Effects of Elevated Core Temperature and Normoxic 30% Nitrous Oxide on Control of Human Breathing during Short Duration, High Intensity Exercise

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Assaf Yogev

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Name: **Assaf Yogev**

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Examining Committee: Chair: David C. Clarke
Assistant Professor

Matthew D. White
Senior Supervisor
Associate Professor _____

Michael L. Walsh
Supervisor
Lecturer _____

Andrew T. Lovering
External Examiner
Associate Professor
Human Physiology
University of Oregon _____

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Abstract

It is unresolved how pulmonary ventilation (V_E) is influenced by normoxic 30% nitrous oxide (N_2O) breathing and hyperthermia during supramaximal intensity exercise. It was hypothesized that normoxic N_2O will suppress and hyperthermia will increase exercise ventilation, timing and ventilatory drive during supramaximal intensity exercise. Seven college-aged males volunteered for 4 separate 30 s Wingate cycle ergometer tests. The studies included a 2 x 2 design with factors of Thermal State (normothermia or hyperthermia) and Gas Type (Air or normoxic 30% N_2O). A significant interaction ($F=8.4$, $p=0.03$) between these 2 factors for V_E was explained by a V_E from 85 ± 27 L/min ($p=0.06$) during normothermia with N_2O to a V_E of 104 ± 23 L/min in hyperthermia with N_2O. There were no main effects or interactions for Thermal State and Gas Type for timing components and ventilatory drive. In conclusion, an interaction of Thermal State and Gas Type on V_E was explained by its suppression during normothermia relative to its rate in hyperthermia, both during normoxic N_2O breathing during supramaximal intensity exercise.

Keywords: hyperthermia; nitrous oxide; temperature; ventilation

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List of Acronyms

AT	anaerobic threshold
ATA	atmospheric pressure absolute
ATP-CP	adenosine triphosphate creatine phosphate
CNS	central nervous system
CR	carotid rete
CSF	cerebral spinal fluid
f_B	frequency of breathing
HCO_3^-	bicarbonate
LGIC	ligand-gated ion channel
MCA_V	middle cerebral artery mean blood velocity
N_2O	nitrous oxide
NMDA	N-methyl-D-aspartate receptor
NO	nitric oxide
NOS	nitric oxide synthase
P_aCO_2	arterial pressure of carbon dioxide
P_{ETCO2}	end-tidal partial pressure of carbon dioxide
SBC	selective brain cooling
T_{core}	core temperature
T_E	expiratory time
T_{ES}	esophageal temperature
T_I	inspiratory time
T_I/T_{TOT}	inspiratory duty cycle
T_{SK}	skin temperature
T_{TOT}	total time of one complete breath
V_E	expired pulmonary ventilation rate
V_I	ventilatory flow at rest
VO_2	volume of oxygen
VO_2max	maximal volume of oxygen
V_T	tidal volume
V_T/T_I	ventilatory drive
VT_1	first ventilatory threshold , also known as anaerobic threshold
VT_2	second ventilatory threshold

Glossary

Acidosis	Increased hydrogen ion concentration of arterial blood plasma.
Alkalosis	Reduced hydrogen ion concentration of arterial blood plasma.
Alloxan Chlorine	A toxic glucose analogue, coupled with a strong oxidizing agent that is known to cause pulmonary edema and lung congestion.
Anaesthesia	A temporary state consisting of unconsciousness, loss of memory, lack of pain, and muscle relaxation.
Analgesia	Absence of sensation while remaining conscious.
Bicarbonate	An intermediate form in the deprotonation of carbonic acid.
Chemosensitivity	The sensitivity of a physiological response to a chemical stimulus such as ventilation responding to carbon dioxide partial pressures.
Core Temperature	The mean temperature of the thermal core.
Eccrine Sweating	Response of eccrine sweat gland to a thermal stimulus.
Glycogenolysis	A breakdown of glycogen to glucose-1-phosphate and glycogen.
Glycolysis	Metabolic pathway that converts glucose, into pyruvate.
Hyperoxia	An elevated arterial partial pressure of oxygen with respect to normoxia.
Hyperthermia	A core temperature that is elevated above its resting value.
Hyperventilation	An increase in pulmonary ventilation rate that reduces P_aCO_2 below normal.
Hypoxia	A reduced arterial partial pressure of oxygen with respect to normoxia.
Metabolic Acidosis	A condition that occurs when the body produces too much acid or when the kidneys are not removing enough acid from the body.
Normoxia	Normal arterial partial pressure of oxygen.
Perfluorocarbon	An organofluorine compound.
Pulmonary Edema	Fluid accumulation in the air spaces and parenchyma of the lungs.
Respiratory Alkalosis	A medical condition in which increased respiration elevates the blood pH.
Respiratory Control Centre	Autonomic nuclei located in the reticular formation of the medulla oblongata and pons that control respiration.

