and dehumidified before measured. Mixed expired oxygen and carbon dioxide were measured using calibrated gas analyzers (S-3A/I and CD-3Am, respectively; Applied Electrochemistry, Bastrop, TX). FiO_2 was measured using a second calibrated gas analyzer (S-3A/I; Applied Electrochemistry, Bastrop, TX). The gas analyzers were calibrated using a gas mixture of 15% O_2 and 5% CO_2.

4.9 Data Analysis

Mixed $\dot{V}O_2$ and $\dot{V}CO_2$ was continuously sampled from the mixing chamber and mixed $\dot{V}O_2$ was determined as the difference between the percent inspired and expired oxygen, using $\dot{V}_I$

and $\dot{V}_E$, and calculated using the continuously sampled FiO_2 channel, during the hypoxic visit. See Equation 2 and 3 below. The FiO_2 channel was smoothed using a Triangular (Bartlett) window with a 5 second window width. $\dot{V}O_2$ and $\dot{V}CO_2$ was be expressed in STPD and determined by a 30 second average maximal response at end exercise. Humidity was set to zero during both visits to account for the dry gas tanks. The decreased atmospheric CO_2 pressure at high altitude was corrected for by decreasing the CO_2 component of the $\dot{V}_E$ and $\dot{V}CO_2$ calculations from 0.03/100 to 0. The 30 second average maximal response at end exercise was used to determined other cardiopulmonary measures (i.e., RER, $\dot{V}_E$, SpO_2 and HR).

Equation 2. Mixed $\dot{V}O_2$ formula = ((FIO_2/100)*($\dot{V}_I$*((273/310)*((Barometric Pressure - 47)/760)))) - (((FEO_2)/100)*($\dot{V}_E$*((273/310)*((Barometric Pressure-47)/760))))

Equation 3. Mixed $\dot{V}CO_2$ formula = (($FECO_2$/100)*($\dot{V}_E$ *((273/310)*((Barometric Pressure-47)/760)))) - (($FICO_2$/100)* ($\dot{V}_I$ *((273/310)*((Barometric Pressure-47)/760))))

4.10 Statistical Analysis

Data was analyzed using RStudio and GraphPad Prism. To make inferences on interchangeable methodology, equivalence testing was used to determine the similarity of $\dot{V}O_2$ between incremental and supramaximal tests in acute hypoxia and normoxia, rather than statistical differences. Equivalence testing rejects the presence of a difference that is large enough to be relevant practically, defined by the upper and lower equivalence margin. Equivalence was based on investigator-determined equivalence bounds of ±1 mL/kg/min. Acceptable rates of Type I errors were set at $\alpha = 0.05$ for all analyses. Equivalence is investigated using a "two one-sided tests" procedure with data tested against an upper and lower bound in two one-sided tests, each carried out at $\alpha = 0.05$ (75) . Equivalence is concluded if both tests statistically reject the presence of effect equal to or larger than the equivalence margins (76). The greater p-value of the two one sided tests is commonly the only reported p-value since the greater p-value is also the p-

value for the overall equivalence (77). A two-tailed, dependent samples t-test was used to determine if a difference existed between participant demographics (age, height, weight, and BMI), test duration and power as well as incremental and supramaximal bouts in normoxia and hypoxia. A Levene test was conducted and determined variance equality in all samples. Significance was set at $\alpha = 0.05$. An analysis of variance (ANOVA) with repeated measures was used to determine if a significant difference existed between the 0%, 25%, 50% 75% and 100% supramax duration in normoxia and hypoxia vs. dependent variables ($\dot{V}O_2$, $\dot{V}_E$, etc.)

5. RESULTS

5.1 Participants

Table 1 summarizes descriptive characteristics of 20 participants (n = 7 female, n = 13 male) from the normoxic and hypoxic visit.

Table 2. Participant demographics (n = 20)

	Normoxia	Hypoxia
Age (years)	24.9 ± 4.5	24.9 ± 4.5
Height (m)	1.74 ± 0.1	1.74 ± 0.1
Weight (kg)	74.2 ± 10.8	74.1 ± 10.8
BMI (kg/m^{-2})	24.6 ± 2.7	24.5 ± 2.8

BMI, body mass index; Values as mean ± SD.

5.2 Pulmonary Function Testing

Table 3 displays the pulmonary function data pre- and post- incremental and supramaximal exercise. There was no significant difference ($p > 0.05$) between the normoxic FVC pre-and post exercise, but post-exercise FVC was significantly lower ($p = 0.02$) than pre-exercise FVC in hypoxia. There was no significant difference ($p > 0.05$) between normoxic or hypoxic pre- and post-exercise FEV1 and FEV1/FVC as well as between normoxic and hypoxic FEV1 and FEV/FVC.

Table 3. Pulmonary function testing

	Normoxia		Hypoxia	
	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise

FVC (l)	4.6 ± 0.9	4.6 ± 0.9	4.5 ± 0.9	4.4 ± 0.9*
% Pred	92 ± 12%	91 ± 13%	89 ± 12%	88 ± 13%
FEV1 (l)	3.9 ± 0.7	3.9 ± 0.7	3.8 ± 0.7	3.8 ± 0.8
% Pred	92 ± 11%	92 ± 12%	90 ± 12%	90 ± 15%
FEV1/FVC (%)	84 ± 7%	84 ± 7%	84 ± 5%	86 ± 7%
% Pred	101 ± 8%	101 ± 8%	101 ± 6%	103 ± 8%

FVC, forced vital capacity FEV1; forced expiratory volume in one second. Values as mean ± SD.*Significant effect of time ($p \leq 0.05$).

5.3 Equivalence testing of normoxic and hypoxic $\dot{V}O_2$

Using *a priori* determined equivalence bounds of ±1 mL/kg/min (78), the normoxic $\dot{V}O_2$ was not sufficiently similar between incremental and supramaximal bouts ($p = 0.84$, 90% confidence interval = [0.73 , 2.0]). The hypoxic $\dot{V}O_2$ was also not sufficiently similar between incremental and supramaximal bouts ($p = 0.83$, 90% confidence interval = [0.68 , 2.1]). Alternatively, when equivalence bounds of ± 150 mL, a widely-accepted primary criterion in determining $\dot{V}O_{2max}$ (64), were used, the incremental and supramaximal $\dot{V}O_2$ were statistically similar in hypoxia ($p = 0.05$, 90% confidence interval = [0.05 , 0.14]), but not normoxia ($p = 0.07$, 90% confidence interval = [0.04 , 0.16]).

5.4 $\dot{V}O_2$ response to incremental exercise

Figure 4 shows the $\dot{V}O_2$ response to the incremental exercise test in normoxia and hypoxia for male and female participants. 13/20 participants achieved $\dot{V}O_{2max}$ in normoxia while 14/20 participants achieved $\dot{V}O_{2max}$ in hypoxia, defined by a ≤150 mL difference in incremental and supramaximal $\dot{V}O_2$. The average incremental $\dot{V}O_2$ in normoxia was 3.87 ± 0.99 L/min and in hypoxia was 3.27 ± 0.81 L/min. The average male $\dot{V}O_2$ was 4.30 ± 0.86 L/min in normoxia and

3.69 ± 0.65 L/min in hypoxia (-15% decrement) while the average female $\dot{V}O_2$ was 3.07 ± 0.70 L/min in normoxia and 2.55 ± 0.61 L/min in hypoxia (-17% decrement). The $\dot{V}O_2$ response to incremental exercise was disaggregated into male and female to better differentiate individual $\dot{V}O_2$ responses. The incremental test duration was not significantly different (p = 0.67) between normoxia (695 ± 214s) and hypoxia (700 ± 178s).

