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The Effect of Central Chemoreceptors on the Peripheral Respiratory Chemoreflex Response to Hypoxia in Humans

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Abstract

We measured the peripheral chemoreflex sensitivity to hypoxia (PChS) at various isocapnic carbon dioxide tensions (PCO_2) to determine the form of the relationship between PChS and central PCO_2. Twenty participants completed three repetitions of modified rebreathing tests with end-tidal PO_2 ($P_{ET}O_2$) clamped at 150, 70, 60, and 45 mmHg. PChS was computed at 1-mmHg intervals of $P_{ET}CO_2$ as follows: the differences in $\dot{V}_E$ between the three hypoxic profiles and the hyperoxic profile ($\Delta\dot{V}_E$) were calculated; three $\Delta\dot{V}_E$ values were plotted against corresponding calculated oxyhemoglobin saturation (S_CO_2); and linear regression determined PChS ($L \cdot min^{-1} \cdot mmHg^{-1} \cdot \%S_CO_2^{-1}$). These processing steps were repeated at each $P_{ET}CO_2$ to produce the PChS vs. isocapnic PCO_2 relationship which was fitted with linear and polynomial functions. Chemoreflex sensitivity ($\dot{V}_ES$) rose ($p<0.001$), mean S_CO_2 fell progressively ($p<0.001$). Despite increasing central chemoreflex activation, in all individuals PChS increased with $P_{ET}CO_2$ and this relationship was best described by a liner model in 15 of the 20 individuals, indicative of an additive interaction.

Keywords: hypercapnia, rebreathing, hypoxic ventilatory response, hyperoxia, respiratory chemoreflex, control of breathing

Summary for Lay Audience

Breathing changes depending on our activity, behavior, and emotional state, yet the blood chemistry, oxygen supply, and the acidity of our tissues remain stable. In humans, this stability is controlled by two rapidly responding sensor organs located within 1) our blood vessels (oxygen sensors) and 2) our brain (carbon dioxide sensors). These sensors respond to increases in acidity in the blood and increase breathing as a response. Often, these oxygen and carbon dioxide sensors are working together, but how or whether they connect with each other in the control of breathing in humans remains under question. Therefore, the goal of this study was to assess the connection between the two sensors by exposing humans to progressively lower oxygen conditions and one level of high oxygen condition while progressively increasing the levels of carbon dioxide. The breathing responses in ten males and ten females was recorded while they completed three repetitions of breathing tests at progressively decreasing levels of oxygen (3 levels) and one level of high oxygen over four laboratory visits. We saw that the breathing response was significantly greater at the low oxygen conditions in comparison with the high oxygen level. We concluded that in 15 of the 20 participants the oxygen and carbon dioxide sensor worked together to control breathing. These findings are significant as they provide information to help the understanding of breathing in different environments, such as at high mountain levels and diseases such as COVID-19.

Co-Authorship Statement

This thesis includes versions of the following manuscript that were submitted for publication in Chapter 2:

1. **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*. Online ahead of print.

Guluzade, N.A., Keir, D.A., and Duffin, J. conceived and designed the study. All data were collected and analyzed by Guluzade, N.A. with collection assistance provided by Huggard, J.D. All authors interpreted the results of the experiment. The original manuscript in Chapter 2 was written by Guluzade, N.A. with feedback provided from all co-authors.

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List of Abbreviations

AIC	Akaike information criterion
ANOVA	analysis of variance
CO_2	carbon dioxide
CSF	cerebrospinal fluid
fB	breathing frequency
FEV_1	forced expiratory volume in 1 second
F_ICO_2	fractional inspired carbon dioxide
FVC	force vital capacity
H^+	hydrogen ion
HCO_3^-	bicarbonate
HSD	honestly significant difference
HVR	hypoxic ventilatory response
IC	inspiratory capacity
MVV	maximal voluntary ventilation
NaCN	sodium cyanide
O_2	oxygen
$PaCO_2$	partial pressure of arterial carbon dioxide
PaO_2	partial pressure of arterial oxygen
P_CCO_2	partial pressure of central carbon dioxide
PChS	peripheral chemoreflex sensitivity to hypoxia
PCO_2	partial pressure of carbon dioxide
PEF	peak expiratory flow rate
$P_{ET}CO_2$	end-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	end-tidal partial pressure of carbon dioxide
P_IO_2	partial pressure of inspired oxygen
PO_2	partial pressure of oxygen
$rrCO_2$	rate of rise of carbon dioxide
SaO_2	arterial oxyhemoglobin saturation
S_CO_2	calculated oxyhemoglobin saturation
SpO_2	pulse oxyhemoglobin saturation
SVC	slow vital capacity
$\dot{V}CO_2$	rate of carbon dioxide output
$\dot{V}_E$	ventilation rate
$\dot{V}_EBSL$	wakefulness drive to breathe
$\dot{V}_ES$	ventilatory sensitivity
$\dot{V}O_2$	rate of oxygen uptake
VRT	ventilatory recruitment threshold
V_T	tidal volume

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Chapter I: Introduction

General Introduction

The ever-changing nature of human behavior, emotion, and metabolism imposes a dynamic challenge to our respiratory system. Breathing must adapt to ensure that the partial pressure of oxygen (PO_2) remains sufficiently high to deliver oxygen (O_2) to our cells and the partial pressure of carbon dioxide (PCO_2) is kept sufficiently low to maintain hydrogen ion concentration ($[H^+]$) of our tissues. The breath-to-breath maintenance of these blood gases and acid base-status are achieved by specialized gas sensors (or "chemoreceptors") within the arteries and brain. The peripheral (carotid body; O_2 and $[H^+]/CO_2$ sensors) and central (brainstem; $[H^+]/CO_2$ sensors) respiratory chemoreceptors play an important role in rapidly responding to the deviations from normal in PO_2 and $PCO_2/[H^+]$ in the arterial blood and cerebrospinal fluid (CSF), respectively, by reflexively activating the respiratory system (i.e., the "chemoreflexes") (Cunningham et al., 1986; Guyenet, 2014).

Alveolar ventilation ($\dot{V}_A$), the product of tidal volume (V_T) and breathing frequency (fB), is the output variable of the respiratory system that is crucial for maintaining adequate gas exchange at the lung and is intimately linked to blood gas homeostasis via the alveolar air equation:

$$\frac{k\ \dot{V}CO_2}{PaCO_2} \leftarrow \dot{V}_A \rightarrow \frac{k\ \dot{V}O_2}{P_IO_2 - PaO_2}$$

Where k (equal to 863) is the constant that accounts for atmospheric conditions such as temperature, pressure, and humidity, $\dot{V}CO_2$ is the rate of carbon dioxide output, $\dot{V}O_2$ is the rate of oxygen uptake, $PaCO_2$ is the partial pressure of arterial carbon dioxide, P_IO_2 is the partial pressure of inspired oxygen, and PaO_2 is the partial pressure of arterial oxygen.

These equations demonstrate that if $\dot{V}_A$ increases relative to $\dot{V}CO_2$ or $\dot{V}O_2$, $PaCO_2$ and PaO_2 must decrease and increase, respectively. Whereas if $\dot{V}_A$ decreases relative to $\dot{V}CO_2$ or $\dot{V}O_2$, $PaCO_2$ and PaO_2 must increase and decrease, respectively. During spontaneous breathing, $\dot{V}_A$ is set by respiratory centres in the brain (termed "wakefulness" drive) but is continuously modulated by the excitatory state of the central and peripheral chemoreceptors which depends on changes in blood gases ($PaCO_2$ and PaO_2) and acidity. Once excited, these receptor groups send afferent signals to the respiratory control center in the medulla to stimulate the respiratory muscles via

phrenic nerve discharge. Activation of the rate and force of respiratory muscle contraction causes swings in pleural pressure that allow the lungs to raise fB and V_T such that $\dot{V}_A$ increases to restore acidity and blood gases to homeostatic levels.

Blood gases (or gas pressures in any physiological fluid compartment) are intimately linked to acid-base status via PCO_2 which is a major determinant of $[H^+]$ as dictated by the modified Henderson-Hasselbach equation (Yee et al., 2022):

$$[H^+] = K' \frac{PCO_2}{[HCO_3^-]}$$

Where *K'* (equal to 24) is a constant including the solubility of CO_2 and dissociation constant of the carbonic acid, $[H^+]$ is the hydrogen ion concentration, PCO_2 is the partial pressure of carbon dioxide, and $[HCO_3^-]$ is the bicarbonate ion concentration. When PCO_2 rises acutely, $[H^+]$ increases and vice-versa. This relationship is approximately linear and, combined with the alveolar air equation above, exemplifies the link between $\dot{V}_A$, PCO_2, and $[H^+]$.

The central and peripheral chemoreceptors are responsible for sensing $[H^+]$ and for dynamically adjusting $\dot{V}_A$, such that PCO_2 (and PO_2) and acid-base status of the compartments they sense are maintained normal. Despite being located in different regions, in situations where O_2 availability or CO_2 clearance are challenged (e.g., during breath-hold, altitude, sleep apnea, swimming, chronic obstructive pulmonary disease) these two chemoreceptor groups may be activated simultaneously. How the respiratory adjustments that are initiated by these sensors work together when engaged at the same time is a contentious topic that remains to be established in humans. This thesis will present and discuss a novel assessment of the interaction between central and peripheral respiratory chemoreflexes in humans. Although $\dot{V}_A$ is the principal output variable of the respiratory system, its measurement necessitates invasive arterial measures, for this reason, minute ventilation ($\dot{V}_E$) is often used (which includes both dead space and alveolar ventilation) and will constitute the efferent arm of the respiratory chemoreflexes for this thesis.

Central chemoreceptor reflex

The central chemoreceptors are located in the medulla, near the ventrolateral surface and scattered within the brain tissue, the retrotrapezoid nucleus, the raphe nucleus, and within the locus coeruleus (Corcoran et al., 2009; Guyenet et al., 2009; Nattie & Li, 2009; Okada et al., 2002;

Putnam et al., 2004). These receptors are sensitive to changes in local [H^+], and when excited, relay afferent signals to the respiratory control centers located in the medulla. The stimulus to the central chemoreceptors is the [H^+] of the CSF (Ainslie & Duffin, 2009; Duffin, 2010). Since [H^+] is directly dependent on the PCO_2 of chemoreceptor tissue, the central chemoreceptors are often thought of as CO_2 receptors (Ainslie & Duffin, 2009). The factors that determine central PCO_2 (P_CCO_2), and thus [H^+], are the metabolic production of CO_2 of the medullary tissue, $PaCO_2$, and medullary blood flow, varying directly with $PaCO_2$ and inversely with medullary blood flow (i.e., $P_CCO_2 = PaCO_2 + (\dot{V}CO_2 \div$ medullary blood flow).

The central chemoreceptors are an integral part of the homeostatic control of local [H^+] through their involvement in a negative feedback loop, referred to as the central chemoreceptor reflex. Higher than normal [H^+] excite central chemoreceptors which reflexively increases pulmonary $\dot{V}_E$ to reduce PCO_2, and thus, [H^+] of medullary tissue (Cunningham et al., 1986; Duffin, 1990; Guyenet, 2014; Guyenet et al., 2008, 2012). For example, when $PaCO_2$ rises, CO_2 will rapidly diffuse across the blood brain barrier and acidify the cerebrospinal fluid (CSF) by combining with water to form H^+ and HCO_3^- (Duffin, 2005). The rise in [H^+] stimulates central chemoreceptors that transmit neuronal signals to the medullary respiratory neurons that modulate phrenic nerve discharge to increase pulmonary $\dot{V}_E$ through activation of the respiratory muscles. The increase in $\dot{V}_E$ allows the elimination of more CO_2 through pulmonary gas exchange to bring down $PaCO_2$ and restore PCO_2, and thus [H^+], of the CSF to homeostatic levels. With the restoration of CSF chemistry, central chemoreceptor activity is suppressed which reduces their input to the medullary respiratory centers in the brain (Duffin, 2005, 2010, 2011). Therefore, through this negative feedback control system, the [H^+] of CSF is maintained. Overall, the central chemoreceptors and their reflexive responses exhibit a linear relationship with central PCO_2. This relationship is depicted in Figure 1 with P_CCO_2 as the input variable and phrenic nerve discharge as the output variable of the central chemoreceptors.

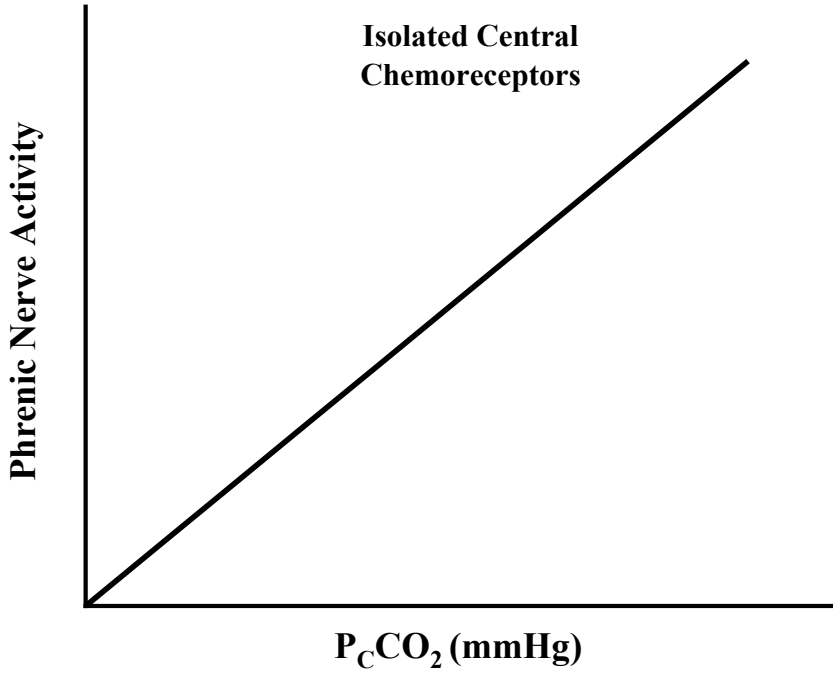


Figure 1: A hypothetical figure of the phrenic nerve activity versus the partial pressure of central carbon dioxide (P_CCO_2) relationship in the isolated central chemoreceptors.

Peripheral chemoreceptor reflex

The peripheral chemoreceptors are a cluster of cells situated in the carotid body located at the bifurcation of the common carotid artery (Cunningham et al., 1986; Duffin, 1990). These receptors are composed of type I glomus cells made up of O_2 and [H^+] sensing elements that continuously monitor the arterial blood leaving the left ventricle. Irrespective of stimulant, when excited, these cells send signals to the central respiratory control center in the medulla via the carotid sinus nerve – a branch of the glossopharyngeal nerve (Duffin, 2010; Gonzalez et al., 1994; Kumar & Bin-Jaliah, 2007; Kumar & Prabhakar, 2012; Nurse & Piskuric, 2013; Prabhakar, 2013). As a result of increased ascending afferent input and central integration, carotid body excitation results in increased descending efferent phrenic nerve discharge to activate respiratory muscles to increase V_T and fB. Like the central chemoreflex, the resultant increase in $\dot{V}_E$ via this reflex arc permits the elimination of CO_2 through exhalation and an increase in O_2 uptake through inhalation.

As mentioned, the peripheral chemoreceptors respond to both increases in arterial [H^+] and falls in arterial PO_2 (PaO_2) (Duffin, 1990; Prabhakar, 2013). With respect to progressive rises in arterial [H^+]/PCO_2, the responsiveness of the carotid body chemoreceptors have consistently been shown to be linear when their output is measured as changes in intracellular calcium of isolated type I cells (Buckler & Vaughan-Jones, 1994; Dasso et al., 2000), discharge rates of intact carotid sinus afferents, phrenic nerve activity of anaesthetized animals (Hornbein & Roos, 1963; Lahiri et al., 1993; Lahiri & Delaney, 1975; Vidruk et al., 2001), or ventilatory responses to CO_2 in humans (Duffin et al., 2000; Keir et al., 2019; Preston et al., 2009). An overall representation of this stimulus-response relationship is displayed in Figure 2A with $PaCO_2$ as the input to peripheral chemoreceptors and phrenic nerve activity as the output variable. With respect to progressive falls in PaO_2, both *in vitro* and *in vivo* preparations demonstrate that the relationship varies hyperbolically (Duffin, 1990; Hanson et al., 1978; Hayes et al., 1976; Keir et al., 2020; Rebuck & Campbell, 1974; Torrance, 1996). Responsiveness accelerates in the hypoxic range (PaO_2 below ~85 mmHg) and decelerates towards zero in the hyperoxic range (PaO_2 above ~125 mmHg) (Kumar & Prabhakar, 2012). Thus, hyperoxia effectively silences the peripheral chemoreceptors (Lloyd & Cunningham, 1963; Mohan & Duffin, 1997). This relationship is also depicted in Figure 2B.

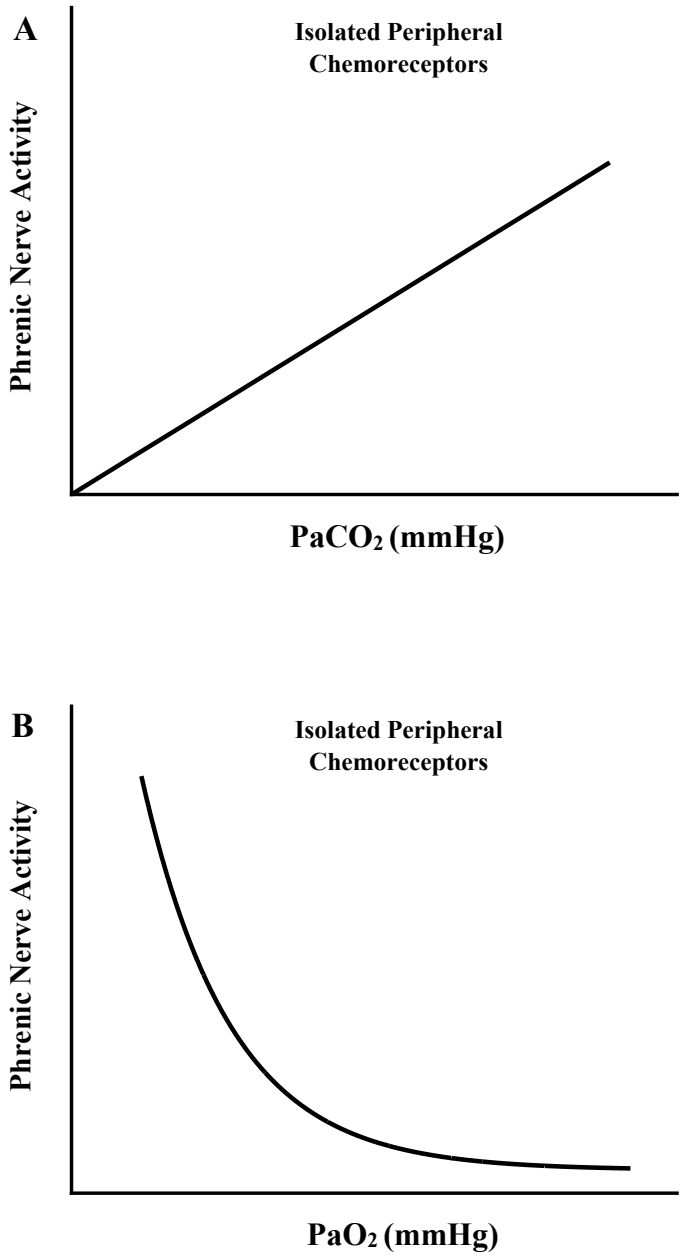


Figure 2: A hypothetical figure of the phrenic nerve activity versus partial pressure of arterial carbon dioxide ($PaCO_2$) and partial pressure of arterial oxygen (PaO_2) in the isolated peripheral chemoreceptors illustrating a linear (Panel A) and a hyperbolic (Panel B) relationship.