Tachypnea	A rapid respiratory frequency accompanied by an increase in respiratory minute volume, and commonly, a decrease in tidal volume to allow heat dissipation.
Thermal Hyperpnea	An increase in tidal volume and frequency of breathing occurring during severe heat stress, which is caused by a large increase in core temperature.

Chapter 1.

Introduction

The introduction of this literature review begins with an overview on measurements of respiratory drive at rest and during different phases of physical exercise. It is followed by a description of what is known about the control of human breathing during elevated core temperature. Next, there is an exploration of the literature concerning the influence of nitrogen narcosis on the control of breathing and its components. This section of the literature review will examine the simulation of nitrogen narcosis using normoxic nitrous oxide gas, and its effects on breathing responses at rest and exercise. Lastly, an integration of these sections to discuss the limitations of the existing literature is made, thus generating a statement of the rationale and hypotheses for the two studies in this thesis.

1.1. Human Pulmonary Ventilation

1.1.1. Control of Breathing

Normal breathing is generated from impulses originating in the brainstem (Legallois 1812). Voluntary changes initiated from the cerebral cortex can override these commands (Euler 1988). Despite a poorly defined understanding of the specific role and location of the respiratory command centers in the brain, three locations according to Lumsden's Three-Part Concept of the 'Respiratory Center', all located within the brainstem, are best regarded as the main controllers of involuntary breathing (Lumsden 1923; Lumsden 1923). In the early 19th century Le Gallois (Legallois 1812), experimented on rabbits' brains in an attempt to localize the location of the respiratory centers. He discovered that breathing in rabbits depended on a small part of the medulla oblongata near the origin of the vagus nerve. The respiratory center located in

the reticular formation of the medulla oblongata, appears to be associated with both inspiration and expiration. The second location is known as the apneustic center. In 1938, Stella (Stella 1938; Stella 1938), displayed the relationship between the stimulation of the apneustic center and changes in inspiration and expiration. From his reports (Stella 1938), he demonstrated that the location of the apneustic center appears to be in the lower pons region. The third respiratory center known as the pneumotaxic center, was found by Cohen in the 1950`s (1958), who showed the location of the pneumotaxic center in the upper pons. Later on, Cohen (1971) displayed the role of the pneumotaxic center through direct electrical stimulation, resulting in inspiratory inhibition and respiratory rhythm regulation.

These three centers are not to be considered as the sole responsible mechanisms for the rhythmic, automatic breathing pattern in humans. However, during normal breathing at rest they have been designated as the primary respiratory control centers (West 2000). Voluntary breathing can be performed to a certain extent, where breathing patterns are being changed via overriding commands from the cerebral cortex. These commands are evident during voluntary hyperventilation and breath-holding (Euler 1988). Emotional state can also vary normal breathing patterns by stress modulators originating in the hypothalamus and limbic system (West 2000).

The signals from the respiratory control center in the brainstem are sent to effector respiratory muscle fibers located in the diaphragm, intercostal and abdominal muscles as well as in the accessory muscles such as the sternomastoids. Synchronicity of these muscles' actions is vital to ensure proper breathing patterns are maintained to prevent potential apnea which may cause hypercapnia and/or severe oxygen deprivation (Braman 1995).

The last components in the control of breathing is the sensors, which feed information to the respiratory control centers in the brainstem, mostly through negative feedback systems, and finally creating the regulatory response system necessary for maintenance of blood gases and pH (Braman 1995). One of the first experimenters to investigate the role of carbon dioxide (CO_2) and oxygen (O_2) on human breathing was Miescher-Riisch (1885), who showed that very small increases in inspired CO_2 produced

significant increases in human ventilation. Also, in his experiments he verified that the contribution of CO_2 was more profound relative to that of O_2. He did this by lowering the O_2 in the lung by an amount greater to which CO_2 had to be raised to create the hyperpnea; despite a greater decrease in partial pressure of O_2 (Po_2) relative to the increase in partial pressure of CO_2 (Pco_2), this resulted in no change in ventilation in the hypoxia condition. Following Miescher-Riisch's work, attempts to discover the specific anatomical locations of chemosensitive tissues and their corresponding mechanisms were made by Leon Fredericq (1890), who carried out a series of experiments between two dogs connected by the carotid artery and jugular vein. He verified that when blood CO_2 was reduced in dog A due to hyperventilation, it was followed by apnea in dog B. However, when dog A breathed hyperoxic mixtures there was no occurrence of apnea in Dog B. Fredericq concluded that the chemoreceptive area for CO_2 was indeed in the head and that CO_2 was the driving component in the central chemoreceptors and the element responsible for changes in ventilation.

