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The ventilatory response to modified rebreathing is unchanged by hyperoxic severity: implications for the hyperoxic hyperventilation paradox

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Abstract

We measured the ventilatory response to hyperoxic CO_2 rebreathing with O_2 tension clamped at increasingly higher pressures. We hypothesized that the $\dot{V}_E$ versus PCO_2 relationship is fixed and independent of PO_2. Twenty participants (10 females; mean± SD age: 24±4 years) performed three repetitions of modified rebreathing in 4, randomized, isoxic-hyperoxic conditions: mild: PO_2=150 mmHg; moderate: PO_2=200 mmHg; high: PO_2=300 mmHg; and extreme: PO_2≈700 mmHg. For each rebreathing trial, the $P_{ET}CO_2$ at which $\dot{V}_E$ rose was identified as the ventilatory recruitment threshold (VRT, mmHg), data before VRT provided baseline $\dot{V}_E$ ($\dot{V}_E$BSL, L·min^{-1}) and the slope of the response above VRT gave the central chemoreflex sensitivity ($\dot{V}_E$S, L·min^{-1}·mmHg^{-1} There were no effects of $P_{ET}O_2$ on $\dot{V}_E$BSL (mild: 7.4±4.2 L·min^{-1}; moderate: 6.9±4.2 L·min^{-1}; high: 6.5±3.7 L·min^{-1}; extreme: 7.5±2.7 L·min^{-1}; p=0.24), VRT (mild: 42.8±3.2 mmHg; moderate: 42.5±2.7 mmHg; high: 42.3±2.7 mmHg; extreme: 41.8±2.7 mmHg; p=0.07), or $\dot{V}_E$S (mild: 4.88±2.6 L·min^{-1}·mmHg^{-1}; moderate: 4.76±2.2 L·min^{-1}·mmHg^{-1}; high: 4.81±2.3 L·min^{-1}·mmHg^{-1}; extreme: 4.39±1.9 L·min^{-1}·mmHg^{-1}; p=0.41). Hyperoxia does not independently stimulate breathing, nor does it affect central chemoreflex sensitivity.

Keywords: Oxygen, ventilation, chemoreflex, chemoreceptor, breathing,

Summary for Lay Audience

It is known that high-oxygen breathing causes people to breathe more than normal. The higher the oxygen level above normal, the harder one breathes, but we do not know the reason for why this happens. Two possible reasons are that high oxygen turns on breathing on its own or that high oxygen causes the body to hold on to carbon dioxide. When carbon dioxide levels rise in the brain, special gas sensors called central chemoreceptors become excited and tell other areas of the brain that are in charge of breathing to increase breathing more. Some people think that high oxygen makes these sensors more excitable. If this is the case, then breathing responses to carbon dioxide should become more vigorous with higher levels of high-oxygen breathing. In this study, we tested this idea by asking 10 males and 10 females to breathe carbon dioxide at 4 levels of higher-than-normal oxygen breathing. Before carbon dioxide levels were raised, we had these volunteers breathe these 4 levels of oxygen when carbon dioxide levels were low and the central chemoreceptors were “turned off”. We found that how hard people breathed did not change regardless of high oxygen level and this was the same when carbon dioxide levels were low and high. Our findings indicate that oxygen on its own or through a sensitization of central chemoreceptors does not explain why high-oxygen breathing causes people to breathe more than normal.

Co-Authorship

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D.A. Keir and J. Duffin conceived and designed the study. J.D. Huggard and N.A. Guluzade acquired the data. All authors interpreted the results of the experiment. J.D. Huggard, N.A. Guluzade, and D.A. Keir analysed the data and prepared the first draft of the manuscript. All authors edited the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify are listed.

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List of Abbreviations

Atm – Standard atmosphere

ANOVA – Analysis of variance

CaO_2 – Arterial oxygen content

CNS – Central nervous system

CSF – Cerebrospinal fluid

CO_2 – Carbon dioxide

COVID-19 – Coronavirus disease

F_iO_2 – Fractional concentration of inspired oxygen

f_B – Breathing frequency

H^+ - Hydrogen ion; proton

HCO_3^- – Bicarbonate

H_2CO_3 – Carbonic Acid

IX – Glossopharyngeal nerve

N_2 – Nitrogen

·NO – Nitric Oxide

O_2 – Oxygen

$\cdot O_2^-$ – Superoxide

$PACO_2$ – Alveolar partial pressure of carbon dioxide

$PaCO_2$ – Arterial partial pressure of carbon dioxide

PAO_2 – Alveolar partial pressure of oxygen

PaO_2 – Arterial partial pressure of oxygen

PCO_2 – Partial pressure of carbon dioxide

PO_2 – Partial pressure of oxygen

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

$P_{ET}O_2$ – End-tidal partial pressure of oxygen

RCPG – Respiratory central pattern generator

ROS – Reactive oxygen species

RNS – Reactive nitrogen species

RTN – Retrotrapezoid nucleus

SD – Standard deviation

$\dot{V}_E$ – Minute ventilation; Pulmonary ventilation

V_T – Tidal volume

$\dot{V}_E$BSL – Basal ventilation; wakefulness drive to breathe

$\dot{V}CO_2$ – Carbon dioxide output

$\dot{V}_E$S – Ventilatory sensitivity to CO_2; central chemoreflex sensitivity

VRT – Ventilatory recruitment threshold

$\dot{V}O_2$ – Oxygen uptake

[ ] – Concentration

Introduction

1 Literature Review

1.1 General Introduction

Breathing is a vital behaviour that is often taken for granted yet necessary to replace oxygen (O_2) mainly utilized in aerobic metabolism to provide energy and remove the byproduct of aerobic metabolism, carbon dioxide (CO_2). Pulmonary ventilation ($\dot{V}_E$) involves the exchange of atmospheric air in and out of the lungs. The inspiratory phase occurs due to active contraction of the diaphragm and external intercostal muscles. Together, their action reduces intrathoracic pressure allowing O_2 rich ambient air to flow into the respiratory tract, through the airways and permit oxygen uptake ($\dot{V}O_2$) in the body via the alveoli. Conversely, in the expiratory phase, passive relaxation of the diaphragm and external intercostals increases intrathoracic pressure which forces air out of the lungs and permits the clearance of carbon dioxide ($\dot{V}CO_2$). The alveoli are capillary-dense and structured specifically to facilitate gas exchange from gas permeable type I alveolar cells (Knudsen & Ochs, 2018). Importantly, the partial pressures of gases in the alveoli are integral for the diffusion of O_2 and CO_2 across the alveolar membrane. With respect to an ambient sea-level environment, the partial pressure of O_2 (PO_2) in the alveoli (PAO_2) is higher than in the surrounding capillary environment (PaO_2). Conversely, the partial pressure of CO_2 (PCO_2) in the capillaries ($PaCO_2$) is higher than in the alveoli ($PACO_2$). Thus, the pressure gradient of the former allows for O_2 diffusion from the alveoli to the capillaries where O_2 binds to hemoglobin within red blood cells, and the latter permits CO_2 to diffuse from capillaries to alveoli whereafter it is expelled during exhalation (Taylor & Weibel, 1981).

Breathing is a labile yet necessary physiological function that fluctuates by voluntary (i.e., behavioural) and involuntary (i.e., acid-base equilibrium, metabolic state) drives to breathe that are functionally integrated (Duffin, 2010). Despite the deceivingly simple nature of breathing, this rhythmic behaviour requires sophisticated programming and control from the respiratory central pattern generator (RCPG) in the brainstem to ensure adequate gas exchange and maintain blood gas homeostasis (Del Negro et al., 2018). Although the PO_2

of ambient air is sufficient for O_2 to diffuse from alveoli to blood and saturate hemoglobin in healthy humans, often in conditions where gas exchange or $\dot{V}_E$ is impaired (e.g., respiratory failure, chronic obstructive pulmonary disorder, at altitude) higher than normal fractional concentrations of inspired oxygen (F_iO_2) (i.e., $F_iO_2 > 20.9\%$) are utilized to raise PO_2 and, thus the diffusive gradient for O_2 across the alveolar-arterial interface (Brugniaux et al., 2018; Horncastle & Lumb, 2019). Given the high prevalence of "hyperoxic" gas breathing in clinical care settings, understanding how raising F_iO_2 can impact the respiratory system and physiological homeostasis is important for clinicians and researchers.

1.2 Hyperoxia

When it comes to the connection between alveoli and hyperoxia, it is important to understand how the alveoli are involved in gas exchange and how they can be affected by high levels of oxygen. Hyperoxia is a state in which cells, tissues, and organs are exposed to an excess supply of O_2 or a higher-than-normal PO_2, typically defined as any F_iO_2 above normal physiological levels (0.21 atm or 21%) (Croal et al., 2015). Hyperoxia increases tissue O_2 delivery by heightening the driving pressure for O_2 diffusion from alveoli to blood and by raising arterial O_2 content (CaO_2) (Brugniaux et al., 2018). A state of hyperoxia can be induced by either increasing total atmospheric pressure (hyperbaric hyperoxia; for example, above 760 mmHg at sea level) or by inspiring an F_iO_2 greater than 21%. The two approaches are fundamentally different with respect to their impact on homeostasis and the compensatory physiological responses that occur (Brugniaux et al., 2018; Singer et al., 2021). Given that most humans would not encounter hyperbaric hyperoxia (unless participating in diving or clinically indicated therapy) this thesis will focus specifically on responses to normobaric hyperoxia.

During its administration in normobaric conditions, hyperoxia is known to impact several physiological systems including the cardiovascular, autonomic, and respiratory systems (Brugniaux et al., 2018). Although considerable investigation has taken place to understand hyperoxia and its potential consequences, how and why it impacts spontaneous breathing remains unresolved (Brugniaux et al., 2018; Dean & Stavitzski, 2022).

1.3 The Ventilatory Response to Hyperoxia

Over the past half-century, many researchers have investigated the effects of high F_iO_2 breathing in humans. Utilizing various methods of administration and observation, the prevailing consensus is that hyperoxia causes $\dot{V}_E$ to rise relative to its rate when breathing room air (Becker et al., 1995; Becker et al., 1996; Dean et al., 2004; Fernandes et al., 2021; Lambertsen, Kough, et al., 1953; Lambertsen, Stroud, et al., 1953; Marczak & Pokorski, 2004; Ren et al., 2000). In particular, Becker et al. (1996) often is cited as support for this assertion. In their study, the effect of different levels of hyperoxia (F_iO_2 = 30, 50, or 75%) on $\dot{V}_E$ was investigated during 30 minutes of quiet breathing during which end-tidal PCO_2 ($P_{ET}CO_2$ – a proxy for $PaCO_2$) was maintained constant (isocapnia) at normal resting pressures (~38 mmHg). The authors found that, on average, $\dot{V}_E$ increased from baseline by 1.8 L/min, 4.5 L/min, and 9.7 L/min, for F_iO_2 of 30, 50 and 75%, respectively. These findings suggest that hyperoxia stimulates breathing in a dose-dependent manner (i.e., the higher the F_iO_2, the greater the ventilatory stimulus). However, in a separate trial within that same experiment, the 75% F_iO_2 condition was repeated but with $P_{ET}CO_2$ allowed to vary (poikilocapnia) such that $P_{ET}CO_2$ could decrease if $\dot{V}_E$ rose and increase if $\dot{V}_E$ fell. In this poikilocapnic hyperoxic condition, $\dot{V}_E$ rose by ~1.3 L/min (~16%) and $P_{ET}CO_2$ decreased by ~4 mmHg compared to the 9.7 L/min (~115%) increase observed in the isocapnic experiment. Thus, at the same extreme level of hyperoxia, the excitatory ventilatory response was reduced by nearly 100% when $P_{ET}CO_2$ was not fixed and permitted to fall as $\dot{V}_E$ rose. These findings suggest that CO_2 and not O_2, *per se*, provided the bulk of the excitatory respiratory stimulus.

In steady-state hyperoxic experiments where $P_{ET}CO_2$ is clamped, one might expect that while arterial PCO_2 is maintained at normal tensions, PCO_2 at the venous and tissue level would rise due to the Haldane effect. The Haldane effect describes a physiological property of hemoglobin whereby heightened PaO_2 reduces the affinity of hemoglobin for CO_2 binding (Christiansen et al., 1914). The reason for this reduced affinity is explained by the heightened plasma PO_2 which causes O_2 molecules to remain bound to hemoglobin as they traverse the tissue capillaries. As a result, less carboxyhemoglobin is formed which reduces the transport of CO_2 from the tissue to the lungs and thus, local tissue PCO_2 rises. This

would be expected occur throughout the body including in the brainstem where central chemoreceptors are housed.

1.4 The Central Respiratory Chemoreceptors and Chemoreflex

Within the central nervous system (CNS) lies a population of neurons that are chemo-sensitive (respond reflexively to chemical stimuli) named the central respiratory chemoreceptors. More specifically, spread within the retrotrapezoid nucleus (RTN) located in the rostral end of the ventrolateral aspect in the medulla oblongata is home to the central respiratory chemoreceptors (Guyenet et al., 2019; Guyenet & Stornetta, 2022). The central chemoreceptors are an important modulator of neural respiratory control that regulate brain hydrogen ion concentration ($[H^+]$) at homeostatic levels by reflexively increasing ventilation (via the central respiratory chemoreflex) when elevations in local tissue $[H^+]$ are sensed (Cunningham, 1986.; Duffin, 1990; Guyenet, 2014; Guyenet et al., 2012, 2019). Both *in vitro* and *in vivo* experiments have demonstrated that central chemoreceptor excitation and the reflexive ventilatory response rise linearly with $[H^+]$ (Duffin et al., 2000; Guyenet et al., 2016; Li & Nattie, 2002; Mohan et al., 1999; Veasey et al., 1995). Because central $[H^+]$ is directly dependent on central PCO_2 these receptors are often referred to as "CO_2 sensors" – henceforth, this thesis will consider them as such (Duffin, 2010). Importantly, the central chemoreceptors are anatomically and functionally distinct from the peripheral chemoreceptors in the carotid body that respond to elevations in arterial $[H^+]$ with a sensitivity that is up-regulated by low PaO_2 and nearly eliminated at high PaO_2 (Cunningham & Lloyd, 1963; Duffin, 1990; Duffin et al., 2000; Mohan & Duffin, 1997; Prabhakar, 2013).