Despite displaying distinct response profiles to changes in $PaCO_2$ and PaO_2 (see Figure 2A vs B, respectively), arterial hypoxia and hypercapnia often occur simultaneous (e.g., during a breath-hold). When exposed to both stimuli, the peripheral chemoreceptor response is not simply superimposed by addition, but it exhibits a multiplicative effect so that the responses to the combined stimuli are greater than the sum of their individual responses (Duffin, 1990; Wilson & Teppema, 2016). With respect to $PaCO_2$, the reflex response slope becomes steeper with lower (hypoxic) PaO_2 and shallower with higher (hyperoxic) PaO_2 (Figure 3A). With respect to PaO_2, the hyperbolic reflex response is shifted up and to the right with high $PaCO_2$ (hypercapnic) and down and to the left with low $PaCO_2$ (hypocapnic) (Figure 3B) (Fitzgerald & Parks, 1971; Kiwull-Schöne et al., 1979; Lahiri & Delaney, 1975; Teppema & Dahan, 2010). Thus, arterial hypocapnia "depresses" the sensitivity of the peripheral chemoreceptors to PaO_2 and arterial hypercapnia "sensitizes" the sensitivity of peripheral chemoreceptors to PaO_2.

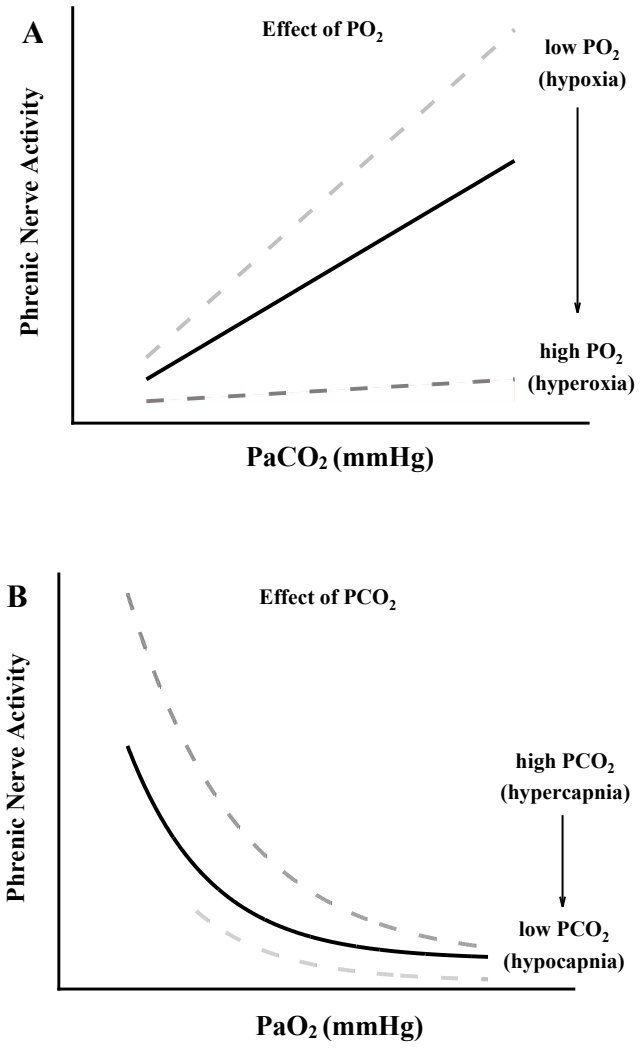


Figure 3: A hypothetical figure of the phrenic nerve activity versus partial pressure of arterial carbon dioxide ($PaCO_2$) at different levels of partial pressure of arterial oxygen (PaO_2; Panel A) and versus PaO_2 at different levels of $PaCO_2$ (Panel B).

Although the "sensitizing" effect of PaO_2 on the response to $PaCO_2$ is obvious and easily quantifiable as a reduction in slope (see Figure 3A), deducing the "sensitizing" effect of $PaCO_2$ on the peripheral chemoreflex response to low oxygen is not easily quantifiable due to the hyperbolic nature of the response and the parameters of this mathematical relationship (Figure 3B). However, when this relationship is instead displayed with arterial oxyhemoglobin saturation (SaO_2) as the independent variable, the sigmoidal relationship between SaO_2 and PaO_2 linearizes the response (Figure 4) and it is easier to see that the stimulus-response slope, or sensitivity of the peripheral chemoreceptors to low O_2 is determined by $PaCO_2$. Interestingly, with central chemoreceptor activation absent or fixed, this response slope to progressive hypoxemia (or peripheral chemoreflex sensitivity to low O_2, PChS, e.g., in phrenic nerve discharge rate per $\%SaO_2$) appears to increase linearly for a standard change in PCO_2 (Irsigler et al., 1980; Riedstra, 1963; Teppema et al., 2010) (see Figure 5). This will be an important feature of the experimental design described below.

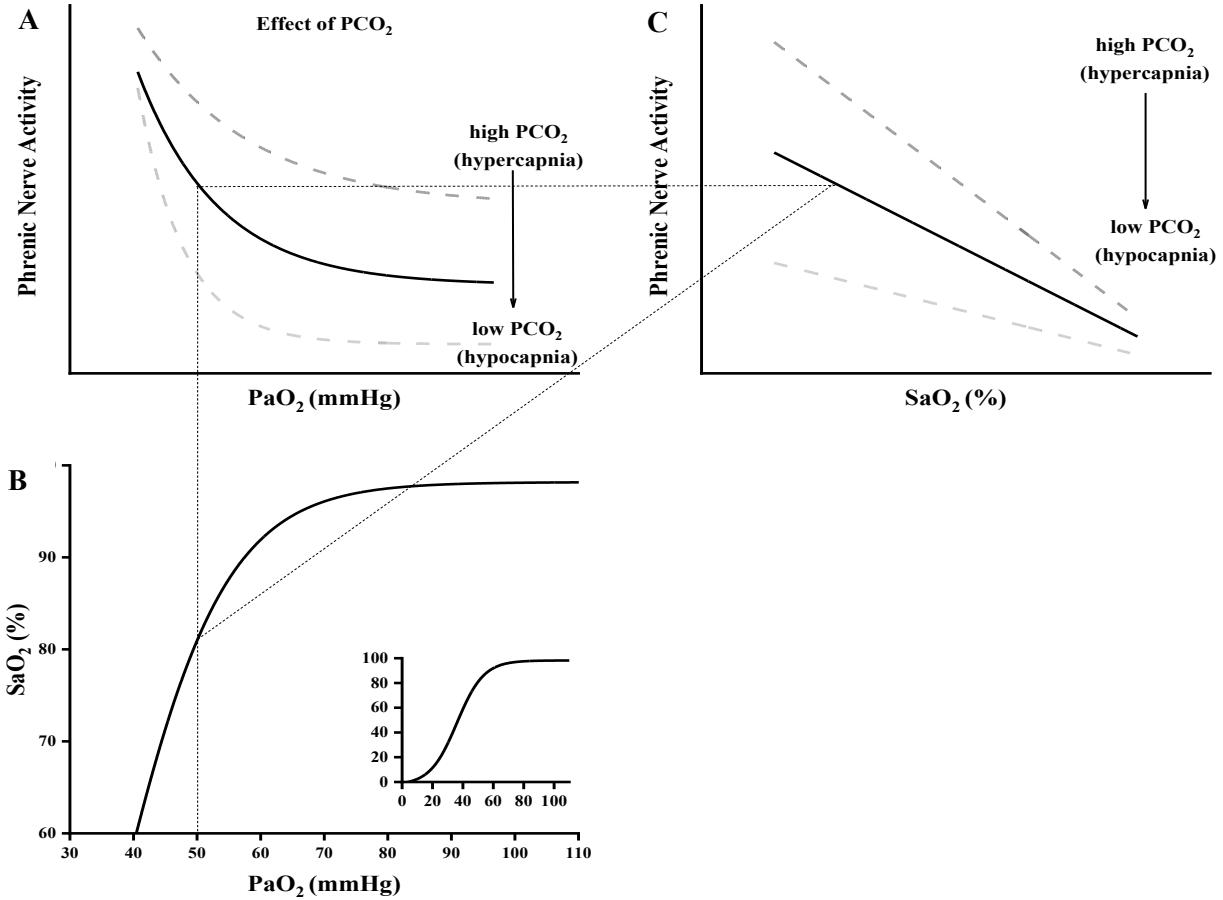


Figure 4: A hypothetical representation of the hyperbolic phrenic nerve activity in relation to partial pressure of oxygen (Panel A) and deriving the relationship against arterial oxyhemoglobin saturation (SaO_2; Panel C) through the oxygen hemoglobin saturation curve (Panel B).

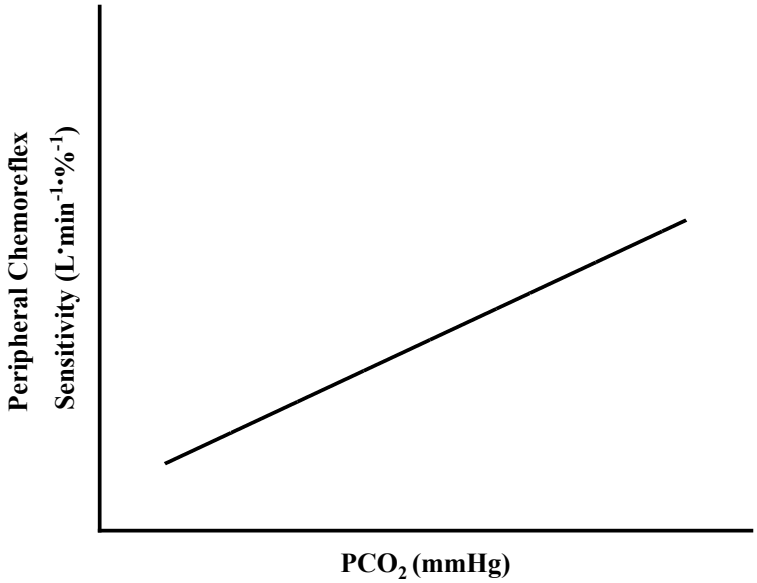


Figure 5: A hypothetical representation of the linear relationship between peripheral chemoreflex sensitivity (PChS) and increasing levels of partial pressure of carbon dioxide (PCO_2).

Respiratory Chemoreflex Interaction

Having previously described how chemoreceptors and the reflexes they initiate behave in isolation, it is important to consider that, in an intact organism, these reflexes operate simultaneously. Numerous animal models and human intervention studies have been conducted to differentiate the chemoreceptors and their subsequent reflexes. Activation or deactivation of the central and peripheral chemoreceptors will increase or decrease ventilation, but how the chemoreceptors interact is controversial with studies in a variety of human and animal experiments concluding three possible interactive effects: hypo-additive (Day & Wilson, 2007, 2009; Wilson & Day, 2013), hyper-additive (Blain et al., 2010; Dahan et al., 2007; Smith et al., 2015; Teppema & Smith, 2013), and additive interaction claims (Clement et al., 1992; Cui et al., 2012; Duffin & Mateika, 2013; St Croix et al., 1996).

Animal Experiments

Anesthetized animal preparations that isolate and control the chemical composition of fluids perfusing the carotid body and medulla suggest that the central and peripheral respiratory chemoreflexes interact hyper-additively in canines (Blain et al., 2010; Smith et al., 2015) and hypo-additively in rats (Day & Wilson, 2007, 2009).

The hyper-additive hypothesis of central-peripheral interaction proposes that there is a synergistic relationship between the central and the peripheral chemoreflex, whereby the stimulation of one chemoreceptor augments the responsiveness of the other to produce a larger ventilatory response than would be anticipated based on their sum (Blain et al., 2010; Smith et al., 2015). This was assessed by Blain et al. (2010) by quantifying the contribution of the peripheral chemoreceptors to the ventilatory response to specific hypercapnic levels at the brain tissue (i.e., central chemoreceptors) in eight unanesthetized and awake dogs. To accomplish this, they used a preparation that provided a separate perfusion of an isolated carotid body. After unilateral carotid body denervation, the contralateral carotid body was completely isolated using an extracorporeal circuit (i.e., out of body) and the peripheral chemoreflex output was abolished. This was confirmed by lack of ventilatory response to systemic injections of sodium cyanide (NaCN), a peripheral chemoreflex stimulant. This set-up allowed the researchers to manipulate the carotid body afferent signals independent of the systemic circulation, and therefore the CNS and central chemoreceptors. Three sets of conditions were setup to assess the ventilatory response to hypercapnia through high CO_2 breathing: 1) carotid bodies were intact with normal endogenous carotid body perfusion (normoxic, normocapnic perfusion); 2) unilateral carotid body denervation with normal carotid body perfusion; 3) during inhibitory (perfused from the extracorporeal circuit with a hyperoxic hypocapnic gas mixture [PO_2 > 500 mmHg and PCO_2 ~20 mmHg]) or stimulatory (perfused from the extracorporeal circuit with a hypoxic normocapnic gas mixture [PO_2 ~40 mmHg]) carotid body perfusion. In each condition, the ventilatory response to $PaCO_2$ was quantified using four progressive levels of systemic arterial hypercapnia induced by increasing inspired CO_2 for seven minutes. Relative to the control condition (normal carotid body conditions), extracorporeal carotid body inhibition by hyperoxic hypocapnia decreased the slope of the ventilatory response to hypercapnia (mediated entirely by central chemoreceptors) by 19% whereas extracorporeal carotid body stimulation by hypoxic normocapnic increased the ventilatory response slope in all dogs by

an average of 223%. Therefore, it was concluded that the central chemoreceptors in dogs were significantly dependent on the peripheral chemoreceptor afferent activity and their sensitivity was augmented by concomitant carotid body activation, i.e., a hyper-additive relationship (Blain et al., 2010).

Postulating that hypoxic versus hypercapnic-stimulation of the carotid body may alter the nature of the peripheral-central chemoreflex interaction, the same group followed up the study of Blain et al. (2010) with an identical preparation (i.e., vascularly isolated carotid body chemoreceptor in awake canines) except the isolated carotid body was perfused with normoxic hypocapnic, normocapnic, or hypercapnic perfusate (Smith et al., 2015). During high F_ICO_2 breathing (sufficient to activate the central chemoreflex), the dogs' carotid body was perfused to match a normal eupneic value, or with hypocapnic (PCO_2 ~20 mmHg) or hypercapnic (PCO_2 ~60-70 mmHg) blood. Despite receiving the same systemic hypercapnic stimulus, the ventilatory response sensitivity to CO_2 ($\Delta\dot{V}_E/\Delta PaCO_2$) of the functionally isolated central chemoreflex was significantly higher when the isolated carotid body was activated by hypercapnia and significantly lower when deactivated by hypocapnia. These findings indicate that concurrent carotid body activation augments central chemoreflex sensitivity and that the peripheral-central chemoreflex interaction is hyper-additive.

Others have observed hypo-additive central-peripheral interaction whereby the stimulation of one chemoreceptor diminishes the responsiveness of the other to produce a smaller ventilatory response than would be anticipated based on their sum (Day & Wilson, 2007, 2009). For example, Day & Wilson (2007) used an *in situ* arterially perfused, vagotomized, decerebrate preparation in which the central and peripheral chemoreceptor compartments were perfused separately. The independent perfusion of central and peripheral compartments was achieved by clamping of the descending aorta (central) and carotid arteries (peripheral), respectively. This permitted the researchers to investigate the two chemoreceptor groups separately by perfusing each through a medium attached to isolated perfusate reservoirs. Phrenic nerve activity was measured by dissecting out the left phrenic nerve with a small piece of diaphragm and it was placed in a plex-glass recording chamber. Two protocols were used to assess the chemoreceptor interaction in 23 preparations. The first protocol consisted of a 5-minute baseline (PO_2 = 100 mmHg), 5-minute specific carotid body hypoxia (PO_2 = 60 mmHg; PCO_2 = 40 mmHg). During the 5-minute baseline

steady-statePCO_2 was held at 25 or 50 mmHg. The magnitude of the change in phrenic nerve activity to a step-change in carotid body hypoxia was greater when brainstem PCO_2 was low (~25 mmHg) compared to high (~50 mmHg). They concluded that the findings were indicative of a negative (hypo-additive) chemoreceptor interaction (Day & Wilson, 2007).

In the follow up study completed by Day and Wilson in 2009, they investigated the phrenic responses to random perturbations of specific carotid body O_2 (isocapnic) and CO_2 (isoxic normoxia) under three different levels of steady-state brainstem PCO_2 (25, 35, and 50 mmHg) and PO_2 (40, 60, 100, 200 and 400 mmHg). 48 preparations were used to quantify the phrenic nerve discharge response to specific carotid body isoxic CO_2 perturbations or isocapnic O_2 perturbations under various levels of steady state brain PCO_2. Following a five-minute baseline period at brainstem PCO_2 of 35 mmHg, the preparations were then exposed to different PCO_2 (25, 35 or 50 mmHg) and then underwent carotid body oxygen perturbations (PO_2 = 40, 60, 100, 200 and 400 mmHg) lasting five minutes each. Overall, they observed that phrenic nerve discharge from peripheral chemoreceptor excitation was greater without concurrent central chemoreceptor stimulation and the stimulation of one chemoreflex attenuated the excitation of the other, resulting in an overall reduction of the chemoreflex response (Day & Wilson, 2009).

Human Experiments

In humans, it is difficult to manipulate arterial or central PCO_2 independently because they are connected via the circulation and there is an approximate 8-10 mmHg difference between these tissue compartments (Eldridge et al., 1987). Experiments are further complicated because medullary PCO_2 depends not only on $PaCO_2$ but is inversely proportional to medullary blood flow which itself is regulated by many factors including $PaCO_2$ and PaO_2 (Ainslie & Duffin, 2009). In humans, any experimental change in $PaCO_2$ and PaO_2 may not translate as a direct proportional change in medullary PCO_2 and PO_2 measurements. However, several experimental designs permit discrimination between the chemoreflexes in humans.

The first is the temporal separation technique (Clement et al., 1992; Cui et al., 2012; St Croix et al., 1996). This method involved the application of "step"- changes in end-tidal PO_2 and PCO_2 by dynamic end-tidal forcing and quantifying the kinetics of the ventilatory response. With this method, changes in $PaCO_2$ are rapid whereas changes in medullary PCO_2 are much slower

(Duffin, 1990) with time constants corresponding to 10 seconds versus 53 seconds, respectively (Eldridge et al., 1987). Therefore, application of step-changes of different duration and quantification of the corresponding ventilatory response provide an estimate of central versus peripheral respiratory chemoreflex responses.

Several studies have applied this technique to assess the central and peripheral respiratory chemoreflexes interaction. Most recently, Cui et al. (2012) measured the ventilatory response to isocapnic hypoxia (i.e., peripheral chemoreflex) at low and high levels of central chemoreceptor excitation. To accomplish this, participants underwent a period of normoxic isocapnic-hypercapnia (partial pressure of end-tidal carbon dioxide [$P_{ET}CO_2$] = 45 mmHg) that was sufficiently long enough to raise central PCO_2 and achieve a steady-state ventilation. After steady-state was achieved, a step-reduction in $P_{ET}O_2$ from normoxia ($P_{ET}O_2$ ~95 mmHg) to hypoxia ($P_{ET}O_2$ =50 mmHg) was performed to derive peripheral chemoreflex response to hypoxia with high central chemoreceptor drive. Next, the experiment was repeated twice except a five-minute period of hyperventilation was performed prior to the initiation of isocapnic-hypercapnia to lower central PCO_2, and thus, central chemoreceptor drive. Following hyperventilation and two minutes of isocapnic-hypercapnia, $P_{ET}O_2$ was permitted to return to normoxic levels (test 1) or was instantaneously reduced to 50 mmHg (test 2). The difference in steady-state ventilation achieved in these experiments provided the peripheral chemoreflex response to hypoxia with low central chemoreceptor drive. The comparison of the hypoxic ventilatory response from normoxia to hypoxia at high and low central PCO_2 were not significantly different, supporting that central and peripheral chemoreflex interact additively. Several other studies that have applied the temporal separation technique in humans report findings in favor of a simple additive response (Clement et al., 1992; Cui et al., 2012; St Croix et al., 1996).

