EFFECTS OF RESPIRATORY MUSCLE ENDURANCE TRAINING ON CEREBRAL OXYGENATION AND HEMODYNAMICS, AND EFFORT PERCEPTIONS DURING MAXIMAL EXERCISE

by

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Abstract

The primary objective of this study was to investigate the effects of a 4-week respiratory muscle endurance training (eRMT) program on the physiological and psychological aspects of central fatigue using, respectively, near-infrared spectroscopy (NIRS) and quantification of effort perceptions during maximal exercise. A secondary objective was to assess any impact of eRMT on respiratory health and exercise performance. This study compared pre- and post-eRMT data from the same group of healthy adults. The results indicated that eRMT did not have any effect on respiratory function, exercise time to exhaustion, or physiological responses to exercise but significantly decreased ratings of perceived exertion (RPE) during exercise. An increase in the concentrations of oxygenated hemoglobin [O_2Hb], deoxygenated hemoglobin [HHb], and total hemoglobin [tHb] during exercise was observed post-eRMT compared to pre-eRMT, and this increase differed by hemisphere. Based on these preliminary findings, we suggest an eRMT-induced left-to-right hemodynamic shift during exercise, consistent with the change from a novel to a learned task.

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Introduction

The basic rhythm of respiration or breathing is regulated and generated by various neuronal nuclei within the brain and spinal cord. Acute exercise, especially endurance exercise and moderate-to-high intensity exercise, induces various changes in ventilation and respiration such as an increase in breathing rate, heart rate (HR), etc., which results in several modifications to these brain regions. Moreover, these exercise-induced physiological changes are believed to impact an individual's tolerance to exercise, that is, their capacity to withstand physical exertion before reaching a state of exhaustion (Coyle, Coggan, Hopper, Walter, 1988).

It is evident that diminished or poor exercise tolerance, often referred to as exercise performance, can impair quality of life or lead to adverse health effects such as an increased risk of illness/mortality, not only in sedentary individuals but also in individuals with moderate fitness levels or even athletes (Marcora & Staiano, 2010). Peripheral fatigue of the respiratory and locomotor muscles may account for poor exercise performance in trained individuals (Harms et al., 1997, 1998; Legrand et al., 2007), however, experimental models of peripheral fatigue are unable to account for all aspects of fatigue. Consequently, research has shifted to the notion that central fatigue—a multifaceted aspect of fatigue associated with physiological and psychological factors in central nervous system (CNS) function—may also play an important role in exercise performance (Kayser, 2003; Nybo & Rasmussen, 2007; Subudhi et al., 2009).

Respiratory muscle training (RMT), which entails breathing exercises designed to target specific inspiratory and/or expiratory muscles, is a technique aimed at improving ventilatory function in diseased (e.g., COPD patients) and healthy (i.e., undiseased) populations as well as augment exercise performance in healthy individuals. RMT has been demonstrated to improve the endurance and strength of respiratory muscles, thereby improving ventilation (Verges et al.,

2009; Illi et al., 2012). Such benefits may also contribute to the attenuation of central fatigue (Caine & McConnell, 1998; Edwards, 2013; Romer, McConnell, & Jones, et al., 2002). Specifically, improving the contractile properties of respiratory muscles causes a reduction in hyperpnoea during exercise. In turn, this can decrease the sense of respiratory exertion or breathlessness, thereby reducing the conscious perception of effort associated with a given exercise task and further delaying the onset of central fatigue (Caine & McConnell, 1998; Edwards, 2013; Romer et al., 2002). Moreover, because central fatigue is in part provoked or modulated by inadequate oxygen (O_2) delivery to the brain (Nybo & Rasmussen, 2007), improving the oxidative metabolism of the respiratory muscles reduces the amount of O_2 the respiratory muscles consume during exercise, possibly resulting in more O_2 available for the brain and other muscles (i.e., respiratory muscle metaboreflex; Dempsey, Romer, Rodman, Miller, & Smith, 2006). Further, the onset of hypoxemia that often accompanies high-intensity exercise may be delayed, thus allowing cerebral O_2 delivery to increase as per usual during conditions of low partial pressure of O_2 (PO_2). Such changes in regional brain oxygenation and hemodynamics can be measured in real-time using near-infrared spectroscopy (NIRS; Jobsis, 1977; Subudhi et al., 2009). NIRS is a non-invasive neuroimaging tool in which changes in the absorption of light are continuously recorded and used to estimate evoked concentration changes of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) that result from local vascular and oxygenation effects that occur during brain activity (Jobsis, 1977). The utilization of NIRS in the prefrontal cortex (PFC) in response to a regular RMT program may offer insight into the central fatigue mechanisms that limit exercise performance.

Literature Review

The overall intent of this literature review is to discuss the potential mechanisms that underlie limitations in exercise tolerance/performance in humans and how respiratory muscle training (RMT) may be able to improve this. However, in order to understand the physiological processes that limit exercise tolerance, the control mechanisms of respiration and the corresponding areas in the brain first must be discussed. The first major focus of this literature review will be the respiratory areas and structures within the brain that currently are believed to play a role in regulating, maintaining, and generating respiration and its rhythmical pattern. The second focus will be the role of several feedback and feedforward mechanisms along with inputs to the brainstem respiratory areas discussed in the first section, which increase the ventilatory response in accordance with exercise. Lastly, mechanisms of peripheral and central fatigue related to exercise tolerance will be considered, along with RMT as a potential technique to enhance exercise tolerance by attenuating central fatigue. An additional focus of this literature review will be near-infrared spectroscopy (NIRS) because it is an inexpensive, non-invasive instrument that has been consistently demonstrated to provide a reliable, real-time measurement of cerebral oxygenation and hemodynamics in humans.

Regulation of Respiration

Respiration Overview

From an anatomical perspective, the human respiratory system is responsible for the movement of air and gases into and out of the lungs through the process of ventilation, commonly referred to as respiration or breathing. The term respiration also refers to cellular respiration, which, in brief, is the process by which living cells produce energy (in the form of adenosine triphosphate; ATP) through the oxidation of organic substances. Although distinct,

these two processes are related because breathing provides the oxygen (O_2) needed in cellular respiration to produce ATP. Moreover, breathing rids the body of carbon dioxide (CO_2), the waste product produced during cellular respiration through ventilation.

The normal (eupneic) periodic motor pattern of breathing typically consists of three main phases—inspiration, post-inspiration, and active/late expiration—although, they often are referred to simply as inspiration and expiration (Richter, 1996). The muscular force required to produce inspiration is a result of spinal motoneurons innervating and stimulating the diaphragm, external intercostals, pectoralis minor, sternocleidomastoid (SCM), and scalenes. Expiration generally is produced by the passive recoil of the lungs and diaphragm, but during loaded breathing, as occurs during exercise, active expiration is assisted by the contraction of the internal intercostal and abdominal muscles (Mateika & Duffin, 1995). Typically, the inspiratory phase lasts two seconds and the expiratory phase lasts approximately three seconds. These cycle continuously to produce a resting eupneic respiratory rate ranging from 12 to 21 breaths per minute, which, for a middle-aged adult, varies depending on the overall health of the individual (e.g., body mass index, stress level, illness, medical conditions, etc.), environmental factors (e.g., air quality), and the assessment technique used (e.g., conventional [visual] vs. objective methods [spirometer, capnometer, impedance pneumograph]; Lapi et al., 2014; Hannon, Pooler, & Porth, 2010; Tobin, Chadha, Jenouri, Birch, Gazerogula, Sackner, 1983).

The control of breathing can be either autonomic or voluntary. The regulation of respiration is typically an autonomic action that occurs mainly through neural inputs to the respiratory centres (discussed further below), but voluntary control is possible. For example, the rate of the respiratory movements can be consciously decreased by deliberate cessation of breathing airflow (i.e., holding one's breath) referred to as voluntary apnea. Likewise, the rate of

respiration can be increased by hyperventilation—a condition of abnormally prolonged and rapid breathing resulting in a state of reduced CO_2 levels in the blood known as hypocapnia. Hyperventilation can be voluntary, in which an individual volitionally over-breathes excessively, or involuntary, which can occur in response to physical and emotional stimuli such as exercise, injury, respiratory disorders, stress, fear, pain, and anxiety. Nevertheless, voluntary apnea, and to a lesser extent, hyperventilation, only can occur for a given period of time before the respiratory centre resumes its normal automatic control of respiration.

Respiratory Areas in the Brainstem

The process of respiration and the muscles involved are fairly well understood; yet the specific neuronal networks and mechanisms responsible for regulating respiratory movements still are debated. It is important to note that in delineating subcortical structures, regions, and neurons, the majority of existing research, and consequently a large portion of the literature discussed in this review, utilizes animal models, particularly rodents and small mammals. Collectively, this evidence indicates that the breathing phases/movements are produced by several networks of respiratory neurons in the medulla oblongata and the pons—referred to as respiratory centres—that coordinate activity of spinal and cranial motoneurons to generate the rhythmic patterns of alternating inspiratory and expiratory airflows (Figure 1 and 2; Rybak, Abdala, Markin, Paton, & Smith, 2007; Smith, Abdala, Borgmann, Rybak, & Paton, 2013; Smith, Abdala, Koizumi, Rybak, Paton, 2007). The respiratory spinal motorneuron outflow that supplies respiratory muscles originates in the medullary respiratory centre (Mateika & Duffin, 1995; Richter & Spyer, 2001). This centre is considered the primary respiratory control centre consisting of two neuronal clusters: the dorsal respiratory groups (DRGr) and the ventral respiratory groups (VRGr; Cohen, 1979; Mateika & Duffin, 1995; Smith et al., 2013). The

second respiratory centre, the pontine respiratory group, may play a role in fine-tuning the

breathing pattern by controlling the timing of the inspiratory/expiratory phase transition,

although it is not believed to be essential for generating respiratory movements (Dutschmann &

Dick, 2012; Dutschmann & Hebert, 2006; Mörschel & Dutschmann, 2009; Song, Yu, & Poon,

2006).
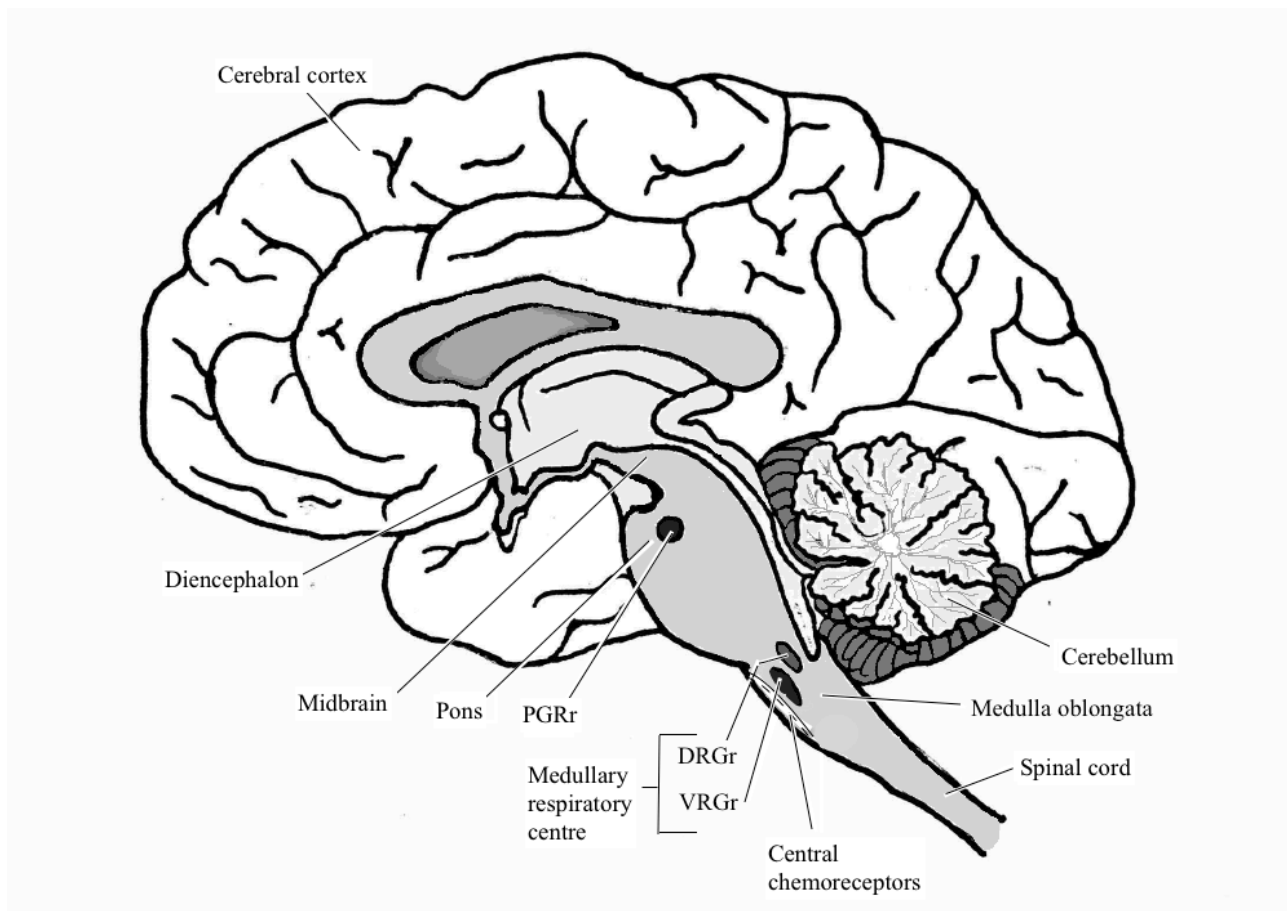
Figure 1. A midsagittal view of the human brain showing some of the major features and respiratory areas in the brainstem. Abbreviations: PRG, pontine respiratory group; DRGr, dorsal respiratory group; VRGr, ventral respiratory group. This image is an orginal work drawn by the author of this thesis.

Dorsal Respiratory Group Neurons. The DRGr, part of the nucleus tractus solitarius (NTS) complex, are composed mainly of inspiratory neurons located bilaterally in the dorsal part of the medulla oblongata (Smith et al., 2013). Aside from their control over voluntary respiration during speaking, laughing, exercise, etc., evidence suggests that the impulses originating in the DRGr neurons regulate the activity of the phrenic nerves to the diaphragm and thus have primary responsibility for inspiration. In addition, the DRGr is responsible for stimulating the external intercostals to further facilitate inspiration (Hannon et al., 2010; Mateika & Duffin, 1995). They receive afferent input from various respiratory-related chemoreceptors and mechanoreceptors via the glossopharyngeal and vagus nerves as well as the spinal cord (Mateika & Duffin, 1995; Palkovits & Zaborszky, 1977). In addition to receiving afferent input from peripheral visceral sources, studies in experimental animals have demonstrated that descending afferents from higher cortical regions such as the medial prefrontal cortex (PFC), insular cortex, and sensorimotor cortex synapse onto neurons located in the NTS (Buchanan, Thompson, Maxwell, & Powell, 1994; Degtyarenko & Kaufman, 2006; M'hamed, Sequeira, Poulain, Bennis, & Roy, 1993; Neafsey, Hurley-Gius, & Arvanitis, 1986). Consequently, these neural inputs from peripheral receptors and supramedullary structures facilitate appropriate modification of respiration.

Ventral Respiratory Group Neurons. In comparison, the VRGr are located in the ventrolateral region of the medulla referred to as the ventral respiratory columns (VRCs; Figure 2). They contain both inspiratory and expiratory neurons that, respectively, are concentrated in regions termed the rostral (rVRGr) and caudal (cVRGr) ventral respiratory groups (Felman & Del Negro, 2006; Smith et al., 2013). The latter stimulate both the abdominal muscles and the internal intercostal muscles via the intercostal nerves, and thus are responsible chiefly for

expiration. That is, they are responsible for the return of the thoracic cage to its compressed size, especially during exercise, when expiration is facilitated by contraction of internal intercostals (Hannon et al., 2010; Mateika & Duffin, 1995; Seeley et al., 2011). Premotor neurons of the rVRGr relay inspiratory drive to spinal and phrenic motoneurons that innervate the diaphragm, and thus are secondarily responsible for initiating inspiration, after the DRGr (Smith, Abdala, Rybak, & Paton, 2009; Smith et al., 2013). Both the rVRGr and cVRGr receive neural input from the DRGr, and evidence suggests that they are driven and inhibited by neuronal rhythmogenic microcircuits located in additional compartments of the VRCs such as the Bötzinger complex (BötC) and pre-Bötzinger complex (pre-BötC), discussed in detail further below (Feldman & Del Negro, 2006; Smith et al., 2009; Smith et al., 2013).
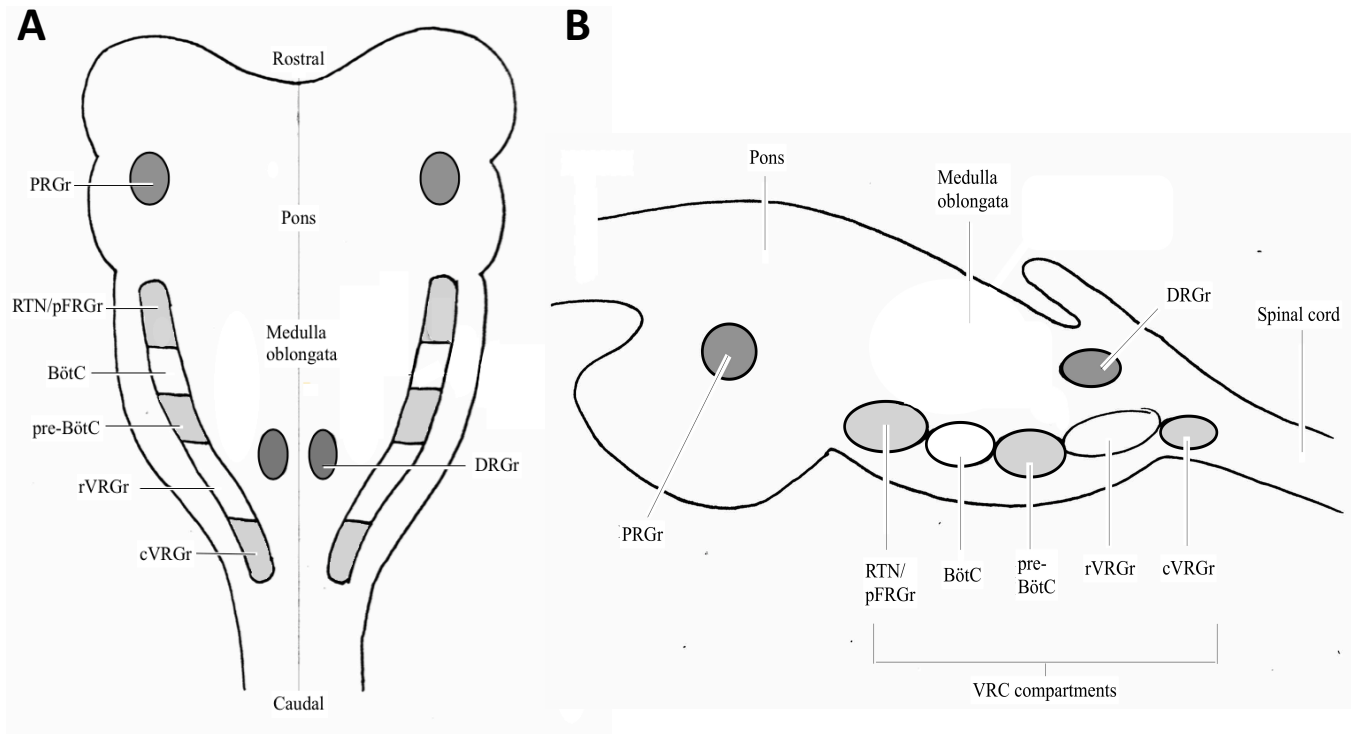
Figure 2. A simplified structural view of the bilaterally compartmentalized microcircuits in the rat brainstem hypothesized to be involved in respiratory rhythm and pattern generation represented neuroanatomically by (A) a horizontal brainstem section and (B) a parasagittal section through one side of the brainstem. Abbreviations: PRG, pontine respiratory group; RTN/pFRGr, retrotrapezoid nucleus/parafacial respiratory group; BötC, Bötzinger complex; pre-BötC, pre-Bötzinger complex; rVRGr, rostral ventral respiratory group; cVRGr, caudal ventral respiratory group; DRGr, dorsal respiratory group; VRC, ventral respiratory column. This image is an orginal work drawn by the author of this thesis.

Pontine Respiratory Group Neurons. Historically, the pontine respiratory centre has been shown to include the apneustic centre and the pneumotaxic centre, both of which are believed to modify the activity of the neuronal clusters of the medullary respiratory centre. The apneustic centre, located in the lower portion of the pons, is thought to have an excitatory effect on inspiration, tending to prolong the activity of the inspiratory neurons by preventing them from being switched off. Likewise, the pneumotaxic centre, which is situated in the dorsal part of the upper pons, is believed to inhibit inspiratory neurons in the DRGr and thus switch off inspiration (Hannon et al., 2010). However, no specific groups of neurons yet have been found in the apneustic centre and, accordingly, many theories regarding this centre have since been abandoned (Kam & Power, 2012).

Although many still refer to these two respiratory centres as separate entities, the pneumotaxic centre is now generally called the pontine respiratory group (PRGr) and is comprised of expiratory and inspiratory neurons in the pontine nuclei (Dutschmann & Hebert, 2006; Ezure, 2004; Kam & Power, 2012; Smith et al., 2009; Song et al., 2006). The precise function of this group is unknown; however, studies have demonstrated that the PRGr has reciprocal connections with the VRGr and DRGr in the medulla (Ezure, 2004; Song et al., 2006). Previous animal experiments have demonstrated that stimulation of the PRGr leads to changes in breathing pattern (Mutolo, Bongianni, Carfi, & Pantaleo, 1998; Okazaki, Takeda, Yamazaki, & Haji, 2002) and, consequently, researchers suggest that the PRGr plays a role in regulating the respiratory transitions between inspiration and expiration, thus calibrating the breathing pattern (Dutschmann & Dick, 2012; Dutschmann & Hebert, 2006; Mörschel & Dutschmann, 2009; Song et al., 2006). However, given that the PRGr does not appear to have any connections with the muscles of respiration (i.e., it does not innervate any respiratory muscles) it is not considered

essential for regulating the basic respiratory rhythm (Dutschmann & Dick, 2012; Mörschel & Dutschmann, 2009).

Taken together, the literature summarized above provides evidence that the respiratory centres—especially the medullary centre—play a vital role in respiration by providing neural outflow to the respiratory muscles. However, the question remains as to how the respiratory rhythm is generated or, more specifically, what neuronal microcircuits generate the rhythmic breathing pattern.

Respiratory Rhythmogenesis

Central Pattern Generators. The generation of innate rhythmic motor behaviours such as respiratory movements are produced by cyclic neural activity generated within neural networks or microcircuits located in the brainstem. These semi-autonomous neural networks, often referred to as central pattern generators (CPG) or rhythm generators, consist of excitatory and inhibitory interneurons that interact to generate rhythmic patterns of motor activity without phasic sensory input from other feedback signals (Barlow & Estep, 2006; Marder & Bucher, 2001). It is widely acknowledged that the role played by CPGs in mammalian respiratory rhythmogenesis is critical to normal function, but there is little consensus concerning the locations and neural mechanisms involved in these circuits. However, modification of the CPGs' motor pattern according to the body's metabolic needs is thought to be controlled by numerous afferent inputs from other brainstem areas/structures including pontine nuclei, raphe nuclei, and the DRGr/NTS as well as ascending neural inputs from various central and peripheral receptors, and descending inputs from higher brain regions (Mateika & Duffin, 1995; Rybak et al., 2007; Smith et al., 2007).

The current understanding is that respiratory CPG microcircuits are located in the VRCs of the medulla. With assistance from the rVRGr and cVRGr, these microcircuit compartments constitute the system—referred to as the pontine-medullary respiratory network—that generates and maintains the rhythmogenic breathing pattern (Rybak et al., 2007; Smith et al., 2007; Smith et al., 2009; Smith et al., 2013). It is hypothesized that these compartments exhibit a spatial and functional organization that extends bilaterally in the rostral-to-caudal direction from the rostral pons to the caudal parts of the medulla, in which each functional compartment is controlled by more rostral compartments (Rybak et al., 2007; Smith et al., 2007; Smith et al., 2013). Although there is considerable debate over the fundamental question of which parts of the VRC are essential, it is postulated that the core circuit components that constitute the neural machinery for generating respiratory rhythmogenesis are distributed among two adjacent structural compartments in the ventrolateral medulla: the pre-BötC (Gray et al., 2010; Gray, Janczewski, Mellen, McCrimmon, & Feldmam, 2001; Gray, Rekling, Bocchiaro, & Feldman, 1999; Smith et al., 2007; Smith, Ellenberger, Ballanyi, Richter, & Feldman, 1991) and the BötC (Jiang & Lipski, 1990; Smith et al., 2007; Tian, Peever, & Duffin, 1999). In addition, it recently has been suggested that the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRGr) also may play a role (Feldman & Del Negro, 2006; Janczewski & Feldman, 2006).

Pre-Bötzinger Complex. The structure hypothesized to be the dominant site of respiratory rhythmogenesis is the pre-BötC, a small region of the ventrolateral medulla (Gray et al., 2010; Gray et al., 2001; Gray et al., 1999; Smith et al., 2007; Smith et al., 1991). The cellular composition of this complex is heterogeneous, containing several types of respiratory neurons including a subset of glutamatergic neurons that exhibit pacemaker-like properties that are proposed to be primarily responsible for generating rhythmic excitatory-inspiratory drive (Gray

et al., 2001; Gray et al., 2010). In addition, subpopulations of GABAergic (Kuwana et al., 2006) and glycinergic neurons (Winter et al., 2009) found in the pre-BötC also may play a small role in coordinating the inspiratory-expiratory pattern formation via inspiratory inhibition (i.e., inhibition of expiratory neurons during inspiration; Smith et al., 2013).