Central PCO_2 is directly related to $PaCO_2$ and metabolic CO_2 production in the medullary compartment, and inversely proportional to medullary blood flow (Ainslie & Duffin, 2009; Duffin, 2010). Thus, central PCO_2 will rise when $PaCO_2$ increases and/or when medullary blood flow decreases. The term central chemoreflex refers to the effect central chemoreceptors have on the control of PCO_2 through a reflexive $\dot{V}_E$ response. Central chemoreceptors are integral to the homeostatic control of PCO_2 (and brain $[H^+]$) via their involvement in a negative feedback loop in which higher PCO_2 stimulates chemoreceptors to increase $\dot{V}_E$ and consequently reduce PCO_2, and thus, $[H^+]$ (Duffin et al., 2000; Duffin,

2010). For example, with a rise in $PaCO_2$ (e.g., during a breath hold), CO_2 will diffuse across the blood brain barrier and combine with water to form carbonic acid (H_2CO_3) which further dissociates into H^+ and bicarbonate (HCO_3^-) consequently creating an acidic environment within the cerebrospinal fluid (CSF) (Duffin, 2005). As a corrective mechanism to maintain desired levels of PCO_2 and $[H^+]$, central chemoreceptors are directly stimulated by the increase in $[H^+]$ in the medullary CSF and send signals to the respiratory centers in the medulla, which, in turn, stimulate the respiratory muscles to increase tidal volume (V_T) and breathing frequency (f_B). The resultant increase in $\dot{V}_E$ – the product of V_T and f_B – permits the elimination of CO_2 through exhalation. As $PaCO_2$ and CSF CO_2 levels decrease, the $[H^+]$ of the CSF starts to decrease to the desired concentration and the central chemoreceptors start to reduce their input to medullary respiratory centers in the brain and central chemoreceptor-mediated respiratory drive decreases (Duffin, 2005, 2010, 2011). Thus, the central chemoreflex arc ensures that changes in PCO_2 are continuously monitored by central chemoreceptors and adjusted via the central chemoreflex to maintain homeostasis in the CSF through a negative feedback control system (Duffin, 2010, 2011).

With respect to heightened PaO_2 induced by high O_2 inhalation, the reduced ability to remove metabolically produced CO_2 (via the Haldane effect) in medullary tissue would cause local tissue PCO_2 to rise and activate central respiratory chemoreceptors (Christiansen et al., 1914) . Consequently, the observation of Becker et al. (1996) of a dose-dependent rise in $\dot{V}_E$ in isocapnic conditions (where $PaCO_2$ is independent of $\dot{V}_E$) could be attributed to heightened central chemoreflex drive and not to the direct affects of hyperoxia, *per se*. That the stimulatory effect of the highest O_2 condition was nearly abolished when $P_{ET}CO_2$ was allowed to vary suggests that CO_2 and not hyperoxia was responsible for the observed rise in $\dot{V}_E$. Thus, an alternative explanation for such "hyperoxic hyperventilation" is warranted.

1.5 Mechanisms of Hyperoxic Hyperventilation

The response to normobaric high O_2 breathing is characterized by an initial depression in $\dot{V}_E$ due to carotid body chemoreceptor inhibition followed ~3-5 min by a progressive rise in $\dot{V}_E$ above pre-hyperoxic levels (Buckler & Vaughan-Jones, 1994; Cunningham, 1987;

Dasso et al., 2000). In addition to the Haldane effect, several other mechanisms have been proposed to explain the hyperventilatory response. These include potential direct (Cragg et al., 1986; Miller & Tenney, 1975) or indirect effects of increased O_2 tension (Becker et al., 1995; Becker et al., 1996; Ciarlone & Dean, 2016b; Dean et al., 2004; Fernandes et al., 2021; Matott et al., 2014; Mulkey et al., 2001). This section provides a summary of these proposed mechanisms.

1.5.1 O_2-specific Mechanisms

With respect to normobaric hyperoxia, a study from 1975 proposed an O_2 specific effect that could be responsible for the hyperoxic hyperventilation phenomenon. Their hypothesis was that PO_2 in the normoxic range (i.e., ~85-100 mmHg) exerts an inhibitory effect on centrally-located respiratory centers and that hyperoxia removes this homeostatic suppression (Miller & Tenney, 1975). Their group compared $\dot{V}_E$ during room air breathing and hyperoxic breathing ($P_AO_2 \approx 425$ mmHg) in unanesthetized cats divided into two groups: normoxic and hyperoxic exposure before and after carotid body removal (i.e., peripheral chemoreflex removal). Before surgery, they found no between-condition differences in $\dot{V}_E$ at 10 minutes. Compared to hyperoxic breathing before surgery, after surgery, $\dot{V}_E$ was higher after 10 minutes, despite a fall in $PaCO_2$. They proposed that, in the absence of carotid body afferent input, normal PaO_2 may constrain central respiratory neuron activity but when hyperoxic PaO_2 is applied, disinhibition of this tonic inhibitory input elicits hyperoxic hyperventilation (Miller & Tenney, 1975).

Alternatively, Cragg et al. (1986) proposed that local elevations in lactate associated $[H^+]$ secondary to histotoxic-hypoxia in the carotid body (i.e., reduced ability of mitochondria to undergo oxidative phosphorylation) might explain hyperoxic hyperventilation. In this scenario, heightened PO_2 would lead to disruption of mitochondrial enzymes necessary for oxidative phosphorylation in regions of high metabolic activity (e.g., the brain and carotid body) necessitating an acceleration of anaerobic glycolysis (Cragg et al., 1986). In their experimental protocol, two groups of anaesthetized rats with or without their carotid sinus nerve sectioned underwent a hyperbaric-hyperoxic (4, 6, or 8 atm) exposure lasting 30-60 minutes. During the 30-60 min period of exposure to hyperbaric hyperoxia, $\dot{V}_E$ rose progressively when administered to intact rats. However, in the carotid sinus-sectioned

rats, the slope of the $\dot{V}_E$ increase vs. time was slightly less than intact rats at 4 atm, identical to intact rats at 6 atm and, at 8 atm, the slope decreased. Thus, the findings in the carotid sinus-sectioned group indicated some contribution of the carotid body to increases in $\dot{V}_E$ during progressive hyperbaric hyperoxia. The authors postulated that, like the carotid body, heightened central PO_2 would also lead to disruption of oxidative phosphorylation in brain tissue and that ensuing lactic acidosis stimulates central chemoreceptors to raise $\dot{V}_E$ (Cragg et al., 1986).

1.5.2 Medullary Blood Flow

It is well-documented that hyperoxic breathing reduces medullary blood flow (Davi et al., 1980; Dean et al., 2004; Lambertsen, Kough, et al., 1953; Ren et al., 2000). Reduced local blood flow is expected to diminish the ability to clear metabolically derived CO_2 (i.e., reduced CO_2 wash-out) and increase central PCO_2. Bulte and colleagues (2007) employed a cerebral perfusion (i.e., flow rate in the cerebral circulation) imaging technique with MRI whilst administering 20% step-increases of O_2 (F_iO_2 = 0.21, 0.4, 0.6, 0.8 and 1.0; respectively) to directly quantify the differences of progressive hyperoxia on cerebral perfusion (Bulte et al., 2007). They found that from each F_iO_2 step-increase cerebral perfusion decreased from normoxia in a dose-dependent manner (F_iO_2 = 0.21, Flow %= 100; 0.4, 97; 0.6, 96; 0.8; 95; 1.0, 93; respectively) further elucidating that hyperoxic bouts appear to reduce regional cerebral blood flow (Bulte et al., 2007). Furthermore, Mattos et al. (2019) demonstrated that isocapnic hyperoxia reduces blood flow through the arteries serving the brain. Compared to isocapnic normoxia, 100% F_iO_2, breathing in isocapnic conditions induced a reduction in blood flow by 6.3 mL/min and 62.9 mL/min in the vertebral and internal carotid arteries, respectively), providing evidence in peripheral arteries that steady-state hyperoxic administration attenuates blood flow.

1.5.3 Reactive Species and Central Chemoreceptor Sensitization

An emerging hypothesis to explain hyperoxic hyperventilation is that O_2 stimulates breathing indirectly via reactive species-induced augmentation of central chemoreceptor excitability in the solitary complex (Ciarlone & Dean, 2016b, 2016b; Matott et al., 2014; Mulkey et al., 2003). Reactive species are a molecule species that contain unpaired

electrons, which have the propensity to attack neurons and disrupt normal function in large concentrations via oxidative and nitrosative stress (Ciarlone & Dean, 2016b; Mulkey et al., 2003). Conversely, in lower concentrations reactive species can even participate in normal physiological signaling processes. (Ciarlone & Dean, 2016b; Mulkey et al., 2003). Most of the experimental work supporting this idea stems from exposure of reduced rat brain preparations to either normobaric or hyberbaric hyperoxia and measuring the electrophysiological characteristics of specific brain regions associated with respiration (Ciarlone & Dean, 2016b; Dean et al., 2004; Matott et al., 2014; Mulkey et al., 2001, 2003). When excess O_2 is given to the mammalian system, it becomes subject to increased amounts of reactive species such as superoxide ($\cdot O_2^-$) and nitric oxide ($\cdot NO$) which are known as reactive oxygen (ROS) and reactive nitrogen species (RNS), respectively. If the concentration of reactive species during hyperoxic exposure persists, this may cause oxidative stress, which occurs when the balance between the production of reactive species and the body's antioxidant defense mechanisms is disrupted (Ciarlone & Dean, 2016b; Dean et al., 2004; Dean & Stavitzski, 2022). Therefore, if hyperoxic stimulus is sufficiently severe and exposures are prolonged, this can result in oxidative damage due to excess production of reactive species ((Dean et al., 2004; Dean & Stavitzski, 2022).

Studies from Ciarlone and Dean have demonstrated that increasing O_2 from 0.4 to 0.95 atm (i.e., 'moderate' hyperoxic to 'severe' hyperoxic PO_2) increases ROS and RNS in rat medullary brain slices, and simultaneously stimulates chemosensitive neurons in the caudal solitary complex (Ciarlone & Dean, 2016b, 2016a). Intracellular recordings of CO_2-sensitive neurons in the solitary complex of rat brain slices exhibited diminished membrane conductance (i.e., heightened excitability) when exposed to one hour of hyperbaric hyperoxia (2-3 atm) (Mulkey et al., 2003). Interestingly, the effects of hyperoxia on the electrical potential of these neurons disappeared in the presence of anti-oxidant and re-established with chemical oxidant. Using this same model, other studies demonstrated similar effects on the firing rate response of excited central chemoreceptors in conditions of normobaric hyperoxia (0.4 to 0.95 atm) (Matott et al., 2014; Mulkey et al., 2001). Collectively, these findings indicate that ROS-inducing hyperoxia modulates the excitability of central chemoreceptors such that their sensitivity to PCO_2 is increased.

Recently in humans, Fernandes et al. explored the effects of ROS on the ventilatory response to poikilocapnic hyperoxia (Fernandes et al., 2021). In this study, they included measures of sonographic vertebral and internal carotid artery blood flow, arterial and intra-venous jugular blood gases, and ventilation. To test the effect of ROS on the hyperventilatory response to poikilocapnic hyperoxia, $\dot{V}_E$ was measured during antioxidant infusion and compared to a sham injection (i.e., saline infusion). In the placebo condition, compared to room air breathing, $\dot{V}_E$ rose by 32% during 100% FiO_2 breathing. However, the increase was reduced to 11% with the infusion of free radical-scavenging ascorbic acid indicating a potential role of ROS in hyperoxic hyperventilation (Fernandes et al., 2021). Interestingly, the venous measurement of jugular PCO_2 (a proxy for central PCO_2) did not change between normoxia or hyperoxia in either saline or ascorbic acid infusion condition (45.7 vs 45.6 mmHg for F_IO_2 of 21% vs 100%, respectively). Thus, the central chemoreceptor stimulus was identical between conditions (Fernandes et al., 2021). These findings provide strong experimental support to the hypothesis that, in humans, the central respiratory chemoreflex becomes sensitized in hyperoxic conditions, that ROS appear to be involved, and that this mechanism may explain a significant proportion (~65%) of the hyperoxic hyperventilatory response. However, this theory of ROS-induced sensitization of the central chemoreflex warrants further exploration in humans. Testing the central respiratory chemoreflex response to varying degrees of hyperoxia could be useful in this regard.

1.6 Duffin's Modified Rebreathing Protocol

The most common method of testing the central respiratory chemoreflex in humans is done by measuring breathing responses to high CO_2 (i.e., hypercapnia) invoked by hyperoxic rebreathing (Read & Leigh, 1967). With this test, a participant breathes from a bag containing 7% CO_2, 50% O_2, and balance nitrogen gas (N_2). In this closed circuit, excretion of CO_2 is blocked, establishing an equilibrium between the PCO_2 in the bag, alveoli, and mixed venous blood. With continued breathing, PCO_2 rises at a rate commensurate with whole body $\dot{V}CO_2$ and the initially high FiO_2 in the bag (i.e., 50%) ensures that PaO_2 remains sufficiently high to eliminate carotid body (peripheral chemoreflex) involvement and isolate the central respiratory chemoreflex. With the rise in PCO_2 throughout

rebreathing, $\dot{V}_E$ increases linearly with a $\dot{V}_E$-PCO_2 slope that relates to central respiratory chemoreflex sensitivity (Read & Leigh, 1967).