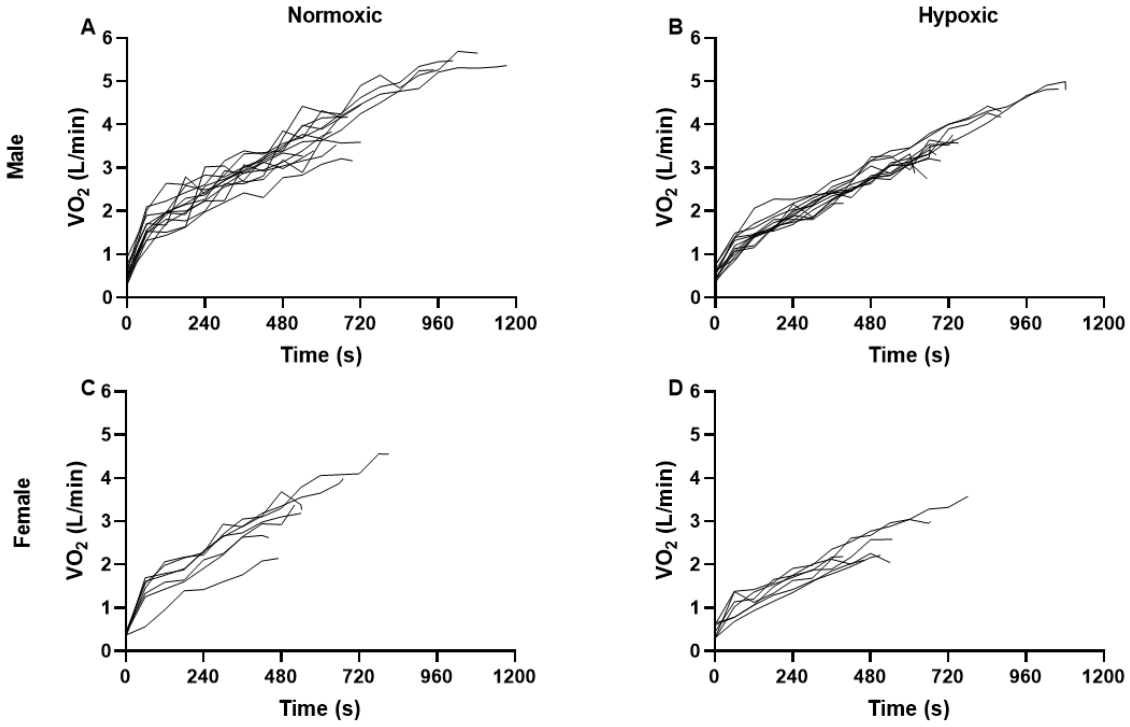

Figure 4. Individual $\dot{V}O_2$ response to incremental exercise in normoxia and hypoxia. The columns represent normoxic and hypoxic conditions while the rows represent male and female $\dot{V}O_2$ response to incremental exercise. $\dot{V}O_2$, oxygen uptake.

Table 4 displays participant's end exercise power and test classification as a max or peak defined by a ≤150 mL difference in incremental and supramaximal $\dot{V}O_2$.

Table 4. Incremental test power and classification

	Normoxic		Hypoxic	
Participant	**Power (W)**	**Max/Peak**	**Power (W)**	**Max/Peak**
1	184	Max	152	Max

2	261	Max	232	Max
3	416	Max	333	Max
4	234	Max	191	Peak
5	207	Max	202	Peak
6	295	Max	258	Max
7	313	Peak	272	Max
8	240	Peak	200	Peak
9	271	Peak	245	Peak
10	470	Peak	403	Peak
11	290	Max	268	Max
12	320	Max	285	Max
13	307	Max	290	Max
14	300	Max	267	Max
15	263	Peak	245	Max
16	317	Max	284	Max
17	395	Peak	334	Max
18	178	Max	174	Max
19	330	Max	291	Max
20	440	Peak	392	Peak

Values are reported as absolute watts at the end of the incremental test and the test classified as a max or peak defined by a difference in incremental and supramaximal $\dot{V}O_2 \leq 150$ mL.

5.5 Normoxic vs. hypoxic incremental test

When comparing normoxic and hypoxic incremental tests in Table 5, $\dot{V}O_2$ (L/min), $\dot{V}O_2$ (mL/kg/min), $\dot{V}CO_2$ (L/min), FiO_2 (%), PO_2 (mmHg), SpO_2 (%) and Power (W) was significantly higher in the normoxic test ($p = 0.001$ for all variables) while RER, $\dot{V}_E/\dot{V}O_2$, and $\dot{V}_E/\dot{V}CO_2$ was significantly lower in normoxia ($p = 0.001$ for all variables). The $\dot{V}O_2$ was -15 ± 7.0% lower in hypoxia compared to normoxia. The percent difference between the normoxic and

hypoxic average maximal incremental variables is presented in Table 6. A significant positive relationship ($p = 0.002$, $r = 0.66$) exists between the hypoxic $\dot{V}O_2$ decrement, shown as the difference between normoxic and hypoxic $\dot{V}O_2$ over normoxic $\dot{V}O_2$, seen in Figure 5A, while Figure 5B displays also displays a significant positive relationship ($p = 0.006$, $r = 0.70$) expressed as relative $\dot{V}O_2$ values. So, the higher the normoxic $\dot{V}O_2$, the greater the absolute $\dot{V}O_2$ difference experienced in hypoxia. The relationship shown in Figure 5 is expected as participants with a larger absolute $\dot{V}O_2$ were likely to have larger absolute differences between normoxic and hypoxic $\dot{V}O_2$. To examine this relationship, Figure 6 plotted the individual percent differences between normoxic and hypoxic $\dot{V}O_2$ (%) against the normoxic $\dot{V}O_2$ (mL/kg/min) and suggested that higher normoxic $\dot{V}O_2$ was associated with greater percent decline in $\dot{V}O_2$ experienced in hypoxia. However, the relationship was found to be non-significant and weak ($p = 0.18$, $r = -0.31$).

Table 5. 15 second maximal gas exchange values in response to incremental and supramaximal exercise in normoxia and hypoxia

	Normoxia			Hypoxia		
	Incremental	Supramaximal	% Δ	Incremental	Supramaximal	% Δ
$\dot{V}O_2$ (L/min)	3.87 ± 0.99	3.76 ± 0.92*	-2.8%	3.27 ± 0.81†	3.16 ± 0.78$^{\dagger *}$	-3.4%
$\dot{V}O_2$ (mL/kg/min)	52.2 ± 11.2	50.8 ± 10.4*	-2.7%	44.0 ± 8.8†	42.6 ± 8.1$^{\dagger *}$	-3.3%
$\dot{V}CO_2$ (L/min)	4.35 ± 1.1	4.23 ± 1.0*	-3.0%	3.82 ± 0.85†	3.83 ± 0.90†	0.4%
RER	1.13 ± 0.05	1.13 ± 0.09	-0.3%	1.17 ± 0.05†	1.22 ± 0.08$^{\dagger *}$	3.5%†

HR (bpm)	183 ± 7	176 ± 8*	-4.4%	182 ± 6	175 ± 6*	-4.2%
$\dot{V}_E$ (L/min)	164 ± 38	159 ± 41	-3.2%	160 ± 36	162 ± 38	1.5%†
$\dot{V}_T$ (L/min)	2.48 ± 0.6	2.39 ± 0.6*	-3.1%	2.46 ± 0.7	2.41 ± 0.6	-2.0%
Fb (bpm)	61 ± 12	60 ± 14	-0.6%	60 ± 13	62 ± 14*	3.3%
FiO_2 (%)	21.1 ± 0.05	21.1 ± 0.06*	-0.2%	14.7 ± 0.5†	14.7 ± 0.5†*	-0.1%
PO_2 (mmHg)	159.8 ± 1.0	159.6 ± 1.0*	-0.2%	111.5 ± 3.9†	111.5 ± 4.1†	0.0%
SpO_2 (%)	94.7 ± 2.5	94.5 ± 4.8	-0.2%	82.2 ± 4.7	84.9 ± 4.1†*	3.2%†
$\dot{V}_E/\dot{V}O_2$	42.8 ± 4.5	42.4 ± 6.7	-0.9%	49.5 ± 6.0†	52.0 ± 7.8†*	4.9%†
$\dot{V}_E/\dot{V}CO_2$	38.0 ± 3.6	37.7 ± 4.3	-0.9%	42.2 ± 4.7†	42.7 ± 5.5†	1.4%
Test Duration (s)	695 ± 214	132 ± 14*	-80%	700 ± 178	140 ± 20*	-80%
Power (W)	302 ± 80	332 ± 88*	10%	266 ± 66†	292 ± 73†*	10%

Values are mean ± SD. $\dot{V}O_{2max,}$ maximal oxygen uptake; $\dot{V}CO_2$, carbon dioxide uptake; RER, respiratory exchange ratio; HR, heart rate; $\dot{V}_E$, expiratory ventilation; $\dot{V}_T$, tidal volume; F_b, breathing frequency; FiO_2, fraction inspired oxygen; PO_2, partial pressure oxygen; SpO_2, oxygen saturation; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent carbon dioxide. *Significant effect of test ($p \leq 0.05$). †Significant effect of condition ($p \leq 0.05$).