This notion of the pre-BötC as the fundamental respiratory rhythm generator originated from *in vitro* experiments and was widely disseminated in the early 1990's especially after Suzue (1984) reported that an *in vitro* preparation of the neonatal rat brainstem and spinal cord (i.e., *en bloc* preparation) generates a spontaneous rhythmic motor output. Since then, a variety of evidence from both *in vivo* and *in vitro* studies has supported the hypothesis concerning the pre-BötC as the dominant respiratory generator. Smith and colleagues (1991) demonstrated, by performing successive microsections in the newborn rat brainstem *en bloc* preparations, that perturbations in respiratory rhythm occurred when the area just caudal to the level of the retrofacial nucleus was reached. Removal of this region resulted in elimination of rhythmic inspiratory activity (Smith et al., 1991). Gray and colleagues (2001) demonstrated that near complete bilateral lesion of neurokinin-1 receptor neurons—a subclass of glutamatergic pre-BötC neurons—in intact, conscious adult rats induced an irregular, ataxic breathing pattern. Moreover, reduction of pre-BötC excitatory neuron activity by microinjection of cyanquixaline (or CNQX, an antagonist to excitatory NDMA activity) into the pre-BötC in medullary slices abolishes respiratory rhythm (Smith et al., 1991). Likewise, *in vivo* studies also demonstrated that injection of excitatory amino acid antagonist drugs in the pre-BötC of the cat (Abrahams, Hornby, Walton, Taveira, & Gills, 1991) and rat (Tan, Janczewski, Yang, Shao, Callaway, & Feldman, 2008) leads to perturbed rhythmogenesis and occasionally induces apnea.

Bötzinger Complex. Although most existing evidence points to the pre-BötC as the centre of integration and generation of respiratory rhythm, additional research suggests that the rhythmogenic microcircuits of the BötC may also play a role in rhythmogenesis (Jiang & Lipski, 1990; Tian et al., 1999). Animal experiments have shown that the BötC is located in the rostral part of the VRC, near the retrofacial nucleus, and contains mainly expiratory neurons with an augmenting or ramping pattern of discharge in which the amplitudes progressively grow (Grélot, Bianchi, Iscoe, & Remmers, 1988; Tian et al., 1999, Smith et al., 2007). Consequently, the BötC is considered to have an exclusive role in expiratory pattern generation (Smith et al., 2009; Smith et al., 2013). Moreover, these BötC expiratory neurons, via axonal projections and synaptic connections, inhibit various inspiratory neurons in the VRGr and other VRC compartments during the late part of expiration suggesting that the BötC neurons may be involved in the control of the phase alternation during normal breathing (Jiang & Lipski, 1990; Smith et al., 2007; Tian et al., 1999).

When examining the pontine-medullary respiratory network collectively, transections or ablations of specific respiratory regions of the network can transform the eupneic three-phase respiratory pattern to other rhythmic patterns with fewer active phases. This has been demonstrated by Smith and colleagues (Rybak et al., 2007; Smith et al., 2007) who performed a series of sequential rostral-to-caudal transections on perfused juvenile rat *en bloc* preparations. The removal of rostral pontine circuits converted the eupneic three-phase respiratory rhythm into a two-phase inspiratory-expiratory pattern (lacking the post-inspiratory phase) generated by alternating activity of intact BötC and pre-BötC circuits. This two-phase rhythm, in turn, transformed to one-phase inspiratory pattern originating within the pre-BötC circuits once the BötC region was removed. Lastly, removal of the pre-BötC eliminated the rhythmic inspiratory

activity, as originally demonstrated by Smith et al. (1991). Therefore, it appears that pontine input to medullary circuits contributes to post-inspiratory activity, while the BötC and pre-BötC, respectively, contribute significantly to the expiratory and inspiratory activity of respiration (Rybak et al., 2007; Smith et al., 2007).

  Retrotrapezoid Nucleus and Parafacial Respiratory Group. Despite the fact that the majority of literature suggests that the neuronal clusters of the pre-BötC and BötC as the fundamental circuit components for generating respiratory rhythm, a developing view concerning an alternate region of respiration-regulating nuclei has emerged. This view holds that two anatomically overlapping neuronal groups in the parafacial region—namely, the retrotrapezoid nucleus (RTN) and the parafacial respiratory group (pFRGr)—contain functionally distinct populations of neurons involved in several interrelated basic respiratory functions including rhythmogenesis and chemoreception (Mulkey et al., 2004; Onimaru & Homma, 2003; Onimaru, Kumagawa, & Homma, 2006; Smith et al., 2009). The RTN was discovered via retrograde tracing experiments in adult rats and cats (Smith, Morrison, Ellenberger, Otto, & Feldman, 1989), in which it initially was identified as a cluster of neurons that innervates the respiratory neurons in the lower medulla. As a result of its connections and location (i.e., close proximity to the BötC), the RTN was proposed to possess interesting respiratory characteristics (Connelly, Ellenberger, & Feldman, 1989; Smith et al., 1989). Experiments further examining this area in neonatal rats found neuronal clusters of pre-inspiratory neurons and some inspiratory neurons located in the superficial region ventrolateral to the facial nucleus, which was named, accordingly, the pFRGr (Mellen, Janczewski, Bocchiaro, & Feldman, 2003; Onimaru & Homma, 2003; Onimaru et al., 2006).

Additional research discovered that the pre-inspiratory neurons are paired-like homeobox 2b (Phox2b)-expressing glutamatergic neurons (Onimaru, Ikeda, & Kawakami, 2008; Stornetta et al., 2006) that, during the neonatal period in rats, have central respiratory chemoreceptor properties as they are directly and selectively activated by both central and peripheral chemoreceptor stimulation (Ominaru & Homma, 2003; Onimaru, Kumagawa, & Homma, 2006; Onimaru et al., 2008). Furthermore, it has been proposed that these Phox2b-expressing glutamatergic neurons have intrinsic inspiratory rhythmogenic properties. Mellen and colleagues (2003) demonstrated through opioid-induced quantal (i.e., step-like) slowing of inspiratory rhythm that the pre-inspiratory neurons in the RTN/pFRGr region as well as the pre-BötC pre-inspiratory neurons are rhythmically active in neonatal rat *en bloc* preparations. They state that the observed "quantal slowing likely arises out of the mutual coupling of these two oscillatory networks" (Mellen et al., 2003, p. 821-822). Additional support for this proposed rhythmogenic function stems from partial bilateral lesioning of the RTN/pFRGr region in *en bloc* preparations, which causes a distinct reduction in the respiratory rate (Ominaru & Homma, 2003). It also has been shown that when mice are transgenically modified to bear a *PHOX2B* mutation—that in humans causes congenital central hypoventilation syndrome (CCHS)—these mice lack Phox2b-expressing neurons in the parafacial region and have lethal breathing deficits at birth (Dubreuil et al., 2008). Consequently, it has been proposed that the RTN/pFRGr activity represents an expiratory oscillator that interacts with and is coupled with the inspiratory oscillator in the pre-BötC to generate coordinated breathing patterns of inspiratory and expiratory activity, a similar role to that proposed for the BötC (Felman & Del Negro; Janczewski & Feldman, 2006; Onimaru & Homma, 2003). Other investigators have proposed that the neurons within the RTN/pFRGr

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region provide a primary rhythmic excitatory drive that entrains the pre-BötC inspiratory oscillator (Onimaru et al., 2006).

The aforementioned studies demonstrate that a rhythmogenic function may indeed be present in the RTN/pFRGr neurons in neonatal rats, yet it is unclear whether this function persists in adulthood (Guyenet et al., 2005). For example, Abbott and colleagues (2009, 2011) observed increases in breathing rate, inspiratory activity, and active expiration elicited by continuous high frequency photostimulation of the RTN-Phox2b neurons in conscious, anesthetized adult rats under normocapneic conditions; these effects were occluded by hypercapnic conditions. This tonic activation of the respiratory network, however, decays with a ten-second time constant that, on the premise of excessively slow synaptic transmission, rules out a "respiratory rhythmogenic role in adult rodents in which the respiratory cycle lasts from 0.5 to 1 second" (Abbott et al., 2011 p. 16416). To further explore this, Abbott and colleagues (2011) tested whether phasic activation of the RTN neurons has the ability to entrain the respiratory rhythm in the adult. They found that, although the tonic activation of the RTN-Phox2b neurons mentioned above produces far greater breathing stimulation, the RTN-Phox2b neurons' respiratory rhythm can be set by phasically activating them, too. Thereby the Phox2b neurons in adult rats retain their central chemoreceptor properties and discharge tonically, but likely do not retain their rhythmogenic properties, although it cannot be ruled out entirely (Abbott et al., 2009; Abbott et al., 2011; Mulkey et al., 2004). Considering the evidence to date, it can therefore be argued that the rat RTN contains neurons described as central chemoreceptors in the adult and respiratory rhythm-generation pacemakers only in neonates (i.e., pFRGr).

Respiration During Exercise

It goes without saying that with exercise, especially of moderate- to high-intensity, the rate and depth of respiration relative to eupnea drastically increases in response to metabolic needs (Mateika & Duffin, 1995). Such modifications are thought to be the result of several intricate neural circuits, and feedback and feedforward mechanisms that relay information to the previously discussed respiratory areas and neuronal clusters within the brain (Mateika & Duffin, 1995; Williamson et al., 2006). This current section aims to summarize the ventilation changes that occur during exercise and then to discuss several different mechanisms that are capable of controlling respiration during exercise.

Ventilation Phases and Gas Exchange

Exercise entails an increased transformation rate of substrate free energy into mechanical energy for muscle contraction, during which requirements for O_2 and substrate in skeletal muscle are increased, as are the removal of CO_2 and metabolites. In order to maintain homeostasis by replenishing the O_2 extracted from the blood by working muscles, while preventing an accumulation of CO_2, alveolar ventilation (V_A, i.e., the exchange of gas between the alveoli and the external environment) must increase proportionally with exercise; thereby increasing the rate and depth of respiration drastically as the inspiratory and expiratory phases shorten (Mateika & Duffin, 1995; Seeley et al., 2011). The physiological response to exercise is dependent on various factors such as exercise characteristics (intensity, duration, etc.), environmental conditions (air quality, elevation level, etc.), and individual health characteristics (lifestyle, diet, etc.).

A review by Mateika and Duffin (1995) described three distinct phases in ventilation following the transition from rest to moderate intensity constant-load exercise in healthy

subjects. The first phase, considered the fast response, occurs at the onset of exercise and is characterized by a rapid increase in ventilation (Mateika & Duffin, 1995; Prabhakar & Pang, 2004). This rise in V_A parallels a simultaneous increase in the pulmonary gas exchange of O_2 and CO_2. An increase in pulmonary capillary blood flow and blood volume are observed and the alveolar-arterial gradient for O_2 widens (i.e., the difference between the alveolar concentration of O_2 and the arterial concentration of O_2 becomes larger). Despite this widening, the partial pressures of oxygen and carbon dioxide (P_{O_2} and P_{CO_2}, respectively) and, thus, the respiratory exchange ratio remain relatively constant (Wetter & Dempsey, 2008). Next a gradual increase in ventilation, O_2 uptake, and CO_2 elimination occur over a 2-3 minute period—this constitutes the second, slow response phase. The third phase is characterized by a steady-state, nonlinear increase in ventilation and a pulmonary gas exchange that matches the metabolic rate. The conclusion of exercise is represented by a fourth, recovery phase during which ventilation decreases abruptly and then declines in an exponential manner back to resting levels (Mateika & Duffin, 1995; Prabhakar & Pang, 2004).

During prolonged moderate intensity exercise or during heavy constant-load exercise, gas exchange generally is well maintained and the first two ventilation phases remain relatively similar to those previously discussed. Occasionally, however, the normally steady-state phase (i.e., the third phase) is characterized by an increase in ventilation referred to as hyperpnoea, which occurs to meet the increasing metabolic demands (Mateika & Duffin, 1995; Wetter & Dempsey, 2008). If this hyperpnoea becomes extremely fast-paced and the V_A becomes excessive (i.e., CO_2 elimination exceeds the body's metabolic production of CO_2), hyperventilation develops and, if persistent, leads to hypocapnia. Consequently, this state of decreased arterial P_{CO_2} leads to respiratory alkalosis—a condition of elevated blood pH resulting

from hyperpnoea—which although is normally well tolerated, can have profound metabolic

effects such as cerebral vasoconstriction that impact exercise tolerance (discussed further below;

Hellstrom, Fischer-Colbrie, Wahlgren, & Jogestrand, 1996; Moraine, Lamotte, Berré, Niset,

Leduc, & Naeijel, 1993; Nybo & Rasmussen, 2007; Ogoh & Ainslie, 2009).

Neural Inputs to the Respiratory Areas

The aforementioned phases in ventilation during exercise are primarily mediated by

alterations in parasympathetic and sympathetic neural activity; however, the neurophysiological

basis for these changes in autonomic neural outflow remains obscure (Williamson et al., 2006).

Literature suggests that these exercise-induced modifications—hyperpnoea in particular—are

mediated via the combined effect of multiple neural mechanisms and inputs to the respiratory

centres (Mateika & Duffin, 1995; Wutilliamson et al., 2006). These mechanisms can be divided

into ascending inputs involving various receptor mechanisms and descending inputs involving

central mechanisms within higher brain regions (Mateika & Duffin, 1995).

Ascending Inputs. Ascending inputs, said to play a role in detecting and generating an

appropriate cardiorespiratory response to exercise, include feedback mechanisms from

chemoreceptors as well as other receptors including proprioceptors, mechanoreceptors, and

nociceptors located throughout the body in muscles, lungs, chest wall, heart, etc. (Mateika &

Duffin, 1995).

Central and Peripheral Chemoreceptors. Chemoreceptors, which include central and

peripheral chemoreceptors, monitor and respond to changes in the partial pressures of arterial O_2

and CO_2 as well as shifts in the blood hydrogen ion concentration (i.e., pH). They are an

important sensory component of the negative feedback loop that aims to control respiratory

activity by indirectly adjusting ventilation according to the metabolic demands of the body in an

attempt to maintain relatively constant partial pressures/concentrations of blood molecules. The central chemoreceptors are located on the ventrolateral surface of the medulla, somewhat remote from the medullary respiratory centre to which they are connected by afferent pathways. These neurons are sensitive to pH changes of their immediate environment—the brain extracellular fluid (bECF). Such changes can be produced by fluctuations in the arterial P_{CO_2} and P_{O_2} since the bECF is composed partially of gases amongst other ions, amino acids, metabolites, and waste products as well as changes in the composition of cerebrospinal fluid (CSF; Mateika & Duffin, 1995; Maurer, 2010). The bECF and CSF—a clear fluid that fills the ventricular system of the CNS and surrounds the brain and spinal cord—are produced independently but are in direct communication with one another so that changes in the composition of one are generally reflected in the composition of the other (Maurer, 2010; Walker, Tong, Perry, Alaveijeh, & Patsalos, 2000). In contrast, peripheral chemoreceptors are situated near the bifurcation of the common carotid arteries and the arch of the aorta, and are commonly called carotid and aortic bodies, respectively (Mateika & Duffin, 1995; Prabhakar & Peng, 2004). Although these chemoreceptors are sensitive to arterial O_2 and CO_2, and blood pH, they are extremely sensitive to abnormally low levels of arterial O_2. Prabhakar and Peng (2004) explain that as the arterial P_{O_2} declines, peripheral chemoreceptors send increasingly rapid afferent impulses to the medullary respiratory centre, primarily the DRGr (i.e., the inspiratory centre), yielding an increased respiratory drive that improves V_A and returns the P_{O_2} back to homeostatic levels.

Additional Sensory Receptors. Various other sensory receptors located throughout the body provide inputs to the respiratory control system to stimulate an increased ventilatory response to exercise. Although the types of receptors are extensive, a common type believed to help with the regulation of ventilation includes proprioceptive reflexes from muscles spindles,

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Golgi tendon organs, and joint pressure receptors. At the onset of exercise, contraction-induced

mechanical and chemical stimuli begin to activate these receptors, which are located on the

terminal ends of thinly myelinated Aδ fibres (commonly referred to as group III afferents) and

unmyelinated C fibres (group IV afferents) with their receptive fields located within skeletal

muscle and joints (Amann, Sidhu, Weavil, Mangum, & Venturelli, 2015; Mateika & Duffin,

1995). This exercise-induced activation increases the spontaneous discharge of the thin fibre

muscle afferents to various spinal and supraspinal sites within the CNS, which, in turn, adjust the

ventilatory response accordingly (Amman et al., 2015). Likewise, proprioceptor and

mechanoreceptor inputs arise from stretch receptors and Golgi organs located within various

respiratory muscles, which provide feedback regarding the position of the thoracic wall to the

medullary respiratory centre. Other receptors that play a role in hyperpnoea during exercise

include receptors responsible for detecting body temperature increases (i.e., thermoreceptors)

and those that are stimulated by circulating catecholamines such epinephrine and norepinephrine

(i.e., adrenergic receptors; Mateika & Duffin, 1995; Seeley et al., 2011).

The contribution of the aforementioned feedback receptor mechanisms to ventilation is

low in resting humans; exercise, however, influences this. During intense exercise, for example,

mechanoreceptors and proprioceptors of muscles and joints are activated increasingly, central

thermoreceptors are activated by increases in body temperature, arterial partial pressure changes,

and decreased pH due to production and accumulation of lactic acid in the blood are detected by

chemoreceptors; all of which can add to an increased ventilatory response. Despite numerous

studies providing support for the role of central and peripheral chemoreceptors and other sensory

receptors in mediating the exercise-induced ventilatory response, various studies have

demonstrated that the removal or loss of putative peripheral drive in patients following carotid

body resection (Wasserman, Whipp, Koyal, & Cleary, 1975), orthotopic cardiac transplantation (Banner, Lloyd, Hamilton, Innes, Guz, & Yacoub, 1989) and spinal cord transection (at or above the level of T6; Adams, Frankel, Garlick, Guz, Murphy, & Semple, 1984) does not significantly affect the normal ventilatory response to steady-state exercise. Moreover, it has been shown that children with congential central hypoventilation syndrome (CCHS)—a rare condition characterized by ineffective or functionally absent central and peripheral chemoregulation of breathing and consequent life-threatening hypoventilation—have a normal ventilatory response during aerobic exercise as compared to controls (Shea, Andres, Shannon, & Banzett, 1992). This indicates that arterial chemoreception and/or mechanoreception may not be essential for an appropriate ventilatory response to exercise. Consequently, alternative hypotheses that focus on descending neural inputs have been proposed to explain exercise-induced hyperpnoea.

Descending Inputs. The notion of descending neural inputs involving mechanisms within higher brain regions focuses heavily on feed-forward concepts that require activation of the respiratory muscles from higher CNS structures. They are of particular interest as experimental evidence has indicated that the proportional changes in respiration and circulation that occur during exercise can be independent of afferent receptor feedback (Eldridge, Millhorn, Killey, & Waldrop, 1985; Eldridge, Millhorn, & Waldrop, 1981).

Central Command. A well-studied and widely accepted neural control mechanism thought to play a primary role in the autonomic adjustments to exercise is the concept of central command (Mateika & Duffin, 1995; Nobrega et al., 2014; Secher, 2007; Williamson et al., 2006), previously called cortical irradiation (Krogh & Lindhard, 1913). Central command refers to a feed-forward mechanism of descending neural signals from higher brain centres that are capable of parallel stimulation of cardiovascular responses such as respiration and ventilation as

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well as exercise movement (discussed below; Mateika & Duffin, 1995; Nobrega et al., 2014; Secher, 2007; Williamson et al., 2006). It has been proposed that central command involves the simultaneous activation of two distinct networks—one for cardiovascular control and one for motor control—the latter of which will be discussed later on (Williamson et al., 2006). This notion is based on findings indicating that the magnitude of a central command-mediated cardiovascular response during exercise appears to be independent of force production (i.e., workload) and directed more so by an individual's conscious perception of effort required to accomplish a specific motor task such as exercise. For example, mental imagery and hypnotic manipulation used to increase the perception of muscular effort/work during 'imaginary' exercise lead to increases in respiration rate, ventilation, HR, blood pressure, and perceived exertion (Decety, Jeannerod, Durozard, & Baverel, 1993; Thornton et al., 2001; Williamson et al., 2002). Moreover, studies employing partial and complete neuromuscular blockade via curarization of the exercising muscles have reported increased ventilation during cycle ergometry at a given O_2 consumption; possibly resulting from a greater level of central command as indexed by an individual's perceived effort (Asmussen, Johansen, Jørgensen & Nielsen, 1965; Gandevia et al., 1993; Galbo, Kjaer, & Secher, 1987). Similarly, Innes, DeCort, Evans, and Guz (1992), demonstrated that dynamic exercise in participants with painless, unilateral leg weakness (from orthopaedic/neuromuscular disorders, or deliberately induced by anaesthetic nerve blocks) results in ventilatory, blood pressure, and HR responses that are exaggerated out of proportion to the work achieved and to the responses seen at the same metabolic rate when using the normal (contralateral) leg. Therefore, in addition to signifying that the modulation of autonomic ventilation is effort-induced, the aforementioned studies indicate that parallel motor activation is not a requisite component of the central command response.

The concept of central command has been well recognized for over a century, yet the specific higher brain region(s) and the descending pathways involved in this exercise-induced ventilation adjustment remain speculative. This is partially due to the fact that examining subcortical cerebral structures in exercising humans requires rather invasive, neurosurgical methods performed while the participant is awake; or imaging techniques that require an immobile head, a condition that is difficult to achieve during most dynamic exercises. Hence, most research to date examining subcortical involvement during exercise comes from animal studies. A substantial amount of research has suggested that the central command mechanism originates from various subcortical regions in the diencephalon, mainly the hypothalamus and subthalamus (DiMarco, Romaniuk, Von Euler, & Yamamoto, 1983; Eldridge et al., 1985; Eldridge et al., 1981; Kuwaki, 2010; Machado, Bonagamba, Dun, Kwok, & Dun, 2002; Samson, Bagley, Ferguson, & White, 2007; Samson, Gosnell, Chang, Resch, & Murphy, 1999; Shirasaka, Nakazato, Matsukura, Takasaki, & Kannan, 1999; Williams & Burdakov, 2008), although some have pointed to the superior portion of the brainstem, the midbrain, or the mesencephalon (Bedford, Loi, & Crandall, 1992; DiMarco et al., 1983; Green et al., 2007; Matsukawa, Nakamoto, & Liang, 2011). The midbrain connects the forebrain (diencephalon and cerebral cortex) to the hindbrain (pons, medulla, and cerebellum) and contains a cluster of neurons that produce dopamine called the substantia nigra. A relationship between the subthalamus and the midbrian exists as evidence has shown that the subthalamic nucleus, whose principal neurons are glutamatergic, exerts an excitatory effect on the substantia nigra (Robledo & Féger, 1990; Windels et al., 2000)

Research in decorticate and decerebrate cats, where descending central inhibition has been removed, has shown that locomotion induced by electrical stimulation of the hypothalamus

(DiMarco et al., 1983; Eldridge et al., 1981), subthalamus (Millhorn, Eldridge, Waldrop, Kiley,

1987), and the midbrain region (Bedford et al., 1992; Matsukawa et al., 2011) elicits proportional

increases in cardiorespiratory responses (e.g., ventilation rate, HR, blood pressure, and so forth)

similar to those occurring during volitional, natural exercise. Likewise, the injection of

picrotoxin, a GABA antagonist, to stimulate the hypothalamus and subthalamic locomotor area

has been shown to elicit the same response to electrical stimulation (Eldridge et al., 1985;

Millhorn et al., 1987). However, these authors failed to ablate the feedback receptors located in

the locomotor muscles and/or in the cardiorespiratory structures, and could, therefore, have

provided the stimulus to the associated cardiorespiratory changes (Mateika & Duffin, 1995).

Eldridge et al (1985) overcame this limitation by examining the response to hypothalamic

electrical and chemical stimulation in anaesthetized and paralyzed cats, and found similar results

to those observed during spontaneous or evoked locomotion. Additionally, studies utilizing

retrograde horseradish peroxidase and autoradiograph tracing methods have demonstrated that

direct and indirect efferent projections from the hypothalamus to the spinal cord, and to regions

of the medullary respiratory centre exist in the cat and rat brain (Berk & Finkelstein, 1982;

Holstege, 1987; Machado et al., 2002).

More recent literature has suggested that the electrical impulses from the hypothalamus

that stimulate breathing may be mediated, at least in part, by hypothalamic neurons that contain

orexin neuropeptides (oxerin-A and –B) commonly referred to as hypocretins (Kuwaki, 2010;

Machado et al., 2002; Samson et al.1999; Samson et al., 2007; Shirasaka et al.,1999; Williams &

Burdakov, 2008). Results from several studies indicate that injection of oxerin-A into the rat

hypothalamus accelerates the cardiovascular response by activating sympathetic outflow,

increasing HR and blood pressure (Machado et al., 2002; Samson et al.1999; Samson et al., 2007; Shirasaka et al.,1999).

As explained above, the cerebral cortex is involved in the voluntary control of breathing as neuronal groups in the brainstem—mainly the DRGr—synapse onto neurons to various regions in the cerebral cortex. This direct control occurs via the lateral corticospinal tract, which begins in the cerebral cortex, decussates in the pyramidal decussation of the lower medulla, and proceeds down the contralateral side of the spinal cord where it synapses onto spinal motor neurons that innervate and thus control the respiratory muscles under voluntary drive (Guz, 1997). Therefore, it has been suggested that several regions of the cerebral cortex may serve as the origin of descending central command signals responsible for cardiovascular changes and may operate independently or together with the aforementioned subcortical structures. Various neuroimaging studies investigating the neuroanatomical structures responsible for the cardiovascular central command mechanism have identified several regions activated in the human brain during isometric exercise and imagination of exercise under hypnosis some of which include the (i) insular cortex (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000; Williamson et al., 2002; Williamson, McColl, & Mathews, 2003), (ii) dorsolateral and medial PFC (Critchley et al., 2000; Thornton et al., 2001), (iii) motor cortex (Thornton et al., 2001), and (iv) anterior cingulate cortex (Critchley et al., 2000; Williamson et al., 2002). The medial PFC has been shown to receive multiple limbic sensory inputs and appears to play a significant role in stress-related modulation of sympathetic outflow (Verberne & Owens, 1998) while the insular cortex has been documented to have numerous efferent pathways to the aforementioned medullary respiratory control centre (Yasui, 1991). It has been suggested, further, that the medial PFC and insular cortex interact given the reciprocal connections that exist between them, may

function in concert or independently to interpret sensory input and elicit appropriate autonomic adjustments (Williamson et al., 2006). Evidence for regions of the motor cortex involved in respiratory regulation stem from various anterograde and retrograde tracing studies that provide evidence that direct connections from the motor cortex to the respiratory motoneurons in the spinal cord exist (Cheema, Rustioni, & Whitsel,1984; Rikard-Bell, Bystrzycka, & Nail, 1985). Lastly, the anterior cingulate cortex, based on its capacity for modulation of autonomic functions such as regulating blood pressure and HR, has been described as suited for the central command role (Williamson et al., 2002).