Today, it is accepted that the central chemoreceptors are located near the exit of the 9^{th} and 10^{th} nerves, and that they are responsible for breathing stimulation (Loeschcke, De Lattre et al. 1970). Loeschcke's group studied this through local application of H^+ or dissolved CO_2 to these areas. This location was first detected by Mitchell and his group in the 1960's (Mitchell, Loeschcke et al. 1963), when they discovered paired respiratory chemosensitive areas on the ventrolateral surface of the medulla in anesthetized cats. Mitchell and his colleagues (Mitchell, Loeschcke et al. 1963) reported that the area is surrounded by extra cellular fluid and its composition is mostly governed by cerebral spinal fluid (CSF), local blood flow and local metabolism. In this area the CSF predominantly adjudicates the magnitude of chemosensitivity. Once an increase in Pco_2 is evident in the body, the diffusion of molecular CO_2 through the blood brain barrier to the CSF rises, liberating H^+ ions, thus increasing its acidity and giving a lower pH. The end result is a of hyperventilation that gives respiratory alkalosis, with a lowering of Pco_2, which in turn removes CO_2 from the CSF and assists in maintenance of pH at ~7. At the same time, Loeschcke et al. (Loeschcke, De Lattre et al. 1970), found the location most sensitive to H^+ stimulation in the fourth ventricle or in the nearby subarachnoid space at the roots of the 8th, 9th and 10th cranial nerves.

The CSF has a much lower buffering capacity than the blood, due to smaller protein content. Therefore it is much more sensitive to changes in pH than other fluids in the body. In cases where increased ventilation does not result in alkalosis in the CSF, and pH of the CSF is displaced for a prolonged period, compensatory production of bicarbonate (HCO_3^-) occurs, thus restoring the pH over a period of 24-36 hours (Baker, Goode et al. 1996). The importance of respiratory alkalosis and central chemoreceptors sensitivity in the role of homeostatic pH maintenance, relative to a much slower renal compensation, is far greater during acute metabolic acidosis, such as that brought on by intense exercise (Dempsey, Vidruk et al. 1985; West 2000; Duffin 2005).

Another very important sensor system is known as the peripheral chemoreceptors (Nielsen and Smith 1951). In the 1930`s, Heymans (Heymans 1967), presented the role of peripheral chemosensitivity in dogs after removal of the Hering's nerve. He showed that by injecting potassium cyanide to both sides of the carotid sinus baroreceptor at different times, which is known to powerfully excite arterial chemoreceptors but has no action on baroreceptors in the carotid sinus (McQueen 1980), ventilation increased during exposure on the uncut nerve of the carotid sinus, but not when injected on the denervated nerve. Following his findings, investigations of the role of peripheral chemoreceptors in altering ventilation began. The specific locations of the receptors are at the bifurcation of the common carotid arteries and in the aortic bodies above and below the aortic arch (West 2000). They respond to reduction in arterial partial pressure of oxygen (Pa_{O_2}) and pH, and increases in arterial Pco_2 (Pa_{CO_2}). Carotid bodies are sensitive to changes in Pa_{O_2} (Nielsen and Smith 1951) in part due to the high blood flow they transfer relative to their size (20 ml/min/gm tissue), thus having a very small arterial-venous O_2 difference in spite of a high metabolic rate (West 2000). Their sensitivity to changes in Pa_{O_2} begins around 500 mm Hg, but remains relatively stagnate until a reduction of Pa_{O_2} to ~50 mm Hg, at which point impulses traveling to the CNS via the carotid chemoreflex pathways increase (Poon 2005). This shows the partial pressure at which the sensitivity of the peripheral receptors begins to influence breathing during hypoxemia. Peripheral chemoreceptors are predominantly responsible for an increase in ventilation during hypoxemia, and examples of complete loss of hypoxic

ventilatory drive has been shown in patients with bilateral carotid body resection (Honda, Myojo et al. 1979; Honda 1992).