In addition to temporal separation, carotid body removal also permits discrimination between central and peripheral chemoreflexes. With afferent input from the carotid bodies removed, whether and how the central respiratory chemoreflex changes before versus after resection can provide insight into the nature of chemoreceptor interaction. Dahan et al. (2007) assessed respiratory responses to hypoxia and hypercapnia in three patients (two males and one female) who underwent bilateral carotid body resection to treat carotid body tumors. Patients were subjected to hypoxia and hypercapnia before and at several time points over a three-year period.

The authors found that ventilatory responses to CO_2 in hyperoxic conditions (a measure of central chemoreflex) fell after the carotid body was removed indicating that the peripheral chemoreceptors augment the central chemoreflex response, with excitatory effect on the central chemoreflex disappearing after two years (Dahan et al., 2007). This supports the hyper-additive interaction hypothesis (Dahan et al., 2007).

Rebreathing

In 1967, Read established a rebreathing technique to measure the ventilatory response to CO_2 mediated by the central chemoreceptors. The rebreathing technique was introduced to assess the regulation of ventilation by generating a progressively increasing CO_2 stimulus (hypercapnic response). Rebreathing is initiated from a bag containing 43% O_2 and 7% CO_2 (Read & Leigh, 1967). With this set-up, bag, alveolar and arterial PCO_2 rapidly equilibrate and rise in unison at a rate commensurate with whole body CO_2 production. Thus, with this test, $P_{ET}CO_2$ is approximately equal to $PaCO_2$. Although the gap between brain, venous, and arterial PCO_2 is expected to narrow, all are anticipated to rise at a similar rate. This test results in a linear $\dot{V}_E$ vs $P_{ET}CO_2$ response relationship with a slope that characterizes the central chemoreflex sensitivity plus peripheral chemoreflex input if PaO_2 falls below ~85 mmHg. The limitation with this technique is that arterial and central PCO_2 are unlikely to be in equilibrium, peripheral chemoreflex excitation is likely changing, and that rebreathing is initiated at a relatively hypercapnic level (Read & Leigh, 1967).

In 1988, James Duffin introduced a modified version of Read's (1967) rebreathing protocol to measure the ventilatory response to CO_2 mediated by the peripheral chemoreceptors with the use of hypoxia (Duffin & McAvoy, 1988). The modified rebreathing protocol uses a preceding period of hyperventilation (deep breathing) to lower alveolar, arterial, and venous PCO_2 such that upon the initiation of rebreathing, these compartments rapidly equilibrate at a hypocapnic pressure that is equivalent to mixed-venous PCO_2. Thus, the PCO_2 gradient between central and peripheral chemoreceptors is nearly abolished, exposing both to the same 'PCO_2 ramp' stimulus (Duffin & McAvoy, 1988) (see Figure 6). In addition, the prior hyperventilation period ensures that rebreathing is initiated at a relatively hypocapnic PCO_2. Unlike Read's rebreathing, this permits a period of time after the onset of rebreathing where $\dot{V}_E$ remains stable (termed $\dot{V}_E$ baseline, $\dot{V}_E$BSL). As PCO_2 rises, it eventually reaches a pressure that is sufficient to activate the chemoreflex. This "PCO_2 threshold" (referred to as ventilatory recruitment threshold, VRT) signifies the PCO_2 at

which the central and peripheral respiratory chemoreflexes begin to raise ventilation linearly. Thus, the steady-state $\dot{V}_E$ achieved during $\dot{V}_E$BSL is attributed to non-chemoreflex drives to breathe and provides an estimation of basal "wakefulness" drive. The slope of the rise in $\dot{V}_E$ vs $P_{ET}CO_2$ beyond the VRT is referred to as the chemoreflex ventilatory sensitivity ($\dot{V}_E$S). These parameters are displayed in Figure 6.

Assuming that central chemoreceptors are not O_2 sensitive (Lloyd & Cunningham, 1963; Mohan & Duffin, 1997) and that the peripheral respiratory chemoreflex is largely attenuated by hyperoxia (Lloyd & Cunningham, 1963; Mohan & Duffin, 1997), Duffin also recommended that rebreathing is performed with O_2 clamped (i.e., "isoxic") at hyperoxic ($P_{ET}O_2 \geq 150$ mmHg) and hypoxic ($P_{ET}O_2 \leq 70$ mmHg) PO_2 values. Therefore, the ventilatory response to isoxic-hyperoxia characterizes the central respiratory chemoreflex and the isoxic-hypoxia provides the sum of central plus peripheral chemoreflexes such that the difference between tests, approximates peripheral chemoreflex sensitivity (Clement et al., 1992; St Croix et al., 1996). In response to simultaneous stimulation of both chemoreceptor groups by isoxic-hypoxic rebreathing, the net ventilatory response (reflecting contributions from both reflex arcs) is consistently linear (Duffin et al., 2000; Keir et al., 2019; Preston et al., 2009). Such linearity is incompatible with a hypo- or hyper-additive central-peripheral chemoreflex response (Duffin & Mateika, 2013). However, this only provides indirect evidence supporting an additive response.

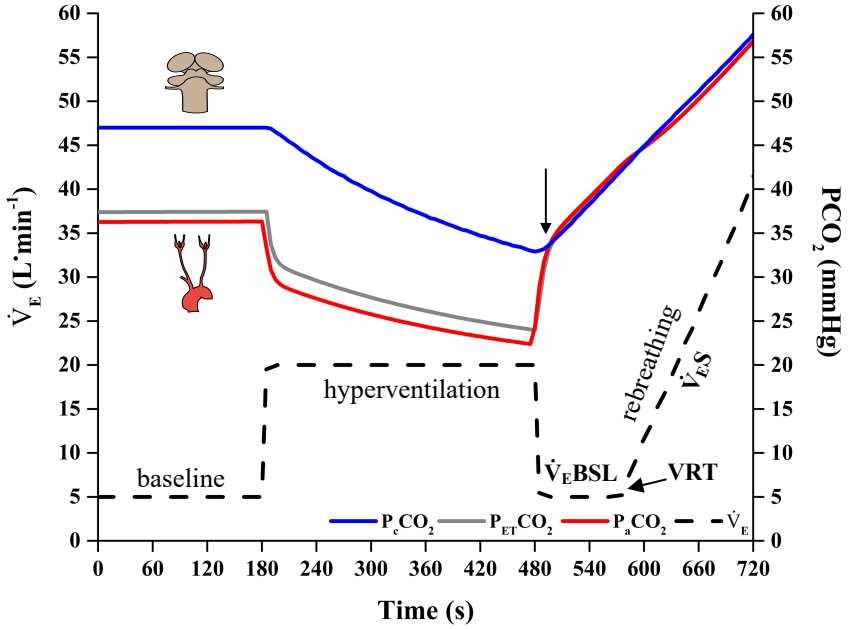


Figure 6: A simulated schematic of Duffin's modified rebreathing protocol. The protocol consists of a 3-minute baseline, 5-minute hyperventilation period where rebreathing is initiated at the end of the 5 minutes. The minute ventilation ($\dot{V}_E$; L·min^{-1}; dashed black), partial pressure of central (P_CCO_2; blue), end-tidal ($P_{ET}CO_2$; grey), and arterial carbon dioxide ($PaCO_2$; mmHg; red), and minute ventilation is displayed on the left and right y-axes respectively plotted against time (seconds). $\dot{V}_E$BSL, the wakefulness drive to breathe at the beginning of rebreathing and prior to reaching the ventilatory recruitment threshold (VRT). $\dot{V}_E$S, the chemoreflex ventilatory sensitivity after the VRT.

The ventilatory parameters determined (wakefulness drive to breathe, $\dot{V}_E$BSL; VRT; ventilatory sensitivity $\dot{V}_E$S) from modelling the $\dot{V}_E$ vs $P_{ET}CO_2$ response to modified rebreathing provide a quantification of the characteristics of the central and peripheral chemoreflexes. Compared to an isoxic hyperoxic condition, the ventilatory sensitivity to modified rebreathing becomes steeper with increasing severity of isoxic hypoxia (Mohan & Duffin, 1997) (similar to Figure 3A). This highlights the modulatory effect of PCO_2 on the peripheral chemoreflex sensitivity to O_2 (similar to what is depicted in Figure 3A, except with $\dot{V}_E$ as the dependent variable and with simultaneous central chemoreflex activation).

These findings highlight two important properties of the peripheral respiratory chemoreflex. First, relative to hyperoxia (i.e., central chemoreflex only) at any fixed PCO_2, a reduction in PO_2 will be associated with a $\Delta\dot{V}_E$ (i.e., the hypoxic ventilatory response, HVR) that is determined by the sensitivity of the carotid body and the prevailing PO_2. The lower the PO_2, the greater the $\Delta\dot{V}_E$. This indicated a dose-response relationship of peripheral chemoreflex response to low O_2. When $\Delta\dot{V}_E$ (HVR) of multiple degrees of hypoxia varying in severity are plotted relative to arterial oxyhemoglobin saturation (SaO_2) a linear relationship was observed (Keir et al., 2020; Rebuck & Campbell, 1974); rather than the hyperbolic relationship exhibited relative to PO_2 (similar to Figure 4C except with $\dot{V}_E$ as the dependent variable). The slope gives a measure of the peripheral chemoreflex sensitivity to low O_2 (PChS in $L{\cdot}min^{-1}{\cdot}\%^{-1}$). Second, as isocapnic PCO_2 is fixed at higher pressures, the $\Delta\dot{V}_E$ (HVR) response to a standardized fall in PO_2 rises. These findings indicates that higher isocapnic PCO_2 sensitizes the PChS (Keir et al., 2020; Rebuck & Woodley, 1975). For this reason, the PChS slope is steeper at a higher compared to lower isocapnic PCO_2. In the absence of central chemoreflex input, the linearized expression of PChS (i.e., Δ ventilation [$\dot{V}_E$] versus Δ arterial oxyhemoglobin saturation [SaO_{2sat}] slope) rises linearly with $PaCO_2$ (Irsigler et al., 1980; Riedstra, 1963; Teppema et al., 2010). Whether this relationship remains linear with different levels of central chemoreceptor excitation is unknown. We reasoned that modified rebreathing could be used to explore this relationship for the first time in humans.

Purpose and hypothesis

The objective of this study was to assess the impact of central chemoreflex activation on peripheral chemoreflex sensitivity to low O_2 in humans. This study quantified respiratory peripheral chemoreflex sensitivity to low O_2 at progressively higher $PaCO_2$ and central PCO_2. It was hypothesized that the respiratory peripheral chemoreflex sensitivity (PChS), as measured by the slope of the ventilatory response to arterial oxyhemoglobin desaturation (O_{2sat}) (or the PChS), will increase linearly with $PaCO_2$ and will be unaffected by heightened central PCO_2 (i.e., an "additive" response). Alternatively, if the PChS increases or decreases curvilinearly with increasing $PaCO_2$ it will indicate that the central chemoreflexes interact hyper- or hypo-additively, respectively (Figure 7).

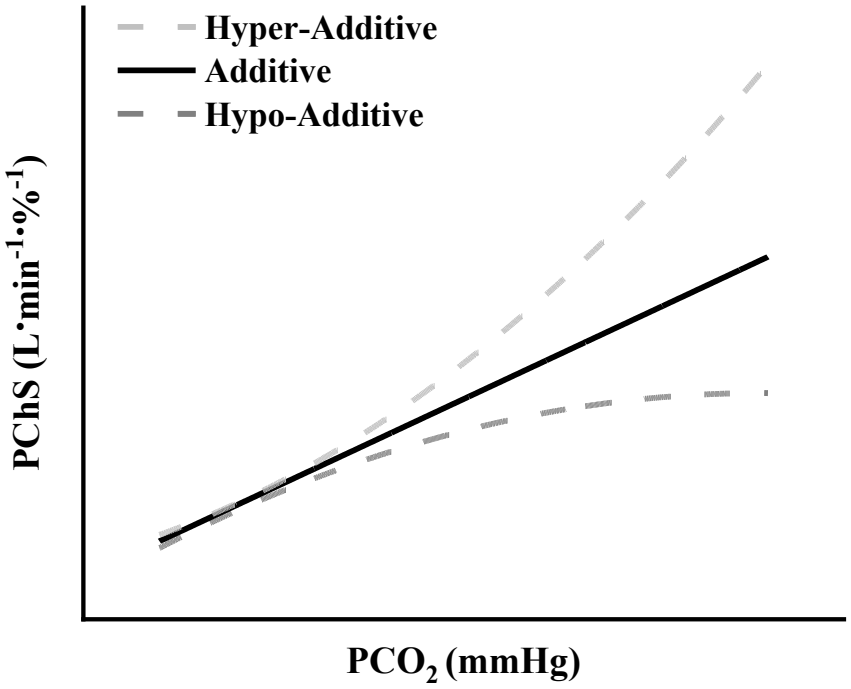


Figure 7: A hypothetical diagram displaying relationship between peripheral chemoreflex sensitivity to hypoxia (PChS) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$). The null hypothesis (black line) and alternate hypotheses (hyper- and hypo- additive; dashed light and dark grey line, respectively) are depicted.

Chapter II: A test of the interaction between central and peripheral respiratory chemoreflexes in humans

Introduction

Breathing fluctuates rapidly depending on our activity, behaviour, and emotional state, yet the partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) and pH of our tissues remain stable. This control is primarily governed by rapidly responding chemoreceptors located within the carotid bodies (peripheral) and brainstem (central) (Cunningham et al., 1986). Peripheral chemoreceptors sense increases in arterial hydrogen ion concentration ($[H^+]$) with a sensitivity that depends on the partial pressure of arterial oxygen (PaO_2) whereas central chemoreceptors sense local changes in tissue PCO_2 (Duffin, 1990). When excited, both central and peripheral chemoreceptors reflexively activate breathing in ways that counteract rapid rises in local PCO_2 or falls in PaO_2 (Guyenet et al., 2012). Independent characterization of these central and peripheral respiratory chemoreflexes is therefore valuable for understanding control of breathing. Often, central, and peripheral chemoreflexes are engaged simultaneously but how or whether they interact with each other in the control of breathing in humans remains contentious (Guyenet, 2014).

The exact nature of chemoreflex interactions is controversial, with studies in a variety of experiments claiming additive (Duffin & Mateika, 2013), hyper-additive (i.e., positive interaction) (Teppema & Smith, 2013), or hypo-additive (i.e., negative interaction) (Wilson & Day, 2013) effects on the control of breathing. Isolation experiments performed in non-human species, support the views that central and peripheral chemoreflexes interact hyper-additively (Blain et al., 2010; Smith et al., 2015) and hypo-additively (Day & Wilson, 2007, 2009). In humans, studies support both additive and hyper-additive central-peripheral interactions, but the balance of findings is equivocal. Common to most studies in humans is the application of rapid changes in end-tidal PO_2 and/or PCO_2 by dynamic end-tidal forcing and quantification of the fast (peripheral) and slow (central) components of the ventilatory response kinetics. With this method, changes in $PaCO_2$ are rapid whereas changes in medullary PCO_2 are much slower. Estimations of central versus peripheral contributions to ventilation by this technique have consistently supported an additive response (Clement et al., 1992; Cui et al., 2012; St Croix et al., 1996). However, when similar experiments were performed in three patients before and after bilateral carotid body removal,

ventilatory responses to CO_2 in hyperoxic conditions (a measure of central chemoreflex) fell immediately after resection, indicating the loss of a tonic hyper-additive effect of peripheral chemoreceptors on the central chemoreflex response. Notably, the excitatory effect of the carotid body on the central chemoreflex disappeared overtime (Dahan et al., 2007).

In addition to the temporal differences between arterial and central compartments, a major limitation of dynamic end-tidal forcing is the assumption that the magnitude of a rapid change in arterial PCO_2 (inferred from end-tidal measures) is equivalent to that of central PCO_2 and fixed under different experimental conditions (e.g., hypoxia versus hyperoxia) and interventions (e.g., before versus after carotid body removal). In humans, it is difficult to manipulate arterial or central PCO_2 independently because they are connected via the circulation and the approximate 8-10 mmHg difference between these tissue compartments is not fixed especially during rapid changes to respired gas tensions. Although central PCO_2 depends on $PaCO_2$ it may, or may not (Cleary et al., 2020), be inversely proportional to medullary blood flow which itself is regulated by many factors including $PaCO_2$ and PaO_2 (Ainslie & Duffin, 2009; Carr et al., 2021).

To overcome such challenges, Duffin introduced a modified rebreathing protocol that uses a preceding period of deep breathing to lower PCO_2 at the central and peripheral chemoreceptors and rapidly equilibrate these compartments with those of the rebreathing bag and lungs at the onset of rebreathing. Thereafter, it is assumed that the arterial-central PCO_2 gradient is largely reduced and both central and peripheral chemoreceptors are exposed to approximately the same PCO_2 'ramp' stimulus, both increasing at a rate commensurate with metabolic CO_2 production (Duffin et al., 2000; Read & Leigh, 1967). Assuming that central chemoreceptors are not O_2 sensitive (Duffin, 2007) and that the peripheral respiratory chemoreflex is largely attenuated by hyperoxia (Lloyd & Cunningham, 1963; Mohan & Duffin, 1997), rebreathing is performed twice with O_2 clamped (i.e., "isoxic") at hyperoxic ($P_{ET}O_2 \geq 150$ mmHg) and hypoxic ($P_{ET}O_2 \leq 70$ mmHg) PO_2. Therefore, the ventilatory response to isoxic-hyperoxia characterizes the central respiratory chemoreflex and the isoxic-hypoxia provides the combination of central and peripheral chemoreflexes (Mohan & Duffin, 1997). In response to simultaneous stimulation by isoxic-hypoxic modified rebreathing, the net ventilatory response (reflecting contributions from both reflex arcs) is consistently linear (Duffin et al., 2000; Keir et al., 2019; Lahiri & Delaney, 1975a; Preston et al., 2009). Such linearity is incompatible with a hypo- or hyper-additive central-

peripheral chemoreflex response (Duffin & Mateika, 2013; Keir et al., 2020). However, this observation provides only indirect evidence supporting an additive interaction between central and peripheral drives to breathing.

Compared to an isoxic hyperoxic condition, isoxic hypoxic experiments using modified rebreathing have shown that the ventilatory response slope becomes steeper with increasing severity of isoxic PO_2 (Mohan & Duffin, 1997). These finding highlights two important properties of the peripheral respiratory chemoreflex. First, at any fixed PCO_2, $\Delta\dot{V}_E$ (i.e., the hypoxic ventilatory response, HVR) is determined by the prevailing PO_2. The lower the PO_2, the greater the $\Delta\dot{V}_E$. This indicates a dose-response relationship of peripheral chemoreflex response to low O_2. When $\Delta\dot{V}_E$ (HVR) of multiple degrees of hypoxia varying in severity are plotted relative to calculated oxyhemoglobin saturation (S_CO_2), a linear relationship is observed (Keir et al., 2020; Rebuck & Campbell, 1974); rather than the hyperbolic relationship exhibited relative to PO_2. The slope gives a measure of the peripheral chemoreflex sensitivity to low O_2 (PChS). Second, as isocapnic PCO_2 is fixed at higher pressures, the $\Delta\dot{V}_E$ (HVR) response to a standardized fall in PO_2 rises. This finding indicates that higher isocapnic PCO_2 sensitizes the PChS (Keir et al., 2020; Rebuck & Woodley, 1975). For this reason, the PChS slope is steeper at a higher compared to lower isocapnic PCO_2. In the absence of central chemoreflex input, the linearized expression of PChS (i.e., Δ ventilation [$\dot{V}_E$] versus Δ arterial O_2 saturation [SaO_{2sat}] slope) rises linearly with $PaCO_2$ (Irsigler et al., 1980; Riedstra, 1963; Teppema et al., 2010). Whether this relationship remains linear with different levels of central chemoreceptor excitation is unknown.