A significant negative relationship exists ($p = 0.003$, $r = -0.63$) between the Δ normoxic and hypoxic incremental SpO_2 and $\dot{V}O_2$ expressed in Figure 7 as the individual percent differences between normoxic and hypoxic incremental SpO_2 (%) plotted against the percent change between normoxic and hypoxic incremental $\dot{V}O_2$ (%).

Table 6. Percent difference between the normoxic and hypoxic average maximal incremental variables

$\dot{V}O_2$ (L/min)	-15 ± 7%

$\dot{V}O_2$ (mL/kg/min)	-15 ± 7%
$\dot{V}CO_2$ (L/min)	-12 ± 8%
RER	4 ± 3%
HR (bpm)	-1 ± 3%
$\dot{V}_E$ (L/min)	-2 ± 9%
$\dot{V}_T$ (L/min)	0 ± 8%
Fb (bpm)	-1 ± 9%
FIO_2 (%)	-31 ± 2%
PO_2 (mmHg)	-30 ± 3%
SpO_2 (%)	-13 ± 6%
$\dot{V}_E/\dot{V}O_2$	16 ± 8%
$\dot{V}_E/\dot{V}CO_2$	11 ± 7%
Power (W)	-11 ± 5%

Values are mean ± SD. $\dot{V}O_{2max,}$ maximal oxygen uptake; $\dot{V}CO_2$, carbon dioxide uptake; RER, respiratory exchange ratio; HR, heart rate; $\dot{V}_E$, expiratory ventilation; $\dot{V}_T$, tidal volume; F_b, breathing frequency; FiO_2, fraction inspired oxygen; PO_2, partial pressure oxygen; SpO_2, oxygen saturation; $\dot{V}_E/\dot{V}O_{2,}$ ventilatory equivalent oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent carbon dioxide.

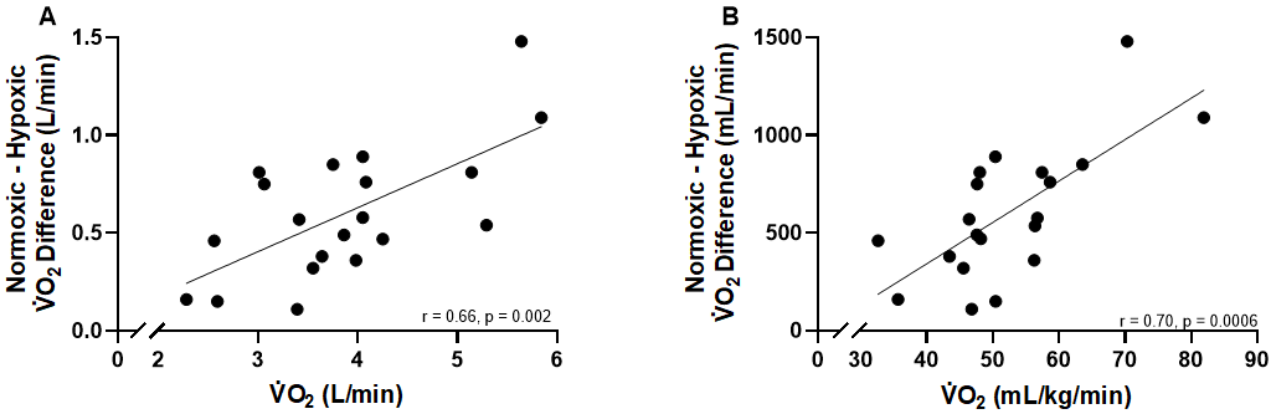

Figure 5. Relationship between the hypoxic $\dot{V}O_2$ decrement and normoxic $\dot{V}O_2$.Individual differences between normoxic and hypoxic $\dot{V}O_2$ plotted against $\dot{V}O_2$ in absolute (Panel A) and relative (Panel B) values.$\dot{V}O_{2max,}$ maximal oxygen uptake.

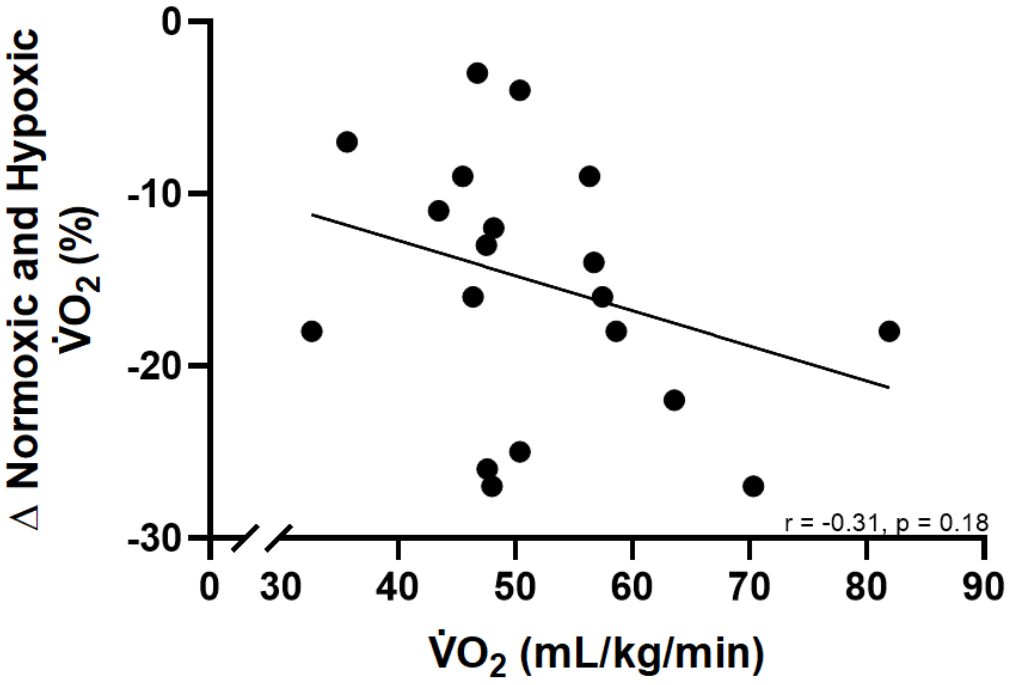

Figure 6. Relationship between the percent change in normoxic and hypoxic $\dot{V}O_2$ and normoxic $\dot{V}O_2$. Individual percent differences between normoxic and hypoxic $\dot{V}O_2$ (%) plotted against the normoxic $\dot{V}O_2$ (mL/kg/min). $\dot{V}O_2$, oxygen uptake.