However, James Duffin applied some modifications to Read's rebreathing protocol to increase the amount of information that can be discerned. With respect to central chemoreflex characterization, two important modifications include the addition of a 5-minute voluntary hyperventilation period prior to rebreathing (Figure 1) and maintenance of a target $P_{ET}O_2$ (i.e., isoxia) throughout rebreathing (Duffin et al., 2000). Prior hyperventilation allows rebreathing to commence from a hypocapnic PCO_2 (see $PcCO_2$ in Figure 1) and the maintenance of hyperoxic isoxia, ensures that $P_{ET}O_2$ remains sufficiently high to diminish carotid body involvement (Duffin et al., 2000; Lloyd et al., 1958; Mohan et al., 1999). In contrast to Read's rebreathing, $\dot{V}_E$ does not begin to rise immediately, rather the $\dot{V}_E$ versus $P_{ET}CO_2$ relationship exhibits a "hockey stick" like profile where $\dot{V}_E$ remains stable until a specific $P_{ET}CO_2$ beyond which it begins to ascend linearly (see $\dot{V}_E$ in Figure 1).

Modelling of the ensuing $\dot{V}_E$ versus $P_{ET}CO_2$ profile permits the identification of three parameters that describe the central chemoreflex. The initial stable or "steady-state phase termed basal $\dot{V}_E$ (i.e., $\dot{V}_E$BSL) describes a period where chemoreflex drive is negligible and "wakefulness" drive dictates $\dot{V}_E$ (Casey et al., 1987; Duffin et al., 2000; Fink, 1961). After a period of stability, $\dot{V}_E$ eventually beings to rise. The $P_{ET}CO_2$ at which this occurs termed the ventilatory recruitment threshold (VRT) describes the PCO_2 at which central chemoreceptor drive begins to contribute to net respiratory output. As PCO_2 continues to rise, central chemoreceptors become increasingly excited causing $\dot{V}_E$ to increase linearly with PCO_2. The slope of this rise in $\dot{V}_E$ beyond the VRT describes the central respiratory chemoreflex sensitivity (ie., $\dot{V}_E$S) (Mohan et al., 1999).

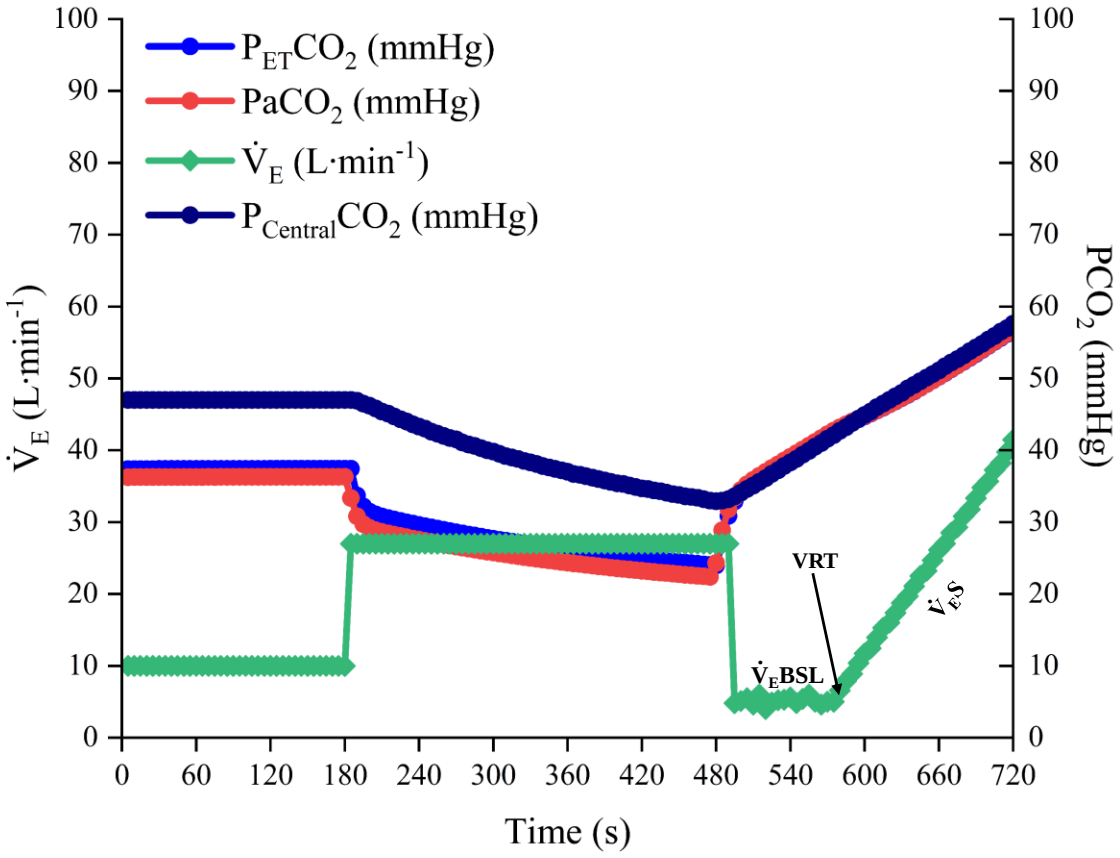

Figure 1: Schematic representation of breathing and PCO_2 changes during modified rebreathing. A simulation of a single modified rebreathing trial is displayed. Phases include: from 0 to 180 s resting spontaneous breathing, from 180 to 480 s volitional hyperventilation, and from 480 to 720 s rebreathing. Continuous tracings of simulated ventilation ($\dot{V}_E$, green) in L·min^{-1} and partial pressures of CO_2 corresponding to end-tidal ($P_{ET}CO_2$, blue), arterial ($PaCO_2$, green) and central PCO_2 ($PcCO_2$, violet) in mmHg are displayed. During rebreathing (480-720s) the model provides chemoreceptor independent basal $\dot{V}_E$ in the period prior to VRT ($\dot{V}_E$BSL), the ventilatory recruitment threshold (VRT, PCO_2 at which $\dot{V}_E$ rises above baseline), and central chemoreceptor sensitivity ($\dot{V}_E$S, slope of the linear rise in $\dot{V}_E$ above VRT).

The $\dot{V}_E$ versus $P_{ET}CO_2$ response to isoxic-hyperoxic modified rebreathing and model parameters estimates have been shown to exhibit excellent within and between-day test-

retest reproducibility in healthy young adults (Guluzade et al., 2022; Jensen et al., 2010). Nevertheless, we recently demonstrated that confidence in measured parameters of central respiratory chemoreflex characterization could be enhanced by averaging of repeated tests (Guluzade et al., 2022). Therefore, this test and the parameters it provides could be applied to better understand the impact PO_2 on $\dot{V}_E$ in the absence (i.e., $\dot{V}_E BSL$) and presence (i.e., $\dot{V}_E S$) of central chemoreflex activation.

1.7 Purpose and Hypothesis

The objective of this study was to compare the $\dot{V}_E$ vs $P_{ET}CO_2$ relationships during modified rebreathing at increasingly higher levels of hyperoxia. We reasoned that if the parameters of the $\dot{V}_E$ vs $P_{ET}CO_2$ relationship (i.e., $\dot{V}_E BSL$, VRT, and $\dot{V}_E S$) do not change with PO_2, then hyperoxia does not stimulate $\dot{V}_E$ on its own or via sensitization of the central respiratory chemoreflex. Alternatively, if $\dot{V}_E BSL$ and/or $\dot{V}_E S$ increase(s) with PO_2, data would indicate that hyperoxia does stimulate $\dot{V}_E$ in a dose-dependent manner and that hyperoxia sensitizes the central chemoreflex, respectively. We hypothesized that the $\dot{V}_E$ versus PCO_2 relationship is fixed and independent of PO_2. (See Figure 2).

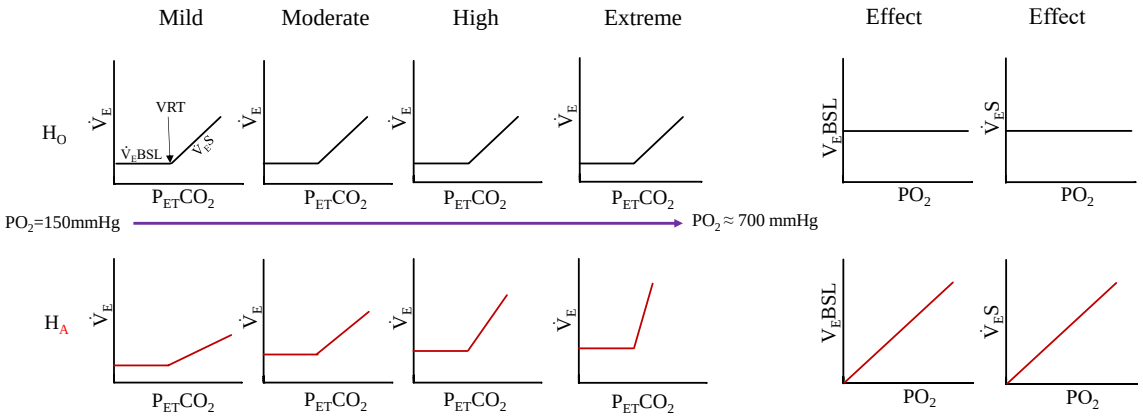

Figure 2: Visual schematic of predicted outcomes.

Chapter 2

2 The ventilatory response to modified rebreathing is unchanged by hyperoxic severity: implications for the hyperoxic hyperventilation paradox

2.1 Introduction

When humans breathe oxygen (O_2)-enriched gas at normobaric pressures, minute ventilation ($\dot{V}_E$) increases above basal levels after ~2-3 minutes (Becker et al., 1995; Becker et al., 1996; Fernandes et al., 2021; Lambertsen, Kough, et al., 1953; Lambertsen, Stroud, et al., 1953; Marczak & Pokorski, 2004). This stimulatory effect on respiration has been attributed to O_2-dependent mechanisms or secondary to the effects of carbon dioxide (CO_2) accumulation in brain tissue (Dean et al., 2004). Definitive mechanisms responsible for the rise in $\dot{V}_E$ with normobaric hyperoxia remain unclear.

Exposing participants to different fractions of inspired O_2 (F_IO_2 = 30, 50, or 75%), Becker et al. (1996) demonstrated during 30 minutes of quiet breathing with end-tidal PCO_2 ($P_{ET}CO_2$ – a proxy for arterial PCO_2) maintained normocapnic, that hyperoxia stimulated $\dot{V}_E$ in a dose-dependent manner. On average, the higher the F_IO_2 the greater the rise in $\dot{V}_E$ above baseline. However, in the same experiment, when the 75% F_IO_2 condition was repeated but with $P_{ET}CO_2$ allowed to vary (poikilocapnia), the excitatory ventilatory response was nearly abolished; when $P_{ET}CO_2$ was permitted to fall as $\dot{V}_E$ rose. This finding indicates that mechanisms related to CO_2 and not hyperoxia provided the bulk of the excitatory respiratory stimulus.

In steady-state hyperoxic experiments where $P_{ET}CO_2$ is clamped, one might expect that while arterial PCO_2 ($PaCO_2$) is maintained normal, PCO_2 at the tissue level will rise due to the Haldane effect whereby heightened arterial PO_2 (PaO_2) reduces the affinity of hemoglobin for CO_2 binding, ultimately raising local PCO_2 via reduced CO_2 removal from tissue (Christiansen et al., 1914). This would occur throughout the body including in the brainstem where the central chemoreceptors are located. The central chemoreceptors are an important modulator of neural respiratory control that regulate brain pH at homeostatic

levels by reflexively increasing $\dot{V}_E$ when elevations in local tissue hydrogen ion concentration ($[H^+]$) are sensed (Cunningham et al., 2011; Duffin, 1990; Guyenet, 2014; Guyenet et al., 2012, 2019; Nattie & Li, 2012). These receptors are often referred to as 'CO_2 receptors' because central $[H^+]$ is directly dependent on PCO_2 (Eldridge et al., 1985). With respect to Becker et al. (1996), it might be expected that central PCO_2 increased with F_IO_2 particularly because the maintenance of isocapnia would reduce the ability to expire excess CO_2. Consequently, the observed O_2 dose-dependent rise in $\dot{V}_E$ could be attributed to heightened central chemoreflex drive and not to the direct affects of hyperoxia, *per se*. Thus, an alternate explanation is that the heightened $\dot{V}_E$ accompanying higher inspired O_2 pressures arose due to progressively greater central chemoreceptor stimulation.

Recently, Fernandes et al. (2021) observed a 32% increase in $\dot{V}_E$ during 10 min of 100% F_IO_2 breathing with PCO_2 allowed to vary (i.e., poikilocapnia). However, compared to spontaneous room air breathing, intravenous jugular PCO_2 (a proxy of central PCO_2) did not differ in the hyperoxic condition (45.7 vs 45.6 mmHg for F_IO_2 of 21% vs 100%, respectively) suggesting that the stimulus for central chemoreceptor excitation was equivalent. Interestingly, when ascorbic acid was infused in a separate set of experiments, the rise in $\dot{V}_E$ with 100% F_IO_2 breathing was reduced to ~11%. The authors attributed this effect, in part, to an ascorbic acid-mediated reduction in reactive oxygen species (ROS) concentration which, in excess, have been shown to heighten the sensitivity of the central chemoreceptors in the brainstem of rats (Mulkey et al. 2003; Dean et al. 2004). Therefore, it remains unclear as to whether hyperoxic breathing stimulates $\dot{V}_E$ directly, or indirectly through heightened central PCO_2 or via central chemoreflex sensitization.