To assess the impact of central chemoreflex activation on peripheral chemoreflex sensitivity to hypoxia in humans, we applied Duffin's modified rebreathing technique in isoxic hyperoxic conditions (central only) and three isoxic hypoxic conditions (O_{2sat} ~ 80%, 90% and 94%; combined central and peripheral). By subtracting the $\dot{V}_E$ response to PCO_2 of the hyperoxic test from each of the hypoxic tests, three $\Delta\dot{V}_E$ (i.e., HVR) corresponding to three levels of arterial oxyhemoglobin saturation are obtained, and PChS can be determined by linear regression over a range of isocapnic PCO_2. We reasoned that the resultant PChS versus PCO_2 relationship would therefore quantify the respiratory peripheral chemoreflex sensitivity to hypoxia at progressively higher $PaCO_2$ *and* central PCO_2. We hypothesized that PChS would increase linearly with PCO_2, unaffected by the increased central PCO_2 (i.e., an "additive" response). Alternatively, a curvilinear

relationship of PChS with increasing PCO_2 would be interpreted as the hyper- or hypo-additive effect of central chemoreflex activation, respectively (Figure 7).

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) and conformed to the standards set by the *Declaration of Helsinki*, except for registration in database.

Participants

Twenty healthy young participants (ten females; age, 24 ± 4 years; body mass, 65.0 ± 14.6 kg; height, 169.8 ± 12.7 cm) with no history of cardiorespiratory and vascular disease completed the study. All participants were screened to ensure that they were non-smokers and were not taking medication that has known respiratory or cardiovascular actions. All participants provided written informed consent prior to their first visit. Participant characteristics are listed in Table 1.

Study Design

To establish and corroborate the PChS vs PCO_2 relationship within each individual, the research protocol involved 5 laboratory visits: one "familiarization session" with baseline measurements and four "rebreathing sessions" with 3 modified rebreathing tests in fixed isoxic-hypoxic and hyperoxic conditions. To minimize within- and between-day data fluctuations, volunteers abstained from strenuous exercise, recreational drug use, alcohol, and caffeine for $\geq$ 12 hours before each visit. The visits were completed within 10 days for males to minimize any time-related changes in participant's physiology. In females, ventilatory responsiveness to hypoxia and hypercapnia are affected by alterations in sex hormones that occur throughout the menstrual cycle (Assadpour et al., 2020; Usselman et al., 2013). To minimize the effect of fluctuating sex hormones on respiratory variables, female participants were studied during their low hormone phase (i.e., during their menses) or during the placebo phase, if taking oral contraception.

Experimental Protocol

Each of the five laboratory visits were separated by a minimum of 24 hours. The first visit (Day 0) familiarized participants with the modified rebreathing procedure and characterized participants' anthropometrics and cardio-respiratory fitness and function. The Day 0 visit consisted of three parts: 1) resting metabolic and cardiovascular measurements; 2) pulmonary function tests (spirometry); and 3) two, normoxic (familiarization; $P_{ET}O_2$ = 90 mmHg) rebreathing tests. Blood pressure, oxyhemoglobin saturation, and heart rate were measured during a period of seated rest. During this time resting rates of oxygen uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were measured by a metabolic cart (Quark, CPET, COSMED, Rome, Italy). Next, the spirometry test feature of the metabolic cart was used to assess lung function. Participants completed three trials of forced vital capacity (FVC), slow vital capacity (SVC) and maximal voluntary ventilation (MVV) tests (Koegelenberg et al., 2013). The trial with the highest percent-predicted value was used to assess pulmonary function. Thereafter, two rebreathing tests were performed with $P_{ET}O_2$ held normoxic at 90 mmHg during the rebreathing phase (see "Modified Rebreathing Procedure" section below for details) to familiarize participants with the protocol.

After Day 0, participants returned to the laboratory for four more visits (Days 1-4). We recently demonstrated that signal averaging of multiple modified rebreathing trials increases the signal-to-noise ratio of ventilatory responses to PCO_2 (Guluzade et al., 2022). On each of these days, participants completed three repetitions of modified rebreathing separated by 20 minutes, in either hyperoxic ($P_{ET}O_2$ = 150 mmHg) or hypoxic ($P_{ET}O_2$ = 45, 60, and 70 mmHg) conditions. Days 1, 2 and 3 were assigned three consecutive hypoxic tests at $P_{ET}O_2$ = 45, 60, and 70 mmHg, respectively and day 4 was assigned three consecutive hyperoxic tests at $P_{ET}O_2$ = 150 mmHg. Days 1-4 were not completed sequentially. Rather, they were completed in a randomized order such that some participants completed Day 4 on their first visit to the lab after familiarization on Day 0.

Modified Rebreathing Procedure

While seated in a semi-recumbent position on a padded, reclining chair, participants breathed through a facemask (V2 Mask, Hans Rudolph Inc, Kansas, USA) connected in series to a pneumotach (3810 Series, Hans Rudolph Inc) 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 the

third port was connected to a 6-L rebreathing bag (filled with 3-5 L of premixed gas). This arrangement permits 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 sampled continuously at the mouth 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) and inspired and expired volumes for each breath were derived from integration of the flow signal. Respiratory flows and fractional gas concentration data were collected at 50 Hz via a 16-bit analog-to-digital converter (National Instruments, Austin, USA). Pulse oxyhemoglobin saturation (SpO_2) was continuously measured with a pulse oximeter attached to the participant's earlobe (Nonin 7500, Plymouth, USA). Due to potential inaccuracies associated with temporal delays and rolling averaging associated with SpO_2 measures (Carter et al., 1998; Pfoh et al., 2016; Severinghaus et al., 1989; Trivedi et al., 1998), arterial oxyhemoglobin saturation was calculated (S_CO_2) using the equation described by Severinghaus (Severinghaus, 1979). This value was used in the determination of PChS (see *Data Analysis*).

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 $P_{ET}O_2$, V_T, fB, and $\dot{V}_E$.

With the three-way valve open to room air, each rebreathing test began with three minutes of quiet spontaneous breathing. Following this initial baseline period, participants were instructed to breathe deeply for five minutes by raising their tidal volume such that they rapidly achieved and maintained a $P_{ET}CO_2$ of ~20-25 mmHg. Following the five-minute deep breathing period, the three-way valve was quickly switched from room air to the rebreathing bag at the end of a full expiration. Participants were then instructed to take three 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 2.5% O_2, 6% CO_2, balance nitrogen, 4% O_2, 6% CO_2, balance nitrogen, 6% O_2, 6% CO_2, balance nitrogen, and 28% O_2, 6% CO_2, balance nitrogen for the hypoxic ($P_{ET}O_2$ = 45, 60, and 70 mmHg) and hyperoxic ($P_{ET}O_2$ = 150 mmHg) tests, respectively. To maintain $P_{ET}O_2$ at the target pressure throughout rebreathing, 100% O_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 compared 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 sufficient data had been acquired to characterize the respiratory chemoreflex response, when $P_{ET}CO_2$ reached 60 mmHg, or when $\dot{V}_E$ exceeded 100 $L \cdot min^{-1}$. Following rebreathing, participants began their recovery phase of breathing on the apparatus for 3 minutes. Each test lasted ~15-20 minutes.

Data Processing

For each participant, breath-by-breath $\dot{V}_E$ from the rebreathing period were edited by removing aberrant data caused by coughs, sighs, or swallows. After excluding the first 3-4 breaths used for equilibration, repeat-trials were linearly interpolated at 0.1 mmHg intervals, then averaged into 1 mmHg bins of $P_{ET}CO_2$ intervals (Guluzade et al., 2022) such that a single ventilatory response profile was established in each of the four conditions.

Data Analysis

The $P_{ET}CO_2$ vs time relationship was fitted with a linear regression line. The equation of the line was used to predict the $P_{ET}CO_2$ versus time relationship during rebreathing. Thereafter, ensemble-averaged $\dot{V}_E$ was plotted relative to predicted $P_{ET}CO_2$ and fitted with either a double-linear (f(x)) or exponential-decay linear (g(x)), model. The double linear (f(x)) model had the form:

$$f(x) = \begin{cases} i_1 + S_1 x, & x < VRT \\ i_1 + (S_1 \cdot VRT) + S_2 \cdot (x - VRT), & x \geq VRT \end{cases}$$

Where: f is $\dot{V}_E$, x is $P_{ET}CO_2$, VRT is the $P_{ET}CO_2$ corresponding to the interception of the two regression lines, i_1 is the intercept of the first linear function, and S_1 and S_2 are the slopes. The

S_1 parameter is fixed at "zero" and therefore i1 gives basal $\dot{V}_E$. S_2 determines the chemoreflex sensitivity to hypercapnia ($L \cdot min^{-1} \cdot mmHg^{-1}$).

The exponential-decay linear (g(x)), model had the form:

$$g(x) = \begin{cases} y_0 + A^{(-\frac{(x-x_0)}{\tau})}, & x < VRT \\ y_0 + A^{(-\frac{(VRT-x_0)}{\tau})} + m \cdot (x - VRT), & x \geq VRT \end{cases}$$

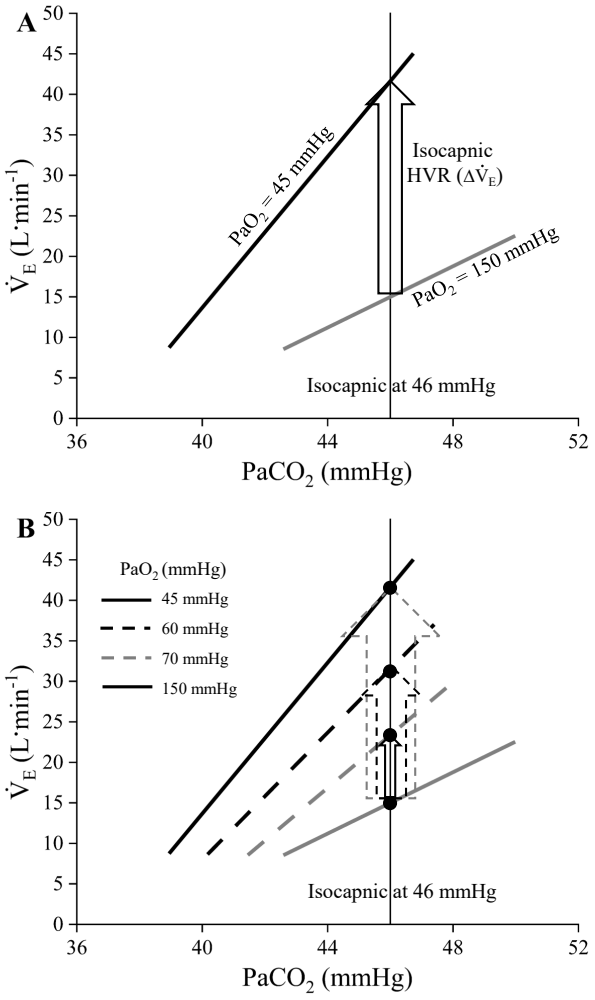
Where: g is $\dot{V}_E$, x is $P_{ET}CO_2$, VRT is the $P_{ET}CO_2$ corresponding to the interception of the exponential decay and the regression line, y_0 is the exponential plateau, A is the amplitude between the first data point y_0, x_0 parameter is the initial $P_{ET}CO_2$, and τ is the time constant. The $\dot{V}_ES$ is determined by m ($L \cdot min^{-1} \cdot mmHg^{-1}$).

Model parameter estimates for each participant were calculated by linear least-square regression analysis that found the minimal sum of squared residuals between the double-linear model (or exponential-decay linear model) and the experimental data (Origin 2022, Northampton, USA). The slope of the line from the hyperoxic rebreathing test (i.e., $\dot{V}_ES$) provided an estimate of central respiratory chemoreflex sensitivity (in $L \cdot min^{-1} \cdot mmHg^{-1}$) and the hypoxic test provided the combined central and peripheral chemoreflex responsiveness. For illustrative and comparative purposes, each individual's ensemble-averaged trial was partitioned into octiles.

The PChS was determined as follows (see Figure 8):

1. HVR represented the difference in $\dot{V}_E$ between an isoxic hypoxic test and the isoxic hyperoxic test ($\Delta\dot{V}_E$) at a single PCO_2 (Figure 8A). Thus, HVR was mediated by the peripheral chemoreflex plus any interaction effect with the central chemoreflex.
2. To determine the PChS at a single isocapnic PCO_2, the $\dot{V}_E$ vs. $P_{ET}CO_2$ relationship for each of the three hypoxic trials and the hyperoxic trial were plotted together and the $\Delta\dot{V}_E$ (HVR) was computed between the isoxic hyperoxic trial and each of the isoxic hypoxic trials yielding three HVR at that $P_{ET}CO_2$ (Figure 8B).

3. When plotted against PO_2 the relationship between the three HVR at a common PCO_2 was hyperbolic (Figure 8C). However, when plotted against SaO_2, the relationship became linear with a slope measuring the peripheral chemoreflex sensitivity to hypoxia (i.e., PChS) at an isocapnic PCO_2 (Figure 8C).
4. Steps 1-3 were repeated at 1-mmHg intervals of isocapnia to generate PChS values over a range of isocapnic PCO_2 (Figure 8D). This relationship was fitted with a linear and a polynomial function which allowed us to reject or fail to reject the hypothesis as depicted in Figure 8.


A
B
$\dot{V}_E$ (L·min^{-1})
PaCO$_2$ (mmHg)
PaO$_2$ = 45 mmHg
PaO$_2$ = 150 mmHg
Isocapnic HVR ($\Delta\dot{V}_E$)
Isocapnic at 46 mmHg
PaO$_2$ (mmHg)
45 mmHg
60 mmHg
70 mmHg
150 mmHg

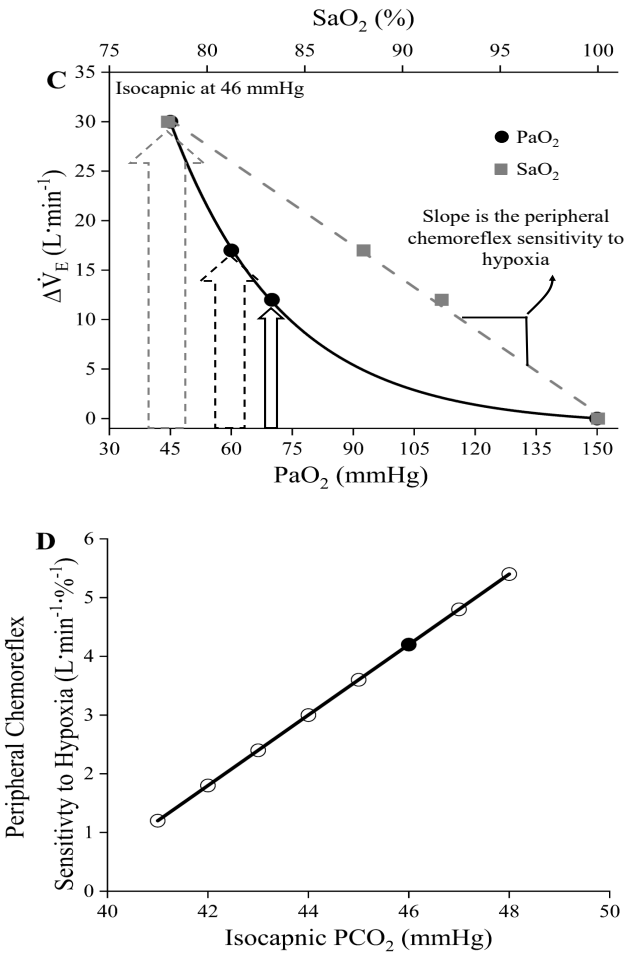


Figure 8: A) A hypothetical ventilatory rebreathing response at two isoxic partial pressure of oxygen (PO_2) (45 mmHg and 150 mmHg) is used to calculate a single hypoxic ventilatory response (HVR) at an isocapnic partial pressure of carbon dioxide (PCO_2); where HVR is the change in minute ventilation ($\Delta\dot{V}_E$). **B)** Three HVRs are calculated using ventilatory rebreathing responses at four isoxic PO_2 at an isocapnic PCO_2. **C)** The HVR vs PO_2 relationship is plotted to demonstrate the peripheral chemoreflex ventilatory response to PCO_2 at an isocapnic PCO_2 with the rectangular hyperbolic relationship being the PO_2 and the linear relationship is vs. oxyhemoglobin saturation (SaO_2). The slope of the linear relationship yields the peripheral chemoreflex sensitivity to CO_2 at an isocapnic PCO_2. **D)** The peripheral chemoreflex sensitivity to hypoxia is plotted against CO_2 at increasing PCO_2 which describes the variation with central chemoreflex PCO_2 and fitted with a linear function supporting the null hypothesis.

Statistical Analysis

Data are presented as means ± SD. A Student t-test was used to determine sex differences in physical characteristics and baseline measures. A two-way (sex x $P_{ET}O_2$ condition) repeated measures analysis of variance (ANOVA) with Tukey's HSD post hoc test consisting of two groups (males and females) and four isoxic conditions ($P_{ET}O_2$ of 45, 60, 70 and 150 mmHg) was used to examine between-condition and between-group differences in rebreathing responses. Where no significant main effects or interactions with sex were found, data was collapsed across sexes and a one-way (condition) repeated measures ANOVA was used to evaluate between- condition differences. A two-way ($P_{ET}O_2$ condition x $P_{ET}CO_2$ condition) repeated measures ANOVA of four conditions and select respiratory variables averaged into octiles of PCO_2 was used to examine the effect of condition and PCO_2. Statistical significance was determined at a p-value of 0.05.

"Linear" (i.e., additive interaction) versus "polynomial" (i.e., interaction) model fits of each participant's PChS vs $P_{ET}CO_2$ relationship were compared by computing the change in corrected Akaike Information Criterion (ΔAICc; where "c" indicates that the AIC reported is a corrected score for small sample size, i.e., <40 data points). The ΔAICc score is a method of comparing regression models that vary in the number of parameters (Cavanaugh & Neath, 2019). The ΔAICc was computed by subtracting the AICc of the "polynomial" model from the ΔAICc of the "linear" model. Since the model with the lower AICc is more likely to characterize the data, a negative ΔAICc value favours the linear model whereas a positive value favours the polynomial model. The level of evidence in support of one model versus the others was considered as "weak" for $|\Delta AIC| < 2$, "moderate" for $|\Delta AIC|$ between 2 and 7, "strong" for $|\Delta AIC| > 7$ (Burnham & Anderson, 2004). In addition, the Akaike weight ratio was computed to determine the relative likelihood that the favoured model best characterized the data. All statistical analyses were performed using Rstudio Version 1.4.1717; with AICc analysis completed using the "AICcmodavg" package.

Results

Participant physical characteristics, spirometry, and resting respiratory measurements are displayed in Table 1. There were no sex differences evident between iso-oxic conditions for the ventilatory responses to rebreathing. A representative figure showing the determination of the sensitivity of the peripheral chemoreflex response to hypoxia is displayed in Figure 9.