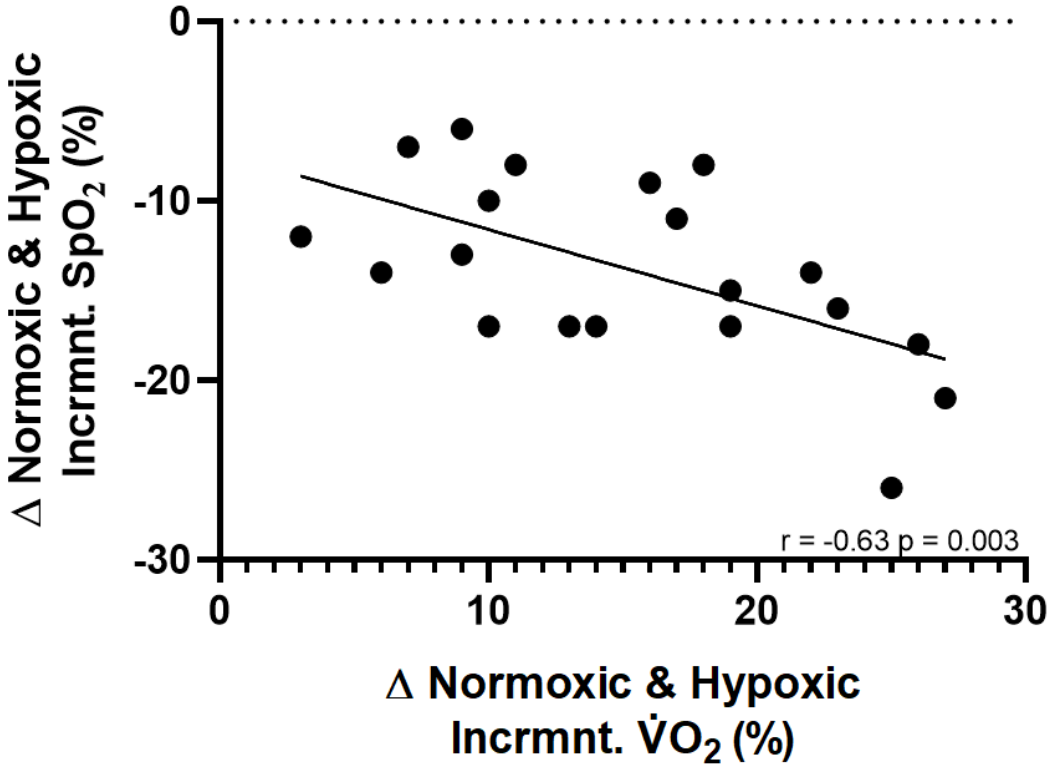

Figure 7. Relationship between the Δ normoxic and hypoxic incremental SpO_2 and $\dot{V}O_2$. Individual percent differences between normoxic and hypoxic incremental SpO2 (%) plotted against the percent change between normoxic and hypoxic incremental V̇O2 (%). $\dot{V}O_2$, maximal oxygen uptake; SpO_2, oxyhemoglobin saturation.

5.6 Incremental vs. supramaximal exercise

The $\dot{V}O_2$ was significantly lower in the supramaximal, compared to incremental, test in normoxia ($p = 0.002$) and hypoxia ($p = 0.001$). Heart rate was also significantly lower ($p = 0.0001$) during the supramaximal test than incremental in both conditions, but HR was not different between the incremental tests ($p = 0.19$) or supramaximal tests ($p = 0.16$). Tidal volume was significantly lower in the supramaximal test, compared to incremental, in normoxia ($p = 0.04$). The fraction of inspired oxygen was significantly lower in the supramaximal test than incremental test in both normoxia ($p = 0.04$) and hypoxia ($p = 0.001$). The supramaximal PO_2 and SpO_2 were significantly lower ($p = 0.001$ and $p = 0.002$ for PO_2 and SpO_2, respectively)

compared to incremental in both conditions. Supramaximal duration was significantly lower than incremental duration in normoxia ($p = 0.001$) and hypoxia ($p = 0.0001$). Supramaximal power was significantly higher than incremental power in normoxia ($p = 0.001$) and hypoxia ($p = 0.001$).

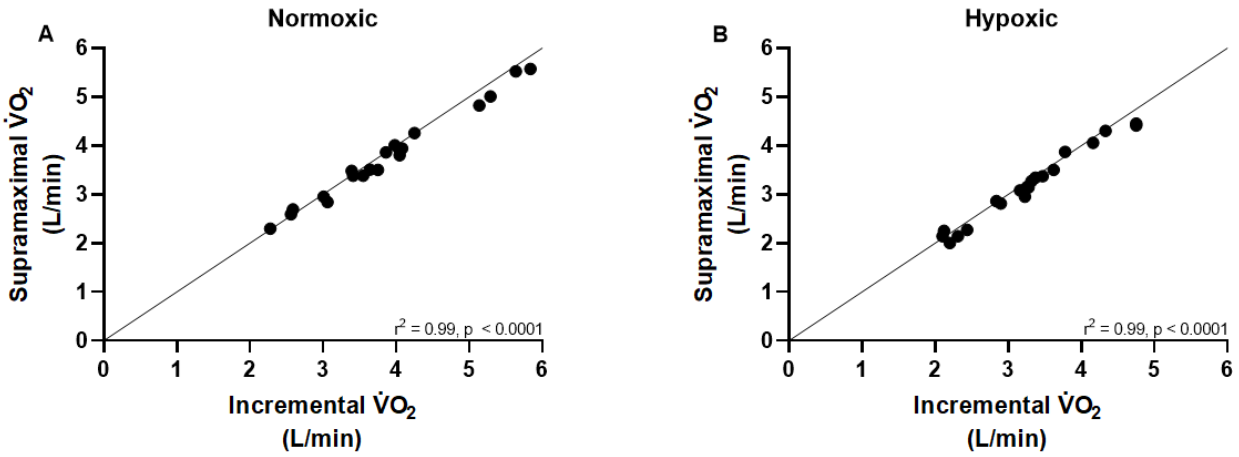

Figure 8. Relationship between incremental and supramaximal $\dot{V}O_2$ in normoxia and hypoxia. Individual supramaximal $\dot{V}O_2$ (L/min) plotted against incremental $\dot{V}O_2$ (L/min) in normoxia (4A) and hypoxia (4B). $\dot{V}O_2$ = oxygen uptake.

Figure 8 shows a significant and strong positive relationship ($p < 0.001$, $r^2 = 0.99$) between incremental and supramaximal $\dot{V}O_2$ in normoxia (8A) and hypoxia (8B) indicating that almost all (99%) if the variation in supramaximal testing can be explained by the incremental $\dot{V}O_2$. Figure 8 also illustrated $\dot{V}O_2$ is underestimated in the supramaximal testing since most of the values fall below the trendline.

A Bland-Altman plot of the incremental and supramaximal $\dot{V}O_2$ differences plotted against their individual means is show in Figure 9. The horizontal dashed lines represent the upper and lower bounds of the coefficient of variation in $\dot{V}O_{2max}$ testing as well as the bias. Previous literature has found the variation of $\dot{V}O_{2max}$ testing to be ± 5.6% with ~90% of the variation attributed to biological variability and less than 10% technical error (79). Therefore,

our differences in incremental and supramaximal $\dot{V}O_2$ in normoxia and hypoxia fall within the coefficient of variation of $\dot{V}O_{2max}$ testing as seen by all 20 individual values in the normoxic plot within the variation in $\dot{V}O_{2max}$ testing while 16/20 fall within the variation in hypoxia.

Figure 10 displays the difference in incremental and supramaximal $\dot{V}O_2$ plotted against incremental $\dot{V}O_2$ in normoxia and hypoxia. A significant, negative relationship ($p = 0.004$, $r = -0.61$) exits between the delta $\dot{V}O_2$ and incremental $\dot{V}O_2$ in normoxia. In hypoxia, a nonsignificant, negative relationship ($p = 0.09$, $r = -0.39$) exists between delta $\dot{V}O_2$ and incremental $\dot{V}O_2$.