Unlike central chemoreceptors that do not respond to lowered PaO2, peripheral chemoreceptors sensitivity to Pa_{O_2} begins around 500mm Hg, and hypoxia increases the response of pulmonary ventilation to increased CO_2 or reduced pH (Nielsen and Smith 1951; West 2000). Nevertheless, because of their sensitivity to changes in O_2 partial pressure they may assist in matching ventilation to abrupt changes in Pa_{CO_2} in hypoxic conditions (Nielsen and Smith 1951; West 2000). With regards to changes in pH and increased acidity, the carotid bodies display greater afferent signalling to increase ventilation in normal breathing and hypoxia (Baker, Goode et al. 1996). Thus, increases in Pa_{CO_2} and decreases in pH potentiate the chemoreceptors activity despite reduced Pa_{O_2}.

Additional types of receptors that influence pulmonary ventilation are located in different parts of the human body. Outside of the afferent receptors mentioned previously, the largest concentration of receptors is located within the lung (Widdicombe 1985). The first of which are the pulmonary stretch receptors, believed to be located within airway smooth muscle (Widdicombe 1985). They discharge afferent signals in response to the distension of the lung and their activity is sustained with lung inflation. Once stimulated they show a slowing of respiratory frequency due to increased expiratory time (Widdicombe 2009). The second set of receptors of the lung are the irritant receptors (Knight and Holgate 2003). Thought to be located between the airway epithelial cells, they are stimulated by noxious gases, cigarette smoke, inhaled dusts and cold air. Their effects include bronchoconstriction and hyperpnea. Also, failure of appropriate growth and differentiation of airway epithelial cells can cause mucosal injury and asthma (Knight and Holgate 2003). The last main known set of receptors reviewed here that are in the lung are known as J-receptors (Paintal 1969). Their role has been investigated in the late 1960's by Paintal (Paintal 1969), on anesthetized cats. He showed that the J-receptors are located in the alveolar walls close to the capillaries and that their effects are to induce rapid, shallow breaths, and even apnea in anesthetized cats under the influence of localized injection of alloxan coupled with chlorine inhalation, which are known to produce pulmonary edema and lung congestion (Paintal 1969).

Other receptors outside the lungs that influence pulmonary ventilation include those in the nose and upper airways that respond to mechanical and chemical stimulation. Several common responses associated with nose and upper airways receptors are sneezing, coughing and bronchoconstriction. Another sensory system is the gamma system located in the intercostal muscles and diaphragm, and it is sensitive to elongation of the muscle and used as reflex control of the contractile intensity. Arterial baroreceptors are another set of receptors located in the aortic and carotic sinus, and can increase or decrease ventilation with respect to increased or decreased arterial blood pressure (Widdicombe 2009). Lastly, receptors located in joints and skeletal muscle were found (Kaufman 2010) to send impulses from the moving limbs to stimulate ventilation during exercise (Kaufman 2010).

The integrated responses of breathing depend on several key elements (Nielsen and Smith 1951). Primarily, Pa_{CO_2}, which generates the greatest ventilatory drive in the respiratory system and is considered as the main factor for the control of breathing at rest (Nielsen and Smith 1951). The sensitivity to changes in Pa_{CO_2} is remarkable. The range in which Pa_{CO_2} remains during the course of daily activity, in rest and exercise is held within 3 mm Hg (Nielsen and Smith 1951). The main stimulus to increase ventilation after an increase of Pa_{CO_2}, is from central chemoreceptor receptors to elevated H^+ concentration in the CSF, and from the peripheral chemoreceptors sensitivity to elevated Pco_2 and lower pH (Baker, Goode et al. 1996). Decreased Po_2 adds additional stimulus to ventilation by reflexive response of the carotid bodies and aortic body chemoreceptors (Nielsen and Smith 1951; Nielsen and Smith 1951). However, the magnitude of hypoxemic reflex is smaller relative to Pco_2. Nevertheless, the combined effect of increased Pco_2 concentration and hypoxia, exceeds the sum of each stimulus provided separately (Nielsen and Smith 1951).

Blood pH, as described previously, has a potent effect on pulmonary ventilation. An acute response to reduced CSF pH and reduced blood pH is compensated by hyperventilatory response until Pa_{CO_2} is resumed to a normal resting partial pressure. During chronic low pH combined with elevated Pco_2, which can be seen in patients with diabetes mellitus (Souto, Donapetry et al. 2011), renal compensation with retention of

HCO_3^- will be the primary regulator to buffer the H^+ concentration following acute respiratory alkalosis from hyperventilation that relieves this acidity (Souto, Donapetry et al. 2011).