Table 1: Participant characteristics.

	All	Males	Females
Physical Characteristics			
n	20	10	10
Age (years)	24 ± 4	25 ± 4	23 ± 3
Mass (kg)	65.0 ± 14.6	74.1 ± 12.7	55.8 ± 10.1*
Height (cm)	169.8 ± 12.7	180.3 ± 7.1	159.4 ± 6.9*
BMI ($kg \cdot m^2$)	22.2 ± 2.6	22.7 ± 2.4	21.9 ± 2.8
Cardiovascular Characteristics			
Systolic Blood Pressure (mmHg)	121 ± 6	124 ± 5	118 ± 5*
Diastolic Blood Pressure (mmHg)	79 ± 6	80 ± 8	77 ± 4
Heart Rate (bpm)	70 ± 13	67 ± 14	73 ± 10
Oxyhemoglobin Saturation (%)	100 ± 0	100 ± 0	100 ± 0
Pulmonary Function			
FVC (L) (% predicted)	4.5 ± 1.4 (98.3 ± 12.0)	5.7 ± 0.8 (102.1 ± 8.3)	3.3 ± 0.5* (94.4 ± 14.3)
FEV_1 (L) (% predicted)	3.5 ± 1.1 (89.5 ± 13.7)	4.5 ± 0.6 (95.3 ± 8.5)	2.5 ± 0.4* (83.7 ± 15.8)
FEV_1/FVC (%)	78.6 ± 8.2	78.7 ± 7.6	78.6 ± 9.1
IC (L)	2.9 ± 0.9	3.6 ± 0.7	2.1 ± 0.4*
PEF ($L \cdot s^{-1}$)	6.3 ± 2.5	8.3 ± 1.8	4.3 ± 1.3*
MVV ($L \cdot min^{-1}$)	145.8 ± 44.2	182.6 ± 21.8	108.9 ± 25.2*
Resting Respiratory Parameters			
$\dot{V}_E$ ($L \cdot min^{-1}$)	7.9 ± 2.3	8.4 ± 2.6	7.4 ± 1.9
fB ($breath \cdot min^{-1}$)	14 ± 5	12 ± 3	16 ± 6
V_T (L)	0.62 ± 0.20	0.74 ± 0.2	0.50 ± 0.1*
$\dot{V}O_2$ ($L \cdot min^{-1}$)	0.25 ± 0.05	0.28 ± 0.05	0.22 ± 0.04*
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	0.21 ± 0.06	0.25 ± 0.04	0.18 ± 0.05*
$P_{ET}O_2$ (mmHg)	99.2 ± 4.8	98.9 ± 3.9	99.6 ± 5.7
$P_{ET}CO_2$ (mmHg)	34.9 ± 3.3	36.4 ± 2.7	33.4 ± 3.3

Values are mean ± SD. BMI, body mass index; FVC, force vital capacity; FEV_1, forced expiratory volume in 1 s; IC, inspiratory capacity; PEF, peak expiratory flow rate; MVV, maximum voluntary ventilation; $\dot{V}_E$, minute ventilation; fB, breathing frequency; V_T, tidal volume; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide output; $P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide. *Significantly different from their group counterpart ($p < 0.05$).

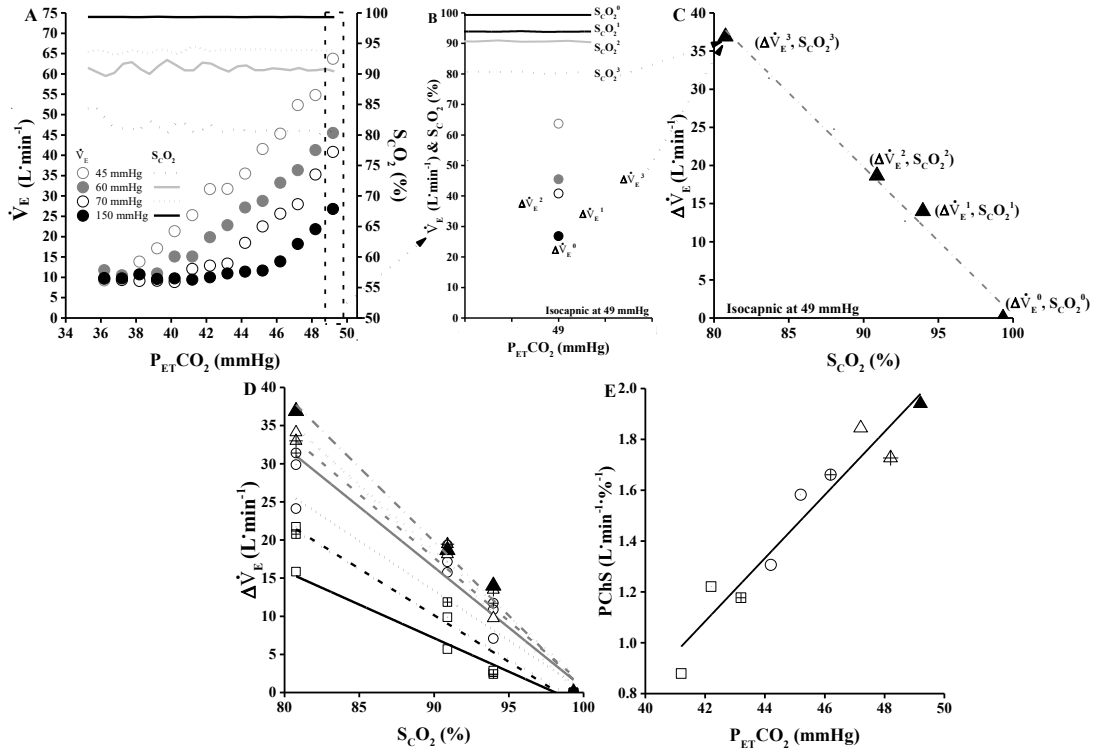


Figure 9: A) A representative figure demonstrating the minute ventilation ($\dot{V}_E$) and calculated oxyhemoglobin saturation (S_CO_2) to increasing end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) and isoxic end-tidal partial pressure of oxygen ($P_{ET}O_2$) levels. **B)** The difference in ventilation ($\Delta\dot{V}_E$) quantified from the subtraction of the $\dot{V}_E$ from the $\dot{V}_E$ vs. $P_{ET}CO_2$ relationship of the hyperoxic trial from three hypoxic trials. **C)** $\Delta\dot{V}_E$ is plotted versus calculated oxyhemoglobin saturation (S_CO_2) at four saturation levels corresponding to the four isoxic conditions, where the slope of the relationship (i.e., PChS) at $P_{ET}CO_2$ of 49 mmHg is 1.94 $L{\cdot}min^{-1}{\cdot}mmHg^{-1}$. **D)** PChS at 1 mmHg intervals of isocapnic $P_{ET}CO_2$. **E)** The PChS of each regression lines in Panel D are plotted versus isocapnic $P_{ET}CO_2$ and fitted with a linear or a polynomial function to demonstrate an additive (linear) or hyper/hypo- additive relationship between central and peripheral respiratory chemoreflexes.

The group mean rebreathing responses of $\dot{V}_E$, V_T, and fB are displayed in Figure 10. In each condition the $\dot{V}_E$, V_T, and fB began from a common baseline and, after a period of stability, increased linearly with $P_{ET}CO_2$ ($\dot{V}_E$: $p<0.001$; V_T: $p<0.001$; fB: $p<0.001$; see Figure 10). Overall, there was a main effect of $P_{ET}O_2$ ($\dot{V}_E$: $p<0.001$; V_T: $p<0.001$; fB: $p<0.001$; see Figure 10) such that after baseline, respiratory variables were elevated progressively as $P_{ET}O_2$ fell. The interaction effect was statistically significant for $\dot{V}_E$, V_T, fB ($\dot{V}_E$: $p <0.001$; V_T: $p<0.001$; fB: $p<0.001$; see Figure 10) with increasingly higher $P_{ET}CO_2$ and $P_{ET}O_2$ levels. Furthermore, the most severe hypoxic condition (i.e., $P_{ET}O_2$ = 45 mmHg) had significantly higher ($p<0.001$) $\dot{V}_E$, V_T, fB with increasingly higher $P_{ET}CO_2$ in comparison to the hyperoxic condition.

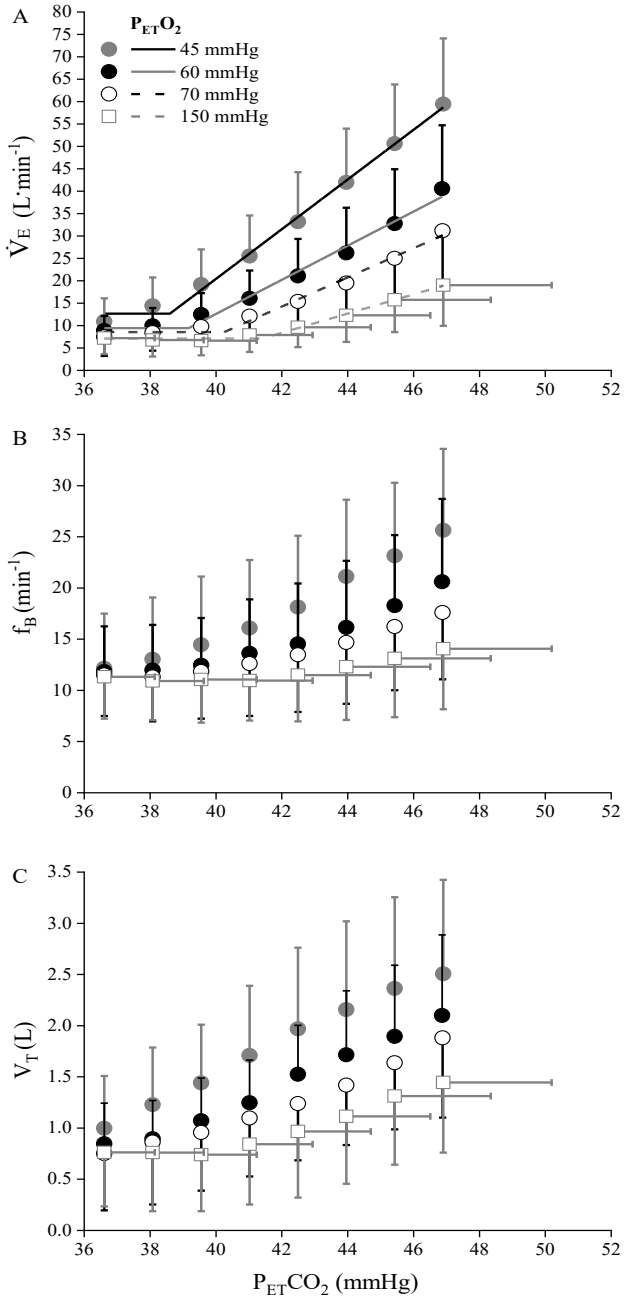


Figure 10: Group mean (± SD) data for minute ventilation ($\dot{V}_E$) (Panel **A**), breathing frequency (fB) (Panel **B**) and tidal volume (V_T) (Panel **C**) versus end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) for three hypoxic trials ($P_{ET}O_2$ = 45, 60, 70 mmHg) and one hyperoxic trial ($P_{ET}O_2$ = 150 mmHg). Fitted double-linear models for $\dot{V}_E$ are superimposed over the data ($P_{ET}O_2$ = 45 mmHg, grey filled circle, and straight black line; 60 mmHg, black filled circle, and straight grey line; 70 mmHg, open circle, and dashed black line; 150 mmHg, open square, and dashed grey line).

Comparisons of ventilatory responses to isoxic hypoxic (45 mmHg, 60 mmHg, 70 mmHg) and hyperoxic (150 mmHg) rebreathing trials and parameter estimates of modelled responses are displayed in Table 2. The $P_{ET}O_2$ was maintained isoxic at 150, 70, 60 and 45 mmHg achieving mean S_CO_2 that fell from 99±0% in the hyperoxic condition to 94±0.1%, 90±0.1%, and 81±0.1% for $P_{ET}O_2$ of 70, 60, and 45 mmHg, respectively; $p<0.001$. Notably, compared to the isoxic hyperoxic condition, both VRT decreased ($p<0.01$) and $\dot{V}_ES$ ($p<0.001$) rose progressively as isoxic $P_{ET}O_2$ was reduced. The $\dot{V}_EBSL$ was significantly different on average when comparing the different isoxic conditions ($p=0.009$). Post hoc analysis showed that the most severe hypoxic condition was significantly different than the hyperoxic condition. Furthermore, on average, the VRT was different for all conditions ($p=0.001$).

Table 2: Group ensemble averages of the ventilatory response to isoxic hypoxic and hyperoxic modified rebreathing tests (n=20).

Variable	Condition				p
	150 mmHg	70 mmHg	60 mmHg	45 mmHg	
$P_{ET}O_2$ (mmHg)	150.2 ± 0.3	69.6 ± 0.3[d]	59.6 ± 0.2[c,d]	45.0 ± 0.3[b,c,d]	<0.001
S_CO_2 (%)	99.3 ± 0	93.7 ± 0.1[d]	90.4 ± 0.1[c,d]	80.5 ± 0.1[b,c,d]	<0.001
$rrCO_2$ (mmHg·min^{-1})	3.88 ± 0.16	3.76 ± 0.14	3.68 ± 0.23	3.52 ± 0.13	0.07
$\dot{V}_E$BSL (L·min^{-1})	6.8 ± 3.9	7.3 ± 3.4	8.1 ± 3.5	8.8 ± 3.8[d]	0.009
VRT (mmHg)	43.0 ± 3.0	40.8 ± 3.1[d]	39.7 ± 3.1[c,d]	37.8 ± 2.7[b,c,d]	<0.001
$\dot{V}_E$S (L·min^{-1}·mmHg^{-1})	3.70 ± 1.5	4.44 ± 1.8[d]	4.99 ± 1.6[d]	6.03 ± 2.2[b,c,d]	<0.001
Peak $P_{ET}CO_2$ (mmHg)	54.9 ± 0.5	52.5 ± 0.8[d]	51.9 ± 0.5[d]	48.8 ± 0.5[b,c,d]	<0.001

Values are mean ± SD. $rrCO_2$, rate of rise of carbon dioxide; $\dot{V}_E$BSL, ventilation representing the wakefulness drive to breath; VRT, ventilatory recruitment threshold; $\dot{V}_E$S, chemoreflex sensitivity; $P_{ET}O_2$, end-tidal partial pressure of oxygen; S_CO_2, calculated oxyhemoglobin saturation; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide at the end of rebreathing. Post Hoc, [a-f] indicate significant differences between conditions ($p<0.05$). "[a]" indicates difference from "45 mmHg", "[b]" indicates difference from "60 mmHg", and so forth.

The PChS vs $P_{ET}CO_2$ profiles of each participant are displayed in Figure 11. Of the 20 participants, the PChS vs $P_{ET}CO_2$ response of 15 were best described by a linear relationship, 4 by a decelerating polynomial, and 1 by an accelerating polynomial. The AICc coefficients for both linear and polynomial fits of each participant's data are displayed in Table 3. Plots of PChS versus $P_{ET}CO_2$ for V_T ($L \cdot \%S_CO_2$) and fB (breaths per $min \cdot \%S_CO_2$) are displayed in Figures 12 and 13.

Table 3: Akaike Information Criterion (AICc) values for all 20 participants.

Participant	Sex	AICc (linear)	AICc (polynomial)	ΔAICc	Akaike Weight Ratio	Category	Level of Evidence
1	male	-32.1	-30.1	-2.0	2.66	additive	moderate
3	male	-24.8	-15.8	-9.0	88.4	additive	strong
5	male	-8.1	16.6	-24.7	$23.0 \cdot 10^4$	additive	strong
6	male	-5.8	19.6	-25.4	$33.8 \cdot 10^4$	additive	strong
8	male	-19.1	6.5	-25.6	$36.1 \cdot 10^4$	additive	strong
12	male	-28.6	-28.0	-0.6	1.40	additive	weak
13	male	-25.3	-25.1	-0.2	1.08	additive	weak
15	male	-28.9	-27.6	-1.3	1.34	additive	weak
16	male	-66.4	-73.2	6.8	29.4	hyper-additive	strong
18	male	-66.5	-63.1	-3.4	5.67	additive	moderate
2	female	-21.5	-9.6	-11.9	378	additive	strong
4	female	-35.0	-33.9	-1.1	1.68	additive	weak
7	female	-32.5	-27.5	-5.0	12.04	additive	strong
9	female	-52.9	-43.8	-9.1	94.7	additive	strong
10	female	-45.6	-92.5	46.9	$1.47 \cdot 10^4$	hypo-additive	strong
11	female	-13.2	-12.7	-0.5	1.31	additive	weak
14	female	-31.8	-27.2	-4.6	10.3	additive	strong
17	female	-35.2	-50.9	15.7	2580	hypo-additive	strong
19	female	-56.2	-62.3	6.1	20.3	hypo-additive	strong
20	female	-46.5	-55.2	8.7	77.2	hypo-additive	strong

Akaike Weight Ratio represents the ratio between the ratio of each model's Akaike weight. Akaike weight is a metric that represents the relative likelihood of a model that is best fit. The ratio provides the factor by which the model with the lower AICc value is more likely to be best fit. For example, for participant 11, this value was 1.31 indicating that Model 1 (linear is 1.31 times more likely to be correct compared to Model 2. For participant 12, this value was 1.40. ΔAICc is the polynomial AICc coefficient subtracted from the linear AICc coefficient in which a negative ΔAICc value favours the linear model whereas a positive value favours the polynomial model.

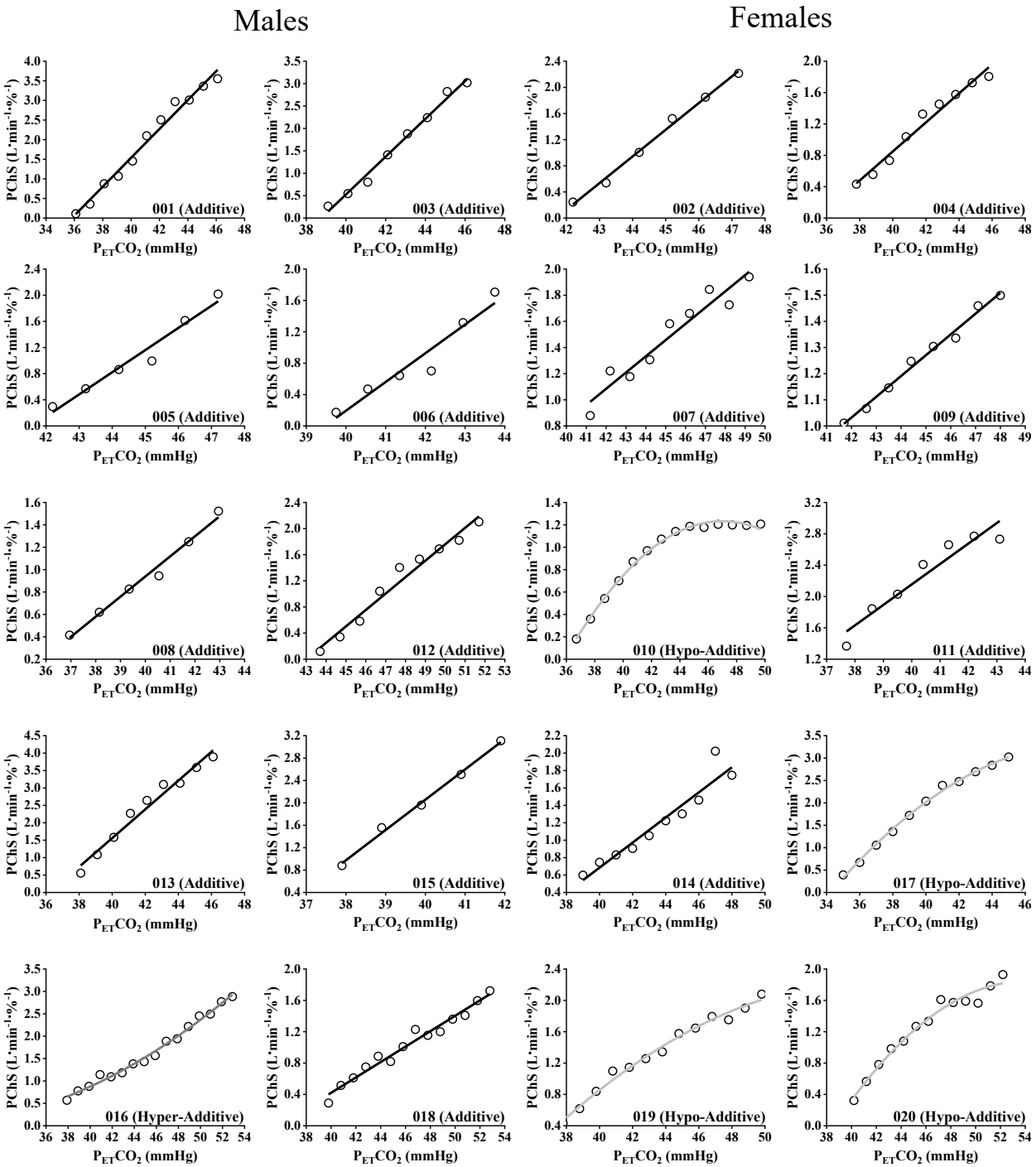


Figure 11: Peripheral chemoreflex sensitivity to hypoxia (PChS) versus end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) for 10 males (first two columns) and 10 females (last two columns). Best fit models are superimposed over the data. Linear (black) is indicative of an 'additive'

relationship and polynomial (grey) indicative of either ‘hypo-additive’ (decelerating) or ‘hyper-additive’ (accelerating).