Limits to Exercise Tolerance and Exercise-Induced Fatigue

Exercise is categorized into three different intensity levels—light or low, moderate, and high or vigorous—which can be expressed in terms of resting oxygen requirement (metabolic equivalent of task [METs]; Canadian Society for Exercise Physiology [CSEP], 2013). Light exercise denotes activities requiring less than 3 METs, moderate exercise denotes activities requiring 3 to 6 METs such as brisk walking, and high-intensity denotes activities such as running requiring more than 6 METs (CSEP, 2013). Exercise of moderate-to-high intensity and of prolonged duration challenges the capability of most muscles and organs of the body. The vital functions of the brain, heart, and respiratory system can become affected as a result of the aforementioned ventilatory changes, and the skeletal muscles become progressively fatigued (Dempsey, Romer, Rodman, Miller, & Smith, 2006). Such physiological changes often result in early exercise termination or task failure prior to exceeding a critical homeostatic state. The mechanisms determining exercise performance and exercise tolerance—the capacity to sustain physical exertion and thus delay exercise termination—have been intensely studied for well over a century and have proven to be a complex area of research that is still heavily debated

(McKenna & Hargreaves, 2008). The majority of literature suggests that peripheral fatigue of the respiratory and locomotor muscles involved during exercise, as a result of various physiological/metabolic processes, contributes to exercise tolerance. However, these peripheral mechanisms alone, cannot account for the decrease in maximal voluntary force that occurs during muscle fatigue, and thus a supplemental model concerning modifications within the CNS (i.e., central fatigue) has been postulated.

Exercise-induced muscle fatigue—defined as a transient reduction in the ability to exert maximal voluntary force or power of a single muscle or muscle group, whether or not the task can be sustained (Gandevia, 2001)—contributes to exercise tolerance limitations in humans; yet it remains elusive exactly how and by what physiological mechanisms fatigue occurs (Gandevia, 2001). It may be driven by changes occurring anywhere along the motor pathway between the brain and the muscle fibres (Gandevia, 2001). It is proposed that it results from two distinct types of fatigue—peripheral and central—which, although distinct, are intrinsically related given that "recruitment of peripheral motoneurons depends on descending drive from supraspinal sites, whereas central drive is modulated by a combination of excitatory and inhibitory [feedback] inputs from peripheral muscles, joints, tendons, and cutaneous afferents" (Oliveira et al., 2014, p. 2).

Peripheral Muscle Fatigue

Peripheral fatigue is produced by changes at or distal to the neuromuscular junction and is typically described as a perturbation in the force-generating capacity of a muscle. It can be demonstrated by a reduction in force evoked by peripheral supramaximal nerve stimulation. Although peripheral fatigue encompasses a wide range of muscles and muscle groups, for the

purpose of this review only those involved with exercise (locomotor and respiratory) will be discussed.

      Locomotor Muscle Fatigue. Skeletal muscles are susceptible to fatigue depending on the demands placed on them (e.g., forces produced, velocity of muscle shortening, length changes) as well as numerous physiological factors such as fibre composition, recruitment order, and oxidative capacity (Fitts, McDonald, & Schluter, 1991; Millet & Lepers, 2004). Various studies examining the physiological mechanisms contributing to peripheral fatigue have indicated that fatigue of the locomotor muscles occurs during sustained exercise (Amann & Dempsey, 2008; Amann, Eldridge, Lovering, Stickland, Pegelow, & Dempsey, 2006; Millet & Lepers, 2004) and it is commonly thought to directly limit exercise tolerance (Sejersted & Sjøgaard, 2000). Exactly how locomotor muscle fatigue plays a role in exercise tolerance is poorly understood, although research in healthy adults demonstrates that the physiological mechanisms (discussed below) contribute to the experience of muscle aches and pain (Borg, Ljunggren, & Ceci, 1985; Dannecker, Liu, Rector, Thomas, Fillingim, & Robinson, 2012; Ljunggren, Ceci, & Karlsson, 1987; Miles & Clarkson, 1994). In turn, this exercise-induced pain, which is often an indicator of muscle damage (Dannecker et al., 2012) can decrease exercise performance and, if intolerable, lead to exercise termination. Cook and colleagues (1997) performed a series of experiments in which pain ratings were obtained during bouts of cycle ergometry of various combinations of duration and intensity and found that volitional exhaustion was associated with a 'very strong' pain rating. Moreover, alternative views have suggested that fatigue of the muscles involved in respiration (i.e., respiratory muscle fatigue), as a result of the accompanying hyperpnoea, may also limit exercise tolerance.

Respiratory Muscle Fatigue. In spite of their specific task, which does not allow them to rest during their entire life, the respiratory muscles have the same basic structure and function as other limb and trunk muscles, yet they are more resistant to developing fatigue than limb muscles both *in vivo* and *in vitro* (Gandevia, McKenzie, Neering, 1983; McKenzie & Gandevia, 1991). It is well known that skeletal muscle fibres are heterogeneous and their fatigue resistance is based on specific fibre characteristics. In particular, the diaphragm, which carries out most of the respiratory work during quiet breathing and is thus engaged in a continuous rhythmic activity, is very resistant to fatigue. For example, the diaphragm has a high oxidative capacity as a result of its fibre composition (Dempsey et al., 2006; Polla, d'Antona, Bottinelli, & Reggiani, 2004). It is estimated that the human diaphragm contains approximately 55% type I (slow oxidative) fibres, 21% type IIa (fast oxidative-glycolytic) fibres, and 24% type IIb (fast glycolytic) fibres (Lieberman, Faulkner, Craig, & Maxwell, 1973; Mizuno, 1991), which supports the idea that the diaphragm is a fatigue resistant muscle. Likewise, although information is fragmentary, it is estimated that the proportion of slow fibres in humans is above 60% in both the internal and external intercostals (Mizuno, 1991). In addition, the muscle fibres of the diaphragm generally have smaller cross-sectional areas in comparison to other limb muscles and accessory respiratory muscles such as the sternocleidomastoid (SCM; Green, Plyley, Smith, & Kile, 1989; Guido, Campos, Neto, Marques, & Minatel, 2010), and thus the capillary-to-mitochondrial diffusion distance for O_2 is reduced (Mizuno, 1991).

The neural control of inspiratory and expiratory muscle function also contributes to their fatigue resistance. With progressively increasing exercise intensity, the activation of the expiratory muscles reduces end-expiratory lung volume and, thus, spares the inspiratory muscle function by allowing the diaphragm and other inspiratory muscles to lengthen and operate near

their optimal length for force generation (Dempsey et al., 2006; Johnson et al., 1993). In addition to triggering the respiratory muscles to contract more forcefully (in comparison to quiet breathing), exercise-induced ventilation further results in the contraction of additional accessory muscles such as the SCM and scalenes to allow additional expansion and elevation of the thoracic cavity (Hudson, Gandevia, & Butler, 2007). Such accessory muscles are progressively recruited with increasing ventilatory demand, thereby sharing the load needed to support exercise hyperpnoea (Dempsey et al., 2006; Seeley et al., 2011).

Despite the aforementioned fibre characteristics of the respiratory muscles that aim to allow them work to without becoming fatigued, respiratory muscle fatigue can occur, largely as a consequence of high-intensity exercise. Studies have found significantly decreased levels of maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and/or maximal voluntary ventilation (MVV) following high-intensity exercise, which may be indicative of fatigue (Loke, Mahler, & Virgulto, 1982; McConnell, Caine, & Sharpe, 1997). It also has been suggested that fatigue resistance of the respiratory muscles is related to the baseline (pre-exercise) strength of the inspiratory muscles, since evidence indicates that participants with weaker inspiratory muscles exhibit significantly less fatigue resistance than those with stronger inspiratory muscles (Coast, Clifford, Henrish, Stray-Gundersen, & Johnson, 1990; McConnell et al., 1997). Interestingly, however, a study comparing highly trained cross-country skiers with untrained, sedentary college students found no statistically significant difference in the baseline inspiratory muscle strength of the trained and untrained groups. After exercise, though, the untrained participants experienced significant decreases in MIP compared to baseline (pre-exercise) values while the trained participants did not (Coast et al., 1996). Additional evidence has indicated that exercise sustained to volitional exhaustion at intensities greater than 80% of

maximal oxygen consumption (VO_{2max}, a measurement of aerobic fitness) causes the diaphragm to become significantly fatigued (Babcock, Pegelow, Harms, & Dempsey, 2002; Johnson, Babcock, Suman, & Dempsey, 1993; Mador, Magalang, Rodis, & Kufel, 1993). This has been demonstrated using the technique of supramaximal bilateral phrenic nerve stimulation to elicit twitches of the diaphragm in healthy humans. This technique demonstrates that, following intensive exercise, the transdiaphragmatic pressure—a measure of maximal diaphragmatic force—is reduced by approximately 20% compared to pre-exercise baseline levels, and then takes well over an hour to recover (Babcock et al., 2002; Johnson et al., 1993; Mador et al., 1993). Having defined and outlined the potential contribution of locomotor and respiratory muscle fatigue to exercise tolerance, the physiological mechanisms behind them must be explored.

Physiological Mechanisms. The origins and mechanisms of peripheral fatigue have been well studied, ranging from impairments in neuromuscular transmission and propagation down the sarcolemma to dysfunctions within the sarcoplasmic reticulum (e.g., calcium release and reuptake issues) and actin-myosin cross-bridge interactions. The classic model, however, concerning the availability of metabolic substrates and accumulation of metabolites such as lactic acid is the most common and, for the purpose of this review, will be the only model of peripheral muscle fatigue discussed to provide a brief overview (Davis & Bailey, 1997).

Cardiorespiratory Capacity and Biomarkers. This model holds that endurance performance is determined by the maximum capacity of the heart to pump blood (i.e., cardiac output) and O_2 to the muscles (Kayser, 2003). When cardiorespiratory capacity or, more specifically, "when substrate availability decreases, when oxygen transport capacity is reached, and when muscle metabolic capacity is fully used," (Kayser, 2003, p. 411) fatigue of the muscles

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occurs and the effort cannot be maintained any further. In other words, fatigue is thought to result when the capacity of the cardiorespiratory system to provide O_2 to exercising muscles falls behind their demand, thereby inducing anaerobic metabolism (Noakes, 2000). The majority of studies examining this model discuss issues such as VO_{2max}, aerobic enzyme capacity, cardiac output, muscle glycogen stores, and so forth.

A biological marker or biomarker—an ojectively measured substance in an organism whose presence is indicative of a normal biological or pathogenic process—commonly is used to offer insights into mechanisms of fatigue during exercise (National Institutes of Health, 2001). Although numerous, only a few prevalent, yet heavily debated, mechanisms and their corresponding biomarkers will be discussed. Fatigue of the muscles results, in part, from ATP metabolism and the rapid accumulation of ATP hydrolysis products (inorganic phosphate $[P_i]$ and hydrogen ion $[H^+]$) that accompanies the increased rate of ATP utilization during exercise (Fitts, 1994). Accordingly, these ATP metabolism products are often used as biomarkers. Evidence from skinned single muscle fibres demonstrates that elevated P_i levels significantly reduce force (Millar & Homsher, 1990; Pate & Cooke, 1989), which is consistent with the idea that the accumulation of P_i may interfere with cross-bridge cycling in sarcoplasm, and thus impact the contractile properties of skeletal muscle. These experiments however, were performed at low temperatures and evidence suggests that the depressive effect of P_i may differ at more physiological temperatures. For example, Dantzig and colleagues (Dantzig, Goldman, Millar, Lacktis, & Homsher, 1992) found a smaller force-depressing effect of P_i at 20°C than at 10°C. In contrast, Pate and colleagues (Pate, Wilson, Bhimani, & Cooke, 1994) demonstrated that the effect of the P_i analogue orthovanadate displayed a similar reduction on maximum shortening velocity at both 5°C and 25°C but had no effect at high temperatures (≥25°C).

Serum lactate or lactic acid is another well-known, easily attainable biomarker of muscle fatigue derived from ATP metabolism (Finsterer, 2012). Various literature indicates that serum lactate concentration increases with the intensity of exercise in animals (Schuback, Essen-Gustavsson, & Persson, 1999), healthy and diseased humans, and trained and untrained individuals (Siegel et al., 2008). Based on Hill and associates (Hill, Long, & Lupton, 1924) initial proposal of the contribution of lactate to muscle fatigue, literature still indicates that during intense exercise an inadequate O_2 supply causes the production of ATP to shift from aerobic processes to anaerobic glycolysis, which results in the production of lactic acid, primarily within the contracting skeletal muscle (Juel, 1998). In order to prevent lactate from accumulating inside the muscles, as per the Cori cycle, lactate enters the bloodstream for transportation to the liver where it is converted to glucose (i.e., gluconeogenesis). However, because the Cori cycle functions more efficiently when muscle activity has ceased, continual, high-intensity exercise, can cause an increased serum $[H^+]$ that is indicative of systemic metabolic acidosis.

Acidosis, defined as an increased intracellular $[H^+]$, is one of the oldest putative agents of peripheral muscle fatigue that also is thought to contribute to muscle fatigue by direct inhibition of the cross-bridge leading to a reduction in force and velocity (Knuth, Dave, Peters, & Fitts, 2006). Experiments performed on isolated animal fast- and slow-twitch muscle fibres have shown that low pH significantly decreases force production and shortening velocity at low temperatures (10-15°C; Pate, Bhimani, Franks-Skiba, & Cooke, 1995; Metzger & Moss, 1990; Westerblad, Bruton, & Lännergren 1997). However, there is little consensus concerning the effect of acidosis on the contractile function of muscle at physiological temperatures. Several studies have shown that, similar to high $[P_i]$, low pH has little effect on isometric force

production and shortening velocity (Pate et al., 1995; Westerblad, 1997). More recently, Knuth and colleagues (2006) also demonstrated minimal effect on force production, but they noted significant decreases in the shortening velocity and power-generating capacity in slow type I fibres under the influence of increased [H^+]. This disparity in findings may be due to poor determination of fibre type proportions (i.e., slow- vs. fast-twitch) in the former studies; nonetheless, it is evident that further research is needed.

In addition to the aforementioned mechanisms associated with the depletion of ATP due to its increased consumption, mechanisms concerning oxidative stress are also important to understanding the origins of muscle fatigue. As assessed by various lipid peroxidation (e.g., malondildehyde or thiobarbituric acid) and antioxidant capacity (e.g., glutathione) biomarkers, exercise enhances the production of reactive oxygen species (ROS)—oxygen-containing free radicals—by contracting skeletal muscles in healthy sedentary subjects performing dynamic exercise (Alessio, Hagernman, Fulkerson, Ambrose, Rice, & Wiley, 2000; Ashton et al., 1998; Jammes, Steinberg, Mambrini, Bregeon, & Delliaux, 2005; Sahlin et al., 2010; Steinberg, Delliaux, & Jammes, 2006) or static muscle contractions (Alessio et al., 2000; Steinberg et al., 2006). As a result of the unpaired electron(s) they contain, ROS can promote oxidative damage to various proteins and lipids in contracting muscle fibres (Finsterer, 2012; Powers, Ji, Kavazis, & Jackson, 2011). The mechanism concerning how exercise-induced ROS production influences muscle fatigue remains elusive, however, Reid and colleagues (Reid, Khawli, & Moody, 1993) were the first to develop a theoretical model that assumes that the muscle redox state in muscle fibres is a physiological variable that is balanced by matching the rates of ROS production with cellular antioxidant buffering capacity. A loss of muscle force production and thereby fatigue results when there is a deviation from the optimal redox balance as has been demonstrated to

occur during intense exercise (Reid et al., 1993). Furthermore, Brotto and Nosek (1996) suggest that ROS (hydrogen peroxide) may damage one or more proteins involved in the excitation-contraction coupling process in skeletal muscles, which produces a disruption in function that may account, at least in part, for the effects of fatiguing stimulation.

   Respiratory Muscle Metaboreflex. Fatigue of the locomotor muscles and possibly the respiratory muscles may be explained by evidence of a metaboreflex whose activation results in the redistribution of available blood flow between these two primary muscle groups involved during exercise (Dempsey et al., 2006; Dershak, Sheel, Morgan, & Dempsey, 2002; Harms et al., 1997, 1998; Legrand, Marles, Prieur, Lazzari, Blondel, & Mucci, 2007; Shadgan, Guenette, Sheel, & Reid, 2011; Sheel, Derchak, Pegelow, & Dempsey, 2002; St Croix, Morgan, Wetter, & Dempsey, 2000). This theory proposes that during high levels of exercise-induced respiratory muscle loading, fatiguing contractions and the detection of increased accumulation of local metabolites (e.g., lactate concentration) by chemoreceptors in the expiratory and inspiratory muscles activates unmyelinated group IV and myelinated group III afferent nerve fibres (Amann, 2012; Amann et al., 2015; Hill, 2000). In turn, this triggers a reduction in blood flow (i.e., increase in sympathetic vasoconstrictor outflow) to the locomotor muscles and an increase in respiratory muscle circulation often referred to as blood "stealing" (Dershak et al., 2002; Harms et al., 1997; Legrand et al., 2007; Sheel et al., 2002; St Croix et al., 2000). Consequently, peripheral locomotor muscle fatigue is exacerbated and the conscious perception of exertion is amplified via feedback from the periphery (Dempsey et al., 2006). Although this reflex has been postulated to be a mechanism to forestall respiratory muscle fatigue, Dempsey and colleagues (2006) suggest that because the respiratory muscles must compete with locomotor muscles for

available blood flow during exercise, it thereby promotes an inadequate O_2 transport and can lead to further fatigue of the muscles involved in respiration.

The majority of literature has indicated that the inspiratory muscles (Harms et al., 1997, 1998) including the diaphragm (Dempsey et al., 2002), external intercostals (Dershak et al., 2002), and SCM (Shadgan et al., 2011) display this blood stealing phenomenon. The involvement of expiratory muscles however, is less clear. Results from several NIRS (discussed in detail further below) studies do not support this blood stealing hypothesis for the intercostal muscles (De Bisschop, et al., 2014; Vogiatzis et al., 2009). De Bisschop and colleagues (2014) found that tissue oxygen saturation (SO_2) values of the intercostal muscles and vastus medialis in healthy, lowlander adults decreased similarly with incremental exercise up to 80% VO_{2max} regardless of altitude (sea level vs. 4350m altitude). The slope of decrease in SO_2 was steeper from 80% VO_{2max} to 100% VO_{2max} and was significantly lower in the intercostal than the vastus medialis. Moreover, the tissue hemoglobin concentration ([Hb]) between the intercostal muscles and vastus medialis did not differ at sea level (De Bisschop et al., 2014). Furthermore, Vogiatzis and associates (2009) demonstrated that during exercise above 80% maximal intensity in trained cyclists, intercostal muscle blood flow and vascular conductance are less than during resting hyperpnoea at the same work of breathing, which suggest that the intercostals do not steal blood flow from the exercising legs to support increased respiration.

Central Fatigue

Via feedback from the fatiguing locomotor and respiratory muscles to the supraspinal respiratory centres, the aforementioned peripheral fatigue mechanisms are thought to lead to intensification of perceived effort, causing reduced motor output to the locomotor muscles and thereby affecting exercise tolerance. This phenomenon, referred to as central fatigue, can be the

result of a dysfunction at any step in the continuum from the brain to the muscles (Davis & Bailey, 1997). In contrast to peripheral fatigue, which has a relatively distinct, uniform definition, central fatigue has a range of definitions. Typically, it is defined as a decrease in muscle force due to a progressive reduction or failure in voluntary muscle activation, which can originate at the spinal and/or supraspinal levels (Kayser, 2003; Gandevia, 2001; Oliveira et al., 2014; Rupp & Perrey, 2008). However, because central fatigue is considered to be a multifaceted phenomenon, Davis and Bailey (2007) suggest a broader definition that allows the likelihood that psychological factors (e.g., perception, motivation, etc.) are important in fatigue, and they extend the definition of central fatigue as follows: "a subset of fatigue (failure to maintain the required or expected force or power output) associated with specific alterations in CNS function that cannot be reasonably explained by dysfunction within the muscle itself" (p. 45).

Psychological Mechanisms. As discussed further below, central fatigue during exercise traditionally has been associated with physiological mechanisms that result in a reduction in corticospinal impulses reaching motoneurons while the psychological mechanisms are generally less discussed. However, our understanding of the psychological workings of the CNS has improved over the years but experimental support for such a specific role of central fatigue is limited, in part, because of a relative lack of objective measures due to the human body being in a constant state of flux (Davis & Bailey, 1997; Kayser, 2003). As stated by Davis and Bailey (1997), "central fatigue is often only accepted by default when experimental findings do not support the hypothesis of muscle dysfunction [and] even then it is often dismissed as a lack of motivation or an unfamiliarity with the experimental design" (p. 45). Consequently, the specific psychological mechanisms that can affect the magnitude of descending motor drive have received the least experimental attention as a possible factor in exercise-induced muscle fatigue.

Central Command/Governor. The first indication that a muscle is not able to maintain a contraction is related to changes in the perception of effort associated with a given task (e.g., an increased sense of respiratory exertion or breathlessness) as opposed to a physical inability to exert the necessary physical force (Cafarelli, 1988). Consequently, it has been argued that exercise ultimately starts and ends in the brain as the initiation of exercise begins with a conscious decision to start a voluntary effort, which results in the contraction of muscle tissue through an increase in spatial and temporal recruitment of motor units. Likewise, the volitional termination of exercise occurs when the perception of effort and/or other sensations such as pain become intolerable forcing the de-recruitment of motor units (Baron et al., 2007; Davis & Bailey, 2003; Kayser, 2003). The conscious decision to terminate exercise may be a consequence of a functional entity residing in the brain, dubbed the central governor. (Baron et a. 2007; Hill, Long, & Lupton, 1924; Kayser, 2003; Noakes, 2000; Noakes, Peltonen, Rusko, 2001). This model of integrative central neural regulation is essentially an elaborate concept of the previously discussed central command in that it proposes that exercise performance is regulated by homeostatic feedforward control mechanisms, or a governor in the brain. More specifically, this model holds that the brain continuously paces the body in response to afferent feedback from various previously mentioned peripheral systems to preserve homeostasis and prevent catastrophic damage or failure to vital organs (Baron et a. 2007; Kayser, 2003; Noakes, 2000; Noakes et al., 2001). The increasing perception of fatigue, which is the conscious interpretation of these subconscious feedforward control mechanisms, reduces the conscious desire to override this control mechanism, subsequently leading to reduced exercise intensity or exercise termination (Baron et al., 2007).

Considering that the decision to start, reduce, or stop exercising requires conscious effort, it likely occurs at the cortical level with input from subcortical structures (Kayser, 2003). Unlike the lack of agreement concerning the areas related to the cardiovascular component of central command, regions of the higher brain involved in the motor component have been more readily and consistently identified. Findings from neuroanatomical, electrophysiological, and functional neuroimaging studies have shown several key regions of the cerebral cortex that are involved in voluntary movement(s) including the primary motor cortex (Cunnington, Windischberger, Deecke, & Moser, 2002; Hanakawa, Immisch, Toma, Dimyan, Van Gelderen, & Hallett, 2003), premotor cortex (Christensen, Lundbye-Jensen, Geertsen, Petersen, Paulson, & Nielsen, 2007; Deiber, Passingham, Colebatch, Friston, Nixon, & Frackowiak, 1991; Picard & Strick, 2001; Thornton et al., 2001), and supplementary motor area (Cunnington et al., 2002; Babiloni et al., 1999; Deiber et al., 1991; Erdler et al., 2000; Ikeda, Luders, Burgess, & Shibasaki, 1992; Lang, Cheyne, Kristeva, Beisteiner, Lindinger, & Deecke, 1991; Nowak, Olsen, Law, Holm, Paulson, & Secher, 1999; Roland, Larsen, Lassen, & Skinhoj, 1980; Thornton et al., 2001). Despite the recognition of the involvement of these three cortical regions in voluntary movements, their precise functions have proven quite diverse and consequently still are debated. Nonetheless, the primary motor cortex is the main contributor to generating neural impulses that function in the execution of intended movements and mediation of specific reflex responses. Specifically, it decodes program instructions from other cortical and non-cortical areas and translates them into output command signals that effect target muscles accordingly (Cheney, 1985). Contemporary understanding of these functional characteristics is supported by early 20th century studies in which surgical lesions to the primary motor cortex in nonhuman primates results in flaccid paralysis of the contralateral limbs (Leyton & Serrington, 1917; Mott & Halliburton, 1908. See

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Wiesendanger, 2011 for review). Located anterior to the primary motor cortex, the premotor cortex is involved in integrating multisensory movement-relevant information and thereby organizing motor movements before they are initiated in the primary motor cortex (Gentile, Petkova, & Ehrsson, 2011; Marconi et al., 2001). However, because the premotor cortex contains direct projections to the spinal cord, it may also play a role in the direct control and execution of motor behaviours (Dum & Strick, 2002). Despite inconsistencies concerning the precise role of the supplementary motor area in humans, studies have shown increased cortical activity within the supplementary motor area beginning prior to volitional movement onset (Cunnington et al., 2002; Lang et al., 1991; Ikeda et al., 1992). These findings are consistent with the notion that the supplementary motor area is involved in planning and preparing voluntary movements (Passingham, Bengtsson, & Lau, 2010).