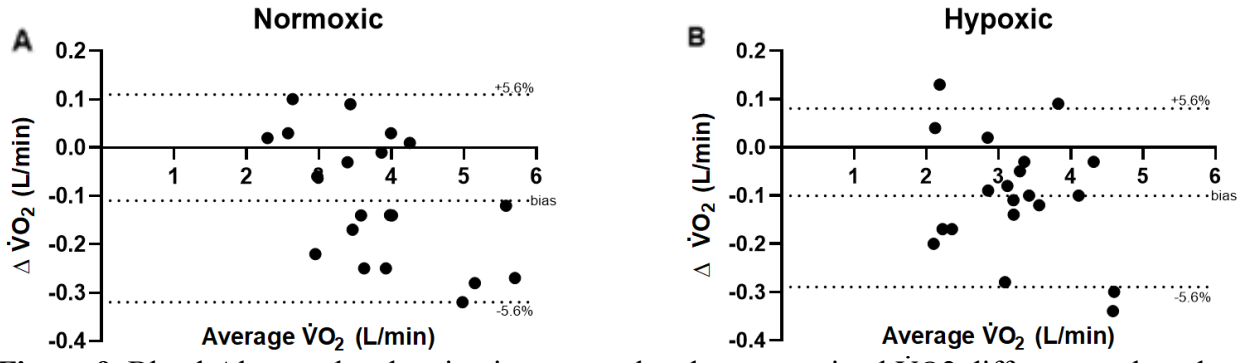

Figure 9. Bland-Altman plot showing incremental and supramaximal V̇O2 differences plotted against normoxic (A) and hypoxic (B) V̇O2. The horizontal dashed lines represent the upper and lower bounds of the coefficient of variation in V̇O2max testing (78). V̇O2

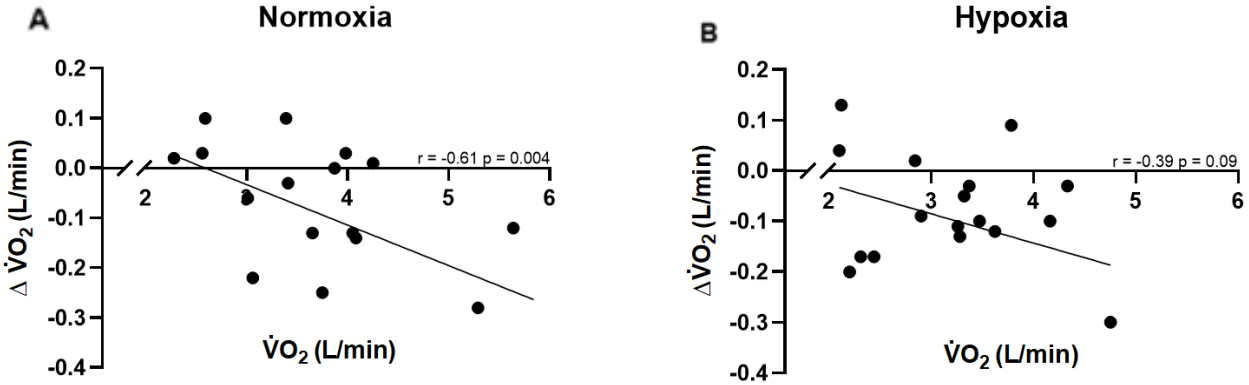

Figure 10. Relationship between the difference in incremental and supramaximal $\dot{V}O_2$ over incremental $\dot{V}O_2$ in normoxia (Panel A) and hypoxia (Panel B). $\dot{V}O_2$ = oxygen uptake.

5.7 Supramaximal exercise test

Figure 10 shows the individual $\dot{V}O_2$ response to supramaximal exercise testing in normoxia (black) and hypoxia (grey) as a percent of supramaximal duration. The orange line represents the maximal average incremental $\dot{V}O_2$ in normoxia and hypoxia. The red line represents the average supramaximal $\dot{V}O_2$ in normoxia and hypoxia. The maximal average supramaximal $\dot{V}O_2$, was significantly greater ($p = 0.001$) in normoxia (3.76 ± 0.92 L/min) than hypoxia (3.16 ± 0.78 L/min). The supramaximal test duration was significantly greater ($p = 0.02$) in hypoxia (140 ± 20s) than normoxia (132 ± 14s). The $\dot{V}O_2$ was significantly higher ($p < 0.001$) in the 25% duration compared to the 0% duration as well as the 50% compared to the 25% in both conditions. No other duration, in either condition, was significantly different ($p > 0.05$). The hypoxic $\dot{V}O_2$ was similar ($p = 0.001$) in the 75% and 100% supramaximal duration, 90% confidence interval [-0.59, 0.11]. In normoxia, $\dot{V}O_2$ was not similar ($p = 0.45$) between the 75% and 100% duration. VO_2 was also not similar in any previous comparisons in normoxia ($p > 0.05$).

$\dot{V}CO_2$ was significantly higher ($p < 0.001$) in the normoxic supramaximal test compared to the hypoxic test. The RER was significantly higher ($p = 0.02$) in the hypoxic than normoxic supramaximal test. Metabolic equivalents ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$) were also significantly higher ($p = 0.001$) in the supramaximal test in hypoxia than normoxia. The FIO_2 and PO_2 were significantly higher ($p = 0.001$) in the normoxic supramaximal test than the hypoxic test. Oxyhemoglobin saturation was significantly higher ($p < 0.001$) in the normoxic compared to hypoxic supramaximal test.

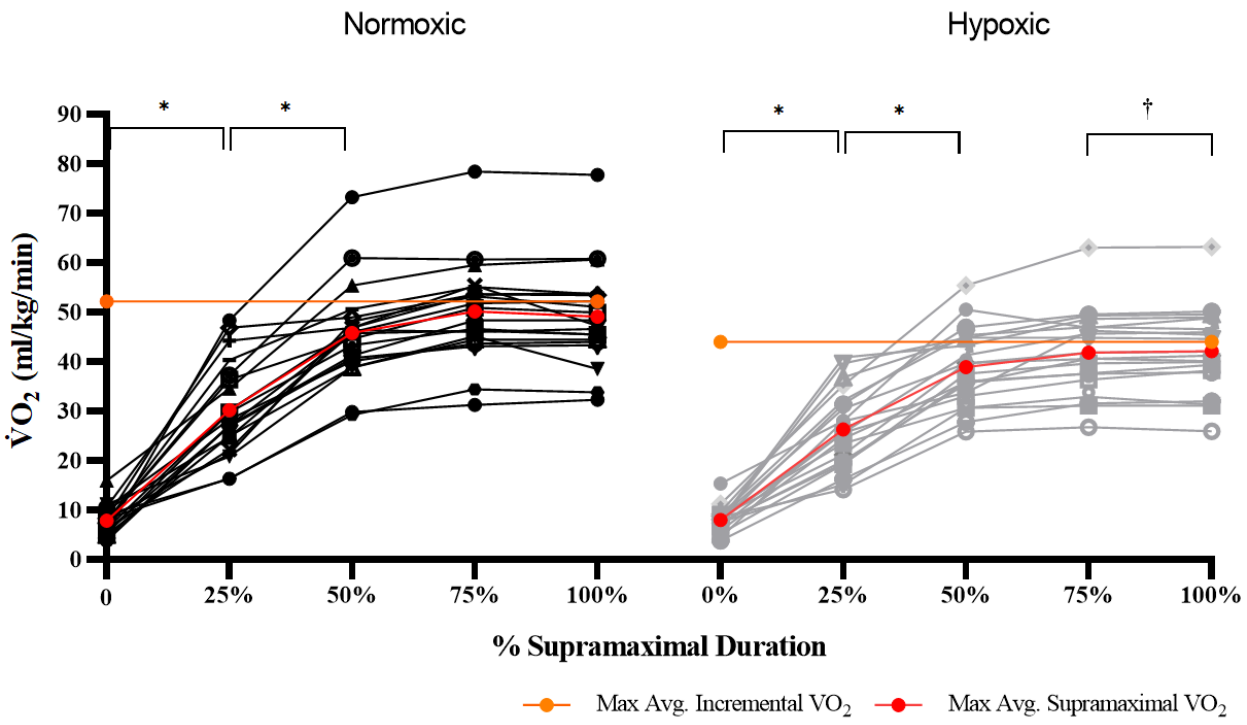

Figure 11. $\dot{V}O_2$ response to supramaximal exercise in normoxia and hypoxia. Normoxia is represented in the left, black figure while hypoxia is represented in the right, grey figure. The orange line represents the average incremental $\dot{V}O_2$ while the red line represents the average supramaximal $\dot{V}O_2$. $\dot{V}O_2$, oxygen uptake. * Significantly different between stage ($p < 0.05$). † Sufficiently similar between stages ($p < 0.05$).