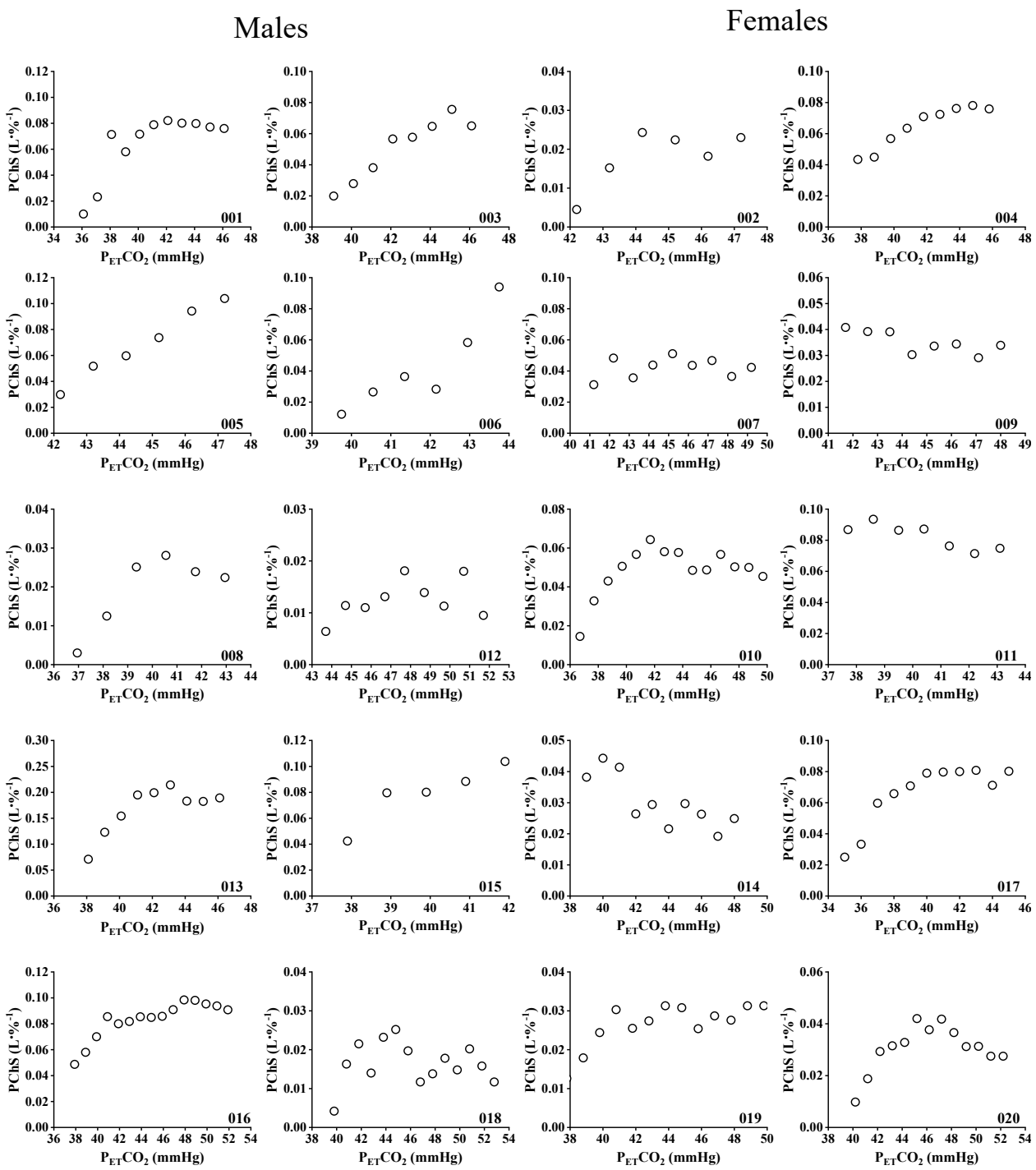


Figure 12: Peripheral chemoreflex sensitivity to hypoxia (PChS; as assessed by tidal volume, V_T) versus end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) for 10 males (first two columns) and 10 females (last two columns).

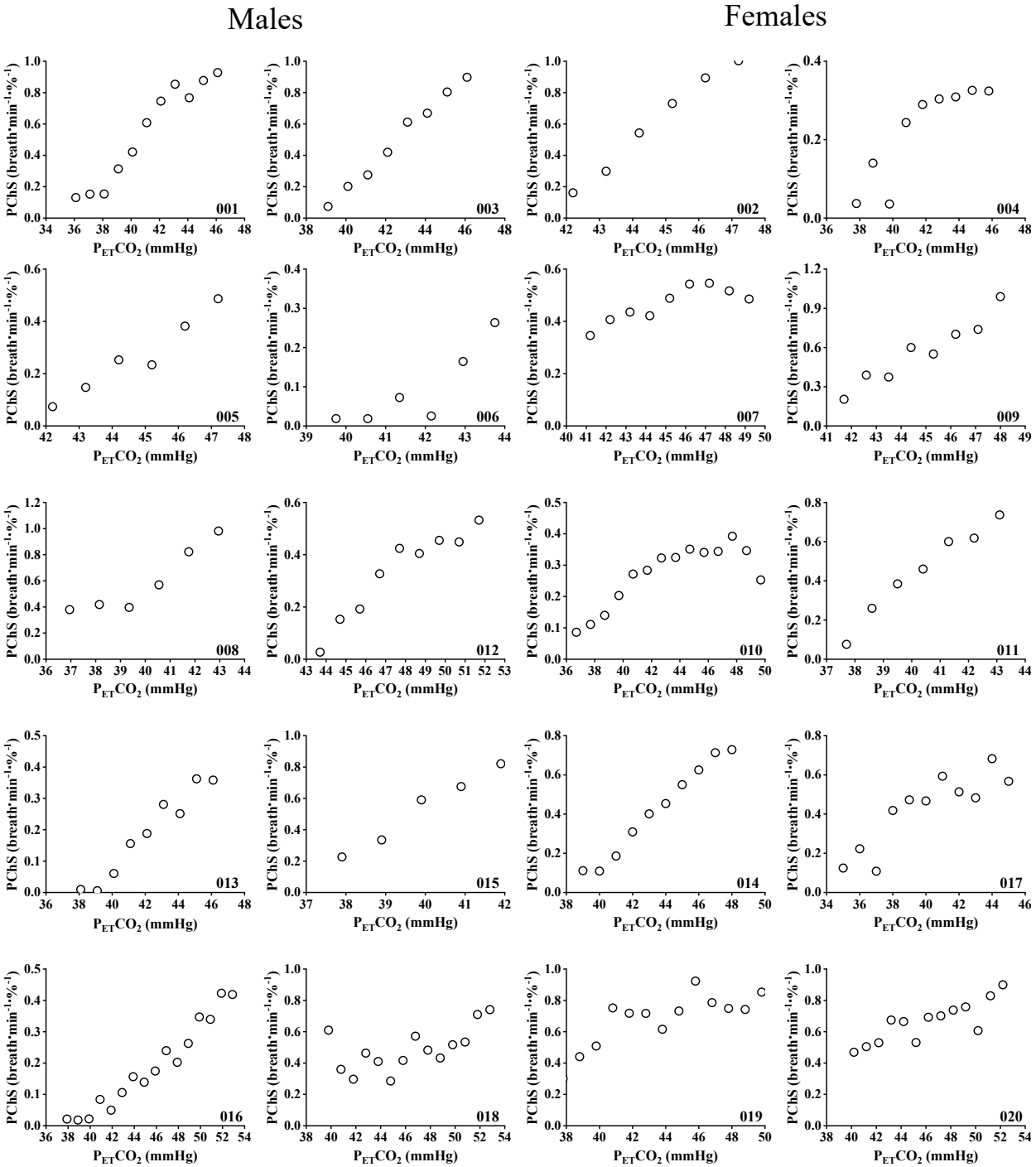


Figure 13: Peripheral chemoreflex sensitivity to hypoxia (PChS; as assessed by breathing frequency, fB) versus end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) for 10 males (first two columns) and 10 females (last two columns).

Chapter III: Discussion and Summary

Discussion

To test the hypothesis that both central and peripheral chemoreflexes drives to breathe are additive, we quantified peripheral chemoreflex sensitivity to hypoxia at a progressively greater arterial and central PCO_2 to discern whether the PChS vs $P_{ET}CO_2$ relationship would retain linearity in the presence of increasing central chemoreceptor stimulation. The novel finding was that in 15 of 20 participants, the PChS vs isocapnic PCO_2 relationship was best characterized by a positive linear function. Of the remaining five participants, the most appropriate model was a decelerating polynomial in four and accelerating polynomial in one. Thus, in most participants, there did not appear to be a hypo- or hyper-additive effect of central chemoreceptors on the peripheral respiratory chemoreflex; the interaction was additive.

As anticipated, for all participants, isoxic PO_2 altered the ventilatory response to PCO_2 (Mohan & Duffin, 1997). On average, when compared to the isoxic hyperoxic trial, as isoxic PO_2 was reduced, the sub-VRT baseline $\dot{V}_E$ (i.e., $\dot{V}_E$BSL) increased, the $P_{ET}CO_2$ associated with VRT fell, and the ventilatory response slope above VRT (i.e., $\dot{V}_E$S) became steeper (see Figure 10). These heightened ventilatory responses – which are evident in Figure 10A – exemplify the synergistic effect of PCO_2 and PO_2 on the peripheral chemoreflex. First, at any isocapnic PCO_2, the lower the PO_2 the greater the $\Delta\dot{V}_E$ (or HVR, e.g., see Figure 9 and 10A). When the HVR of varying doses of hypoxia are plotted relative to S_CO_2, a linear relationship is observed (Keir et al., 2020; Rebuck & Campbell, 1974) with a slope that gives PChS (e.g., see Figure 8C and 9D). Second, as isocapnic PCO_2 is increased, the PChS is steeper (i.e., synergistic PCO_2-PO_2 effect at the carotid body) (Keir et al., 2020; Rebuck & Woodley, 1975) or increases in sensitivity (e.g., see Figures 8D and 9E)

When central chemoreceptor excitation is fixed or absent, the synergistic effect of O_2 and CO_2 at the peripheral chemoreceptors yields a PChS that increases linearly with PCO_2 (Irsigler et al., 1980; Riedstra, 1963; Teppema et al., 2010). In the present study, central chemoreceptor excitation was not fixed but increased progressively with PCO_2. To assess the central-peripheral interaction (Duffin, 2007; Guyenet et al., 2012), we subtracted, at intervals of 1 mmHg of PCO_2, the ventilatory response at a PO_2 of 150 mmHg from those at 45, 60, and 70 mmHg (Figure 9A

and B) to obtain the PChS across a wide range of PCO_2 (Figure 9E). Importantly, with this method, we reasoned that the central chemoreflex contribution to $\dot{V}_E$ is removed but the potential influence on the peripheral chemoreflex response would be retained (interaction) and possibly vary with PCO_2. With this method, Figure 7 illustrates the three possible relationships that describe the interaction between the central and peripheral chemoreflexes. Notably, the PChS vs $P_{ET}CO_2$ relationship in 15 of the 20 participants was best described by a linear fit (see Figure 11 and Table 3) indicating that the progressive activation of central chemoreceptors did not affect the sensitivity of the peripheral chemoreflex to low O_2 (i.e., the PChS) beyond the anticipated effect of heightened $PaCO_2$ at the carotid body (Irsigler et al., 1980; Riedstra, 1963; Teppema et al., 2010). Such findings are consistent with previous investigations in healthy humans (Clement et al., 1992; Cui et al., 2012; St Croix et al., 1996) supporting the hypothesis that central and peripheral chemoreflexes do not interact.

Several studies in humans and animals have supported a hyper-additive relationship between central and peripheral chemoreceptors. For example, Dahan et al., (2007) found that ventilatory responses to CO_2 in hyperoxic conditions fell immediately after the carotid body was removed in three patients indicating that peripheral chemoreceptors may augment the central chemoreflex response. A hyper-additive relationship has also been observed in anaesthetized awake dogs in whom ventilatory responsiveness to central hypercapnia were augmented by concurrent stimulation of a vascularly isolated carotid body (Blain et al., 2010; Smith et al., 2015). Notably, only one of 20 participants exhibited a PChS vs $P_{ET}CO_2$ relationship consistent with a hyper-additive interaction. Two other observations also refute the hyper-additive hypothesis. First, within each isoxic hypoxic condition (reflecting combined central and peripheral chemoreflex responses), progressive peripheral excitation by increasing PCO_2 should progressively augment the central chemoreflex gain. This progressive augmentation would then cause the net $\dot{V}_E$ vs $P_{ET}CO_2$ relationship to accelerate upward. However, such accelerations were not observed; individual rebreathing tests were well characterized by a linear model after VRT (e.g., see Figure 9A and Figure 10). Second, had this synergistic effect occurred, it is difficult to reconcile how the PChS vs $P_{ET}CO_2$ relationship would exhibit a linear response in the majority of participants. In such a case, the hyper-additive peripheral-central effect would need to be perfectly balanced by a hypo-additive central-peripheral effect to maintain a linear PChS vs $P_{ET}CO_2$. Thus, the linear relation of peripheral chemoreflex sensitivity to hypoxia versus isocapnic PCO_2 not only provides

evidence that central chemoreceptors do not affect the peripheral chemoreflex but also suggest that a carotid body effect on the central chemoreflex is unlikely in young adults.

Applying a different approach to test whether central chemoreceptor activation affected peripheral respiratory chemoreflex sensitivity, Milloy et al. (2022) performed transient HVR tests immediately after an 8-min period of breathing inspired CO_2 fractions of 0, 2, and 4%. Importantly, sufficient time was provided to allow central chemoreceptors to establish a steady-state of local PCO_2 and $\dot{V}_E$, and the HVR stimulus was initiated in all three conditions from a common $P_{ET}CO_2$ (and presumably $PaCO_2$). On average, there was no difference in HVR across all conditions indicating that the degree of central chemoreflex activation does not influence peripheral chemoreflex-mediated HVR. At the individual level, however, the HVR remained the same (i.e., additive) in 3 of 16 participants, fell (hypo-additive) in 7, rose (hyper-additive) in 2 and exhibited no discernable pattern in 4. Much less between-participant variability was present in our data with the majority exhibiting additive central-peripheral interactions. Rather than utilizing a traditional approach to quantifying PChS that relies on the average of several, transient, highly variable, single-breath peaks in response to arterial desaturation (Pfoh & Day, 2016; Prasad et al., 2020), every $\Delta\dot{V}_E$ data point used to quantify HVR at each $P_{ET}CO_2$ interval was a multi-breath (~5-6 breaths) average of three repeated trials. Given that modified rebreathing has been shown to have excellent test-retest reproducibility (Guluzade et al., 2022; Jensen et al., 2010), we can be very confident in our estimates of HVR and calculated PChS. Indeed, inspection of each panel in Figure 11 reveals the high goodness of fit of each best fit model. An additional advantage of using modified rebreathing is that in this closed loop condition the arterial and central PCO_2 gradient is largely reduced and end-tidal PCO_2 provided a much more accurate estimate of their absolute values throughout rebreathing experiments compared to steady-state experiments (Duffin, 2010). Thus, we can be confident that the rise in $P_{ET}CO_2$, observed with rebreathing in all conditions reflected a concomitant increase in both arterial and central PCO_2.

Animal studies performed in rodents have supported a hypo-additive central-peripheral interaction (Cummings, 2014; Day & Wilson, 2009; Tin et al., 2012). However, only four of our participants' PChS vs $P_{ET}CO_2$ relationships were consistent with this type of interaction. Interestingly, all four of these individuals were female. Of the six females with an additive PChS vs $P_{ET}CO_2$ response, four were on oral contraception. The remaining females were not on oral

contraception including the four participants with a hypo-additive response. However, this observation may be coincidental because ventilatory responses to modified rebreathing in females on monophasic oral contraceptives are known not to change across the menstrual cycle (Nettlefold et al., 2007). Alternatively, female participants were smaller in size and exhibited significantly lower FVC (3.3 ± 0.5 L), IC (2.1 ± 0.4 L), and MVV (108.9 ± 25.2 $L \cdot min^{-1}$) compared to males (FVC = 5.7 ± 0.8 L; IC = 3.6 ± 0.7 L; MVV = 182.6 ± 21.8 $L \cdot min^{-1}$; see Table 1). Such morphological differences have been shown to result in a greater mechanical work of breathing and (possibly) earlier onset of respiratory muscle fatigue in females at a given $\dot{V}_E$ during exercise (Dominelli et al., 2015; Guenette et al., 2007; Wanke et al., 1991). However, the four females exhibiting "hypo-additive" responses attained only 58% of their MVV at the end of rebreathing. Alternatively, sex-specific differences in lung morphology and breathing mechanics may contribute to differences in neuronal adaptations associated with the chemoreflex arc.

The reason why our finding of an additive central-peripheral chemoreflex interaction in humans' contrasts with the majority of experiments in non-human species (Blain et al., 2010; Day & Wilson, 2007, 2009; Smith et al., 2015) could be due to innate differences in respiratory control requirements. For example, unlike humans, animals perceive very low levels of ambient CO_2 via the olfactory system (Hu et al., 2007) and rely on breathing to dissipate heat under thermal strain (Robertshaw, 2006). These attributes could necessitate more complex central-peripheral interactions to maintain blood gas homeostasis. In contrast, humans may differ with respect to chemoreflex interaction because of their dependence on regulating ventilation for speech. It is also worth considering that our participants were awake and breathing spontaneously whereas relative contributions of central and peripheral chemoreflexes to control of breathing may differ in anesthetized or reduced preparations (Guyenet, 2014).