An alternative means of investigating this concept is to explore the effects of hyperoxia on the central respiratory chemoreflex response. The most common method of testing the central respiratory chemoreflex in humans is done by measuring breathing responses to high CO_2 (i.e., hypercapnia) invoked by hyperoxic rebreathing (Read & Leigh, 1967). Progressive hypercapnia is applied to stimulate the central chemoreflex (Duffin, 2011) and hyperoxia is applied (typically F_IO_2 > 90%) to minimize the influence of other H^+/CO_2 sensitive receptors within the carotid body that also reflexively increase breathing (i.e., the peripheral chemoreflex) (Duffin, 1990, 2010, 2011). With modified rebreathing, a 5 min

period of hyperventilation precedes the transition to rebreathing such that upon transition to rebreathing, PCO_2 rises linearly from a hypocapnic PCO_2 (Duffin, 2011). This permits identification of the PCO_2 at which the central chemoreflex is initiated (ventilatory recruitment threshold, VRT). Importantly, in this paradigm, drive to breathe in the period prior to VRT ($\dot{V}_E$BSL) is chemoreceptor-independent reflective of basal ventilation and the slope of the compensatory and progressive rise in $\dot{V}_E$ after VRT ($\dot{V}_E$S) provides the central respiratory chemoreflex sensitivity is assessed by quantifying the linear relationship between $\dot{V}_E$ and $P_{ET}CO_2$ (see Figure 3).

The objective of this study was to compare the $\dot{V}_E$ vs $P_{ET}CO_2$ relationships during modified rebreathing at increasingly higher levels of hyperoxia. We reasoned that if the parameters of the $\dot{V}_E$ vs $P_{ET}CO_2$ relationship (i.e., $\dot{V}_E$BSL, VRT, and $\dot{V}_E$S) do not change with PO_2, then hyperoxia does not stimulate $\dot{V}_E$ on its own or via sensitization of the central respiratory chemoreflex. Alternatively, if $\dot{V}_E$BSL and/or $\dot{V}_E$S increase(s) with PO_2, data would indicate that hyperoxia does stimulate $\dot{V}_E$ in a dose-dependent manner and that hyperoxia sensitizes the central chemoreflex, respectively. We hypothesized that the $\dot{V}_E$ versus PCO_2 relationship is fixed and independent of PO_2.

2.2 Methods

Ethics Approval

The study protocol and consent form were approved by The University of Western Ontario's Health Sciences Research Ethics Board (WREM: 119281). All participants completed a screening questionnaire and provided written informed consent prior to their first visit.

Participants

Twenty healthy male (n=10) and female (n=10) volunteers between the ages of 18 and 40 years were recruited for this cross-sectional, case-controlled study. Participants were non-smokers, free of asthma, chronic lung, kidney, and/or cardiovascular disease, without sleep apnea, nor had dependence on recreational drugs or alcohol within the past year.

To minimize physiological variability within and between visits, participants arrived rested and having refrained from any caffeine, alcohol, and/or recreational drug intake for at least 12 hours prior to each visit. Additionally, participants were asked to abstain from strenuous activity at least 12 hours before each visit. All testing sessions were completed within 2 weeks for males to minimize any time-related changes in participants' physiology. To minimize potential confounding effects of fluctuating hormones on respiratory variables (Assadpour et al., 2020; Usselman et al., 2013), female participants were assessed during the low hormone phase of their menstrual cycle (i.e., 4 consecutive visits within the first 5 days after onset of menses or placebo phase if using oral contraception).

Experimental Protocol

Participants visited the laboratory on 5 separate occasions (1 familiarization, 4 experimental visits). On the first day ('Day 0') participant anthropometrics, resting metabolic and cardiovascular measurements, and spirometry data were acquired. Participants were also familiarized with the modified rebreathing protocol. Resting in a dental chair equipped with a heart rate (HR) monitor (Polar USA, Model H10), flowmeter, and arm cuff, resting HR, blood pressure (BP), oxygen uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were measured simultaneously by sphygmomanometer and a metabolic cart (Quark, CPET, COSMED, Rome, Italy). An average of 3 BP measurements was used for the participants resting BP and an average of the $\dot{V}O_2$ and $\dot{V}CO_2$ over a five-minute period of steady-state spontaneous breathing was computed to quantify resting metabolic rate. Thereafter, a minimum of three trials of the following spirometric evaluations were administered: forced vital capacity (FVC), slow vital capacity (SVC), and maximal voluntary ventilation (MVV). The trial with the highest percent-predicted value was selected to represent pulmonary function. Upon completion of the resting measurements participants were familiarized with the modified rebreathing protocol by completing two repetitions of the test in isoxic-normoxic ($P_{ET}O_2$ = 90 mmHg) conditions (see Modified Rebreathing Procedure below).

After familiarization, participants visited the laboratory on 4 more occasions to perform 3 repetitions of modified rebreathing with $P_{ET}O_2$ maintained by a computer-controlled system at one of 4 hyperoxic pressures: i) mild (PO_2 = 150 mmHg); ii) moderate (PO_2 = 200 mmHg); iii) high (PO_2 = 300 mmHg); and extreme ($PO_2 \approx$ 700 mmHg). Isoxia was not maintained in the extreme condition to mimic the traditional method by which central chemoreflex sensitivity is assessed (Bain et al., 2017; Rodman et al., 2001; Sayegh et al., 2022). Three like-trials were performed in each session and only one hyperoxic tension was administered per visit. The order of hyperoxic pressures was randomized. Throughout each protocol, participants wore a facemask (V2 Mask, Hans Rudolph Inc, Kansas, USA) and $\dot{V}_E$, $P_{ET}CO_2$, and $P_{ET}O_2$ were assessed breath-by-breath using a heated pneumotach (3810 Series, Hans Rudolph Inc) and dual gas analyzer (VacuMed, Model 17500 Ventura, USA), respectively.

Modified Rebreathing Procedure

While seated in a semi-recumbent position on a dental chair, participants breathed through a facemask connected in series to a bidirectional pneumotach and a 3-way T-shaped valve (2870 Series, Hans Rudolph Inc., Kansas, USA). The second port on the 3-way valve was open to room air and third port was connected to a 6-L rebreathing bag (filled with 3-5 L of premixed gas set ~ to FVC). This arrangement permitted the investigator to switch participants from breathing room air (open circuit) to breathing from the rebreathing bag (closed circuit, i.e., rebreathing). Respired air was continuously sampled at the mouth using a sampling line (Nafion, H_2O absorbing tube, VacuMed, Ventura, USA) attached to the breathing apparatus and analyzed for fractional concentrations of O_2 and CO_2 (model 17500, VacuMed, Ventura, USA). Inspiratory and expiratory pressures were sampled continuously using the heated pneumotach (Heat Controller, Hans Rudolph Inc., Kansas, USA). Analog signals were amplified and converted to flow via an amplifier (Pneumotach Amplifier 1, Hans Rudolph Inc., Kansas, USA). Inspired and expired volumes for each breath were derived from integration of the flow signal. Saturation of oxygen (SpO_2) was continuously measured with a pulse oximeter attached to the participant's earlobe (Nonin 7500, Plymouth, USA). Respiratory flows and fractional gas concentration data were sampled at 50 Hz via a 16-bit analog-to-digital converter (National Instruments, Austin,

USA). Custom software aligned gas concentrations and flow signals as measured by the pneumotach and executed a peak-detection program to determine breath-by-breath pressures of $P_{ET}CO_2$ and $P_{ET}O_2$, tidal volume (V_T), breathing frequency (f_B) and $\dot{V}_E$.

With the three-way valve open to room air, each rebreathing test began with 3-minutes of quiet spontaneous breathing. Following this initial baseline period, participants were coached to breathe deeply for 5 minutes, and raise their tidal volume such that they rapidly achieved and maintained a $P_{ET}CO_2$ of ~20-25 mmHg. Following the 5-min deep breathing period, the 3-way valve was quickly switched from room air to the rebreathing bag at the end of a full expiration. Participants then took 3 deep breaths to produce a rapid equilibration of PCO_2 in the bag, lungs, and arterial blood to that of mixed venous blood and then to breathe freely. Equilibration was verified by observation of a plateau in the continuous PCO_2 signal, and this was a prerequisite for continuing the test.

The rebreathing bag contained 28% O_2, 6% CO_2, balance nitrogen (N_2) for mild, 35% O_2, 6% CO_2, balance N_2 for moderate, 60% O_2, 6% CO_2, balance N_2 for high, and 94% O_2, 6% CO_2 for extreme, respectively. To maintain $P_{ET}O_2$ at the target pressure throughout rebreathing in the mild, moderate, and high O_2 conditions, a gas mixture of 94% O_2 and 6%CO_2 was periodically fed into the circuit by a small tube attached at the connection between the three-way valve and the rebreathing bag. The flow of O_2 was controlled by a program (LabVIEW, National Instruments Inc, TX, USA) that compares the actual and desired $P_{ET}O_2$ values to determine the amount of O_2 required to maintain isoxia.

Rebreathing was terminated at the participant's discretion, when $P_{ET}CO_2$ reaches 60 mmHg, or when $\dot{V}_E$ exceeds 100 $L \cdot min^{-1}$. Following rebreathing, participants continued to breathe on the apparatus for 3 minutes. Each test lasted ~15-20 minutes.

Data Processing

For each rebreathing trial, raw breath-by-breath data were edited by removing aberrant breaths associated with coughs, sighs, sneezes, or swallows. During the rebreathing period of the trial, the first 3-4 deep equilibration breaths were excluded and the entire $P_{ET}CO_2$ versus time relationship of this phase was fitted with a linear regression line. The equation of the line generated was utilized to predict the $P_{ET}CO_2$ versus time relationship during the

rebreathing period. Further, the $\dot{V}_E$ was plotted relative to predicted $P_{ET}CO_2$ to determine parameters describing the central respiratory chemoreflex. For visual display purposes, like-trials from each hyperoxic condition were processed, interpolated to 0.1 mmHg bins of $P_{ET}CO_2$, ensemble-averaged as previously described (Guluzade et al., 2022), and further binned into octiles of $P_{ET}CO_2$.

Data Analysis

The $\dot{V}_E$ versus $P_{ET}CO_2$ were plotted and fit with either a double-linear (f(x)) or exponential decay-linear (g(x)) models. The double-linear model had the form:

$$f(x) = \begin{cases} i1 + S1x, & x < VRT \\ i1 + (S1 \cdot VRT) + S2 \cdot (x - VRT)\, x, & x \geq VRT \end{cases}$$

Where f is the double-linear function, x is $P_{ET}CO_2$ and f is $\dot{V}_E$, VRT (i.e., ventilatory recruitment threshold) is the $P_{ET}CO_2$ corresponding to the interception of the two regression lines, i_1 (i.e., $\dot{V}_E$BSL) is the intercept of the first linear function, and S_1 and S_2 are the slopes. The S_1 parameter will be fixed at "zero" and thus i_1 gives basal $\dot{V}_E$ or wakefulness drive to breathe. S_2 gives the chemoreflex sensitivity to hypercapnia ($L \cdot min^{-1} \cdot mmHg^{-1}$). The exponential decay-linear model had the form:

$$g(x) = \begin{cases} y0 + A^{\left(-\frac{(x-x0)}{\tau}\right)}, & x < VRT \\ y0 + A^{\left(-\frac{(VRT-x0)}{\tau}\right)} + m \cdot (x - VRT), & x \geq VRT \end{cases}$$

Where: g is $\dot{V}_E$, x is $P_{ET}CO_2$, VRT is the $P_{ET}CO_2$ corresponding to the interception of the exponential decay and the regression line, y0 is the exponential plateau (i.e., $\dot{V}_E$BSL), A is the amplitude between the first data point y0, x0 parameter is the initial $P_{ET}CO_2$, and τ is the time constant. The chemoreflex sensitivity to hypercapnia is determined by m ($L \cdot min^{-1} \cdot mmHg^{-1}$).

Model parameter estimates for each trial were determined by linear least-square regression that identified the minimal sum of squared residuals between the selected model and the experimental data using OriginLab Pro 2022 (OriginLab, Northampton, MA, USA).

For each condition, $\dot{V}_E$BSL ($L \cdot min^{-1}$), VRT (mmHg), and $\dot{V}_E$S ($L \cdot min^{-1} \cdot mmHg^{-1}$) were computed as the average of the three repeated trials as previously described (Guluzade et al., 2022).

Statistical Analysis

Data are presented as means ± SD. Between-condition differences in measured respiratory parameters were assessed by a two-way (sex x $P_{ET}O_2$ condition) repeated measures analysis of variance (ANOVA). Where main effects were observed, Tukey's HSD post hoc was applied to identify pairwise differences. Statistical significance was determined at a p-value of 0.05. The minimal detectable change (MDC) in respiratory parameters for between-day assessments of iso-oxic hyperoxic rebreathing involving at least three repeated trials in healthy young adults is 3.08 $L \cdot min^{-1}$ for $\dot{V}_E$BSL; 2.2 mmHg for VRT; and 1.54 $L \cdot min^{-1} \cdot mmHg^{-1}$ for $\dot{V}_E$S (Guluzade et al., 2022). Mean differences between each condition for $\dot{V}_E$BSL, VRT, and $\dot{V}_E$S were compared to a hypothesized mean of their respective MDC using two separate one-tailed sample t-tests to assess equivalence between $P_{ET}O_2$ conditions. Bland-Altman plots were used to assess the variability of measured parameters at the group level across $P_{ET}O_2$ conditions.

2.3 Results

A summary of participants' physical characteristics, spirometry, and resting cardiovascular, respiratory, and metabolic data are displayed in Table 1. There were no between-condition differences in end-tidal and ventilatory variables during the resting and hyperventilation periods that preceded rebreathing in all conditions (Table 2). For all trials, regardless of hyperoxic condition, the $\dot{V}_E$ versus $P_{ET}CO_2$ relationships were well represented by either the double-linear or exponential decay-linear models. Exemplar data of a male and female participant for each hyperoxic condition are displayed in Figure 3. On average, males had a significantly higher VRT ($p<0.05$), but no between-sex differences were observed for $\dot{V}_E$BSL ($p=0.49$) and $\dot{V}_E$S ($p=0.054$; see Table 3). In addition, there were no significant sex x $P_{ET}O_2$ condition interactions (Table 3). For this reason, males and females were considered together to assess the effect of hyperoxia on the respiratory response to iso-oxic rebreathing.