5.8 Ventilatory response to supramaximal exercise testing

Figure 11 shows the ventilatory response to supramaximal exercise testing, expressed as a percent of total supramaximal duration. Panel A displays the average ventilatory response to supramaximal exercise with the grey line representing the maximal average incremental $\dot{V}_E$. The supramaximal $\dot{V}_E$ was significantly higher ($p < 0.05$) when compared to the previous % supramax duration in both normoxia and hypoxia. The maximal average incremental and supramaximal $\dot{V}_E$ were not different in normoxia ($p = 0.16$) and hypoxia ($p = 0.19$). Panel B in Figure 11 displays the average breathing frequency and tidal volume response to supramaximal exercise. The orange line represents the maximal average incremental V_T in normoxia and

hypoxia while the red line represents the maximal average incremental F_B in normoxia and hypoxia. The maximal average supramaximal V_T was not significantly different ($p = 0.70$) between normoxia and hypoxia. The V_T was significantly lower in the 0% vs. 25% ($p < 0.05$) and 25% vs. 50% ($p < 0.05$) in normoxia and hypoxia. The maximal average F_B was not statistically different ($p = 0.33$) between supramaximal bouts in normoxia and hypoxia. The F_B was significantly lower in each successive comparison of % supramaximal duration ($p < 0.05$) (i.e., 0% lower than 25% and 25% lower than 50%). The maximal average F_B was not significantly different between incremental and supramaximal tests in normoxia ($p = 0.78$) and hypoxia ($p = 0.09$). The maximal average V_T was significantly lower ($p = 0.04$) in supramaximal test than the incremental test in normoxia (2.39 ± 0.6L vs. 2.48 ± 0.6L for supramaximal and incremental, respectively) but not significantly different between tests in hypoxia ($p = 0.19$).

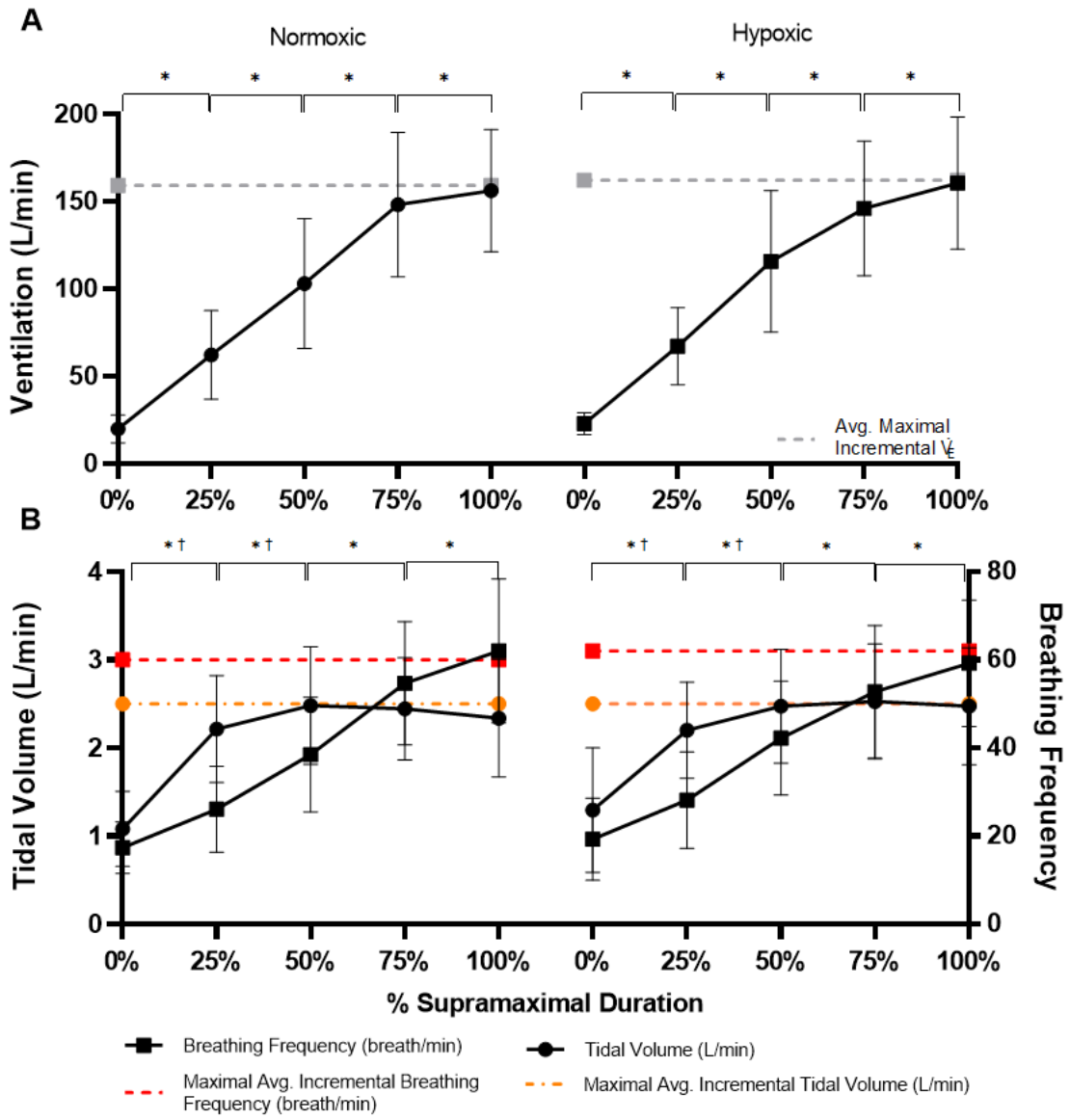

Figure 12. Ventilatory response to supramaximal exercise in normoxia and hypoxia. The supramaximal test is expressed as a percentage of the supramaximal test duration. Panel A displays the ventilatory response to supramaximal exercise. The grey line represents the maximal average incremental $\dot{V}_E$ in each condition. Panel B displays the V_T and F_B response to supramaximal exercise. The orange line represents the maximal average incremental V_T, and the red line represents the maximal average incremental F_B. $\dot{V}_E$, expired ventilation; V_T, tidal volume; F_B, breathing frequency. Panel A: * Ventilation significantly different from previous stage ($p < 0.05$). Panel B: † Tidal volume is significantly different ($p < 0.05$). * Breathing frequency is significantly different ($p < 0.05$).

6. DISCUSSION

6.1 Major Findings

This thesis sought to determine the efficacy of supramaximal exercise testing in acute hypoxia to verify $\dot{V}O_{2max}$ in healthy adults. The results indicate that supramaximal exercise testing yielded significantly lower $\dot{V}O_2$ than incremental testing in normoxia ($p = 0.002$) and hypoxia ($p = 0.001$). Equivalence testing further supported this finding, indicating that supramaximal $\dot{V}O_2$ exceeded the equivalence bounds of ±1 mL/kg/min when compared to incremental testing. These findings contradict those of Nolan et al., (2004) and Astorino et al., (2009), who found that supramaximal verification testing performed 20-minutes after incremental exercise produced nonsignificant differences in $\dot{V}O_2$.

6.2 Incremental and supramaximal testing

Significantly lower $\dot{V}O_2$ in supramaximal testing could be partly explained by the significantly lower heart rates ($p = 0.001$) measured in supramaximal testing than in incremental testing, because heart rate is the main modulator of cardiac output in high intensity exercise (3). Indeed, both maximal $\dot{V}O_2$ and maximal heart rate may not have been reached in supramaximal testing because volitional fatigue could have occurred before $\dot{V}O_{2max}$ could be achieved (77). Previous literature has also found heart rate to be significantly lower ($p = 0.001$) in supramaximal testing than in incremental testing, suggesting the former was inadequate in eliciting maximal values (80).

Another explanation for a significant difference between incremental and supramaximal $\dot{V}O_2$ could be the significant effect of the $\dot{V}O_2$ bias, as the supramaximal test underestimates $\dot{V}O_2$ in nearly all subjects and in both conditions (see Figure 9). Indeed, a positive bias (0.11 for both normoxia and hypoxia, respectively) favored the incremental test in both conditions. However,

this could be the result of our *a priori* determination of -1 and 1 mL/kg/min equivalence bounds (78) being too strict and potentially underestimate the incidence of $\dot{V}O_2$ verification using supramaximal exercise testing. Alternatively, when equivalence bounds of ±2.1 mL/kg/min, a widely-accepted primary criterion in determining VO_{2max} (64), were used, the incremental and supramaximal $\dot{V}O_2$ were statistically similar in both normoxia ($p = 0.04$) and hypoxia ($p = 0.05$). It would thus be advisable to carefully consider the equivalence bounds and their basis used in future testing.