Changes in human breathing, or human pulmonary ventilation (V_E, L), are comprised of several components which are indicative of respiratory and neuromuscular actions in the respiratory system (Milic-Emili and Grunstein 1976; Milic-Emili, Whitelaw et al. 1981; West 2000). These components, amongst others, include tidal volume (V_T, L) and frequency of breathing (f_B, breaths/min). The product of tidal volume and breathing frequency, give pulmonary ventilation and these two components can be assessed on a breath-by-breath basis. Until V_T reaches half of the vital capacity, there is a linear relationship between V_T and V_E, whereas the increase in frequency of breathing (f_B) accounts for the remaining increase in V_E while V_T remains constant (Hey, Lloyd et al. 1966). Individual expressions of V_T and f_B provide limited attention to central command response and inspiratory - expiratory durations from the total breath cycle (Milic-Emili, Whitelaw et al. 1981). Therefore, separate observations made to different components of timing of breathing (Milic-Emili and Grunstein 1976; Milic-Emili, Whitelaw et al. 1981) which include expiratory time (T_E, s) and inspiratory time (T_I, s), provide additional information to the breathing cycle, respiratory motoneuron relay from the CNS to respiratory muscles and mechanical work rate of the respiratory and accessory muscles (Milic-Emili and Grunstein 1976; Milic-Emili, Whitelaw et al. 1981). In addition, both timing components can be added to result in the total respiratory cycle duration ($T_{TOT,}$ s), a measurement of the sum of the total inspiratory and expiratory portions during a breathing cycle (Milic-Emili, Whitelaw et al. 1981).

Further computation of the timing components provide additional indices to the relative portion of inspiration from the total breathing cycle, known as the inspiratory duty cycle ($T_I/T_{TOT,}$ unitless) (Milic-Emili, Whitelaw et al. 1981). The inspiratory duty cycle is used to measure the respiratory muscle contraction duration from the total respiratory time to express the active portion of the breathing cycle (Milic-Emili, Whitelaw et al. 1981). Furthermore, mean inspiratory flow rate which is equal to the tidal volume divided by inspiration time (V_T/T_I, L/s), is an index of inspiratory drive that can be obtained from breath-by-breath analysis (Milic-Emili, Whitelaw et al. 1981). Inspiratory

drive is used as an index of the neural drive of the respiratory muscles (Milic-Emili, Whitelaw et al. 1981).

The mechanisms responsible for the control of human breathing at rest become more complex during exercise. Many of the inputs such as Pa_{CO_2}, Pa_{O_2} and arterial pH (pH_a) during rest, do not appear to influence ventilation to the same extent during exercise (Wasserman 1978). In healthy humans, exercise V_E can increase 15 fold relative to normal resting rates (Wasserman 1978). The causes for this dramatic increase in V_E and its components still remain unresolved (Wasserman 1978; Lambertsen 1980; Dempsey, Vidruk et al. 1985). Despite the lack of understanding of the mechanisms involved in the control of exercise V_E responses, there is a systemic breathing pattern during exercise which has been divided into 3 different phases (Dejours 1964) to represent different neurological, chemical and metabolic processes (Wasserman 1967; Wasserman, Whipp et al. 1975; Wasserman 1977; Wasserman 1978; Kaufman 2010).

1.1.2. Exercise Ventilation

Pulmonary ventilation response to exercise displays an increase in respiratory drive, resulting from a preparatory increase in CNS signal relay to the respiratory muscles, followed by an increase in metabolic demand and changes in chemosensitivity (West 2000). This was presented in the early 1960's, by Pierre Dejours, following a series of treadmill exercise tests completed on human participants (Dejours 1964).

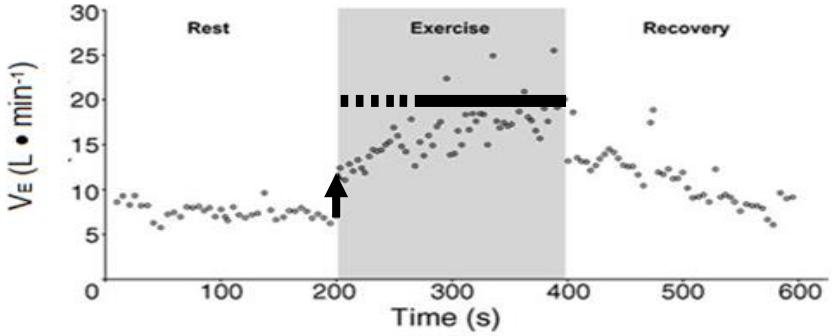

Figure 1.1. V_E (L/min), responses during rest, submaximal exercise and recovery. The black arrow indicates phase 1 response of breathing at the onset of the exercise. The broken line represents phase 2 response of breathing during the transitional mode of exercise. The solid line represents phase 3 of breathing during constant load exercise mode. Adapted from Bell (Bell 2006).