As designed, the group mean $P_{ET}O_2$ for the mild, moderate, and high iso-oxic conditions increased from 150 ± 1 mmHg to 199 ± 2 mmHg and 301 ± 2 mmHg, respectively. The $P_{ET}O_2$ of the extreme condition was not recorded because it exceeded the maximum

detectable PO_2 of the gas analyzer (400 mmHg). However, during none of the extreme hyperoxic trials did the PO_2 signal fall below the "saturation line" indicating that $P_{ET}O_2$ was well above 500 mmHg even at the end of each rebreathing test. In addition, the fall in $P_{ET}O_2$ during hyperoxic rebreathing averages ~25 mmHg/min (Rapanos & Duffin, 1997). Total rebreathing time in the extreme condition averaged 5.1±1.1 minutes. Thus, it is unlikely that $P_{ET}O_2$ fell below 550 mmHg in any participant. Group mean data for $\dot{V}_E$, V_T, and breathing frequency (f_b) as a function of $P_{ET}CO_2$ between $P_{ET}O_2$ conditions are displayed in Figure 4. On average V_T, and f_b versus $P_{ET}CO_2$ profiles were similar amongst $P_{ET}O_2$ conditions.

All statistical and group mean data from ANOVA are displayed in Table 4. There was no effect of $P_{ET}O_2$ on $\dot{V}_E$BSL ($p=0.17$), VRT ($p=0.07$), or $\dot{V}_E$S ($p=0.39$). Group mean data as well as individual between-condition values are depicted in Figure 5. For all conditions, the $\dot{V}_E$BSL was lower ($p<0.05$) compared to the resting $\dot{V}_E$ measured during the period of spontaneous breathing that preceded hyperventilation (9.1 ± 0.7 versus 7.1 ± 0.5 $L \cdot min^{-1}$, respectively; no interaction $p=0.29$).

Bland–Altman comparisons of $\dot{V}_E$S and $\dot{V}_E$BSL between all hyperoxic conditions are displayed in Figure 6. Bland-Altman analysis allowed for the between-condition comparison of $\dot{V}_E$S and $\dot{V}_E$BSL to test for equivalence across conditions. Between each hyperoxic condition, the mean bias was not significantly different from zero (all $p<0.05$; see Figure 4 for exact p-values). In addition, the mean difference between each condition for $\dot{V}_E$BSL, VRT, and $\dot{V}_E$S were not different ($p>0.05$ for both one-tailed t-tests, for each variable, and amongst all conditions) from pre-established MDC boundaries for $\dot{V}_E$BSL (-3.08, 3.08 L·min-1), VRT (-2.2, 2.2 mmHg), and $\dot{V}_E$S (-1.54, 1.54 L·min-1·mmHg-1) indicating that mean differences for each between-condition comparison, and rebreathing parameter were equivalent.

Table 1: Group Average of Participant Characteristics (n=20)

Physical Characteristics			
	All (n=20)	**Male (n=10)**	**Female (n=10)**
Age (years)	24 ± 4	26 ± 5	22 ± 2
Mass (kg)	67.2 ± 12.8	76.0 ± 9.3	58.4 ± 9.4*
Height (cm)	171.3 ± 10.3	179.5 ± 5.2	163 ± 6.6*
BMI ($kg \cdot m^2$)	22.7 ± 2.6	23.5 ± 2.3	21.8 ± 2.8
Cardiovascular Data			
Systolic Blood Pressure (mmHg	122 ± 6	124 ± 7	119 ± 5
Diastolic Blood Pressure (mmH	78 ± 8.1	77 ± 10.8	78 ± 4.6
Heart Rate (bpm)	71 ± 11	68 ± 10.8	75 ± 9.7
Oxygen Saturation (%)	100 ± 0	99± 0	98 ± 1
Spirometry Data			
FVC (L)	4.5 ± 1.2	5.4 ± 0.9	3.7 ± 0.6*
FVC (%predicted)	99 ± 10	100 ± 11	97 ± 9
VC (L)	4.4 ± 1.2	5.2 ± 1.0	3.6 ± 0.7*
FEV_1 (L)	4.9 ± 6.0	4.2 ± 0.8	5.5 ± 8.8
FEV_1 (% predicted)	(88.0 ± 12.1)	(92.11±10.01)	(85.2 ± 13)
FEV_1/FVC (%)	77.8± 8.0	78.2 ± 7.2	77.2 ± 9.0
PEF ($L \cdot s^{-1}$)	6.6 ± 2.7	8.6 ± 2.1	4.5 ± 1.3*
MVV ($L \cdot min^{-1}$)	143.1 ± 38.3	174.6 ± 18.3	111.6 ± 23.5*
Resting Metabolic Data			
$\dot{V}_E$ ($L \cdot min^{-1}$)	9.1 ± 0.3	9.3 ± 1.5	8.9 ± 1.8
V_T (L)	0.68 ± 0.03	0.76 ± 0.17	0.59 ± 0.2
f_b (min^{-1})	15 ± 0	13 ± 3	16 ± 5
$\dot{V}O_2$ ($mL \cdot min^{-1}$)	265 ± 57	297 ± 52	234 ± 45*
$\dot{V}CO_2$ ($mL \cdot min^{-1}$)	222 ± 55	252 ± 41	191 ± 50*
$P_{ET}CO_2$ (mmHg)	35.7 ± 0.2	36.6 ± 1.3	34.8 ± 1.9*
$P_{ET}O_2$ (mmHg)	99.7 ± 1.8	98.1 ± 2.3	103.1 ± 3.8*

Abbreviations: forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow rate; MVV, maximal voluntary ventilation; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; $P_{ET}O_2$, end-tidal partial pressure of oxygen. '*' Indicates difference from males.

Table 2: Group averages for ventilatory and end-tidal values during resting and hyperventilation periods preceding rebreathing

Parameters	Mild	Moderate	High	Extreme	p-value
		Spontaneous breathing			
$P_{ET}O_2$ (mmHg)	100.7 ± 4.7	100.9 ± 5.8	100.1 ± 4.0	100.8 ± 3.7	0.82
$P_{ET}CO_2$ (mmHg)	35.6 ± 2.1	35.8 ± 2.4	35.7 ± 1.8	35.9 ± 1.9	0.79
$\dot{V}_E$ ($L \cdot min^{-1}$)	9.0 ± 2.0	9.4 ± 1.6	8.8 ± 1.7	9.3 ± 1.8	0.23
F_b (min^{-1})	15 ± 4.6	15 ± 4.8	16 ± 4.3	15 ± 4.5	0.48
V_T (L)	0.66 ± 0.06	0.72 ± 0.05	0.60 ± 0.07	0.69 ± 0.06	0.24
		Hyperventilation			
$P_{ET}O_2$ (mmHg)	127 ± 3.0	127.8 ± 3.0	127.2 ± 3.0	127.2 ± 2.5	0.47
$P_{ET}CO_2$ (mmHg)	23.7 ± 2.4	23.2 ± 2.1	23.2 ± 2.0	23.7 ± 2.1	0.23
$\dot{V}_E$ ($L \cdot min^{-1}$)	23.7 ± 4.7	24.8 ± 5.2	24.3 ± 5.3	24.8 ± 5.4	0.34
F_b (min^{-1})	8 ± 1*	8 ± 2	9 ± 2†	8 ± 2	$p<0.05$
V_T (L)	2.98 ± 0.66	3.09 ± 0.80	2.98 ± 0.76	3.07 ± 0.73	0.45

Values are presented as: mean ± SD. Abbreviations: $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; $P_{ET}O_2$, end-tidal partial pressure of oxygen. $\dot{V}_E$, minute ventilation. "$^{a\text{-}c}$" represents significant differences between hyperoxic conditions ($p<0.05$) "†" indicates difference between "mild" and its respective condition, and "*" indicates difference between "high" and its respective condition. Average data corresponding to hyperventilation were determined from the last 2 minutes of the 5-minute period.

Table 3: Simple averages of respiratory parameters from the ventilatory response to isoxic-hyperoxic modified rebreathing tests (n=20) grouped by biological sex.

Parameters	**Mild**		**Moderate**		**High**		**Extreme**		**p value**		
	M	F	M	F	M	F	M	F	Sex	Condition	Interaction
$P_{ET}O_2$ (mmHg)	$149 \pm 1^{b\text{-}d}$	$150 \pm 1^{b\text{-}d}$	$198 \pm 1^{a,c,d}$	$200 \pm 1^{a,c,d}$	$300 \pm 2^{a,b,d}$	$302 \pm 2^{a,b,d}$	-	-	<0.005	<0.001	0.42
$\dot{V}_E$BSL ($L \cdot min^{-1}$)	8.01 ± 3.05	6.8 ± 5.2	7.5 ± 4.2	6.4 ± 4.3	7.2 ± 3.5	5.8 ± 4.0	8.09 ± 4.65	7.0 ± 4.3	0.49	0.24	0.99
VRT (mmHg)	44.6 ± 2.93	41.0 ± 2.5	43.9 ± 2.8	41.1 ± 1.9	44.1 ± 1.9	40.6 ± 2.1	43.13 ± 2.6	40.4 ± 2.1	<0.05	0.07	0.59
$\dot{V}_E$S ($L \cdot min^{-1} \cdot mmHg^{-1}$)	6.06 ± 3.13	3.69 ± 1.38	5.83 ± 2.51	3.69 ± 1.28	5.60 ± 2.8	4.01 ± 1.45	5.1 ± 2.15	3.73 ± 1.35	0.054	0.41	0.30
Peak $P_{ET}CO_2$ (mmHg)	55.4 ± 3.0	53.4 ± 2.9	55.1 ± 3.0	54 ± 2.5	55.3 ± 3	52.9 ± 2.7	53.6 ± 2.5	53.3 ± 2.8	<0.05	0.09	0.27
rrCO2 ($mmHg \cdot min^{-1}$)	3.7 ± 0.5	3.5 ± 0.6	3.5 ± 0.4	3.5 ± 0.5	3.8 ± 0.5	3.5 ± 0.5	$4.1 \pm 0.5^{a,b}$	$4.02 \pm 0.5^{a,b}$	0.53	<0.01	0.79

Values are presented as: mean ± SD. Abbreviations: $P_{ET}O_2$, end-tidal partial pressure of oxygen $\dot{V}_E$BSL, ventilation representing the wakefulness drive to breathe; VRT, ventilatory recruitment threshold; $\dot{V}_E$S, central respiratory chemoreflex sensitivity; peak P_{ET} CO_2, peak end-tidal partial pressure of carbon dioxide at the end of rebreathing. $rrCO_2$, rate of rise of carbon dioxide "[a-d]" represent significant differences between hyperoxic conditions ($p<0.05$) "[a]" indicates difference between "mild" and its respective condition, "[b]" indicates difference between "moderate" and its respective condition, and so forth. "-" is indicated in the extreme column as a single isoxic value cannot be assigned.

Table 4: Group mean parameters from the ventilatory response to hyperoxic modified rebreathing (n=20)

Parameters	Mild	Moderate	High	Extreme	p value
$P_{ET}O_2$ (mmHg)	$150 \pm 1^{b\text{-}d}$	$199 \pm 2^{a,c,d}$	$301 \pm 2^{a,b,d}$	$-^{a,b,c}$	p<0.001
$\dot{V}_E$BSL ($L \cdot min^{-1}$)	7.4 ± 4.2	6.9 ± 4.2	6.5 ± 3.7	7.5 ± 2.7	0.17
VRT (mmHg)	42.8 ± 3.2	42.5 ± 2.7	42.3 ± 2.7	41.8 ± 2.7	0.07
$\dot{V}_E$S ($L \cdot min^{-1} \cdot mmHg^{-1}$)	4.88 ± 2.6	4.76 ± 2.2	4.81 ± 2.3	4.39 ± 1.9	0.39
Peak $P_{ET}CO_2$ (mmHg)	54.4 ± 3.1	54.5 ± 2.7	54.1 ± 3.0	53.4 ± 2.6	0.17
rrCO2 ($mmHg \cdot min^{-1}$)	3.6 ± 0.6^{d}	3.5 ± 0.5^{d}	3.7 ± 0.5^{d}	4.0 ± 0.5^{a}	p<0.001

Values are presented as: mean ± SD. Abbreviations: $P_{ET}O_2$, end-tidal partial pressure of oxygen $\dot{V}_E$BSL, ventilation representing the wakefulness drive to breathe; VRT, ventilatory recruitment threshold; $\dot{V}_E$S, central respiratory chemoreflex sensitivity; peak $P_{ET}CO_2$, peak end-tidal partial pressure of carbon dioxide at the end of rebreathing. $rrCO_2$, rate of rise of carbon dioxide "[a-d]" represent significant differences between hyperoxic conditions ($p<0.05$) "[a]" indicates difference between "mild" and its respective condition, "[b]" indicates difference between "moderate" and its respective condition, and so forth. "-" is indicated in the extreme column as a single isoxic value cannot be assigned.