Subcortical structures, mainly the cerebellum, also have been proven to play a role in voluntary motor control (Bareš, Lungu, Husárová, & Gescheidt, 2010; Coco & Perciavalle, 2015; Manto et al., 2012; Nowak et al., 1999; Spencer, Zelaznik, Diedrichsen, & Ivry, 2003; Stoodley, Valera, & Schmahmann, 2012; Thornton et al., 2001). In addition to its other roles in cognitive tasks and the maintenance of balance and posture, literature holds that the cerebellum is responsible for regulating movement as opposed to producing it (Coco & Perciavalle, 2015; Spencer et al., 2003; Stoodley et al., 2012). The majority of evidence supporting the motor role of the cerebellum comes from examining the consequences of damage to it. Studies have demonstrated that humans with cerebellar damage or dysfunction have problems with the temporal properties of voluntary movements including increased variability between trials, impaired timing, and overall slowness as well as overshooting/undershooting and increased curvature of trajectories (Bares et al., 2010; Ivry, Keele, & Diener, 1988; Spencer et al., 2003;

Timmann, Citron, Watts, & Hore, 2001). Spencer and colleagues (2003) further demonstrated that patients with cerebellar damage exhibit temporal variability when producing discontinuous movements (intermittent circle drawing) but not when producing continuous movements (continuous circle drawing). These findings are consistent with the notion that the cerebellum is part of an internal timing system that regulates the sequence and duration of elementary movements, which together, with input from other control parameters such as the motor cortices, produces larger, more complex segments of movements (Spencer et al., 2003).

Physiological Mechanisms.

Neurotransmitters. To date, the majority of literature examining the supposed physiological causes of central fatigue during exercise have focused primarily on changes in extracellular neurotransmitter levels or exercise-induced alterations in the function of various neurotransmitter systems such as serotonin, acetylcholine, and dopamine. However, these mechanisms are not the focus of this review and thus will not be discussed in any further detail (for a review see Davis & Bailey, 1997).

Cerebral Blood Flow and Oxygen Delivery. Despite accounting for only approximately 2% of total body weight, the human brain is estimated to use 20% of total body O_2 consumed under normal conditions (Bor-Seng-Chu et al., 2012). During normal conditions or low- to moderate-intensity exercise, the brain obtains the majority of its energy almost exclusively from aerobic metabolism. Therefore, it is evident that regulation of cerebral blood flow (CBF) and oxygenation is critical to survival, and any impairment in the supply of O_2 and other nutrients to the brain can cause cellular damage (Bor-Seng-Chu et al., 2012). Strenuous, high-intensity exercise is one such condition that has been shown to cause a critical reduction in CBF and thereby a reduction in O_2 delivery. This impairment may pose a central fatigue limitation to

exercise tolerance by influencing the function of neurons and thus the ability to maintain motor activation (Amann, Eldridge, Lovering, Stickland, Pegelow, & Dempsey, 2006; Calbet, Boushel, Rådegran, Søndergaard, Wagner, & Saltin, 2003; Imray et al., 2005; Kayser, 2003; Subudhi, Dimmen, Roach, 2007; Noakes et al., 2001; Nybo & Rasmussen, 2007; Rasmussen, Dawson, Nybo, Van Lieshout, Secher, & Gjedde, 2007).

A reduction in CBF occurs during exercise at high altitudes (Imray et al., 2005) and may also arise as a consequence of exercise-induced arterial hypoxemia or exercise conditions in which hyperventilation-induced hypocapnia lowers CBF (Hellstrom et al.,1996; Moraine, et al., 1993). Hypoxemia, an abnormally low concentration of O_2 in the blood (i.e., P_{O_2}), can be induced by a diminished availability of O_2 to the body tissues referred to as hypoxia. The brain is protected against hypoxia-induced reductions in arterial O_2 delivery during resting conditions and low- to moderate-intensity exercise because CBF increases when the arterial P_{O_2} becomes low. However, during strenuous, high-intensity exercise, which often accompanies hypoxemia, hyperventilation-induced reductions of arterial P_{CO_2} may become so pronounced that they blunt the increase in CBF by constricting the arterioles of the brain, thereby diminishing CBF (Hellstrom et al., 1996; Moraine et al., 1993; Nybo & Rasmussen, 2007; Ogoh & Ainslie, 2009). Consequently, increased perfusion fails to compensate for the lower arterial O_2 content (Imray et al., 2005; Nybo & Rasmussen, 2007; Subudhi et al., 2007). This compromised O_2 delivery may further cause the cerebral mitochondrial P_{O_2} to decline, which, given the fact that the brain is critically dependent on a continuous supply of O_2 as well as glucose, is critical for cerebral function. In turn, this may influence the function of neurons, causing them to fire less rapidly. Since prolonged dynamic exercise requires continual or frequent repetitive neuronal firing in several regions of the brain (Davis & Bailey, 1997; Gandevia, 2001), the compromised function

of neurons due to a deficit in ATP could inhibit cortical/subcortical activation of efferent motoneurons, hence leading to the development of muscle fatigue and subsequently exercise termination (Nybo & Rasmussen, 2007; Subudhi et al., 2007).

Cerebral Hemodynamics During Exercise and Respiratory Muscle Training

In order for the circulatory system to carry out its primary function of bringing blood close to cells so that the exchange of gases can occur via diffusion, the blood must be able to flow through networks of blood vessels in the various organs. The forces involved in the circulation of blood typically are referred to as hemodynamics, which includes but is not limited to blood flow, pressure, volume, and resistance. Moreover, because red blood cells (RBCs) or, more specifically, a protein carried within RBCs called hemoglobin (Hb), facilitates the transportation of O_2 within the body, hemodynamics and oxygenation often go hand-in-hand with one another. This relationship between hemodynamics and oxygenation has been experimentally tested and demonstrated by various cerebral studies (e.g., Buxton, Wong, & Frank, 1998; Malonek, Dirnagl, Lindauer, Yamada, Kanno, & Grinvald, 1997; Molinari, Liboni, Grippi, & Negri, 2006). Hemodynamics and oxygenation are also closely coupled to tissue metabolic activity in most organs of the body. For example, an increase in tissue metabolism as occurs during muscle contraction, leads to an increase in blood flow in order to supply the corresponding muscles with O_2 and other nutrients. Therefore, hemodynamics are critical to maintaining homeostasis and ultimately survival.

The aforementioned notion of reduced or inadequate cerebral O_2 delivery during intense exercise is supported by anecdotal evidence from athletes who have experienced fainting or syncope, which refers to a sudden loss of consciousness and postural tone, immediately following completion of maximal exercise (Williams & Bernhardt, 1995). However, the potential

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mechanisms underlying the hypothesis that central fatigue contributes to limitations in exercise tolerance are poorly understood, in part because scientific evidence concerning specific, regional measurements of cerebral hemodynamics and oxygenation are difficult to obtain during intense exercise. Studies utilizing functional imaging modalities such as positron emission tomography (PET), single photon emission computed topography (SPECT), magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) have shown regional differences in cerebral oxygenation during submaximal exercise (Fink et al., 1995; Williamson et al., 1997), yet the temporal resolution of these tools and their intolerance to head motion preclude studies of high-intensity exercise (Subudhi, Miramon, Granger, & Roach, 2009). In addition, such assessment techniques are often invasive, expensive, and not easily transported.

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS), however, offers a noninvasive, real-time measurement of tissue oxygenation and hemodynamics that can overcome these obstacles. Initially introduced by Jobsis (1977), this inexpensive, portable, optically-based instrumentation has a high temporal resolution and uses low levels of near-infrared (NIR) light (wavelength region of 650-900 nm) to measure evoked concentration changes of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) that result from local vascular and oxygenation effects that occur during brain activity (Jobsis, 1977; Meek et al., 1995). In patients with symptomatic and asymptomatic steno-occlusive internal carotid arteries, Zirak and colleagues (Zirak, Delgado-Mederos, Dinia, Martí-Fàbregas, & Durduran, 2014) demonstrated that cerebral microvascular oxygenation as measured by NIRS was significantly correlated with microvascular CBF, measured by diffuse correlation spectroscopy, but was not correlated with macrovascular transcranial Doppler (TCD) CBF velocity. This finding is consistent with the idea that NIRS, as

opposed to TCD, primarily is sensitive to capillary Hb (Yamaguchi, Yamauchi, Hazama, Hamamoto, & Inoue, 1999; Zirak et al. 2014).

NIRS devices allow interrogation of the cerebral cortex via optodes, light transmitting and detecting devices, placed on the scalp. Near-infrared light passes easily through skin and the skull but it is variably absorbed by tissues containing blood. This is determined primarily by chromophores in tissue whose capacity to absorb NIR light is dependent on O_2 content, such as O_2Hb and HHb. However, only a small proportion of the transmitted NIR light returning from the tissues reaches the detectors while the rest is either lost due to scattering or is absorbed by other compounds. The changes in chromophore absorption at distinct wavelengths can be converted into relative concentration changes for both O_2Hb and HHb by applying a modified Beer-Lambert law (Delpy & Cope, 1997). Moreover, because frequency domain NIRS devices can provide absolute values of tissue Hb saturation in real-time, NIRS can further provide both the total hemoglobin (tHb) and the percentage O_2 saturation of Hb in tissue referred to as tissue oxygenation index (TOI). The [tHb] is the summation of changes in [O_2Hb] and [HHb], and thereby reflects blood volume changes, which can be interpreted as changes in cerebral perfusion (León-Carrion et al., 2008; Hoshi, Kobayashi, & Tamura, 2001). By comparison, TOI is an estimation of the balance between O_2 delivery and O_2 utilization (Smith, 2008). The parameters measured by NIRS include [O_2Hb], [HHb], [tHb], and TOI%.

NIRS and PFC studies. Cerebral NIRS measurements during exercise to exhaustion have, for the most part, focused primarily on the prefrontal cortex (PFC Perrey, 2008). Reasoning for this relates to the facts that the frontal bone is relatively thin, there is generally less head hair in this region, and the NIRS optodes can easily be placed bilaterally on the forehead. The PFC is known to project to the premotor areas and is responsible for planning and

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strategizing movement and pacing as well as executive functions such as decision-making (Miller & Cohen, 2001; Perrey, 2008). Reductions in prefrontal oxygenation near maximal exertion are associated with reduced muscle force generation (Rasmussen et al., 2007). Consequently, it has been suggested that reductions in prefrontal oxygenation near maximal exertion may limit exercise tolerance by impairing executive functions that influence the decision to stop exercising (Keramidas et al., 2011; Rupp et al., 2013; Subudhi et al., 2009).

Exercise Intensity. Muscle oxygenation decreases progressively with increased exercise intensity, reaching a plateau near maximal aerobic power output (Berlardinelli, Barstow, Porszasz, & Wasserman, 1995; Berlardinelli, Georgiou, Barstow, 1995; Bhambhani, Mailkala, & Esmall, 2001; Bhambhani, 2004), but the exercise-induced changes in cerebral oxygenation have not been as well defined. Results from NIRS studies indicate that PFC oxygenation is maintained or increased slightly during submaximal exercise and remains elevated or increases at maximal-intensity exercise (González-Alonso et al., 2004; Jensen et al., 2002), but several other studies indicate that the PFC oxygenation decreases at maximal-intensity exercise (Bhambhani et al., 2007; Ide, Horn, & Secher, 1999; Imray et al., 2005; Nielsen, Boesen, Secher, 2001; Rupp & Perrey, 2008; Subudhi et al., 2007; Subudhi et al., 2009). However, these differences at maximal intensity can be attributed mainly to the differences in defining/categorizing exercise intensities (e.g., maximal/supramaximal, moderate/hard/very hard). Indeed, closer examination of the results of these studies indicates that cerebral oxygenation increases progressively with increased exercise intensity until the point of exhaustion, at which time there is a decline in oxygenation. The results from a systematic review and meta-regression analysis by Rooks, Thom, McCully, and Dishman (2010) indicate that in 291 healthy participants in 21 studies, acute incremental exercise is accompanied by moderate-to-large PFC increases in [O_2Hb]—or other measures of O_2

levels—as well as in HHb, and tHb between low (<30% VO_{2peak}) and moderate ($\geq$30% to <60% VO_{2peak}) intensities. Cerebral oxygen values remain relatively stable at moderate-to-hard ($\geq$60% VO_{2peak}) intensities and then decrease towards values similar to those observed during low intensity exercise at very hard, exhaustive ($\geq$60% VO_{2peak}) intensities (Rooks et al., 2010). These findings are consistent with the aforementioned central governor and inadequate O_2 delivery hypotheses.

Additional support for the idea of a central limitation to exercise performance comes from Nielsen and colleagues (1999) who demonstrated via NIRS that an elevated inspiratory O_2 fraction through supplemental O_2 maintains cerebral oxygenation and increases exercise time trial performance without affecting muscle oxygenation.

Training Status. Further evidence has indicated that cerebral hemodynamic responses during varying intensities of exercise differ between people of different training history and/or health status. In their systematic review, Rooks and colleagues (2010) defined two types of training status, trained and untrained. Trained athletes such as elite cyclists, triathletes, and soldiers were described as having above-average aerobic fitness with an average VO_{2peak} ($M =$ 62.7, $SD =$ 7.9 mL·min^{-1}·kg^{-1}) while untrained individuals had a mean VO_{2peak} ($M =$ 40.3, $SD =$ 10.4 mL·min^{-1}·kg^{-1}) and were defined as having average aerobic fitness. They found that at low and moderate exercise intensities, aerobically trained athletes had lower prefrontal O_2Hb and tHb than the untrained individuals (Rooks et al., 2010). During hard intensity exercise, compared to untrained individuals, aerobically trained athletes had significantly lower O_2Hb levels, and significantly higher HHb and tHb levels (Rooks et al., 2010). At very hard intensities, athletes attained higher levels of O_2Hb, HHb, and tHb than untrained people. These findings may be the result of adaptations to exercise training (Gandevia, 2001). Specifically, exercise training may

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reduce somatosensory input to the motor cortex or other areas directly involved with central command by down-regulating afferent signals from peripheral sources, such as nociceptors, mechanoreceptors, and proprioceptors of muscles and joints, to the CNS (Gandevia, 2001). In turn, this may cause the conscious perception of effort required to accomplish the given exercise task to decline, thereby increasing exercise tolerance (Gandevia, 2001).

Respiratory Muscle Training

One approach to improving exercise tolerance is respiratory muscle training (RMT), a technique that is designed to improve the endurance and strength of the respiratory muscles through specific exercises (Illi, Held, Frank, & Spengler, 2012). It consists of a series of breathing and related exercises designed to target specific inspiratory and expiratory muscles, thereby improving ventilation. Currently, there are two primary, distinct forms of RMT: (i) respiratory muscle endurance training (eRMT; also known as ventilatory muscle training [VMT] and voluntary isocapnic hyperpnoea [VIH]) and (ii) respiratory muscle strength training (sRMT; also referred to as inspiratory muscle training [IMT], inspiratory resistive loading [IRL], and resistive/resistance respiratory muscle training [rRMT]; Illi et al., 2012; McConnell & Romer, 2004). Generally, eRMT is implemented in the form of voluntary normocapneic hyperpnoea and involves high-speed, low-resistance contractions of both inspiratory and expiratory muscles. In contrast, sRMT, is performed by breathing against an external resistance or threshold load and includes low-speed, high-resistance contractions targeted at increasing the force-generating capacity of generally the inspiratory muscles although the choice of respiratory muscles targeted depends on the desired outcome (Illi et al., 2012; Sapienza, Troche, Pitts, & Davenport, 2011; Verges, Renggli, Notter, & Spengler, 2009). It is unclear which form of RMT is more effective in terms of improving exercise performance but, from a physiological point of view, it seems

logical that the combined inspiratory/expiratory eRMT technique should be superior. However, in a systematic review and meta-analysis of RMT, Illi and colleagues (2012) undertook a detailed analysis of some 46 original RMT studies and concluded that RMT improves endurance exercise performance in healthy participants independent of the type of RMT. Not surprisingly, they also noted greater improvements in less fit individuals than in highly trained athletes.

Arguably, exercise performance is imperative for athletes, especially those involved in endurance-type sports, as athletes that are unable to meet the required exercise demands may face undesirable consequences including increased bench time or removal from competition (Coyle, Coggan, Hopper, & Walters, 1988). Rigorous cardiovascular and peripheral muscle strength training using partial or full-body exercises have traditionally been used to attain high performance; such training however, often inevitably reaches a plateau (HajGhanbari et al., 2013). Once this plateau has been reached, which may be the result of any one or more of the several above-mentioned mechanisms of exercise-induced fatigue, maximal exercise terminates. RMT is one technique that has been implemented by various athletes in an attempt to overcome this plateau and thereby improve exercise performance. Various literature has demonstrated that RMT does indeed have an ergogenic effect (i.e., enhances physical performance) as it has been shown to improve exercise performance times (Lemaitre et al., 2013; McConnell, 2009; McMahon, Boutellier, Smith, & Spengler, 2002; Romer et al., 2002; Wylegala, Pendergast, Gosselin, Warkander, & Lundgren, 2007) although some studies have demonstrated minimal impacts of RMT on performance (Fairbarn, Coutts, Pardy, & McKenzie, 1991; Hanel & Secher, 1991).

The ability to delay the termination of exercise and withstand physical exertion, and thereby improve tolerance, is important not only for athletic performance, but also for

maintaining or improving health as well as managing disease and illness (Marcora & Staiano, 2010). Indeed, RMT has been shown to help improve the quality of life for people living with respiratory diseases and related conditions such as asthma (Duruturk, Acar, & Dogrul, 2015; Weiner, Magadle, Massarwa, Beckerman, & Berar-Yanay, 2002) and chronic obstructive pulmonary disease (COPD; Charususin, Langer, Demeyer, Topalovic, Decramer, & Gosselink, 2015; Crisafulli, Costi, Fabbri, & Clini, 2007; Majewska-Pulsakowska, Wytrychowski, & Rozek-Piechura, 2016; Ramírez-Sarmiento et al., 2002; Riera et al., 2001). The inclusion of a specific RMT modality in a program focused on rehabilitation of symptomatic COPD has been demonstrated to improve patients' health-related quality of life (Riera et al., 2001).

Despite the general consensus that, over time, RMT improves exercise performance in both diseased and healthy populations, it is unclear exactly how or by what physiological mechanisms it accomplishes this. Evidence in humans suggests the RMT induces structural adaptations within the respiratory muscles. Specifically, using open biopsies of the external intercostal muscles of COPD patients, Ramírez-Sarmiento and colleleauges (2002) found statistically significant increases in both the proportion of type I fibres (38%) and the size of type II fibres (21%) after a 5-week IMT program. These structural changes were associated with increases in the strength and endurance of the inspiratory muscles and presumably represent adaptive effects with the genuine remodeling of respiratory muscle structure during RMT (Polla et al., 2004; Ramírez-Sarmiento et al., 2002). Enhanced exercise performance following RMT may also be due to the incorporation, over time, of more elastic tissue throughout the muscular and connective tissues to facilitate the efficient expansion and inflation of the thoracic cavity (Powers et al., 1990; Powers, Criswell, Lieu, Dodd, & Silverman, 1992). This notion is supported by athletes who have demonstrated improvements in chest expansion and ventilatory

function parameters such as forced vital capacity (FVC), forced expiratory volume (FEV), MIP, and MEP following a minimum 4-week RMT period (Lemaitre et al., 2013; Wylegala et al., 2007). It is well known that long-term, consistent endurance training (mainly cycling or running) can alter the oxidative metabolic machinery in the respiratory muscles as shown in animal studies (Powers et al., 1990; Powers, Criswell, Lieu, Dodd, & Silverman, 1992); however, whether RMT improves whole body endurance capacity remains controversial with many authors arguing that RMT does not influence VO_{2max} (Edwards, 2013; Markov, Spengler, KnoÈpfli-Lenzin, Stuessi, Boutellier, 2001; Powers, Coombes, & Demirel, 1997). Moreover, since RMT has been shown to reduce the development of respiratory muscle fatigue (Verges et al., 2009), blood lactate concentration (Spengler, Roos, Laube, & Boutellier, 1999; Verges et al., 2009), and sympathetic activation (Witt, Guenette, Rupert, McKenzie, & Sheel, 2007), it may improve exercise performance by a reduction or delay of the respiratory metaboreflex previously described (e.g., Dempsey et al., 2006).

It has been proposed further that RMT may increase exercise tolerance by altering the psychological and physiological mechanisms of central fatigue. For example, perhaps by improving the contractile properties of respiratory muscles (i.e., delaying the onset of respiratory muscle fatigue; Verges et al., 2009), RMT causes an overall abatement in hyperpnoea during exercise and, as previously mentioned, reduces the participants' perception of effort (Romer et al., 2002). This has been demonstrated by a few studies examining 4-6 week RMT programs that found increases in participants' time to volitional exhaustion along with decreases in their ratings of perceived exertion (Caine & McConnell 1998; Edwards, 2013; Romer et al., 2002). Specifically, Caine and McConnell (1998) reported improvements in endurance trained athlete's cycling performance following 4 weeks of IMT, consisting of 30 inspiratory efforts (against a

resistance equivalent to 50% peak MIP) performed twice daily. Edwards (2013) observed

significant improvements in time to volitional exhaustion in healthy adults after 4 weeks of IMT

(30 consecutive inspiratory efforts at 55% of MIP). Lastly, following 6 weeks of IMT (30

dynamic inspiratory efforts twice daily against pressure-threshold load equivalent to 50% MIP),

Romer and colleagues (2002) observed improved performance amongst trained cyclists in both

simulated 20 and 40 km time-trials. The authors, therefore, suggest that RMT may mediate the

intensity of effort perceptions via peripheral mechanisms (Caine & McConnell, 1998; Edwards,

2013; Romer et al., 2002).

RMT and PFC Studies. Despite the few aforementioned studies that examined the

effects of RMT on the psychological aspect of central fatigue during exercise, studies examining

the physiological (hemodynamic and oxygenation) response of the PFC to RMT are limited. A

study by Nielsen, Boesen, and Secher (2001) reported that moderate and intense resistive load

breathing during a submaximal exercise test increased [O_2Hb], [HHb], and [tHb] due to elevated

production of CO_2 and enhanced arterial P_{CO_2}. Moreover, Keramidas and colleagues (2011)

examined the effect of a single, short-term (30 minutes) RMT task on cerebral hemodynamics

during a subsequent constant-power test on a stationary bike. They found similar patterns in the

NIRS parameters of both the experimental test and control test (power test with no preceding

eRMT). Specifically, the [O_2Hb] remained relatively constant throughout each test, [HHb]

significantly increased after 60% of performance time compared to resting values, and [tHb] fell

below resting values upon initiation of exercise and then increased at the end of exercise. The

authors conclude that the cerebral oxygenation of the PFC was not affected by the previously

performed eRMT task as there were no significant differences in any of the NIRS parameters

between the two tests. During the eRMT task, however, the cerebral [tHb] and [O_2Hb] were

significantly increased compared to resting values. Interestingly, Keramidas and colleagues (2011) found that the failure of participants to continue the eRMT task due to an inability to maintain the requested breathing frequency, and consequently having short breaks, is associated with a considerable decrease in cerebral [O_2Hb]. This could be due, in part, to fatigue of the respiratory muscles, which may directly or indirectly affect the hemodynamics of the cerebral cortex, or it could be the result of reduced central command.

Gaps in the Literature

The two NIRS studies summarized in the preceding paragraph focused on cerebral hemodynamics during exercise *after* participants had completed a single, acute RMT task. To date, no NIRS studies have examined the effects of chronic eRMT on cerebral hemodynamics and oxygenation. Hence, in addition to effort perceptions, investigating oxygenation and hemodynamics of the PFC in response to a regular RMT program may further identify the central fatigue mechanisms that limit exercise performance.

Objectives and Hypotheses

Purpose

The purpose of this study was to address some of the gaps in the existing literature regarding RMT, central fatigue, and cerebral oxygenation and hemodynamics. Specifically, the primary goal of this study was to investigate the effects of a 4-week respiratory muscle endurance training (eRMT) program (20-minute sessions, 3 times per week) on physiological aspects of central fatigue by examining near-infrared spectroscopy (NIRS) measurements in the PFC of healthy adults during maximal exercise. The secondary goal of this study was to examine the impact of eRMT on psychological aspects of central fatigue by investigating effort

perceptions during maximal exercise. Lastly, this study examined whether or not respiratory function and exercise performance are influenced by eRMT.

Hypotheses

To achieve these research objectives, this study tested several hypotheses. Firstly, it was hypothesized that 4 weeks of eRMT would lead to increases in respiratory pressures (maximum inspiratory pressure [MIP] and maximum expiratory pressure [MEP]) and spirometry values (forced vital capacity [FVC] and forced expiratory volume in 1 second [FEV_1]). Secondly, it was hypothesized that slight or negligible declines in Storer estimated maximal oxygen consumption (VO_{2max}) values, slight reductions in end-tidal carbon dioxide ($ETCO_2$) levels, and improvements in time to volitional exhaustion, would be observed during the post-eRMT (i.e. follow-up) exercise test. Thirdly, it was hypothesized that during the post-eRMT exercise test, participants would exhibit a decrease in maximal heart rate (HR) at a given resistance level compared to baseline. Fourthly, it was hypothesized that declines in participants' self-perceived exertion during the post-eRMT exercise test would be observed. Lastly, it was hypothesized that eRMT would lead to slight increases in prefrontal oxygenation and blood volume during maximal exercise testing.

<div align="center">

Methods

</div>

Participants

Participants were recruited via flyers located at UNBC (Prince George campus) and the Northern Sport Centre (see Appendix A: Participant Recruitment Flyer). Inclusion criteria for participants included: (i) be 18-55 years old; (ii) be a non-smoker; (iii) be recreationally active (exercise at least 3 days a week at a moderate-to vigorous-intensity for 30 minutes); (iiii) not be a competitive or elite athlete (exercise more than 60 min/day for at least 6/day week); (iv) have

normal lung function based on their age and standardized values as defined by the FVC, FEV_1, MIP, and MEP; (v) have no known history of respiratory, cerebral, or cardiovascular dysfunction; and (vi) be able to provide informed consent. Participants were given a $50 honorarium to compensate for their time. Informed written consent was obtained from each participant. Participant confidentiality was ensured by assigning a participant with a randomly generated three-digit numeric code. Participant information, data documents, and questionnaires were protected at all times in secured computers and/or locked in a filing cabinet to which only the principal investigators had access. The Research Ethics Board of UNBC reviewed and approved this study prior to commencement (REB # E2016.1013.079.00). Instrumentation, testing, training, and data collection, as described below, were conducted in the Northern British Columbia Near-Infrared Spectroscopy (NIRS) Research Laboratory located at UNBC.