Ventilation during steady state exercise is characterized by reproducible (Miyamoto and Niizeki 1995) transition between 3 key phases. The first of Dejours' phases appears at the onset of exercise and is characterized by a sharp increase in V_E, from an increase in V_T (Dejours 1964; Whipp 1983; Bell 2006) (Fig. 1.1). This phase is short and normally lasts around 10 to 20 seconds (Dejours 1964). Despite the appearances of this phase during all modes of exercise, the mechanisms for phase one exercise V_E are not fully understood. Kaufman and Forester (Kaufman 2010) suggested the potential afferents from the exercising skeletal muscle receptors as a potential explanation to the rapid increase of V_E displayed in this phase (Whipp 1983; Kaufman 2010). This mechanism was present during passive limb movement in spinal cord intact humans vs. T5 – T12 paraplegics who did not display a similar sharp increase in V_E (Morikawn, Ono et al. 1989; Kaufman 2010). This suggests that there is no peripheral feedback system to the CNS following thoracic level spinal lesions. Following the sharp increase in V_E, a slower increase in ventilation occurs, known as Dejours' second phase or transition phase of exercise V_E (Dejours 1964; Whipp 1983; Bell 2006; Kaufman 2010). It is characterized by a slower exponential increase in ventilation, with duration of approximately 3 minutes (Dejours 1964; Whipp 1983) (Fig. 1.1) as exercise intensity is changed from a lower to a higher submaximal level. The reason for this gradual increase was suggested by Casaburi and colleagues, who observed changes in pulmonary arterial blood gas tensions during the transition from rest to moderate exercise (Casaburi, Daly et al. 1989). They reported a large increase in

mixed venous P_{CO_2} from 42 to 59 Torr, and a decrease in O_2 saturation from 71% to 41%, and inferred these changes in ventilation were due to the increase in muscle metabolic rate following phase 1 of exercise V_E (Casaburi, Daly et al. 1989). Dejours' third and final phase response to steady state, moderate exercise is a steady-state ventilation (Dejours 1964; Whipp 1983; Mateika and Duffin 1995; Kaufman 2010) (Fig. 1.1). Pulmonary gas exchange in this phase of exercise tends to match the metabolic rate so that the respiratory exchange ratio as well as Pa_{CO_2} and Pa_{O_2} remain close to resting values. Blood lactate concentration also remains near resting values during the steady state of moderate exercise (Dejours 1964; Mateika and Duffin 1995).

During heavy constant load exercise, steady state exercise may be characterized by a gradual increase in ventilation also known as ventilatory drift (Martin, Morgan et al. 1981), where ventilation rises out of proportion to metabolic rate and resulting in reduced Pa_{CO_2} (Mateika and Duffin 1995).

1.1.3. Control of Breathing during Low – Moderate Intensity Exercise

During low to moderate exercise intensities around 30-50 % of maximal work capacity (Dejours 1964), ventilatory responses increase in linear proportion to increases in VO_2 (Wasserman 1978). During moderate to high intensities, however, at approximately 60-75 % of maximal work capacity (Dejours 1964), exercise ventilation reaches a threshold known as VT_1 the first ventilatory threshold, also known as anaerobic threshold. This threshold marks a compensatory hyperventilation at the point when the capacity of bicarbonate to buffer H^+ is exceeded at the end of the isocapnic buffering period. Following VT_1, V_E increases more quickly than VO_2 and VCO_2 (Wasserman 1978). During higher intensity exercise energy transfer relies on both substrate catabolism from both the anaerobic and aerobic energy transfer pathways. A greater proportion of energy transfer at these high intensities, when oxygen delivery is outpaced by its consumption in the working muscles, is from the anaerobic pathway. In turn lactic acid production increases and this gives a metabolic acidosis (Fukuba and Whipp 1995; Mateika and Duffin 1995). Above this threshold VO_2 kinetics are slowed, ventilatory steady state is delayed, and V_E continues to increase excessively to help

compensate for metabolic acid production (Skinner and McLellan 1980; Wasserman 1986; McLellan 1987).