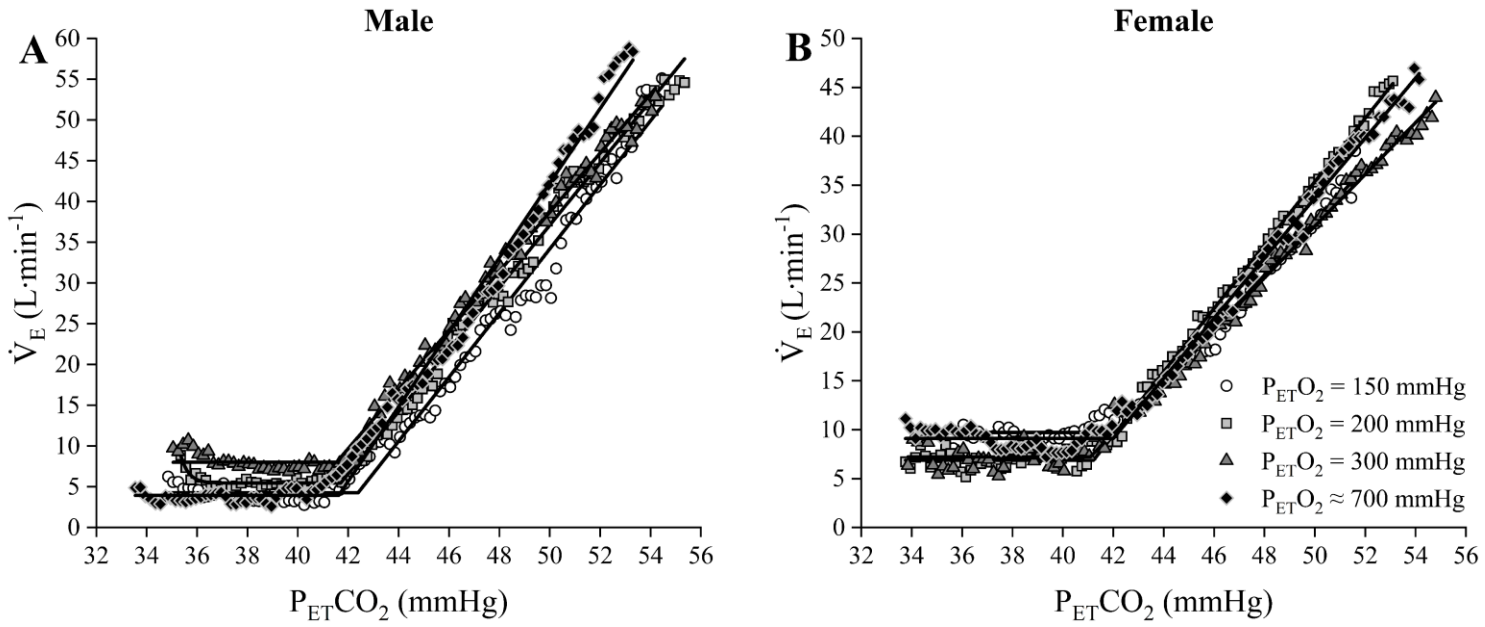

Figure 3: Male and female rebreathing responses to hyperoxia. The $\dot{V}_E$ versus $P_{ET}CO_2$ relationships of a male (A) and female (B) participant in each of the 4 hyperoxic conditions (i.e., mild: $P_{ET}O_2$ = 150 mmHg [circles]; moderate; $P_{ET}O_2$ = 200 mmHg [squares]; high: $P_{ET}O_2$ = 300 mmHg [triangles]; and extreme $P_{ET}O_2 \approx$ 700 mmHg [diamonds]). Data from each condition are ensemble-averages of three repeated trials. Double-linear or exponential decay-linear model best fits are superimposed upon each relationship of the $\dot{V}_E$ versus $P_{ET}CO_2$ relationships.

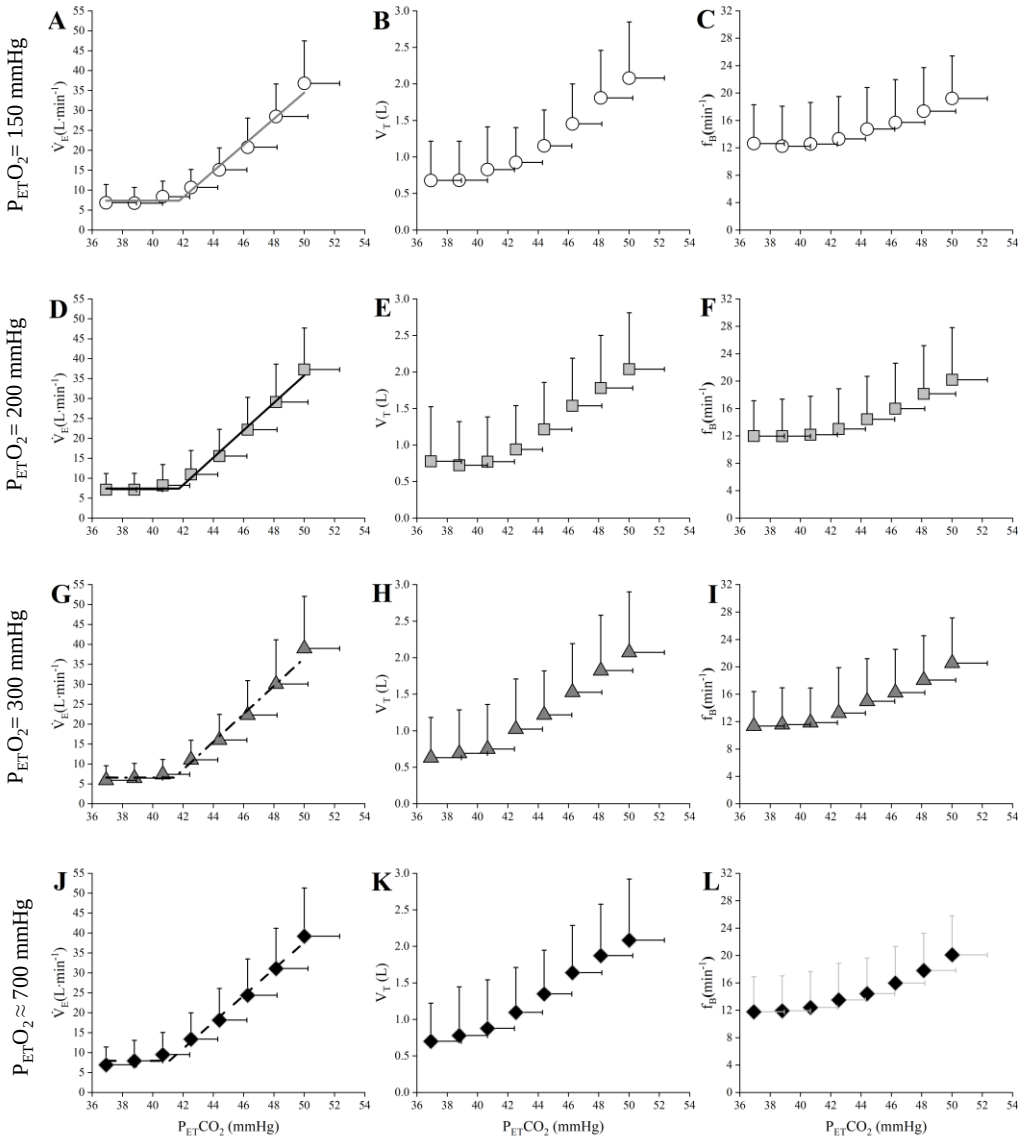

Figure 4: Group mean $\dot{V}_E$, V_T, and f_B data. Group mean (±SD) data for minute ventilation, $\dot{V}_E$ (A, L·min^{-1}), tidal volume, V_T (B, L), and breathing frequency, f_B (C, min^{-1}) in relation to $P_{ET}CO_2$ for each hyperoxic condition.

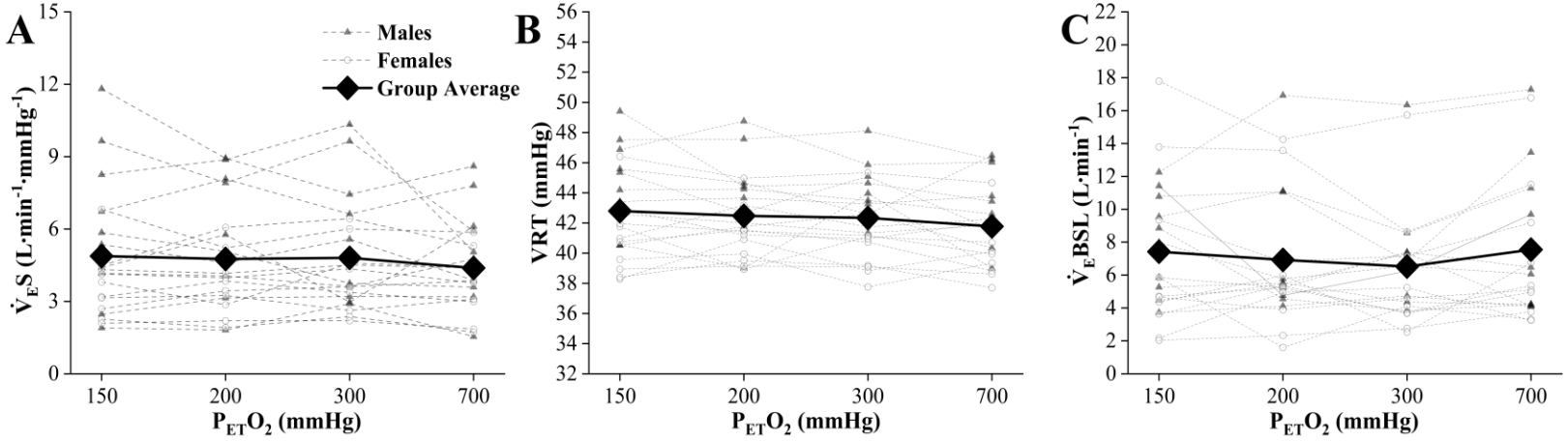

Figure 5: Individual and group central respiratory chemoreflex characteristic data. Individual and group data across $P_{ET}O_2$ conditions for central respiratory chemoreflex sensitivity, $\dot{V}_ES$ (A, L·min^{-1}·mmHg^{-1}); ventilatory recruitment threshold, VRT (B, mmHg); and wakefulness drive to breathe, $\dot{V}_EBSL$ (C, L·min^{-1}).

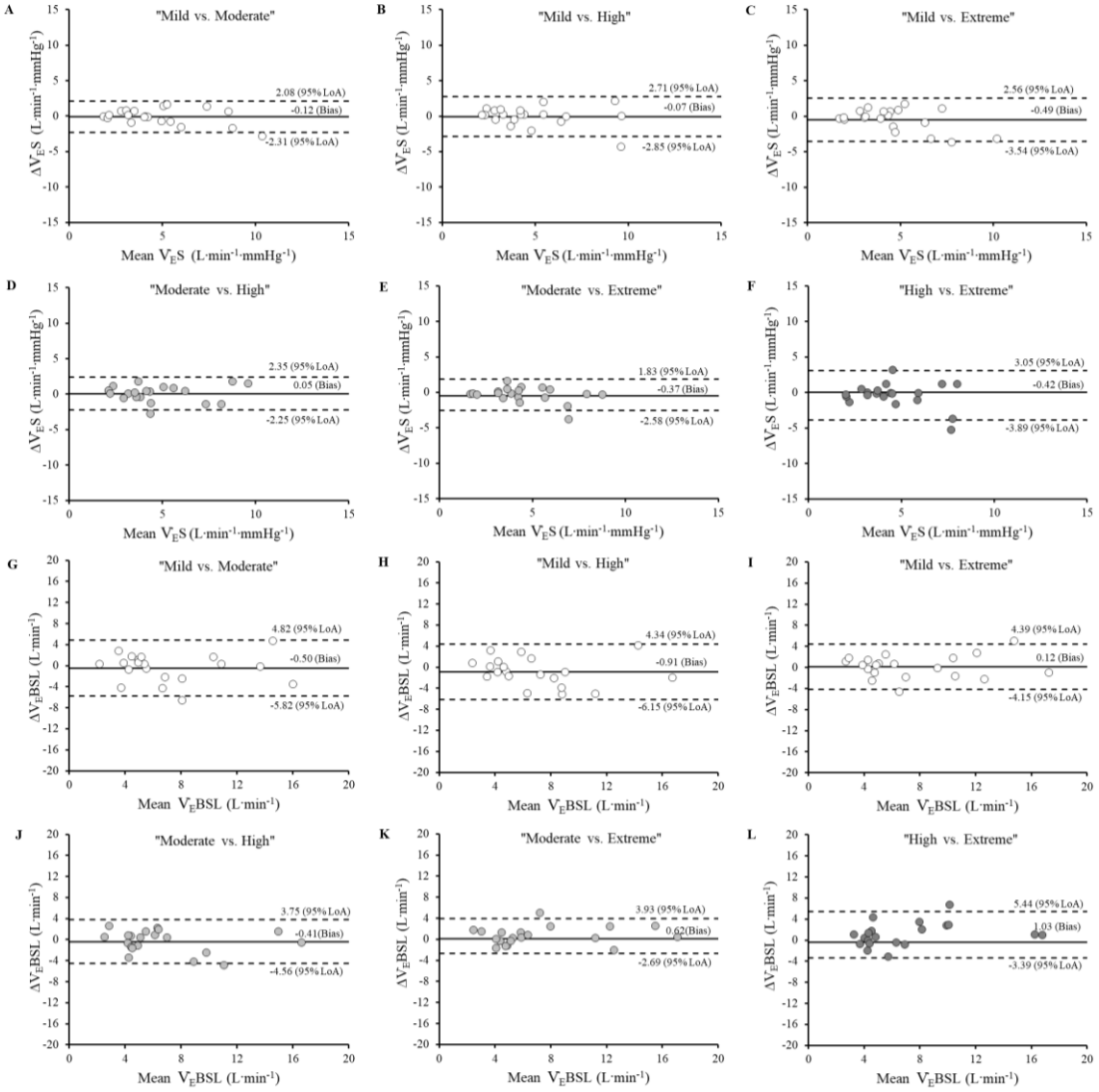

Figure 6: Bland-Altman plots of the central respiratory chemoreflex characteristics. Bland-Altman plots of the central respiratory chemoreflex sensitivity $\dot{V}_ES$ (A-F, $L \cdot min^{-1} \cdot mmHg^{-1}$), and wakefulness drive to breathe $\dot{V}_EBSL$ (G-L, $L \cdot min^{-1}$) group mean response data compared against each different isoxic- and poikiloxic condition.