Material and Outcome Measures

Anthropometrics

Upon initial visit, participants completed a brief screening questionnaire (The Physical Activity Readiness Questionnaire or PAR-Q, revised 2002) from the Canadian Society for Exercise Physiology (CSEP, 2013) to evaluate participants for exercise readiness and any underlying pathologies. Only those who answered 'no' to all of the questions were allowed to participate. Participants were also asked to state their physical activity behaviour as either regular (1-3 hours/week), frequent (3-5 hours/week), or heavy (5-6 hours/week).

Anthropometric measurements were taken based on protocols outlined in the CSEP - Physical Activity Training for Health manual (CSEP, 2013). Participants' height was measured using a portable wall stadiometer (Seca 213, Seca North America, Chino, CA, USA) at peak of a deep inspired breath. Participants' weight was recorded using a digital scale (Health O Meter

844KL Digital Scale, Sunbeam Products, Boca Raton, FL, USA) on a flat surface with footwear removed. Subcutaneous fat thickness of the NIRS sites (sternocleidomastoid; SCM and vastus lateralis; VL) were estimated using standardized measures of skinfold thickness (Heyward, 2006). Specifically, measurements were taken using a skinfold caliper (Live Life Fit Body Fat Caliper, Fitlosophy, Newport Beach, CA, USA) on participants' right side by firmly grasping and lifting the skin between the thumb and index finger. The calipers were placed on the contact surface at 90° to the skinfold, approximately 1 cm below the fingers (Heyward, 2006). Three measurements at each site were taken; the average was recorded.

Blood Pressure

Participants' systolic, diastolic, and mean arterial blood pressures were measured using a vital signs monitor (Carescape V100 Monitor, General Electric, Boston, MA, USA). Participants were seated in a non-rolling arm chair with feet placed on the floor, and the cuff was placed on participants' left arms. Three measurements were taken, the average of which was recorded.

End-Tidal Carbon Dioxide (ETCO$_2$)

Participants breathed into a capnometer (EMMA Capnograph, Masimo, Irvine, CA, USA) for approximately one minute to measure respiration rate (RR) and the resting mean partial pressure of expired carbon dioxide during each tidal breath (i.e. end-tidal CO_2, or ETCO$_2$).

Respiratory Function

Participants completed several respiratory function measures while wearing a nose clip and seated comfortably in a non-rolling chair with arms. Spirometry measures—forced vital capacity (FVC) and forced expiratory volume (FEV$_1$)—were completed simultaneously using a hand-held spirometer (Spirobank II Smart, Medical International Research, Waukesha, WI, USA) by having the participant inhale fully and then exhale as forcefully and rapidly as they

were able (approximately 6 second duration). To measure the strength of the respiratory muscles, maximal inspiratory pressures (MIP) and maximal expiratory pressure (MEP) were measured with an additional handheld device (MicroRPM, CareFusion, Yorba Linda, CA, USA), which the participant inserted the mouthpiece accordingly and inhaled (MIP) or exhaled (MEP) with maximal effort against a closed system (occluded mouthpiece). Specifically, to perform MIP, the participant was instructed to fully exhale and then, once the occlusion valve manually closed using pressure-sensing technology, forcefully inhale against the device with as much effort as possible for as long as possible (minimum 2 second duration). The MEP test was similar except the participant was instructed to perform the opposite—fully inhale and then forcefully exhale against the device with maximal effort for as long as possible (minimum 2 second duration). Three technically correct trials of each measure (FVC including FEV_1, MIP, and MEP) were performed with an adequate recovery period (1 minute) permitted between efforts. The best value of each trial was selected. For FVC with FEV_1 the highest sum of FVC and FEV_1 was selected (Coates et al., 2013). For MIP and MEP the largest values were selected. Participants received visual and verbal feedback/instruction to inspire or expire maximally and as rapidly as possible in order to maximize respiratory efforts. Testing procedures adhered to the guidelines for spirometry of the Canadian Thoracic Society (Coates et al., 2013) as well as of the American Thoracic Society and the European Respiratory Society for MIP and MEP (European Respiratory Society & American Thoracic Society, 2002).

Heart Rate

Participants' HR was continuously monitored using a HR sensor (Polar H10, Polar Electro Canada, Lachine, QC, Canada) worn around the chest, positioned just above the level of the xiphoid process.

NIRS

Participants were fitted with two optodes from a benchtop frequency-domain NIRS instrument (OxiplexTS, ISS Inc., Champaign, IL, USA) placed over the right (optode A) and the left (optode B) prefrontal cortices, just above the supraorbital ridge and as laterally as possible to the cerebral longitudinal fissure (Bhambhani et al., 2007; Obrig et al., 1996; Oussaidene et al., 2015). These optodes consisted of one detector and four fiber optic sources with minimum and maximum source-detector separations of 1.90 cm to 3.51 cm (optode A) and 1.91 cm to 3.50 cm (optode B). The optodes were secured with a self-adhering medical bandage wrapped around the forehead. The optodes emitted light into the tissue at wavelengths of 684 and 830 nm, with a modulated frequency of 110 MHz. Cerebral frequency domain NIRS data were recorded using data acquisition software specific to the OxiplexTS (OxiTS, ISS Inc., Champaign, IL, USA). Additionally, two wireless, handheld, two-channel, spatially resolved NIRS optodes (OxiTor Mk II, Pathonix Innovation Inc., Vancouver, BC, Canada) were secured over the VL and SCM muscles on participants' right side using, respectively, a self-adhering medical bandage and medical grade silicone adhesive tape. Skeletal muscle spatially resolved NIRS data were recorded using data acquisition software specific to the OxiTor Mk II (Atrasoft, Pathonix Innovation Inc., Vancouver, BC, Canada). These data were analyzed by another investigator for reporting elsewhere.

The NIRS parameters collected directly or calculated using OxiTS and Atrasoft were oxygenated hemoglobin concentration ($[O_2Hb]$), deoxygenated hemoglobin concentration ($[HHb]$), total hemoglobin concentration ($[tHb]$), and tissue oxygenation index in percentage or tissue oxygen saturation or (TOI% or Sat%). Changes in $[O_2Hb]$ and $[HHb]$ reflect tissue oxygenation, and changes in tissue blood volume within the illuminated area are indicated by

[tHb] (Hoshi et al., 2001). Tissue oxygen saturation is an assessment of the average O_2 saturation of the underlying tissue (Smith, 2008). Hemoglobin concentrations measured by the OxiplexTS were recorded as absolute values of μM, and those measured by the OxiTor Mk II were recorded as relative values of μM•mm. Before collecting data, the instruments were warmed up for a minimum of 30 minutes.

Exercise Testing

In order to assess exercise performance, physiologic outcome variables (NIRS parameters, HR, $ETCO_2$, VO_{2max}), and perceived exertion ratings (see below), the participants completed maximal incremental exercise tests to volitional exhaustion on a leg cycle ergometer (i.e., stationary bicycle; Ergomedic 828E, Monark, Vansbro, Sweden). For each participant, two separate ergometer tests to voluntary exhaustion were conducted: one test was completed before the four-week training intervention (described below) and one test was completed after the four-week training intervention. Approximately 48 hours prior to each of these two tests, participants were instructed to abstain from: (i) exercise during the 24 hours prior to testing; (ii) caffeinated beverages during the 4 hours before testing; and (iii) a heavy meal during the 1 hour prior to testing.

Each participant was familiarized with the cycle ergometer, and the handlebar and seat heights were adjusted according to each participant's comfort. The testing protocol, Storer Maximal Bicycle Test (Storer, Davis, & Caiozzo, 1990), began by having participants complete a warm-up period of cycling at zero resistance for 4 minutes, which was intended to provide baseline measurements (NIRS, HR). After completion of the warm-up, the power was increased by 15 W every minute starting from 50 W. Cycling for 1 min at 50 W was later selected as the baseline period for each incremental exercise test because participants consistently reported that

cycling against zero resistance to be more difficult. Participants were instructed to maintain a pedaling cadence of 60 rpm based on visual feedback from the ergometer's digital readout and to remain seated with hands placed on the handlebars for the duration of the test. The test was terminated either when volitional exhaustion was achieved or when the pedal cadence fell below 60 rpm for a duration greater than 15 seconds. Immediately following exercise termination, participants breathed into the capnometer.

Participants' ratings of perceived exertion (RPE) were queried at the end of each incremental stage (every minute) using the Borg 15-point RPE scale (Borg, 1982), a modified 6-20 point scale commonly used to quantitatively measure the level of physical strain or perceived exertion during exercise (Edwards, 2013; Eston, 2012; Morishita, Yamauchi, Fujisawa, & Domen, 2013). Prior to testing, participants were familiarized with the scale and its location directly in front of them—both on the bike and on a wall. During testing, participants were asked to rate their exertion on the scale, combining all feelings and sensations of physical fatigue. They were asked to disregard any one factor such as shortness of breath or leg discomfort and to try to focus on the whole feeling of exertion.

Participants' predicted VO_{2max} values were calculated using previously published equations specific to the Storer Maximal Bicycle Test (Storer et al., 1990). The multiple linear regression equations predict VO_{2max} ($mL \cdot min^{-1}$) from the independent variables of maximum Watts achieved during exercise (Watt level attained while completing the full 1 minute cycle increment), body weight (kg), and age (year). The sex specific equations were as follows. Males:

$$VO_{2max} \ (mL \cdot min^{-1}) = [(10.51 \times watts) + \ (6.35 \times weight) - (10.49 \times age) + 519.3]$$

Females:

$$VO_{2max} (mL \cdot min^{-1}) = [(9.39 \times watts) + (7.7 \times weight) - (5.88 \times age) + 136.0]$$

Training Intervention

Once HR, blood pressure, and NIRS parameters returned to resting after the baseline exercise test, participants were instructed on the proper technique for training with the eRMT device (SpiroTiger© SMART, FaCT Canada, Prince George, BC, Canada) to be used over the duration of the 4-week eRMT period. Participants were given approximately one hour to practice the eRMT technique through trial runs.

The eRMT device (Figure 3) consists of a handheld unit with one breathing channel to the atmosphere and one channel to a rebreathing bag and a base station. The base station computer sets threshold limits for breathing patterns, monitors the breathing frequency (f_b), and displays visual and acoustic feedback so as to allow the participant to breathe within the threshold values for isocapnia. A patented magnetic valve adjusts how much air is inspired from the atmosphere versus the rebreathing bag, depending on the cadence of ventilation. This valve allows training of both the inspiratory and expiratory muscles and ensures that isocapnic hyperpnoea is maintained without metabolic hyperventilation.
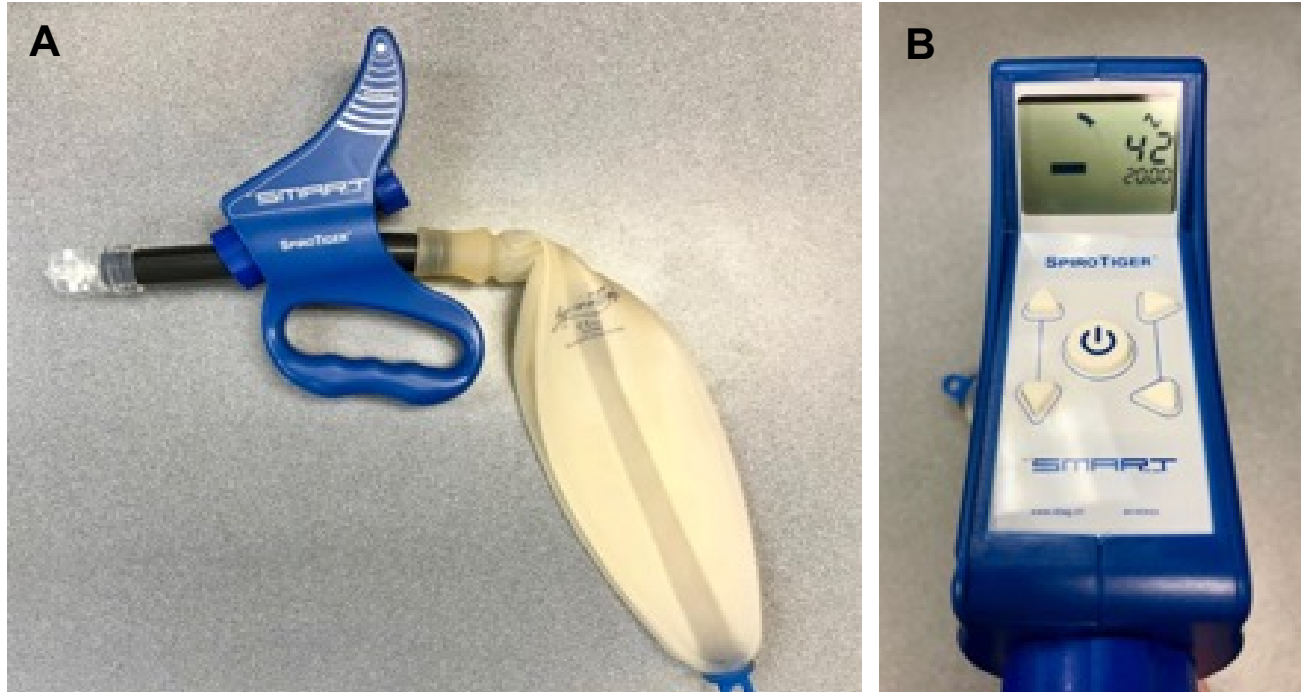
Figure 3. The SpiroTiger Smart device (A) consisting of the base unit (blue), a detachable mouthpiece, air-guiding parts, and a customized breathing bag. (B) The base unit screen and control panel.

In accordance with other studies (Bernardi et al., 2015; Campbell, 1982; Edwards, 2013; Wylegala, Pendergast, Gosselin, Warkander, & Lundgren, 2007) the size of the bag (V_{bag}) was adjusted to 55% of the participant's baseline FVC. The breathing frequency (f_b) of the first session was determined by dividing 55% of maximum voluntary ventilation (MVV; calculated as 35 times FEV_1) by the bag volume such that $f_b = MVV (0.55)/V_{bag}$. Throughout the four-week training intervention, if participants 'successfully' finished a training session (i.e., ≤ 2 breaths per minute below target f_b), participants were instructed to increase f_b by 3 breaths per minute for the succeeding session. If participants did not successfully finish a session, f_b remained the same for the succeeding session.

During the 4-week training period, participants performed a total of 20 minutes of eRMT (not including any breaks) in each of three separate sessions per week, with breaks during each session as needed. The session duration and amount of sessions per week were chosen based on recommendations from a SpiroTiger Breathing Specialist and guidelines outlined in the SpiroTiger Brochure (SpiroTiger, n.d., p.13). While training, participants remained seated and wore a nose clip to ensure breathing occurred exclusively through the eRMT device. The majority of sessions were evenly spaced with a one-day break between sessions, although some sessions were on back-to-back days to accommodate participants' schedules. This was a total of four hours of training spread across a total of 12 sessions. Participants were instructed to refrain from both rigorous exercise and a heavy meal 1 hour prior to eRMT.

During each eRMT session, HR data was continuously collected, and RPE were queried at the end of the session. Participants performed the respiratory function measurements and NIRS measurements described above at the beginning of the pre- and post-eRMT sessions during which the ergometer tests to voluntary exhaustion were conducted. Respiratory function measurements and NIRS measurements were also conducted during a separate mid-intervention session after two weeks of eRMT. At the end of each week, participants were asked to report their weekly exercise information (e.g., days, duration, and intensity). All measurements and tests were performed in a standardized manner and sequence.

Procedure

Visit 1

After querying and ensuring participants met inclusion criteria again (initially asked via email communication), participants were given an overview of what the study entailed, consented to participate, and completed the PAR-Q. Baseline (i.e., pre-eRMT) anthropometric

and demographic data as well as respiratory function measures were collected. Participants then underwent the baseline incremental exercise test. Following the exercise test, participants were instructed on the proper technique for training with the eRMT device.

Visits 2-13

Each visit, participants performed a 20-min session of eRMT. Visit 7, the mid-point (i.e., 2-week) visit, respiratory function measures were collected followed by a 20-minute eRMT session.

Visit 14

Follow-up (i.e., post-eRMT) anthropometric data and respiratory function measures were collected. Participants then completed the follow-up incremental exercise test. Participants were debriefed and given their honorarium.

Data Analysis

Data were first summarized using descriptive statistics and the D'Agostino-Pearson omnibus test was used to assess normality. After assessing normality, to make comparisons between the pre- and post-eRMT data, a paired t-test, or repeated measures one-way or two-way analysis of variance (ANOVA) was used as the parametric test when the data fit a Gaussian distribution; the Friedman test was used for nonparametric data. To provide a more valid critical F-value (i.e., reduce the increase in Type I error), violations of sphericity were corrected using the Geisser-Greenhouse correction for repeated measures ANOVAs.

Specifically, paired t-tests were used to compare the pre- and post-eRMT mean values of anthropometric characteristics, time to exhaustion, and estimated VO_{2max}. A repeated measures one-way ANOVA or the Friedman test was used to evaluate the effects of the 4-week eRMT program on respiratory function measures. Specifically, a repeated measures one-way ANOVA

was used to evaluate pre-, 2-week, and post-eRMT changes in FVC, FEV_1, MIP, and MEP, and the Friedman test for changes in FEV_1/FVC. Repeated measures one-way ANOVAs were also used to compare pre-, 2-week, and post-eRMT spirometry values (FVC, FEV_1, FEV_1/FVC) to reference values as per Quanjer (1993). Repeated measures one-way ANOVAs and the Friedman test were used to compare pre-, 2-week, and post-eRMT MIP and MEP values, respectively, to reference values as per Evans and Whitelaw (2009). Repeated measures two-way ANOVAs were used to evaluate the effects of the 4-week eRMT program on Borg exertion ratings, HR, and percentage of heart rate reserve (%HRR). Cohen's d_{av} effect sizes, commonly used for within-subject designs, were determined for resulting within-time point differences and then, given the small sample size, Hedges' correction was applied to generate Hedges's g_{av} (i.e., the corrected effect size; Lakens, 2013). Due to instrument errors, end-tidal carbon dioxide ($ETCO_2$) levels were displayed inconsistently and/or inaccurately; therefore, no $ETCO_2$ data was analyzed.

The Bonferroni test (ANOVA) or Dunn's test (Friedman) was used to correct for multiple comparisons and the multiplicity adjusted *p*-value for each comparison was reported. The selected post-hoc tests were chosen based on the experimental design and the structure of the specific comparisons of interest. Given the pre- to post-eRMT experimental design of our study, selected pairs of means were compared using a one-way ANOVA (i.e., comparison of the pre-eRMT mean to the post-eRMT mean), for which comparisons the Bonferroni post-hoc test is appropriate (Motulsky, July 21, 2020-a). Likewise, when the data were organized in three or more columns and the means within each row were compared using a two-way ANOVA, the Bonferroni was best suited to making post hoc comparisons (Motulsky, July 21, 2020-a). Further, the Bonferroni method was suitable for both the one-way and the two-way ANOVAs in this study because experimental means were not compared to a control mean (in which case

Dunnett's method would have been appropriate) nor was every mean compared to every other

mean (in which case Tukey's method would have been appropriate; Motulsky, July 21, 2020-a).

Based on the aforementioned conditions, Sidak's test would have yielded more statistical power

than the Bonferroni method; however, Sidak's test assumes that each comparison is independent

of the others (Motulsky, July 21, 2020-a). The comparisons in this study's experimental design

are not independent (e.g., see pg 79) and so the Bonferroni method was the best choice. For non-

parametric data that were analyzed using Friedman's omnibus test, Dunn's post hoc test was

selected because it is well suited to the following conditions that apply to the experimental

design and the goals of our analyses (Motulsky, accessed July 21, 2020-b): every mean was not

compared to every other mean, experimental means were not compared to a control mean, and,

unlike for the one-way ANOVA, selected pairs of means were not compared (e.g., see pg 71). It

is worth noting that GraphPad Prism does not even offer any alternatives to this test when faced

with the need for post-hoc comparisons following Friedman's test because Dunn's test is the

most commonly used option (Motulsky, July 21, 2020-c).

Data from the OxiplexTS system were processed in MATLAB (The MathWorks Inc.,

Natick, MA, USA). Specifically, the loading cycles were identified and the averages of the first

and last 30 full revolutions or cycles (~30 seconds) were calculated. For comparison of the

OxiplexTS data pre- and post-eRMT, a baseline adjustment to normalize optode placement and

intra-participant variation (e.g., skin tone, skin thickness, vasculature, etc.) was performed.

Specifically, a delta score was calculated by subtracting the average of the first 30 full cycles

during the 50 W stage of the cycle ergometer test from the average of the last 30 cycles before

task failure (i.e., exercise termination), providing relative values. Using GraphPad Prism

(GraphPad Software Inc., La Jolla, CA, USA), outliers were then identified and removed using

the ROUT method (robust regression followed by outlier identification) with the maximum False Discovery Rate (FDR), or coefficient Q, set to the default value of 1% (Motulsky & Brown, 2006). One participant was completely eliminated as a result of outlier identification. A mixed model with two fixed effects (PFC hemisphere [left and right] and intervention [pre- and post-eRMT]) and one random effect (participant) was performed to examine the difference in the pattern changes of NIRS variables. This model, as opposed to a traditional repeated measures two-way ANOVA, was chosen because of its advantage in dealing with missing data; Sidak's test was used to compare means.

Statistical analyses were performed using GraphPad Prism. Data are presented as mean and standard error unless specified otherwise. Results were considered significant at $p < 0.05$. Effects sizes were considered large when Hedge's g_{av} values exceeded 0.8.

Results

Descriptive Characteristics

Participants ($n = 8$) were recruited from UNBC (Prince George campus) and the Northern Sport Centre. Despite best efforts to obtain twenty participants, time and resource limitations prevented us from obtaining additional participants. Participants' anthropometric characteristics are reported as mean and standard deviation, shown in Table 1. Participants were male ($n = 4$) and female ($n = 4$), young adults ($M = 24.6$ years, $SD = 4.6$) of normal body mass index ($M = 22.91$ kg/m², $SD = 2.68$) and skinfold thickness (SCM; $M = 2.13$ cm, SD $= 0.84$, VL; $M = 7.50$ cm, $SD = 4.41$). There were no changes in anthropometric variables over the 4-week eRMT period. Participants self-reported their physical activity behaviour as either regular (1-3 hours/week; 37.5%), frequent (3-5 hours/week; 50%), or heavy (5-6 hours/week; 12.5%). No changes in weekly physical activity behaviour were reported throughout the study. All

participants were non-smokers and had no known history of respiratory, cerebral, or

cardiovascular dysfunction. All participants adhered to the RMT intervention, completing all 12

eRMT sessions.

Table 1

Sample and anthropometric characteristics of participants

	Pre-eRMT (*n*=8)	Post-eRMT (*n*=8)	Δ (%)
Age (years)	24.6 (4.6)	n/a	
Sex; *n* (%)			
Male	4 (50)	n/a	
Female	4 (50)	n/a	
Physical Activity Behaviour; *n* (%)			
Regular (1-3hr/week)	3 (37.5)	3 (37.5)	0
Frequent (3-5hr/week)	4 (50)	4 (50)	0
Heavy (5-6hr/week)	1 (12.5)	1 (12.5)	0
Weight (kg)	69.13 (13.21)	69.59 (13.55)	0.46 (0.66)
Body Mass Index (kg/m^2)	22.91 (2.68)	23.06 (2.78)	0.15 (0.65)
Skinfold Thickness (cm)			
SCM	2.13 (0.84)	2.13 (0.84)	0
VL	7.50 (4.41)	7.50 (4.41)	0

Note. Values are mean (± *SD*). Abbreviations: SCM, sternocleidomastoid; VL, vastus lateralis.

Respiratory Function Measures

The changes in respiratory function variables measured at baseline (pre-eRMT), mid-point (2-weeks of eRMT), and follow-up (after 4 weeks of eRMT; post-eRMT) are shown in Table 2. Figure 4 shows individual changes in FVC, FEV_1 and FEV_1/FVC. Repeated measures one-way ANOVAs showed there were no significant changes in either FVC ($F(1.95, 13.65) = 2.250, p = 0.1438$) or FEV_1 ($F(1.305, 9.135) = 0.9749, p = 0.3748$) over the 4-week eRMT period. A Friedman test showed that the effect of eRMT on FEV_1/FVC was not significant ($X^2 = 4.75, p = 0.1197$).

The observed FVC, FEV_1 and FEV_1/FVC values at baseline, mid-point, and follow-up were compared to reference values predictions calculated from the regression equations identified by Quanjer (1993; Table 2). Overall, compared to reference values, there was a statistically significant difference in observed FVC ($F(1.887, 13.21) = 13.40, p = 0.0008$) and FEV_1 values ($F(1.95, 13.65) = 8.082, p = 0.0050$) but not FEV_1/FVC ($X^2 = 7.650, p = 0.0538$). Bonferonni's multiple comparisons test indicated that FVC was significantly higher than the reference values ($M = 4.54$ L, $SE = 0.35$) at baseline ($\Delta = 0.80$; $t(7) = 3.590, p = 0.0266, g_{av} = 0.598$), mid-point ($\Delta = 1.01$; $t(7) = 3.983, p = 0.0159, g_{av} = 0.716$), and follow-up ($\Delta = 1.06$; $t(7) = 4.757, p = 0.0062, g_{av} = 0.791$). The mean observed FEV_1 was also significantly higher than the reference value ($M = 3.88$ L, $SE = 0.25$) at baseline ($\Delta = 0.59$; ($t(7) = 3.22, p = 0.0440$), $p = 0.0356, g_{av} = 0.573$), mid-point ($\Delta = 0.72$; $t(7) = 3.45, p = 0.0322, g_{av} = 0.670$), and follow-up ($\Delta = 0.79, t(7) = 3.37, p = 0.0355, g_{av} = 0.748$). Dunn's test showed that FEV_1/FVC at baseline was significantly higher than the reference value ($\Delta = 1.15$; $Z = 2.711, p = 0.0201, g_{av} = 0.430$), but was not significantly different from the reference value at mid-point ($\Delta = 0.66$; $Z = 0.9682, p = 0.9988, g_{av} = 0.150$) or follow-up ($\Delta = 0.77$; $Z = 0.9682, p = 0.9988, g_{av} = 0.167$).