Several alternative hypotheses for VT_1 have been proposed, but no single hypothesis has been able to clearly identify the mechanism or mechanisms involved in the greater increase in ventilation relative to those increases for VO_2 or VCO_2. Four main hypotheses for VT_1 are the lactate hypothesis (McLellan and Skinner 1985; Loat and Rhodes 1993), the hydrogen ion hypothesis (Oelberg, Evans et al. 1998), the CO_2 flow hypothesis (Wasserman 1977), and the carotid body stimulation hypothesis (Wasserman, Whipp et al. 1975; Honda, Myojo et al. 1979; Mitchell, Smith et al. 1984; Honda 1985).

Lactic acid and its accumulation in the extracellular fluids including the plasma is considered to be one of the primary mechanisms for VT_1 in steady state exercise ventilation, and it is also known as the anaerobic lactate threshold (McLellan and Skinner 1985; Loat and Rhodes 1993). This is the point where the rate of entry of H^+ into the extracellular fluids surpasses the rate of removal by the buffer HCO_3^- production. According to the Henderson-Hasselbach equation (Fig 1.2), where the hydration of CO_2 is catalyzed by carbonic anhydrase (CA); when there is an increase in H^+ as the equilibrium shifts to the left producing more CO_2.

$$CO_2 + H_2O \overset{CA}{\leftrightarrows} H_2CO_3^- \leftrightarrows HCO_3^- + H^+$$

Figure 1.2. Henderson-Hasselbach equation. Adapted from West (West 2000).

Above AT or VT_1, decreased CO_2 tension in the plasma causes a reduction in the rate of CO_2 diffusion into the CSF, but the ventilatory response gives a hyperventilation and a respiratory alkalosis, which continues to reduce CO_2 and this subsequently elevates pH in the CSF (Nielsen and Smith 1951). The cause for continued increases in ventilation above VT_1, despite a drop in CO_2 partial pressure remains controversial.

The lactate threshold hypothesis as an explanation for the VT_1 was criticized by Glass et al. (Glass, Knowlton et al. 1997) who found that glycogen depleted volunteers displayed a lactate threshold at a higher VO_2 relative to the control group. From their

findings they suggested that lactate accumulation may not be the main explanation for VT_1 during exercise in humans.

Another proposed hypothesis suggests that the reduction in muscle pH is the cause for the increase in ventilation at VT_1. Measurements were made of intracellular (Oelberg, Evans et al. 1998) and extracellular (Oelberg, Evans et al. 1998) muscle pH during steady state exercise, while occluding blood flow to the working muscle. This showed that an increase in exercise V_E was delayed when occlusion of the blood supply to the working muscles was performed, despite reduced pH_a. Therefore, it was claimed that the increase in exercise V_E at VT_1 is stimulated by decreased pH in the working muscle's intra and extra cellular fluids, rather than at the arterial chemoreceptors (Oelberg, Evans et al. 1998). This mechanism, however, remains unsupported in some populations (Brice, Forster et al. 1988). Brice et al. 1988 (Brice, Forster et al. 1988) demonstrated for paraplegics where electric stimulation was used to evoke exercise, that there was a similar ventilatory responses to that of normal humans. This example does not contradict the existence of cellular fluid pH influence on exercise ventilation, but it provides uncertainty as to how much influence muscle pH has on VT_1 relative to other proposed mechanisms (Oelberg, Evans et al. 1998).

Wasserman and colleagues (Wasserman 1977) proposed several mechanisms that may cause VT_1. They suggested that CO_2 flow across the lungs is proportional to the increase in ventilation and independent of Pa_{CO_2} changes during exercise. This they hypothesize is a potential explanation for VT_1 (Wasserman 1977). Their hypothesis involves CO_2 flow receptors in the pulmonary circulation that have yet to be found. They suggest that the proportional increase in ventilation is due to yet-to-be-identified receptor`s sensitivity to CO_2 flow through the pulmonary circulation. They also suggest that there is little involvement from either the peripheral or central chemoreceptors in the explanation of VT_1. (Wasserman 1977).