Finally, the accumulation of H^+ due to lactic acid accumulation may further impair performance in hypoxic supramaximal testing compared to incremental testing. The increased reliance on anaerobic energy pathways in hypoxia to support the onset of the supramaximal exercise should theoretically increase the oxygen deficit more than the incremental test and result in greater blood lactate and RER values because of the increased buffering of lactic acid (81). This is consistent with this thesis' finding that RER values were significantly higher ($p = 0.02$) in the hypoxic supramaximal test than incremental test. Hypoxia results in increased reliance on the glycolytic system during exercise due to the reduction in systemic oxygen content and the increased ATP synthesis via anaerobic pathways, which increases the H^+ ions and decreases pH relative to normoxia (82, 83). During exercise, the accumulation of H^+ decreases bicarbonate and pH and causes metabolic acidosis. Due to a progressive decrease in $[HCO_3^-]$, arterial pH progressively decreases over the duration of the incremental exercise test (84). The increased hydrogen ion concentration in the blood will stimulate the peripheral and central chemosensors to increase ventilation to expire CO_2, decrease PCO_2, and increase pH (36, 37) Prolonged hypoventilation can occur post-exercise to increase PCO_2 in response to sustained metabolic acidosis. Bicarbonate remains significantly lower, and pH remains significantly more acidic 20

minutes post incremental exercise test (84). Therefore, the blood buffering capacity of H^+ will be reduced beginning the supramaximal exercise test, possibly explaining the decreased exercise performance that caused the lower $\dot{V}O_2$ values reported by this thesis.

The significant, negative relationship ($p = 0.004$, $r = -0.61$) found between the delta $\dot{V}O_2$ and incremental $\dot{V}O_2$ in normoxia (Figure 10A) suggest individuals with greater $\dot{V}O_2$ experienced greater $\dot{V}O_2$ differences between incremental and supramaximal bouts. However, this relationship was not similar in hypoxia ($p = 0.09$, $r = -0.39$) (Figure 10B). Individual differences between exercise bouts in normoxia and hypoxia were compared and revealed two thirds of participants had a similar difference between the incremental and supramaximal bouts in both normoxia and hypoxia. The non-significant relationship found in hypoxia may be underpowered due to a few participants having dissimilar responses to incremental and supramaximal bouts in normoxia and hypoxia and may become significant with the addition of participants.

A reduction in $\dot{V}O_{2max}$ during acute hypoxia has been associated with a decreased arterial oxygen content and SaO_2 at max exercise due a diffusion limitation across the blood gas barrier. Previous literature determined virtually all the change in $\dot{V}O_{2max}$ observed between normoxia and hypoxia ($FiO_2 = 0.14$) was found to result from reductions in SaO_2 and thus CaO_2 (44). However, this may not be the case in the present study. In normoxia, the $\dot{V}O_2$ and HR were significantly greater in the incremental test than the supramaximal test while the saturation was only 0.2% lower during the supramaximal test. In hypoxia, the incremental $\dot{V}O_2$ and HR were also significantly greater than the supramaximal test while the saturation was 3.2% higher during the supramaximal test. The difference in incremental and supramaximal $\dot{V}O_2$ weren't all that different between conditions (-2.8% in normoxia and -3.4% in hypoxia). This would suggest that

the change in SpO_2 wasn't large enough or does not have a strong of an effect as expected on $\dot{V}O_2$ in the present study.

Differences in hypoxic oxyhemoglobin decrements may be useful in predicting normoxic $\dot{V}O_2$. For instance, Figure 5B shows a significant positive relationship ($p = 0.006$, $r = 0.70$) between normoxic $\dot{V}O_2$ and delta $\dot{V}O_2$ between normoxia and hypoxia, in which, the participants with a higher $\dot{V}O_2$ had a greater drop in $\dot{V}O_2$ between conditions. Previous literature has found the relationship between normoxic $\dot{V}O_2$ and hypoxic $\dot{V}O_2$ decrement to range from $r = 0.54$ to $r = 0.94$ (44, 85, 86) which support our finding ($r = 0.70$). Figure 7 displays the significant positive relationship between the percent change in $\dot{V}O_2$ and SpO_2 between normoxia and hypoxia, in which, participants with a greater percent change in $\dot{V}O_2$ had greater changes in SpO_2 in hypoxia. Therefore, the larger hypoxic $\dot{V}O_2$ decrement, indicative of a higher normoxic $\dot{V}O_2$ (Figure 5), the greater difference in SpO_2 between normoxia and hypoxia (Figure 7). Thus, predictions of normoxic $\dot{V}O_2$ may be inferred by observing differences in hypoxic oxyhemoglobin decrements.

6.3 Hypoxic $\dot{V}O_2$ decrements

The partial pressure of oxygen decreases as altitude increases or as the fraction of inspired oxygen decreases (87), causing a reduction in the partial pressure of oxygen throughout the oxygen cascade, hindering oxygen delivery and reducing $\dot{V}O_{2max}$ relative to normoxia (88). Decrements in $\dot{V}O_{2max}$ as high as 7% have been observed at altitudes as low as 580m (45) with $\dot{V}O_2$ decrements reaching nearly 50% at 5,300m (89). During exercise in normoxia and moderate altitude (<4,100m), peripheral fatigue accumulates during exercise until a critical threshold is reached (90, 91). Once the critical threshold is met, strong inhibitory neural feedback inhibits central motor output to limit the peripheral fatigue beyond the critical threshold and exercise does not continue, despite improved oxygenation via hyperoxic gas (92). At severe altitude

(>5100m), arterial hypoxemia during exercise can pose a severe threat to the brain and thus central motor feedback is limited regardless of the peripheral fatigue threshold. When oxygenation is improved at the end of exercise, time to exhaustion is improved (+170%) and exercise continues until the peripheral fatigue threshold is reached (92).

Since $\dot{V}O_2$ has been shown to progressively decrease with stepwise reductions in PO_2 (44, 93), it can be determined that reductions in $\dot{V}O_2$ correspond to reductions in PO_2 (44). As a result, predictive equations can be derived to estimate the hypoxic $\dot{V}O_2$ decrement (94). For instance, our estimated hypoxic $\dot{V}O_2$ was 45.9 mL/kg/min while our actual hypoxic $\dot{V}O_2$ was 44.0 mL/kg/min. This model was fairly accurate (root-mean-squared error = 3.9 mL/kg/min^{-1}) in predicting hypoxic $\dot{V}O_2$ and explained about 80% of the variance in the model (94). A linear relationship ($r = 0.94$, $p < 0.05$) exists between the normoxic $\dot{V}O_2$ and the relative change in $\dot{V}O_2$ between normoxia and hypoxia (44). So, an individual with a $\dot{V}O_2$ of 52ml/kg/min should have a relative $\dot{V}O_2$ reduction of ~8 ml/kg/min at $FIO_2 = 0.14$ (44). In the present study, we observed a $\dot{V}O_2$ reduction of 8.1ml/kg/min at $FIO_2 = 0.146$. Therefore, our hypoxic decrement is like the hypoxic decrement observed in Lawler et al., (1988), and similar to the predictive equation reported in MacInnis et al., (2015).