Overall, there was no statistically significant difference in the MIP ($F(1.487, 10.41) =$ 1.610, $p = 0.2422$) and MEP ($F(1.801, 12.61) = 3.201, p = 0.0789$) values produced at 4 weeks post-eRMT compared to pre-eRMT and 2 weeks of eRMT (Table 2). Figure 5 shows individual changes in MIP and MEP. The MIP and MEP reference values per Evans and Whitelaw (2009) were calculated and compared to the observed MIP and MEP values at baseline, mid-point, and follow-up (Table 2). No statistically significant changes were observed for MIP ($F(1.724, 12.07)$ $= 1.417, p = 0.2766$). Post hoc analyses using Bonferroni's indicated that the reference value was not significantly different from the observed MIP values at follow-up, however, the effect was of large size, as indicated by the Hedge's g_{av} value ($\Delta = 17.4, t(7) = 2.81, p = 0.0786, g_{av} = 0.894$). A Friedman test showed that the difference between observed and reference MEP values was significant ($X^2 = 8.620, p = 0.0348$). However, post hoc analyses using Dunn's test indicated that compared to the reference value, the mean MEP value was not significantly different at baseline ($\Delta = 9.2, Z = 1.840, p = 0.1975$ $g_{av} = 0.254$), mid-point ($\Delta = 6.6; Z = 1.743, p = 0.2441, g_{av} =$ 0.178), or follow-up ($\Delta = -6.4; Z = 0.48, p = >0.9999, g_{av} = 0.157$).

Table 2

Pre-, midpoint, and post-eRMT respiratory function variables of participants, and reference spirometry values per Quanjer (1993) and MIP and MEP reference values per Evans and Whitelaw (2009)

	Pre-eRMT (*n*=8)	2-week eRMT (midpoint; *n*=8)	Post-eRMT (*n*=8)	Reference value
FVC (L)	5.34 (0.51)*	5.55 (0.55)*	5.60 (0.51)*	4.54 (0.32)
FEV_1 (L)	4.48 (0.39)*	4.60 (0.41)*	4.67 (0.40)*	3.88 (0.25)
FEV_1/FVC (%)	84.35 (1.32)*	83.86 (2.38)	83.97 (2.48)	83.21 (0.31)
MIP (cmH_2O)	105.4 (13.95)	107.5 (9.71)	118.9 (8.69)	101.5 (3.29)
MEP (cmH_2O)	122.5 (13.89)	125.1 (14.34)	138.1 (16.79)	131.7 (8.34)

Note. Values are mean (± *SE*). Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume during first second of expiration; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure.

* denotes significant intervention (pre-, mid-point, and post-eRMT) to reference value effect, *p* = <0.05.

A

B

C
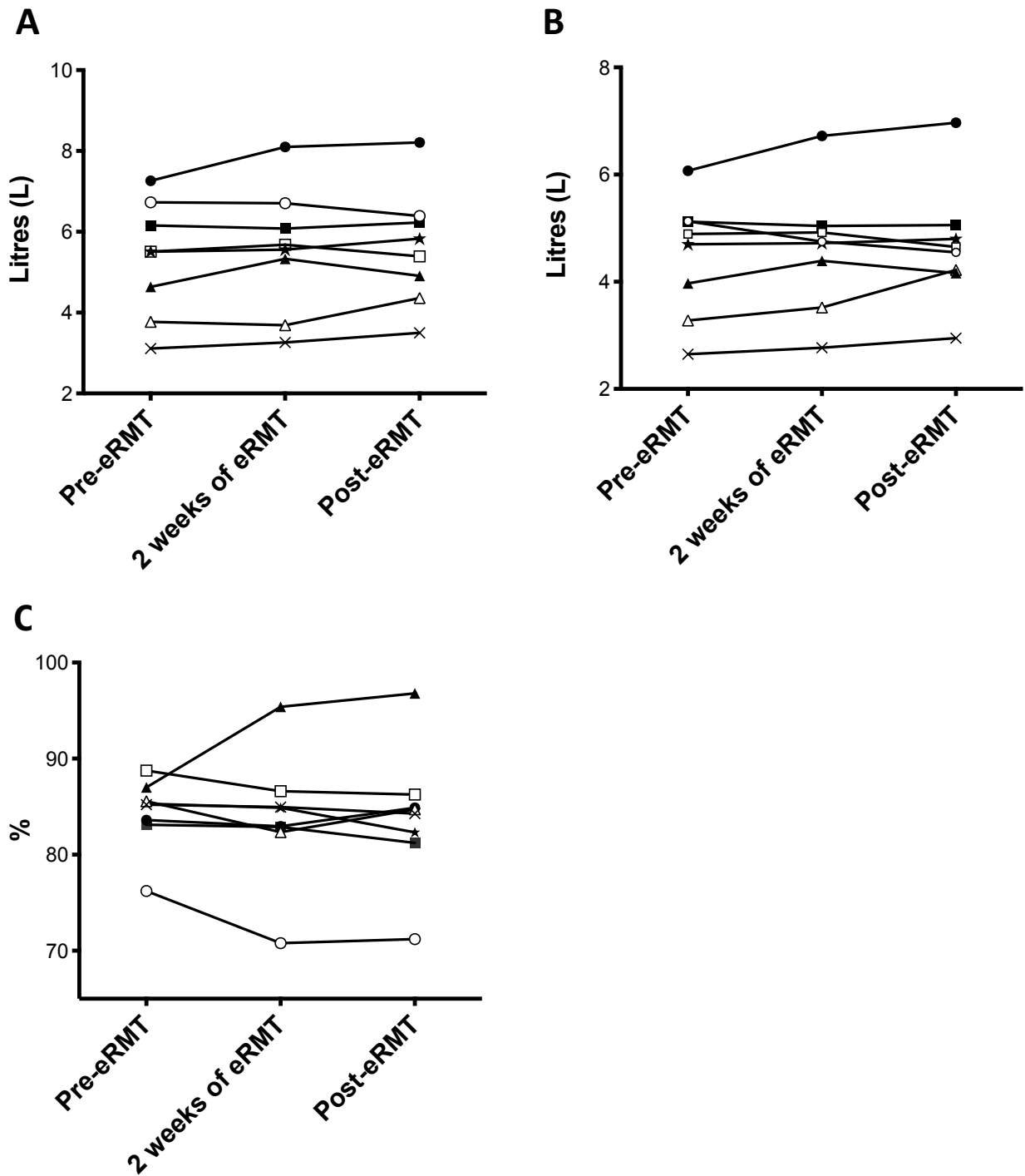
Figure 4. Spirometry values for FVC (A), FEV_1 (B), and FEV_1/FVC (C) before, at 2-weeks, and after 4-weeks of respiratory muscle endurance training (eRMT). Each symbol represents a different participant.
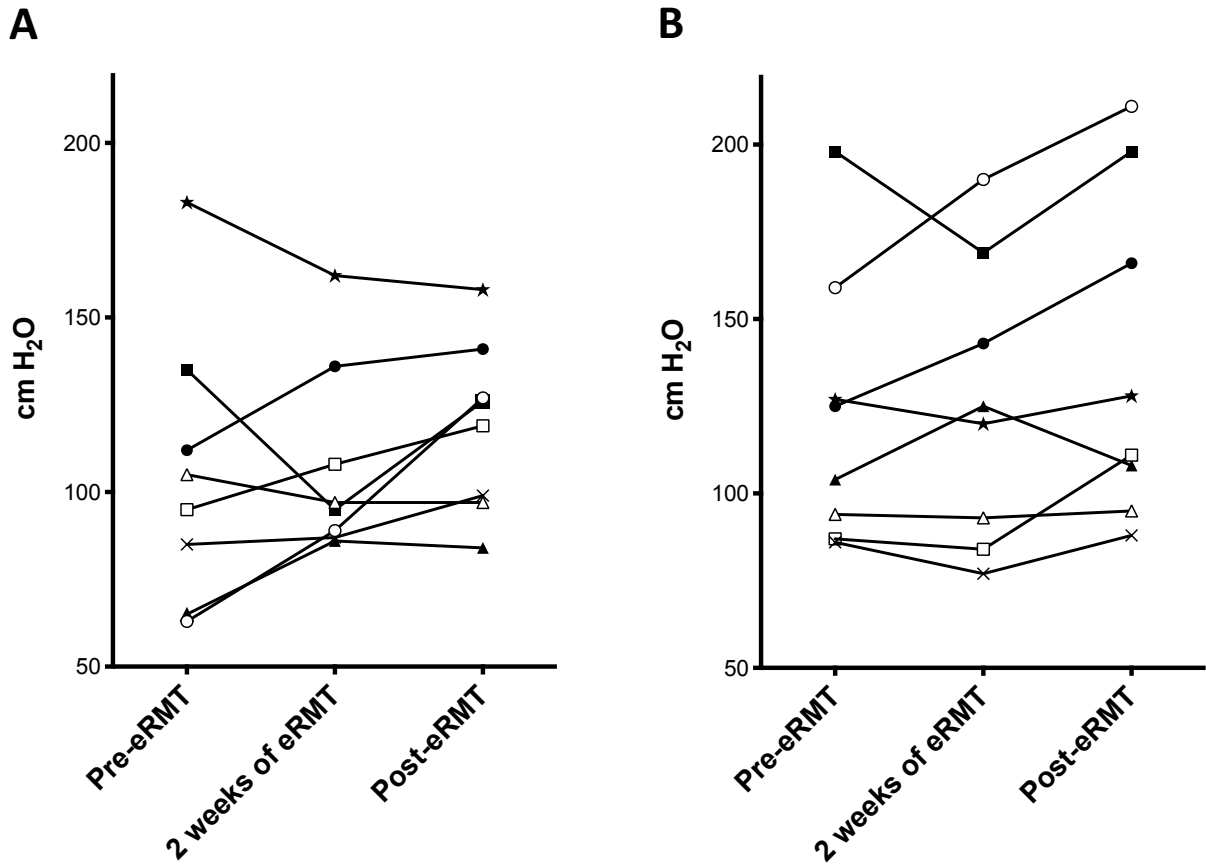
Figure 5. MIP (A) and MEP (B) values before (pre-), at 2-weeks, and after (post-) 4-weeks of respiratory muscle endurance training (eRMT).

Estimated VO$_{2\text{max}}$, Time to Exhaustion, and Heart Rate

The physiological responses to the incremental exercise tests before and after eRMT are shown in Table 3. There was no statistically significant change in the Storer estimations of VO$_{2\text{max}}$ over the 4-week eRMT period ($t(7) = 0.38$, $p = 0.7138$, $g_{av} = 0.036$). No statistically significant changed in the mean time to exhaustion (volitional or task failure) was observed post-eRMT ($t(7) = 1.36$, $p = 0.2174$, $g_{av} = 0.118$).

Figure 6 shows the progression of HR during the pre- and post-eRMT incremental exercise test that was sustained by all participants. Heart rate during the exercise tests were subjected to a repeated measures two-way ANOVA with two levels of intervention (pre- and post-eRMT) and four levels of exercise power increment (50 W, 95 W, 125 W, and final W before fatigue). The HR values at 50 W, 95 W, and 125 W of the incremental exercise tests as well as the HR at peak (HR$_{peak}$; i.e., final) intensity were chosen to ensure all participant ($n = 8$) data was included. The ANOVA results indicated that the main effect of intervention was not significant ($F(1,7) = 2.061$, $p = 0.1943$) but the main effect of exercise power (W) increment was significant ($F(1.066, 7.465) = 57.89$, $p < 0.0001$). However, post hoc comparisons using Bonferroni's multiple comparisons test indicated that the mean HR values were not significant at the 50 W ($p = >0.9999$, $t(7) = 0.83$, $g_{av} = 0.133$), 95 W ($p = 0.8166$, $t(7) = 1.40$, $g_{av} = 0.104$), 125 W ($p = 0.2850$, $t(7) = 2.12$, $g_{av} = 0.147$), or the final W ($p = >0.9999$, $t(7) = 0.08$, $g_{av} = 0.083$) increment of the exercise test. The interaction effect was non-significant ($F(1.421, 9.944) = 0.5698$, $p = 0.5266$). Mean and standard error HR data from the pre- and post-eRMT exercise tests are presented in Table 3.

To more accurately reflect the relative exercise intensity, the HR values at 50 W, 95 W, and 125 W of the exercise test were also expressed as a percentage of heart rate reserve (%HRR)

and evaluated using a repeated measures two-way ANOVA with two levels of intervention (pre- and post-eRMT) and four levels of exercise power increment (50 W, 95 W, 125 W, and final W before fatigue). The main effect of intervention was not significant ($F(1, 7) = 1.388, p = 0.2773$). The ANOVA results indicated that the main effect of exercise power (W) increment was significant ($F(1.144, 8.006) = 76.80, p < 0.0001$) but Bonferroni's multiple comparisons test indicated that the %HRR values were not significant at the 50 W ($p = >0.9999, t(7) = 0.60, g_{av} = 0.173$), 95 W ($p >0.9999, t(7) = 1.11, g_{av} = 0.129$), 125 W ($p = 0.4165, t(7) = 1.87, g_{av} = 0.176$), or the final W ($p = >0.9999, t(7) = 0.18, g_{av} = 0.068$) increment of the exercise test. There was no statistically significant interaction between the effects of intervention and exercise power increment on %HHR ($F (1.309, 9.165) = 0.3185, p = 0.6454$). Mean and standard error %HRR from the pre- and post-eRMT exercise tests are shown in Table 3.

Table 3

Pre- and post-eRMT physiological responses to the incremental exercise tests

	Pre-eRMT (*n*=8)	Post-eRMT (*n*=8)
Storer estimated VO_{2max} (mL·min^{-1}·kg^{-1})	39.2 (3.5)	39.6 (3.9)
Time to exhaustion (min)	16.9 (4.3)	17.5 (4.5)
HR at 50 W (beats·min^{-1})	102 (7.0)	99 (7.9)
HR at 95 W (beats·min^{-1})	123 (8.9)	120 (9.5)
HR at 125 W (beats·min^{-1})	139 (10.0)	134 (10.3)
HR_{peak} (beats·min^{-1})	179 (3.86)	178 (3.54)
%HRR at 50 W (%)	22 (0.04)	20 (0.04)
%HRR at 95 W (%)	40 (0.05)	38 (0.05)
%HRR at 125 W (%)	54 (0.06)	50 (0.06)
%HRR at HR_{peak} (%)	86 (0.02)	86 (0.02)

Note. Values are mean (± *SE*). Abbreviations: HR, heart rate; HR_{peak}, heart rate at peak intensity; %HRR, percentage of heart rate reserve.
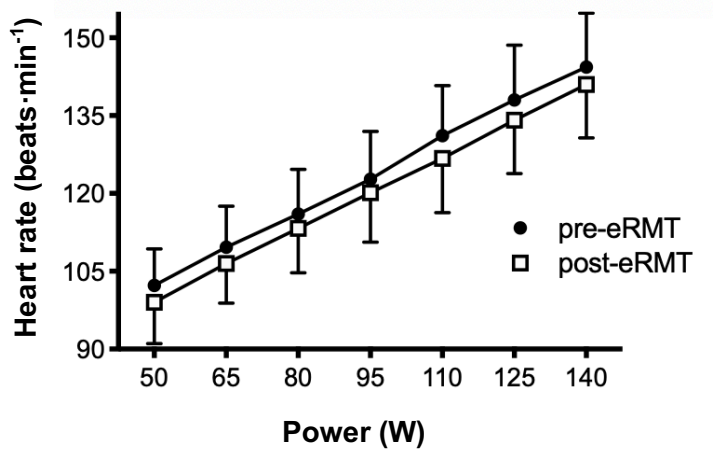
Figure 6. Progression of heart rate during incremental exercise test to exhaustion before (pre-) and after (post-) respiratory muscle endurance training (eRMT) that was sustained by all participants (*n*=8). Data are means mean (± *SE*).

Rating of Perceived Exertion

A repeated measures two-way ANOVA that examined the effect of intervention (i.e., the 4-week eRMT program) and exercise time points on Borg ratings of perceived exertion (RPE) was conducted. The Borg RPE evaluations at the end of the fifth (t5), eighth (t8), and tenth (t10) minute of the incremental exercise tests as well as the final RPE at exhaustion (tFinal) were chosen to ensure all participant ($n = 8$) data was included. The main effect of intervention yielded an F ratio of $F(1, 7) = 25.84, p = 0.0014$, indicating that the mean Borg rating was significantly decreased after the 4-week eRMT program than before the eRMT program. The main effect of exercise time point was also significant ($F(1.855, 12.98) = 129.0, p = < 0.0001$). Bonferroni's multiple comparisons test indicated that the Borg RPE evaluations were significant at t5 ($t(7) = 3.81, p = 0.0264, g_{av} = 0.627$) but not significant at t8 ($t(7) = 3.06, p = 0.0738, g_{av} = 0.560$), t10 ($t(7) = 2.76, p = 0.1126, g_{av} = 0.567$), or tFinal ($t(7) = 1.821, p = 0.4457, g_{av} = 1.12$). While these Borg RPE evaluations at t8 and t10 were not statistically significant, both effects were of medium size, as indicated by the Hedge's g_{av} values. There was no statistically significant interaction effect ($F(1.579, 11.05) = 0.3379, p = 0.6713$). Shown in Table 4 and Figure 7, mean RPE evaluations at t5 of the incremental exercise test decreased from 8.5 pre-eRMT to 7.4 post-eRMT (Δ 12.9%). At t8 and t10, the mean RPE evaluations decreased from 12.1 pre-eRMT to 11.1 post-eRMT (Δ 8.3%) and from 14.3 to 13.0 pre- to post-eRMT (Δ 9.1%). An RPE evaluation of 20 at pre-eRMT to 19.3 post-eRMT was observed immediately at the exercise test conclusion (tFinal; Δ 3.5%).

Table 4

Rating of perceived exertion (RPE: 6-20) in response to the incremental exercise test protocol at three time points and at exhaustion

	Pre-eRMT (*n*=8)	Post-eRMT (*n*=8)
RPE (t5)†	8.5 (0.5)	7.4 (0.46)*
RPE (t8)	12.1 (0.55)	11.1 (0.55)*
RPE (t10)	14.3 (0.68)	13.0 (0.68)*
RPE (tFinal)	20 (0)	19.3 (0.41)

Note. Values are mean (± *SE*). Abbreviations: (t5), minute 5 of exercise test; (t8), minute 8 of exercise test; (t10), minute 10 of exercise test; (tFinal), immediately at test conclusion.
* denotes significant pre-/post-eRMT main effect, $p = <0.05$;
† denotes significant exercise time point main effect, $p = <0.05$.
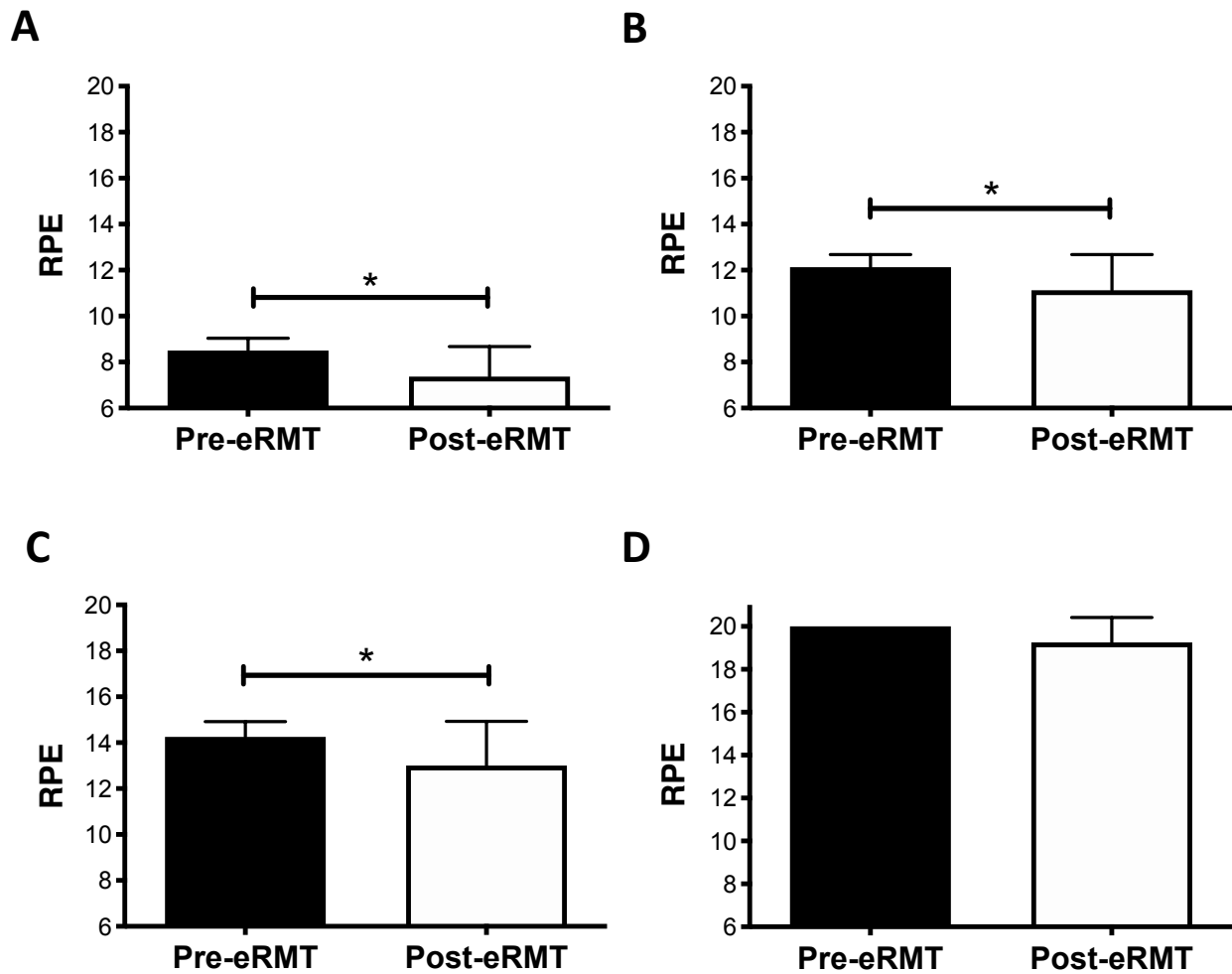
Figure 7. Rating of perceived exertion (RPE: 6-20) before (pre-) and after (post-) respiratory muscle endurance training (eRMT) at the end of the fifth (A), eighth (B), and tenth (C) minute of incremental exercise, and at test conclusion (D). Data are mean (± *SE*). * denotes significant effect between pre-/post-eRMT, $p < 0.05$.

NIRS Measurements

 Continuous NIRS measurements of cerebral oxygenation and hemodynamics using the OxiplexTS system were recorded during the pre- and post-eRMT incremental exercise tests. Mixed-effects models with two fixed effects (PFC hemisphere [i.e., left and right] and intervention [i.e., the 4-week eRMT program]) and one random effect (participant) showed that overall there were no statistically significant main effects for any of the hemodynamic variables ($[O_2Hb]$, $[HHb]$, $[tHB]$, and TOI%). There were, however, statistically significant interaction effects between intervention and PFC hemisphere for $[O_2Hb]$, $[HHb]$, $[tHB]$, but not for TOI%. The data are relative values as they were normalized to baseline and are presented in Table 5 and Figure 9.

 For $[O_2Hb]$, the main effect of intervention was not significant ($F(1, 6) = 0.01004, p = 0.9235$) and the main effect of hemisphere was also not significant ($F(1, 6) = 0.2952, p = 0.6065$). However, the interaction effect between intervention and PFC hemisphere was statistically significant ($F(1, 6) = 15.27, p = 0.0079$). As found in Table 5 and Figure 9A, the mean change in $[O_2Hb]$ increased pre-eRMT ($M = 0.59$ µM, $SE = 1.08$) to post-eRMT ($M = 2.31$ µM, $SE = 0.92$) in the right PFC, and decreased pre-eRMT ($M = 1.86$ µM, $SE = 1.67$) to post-eRMT ($M = 0.36$ µM, $SE = 0.72$) in the left PFC. The main effect of intervention on $[HHb]$ was not significant ($F(1, 6) = 1.339, p = 0.2912$) nor was the main effect of PFC hemisphere ($F(1, 6) = 0.2702, p = 0.6218$); the interaction effect, however, was statistically significant ($F(1, 6) = 14.50, p = 0.0089$). The mean change in $[HHb]$ increased pre-eRMT ($M = 1.03$ µM, $SE = 0.40$) to post-eRMT ($M = 1.66$ µM, $SE = 0.39$) in the right PFC and decreased pre-eRMT ($M = 1.89$ µM, $SE = 0.51$) to post-eRMT ($M = 0.52$ µM, $SE = 0.48$) in the left PFC (Table 5 and Figure 9B). For $[tHb]$, both the main effect of intervention ($F(1, 6) = 0.0465, p = 0.8365$) and

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hemisphere ($F(1, 6) = 0.4941$, $p = 0.5084$) were not significant although the interaction effect

was significant ($F(1, 6) = 24.57$, $p = 0.0026$). In the right PFC, the mean change in [tHb]

increased pre-eRMT ($M = 1.62$ μM, $SE = 0.81$) to post-eRMT ($M = 3.97$ μM, $SE = 0.70$) and in

the left PFC, [tHb] decreased pre-eRMT ($M = 3.76$ μM, $SE = 1.78$) to post-eRMT ($M = 0.88$ μM,

$SE = 0.38$; Table 5 and Figure 9C). For TOI%, no statistically significant difference for the main

effect of intervention ($F(1, 6) = 1.177$, $p = 0.3196$) or PFC hemisphere ($F(1, 6) = 0.1157$, $p =

0.7454$) was detected. Likewise, the interaction effect between PFC hemisphere and intervention

was non-significant ($F(1, 6) = 0.7412$, $p = 0.4223$) for TOI%. The mean TOI increased pre-

eRMT ($M = -1.59\%$ μM, $SE = 1.03$) to post-eRMT ($M = -1.35\%$ μM, $SE = 1.00$) in the right PFC

and in the left PFC increased pre-eRMT ($M = -2.25\%$ μM, $SE = 1.17$) to post-eRMT ($M =

-1.10\%$ μM, $SE = 0.99$; Table 5 and Figure 9D).