Although the majority of participants exhibited an additive central-peripheral interaction, 25% of the group did not. This heterogeneity is consistent with other human-based experiments (Clement et al., 1992; Cui et al., 2012; Milloy et al., 2022; St Croix et al., 1996) indicating that different phenotypes may exist amongst humans. The consequence of such differences in unknown. We would speculate that central-peripheral interaction would act to "destabilize" the ventilatory control system and predispose to disordered or unstable breathing. Whether those who exhibited interactive effects are more likely to endure periods of hypopnea and apnea during

spontaneous breathing at rest or during sleep is unknown. Alternatively, it is possible that interactions between these two sites of chemoreception may be inconsequential to maintain adequate gas exchange and blood gas homeostasis in humans (Wilson & Teppema, 2016).

Although PChS at 1-mmHg intervals of $P_{ET}CO_2$ with respect to V_T and fB were computed and are displayed in Figures 12 and 13, we elected not to model the resultant PChS vs $P_{ET}CO_2$ data as was done for $\dot{V}_E$ to test the hypothesis depicted in Figure 7. Although it has been proposed that additive central-peripheral interactions of $\dot{V}_E$, necessitate from a mathematical perspective, linear and hypo-additive relationships of V_T and fB (or vice versa) (Milloy et al., 2022; Wilson & Teppema, 2016), it is our opinion that this may be an oversimplification. Our data support that either more complex, higher-order models and/or strategies to limit variability of these variables are required (e.g., see participants 14 or 18). Indeed, adjustments in $\dot{V}_E$ (or more appropriately alveolar ventilation) are needed to correct error signals in PCO_2 and PO_2, and this may be achieved by numerous combinations of V_T and fB pattern that are influenced by stimuli outside the chemoreflex arc (e.g., rate and degree of pulmonary stretch, air temperature/humidity, stress/anxiety) (Clark & Von Euler, 1972; Tipton et al., 2017).

Our approach to examining the effect of central chemoreceptors on the peripheral chemoreflex response to low O_2 relied on the assumption that hyperoxia silences the peripheral chemoreflex response to CO_2. The relationship between the ventilatory response to PCO_2 relates hyperbolically with PaO_2, such that there is a minimal rise in $\dot{V}_E$ until PaO_2 falls below ~85 mmHg (Duffin et al., 2000; Lloyd et al., 1958). As PO_2 increases above 150 mmHg, further reduction in the CO_2 ventilatory response is evident (Duffin et al., 2000; Lloyd et al., 1958; Mohan & Duffin, 1997). In addition, previous *in vitro* and *in vivo* experiments, show that a PO_2 of 150 mmHg nearly eliminated carotid body type I cell excitation (Buckler & Vaughan-Jones, 1994; Dasso et al., 2000; Duchen & Biscoe, 1992; Fitzgerald & Parks, 1971) and carotid sinus afferent discharge (Hornbein & Roos, 1963; Lahiri et al., 1993; Lahiri & Delaney, 1975; Vidruk et al., 2001) in response to acidosis or hypoxia. Although there may be afferent output from the carotid bodies in humans exposed to hyperoxic conditions, we suggest that it contributes insignificantly to the reflex response to CO_2. In addition, we assumed that the entirety of the ventilatory response to hypoxia is mediated by the peripheral chemoreceptors located in the carotid body and aorta. However, hypoxic sensors in the brainstem, kidney, and spinal cord may also contribute to the PChS as

calculated in this study (Barioni et al., 2022; Bauer & Kurtz, 1989; Getsy et al., 2021; Gourine & Funk, 2017; Neubauer & Sunderram, 2004; SheikhBahaei, 2020). Finally, AICc difference and relative likelihood scores ranged from 0.2 to 46.9 and from 1.08 to >1000, respectively (Table 3). Assigning |ΔAIC| cut-offs of <2, 2 - 7, and >7 as "weak", "moderate", "strong" levels of evidence in support of the selected best-fit model, respectively, 8 of the 15 in whom individuals' PChS vs $P_{ET}CO_2$ took a linear form were classified as "strong", 2 as "moderate" and 5 as "weak". The level of evidence for those displaying hypo- (n=4) or hyper-additive (n=1) relationships were all classified as "strong". Important to consider is that these scores operate on a continuum from 0 to infinity such that evidence of superiority of one model (e.g., linear) versus another (e.g., polynomial) increases from limited to substantial and the ΔAIC cut-offs applied are arbitrary rather than statistically justified (Burnham et al., 2011).

Conclusion

In most participants, the peripheral chemoreflex response to hypoxia maintained a positive linear relationship with $P_{ET}CO_2$ despite increasing central chemoreflex activation. This finding indicated that, in healthy young adults, the central chemoreceptors do not exert a significant effect on the peripheral respiratory chemoreflex response to hypoxia. In addition, that the PChS relationship was linear in most instances also provides evidence that the peripheral chemoreceptors are unlikely impacting the central respiratory chemoreflex. Collectively, these findings refute the hypothesis that central and peripheral chemoreflexes interact in healthy awake humans.

Future directions

The current investigation advanced the understanding of the respiratory central and peripheral chemoreflex interaction in humans. This study was designed to explore if the interaction between central and peripheral chemoreceptor inputs is additive rather than hypo or hyper-additive in humans. We used Duffin's rebreathing technique to mathematically dissect out peripheral and central components of ventilatory chemoreflexes. Our findings suggest that upon exposing the chemoreflexes to increasing levels of PCO_2, the chemoreflex sensitivity increased in a linear manner in 75% of the sample size. This is indicative of an additive interactive effect between the central and the peripheral chemoreflex.

The novelty findings of respiratory control via the chemoreflexes developed from this work will initiate new research towards developing therapies and trials designed to intervene at these chemoreceptors to normalize breathing in conditions (e.g., sleep apnea, COVID-19) and environments (e.g., altitude) in which chemoreflex physiology can become pathological. The methodology that was used in this study was a non-invasive modified rebreathing technique that characterized the respiratory chemoreflex parameters with a few assumptions being made. Future research could incorporate the use of pharmacological modulation of chemoreflex sensitivity to assess the interaction of the central and peripheral chemoreflex. Dose-dependent pharmaceutical injection of dopamine and dobutamine could be used to assess the peripheral chemoreflex sensitivity using the modified rebreathing technique to clearly assess the mechanistic behavior of central and peripheral chemoreflex relationship.

References

Ainslie, P. N., & Duffin, J. (2009). Integration of cerebrovascular CO_2 reactivity and chemoreflex control of breathing: Mechanisms of regulation, measurement, and interpretation. *Am J Physiol Regul Integr Comp Physiol*, *296*, 1473–1495. https://doi.org/10.1152/ajpregu.91008.2008.-Cerebral

Assadpour, E., Ivry, I., Wasef, S., Adeyinka, B., Murray, K. R., & Edgell, H. (2020). Oral contraceptives and menstrual cycle influence autonomic reflex function. *Physiological Reports*, *8*(17). https://doi.org/10.14814/phy2.14550

Barioni, N. O., Derakhshan, F., Lopes, L. T., Onimaru, H., Roy, A., Mcdonald, F., Scheibli, E., Baghdadwala, M. I., Heidari, N., Bharadia, M., Ikeda, K., Yazawa, I., Okada, Y., Harris, M. B., Dutschmann, M., & Wilson, R. J. A. (2022). Novel oxygen sensing mechanism in the spinal cord involved in cardiorespiratory responses to hypoxia. *Sci. Adv*, *8*(12).

Bauer, C., & Kurtz, A. (1989). Oxygen sensing in the kidney and its relation to erythropoietin production. *Annu. Rev. Physiol.*, *51*, 845–856.

Blain, G. M., Smith, C. A., Henderson, K. S., & Dempsey, J. A. (2010). Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO_2. *Journal of Physiology*, *588*(13), 2455–2471. https://doi.org/10.1113/jphysiol.2010.187211

Buckler, K. J., & Vaughan-Jones, R. D. (1994). Effects of hypercapnia on membrane potential and intracellular calcium in rat carotid body type I cells. *The Journal of Physiology*, *478*(1), 157–171. https://doi.org/10.1113/jphysiol.1994.sp020239

Buckler, K. J., & Vaughan-Jones, R. D. (1994). Effects of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type I cells. *The Journal of Physiology*, *476*(3), 423–428. https://doi.org/10.1113/jphysiol.1994.sp020143

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods and Research*, *33*(2), 261–304. https://doi.org/10.1177/0049124104268644

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*(1), 23–35. https://doi.org/10.1007/s00265-010-1029-6

Carr, J. M. J. R., Caldwell, H. G., & Ainslie, P. N. (2021). Cerebral blood flow, cerebrovascular reactivity and their influence on ventilatory sensitivity. *Experimental Physiology*, *106*(7), 1425–1448. https://doi.org/10.1113/EP089446

Carter, B., Carlin, J., Tibballs, J., Mead, H., Hochmann, M., & Osborne, A. (1998). Accuracy of two pulse oximeters at low arterial hemoglobin-oxygen saturation. *Critical Care Medicine*, *26*(6), 1128–1133. https://doi.org/10.1097/00003246-199806000-00040

Cavanaugh, J. E., & Neath, A. A. (2019). The Akaike information criterion: Background, derivation, properties, application, interpretation, and refinements. In *Wiley Interdisciplinary Reviews: Computational Statistics* (Vol. 11, Issue 3). Wiley-Blackwell. https://doi.org/10.1002/wics.1460

Clark, F. J., & Von Euler, C. (1972). On the regulation of depth and rate of breathing. *J. Physiol*, *222*, 267–295.

Cleary, C. M., Moreira, T. S., Takakura, A. C., Nelson, M. T., Longden, T. A., & Mulkey, D. K. (2020). Vascular control of the CO_2/H^+ - Dependent drive to breathe. *ELife*, *9*, 1–23. https://doi.org/10.7554/ELIFE.59499

Clement, I., Bascom, D., Conway, J., Dorrington, K., Painter, R., & Robbins, P. (1992). An assessment of central-peripheral ventilatory chemoreflex interaction in humans. *Respiration Physiology*, *88*, 87–100.

Clement, I., Pandit, J. J., Bascom, D. A., Dorrington, K. L., O'Connor, D. F., & Robbins, P. A. (1995). An assessment of central-peripheral ventilatory chemoreflex interaction using

acid and bicarbonate infusions in humans. *The Journal of Physiology*, *485*(2), 561–570. https://doi.org/10.1113/jphysiol.1995.sp020752

Corcoran, A. E., Hodges, M. R., Wu, Y., Wang, W., Wylie, C. J., Deneris, E. S., & Richerson, G. B. (2009). Medullary serotonin neurons and central CO_2 chemoreception. In *Respiratory Physiology and Neurobiology* (Vol. 168, Issues 1–2, pp. 49–58). https://doi.org/10.1016/j.resp.2009.04.014

Cui, Z., Fisher, J. A., & Duffin, J. (2012). Central-peripheral respiratory chemoreflex interaction in humans. *Respiratory Physiology and Neurobiology*, *180*(1), 126–131. https://doi.org/10.1016/j.resp.2011.11.002

Cummings, K. J. (2014). Interaction of central and peripheral chemoreflexes in neonatal mice: Evidence for hypo-addition. *Respiratory Physiology and Neurobiology*, *203*, 75–81. https://doi.org/10.1016/j.resp.2014.08.016

Cunningham, D., Robbins, P., & Wolff, C. (1986). Integration of respiratory responses to changes in alveolar partial pressures of CO_2 and O_2 and in arterial pH. *American Physiological Society*, 475–528.

Dahan, A., Nieuwenhuijs, D., & Teppema, L. (2007). Plasticity of central chemoreceptors: Effect of bilateral carotid body resection on central CO_2 sensitivity. *Plos Medicine*, *4*(7), 1195–1204. https://doi.org/10.1371/journal

Dasso, L. L. T., Buckler, K. J., & Vaughan-Jones, R. D. (2000). Interactions between hypoxia and hypercapnic acidosis on calcium signaling in carotid body type I cells. *Am J Physiol Lung Cell Mol Physiol*, *279*, 36–42. http://www.ajplung.orgL36

Day, T. A., & Wilson, R. J. A. (2007). Brainstem PCO_2 modulates phrenic responses to specific carotid body hypoxia in an in situ dual perfused rat preparation. *Journal of Physiology*, *578*(3), 843–857. https://doi.org/10.1113/jphysiol.2006.119594

Day, T. A., & Wilson, R. J. A. (2009). A negative interaction between brainstem and peripheral respiratory chemoreceptors modulates peripheral chemoreflex magnitude. *Journal of Physiology*, *587*(4), 883–896. https://doi.org/10.1113/jphysiol.2008.160689

Dominelli, P. B., Molgat-Seon, Y., Bingham, D., Swartz, P. M., Road, J. D., Foster, G. E., & Sheel, A. W. (2015). Dysanapsis and the resistive work of breathing during exercise in healthy men and women. *J Appl Physiol*, *119*, 1105–1113. https://doi.org/10.1152/japplphysiol.00409.2015.-We

Duchen, M. R., & Biscoe, T. J. (1992). Relative mitochondrial membrane potential and $[Ca^{2+}]_i$ in type I cells isolated from the rabbit carotid body. *The Journal of Physiology*, *450*(1), 33–61. https://doi.org/10.1113/jphysiol.1992.sp019115

Duffin, J. (1990). The chemoreflex control of breathing and its measurement. *Canadian Journal of Anaesthesia*, *37*, 933–942. https://doi.org/https://doi.org/10.1007/bf03006641

Duffin, J. (2005). Role of acid-base balance in the chemoreflex control of breathing. *Journal of Applied Physiology*, *99*(6), 2255–2265. https://doi.org/10.1152/japplphysiol.00640.2005

Duffin, J. (2007). Measuring the ventilatory response to hypoxia. *Journal of Physiology*, *584*(1), 285–293. https://doi.org/10.1113/jphysiol.2007.138883

Duffin, J. (2010). The role of the central chemoreceptors: A modeling perspective. *Respiratory Physiology and Neurobiology*, *173*(3), 230–243. https://doi.org/10.1016/j.resp.2010.03.010

Duffin, J. (2011). Measuring the respiratory chemoreflexes in humans. *Respiratory Physiology and Neurobiology*, *177*(2), 71–79. https://doi.org/10.1016/j.resp.2011.04.009

Duffin, J., & Mateika, J. H. (2013). CrossTalk opposing view: Peripheral and central chemoreflexes have additive effects on ventilation in humans. *Journal of Physiology*, *591*(18), 4351–4353. https://doi.org/10.1113/jphysiol.2013.256800

Duffin, J., & McAvoy, G. V. (1988). The peripheral-chemoreceptor threshold to carbon dioxide in man. *The Journal of Physiology*, *406*(1), 15–26. https://doi.org/10.1113/jphysiol.1988.sp017365

Duffin, J., Mohan, R. M., Vasiliou, P., Stephenson, R., & Mahamed, S. (2000). A model of the chemoreflex control of breathing in humans: Model parameters measurement. *Respiration Physiology*, *120*, 13–26. www.elsevier.com/locate/resphysiol

Eldridge, F. L., Kiley, J. P., & Paydarfar, D. (1987). Dynamics of medullary hydrogen ion and respiratory responses to square-wave change of arterial carbon dioxide in cats. *The Journal of Physiology*, *385*(1), 627–642. https://doi.org/10.1113/jphysiol.1987.sp016511

Fitzgerald, R. S., & Parks, D. C. (1971). Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. *Respiration Physiology*, *12*, 218–229.

Getsy, P. M., Coffee, G. A., Hsieh, Y. H., & Lewis, S. J. (2021). The superior cervical ganglia modulate ventilatory responses to hypoxia independently of preganglionic drive from the cervical sympathetic chain. *Journal of Applied Physiology*, *131*(2), 836–857. https://doi.org/10.1152/japplphysiol.00216.2021

Gonzalez, C., Almaraz, L., Obeso, A., & Rigual, R. (1994). Carotid body chemoreceptors: From natural stimuli to sensory discharges. *REVIEWS*, *74*(4).

Gourine, A. V, & Funk, G. D. (2017). On the existence of a central respiratory oxygen sensor. *J Appl Physiol*, *123*, 1344–1349. https://doi.org/10.1152/japplphysiol.00194.2017.-A

Guenette, J. A., Witt, J. D., McKenzie, D. C., Road, J. D., & Sheel, A. W. (2007). Respiratory mechanics during exercise in endurance-trained men and women. *Journal of Physiology*, *581*(3), 1309–1322. https://doi.org/10.1113/jphysiol.2006.126466

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

Guyenet, P. G. (2014). Regulation of breathing and autonomic outflows by chemoreceptors. *Comprehensive Physiology*, *4*(4), 1511–1562. https://doi.org/10.1002/cphy.c140004

Guyenet, P. G., Bayliss, D. A., Stornetta, R. L., Fortuna, M. G., Abbott, S. B. G., & DePuy, S. D. (2009). Retrotrapezoid nucleus, respiratory chemosensitivity and breathing automaticity. In *Respiratory Physiology and Neurobiology* (Vol. 168, Issues 1–2, pp. 59–68). Elsevier B.V. https://doi.org/10.1016/j.resp.2009.02.001

Guyenet, P. G., Stornetta, R. L., Abbott, S. B. G., Depuy, S. D., & Kanbar, R. (2012). The retrotrapezoid nucleus and breathing. *Advances in Experimental Medicine and Biology*, *758*, 115–122. https://doi.org/10.1007/978-94-007-4584-1_16

Guyenet, P. G., Stornetta, R. L., & Bayliss, D. A. (2008). Retrotrapezoid nucleus and central chemoreception. *Journal of Physiology*, *586*(8), 2043–2048. https://doi.org/10.1113/jphysiol.2008.150870

Hanson, M. A., Rao, P. S., & Torrance, R. W. (1978). *Aortic Nerve Chemoreceptors are Sensitive to Changes in $PaCO_2$* (pp. 269–273). https://doi.org/10.1007/978-1-4613-4009-6_29

Hayes, M. W., Maini, B. K., & Torrance, R. W. (1976). Reduction of the responses of carotid chemoreceptors by acetazolamide. In *Morphology and Mechanisms of Chemoreceptors* (pp. 36–45).

Hornbein, T. F., & Roos, A. (1963). Specificity of H ion concentration as a carotid chemoreceptor stimulus. *Journal of Applied Physiology*, *18*, 580–584.

Hu, J., Zhong, C., Ding, C., Chi, Q., Walz, A., Mombaerts, P., Matsunami, H., & Luo, M. (2007). Detection of near-atmospheric concentrations of CO_2 by an olfactory subsystem in the mouse. *Science*, *317*(5840), 953–957. https://doi.org/10.1126/science.1144334

Irsigler, G. B., Stafford, M. J., & Severinghaus, J. W. (1980). Relationship of CSF pH, O_2, and CO_2 responses in metabolic acidosis and alkalosis in humans. *J Appl Physiol Respir Environ Exerc Physiol.*, *48*(2), 355–361. https://doi.org/https://doi.org/10.1152/jappl.1980.48.2.355

Jensen, D., Mask, G., & Tschakovsky, M. E. (2010). Variability of the ventilatory response to Duffin's modified hyperoxic and hypoxic rebreathing procedure in healthy awake

humans. *Respiratory Physiology and Neurobiology*, *170*(2), 185–197. https://doi.org/10.1016/j.resp.2009.12.007

Keir, D. A., Duffin, J., & Floras, J. S. (2020). Measuring peripheral chemoreflex hypersensitivity in heart failure. *Frontiers in Physiology*, *11*. https://doi.org/10.3389/fphys.2020.595486

Keir, D. A., Duffin, J., Millar, P. J., & Floras, J. S. (2019). Simultaneous assessment of central and peripheral chemoreflex regulation of muscle sympathetic nerve activity and ventilation in healthy young men. *Journal of Physiology*, *597*(13), 3281–3296. https://doi.org/10.1113/JP277691

Kiwull-Schöne, H., Kiwull, P., Mückenhoff, K., & Both, W. (1979). The role of carotid chemoreceptors in the regulation of arterial oxygen transport under hypoxia with and without hypercapnia. *Adv Exp Med Biol*, *75*, 469–476. https://doi.org/10.1007/978-1-4684-3273-2_55

Koegelenberg, C. F. N., Swart, F., & Irusen, E. M. (2013). Guideline for office spirometry in adults, 2012. *South African Medical Journal*, *103*(1), 52–61. https://doi.org/10.7196/SAMJ.6197

Kumar, P., & Bin-Jaliah, I. (2007). Adequate stimuli of the carotid body: More than an oxygen sensor? *Respiratory Physiology and Neurobiology*, *157*(1), 12–21. https://doi.org/10.1016/j.resp.2007.01.007

Kumar, P., & Prabhakar, N. R. (2012). Peripheral chemoreceptors: Function and plasticity of the carotid body. *Comprehensive Physiology*, *2*(1), 141–219. https://doi.org/10.1002/cphy.c100069

Lahiri, S., & Delaney, R. G. (1975). Stimulus interaction in the responses of carotid body chemoreceptor single afferent fibers. *Respiration Physiology*, *24*, 249–266.