Chapter 3

3 Discussion and Summary

3.1 Discussion

We assessed the effect of hyperoxic tension on the characteristics of the central respiratory chemoreflex response to CO_2. When modified rebreathing was performed in conditions of mild (PO_2 = 150 mmHg), moderate (PO_2 = 200 mmHg), and high (PO_2 = 300 mmHg) isoxic- and extreme poikiloxic- ($PO_2 \approx$ 700 mmHg) hyperoxic conditions, the $\dot{V}_E$S values were equivalent. In addition, between hyperoxic conditions the VRT was not different nor was the steady-state $\dot{V}_E$ prior to baseline (i.e., $\dot{V}_E$BSL). These observations were consistent between males and females indicating that PO_2 in excess of 150 mmHg does not alter the central respiratory chemoreflex or drive to breathe in the absence of central chemoreflex activation in that the rebreathing parameters quantified remained equivalent throughout each progressive PO_2 condition. Therefore, in healthy young adults, normobaric hyperoxia does not independently stimulate $\dot{V}_E$ nor does it appear to independently activate the central respiratory chemoreflex.

Shortly after the transition from poikilocapnic hyperventilation to hyperoxic rebreathing, $\dot{V}_E$ rapidly stabilizes at a steady-state value prior to VRT (e.g., see Figures 1 and 2) (Mahamed et al., 2004). After stabilization, the $\dot{V}_E$ during this steady-state phase is characterized by the $\dot{V}_E$BSL parameter which is quantified over a duration in which respiratory output receives negligible contributions from chemoreceptor drives (Duffin et al., 2000; Fink, 1961). Thus, the $\dot{V}_E$ measured during this period reflects that which is required to maintain hypopnea under resting metabolic conditions; termed basal ventilation or the "wakefulness drive" to breathe (Casey et al., 1987; Duffin et al., 2000; Fink, 1961). On average, the $\dot{V}_E$BSL was computed over a duration of 2.4 ± 0.1 minutes that was not different amongst conditions because VRT values were consistent independent of PO_2. In addition, across all conditions, $\dot{V}_E$BSL was consistently lower than the $\dot{V}_E$ measured during spontaneous rested breathing by 15-25%, or the approximate contribution of the carotid body to resting $\dot{V}_E$ (Dejours, 1963). Importantly, in the absence of significant central (or peripheral) chemoreceptor drive during this period, $\dot{V}_E$BSL was the same irrespective of

prevailing O_2 conditions. These findings argue against a dose-response relationship between hyperoxic PO_2 and $\dot{V}_E$, or any effect of PO_2 on breathing, for all PCO_2 below the VRT of the central respiratory chemoreflex, at least for the brief duration over which $\dot{V}_E$BSL was computed.

In hyperoxic modified rebreathing, the VRT reflects the PCO_2 at which central chemoreceptor drive begins to contribute to net respiratory output (Duffin, 2010; Duffin et al., 2000). As PCO_2 rises throughout rebreathing above VRT, so too does the activation of central chemoreceptors such that $\dot{V}_E$ rises linearly above $\dot{V}_E$BSL with a slope characterized by $\dot{V}_E$S (see Figures 1 and 2). In our experiments, the $\dot{V}_E$S reflects the sensitivity of the central respiratory chemoreflex plus any added effect of PO_2. Notably, there were no differences in $\dot{V}_E$S between hyperoxic conditions. For all comparisons, the mean bias was not different from zero (see Figure 4) and all between-condition comparisons were well within the established MDC for $\dot{V}_E$S (Guluzade et al., 2022). That $\dot{V}_E$S values were equivalent between conditions indicates that the ventilatory drive arose from central chemoreceptor activation by local elevations of $CO_2/[H^+]$, that a dose-dependent hyperoxic drive was not present, and that central respiratory chemoreflex sensitivity was not affected by prevailing hyperoxic conditions.

In spontaneous, non-rebreathing, conditions, the majority of past work indicates that increases in $\dot{V}_E$ with normobaric hyperoxic breathing rise proportionally with increases in PaO_2 and in a time-dependent manner (Becker et al., 1996; Lambertsen, Kough, et al., 1953; Marczak & Pokorski, 2004; Ren et al., 2000). After a brief period of ventilatory suppression likely due to carotid body inhibition (Cunningham et al., 2011; May, 1957; Watt et al., 1943), $\dot{V}_E$ rises above basal levels. Several mechanisms related directly and indirectly to PaO_2 have been proposed as drivers of the stimulatory or “hyperoxic hyperventilatory” response, many of which may be addressed by our experimental findings.

Several O_2 specific mechanisms have been proposed to contribute to the hyperoxic hyperventilation response. One hypothesis is that PO_2 in the normoxic range (i.e., ~85-100 mmHg) exerts an inhibitory effect on upper respiratory centres and that hyperoxia removes

this homeostatic suppression (Miller & Tenney, 1975). Presumably, hyperoxic disinhibition of respiratory neurons would increase with greater PaO_2. However, we did not observe increases in any measured parameters despite substantial differences in hyperoxic $P_{ET}O_2$ (i.e., 150 mmHg to 700 mmHg). Alternatively, Cragg et al. proposed that local elevations in lactate associated $[H^+]$ secondary to histotoxic-hypoxia might explain hyperoxic hyperventilation. In this scenario, heightened central PO_2 would lead to disruption of mitochondrial enzymes necessary for oxidative phosphorylation in regions of high metabolic activity (i.e., the brain) necessitating an acceleration of anaerobic glycolysis (Cragg et al., 1986). It would be anticipated that with increasing hyperoxic severity, the degree of lactate-associated acidosis would worsen leading to increased activation of central chemoreceptors and heightened drive to breathe at any given PCO_2. Although lactate was not measured, because each of $\dot{V}_E$BSL, VRT, and $\dot{V}_E$S were unaltered with increasing PO_2, our data are also inconsistent with this hypothesis. Thus, it seems unlikely that hyperoxic hyperventilation can be explained by a direct effect of PaO_2 without central PCO_2 and/or chemoreceptor involvement.

An emerging hypothesis is that O_2 stimulates breathing indirectly via ROS -induced augmentation of central chemoreceptor excitability in the solitary complex (SC) (Ciarlone & Dean, 2016a, 2016b; Mulkey et al., 2003). Intracellular recordings of CO_2-sensitive neurons in the solitary complex of rat brain slices exhibited diminished membrane conductance (i.e., heightened excitability) when exposed to one hour of hyperbaric hyperoxia (2-3 atm) (Mulkey et al., 2003). Interestingly, the effects of hyperoxia on the electrical potential of these neurons disappeared in the presence of anti-oxidant and re-established with chemical oxidant. Using this same model, other studies demonstrated similar effects on the firing rate response of excited central chemoreceptors in conditions of normobaric hyperoxia (0.4 to 0.95 atm) (Matott et al., 2014; Mulkey et al., 2001). More recently, in humans Fernandes et al. reported that the increase in $\dot{V}_E$ from room air to 100% FiO_2 breathing without PCO_2 control, was reduced from 32% to 11% with the infusion of free radical-scavenging ascorbic acid infusion (compared to placebo) (Fernandes et al., 2021). Interestingly, in both conditions, intravenous jugular PCO_2 (a proxy of central PCO_2) was not different between conditions suggesting that the stimulus for central

chemoreceptor excitation was equivalent. The authors proposed that between-condition differences could be explained by a sensitization of the central chemoreceptors (and their reflex response to CO_2) secondary to hyperoxic-induced free radical production. Although ROS were not measured in our study, it would be anticipated that their concentration would rise with $P_{ET}O_2$ condition (Fernandes et al., 2021). Despite this, we observed no $P_{ET}O_2$-specific changes in $\dot{V}_ES$ indicating the central chemoreflex sensitivity remained unaltered.

There remain two other mechanisms to explain the normobaric hyperoxic hyperventilation phenomenon both involving heightened medullary PCO_2 relative to room air conditions. The first involves hyperoxia-induced decreases in cerebral blood flow (Davi et al., 1980; Dean et al., 2004; Fernandes et al., 2021; Lambertsen, Kough, et al., 1953; Ren et al., 2000) and the second involves a widening of the arterial-venous CO_2 gradient via the Haldane effect (Lambertsen, Kough, et al., 1953; Ren et al., 2000). Either by a reduced wash-out of metabolically-produced CO_2 or impaired blood CO_2 carriage, these secondary effects of higher-than-normal PO_2 would elevate local PCO_2 and stimulate the central respiratory chemoreflex. In the closed-circuit condition of rebreathing, it would be anticipated that neither of these mechanisms would affect the net ventilatory response because the arterial-tissue gradient of PCO_2 is largely reduced. Nevertheless, our data support the contention that the central chemoreflex to CO_2 provides the majority of the drive to breathe during brief exposures to normobaric hyperoxic conditions. Indeed in paralyzed and chemodenervated cats, exposed to 100% F_iO_2, the increase in phrenic nerve discharge rate was temporally matched to a rise in extracellular cerebrospinal fluid [H^+] (Eldridge & Kiley, 1987).

Ventilatory responses to modified rebreathing in both hypoxic and hyperoxic conditions have been shown to have excellent within and between-day test-retest reproducibility (Guluzade et al., 2022; Jensen et al., 2010). Nevertheless, we recently demonstrated that confidence in measured parameters of central respiratory chemoreflex characterization could be enhanced by averaging of repeated tests (Guluzade et al., 2022). A strength of our study was that three repeated tests were completed for each participant and in each condition to derive parameter estimates. In addition, we employed statistical equivalence

testing using a pre-established minimal detectable change scores (Guluzade et al., 2022), to determine whether each parameter was reliably similar between conditions.

3.2 Limitations

The central chemoreflex sensitization hypothesis (Ciarlone & Dean, 2016b; Dean et al., 2004; Matott et al., 2014; Mulkey et al., 2001, 2003) dictates that heightened ROS concentration are required to heighten the activation of the central chemoreflex. Notably, ROS were not measured in this study. Although we would anticipate that ROS production would be progressively greater the higher $P_{ET}O_2$ conditions (Fernandes et al., 2021), we cannot say for certain that this was the case. We assumed that carotid body contributions to $\dot{V}_E$ during rebreathing were minimized by high $P_{ET}O_2$ and that their contribution, if any, was consistent across all conditions. Our rebreathing trials provided a total hyperoxic exposure time of 5-9 minutes. Whether the properties of the central chemoreflex are altered with longer exposure times in humans is unknown. Most previous work on hyperoxic hyperventilation exposed participants to high F_iO_2 breathing for 10 minutes or longer. Finally, our experiments applied normobaric hyperoxia which is a stimulus unique from hyperbaric hyperoxic exposures and worth considering (Demchenko et al., 2007; Singer et al., 2021).

3.3 Conclusion

Healthy young adult males and females exposed to brief periods of normobaric hyperoxia of increasing severity exhibit no changes to their ventilatory response to CO_2. These findings indicate that neither a direct effect of high PO_2 nor a PO_2-induced sensitization of the central respiratory chemoreflex explain the hyperoxic hyperventilatory response, at least in the context of modified rebreathing. Our data support the contention that the stimulation of breathing by hyperoxia depends on the prevailing central PCO_2, which likely rises in a PO_2 dose-dependent manner as a result of decreased medullary blood flow and the Haldane effect.

3.4 Future Directions

Results from this study have important implications for human testing of the central chemoreflexes which are routinely performed assuming that hyperoxia does not stimulate ventilation. Beyond the mechanistic importance of this work, hyperoxia is used worldwide as an acute clinical treatment of conditions where gas exchange and ventilation are impaired. Future research should incorporate the measurement of ROS during rebreathing, to the behaviour of ROS during isoxic-hyperoxic modified rebreathing. With this additional measurement, the comparison of an antioxidant infusion during rebreathing would help further elucidate the hyperoxic hyperventilation phenomenon. Furthermore, the manipulation of cerebral blood flow via adding a condition of lower body positive pressure administration whilst recording the ventilatory response to hyperoxia is warranted. This way we can investigate what happens to the ventilatory response to isoxic-hyperoxic modified rebreathing when blood is being physically pushed back towards the brain and hyperoxic gas is acting on the cerebrovasculature. Furthermore, iso-oxic hyperoxia could be applied during the baseline and hyperventilation period to extend the duration of hyperoxic exposure.

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Appendices

Appendix A: Ethical Approval

Western Research

Date: [redacted]

To: [redacted]

Project ID: 119281

Study Title: Central and Peripheral Chemoreflex Control of Breathing

Application Type: HSREB Amendment Form

Review Type: Delegated

Meeting Date / Full Board Reporting Date: 14/June/2022

Date Approval Issued: 18/May/2022

REB Approval Expiry Date: 19/Jul/2022

Dear [redacted],

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Protocol_V3	Protocol	17/May/2022	3
Consent Form A_V2	Consent Form	18/May/2022	2
Consent Form B_V2	Consent Form	18/May/2022	2
Consent Form_V4	Consent Form	18/May/2022	4

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
HSREB 119281 - Summary of Changes	Summary of Changes	17/May/2022	1

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

[redacted]

Appendix B: Letter of Information

Western HealthSciences

Kinesiology

LETTER OF INFORMATION AND CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE:	**Central and Peripheral Chemoreflex Control of Breathing (Form B)**
Principal Investigator:	[redacted]
Contact Information:	[redacted]
Co-Investigators:	[redacted]
Funding Sources:	Natural Sciences and Engineering Research Council of Canada
Conflicts of Interest:	There are no conflicts of interest to declare related to this study

INTRODUCTION

We invite you to participate in our research study. We are seeking a total of 65 participants (25 males and 40 females) to help us learn more about how humans adjust their breathing in response to changes in the level of oxygen and carbon dioxide in our bodies. Before you decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. Before you volunteer as a study participant, it is important that you first read this summary of the study's purpose, procedures, possible discomfort and risks, benefits, and precautions. We also describe your right to refuse to participate or withdraw from the study at any time. Before signing this consent form, please ask the study investigator(s) to explain any words that you do not understand and make sure all your questions have been answered to your satisfaction before signing this document.