Table 5

Prefrontal cortex NIRS variables in response to the pre- and post-eRMT incremental exercise tests

	Pre-eRMT (*n*=7)		Post-eRMT (*n*=7)	
	Right PFC	Left PFC	Right PFC	Left PFC
O₂Hb (μM)*	0.59 (1.08)	1.86 (1.67)	2.31 (0.92)	0.36 (0.72)
HHb (μM)*	1.03 (0.40)	1.89 (0.51)	1.66 (0.39)	0.52 (0.48)
tHb (μM)*	1.62 (0.81)	3.76 (1.78)	3.97 (0.70)	0.88 (0.38)
TOI (%)	-1.59 (1.03)	-2.25 (1.17)	-1.35 (1.00)	-1.10 (0.99)

Note. Values are mean (± *SE*). Abbreviations: O₂Hb, oxygenated hemoglobin; HHb, deoxygenated hemoglobin; tHb, total hemoglobin; TOI, tissue oxygenation index.
* denotes significant interaction effect between eRMT intervention and hemisphere, $p = <0.05$
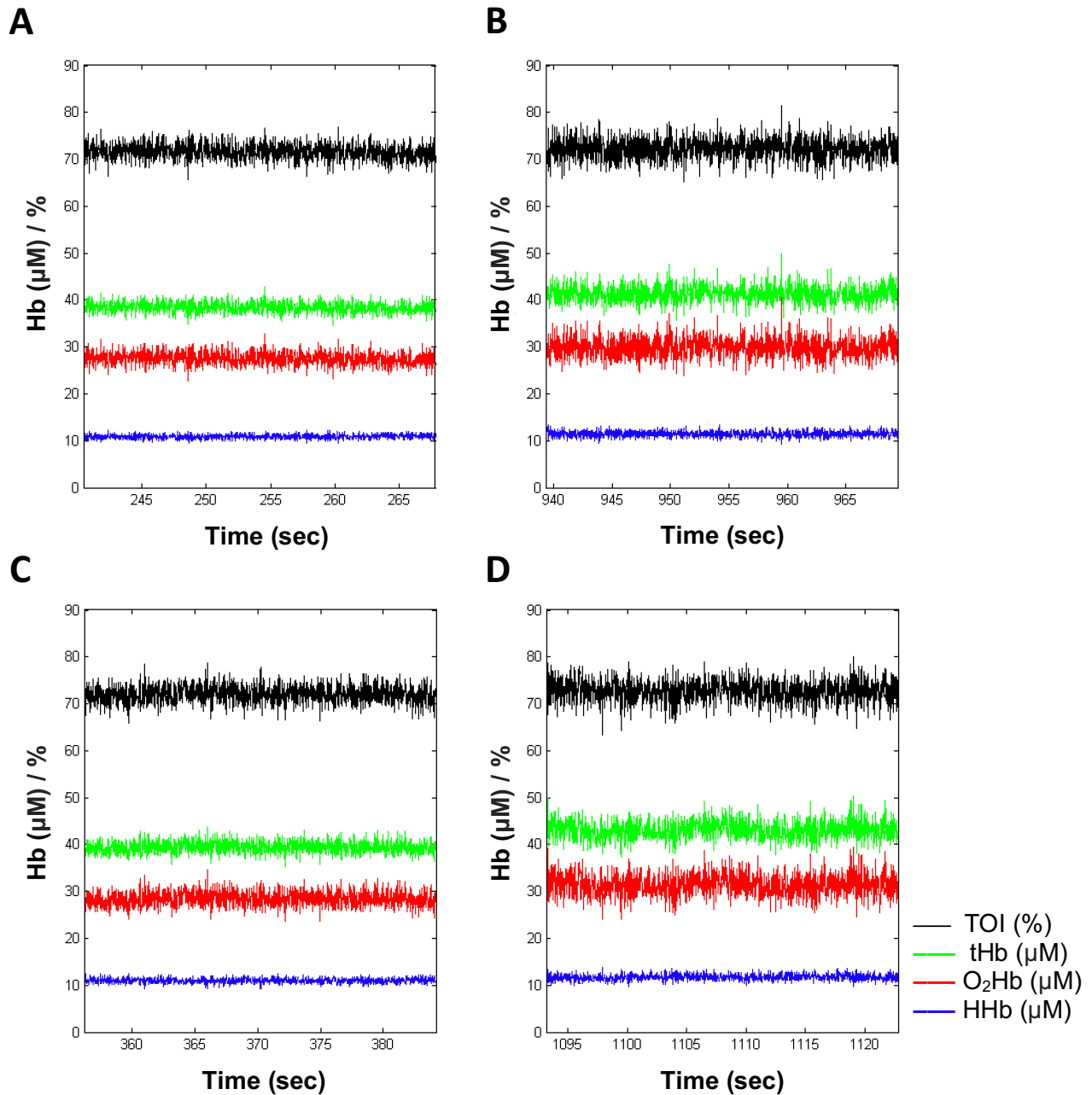
Figure 8. Representative near-infrared spectroscopy (NIRS) trace of the right prefrontal cortex (PFC) hemoglobin changes during: the first 30 cycles of 50 W increment of pre-eRMT incremental exercise test (A), the last 30 cycles before exhaustion of pre-eRMT incremental exercise test (B), the first 30 cycles of 50 W increment of post-eRMT incremental exercise test (C), and the last 30 cycles before exhaustion of post-eRMT incremental exercise test (D).
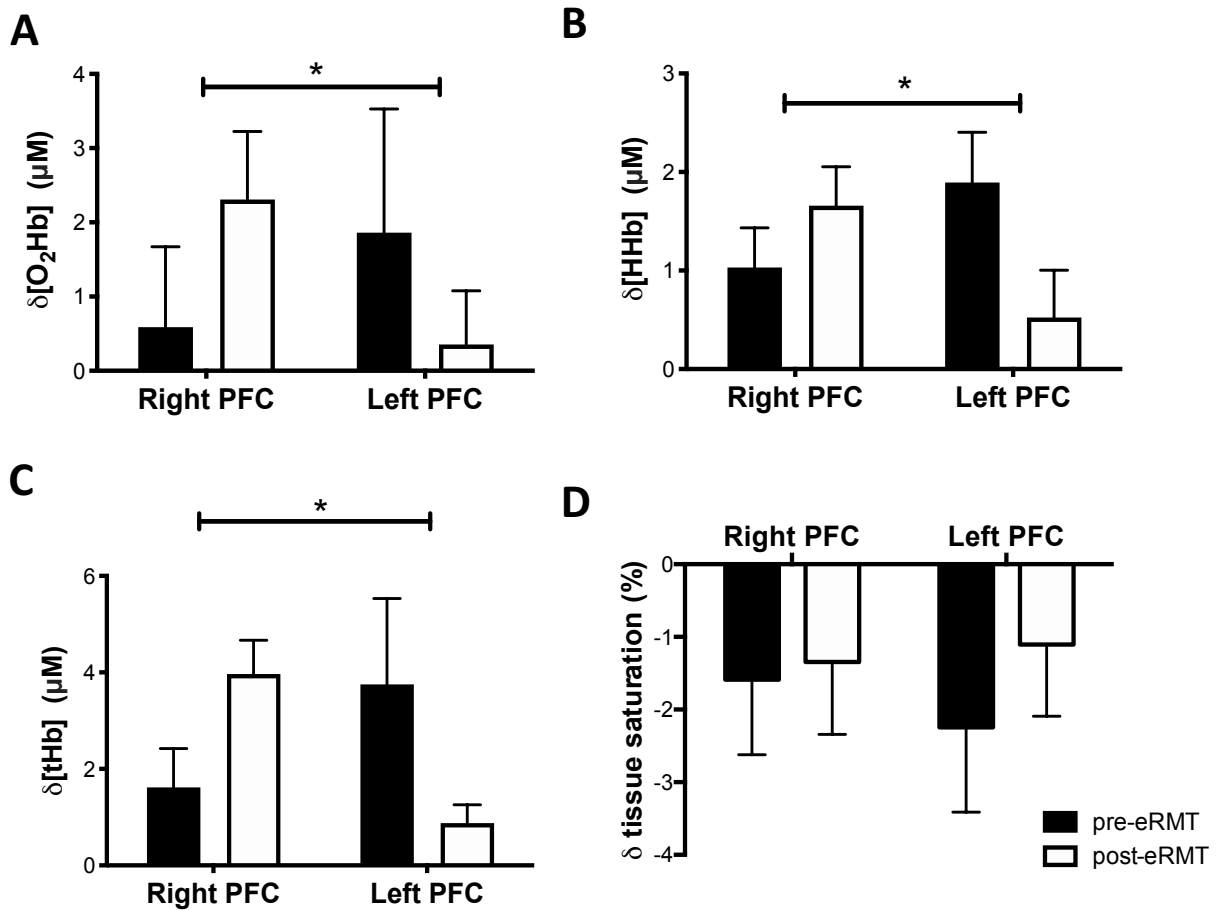
Figure 9. Prefrontal cortex (PFC) oxygenated hemoglobin (A), deoxygenated hemoglobin (B), perfusion (C), and tissue oxygenation (D) changes during incremental exercise test to exhaustion before (pre-) and after (post-) respiratory muscle endurance training (eRMT). Data are mean (± *SE*). * denotes significant interaction between eRMT and hemisphere, $p < 0.05$.

Discussion

Main Findings

The primary goal of this study was to investigate whether a 4-week respiratory muscle endurance training (eRMT) program influences physiological and psychological aspects of central fatigue using, respectively, near-infrared spectroscopy (NIRS) and quantification of effort perceptions. A secondary aim of the study was to assess any impact of the eRMT program on respiratory function and exercise performance in healthy adults. The main findings of the study were as follows. Firstly, eRMT program did not have any statistically measurable effects on respiratory function including spirometry (FVC, FEV_1, FEV_1/FVC) and respiratory muscle strength (MIP and MEP), on the time to exhaustion during the incremental exercise test, or on basic physiological responses to exercise (i.e., HR). As previously mentioned, $ETCO_2$ data was not analyzed due to instrument error and accordingly this hypothesis was not examined. Secondly, the main effects of intervention and exercise time point on participants' self-perceived exertion (RPE) at the fifth (t5) minute of incremental exercise were statistically significant but the interaction effect was not significant. Finally, during the incremental exercise test after 4 weeks of eRMT, compared to before eRMT, a statistically significant interaction between the prefrontal cortex (PFC) hemisphere (i.e., left versus right) and intervention (i.e., pre- and post-eRMT) was observed for three hemodynamic variables: oxygenated hemoglobin concentration ($[O_2Hb]$), deoxygenated hemoglobin concentration ($[HHb]$), and total hemoglobin concentration ($[tHb]$).

Changes in Respiratory Function Measures

Spirometry

Respiratory function is said to be improved by performing various breathing exercises denoted as respiratory muscle training (RMT; Illi et al., 2012). As previously discussed in the literature review, the two distinct forms of RMT used in healthy individuals—respiratory muscle strength training (sRMT) and respiratory muscle endurance training (eRMT)—differ in how they are performed and consequently, the muscle groups they target (Illi et al., 2012; McConnell & Romer, 2004). Respiratory muscle strength training is performed by breathing against an external inspiratory and/or expiratory load and involves high-force, low-velocity contractions, and thereby focuses on increasing the force-generating capacity (i.e., strength) of the respiratory muscles (Illi et al., 2012). Generally, sRMT focuses on increasing strength of the inspiratory muscles but can target the expiratory muscles; it is dependent on the desired outcome (Sapienza et al., 2011). In contrast, eRMT is performed using normocapneic hyperpnoea and includes low-force, high-velocity contractions of the inspiratory and expiratory muscles, and thus aims to improve the endurance of the respiratory muscles (Illi et al., 2012; refer to literature review for discussion on physiological mechanisms of RMT).

Accordingly, we hypothesized that performing breathing exercises by using a eRMT device could influence the endurance of the respiratory muscles and capacity of the lungs, and improve pulmonary function. We hypothesized that spirometry values (FVC and FEV_1,) and respiratory pressures (MIP and MEP, which are surrogate measures for the collective strength of the inspiratory and expiratory muscles) would increase after 4 weeks of eRMT. We found no statistically significant changes in any of the aforementioned variables after 4 weeks of eRMT. The mean FVC and FEV_1 values obtained from the baseline and follow-up sessions increased,

respectively, from 5.34 L to 5.60 L, and from 4.48 L to 4.67 L; and FEV_1/FVC declined from

84.35% pre-eRMT to 83.97% post-eRMT. Our results are similar to those in the existing

literature. Edwards (2013) found that participants ($n = 18$) who performed 30 consecutive

inspiratory efforts (i.e., inspiratory muscle training [IMT]) using an inspiratory pressure-

threshold training device set at 55% of MIP daily for 4 weeks had minor increases from pre- to

post-training of 5.4 L to 5.5 L for FVC, and 5.0 L to 5.1 L for FEV_1, although these findings, like

our own, were not statistically significant. Likewise, Gething, Passfield, and Davies (2004), and

Romer et al. (2002) did not observe any significant changes in FVC, FEV_1, and FEV_1/FVC after

6 weeks of IMT. In the Gething et al. (2004) study, participants in the experimental groups

performed 10 efforts daily at 100% MIP (maximum group; MAX, $n = 22$) or at 80% MIP (sub-

maximum group; SUB, $n = 21$) with 20 seconds recovery between each effort for 6 weeks

(maximal template was reassessed at each IMT session) while the control group (CON, $n = 23$)

did not complete any IMT. No changes in lung function as assessed by FVC, FEV_1, and

FEV_1/FVC were observed in any of the three groups. Participants in Romer et al. (2002)

consisted of healthy trained male cyclists ($n = 16$) who performed 30 dynamic inspiratory efforts

twice daily for 6 weeks against a pressure-threshold load equivalent to 50% MIP (load

periodically increased to a value that permitted participants to only just complete 30 efforts).

However, the participants in these investigations utilized IMT, a type of sRMT intended to target

primarily the inspiratory muscles whereas in the current study, we utilized eRMT designed to

target both the inspiratory and expiratory muscle groups.

Interestingly, Wylegala et al. (2007), who used a self-designed and built RMT device

similar to the SpiroTiger we used (D. Pendergast, personal communication, August 15, 2019),

found small yet statistically significant increases in FVC and FEV_1 in the eRMT group. The

participants ($n = 10$) were experienced male swimmers who underwent self-contained underwater breathing apparatus (SCUBA) training and 4 weeks of fin swim training (3 days/week) prior to eRMT (Wylegala et al., 2007). The eRMT sessions were 30 min/day, 5 days/week, for 4 weeks with the volume of the bag (V_{bag}) set to 55% of participants' slow vital capacity (Wylegala et al., 2007). Such discrepancies in findings may be the result of variances between the duration and frequency of the eRMT sessions used. Participants in Wylegala's and colleagues' study (2007) had a 4-week eRMT period similar to ours, but they performed approximately 10 hours of total eRMT time (30 min/day, 5 days/week, for 4 weeks) while participants in the present study performed the equivalent to approximately 4 hours of total eRMT time (20 min/day, 3 days/week, for 4 weeks). Moreover, the initial breathing frequency (f_b) corresponded to 60% and 55% of maximum voluntary ventilation (MVV) in Wylegala et al. (2007) and our study, respectively. Although it is near impossible to calculate the precise number of respiratory efforts (i.e., breaths) each participant performed, it can be estimated that 2-3 times more efforts were performed by Wyelgala et al. (2007) participants than our participants as they were breathing at a faster intensity and for a longer total duration. Therefore Wylegala et al. (2007) participants performed more than twice the total amount of eRMT time and total number of respiratory efforts than our participants did. This is suggestive that a greater duration of eRMT may result in greater structural and functional respiratory muscle adaptations, and thus improve respiratory function as well as exercise performance (Cheng et al., 2003; Gething et al., 2004; Illi et al., 2012). Therefore, the longer, more frequent training sessions observed in Wylegala and colleague's study (2007) may have contributed to their findings. Differences in RMT program design between the current study and previous studies may account for the lack of significant findings, although the observation that FVC and FEV_1 were essentially unchanged may be

explained by the notion that such parameters are generally understood to be largely dictated by an individual's height—the taller the individual, the larger the lung volume—and less influenced by training (Coates et al., 2013; Gething et al., 2004). This may, in part, be true, however it does not explain the results of Griffiths & McConnell (2007). Griffiths and McConnell (2007) found a decrease in FVC amongst male rowers after a two phase, 10-week RMT period. Specifically, participants performed 4 weeks of inspiratory (IMT, $n = 8$) or expiratory muscle training (EMT, $n = 7$) consisting of 30 inspiratory or expiratory efforts, respectively, twice daily against a pressure load equivalent to their individual 30 repetition maximum, followed by a 6-week period of combined IMT and EMT, which all participants underwent ($n = 15$).

To further examine this, our observed spirometry values were viewed in context of reference values derived from Quanjer et al. (1993; Table 2). It is important to specify, given the importance of ethnicity in lung volume (Coates et al., 2013; Quanjer et al., 1993), that the ethnicity of participants was not queried and therefore equations were used that do not require such information. Moreover, between the ages of 18 and 25 years, there is little if any change in ventilatory function (Quanjer et al., 1993) and therefore, as specified by Quanjer et al. (1993), 25 years was entered into the equations for participants ($n = 3$) in this age range. All of our observed spirometry values were higher than the predicted reference value and thus considered normal; consequently, the lower limit of normal (LLN) values were not calculated. We found that the pre-, mid-point, and post-eRMT observed mean FVC and FEV_1 were statistically different from the reference values further confirming the finding that there was no effect of eRMT on FVC and FEV_1. The mean observed FEV_1/FVC was significantly different from the reference value before eRMT, but was not significantly different after 2-weeks (mid-point) or 4 weeks of eRMT. Although this may demonstrate, indirectly, that there was an effect of eRMT on FEV_1/FVC, the

small effect sizes comparing the observed and reference values for FEV_1/FVC indicate a weak association.

Our lack of statistically significant findings for spirometry changes is likely due, in part, to low statistical power. As a result of the small sample size we obtained, our study is almost certainly underpowered and thereby has a low probability of detecting an effect. More so, our eRMT intervention was likely not sufficient enough (duration and intensity wise) to elicit any such eRMT-induced respiratory changes. Lastly, participants were initially asked if they had any known history of respiratory dysfunctions, to which all answered 'no.' However, participants were not queried about specific respiratory illnesses such as asthma and the use of inhalers. Given the commonality of asthma and the inter- and intra-variability of the disease, as well as the role it can play on spirometry and other lung function tests (i.e., MIP and MEP), this was largely overlooked and should be taken into consideration for future research.

MIP and MEP

We observed non-significant mean MIP values increase from 105.4 cmH_2O pre-eRMT to 118.9 cmH_2O post-eRMT while the mean MEP values increased 122.5 cmH_2O pre-eRMT to 138.1 cmH_2O post-eRMT. While increases in MIP following completion of a RMT program, mainly IMT, is a well-known functional improvement related to RMT (Edwards, 2013; Gething, et al., 2004; Romer et al., 2002; Verges et al., 2009; Wylegala et al., 2007), changes in MEP have less often been reported. Verges and colleagues (2009) compared the effects of IMT and eRMT on respiratory muscle performance in healthy males ($n = 26$). They observed that, after a 4-week IMT intervention, there were significant increases and decreases in, respectively, MIP and MEP. Intriguingly, they also observed the opposite after a 4-week eRMT intervention: decreases and increases in, respectively, MIP and MEP (Verges et al., 2009). As in our study,

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however, these latter eRMT-associated changes were not statistically significant. Verges et al. (2009) findings as well as our finding that MIP and MEP changes were not statistically significant probably demonstrates the specificity of the types of RMT techniques and their corresponding effects on inspiratory and expiratory lung function. As stated by Leith and Bradley (1976), voluntary normocapneic hyperpnoea eRMT, such as what we used in our study, does not improve the maximal pressure generating capacity of the respiratory muscles whereas IMT does.

Furthermore, it has been suggested that the training intensity and resistance load of a RMT program may account for such aforementioned differences in respiratory muscle strength parameters. As previously discussed (refer to literature review), the respiratory muscles are composed of different muscle fibres, which undergo various structural adaptations in response to RMT such as changes in fibre type and hypertrophy (Polla et al., 2004). Training at different intensities and loads will inevitably alter the number of respiratory efforts performed during a RMT session, and therefore the respiratory muscles will undergo adaptations to their structure (and function) that are specific to the training stimulus (Polla et al., 2004). Gething et al. (2004) observed healthy participants ($n = 66$) who trained (IMT) three times per week for 6 weeks. Each participant was randomly assigned to one of three groups—the maximum group (MAX) trained at 100% of MIP, the sub-maximal group (SUB) trained at 80% of MIP, and a control group (CON) who received no training. They reported that both the MAX and SUB training groups improved MIP (29% and 38% improvement, respectively) relative to the CON group, but did not differ between each other (Gething et al., 2004). Interestingly, they also observed a 12% non-significant change in the CON group, which suggests that caution should be exercised when relying on MIP as a measure of inspiratory muscle strength (Gething et al., 2004).

Considering these findings, it is understandable why we did not find any significant changes in the average MIP and MEP values from pre- to post-eRMT. The large variances we observed are presumably an effect not only of the small sample size but also of the inter-individual variation in response to the 4-week eRMT protocol (Figure 5). Thereby, the non-significant MIP and MEP increases, as well as the FVC and FEV_1, were likely not a physiological change but instead may have been the result of participant characteristics that have been considered to influence MIP such as effort or a learning effect (Dimitriadis, Kapreli, Konstantinidou, Oldham, & Strimpakos, 2011; Gething et al., 2004). Given that such tests are volitional and require full cooperation, participants may have been more motivated (for personal competition) to increase their MIP and MEP values during the post-eRMT testing. Likewise, because all participants had never previously performed any form of respiratory function testing, and because MIP and MEP testing requires understanding and coordination, participants may have developed or learned the proper technique with each succeeding effort (Pessoa et al., 2014). However, Dimitriadis et al. (2011) found that only two repetitions per session were needed to ensure MIP and MEP reliability of the MicroRPM, and eliminating the first repetition value increased the reliability further which suggests that a learning effect may only exist on the first repetition. Participants were given a demonstration of the procedure and several trials were permitted beforehand to minimize such effects. Moreover, apparatus set-up and test performance issues including type and fit of mouthpiece, presence of a small leak, and lung volume at the starting point of test performance can affect maximal mouth respiratory pressure values (Evans & Whitelaw, 2009; Pessoa et al., 2014). Regardless, low statistical power may have again played a role in our non-significant findings.

The observed pre- and post-eRMT MIP and MEP values were not significantly different from the reference values per Evans and Whitelaw (2009; Table 2), which provides further support that the eRMT had no effect on participants' inspiratory or expiratory respiratory muscle strength. That being said, however, although not statistically significant ($p = 0.0786$), we did however observe a large effect size ($g_{av} = 0.894$) when comparing the observed post-eRMT MIP value to the Evans and Whitelaw (2009) calculated reference value. With a larger sample size and/or a longer duration training intervention, an effect of eRMT on MIP may have been observed.

Estimated VO$_{2max}$, Exercise Performance, and Heart Rate Changes

Maximal oxygen consumption

The cycle exercise test (Storer et al., 1990) was chosen in part to allow us to estimate participants' relative maximal rate of oxygen consumption (VO$_{2max}$). Using participants' sex, age, body weight, and the maximal work rate completed during exercise, the equations generated by Storer et al. (1990) are said to predict VO$_{2max}$ to within 10% of its true value. Regardless, as hypothesized, we did not detect any significant differences in estimated VO$_{2max}$ after 4 weeks of eRMT compared to before eRMT (Table 3). It is well known that, in response to endurance training, skeletal muscle and cardiorespiratory metabolic adaptations such as an increase in the size and number of mitochondria and augmentation of the myoglobin content in the muscle are responsible for increases in VO$_{2max}$ (Bassett & Howley, 2000). Such adaptations in the oxidative capacity of the endurance-trained muscle and thus increase in VO$_{2max}$ however take several months of consistent and intense training, and vary immensely between individuals (i.e., genetic factors and initial fitness levels; Bassett & Howley, 2000). Moreover, eRMT is not believed to

result in such cardiorespiratory adaptations and thus does not increase VO_{2max} (Markov et al., 2001).

Our results are consistent with current literature. A systematic review by Illi et al. (2012) found that of 22 studies assessing VO_{2max} before and after RMT, 20 found no changes while the other two studies observed significant but opposing changes. Edwards (2013) found that VO_{2peak}, used to predict VO_{2max}, was unchanged following 4 weeks of eRMT. In a study by Markov et al. (2001), neither VO_{2peak} nor substrate utilization (measured using the respiratory exchange ratio) changed during a cycling endurance test performed after 15 weeks of eRMT; however, both did significantly decrease in the endurance trained group.

Exercise performance

Based on previous studies that provide convincing evidence supporting the ergogenic effect of RMT (Caine & McConnell, 1998; Edwards, 2013; Lemaitre et al., 2013; Markov et al. 2001; Romer et al., 2002; Verges et al. 2009), we hypothesized that improvements in the time to volitional exhaustion would be observed following the post-eRMT incremental exercise test as compared to the pre-eRMT test. However, our results did not support this because, while there appeared to be a small increase from 16.9 minutes pre-eRMT to 17.5 minutes post-eRMT, we found no statistically significant change in the average time to exhaustion. In agreement with the non-significant changes in HR and VO_{2max}, the lack of statistical significance in the exercise time to exhaustion data provide further support that our eRMT intervention was not a strong enough stimulus to invoke changes in exercise capacity. Moreover, the maximal incremental cycle ergometer test (Storer et al., 1990) we chose for this study may have also played a role. There is evidence that conventional (non-intermittent) incremental as well as fixed very high-intensity exercise tests (> 85% VO_{2max}) are inappropriate outcome measures of exercise performance and

are insensitive to RMT (Illi et al., 2012; McConnell & Romer, 2004). A meta-analysis including 46 original studies showed that improvements in exercise performance after RMT were significantly greater when tested with intermittent incremental tests (i.e., stepwise increase in exercise intensity with active recovery between steps) or constant load tests below 85% VO_{2max}, maximal workload, or maximal velocity compared with non-intermittent incremental tests (Illi et al., 2012). The reasoning for this relates to the physiology of fixed, very high-intensity exercise and the metabolic energy requirements it demands of skeletal muscle. Such exercise requires a 1000-fold increase in ATP demand compared to that at rest and, consequently, is limited by the inability of the oxygen transport system to maintain ATP re-synthesis and the corresponding accumulation of anaerobic metabolites that lead to the development of fatigue (i.e., muscle contraction failure; Baker, McCormick, & Robergs, 2010; McConnell & Romer, 2004).