6.4 Inspired oxygen and pressure differences

The FIO_2 was significantly lower in the incremental compared to the supramaximal bout in normoxia ($p = 0.04$) and hypoxia ($p = 0.006$). However, the percentage difference between incremental and supramaximal FIO_2 was only 0.2% in normoxia (21.1 ± 0.05% and 21.1 ± 0.06%) and 0.1% in hypoxia (14.7 ± 0.5% and 14.7 ± 0.5%). The supramaximal PO_2 was significantly lower ($p = 0.001$) than the incremental PO_2 in normoxia (mean ± S.D.; 159.8 ± 1.0mmHg vs. 159.6 ± 1.0mmHg). The PO_2 maximally differed by 4.1mmHg during the

normoxic incremental test and 4.2mmHg during the normoxic supramaximal test. Physiologically, the difference in inspired oxygen is trivial and does not reach levels to stimulate a ventilatory response (95, 96). Decreases in PO_2 of 4mmHg regularly occur due to weather changes and elevation changes. For instance, barometric pressure can differ by ~30 mmHg at a given elevation because of standard weather variations and thus alter PO_2 by ~6mmHg (97). At least a 50 mmHg drop in barometric pressure, resulting in ~10 mmHg decrease in PO_2, is required to significantly hinder $\dot{V}O_{2max}$ and reduce SpO_2 in trained adults at 580m (45). Therefore, we do not expect a significant difference in normoxic FIO_2 and PO_2 and hypoxia FIO_2 to physiologically impact the exercise response in healthy adults.

7. PERSPECTIVES

Definitive supramaximal verification testing protocols have not been adopted; therefore, supramaximal verification has confirmed $\dot{V}O_{2max}$ at intensities ranging from 105-130% maximal incremental power (61, 63, 64, 98), test duration ranging from 2-6 minutes (2, 61, 99) and rest periods of 1, 3, 10, 20, 60 - 90min and >24 hours (61, 63, 66, 67). Thoden et al., (1991) suggested a verification test duration of 3 minutes was needed to reach $\dot{V}O_{2max}$ and advised the verification phase should be repeated at the same stage or one below the last completed incremental phase if the duration was less than 3 minutes (100). However, follow-up testing to confirm this recommendation was not conducted (101). Supramaximal test durations as short as 2 minutes have verified $\dot{V}O_2$ (61, 63). Our test duration ranged from 110 – 155s and 110 – 180s in normoxia and hypoxia, respectively. However, $\dot{V}O_2$ was not verified using equivalence bounds of ±1mL/kg/min.

Previous literature has adopted a square-wave exercise profile (63, 80, 99) when conducting supramaximal verification testing. A square-wave approach may not allow enough time for untrained individuals that exhibit slower $\dot{V}O_2$ kinetics (102) to reach maximal $\dot{V}O_2$ before exercise tolerance is reached. This approach may be further exacerbated in supramaximal bouts performed at too high of an intensity (>130%) (62). In the present study, a ramp approach was used to ease participants into the supramaximal workload and prevent a large "drop" of power at the start of the supramaximal bout in attempt to accommodate participants with slower $\dot{V}O_2$ kinetics. However, further testing to determine an appropriate ramp protocol and duration may be needed to optimize verification testing protocols since the $\dot{V}O_2$ was not similar between exercise bouts. A priming bout of exercise prior to the supramaximal test may prove useful in combatting varying rates of $\dot{V}O_2$ kinetics and improve the likelihood of achieving $\dot{V}O_{2max}$ (65).

Verification testing >24hr after incremental testing has shown to have no effect on verifying $\dot{V}O_2$ in sedentary adults (61). Testing on separate days may be advantageous since the participant is less fatigued which may increase the time to exhaustion and $\dot{V}O_2$ in the supramaximal verification bout. However, the utility and practicality of supramaximal testing is relinquished by having the participant complete the verification bout in a separate visit. Also, researchers have increased control of biological variability using same day testing. Since $\dot{V}O_2$ was not verified in the present study, future studies addressing test duration, intensity and rest period may provide insight and standardize verification testing protocols for future testing.

8. CONSIDERATIONS / LIMITATIONS

8.1 Participant recruitment

A limitation of this study is the unequal number of male and female participants which skews the findings toward a more male bias. For instance, the average male hypoxic $\dot{V}O_2$ decrement was -15% while the average female hypoxia decrement was -17%. However, the average hypoxic decrement was 15% due to the skew of the male participants. This study would have been improved by having equal number of men and women to reduce the skew of males and increase the generalizability of our findings.

8.2 Passive vs. active recovery

Blood lactate concentration remains elevated for 20 – 60 minutes after high intensity exercise during passive recovery (103). Compared to passive recovery, active recovery has shown to increase muscle blood flow and aid in removal of H^+ and lactate from the blood (104, 105). Also, active recovery has been shown to increase $\dot{V}O_2$ kinetics and $\dot{V}O_{2peak}$ during supramaximal exercise (110% W_{max}) following active recovery (106). By adjusting our protocol to include a period of active recovery, blood H^+ and lactate may be removed at a greater rate compared to passive recovery and aid $\dot{V}O_2$ kinetics during the supramaximal exercise bout.

8.3 Acute normbaric hypoxia

Due to the limitations of our research facilities, altitude research must be conducted in normbaric hypoxia (NH). Although a PO_2 similar to hypobaric hypoxia (HH) can be achieved in NH, via alterations in FIO_2, physiological differences exist between NH and HH. For instance, increased F_B with lower V_T and $\dot{V}_E$, implying increased dead space ventilation, lead to lower SaO_2 as well as increased hypoxemia, hypocapnia and blood alkalosis in HH (107). The onset and development of acute mountain sickness was found to be greater in HH compared to NH and

could be explained by a combination of the greater hypoxemia, hypocapnia and blood alkalosis and hypobaria (108). The differences between NH and HH could be the result of increased dead space ventilation, potentially related to the reduction of barometric pressure (107).

8.4 Compressed Gas

Compressed gasses were used to lower the FIO_2 and simulate altitude during the hypoxic visit. During the normoxic visit, compressed room air was used to maintain consistency of compressed gas usage. Due to the nature of compressed air being stored as a liquid, the gas is cold and dry when released. Compressed air can be humified by bubbling the gas through water in a sealed reservoir. However, the level of humification is limited to surface area of the gas/water interface. To ensure precision and accuracy in our calculations, the compressed gas was not humified in either trial to avoid potential error in incomplete humidification and/or the potential for an inadequate seal of the bubbling reservoir allowing room air to mix with the compressed gas. A potential limitation of ventilating with dry gas is the loss of moisture in the respiratory tract, particularly in the trachea. In sensitive individuals, such as asthmatics, bronchoconstriction can occur. In attempt to limit the effects of dry gas on the respiratory system, participants with respiratory health conditions were excluded in the study. No significant difference existed between normoxic and hypoxic FVC, FEV1 and FEV1/FVC pre-exercise as well as post-exercise. So, the compressed gasses may have not had a significant effect on participants' pulmonary function.

8.5 Hypoxic Ventilatory Decline

Minutes after hypoxic exposure, a short term ventilatory depression, or hypoxic ventilatory decline, occurs as a result of an overshoot in breathing frequency and works to reduce breathing frequency via reductions in phrenic nerve activity. Hypoxic ventilatory decline can last for a few

hours and may be followed by a progressive rise in $\dot{V}_E$ (109, 110). Therefore, before starting exercise, participants could be experiencing some degree of hypoxic ventilatory decline. The effect of hypoxic ventilatory decline on exercise is not well understood. Therefore, hypoxic ventilatory decline may limit the ventilatory response at max exercise in acute hypoxia. However, no significant difference between maximal ventilation in normoxic and hypoxic was found in the present study.

9. CONCLUSION

In conclusion, this thesis sought to determine the efficacy of supramaximal exercise testing in acute hypoxia to verify $\dot{V}O_{2max}$ in healthy adults. Equivalence testing indicated that supramaximal $\dot{V}O_2$ was not sufficiently similar to incremental testing in acute hypoxia. Supramaximal exercise testing yielded significantly lower $\dot{V}O_2$ than incremental testing in normoxia and acute hypoxia. However, the mean difference in incremental and supramaximal $\dot{V}O_2$ in normoxic and acute hypoxic was less than 150mL/min, and physiologically speaking, should verify supramaximal exercise testing in acute hypoxia. Supramaximal exercise testing may be beneficial in verifying $\dot{V}O_{2max}$ and could provide a viable tool to verify $\dot{V}O_{2max.}$ Future research involving $\dot{V}O_{2max}$ verification testing may be useful to establish a widely accepted supramaximal verification testing criterion.

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