BACKGROUND & PURPOSE

Anyone who has ever held their breath for a long period of time will have experienced an overwhelming urge to breathe. This breathing sensation comes from specialized sensors in the blood vessels and brainstem that send more intense signals to brain regions that regulate breathing when body oxygen levels fall and carbon dioxide levels rise (as occurs when we hold our breath). This breathing "chemoreflex" is critically important for maintaining oxygen supply and normal blood chemistry.

Often, in environments (e.g. poorly ventilated areas), activities (e.g. exercise) and conditions (e.g. sleep) that challenge carbon dioxide removal and oxygen availability, the two chemoreceptor groups in the blood vessels (peripheral) and brainstem (central) are turned on at the same time. The peripheral chemoreflex is "turned off" in high oxygen conditions. For this reason, high oxygen is given to test the central chemoreflex on its own. However, high oxygen is also considered "to turn" on breathing in a way that is separate from the central chemoreflex. The purpose of this research is to assess the influence of high oxygen on breathing.

PARTICIPANT ELIGIBILITY

Inclusion Criteria

You may be included in this study if you are a male of female between the ages of 18 and 40 years

Exclusion Criteria

You are not eligibile to participate if you are pregnant, smoke cigarettes, have asthma or require an inhaler, have a chronic lung, cardiovascular, or kidney disease, have diagnosed sleep apnea, or a dependence on alcohol or drugs within the past year.

STUDY DESIGN & PROCEDURES

If you agree to participate you will be asked visit the Cardiorespiratory Physiology Laboratory [redacted]) on five separate days. Each visit will last ~75 minutes. The first visit will involve testing of your lung function and exercise capacity and the next four visits will each involve three repetitions of the same breathing experiment.

All visits will take place in a quiet, temperature controlled room. Prior to each visit, you need to abstain from **strenuous exercise**, **alcohol, caffeinated beverages (coffee, tea, soft drinks),** and **recreational drug use** for at least 12 hours. All five visits will be performed at least 24 hours apart.

Laboratory Visits

Visit 1: Baseline Measurements

The following tests will be performed on the first visit:

Lung function: This test measures how much air your lungs can take in and how quickly you can move air out of your lungs. You will breathe through a sterile cardboard tube attached to an air flow device. While standing quietly, you will breathe into the tube for ~1 minute. At the end of the 1-minute period, you will slowly breathe in and fill your lungs as much as you can and then empty your lungs a quickly and as much as you can.

Exercise capacity: This test will measure your cardiorespiratory (or "aerobic") fitness. You will perform an exercise test on a cycle ergometer. The exercise intensity will begin at a low level and will be advanced gradually and continue to rise until you are unable to continue. You may be unable to continue because you cannot turn the pedals or because you will perceive the exercise as being too strenuous. During this cycling test you will wear a facemask that covers your nose and mouth (similar to a medical mask) and we will measure the volume of oxygen and carbon dioxide that you breathe in and out.

Breathing Task: This test measures how your chemoreflexes respond to increases in carbon dioxide. The details of the procedure are described in the next section ("Visits 2-5: Breathing Experiments"). The purpose of this test on Visit 1 is to practice the breathing maneuver and to become comfortable with the sensations associated with high and low levels of carbon dioxide.

Visits 2-5: Breathing Experiments

On the next four visits, you will perform three repetitions of a simple breathing task while seated comfortably on a dental chair. The breathing tasks lasts 10-12 minutes and each task will be separated by 15-20 minutes of seated rest.

Breathing Task: A facemask will be secured to your face with a head harness. The facemask will not block your breathing in any way. Attached to the facemask will be a tube-shaped device to measure the volume of air you breathe, a sampling line to measure the oxygen and carbon dioxide quantities of each breath, a valve and a plastic clear bag. You will then perform the following:

- At the beginning of the breathing task, you will be asked to perform a deep breathing exercise (breathe more deeply than you would normally while seated) for 5 minutes while breathing room air. You may feel slightly light-headed during this period or experience tingling in your skin.
- After these 5 minutes we will ask you to take a deep breath out. Then we will switch the valve so that you begin to breathe from a bag. We will ask you to take three deep breaths in and out. After these, you may breathe normally.
- With this set-up you will be breathing in air that you breathed out causing your carbon dioxide levels to rise. When carbon dioxide levels are raised, you will become more aware of your breathing (like you feel after exercise or while holding your breath). You will be able to breathe as hard as you feel necessary without affecting the test. This "rebreathing" will last between 2 to 5 minutes depending on how hard you are breathing and how fast your carbon dioxide levels rise.
- Your mask will be supplied with oxygen by a computer-controlled machine built to control breathing levels of oxygen. This will allow us to give you a standard amount of oxygen to breathe during the "rebreathing" period.
- At the end of the task, we will switch you back to room air breathing and the out of breath feeling (or "breathlessness") will stop within 2 to 3 breaths.

In addition to measuring your breathing responses, during each breathing task that you perform, we will also monitor the following:

- Heart rate will be measured by applying electrodes (sticky patches) to your chest.
- Blood pressure will be recorded by a cuff wrapped around your arm in the usual fashion.
- The amount of oxygen and carbon dioxide in your blood will be measured by a sensor clipped to your ear lobe.
- Oxygen levels in your brain tissue will be measured using near-infrared spectroscopy which projects light into a specific location of your brain and measures the amount of light coming out at another location. A small probe will be placed near your forehead and it will be secured with tape, covered to prevent light from entering or leaving the area, and bound with elastic bandage.
- Blood flow to your brain will be monitored by applying a small spherical probe to the side of your head just in front of your ear. We may also use ultrasound to measure blood flow in the arteries of your neck and in your limbs.

For Visits 2-5, you will repeat this "Breathing Task" three times. The instructions for each breathing task are identical. The only difference between tests will be the content of oxygen in the bag. In all tests, the oxygen levels will be higher than normal. You will not be told ahead of time how high the oxygen level in the bag is.

RISKS AND DISCOMFORTS

Breathing Tasks

The changes in levels of carbon dioxide and breathing are in the range expected in most people during normal living (such as during sleep or exercise). The high oxygen breathing is considered safe.. The breathing exercises and gas manipulations may cause you to faint or feel dizzy, light-headed, or other minor unpleasantness. During parts of the test you WILL feel a need to breathe harder to get enough air than normally required when sitting and you may perceive this as feeling "breathless".

If the sensations that you experience during any part of the breathing tasks cause any discomfort and you wish to stop; the experiment will be stopped immediately, and oxygen will be delivered. Any unpleasant sensations should likely be resolved with two or three breaths after stopping the test.

The adhesive on the electrodes for the ECG may cause allergic reactions, slight redness, and irritation of the skin.

Exercise Test

Although exercise testing is considered a safe procedure, there exists the possibility of certain changes occurring during the exercise test. These include abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instances, acute heart attack or arrest (4 events in every 10,000 tests in those with chronic heart conditions). Every effort will be made to minimize risks by evaluation of preliminary information relating to your health and fitness and by careful observation during testing. All study personnel will be certified in CPR and, thus, will possess the skills needed to recognize and respond to cardiovascular emergencies (including the use of an Automatic Electronic Defibrillator) should they arise.

BENEFITS

You will receive no personal benefit from this study. However, the study will generate knowledge regarding how breathing is controlled which may inform new therapies for targeting chemoreceptors in conditions where breathing becomes irregular.

CONFIDENTIALITY

Your research records will be stored in a secure office at the University of Western Ontario. To further protect your confidentiality, your name will be replaced with a participant ID number on all documents. The master list linking your identity, participant ID number, and contact information will kept in a locked and secured area on the Western campus for a minimum of 7 years.

All information collected during this study, including any personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law or requested by a certified representative of the Western University Health Sciences Research Ethics Board. You will not be named in any reports, publications, or presentations that may come from this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Your participation in this study is voluntary. You may decide not to be in the study, or to be in the study now and then change your mind later. You may leave the study at any time without affecting your status at the University of Western Ontario. If you decide to leave the study, you have the right to request withdrawal or information collected about you.

RIGHTS AS A PARTICIPANT

If you are harmed as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost.

By signing this form you do not give up any of your legal rights against the investigators or involved institutions for compensation, nor does this form relieve the investigators or involved institutions of their legal and professional responsibilities.

You will be given a signed copy of this consent form.

REIMBURSEMENT

We will reimburse you $10 per visit for expenses related to time and travel for a total of $50. This will be given to you in cash at the end of visit 5.

QUESTIONS ABOUT THE STUDY

If you have any questions, concerns or would like to speak to the study team for any reason please contact the principal investigator: [redacted]

If you have any questions about your rights as a research participant or have concerns about this study, call a representative from the Western University Health Sciences Research Ethics Board (HSREB) at [redacted] The REB is a group of people who oversee the ethical conduct of research studies. The HSREB is not part of the study team. Everything that you discuss will be kept confidential.

TITLE: **Central and Peripheral Chemoreflex Control of Breathing (Form B)**

Principal Investigator: [redacted]

CONSENT

This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to the use of my information as described in this form. I agree to take part in this study.

CONTACT FOR FUTURE STUDIES

I AGREE to be contacted for future research studies ☐ **YES** ☐ **NO**

Study Participant Name	Signature	Date

My signature means that I have explained the study to the participant named above. I have answered all questions.

Name of Person Obtaining Consent	Signature	Date

Curriculum Vitae

Joshua Huggard
School of Kinesiology, Faculty of Health Sciences
Cardiorespiratory Physiology Laboratory
The University of Western Ontario
London, Ontario, Canada

Education

2021-2023	**Master of Science (M.Sc.)** Integrative Biosciences, *The University of Western Ontario, London, ON*
2016- 2021	**Honors Bachelor of Arts (B.A.)** Specialization in Kinesiology, *The University of Western Ontario, London, Ontario*

Research

Peer-Reviewed Original Research Publications (2)

1. Guluzade, N. A., **Huggard, J. D.,** Keltz, R. R., Duffin, J., & Keir, D. A. (2022). Strategies to improve respiratory chemoreflex characterization by Duffin's rebreathing. *Experimental Physiology*, *107*(12), 1507–1520. https://doi.org/10.1113/EP090668
2. Guluzade, N. A., **Huggard, J. D.**, Duffin, J., & Keir, D. A. (2023). A Test of the interaction between central and peripheral respiratory chemoreflexes in humans. *J Physiol*. *In Production. DOI: 10.1113/JP284772*

Manuscripts Under Review (1)

1. **Huggard, J. D.,** Guluzade, N. A., Duffin, J., & Keir, D. A. (2023). The ventilatory response to modified rebreathing is unchanged by hyperoxic severity: implications for the hyperoxic hyperventilation paradox. *J Appl Phys.* First revisions requested.

Refereed Abstracts (5)

Guluzade, N. A., Keltz, R. R., **Huggard, J. D**., & Keir, D. A. (2022). A Strategy to Enhance Confidence in Respiratory Chemoreflex Characterization by Modified Rebreathing. The FASEB Journal, 36(S1). https://doi.org/10.1096/fasebj.2022.36.S1.L7734

Guluzade, N., **Huggard, J.D.**, Duffin, J., & Keir, D. (2023). Effect of Central Chemoreceptors on the Peripheral Chemoreflex Response to Hypoxia. Physiology, 38(S1), 5731199. https://doi.org/10.1152/physiol.2023.38.S1.5731199

Huggard, J. D., Guluzade, N. A., Keir, D. A., & Keltz, R. R. (2022). Reproducibility of Respiratory Chemoreflex Characterization by Modified Rebreathing. The FASEB Journal, 36(S1). https://doi.org/10.1096/fasebj.2022.36.S1.L7757

Huggard, J.D., Guluzade, N., Duffin, J., & Keir, D. (2023). Does hyperoxia stimulate breathing? Physiology, 38(S1), 5732953. https://doi.org/10.1152/physiol.2023.38.S1.5732953

Keltz, R. R., Guluzade, N. A., **Huggard, J. D.,** & Keir, D. A. (2022). The Relationship Between Central and Peripheral Chemoreflex Sensitivities and V̇E-V̇CO2 Slope Below and Above the Respiratory Compensation Point of Incremental Exercise. The FASEB Journal, 36(S1). https://doi.org/10.1096/fasebj.2022.36.S1.L7767

Travel Grants and Other Awards

- Kinesiology Graduate Student Travel Award ($500) 2023
- Dean's Honour List: 2019-2021
- Mustang Scholar Athlete 2019-2020
- OUA Academic Achievement Award: 2019-2020

Work & Teaching Experience

Graduate Teaching Assistant

2023/01- 2023/04	Kinesiology 2230B – Introduction to Exercise Physiology, The University of Western Ontario, London, Ontario
2022/09- 2022/12	Kinesiology 3330F – Laboratory in Exercise Physiology, The University of Western Ontario, London, Ontario
2022/01- 2022/04	Kinesiology 2230B – Introduction to Exercise Physiology, The University of Western Ontario, London, Ontario
2021/09- 2021/12	Kinesiology 4412A – Exercise for Specific Populations: Impairment and Aging, The University of Western Ontario, London, Ontario

Research Assistant

2021/09 – 2023/06	Cardiorespiratory Physiology Lab, The University of Western Ontario, London, Ontario