It has been suggested that time trial exercise, with a maximal exercise effort of 75 seconds, is best suited for evaluating exercise performance as it derives approximately equal energy from both aerobic and anaerobic metabolism (Baker et al., 2010; McConnell & Romer, 2004). This has been demonstrated by Romer et al. (2002) who found 6 weeks of IMT improved exercise performance in both the simulated 20 and 40 km time-trials but not in the maximal incremental exercise test. The volitional nature of the intensity during a time trial performance, allows individuals to exert themselves as hard as they feel able, which may allow the benefits of RMT (e.g., reduced effort perceptions) to be expressed and thereby improve exercise performance (e.g., time to exhaustion). Contrarily, the only way individuals performing incremental or fixed high-intensity exercise tests are able to 'influence' the test is by prolonging the exercise to the point of exhaustion (i.e., metabolic energy systems reach the point of failure; McConnell & Romer, 2004). Moreover, psychological factors such as boredom and motivation

may also play a vital role in determining the point of exhaustion in fixed intensity exercise tests (Illi et al., 2012). Therefore, any ergogenic impact of RMT observed during incremental and/or fixed high-intensity exercise tests is likely to be small and hence difficult to measure (McConnell & Romer, 2004).

Heart rate

The Borg 6-20 Category Scale Rating of Perceived Exertion (RPE) evaluation implicates the combination of afferent feedback from cardiorespiratory, metabolic, and thermal stimuli along with feed-forward mechanisms (i.e., central command) to enable an individual to evaluate the work intensity undertaken at any point during an exercise task (Eston, 2012). However, the self-report of exertion is unique to an individual and thus is subjective (Eston, 2012). Moreover, it is moderated by various psychological aspects such as cognition, memory, previous experience, and understanding of the task as well as situational factors such as participant awareness of when the test will end and time-based characteristics of the exercise (Eston, 2012). Therefore, although RPE is a validated marker of exercise intensity, it is important to examine objective measures of intensity such as HR that may provide further insight into central fatigue and, by extension, exercise performance (Eston, 2012). For this reason, as well as to ensure safety, participants in our study had their HR continually monitored during both exercise tests. Maximal HRs at the power outputs of 50 W, 95 W, 125 W, and at peak intensity (HR$_{peak}$) were again chosen for the reason previously mentioned. As shown in Figure 6, HR during the cycling test was compared before and after eRMT for the constant-load period that was able to be sustained by all participants. There is a decrease in maximal HR at each minute of exercise during the post-eRMT test compared to the pre-eRMT test, although no statistically significant changes in HR at any of the specified power outputs were observed. Likewise, the %HRR at the

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50 W, 95 W, and 125 W increments of exercise were decreased post-eRMT compared to pre-eRMT but were not statistically significant. In agreement with the statistical significance, the small effect sizes further suggest that the intervention did not have any effect on HR.

Markov et al. (2001) examined healthy, sedentary adults who were randomized into three groups—RMT group, endurance training (running or cycling) group, and non-training control group—to examine the effects of 15-weeks of eRMT on cardiac stroke volume and HR in an attempt to explain post-eRMT increases in exercise performance. In agreement with our results, they found that HR at the same submaximal workload (60% maximal aerobic power) during an incremental cycling test to exhaustion did not change post-eRMT; stroke volume was also unchanged but exercise performance was increased (Markov et al., 2001). In the control group, no changes were observed in any of these variables, however, the endurance trained group observed increased stroke volume by 17% and reduced HR by 12% (Markov et al., 2001). They concluded that because eRMT prolonged exercise performance but neither lowered HR nor increased stroke volume, the prolongation cannot be attributed to hypothesized cardiovascular adaptations (Markov et al., 2001). Thus, the results provide further evidence that an alternative mechanism such as central fatigue is an exercise-limiting factor.

Rating of Perceived Exertion

In this study, self-reported RPE evaluations at the fifth (t5), eighth (t8), and tenth (t10) minute of the incremental exercise tests were examined, which equated to power levels of 50 W, 95 W, and 125 W, respectively. The RPE taken immediately at the conclusion of the exercise test (tFinal) was also recorded although we did not expect to see any significant differences given that, theoretically, participants should be at a state of maximal exertion (i.e., RPE 20) at exercise termination. Because the duration of the test varied amongst participants, these times were

chosen to ensure all participants were included in statistical analyses. We observed a significant main effect for intervention indicating that participants' Borg RPE evaluations were influenced by eRMT. Our results further suggest that participants RPE evaluations were influenced by the power increment of the exercise test and thereby exercise intensity as we observed significant RPE ratings at t5 (50 W). Although RPE was not statistically significant at t8 or t10, the effect sizes, $g_{av} = 0.560$ and 0.567, respectively suggests that the eRMT intervention had a medium effect on RPE at t8 and t10. However, the interaction effect between intervention and exercise time points was not significant.

Similar to our findings, Edwards (2013) observed reduced effort perceptions in the training group ($n = 18$) during incremental exercise after 4 weeks of IMT (30 consecutive inspiratory efforts at 55% of MIP). Moreover, Romer et al. (2002) recorded respiratory and peripheral exertion ratings during the final 30 seconds of each submaximal exercise stage using Borg's modified CR10 scale. After 6-weeks of IMT (30 consecutive inspiratory efforts twice a day [84 sessions total]), the training group ($n = 8$) experienced a reduction in the perception of both respiratory and peripheral effort while the control group did not (Romer et al., 2002). Interestingly, after 6 weeks of IMT, Gething and colleagues (2004) also observed a significant decrease in RPE in the MAX group (10 efforts daily at 100% MIP, $n = 22$) but not the SUB group (10 efforts daily at 80% MIP, $n = 21$). Our results, in conjunction with the aforementioned studies (Edwards, 2013; Gething et al., 2004; & Romer et al., 2002), suggest that feelings of fatigue were suppressed or experienced later in the post-eRMT incremental exercise compared to pre-eRMT. This provides further support that not only IMT (Caine & McConnell, 1998; Edwards, 2013; Romer, 2002) but eRMT may delay perceived exertion and the onset of central fatigue during exercise.

The precise mechanism(s) by which RMT lowers RPE remains unknown. It has been demonstrated that RPE is related to physiological markers such as VO_{2max}, blood lactate, and HR (Borg et al., 1985; Scherr et al., 2013). A high correlation exists between RPE ratings and HR during exercise, such that both increase linearly as exercise intensity progresses. The non-significant decreases in HR we observed did correspond to significant changes in RPE ratings at t5. At t5 of the post-eRMT exercise test, participants' average HR declined 3 beats·min^{-1} compared to the pre-eRMT exercise test and RPE decreased from 8.5 pre-eRMT to 7.4 post-eRMT (Table 3 and 4). Likewise, at t8 and t10 of the post-eRMT exercise test, average HR decreased by 3 and 5 beats·min^{-1} while RPE decreased from 12.1 to 11.1 and 14.3 to 13.0, respectively (Table 3 and 4).

It has been further suggested that perceived exertion may be related to an altered (reduced) perception of breathing effort (Gething et al., 2004). Gething and colleagues (2004) suggest this may be the result of improvements in the physiological conditioning that RMT brings about. Although we did not observe directly any significant eRMT-induced physiological changes, the finding that FEV_1/FVC was significantly different from the reference value before, but not after, eRMT as well as the significant interactions between intervention and the PFC hemisphere for the NIRS variables—[O_2Hb], [HHb], and [tHb]—suggest otherwise. Moreover, because we did not observe a significant interaction effect between the 4-week eRMT program and exercise time point on RPE, there is no question as to whether eRMT influences RPE independently. Therefore, it may be that the RPE is linked more to PFC hemodynamics than to the eRMT. As discussed in further detail below, the decreased RPE may be the result of an eRMT-induced left-to-right PFC hemodynamic shift consistent with the change from a novel task (i.e., baseline exercise test) to a learned task (i.e., follow-up exercise test). This

hemodynamic shift may occur due to central command (refer to literature review for prior discussion)—a feed-forward mechanism of descending neural signals from higher brain centres that are capable of parallel stimulation of cardiovascular responses and exercise movement (Mateika & Duffin, 1995; Nobrega et al., 2014; Secher, 2007; Williamson et al., 2006), which has been shown to be directed by an individual's perception of effort required (Decety et al., 1993; Thornton et al., 2001; Williamson et al., 2002). Although the origin(s) is ambiguous, evidence has shown that the PFC receives sensory inputs from multiple limbic structures such as the amygdala, hippocampus, and the PFC itself, and plays an imperative role in stress-related modulation of sympathetic outflow (Verberne & Owens, 1998). Various animal studies have demonstrated that introduction to a novel environment causes a significant increase in turnover of neurotransmitters and hormones (e.g., dopamine) in the medial PFC (Davis et al., 1994; Handa, Hejna, & Lorens, 1997). Therefore, we could postulate that as a result of the follow-up exercise test no longer being a novel task and environment, the observed eRMT-induced hemodynamic PFC shift was the result of a central command response-mediated cardiovascular response (i.e., decreased respiration rate). Such a response led participants to believe the effort required to accomplish the exercise test was lessened, resulting in lower RPE evaluations.

Cerebral Oxygenation and Hemodyamic Changes During Exercise

We hypothesized that 4 weeks of eRMT would result in slight increases in PFC perfusion and oxygenation during exercise testing. We found no overall main effects of intervention or PFC hemisphere on the NIRS variables but observed statistically significant crossover interaction effects for O_2Hb, HHb, and tHb. More precisely, during the incremental pre- and post-eRMT exercise tests to exhaustion, we observed an increase in PFC perfusion (i.e., tHb or blood volume), as well as concentrations of O_2Hb and HHb, and this increase differed by PFC

hemisphere (Figure 9). This may suggest that the interaction between the 4-week eRMT program and PFC hemisphere is driven by a eRMT-induced shift in exercise-dependent perfusion, in which cerebral blood shifts in favour of the right PFC at the expense of the left PFC (i.e., a relative redirection of blood flow from the left PFC to the right PFC).

When performing exercise, neurons in the corresponding brain areas are activated. Such an increase in neural activity is linked to an increase in regional blood flow to transport O_2 as well as glucose to meet increased metabolic demands. To avoid a state of O_2 depletion due to O_2 consumption, this, in turn, alters the concentrations of O_2Hb, HHb, and tHB such that an increase in $[O_2Hb]$ and decrease in $[HHb]$ occurs (Bonetti et al., 2018). Therefore, changes in NIRS-determined cerebral oxygenation reflect modulations in cerebral functional activation (Bonetti et al., 2018; Rasmussen et al., 2007; Rupp & Perrey, 2008). As previously discussed (see NIRS and PFC studies section), several studies have reported on PFC oxygenation and hemodynamic changes in response to exercise maintained to exhaustion, yet the majority of these studies only use one cerebral NIRS probe to examine either the left (Bhambhani et al., 2007; Rupp & Perrey, 2008; Subudhi et al., 2007; Subudhi et al., 2009) or right (Imray et al., 2005) hemisphere of the PFC, or they do not differentiate between/specify hemisphere (González-Alonso et al., 2004; Ide et al., 1999; Nielsen et al., 2001; Nielsen et al., 1999). The PFC is well understood to be involved in functions that enable cognitive control—the ability to orchestrate thought and action to yield goal-directed and intelligent behaviors—such as stimulus selection, decision-making, motivational salience, planning a task, maintenance of attention, memory, and others, but these functions are often generalized to the entire PFC (Miller & Cohen, 2001; Ott & Nieder, 2019; Tempest, Eston, Parfitt, 2014). As with many regions of the brain, there are significant

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hemispherical differences within the PFC, which may offer some insight into our findings regarding the interaction between hemisphere and eRMT.

Activation of the left PFC is believed to assist in working memory as well as planning and manipulating the appropriate rules for the brain to accomplish a specified, current goal (Banich et al., 2000; Gabrieli, Poldrack, & Desmond, 1998; Tempest & Reiss, 2019). This has been demonstrated using the colour-word Stroop task. Using NIRS, Yanagisawa et al. (2010) found that healthy, young adults dominantly recruited the left PFC during Stroop task, and that an acute bout of moderate exercise caused increased activation of the left dorsolateral PFC due to Stroop interference. The increased activation coincided with improved cognitive performance in the majority of participants (Yanagisawa et al., 2010). Contrarily, the right PFC is said to have an important role in response inhibition, which allows the suppression of actions, thoughts, and impulses that are inappropriate and/or interfere with goal-directed behaviour (Hege, Preissl, & Stingl, 2014). In the current study, both the exercise task itself and the environment were novel and unfamiliar during the initial, pre-eRMT exercise test. Accordingly, during the test, participants were required to remember and apply the various information regarding the test protocol that they were given prior to commencement (i.e., working memory) in order to complete the defined goal of cycling until task failure or volitional exhaustion. This may explain the increased activation denoted by increased concentrations of O_2Hb, HHb, and tHb in the left PFC we observed during the pre-eRMT. Contrarily, during the post-eRMT exercise test, the right PFC presented an increase in cerebral perfusion, [O_2Hb], and [HHb]. As the task was no longer new but learned, the reduced processing of variables required during the post-eRMT test and any unnecessary thoughts or behaviours that may interfere with the goal could have been repressed, hence activating the right PFC.

Moreover, literature has indicated that distinct PFC regions contribute to different dimensions of emotional stimulus processing such as judgment, perception, and attention (Ueda et al., 2003; Grimm et al., 2006). Using fMRI, Ueda et al. (2003) demonstrated that increased neural activity in the left dorsolateral PFC of healthy adults is associated with emotional judgment, whereas the right dorsolateral PFC is associated with anticipation or attention to emotional judgment. Their findings further suggest that left PFC activation is associated with the expectation of pleasant stimuli and that right PFC activation is associated with the expectation of unpleasant stimuli (Ueda et al., 2003). This is in line with the valence theory of lateralization, which postulates that each half of the brain is specialized for processing particular classes of emotions, with a dominance of the left PFC in processing positive emotions and the right PFC in negative emotions (Ahern & Schwartz, 1979; Wager, Phan, Liberzon, & Taylor, 2003). Consequently, in the current study, it could be argued that compared to the pre-eRMT exercise test, the increased concentrations of O_2Hb, HHb, and tHb in the right PFC during the post-eRMT exercise test are the result of participants' negative intrinsic valence or emotion regarding the exercise task. To the majority of healthy individuals, the dominant affective response to high-intensity exercise is displeasure (Ekkekakis, Hall, & Petruzzello, 2005). This is predominately shaped by the aversive sensations that accompany an accumulation of lactic acid resulting from the transition to anaerobic metabolism (Ekkekakis et al., 2005). Therefore, given that participants had already performed the exercise test prior (i.e., pre-eRMT exercise test), they likely anticipated what to expect from the seemingly unpleasant, exhausting exercise test, possibly resulting in the increased activation in right PFC. Likewise, the majority of participants expressed the physical discomfort and 'mentally taxing' aspect of performing eRMT. The

perceived discomfort of the eRMT may have impacted the cognitive response to the post-eRMT exercise test.

Limitations

As with most, this study is not without limitations. First, the small sample size may have increased the probability that the observed changes were due to chance, and also conversely that real effects may not have been detected due to low power (i.e., a Type II error). Another limitation of the study, as previously mentioned, is the frequency and duration of the eRMT sessions, as well as the intensity at which each eRMT session was performed. A review by McConnell and Romer (2004) stated that voluntary normocapneic hyperpnoea eRMT sessions are typically conducted 3 to 5 times per week at approximately 60-90% of MVV. Participants in our study conducted 3 eRMT sessions per week at 55% of MVV. More frequent sessions and/or at a higher intensity might have produced some respiratory function and strength adaptions, and thus better exercise performance. However, given the already extensive time commitment to the study and the fact that participants were untrained (i.e., not athletes), a longer and/or more frequent eRMT schedule would likely have proven too time consuming and physically demanding. Due to budgetary limitations, we were unable to afford more than two SpiroTiger devices. Consequently, participants had to complete all eRMT sessions in the lab in contrast to better funded studies in which participants complete the training at home (Bernardi et al., 2015; Caine &McConnell, 1998; Wylegala et al., 2007). Moreover, the technique of eRMT we utilized requires a high degree of participant motivation. Although participants were encouraged to complete each training session to the best of their ability as well as the fact that the SpiroTiger provides visual and audio feedback, only the individual using the device is truly aware of their

effort given. Moreover, the inability to accurately measure VO_{2max}, due to budgetary limitations preventing the use of a metabolic cart, was a large limitation of this study.

Perhaps one of the largest denunciations of this study is the lack of any control group, which did not engage in any real respiratory muscle exercise. It is well recognized that not having a control group could undermine the validity of findings since any potential improvements could be attributed to various confounding factors (e.g., a learning effect, motivation differences, intra-individual variation, etc.). However, given that this study is one of the first serious attempts at (1) examining the effects of eRMT on cerebral hemodynamics and oxygenation and (2) assessing the potential utility of normocapneic hyperventilatory eRMT as an option for COPD patients as opposed to the extensively researched resistance/threshold loading type of RMT, we chose to place emphasis on collecting as much data as possible from an experimental group.

The strengths of NIRS are numerous, however, there were several limitations presented by the instrumentation during our study. First, the temporary placement of the OxiplexTS optodes proved difficult. A stable contact between the optode and skin is vital, therefore careful initial placement and adherence to the head was ensured. However, as a result of high-intensity exercise, the majority of participants were perspiring profusely, especially from their heads. This rendered it very difficult to keep the optodes from shifting and may have led to detector contamination with room light, giving a less clean signal. Second, the exact spatial origin of the cortical hemodynamic response and the precise identification of the brain area beneath the optode is not possible using only NIRS. Although we are confident we were examining the PFC, we cannot state with certainty that it was the same location of the PFC that was measured for each participant. Lastly, as stated by Quaresima & Ferrari (2019), the separation of

hemodynamic changes originating either from the cerebral cortex or other tissues/structures such as the scalp, meninges, or frontal sinuses is challenging. We did not account for changes in skin blood flow or temperature during the incremental exercise tests, so our NIRS measurements may not be entirely representative of strictly cerebral tissue.

Future Research

The findings from this study illuminate several recommendations and areas for further investigations. To build on the findings from this 4-week eRMT program, a larger sample size and a longer duration eRMT program and/or more frequent weekly sessions could contribute to understanding eRMT benefits on respiratory health (i.e., function and strength). As previously discussed, an intermittent incremental or time trial exercise test as opposed to the non-intermittent maximal incremental test we chose, may be better suited in evaluating an ergogenic effect of eRMT (McConnell & Romer, 2004). As well, increased understanding of the mechanisms that lead to cerebral hemodynamic shifts as a result of eRMT is needed. For example, further study of the physiological consequences of eRMT on other areas of the brain is required. To supplement NIRS findings as well as combat some of the aforementioned limitations of NIRS, future research may wish to use another non-invasive cerebral monitoring techniques such as an electroencephalogram (EEG) to gain insight about other areas of the brain in response to an eRMT program.

Due to aging populations and higher smoking prevalence in many countries, the burden of debilitating chronic respiratory conditions is estimated to increase in the coming years Mathers & Loncar, 2006; World Health Organization [WHO], 2017). We are hopeful that future research will consider our recommendations and provide further evidence for the feasibility and effectiveness of eRMT especially for patients with COPD. Further study into the physiological

and psychological effects of eRMT in other populations, such as middle-aged adults (>40 years of age) whom the disease generally becomes apparent in should, however, first be undertaken (WHO, 2017).

Conclusion

In summary, the results of the present study provide preliminary evidence for a respiratory muscle endurance training (eRMT)-induced left to right prefrontal cortex (PFC) hemodynamic shift during maximal cycle ergometer exercise, which is consistent with change from a novel to a learned task. Participants' ratings of perceived exertion (RPE) decreased after 4 weeks of eRMT, which we suggest may be related to the PFC perfusion interaction between eRMT and hemisphere. These findings support the utility of NIRS to provide a safe, non-invasive technique capable of measuring PFC hemodynamic and oxygenation changes during cycling exercise that could not be achieved by other means. Moreover, although we did not observe any significant respiratory muscle function or strength changes after eRMT, this study is one of the first systematic investigations of the SpiroTiger and accordingly, one of the first attempts at assessing the potential utility of normocapneic hyperventilatory eRMT in healthy individuals.

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Participant Recruitment Flyer

VOLUNTEERS ARE REQUIRED

to participate in a research study at **UNBC** UNIVERSITY OF NORTHERN BRITISH COLUMBIA

Effects of Respiratory Muscle Training on Cerebral Oxygenation and Hemodynamics, Effort Perceptions, and Respiratory Muscle Adaptations

Purpose: We want to assess physiological changes that are associated with an endurance respiratory training device. This device is currently used in training programs for elite athletes, and it may potentially help people with respiratory diseases. This study will hopefully provide baseline data for future studies of athletes and patients.

This study is not a therapeutic or clinical intervention.

What will happen?	Participants must
You will participate in a respiratory muscle training spanning 4 weeks. We will assess your physiological response to respiratory muscle training using near infrared spectroscopy (measures tissue oxygen content/consumption), electromyography (measures muscle activation), and performance during an exhaustive, incremental cycling test. **Total time commitment:** Approximately 9 hours spread over 4 weeks **Honorarium:** $50 gift card	• be between the ages of 18 and 55 • be a non-smoker • have normal respiratory values • exercise at least 3 days/week at a moderate- to vigorous-intensity for 30 minutes. • wear shorts and a t-shirt during measurement • <u>not</u> be an elite athlete (60 min/day for at least 6 days/week) • <u>not</u> have excessive body fat on the leg, arm or neck • <u>not</u> have any known neuromuscular, respiratory, cerebral or cardiovascular diseases

For more information, please contact
Johnna Somerville
somerville@unbc.ca
Cell: (250) 613-5234

Participant Information Form

Participant Number: _____   Researcher: _____

Participant Information Form:
Effects of Respiratory Muscle Training on Cerebral Oxygenation and Hemodynamics, Effort Perceptions, and Respiratory Muscle Adaptations

Age: _____

Sex: _____

Height: _____

Training background (circle one): Regular: 1-3hr/week
                                 Frequent: 3-5hr/week
                                 Heavy: 5-8 hr/week

Health Parameters					
	Date/time	Weight	Skin-fold thickness (mm)		Blood pressure (mmHg)
			SCM	VL	
Baseline		lb: kg:			
Follow-up		lb: kg:			

Respiratory Function Measures						
	Date/time		FVC	FEV$_1$	MIP	MEP
Baseline		1				
		2				
		3				
		Average				
2 week follow-up		1				
		2				
		3				
		Average				
4-week follow-up		1				
		2				
		3				
		Average				

Participant Number: _____

Baseline Maximal Exercise Test						
Date/time:						
Caffeine consumption 4 hours prior (circle): YES NO						
End of stage #	Power (watt)	Resistance (kp)	Time	Borg rating	Heart rate	Comments
Warm-up	0	0				
1	50	0.85				
2	65	1.10				
3	80	1.36				
4	95	1.61				
5	110	1.87				
6	125	2.12				
7	140	2.38				
8	155	2.63				
9	170	2.89				
10	185	3.14				
11	200	3.40				
12	215	3.65				
13	230	3.91				
14	245	4.16				
15	260	4.42				
16	275	4.67				
17	290	4.93				
18	305	5.18				
19	320	5.44				
20	335	5.69				
21	350	5.95				
22	365	6.20				
23	380	6.46				
24	395	6.71				
25	410	6.97				

Pre-ETCO$_2$ level:

Post-ETCO$_2$ level:

Post-BP:

Time to fatigue:

Test termination (circle):   VOLITIONAL   TASK FAILURE

Estimated VO$_{2max}$ (Storer):

Estimated VO$_{2max}$ (movescount):

2

139

Participant Number: _____

* Bag size will be adjusted to 55% of the participant's baseline FVC and f_b will correspond to 55% of MVV (calculated as 35 times FEV_1/FVC).

If participants can perform 20mins of RMT with no breaks, increase f_b by 5 for the succeeding session.

Respiratory Muscle Training								
Session	Date/time	RMT Duration (min)	Rest Duration (min)	Bag Size (L)	f_b	Borg Rating	Max. HR	Comments
1*				.55 x FVC=	35 x (FEV_1/ FVC) =			Pre-ETCO$_2$: Post-ETCO$_2$:
2								
3								
End of week 1 Weekly physical activity (days/duration/intensity):								
4								
5								
6								Pre-ETCO$_2$: Post-ETCO$_2$:
End of week 2 Weekly physical activity (days/duration/intensity):								
7								
8								
9								
End of week 3 Weekly physical activity (days/duration/intensity):								
10								
11								
12								Pre-ETCO$_2$: Post-ETCO$_2$:
End of week 4 Weekly physical activity (days/duration/intensity):								

3

Participant Number: _____

Follow-Up Maximal Exercise Test						
Date/time:						
Caffeine consumption 4 hours prior (circle): YES NO						
End of stage #	Power (watt)	Resistance (kp)	Time	Borg rating	Heart rate	Comments
Warm-up	0	0				
1	50	0.85				
2	65	1.10				
3	80	1.36				
4	95	1.61				
5	110	1.87				
6	125	2.12				
7	140	2.38				
8	155	2.63				
9	170	2.89				
10	185	3.14				
11	200	3.40				
12	215	3.65				
13	230	3.91				
14	245	4.16				
15	260	4.42				
16	275	4.67				
17	290	4.93				
18	305	5.18				
19	320	5.44				
20	335	5.69				
21	350	5.95				
22	365	6.20				
23	380	6.46				
24	395	6.71				
25	410	6.97				

Pre-ETCO$_2$ level:

Post-ETCO$_2$ level:

Post-BP:

Time to fatigue:

Test termination (circle):   VOLITIONAL   TASK FAILURE

Estimated VO$_{2max}$ (Storer):

Estimated VO$_{2max}$ (movescount):