Lahiri, S., Rumsey, W. L., Wilson, D. F., & Iturriaga, R. (1993). Contribution of in vivo microvascular PO_2 in the cat carotid body chemotransduction. *Journal of Applied Physiology*, *75*, 1035–1043.

Lloyd, B., & Cunningham, D. (1963). A quantitative approach to the regulation of human respiration. In *Regul Hum Respir*.

Lloyd, B., Jukes, M., & Cunningham, D. (1958). The relation between alveolar oxygen pressure and the respiratory response to carbon dioxide in man. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences*, *43*(2), 214–227. https://doi.org/10.1113/expphysiol.1958.sp001319

Milloy, K. M., White, M. G., Chicilo, J. O. C., Cummings, K. J., Pfoh, J. R., & Day, T. A. (2022). Assessing central and peripheral respiratory chemoreceptor interaction in humans. *Experimental Physiology*, *107*(9), 1081–1093. https://doi.org/10.1113/EP089983

Mohan, R., & Duffin, J. (1997). The effect of hypoxia on the ventilatory response to carbon dioxide in man. *Respiration Physiology*, *108*, 101–115. https://doi.org/https://doi.org/10.1016/s0034-5687(97)00024-8

Nattie, E., & Li, A. (2009). Central chemoreception is a complex system function that involves multiple brain stem sites. In *Journal of Applied Physiology* (Vol. 106, Issue 4, pp. 1464–1466). https://doi.org/10.1152/japplphysiol.00112.2008

Nettlefold, L., Jensen, D., Janssen, I., & Wolfe, L. A. (2007). Ventilatory control and acid-base regulation across the menstrual cycle in oral contraceptive users. *Respiratory Physiology and Neurobiology*, *158*(1), 51–58. https://doi.org/10.1016/j.resp.2007.04.005

Neubauer, J. A., & Sunderram, J. (2004). Oxygen-sensing neurons in the central nervous system. *J Appl Physiol*, *96*, 367–374. https://doi.org/10.1152/japplphysiol.00831.2003.-This

Nurse, C. A., & Piskuric, N. A. (2013). Signal processing at mammalian carotid body chemoreceptors. *Seminars in Cell and Developmental Biology*, *24*(1), 22–30. https://doi.org/10.1016/j.semcdb.2012.09.006

Okada, Y., Chen, Z., Jiang, W., Kuwana, S.-I., Eldridge, F. L., Okada, Z., Chen, W., Jiang, S.-N.-I., & Kuwana, F. L. E. (2002). Anatomical arrangement of hypercapnia-activated cells in the superficial ventral medulla of rats. *J Appl Physiol*, *93*, 427–439. https://doi.org/10.1152/japplphysiol.00620

Pfoh, J. R., & Day, T. A. (2016). Considerations for the use of transient tests of the peripheral chemoreflex in humans: The utility is in the question and the context. In *Experimental Physiology* (Vol. 101, Issue 6, pp. 778–779). Blackwell Publishing Ltd. https://doi.org/10.1113/EP085784

Pfoh, J. R., Tymko, M. M., Abrosimova, M., Boulet, L. M., Foster, G. E., Bain, A. R., Ainslie, P. N., Steinback, C. D., Bruce, C. D., & Day, T. A. (2016). Comparing and characterizing transient and steady-state tests of the peripheral chemoreflex in humans. *Experimental Physiology*, *101*(3), 432–447. https://doi.org/10.1113/EP085498

Prabhakar, N. R. (2013). Sensing hypoxia: physiology, genetics and epigenetics. *Journal of Physiology*, *591*(9), 2245–2257. https://doi.org/10.1113/jphysiol.2012.247759

Prasad, B., Morgan, B. J., Gupta, A., Pegelow, D. F., Teodorescu, M., Dopp, J. M., & Dempsey, J. A. (2020). The need for specificity in quantifying neurocirculatory vs. respiratory effects of eucapnic hypoxia and transient hyperoxia. In *Journal of Physiology* (Vol. 598, Issue 21, pp. 4803–4819). John Wiley and Sons Inc. https://doi.org/https://doi.org/10.1113/jp280515

Preston, M. E., Jensen, D., Janssen, I., & Fisher, J. T. (2009). Effect of menopause on the chemical control of breathing and its relationship with acid-base status. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *296*(3). https://doi.org/10.1152/ajpregu.90865.2008

Putnam, R. W., Filosa, J. A., & Ritucci, N. A. (2004). Cellular mechanisms involved in CO_2 and acid signaling in chemosensitive neurons. In *American Journal of Physiology - Cell Physiology* (Vol. 287, Issues 6 56-6). American Physiological Society. https://doi.org/10.1152/ajpcell.00282.2004

Read, D. J., & Leigh, J. (1967). Blood-brain tissue PCO_2 relationships and ventilation during rebreathing. *Journal of Applied Physiology*, *23*(1), 53–70.

Rebuck, A. S., & Campbell, E. J. (1974). A clinical method for assessing the ventilatory response to hypoxia. *The American Review of Respiratory Disease*, *109*(3), 345–350. https://doi.org/10.1164/arrd.1974.109.3.345

Rebuck, A. S., & Woodley, W. E. (1975). Ventilatory effects of hypoxia and their dependence on PCO_2. *Journal of Applied Physiology*, *38*(1), 16–19. www.physiology.org/journal/jappl

Riedstra, J. W. (1963). Influence of central and peripheral PCO_2 (pH) on the ventilatory response to hypoxic chemoreceptor stimulation. *Acta. Physiol. Pharmacol. Neerl.*, *12*, 407–452.

Robertshaw, D. (2006). Mechanisms for the control of respiratory evaporative heat loss in panting animals. *J Appl Physiol*, *101*, 664–668. https://doi.org/10.1152/japplphysiol.01380.2005.-Panting

Severinghaus, J. W. (1979). Simple, accurate equations for human blood O_2 dissociation computations. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *46*(3), 599–602. https://doi.org/https://doi.org/10.1152/jappl.1979.46.3.599

Severinghaus, J. W., Naifeh, K. H., & Koh, S. O. (1989). Errors in 14 pulse oximeters during profound hypoxia. *J Clin Monit*, *5*, 72–81.

SheikhBahaei, S. (2020). Physiology: New insights into central oxygen sensing. *Current Biology*, *30*(17), R1004–R1006. https://doi.org/10.1016/j.cub.2020.06.101

Smith, C. A., Blain, G. M., Henderson, K. S., & Dempsey, J. A. (2015). Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO_2: Role of carotid body CO_2. *Journal of Physiology*, *593*(18), 4225–4243. https://doi.org/10.1113/JP270114

St Croix, C., Cunningham, D., & Paterson, D. (1996). Nature of the interaction between central and peripheral chemoreceptor drives in human subjects. *Can. J. Physiol. Pharmacol.*, *74*, 640–646.

Teppema, L. J., & Dahan, A. (2010). The ventilatory response to hypoxia in mammals: Mechanisms, measurement, and analysis. *Physiol Rev*, *89*, 675–754. https://doi.org/10.1152/physrev.00012.2009.-The

Teppema, L. J., & Smith, C. A. (2013). CrossTalk opposing view: Peripheral and central chemoreceptors have hyperadditive effects on respiratory motor control. *Journal of Physiology*, *591*(18), 4359–4361. https://doi.org/10.1113/jphysiol.2013.256818

Teppema, L. J., Van Dorp, E. L. A., & Dahan, A. (2010). Arterial [H^+] and the ventilatory response to hypoxia in humans: Influence of acetazolamide-induced metabolic acidosis. *Am J Physiol Lung Cell Mol Physiol*, *298*, 89–95. https://doi.org/10.1152/ajplung.00255.2009.-In

Tin, C., Song, G., & Poon, C. S. (2012). Hypercapnia attenuates inspiratory amplitude and expiratory time responsiveness to hypoxia in vagotomized and vagal-intact rats. *Respiratory Physiology and Neurobiology*, *181*(1), 79–87. https://doi.org/10.1016/j.resp.2012.01.008

Tipton, M. J., Harper, A., Paton, J. F. R., & Costello, J. T. (2017). The human ventilatory response to stress: Rate or depth? *Journal of Physiology*, *595*(17), 5729–5752. https://doi.org/10.1113/JP274596

Torrance, R. W. (1996). Chemoreception upstream of transmitters. *Frontiers in Arterial Chemoreception*, 13–38.

Trivedi, N. S., Ghouri, A. F., Lai, E., Shah, N. K., & Barker, S. J. (1998). Pulse oximeter performance during desaturation and resaturation: A comparison of seven models. *Crit Care Med*, *26*(6), 1128–1133.

Usselman, C. W., Luchyshyn, T. A., Gimon, T. I., Nielson, C. A., Van Uum, S. H. M., Shoemaker, J. K., & Shoemaker, J. K. (2013). Hormone phase dependency of neural

responses to chemoreflex-driven sympathoexcitation in young women using hormonal contraceptives. *J Appl Physiol*, *115*, 1415–1422. https://doi.org/10.1152/japplphysiol.00681.2013.-Hormone

Vidruk, E. H., Burt Olson, E., Ling, L., & Mitchell, G. S. (2001). Responses of single-unit carotid body chemoreceptors in adult rats. *Journal of Physiology*, *531*(1), 165–170. https://doi.org/10.1111/j.1469-7793.2001.0165j.x

Wanke, T., Formanek, D., Schenz, G., Popp, W., Gatol, H., & Zwick, H. (1991). Mechanical load on the ventilatory muscles during an incremental cycle ergometer test. *Eur Respir J*, *4*, 385–392.

Wilson, R. J. A., & Day, T. A. (2013). CrossTalk opposing view: Peripheral and central chemoreceptors have hypoadditive effects on respiratory motor output. *Journal of Physiology*, *591*(18), 4355–4357. https://doi.org/10.1113/jphysiol.2013.256578

Wilson, R. J. A., & Teppema, L. J. (2016). Integration of central and peripheral respiratory chemoreflexes. *Comprehensive Physiology*, *6*(2), 1005–1041. https://doi.org/10.1002/cphy.c140040

Yee, J., Frinak, S., Mohiuddin, N., & Uduman, J. (2022). Fundamentals of arterial blood gas interpretation. *Kidney360*, *3*(8), 1458–1466. https://doi.org/10.34067/kid.0008102021

Appendices

Appendix A: Ethical Approval.

Western Research

Date: 18 May 2022

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 Dr. [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 TriC ouncil 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]

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

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 A)**
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. How they work together to determine how much we breathe remains unknown. The purpose of this research is to assess how these two chemoreflexes regulate breathing on their own and when both are activated at the same time.

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 (TH-4120) 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. o 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 some tests, the oxygen levels will be lower than normal (similar to the end of a breath-hold or at altitude) and in other tests the level of oxygen will be higher than normal. You will not be told ahead of time whether the oxygen level in the bag is low or high.

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 low oxygen breathing is considered safe and is like what you might experience on a mountain resort. 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 and giving oxygen.

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.

Initials: _____

Western HealthSciences

Kinesiology

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

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

Nasimi A. Guluzade
School of Kinesiology, Faculty of Health Sciences
Western University
London, Ontario, Canada

Education

2021 – 2023 Master of Science (M.Sc.), Kinesiology (Integrative Biosciences), Western University, London, Ontario.

2017 – 2021 Honors Bachelor of Science (B.Sc.) Specialized, Kinesiology and Health Science, York University, Toronto, Ontario.

Honours: **Summa Cum Laude** Graduate of Specialized Honours Kinesiology and Health Science, Member of Dean's **Honour Roll** (2017-2021), Member of the Golden Key International Honour Society, Student Leadership Certificate.

Research

Peer-Reviewed Original Research Publication (4)

1. **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*. Online ahead of print.

2. **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. *Exp Physiol.* 107(12):1507-1520.

3. Ward, A.M.M., **Guluzade, N.A.**, Kowalchuk, J.M., & Keir, D.A. (2022) Coupling of $\dot{V}_E$ and $\dot{V}CO_2$ kinetics: insights from multiple exercise transitions below the estimated lactate threshold. *Eur J Appl Physiol*. 123(3):509-522.

4. Hwang, M., Nagappa, M., **Guluzade, N.**, Saripella, A., Englesakis, M., & Chung, F. (2022) Validation of the STOP-Bang questionnaire as a preoperative screening tool for obstructive sleep apnea: A systematic review and meta-analysis. *BMC Anesthesiol*. 22(1):366.

Manuscripts Under Review (2)

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

2. Faricier, R., Micheli, L., **Guluzade, N.A.**, Murias, J.M., & Keir, D.A. A modified step-ramp-step protocol to prescribe exercise intensity domain-specific exercise in treadmill running. *Med Sci Sports Exerc. (Under Review).*

Manuscripts in Preparation (2)

1. Micheli, L., Teso, M., **Guluzade, N.A.**, Rizzo, M., Ferri-Marini, C., Lucherinin, F., Keir, D.A., & Pogliaghi, S. The effect of pedalling cadence on the relationship between critical power and respiratory compensation point. *(In progress).*

2. **Guluzade, N.A.**, Golestan, P.M., Kamakh, D., Dobrovetsky, W., Wong, O., & Rotondi, M. The effect of unstable load exercises on muscle fibre activation: A systematic review and meta-analysis. *(In progress).*

Oral Conference Presentation (1)

1. **Guluzade, N.A.**, Ward, A.M.M., Kowalchuk, J.M., & Keir, D.A. (November 2022) Coupling of minute ventilation ($\dot{V}_E$) and carbon dioxide output ($\dot{V}CO_2$) kinetics: Insights from multiple exercise transitions below the estimated lactate threshold. *Canadian Society of Exercise Physiology*.

Publications in Conference Proceedings (8)

1. Faricier, R., Micheli, L., **Guluzade, N.A.**, Murias, J.M., & Keir, D.A. (2023) A Step-Ramp-Step treadmill protocol predicts exercise intensity domain-specific $\dot{V}O_2$ responses in running. *American College of Sports Medicine.*

2. **Guluzade, N.A.**, Huggard, J.D., Duffin, J., & Keir, D.A. (2023) Effect of central chemoreceptors on the peripheral chemoreflex response to hypoxia. *American Physiology Society Summit. Physiology. 38(S1).*

3. Huggard, J.D., **Guluzade, N.A.**, Duffin, J., & Keir, D.A. (2023) Does hyperoxia stimulate breathing? *American Physiology Society Summit. Physiology. 38(S1).*

4. **Guluzade, N.A.**, Ward, A.M.M., Kowalchuk, J.M., & Keir, D.A. (2022) Coupling of minute ventilation ($\dot{V}_E$) and carbon dioxide output ($\dot{V}CO_2$) kinetics: Insights from multiple exercise transitions below the estimated lactate threshold. *Canadian Society of Exercise Physiology*. *Appl. Physiol. Nutri. Metabol. S68.*

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. *Experimental Biology*. *FASEB J. 36(S1)*.

6. Huggard, J.D., **Guluzade, N.A.**, Keltz, R.R., & Keir, D.A. (2022) Data reproducibility of respiratory chemoreflex characterization by modified rebreathing. *Experimental Biology*. *FASEB J. 36(S1).*

7. Keltz, R.R., **Guluzade, N.A.**, Huggard, J.D., & Keir, D.A. (2022) The relationship between central and peripheral chemoreflex sensitivities and $\dot{V}_E$-$\dot{V}CO_2$ slope below and above the respiratory compensation point of incremental exercise. *Experimental Biology*. *FASEB J. 36(S1).*

8. **Guluzade, N.**, Hwang, M., Saripella, A., Nagappa, M., & Chung, F. (2021) Validation of the STOP-Bang questionnaire as a preoperative screening tool for obstructive sleep apnea: A systematic review and meta-analysis. *Society of Anesthesia and Sleep Medicine Annual Meeting*.

Scholarships and Awards

Research Scholarships

2022 – 2023 **Natural Sciences and Engineering Research Council of Canada (NSERC)**, Canadian Graduate Scholarship – Master's (CGS-M) – funded for 3 terms ($17,500).

2022 – 2023 **Ontario Graduate Scholarship (OGS)**, Ministry of Training, Colleges, and Universities – Declined ($15,000).

2022 – 2023 **Western Graduate Research Scholarship**, Western University Institutional Scholarship – funded for three terms ($2,500).

Travel Grants and Other Awards

2023 **Faculty of Health Science Graduate Student Travel Award,** Western University ($250).

2023 **School of Kinesiology Travel Award,** Western University ($500).

2020 – 2021 **York University Continuing Student Scholarship,** York University Institutional Scholarship – funded for three terms ($770).

2019 – 2020 **York University Continuing Student Scholarship,** York University Institutional Scholarship – funded for three terms ($783).

2018 – 2019 **York University Continuing Student Scholarship,** York University Institutional Scholarship – funded for three terms ($550).

2017 – 2018 **HSBC Scholarship,** York University – funded for three terms ($5,000).

2017 – 2018 **York University Automatic Entrance Scholarship,** York University – funded for three terms ($2,500).

2017 – 2018 **York University Student Life Award** – funded for three terms ($500).

Work and Teaching Experience

Undergraduate course guest lecture

2022/11 **Guluzade, N.A.**, Laboratory in Exercise Physiology, Kinesiology 3330A, Western University.

Graduate Teaching Assistant

2023/01 – 2023/04 Kinesiology 2230B – Introduction to Exercise Physiology, Western University.

2023/01 – 2023/04 Kinesiology 4433B – Physiology of Exercise Training, Western University.

2022/09 – 2022/12 Kinesiology 3330A – Laboratory in Exercise Physiology, Western University.

2022/01 – 2022/04 Kinesiology 2230B – Introduction to Exercise Physiology, Western University.

2021/09 – 2021/12 Kinesiology 3339A – Exercise Nutrition, Western University.

Professional Development and Training

2023/04 Phlebotomy Workshop, The Canadian College of Healthcare & Pharmaceutics.

2023/02 Research Workshop: Doing Research with Fitness Tracking, Western University.

2022/11 YSI-2500 Glucose/Lactate Operator Training, Western University.

2022/10 Building Inclusivity through Anti-Racism, Western University.

2021/09 Accessibility for Ontarians with Disability Act (AODA) - Accessibility in Teaching, Western University.

2021/09 WHMIS Training, Western University.

2021/09 Biosafety, Western University.

2021/09 Mental Health at Western, Western University.

2021/09 Laboratory Safety and Hazardous Waste Management, Western University.

2020/09 Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics, Government of Canada.

2019/07 Standard First Aid with CPR-C, LifeSaving Society.