In another study, Wasserman et al. (Wasserman, Whipp et al. 1975) also suggested the chemoreceptors in the carotid body as the modulators for the increase in exercise ventilation at intensities above VT_1. They found humans with resected carotid bodies did not display the same response at VT_1 as the control group (Wasserman,

Whipp et al. 1975). Their additional findings show a relationship between an increase in ventilation and carotid bodies hypoxic stimulation both at rest and exercise in normal patients without in bilateral carotid chemoreceptor resection (Honda, Myojo et al. 1979; Honda 1985). It should be noted that these studies, however, were performed at intensities under VT_1 and therefore are not accounting for the changes that occur in ventilation at that level of intensity (Honda, Myojo et al. 1979; Honda 1985). In an animal study by Mitchell et al. (Mitchell, Smith et al. 1984) it was found in serotonin depleted and carotid body denervated sheep, that an increase in ventilation during exercise was still occurring, mainly due to an increase in tidal volume. This finding contradicts Wasserman group`s findings as it shows that hyperventilatory responses during exercise are not necessarily associated with peripheral chemoreceptor involvement (Mitchell, Smith et al. 1984).

1.1.4. Control of Breathing during High Intensity Exercise

High intensity exercise is described by Dejours as exercise intensity greater than 75 % of maximal work capacity (Dejours 1964). Around this exercise intensity, V_E displays an exponential increase when given as a function of VO_2, following VT_2 or the second ventilatory threshold. There has not been any conclusive evidence to support the mechanisms accounting for the change in exercise V_E at VT_2 (Kaufman 2010). This threshold may in part be dependent on factors that affect oxygen delivery to the tissues but it remained to be determined what mechanisms underlie this response. This VT_2 threshold is increased when oxygen delivery to the tissues increases and reduced when oxygen delivery to the tissues is diminished (Wasserman 1986). VT_2 has also been suggested to be mediated by excitatory nerve impulses of the respiratory centre during exercise, similar to the mechanism seen during the large increase in V_E during phase 1 of exercise V_E (Dejours 1964; Lambertsen 1980; Kaufman 2010). Since the proposition of this mechanism, an attempt to link between neurological factors from the moving limbs to the increase in exercise V_E at VT_2 was made (D'Angelo and Torelli 1971). Despite this attempt, no definitive evidence has yet been reported to provide explanation to the primary mechanisms responsible for the physiological response at VT_2.

Another proposed mechanism for VT_2 is that pulmonary ventilation above this threshold is a thermolytic response (Cabanac 2005; White 2006). This mechanism suggests that the cause for VT_2 may be a result of a CNS temperature controller, which at around 70-85 % of an individual maximal work rate, initiates a thermal hyperpnoea or phase II panting response in the form of a greater increase in V_E (White 1996). However, the reasons for this increase remain to be fully elucidated (White 1996; Nybo and Nielsen 2001; White 2006).

1.1.5. Control of Breathing during Short Duration High Intensity Exercise

During short duration high intensity exercise, ventilatory responses and phases differ from those observed during graded or steady state exercises. These differences are attributed to the short duration and supramaximal intensities of this exercise mode. A commonly used protocol since 1974 to quantify anaerobic capacity is known as a Wingate protocol. The Wingate test assesses supramaximal efforts during 30 second periods on a loaded cycle ergometer (Bar-Or 1987). During this supramaximal exercise, energy expenditure relies predominately on anaerobic glycolysis, ATP-CP molecules and alactic energy transfer (Bar-Or 1987), while O_2 use by the working tissues is minimal (Bar-Or 1987). Despite the short duration of the protocol, the V_E response can reach similar and even greater values than prolonged steady state or graded exercise protocols. A paper by Yoon, (Yoon 2002) on Korean national team wrestlers, showed that the mean result for maximal pulmonary ventilation was 180 $L \cdot min^{-1}$ and that it was achieved in a mean time of 12 seconds from the start of exercise. Also the mean minute ventilation response was 132.5 $L \cdot min^{-1}$. Due to the short duration of the protocol necessary to determine anaerobic responses (Bar-Or 1987), the ventilatory response during this short exercise protocol includes mainly phase 1 and 2 of exercise V_E (Dejours 1964).

In summary, the exact mechanisms responsible for VT_1 or AT, VT_2 and Dejours' phase 1 of exercise ventilation remain unclear. There is a great deal of controversy on the mechanisms underlying VT_1, as shown by the previously discussed four proposed hypotheses, and there are even a smaller number of potential hypotheses and

supporting evidence to explain the physiological mechanism underlying VT_2. Possible changes to many physiological responses before and after VT_2 increase the number of potential mechanisms responsible for this threshold (Skinner and McLellan 1980; Wasserman 1986; McLellan 1987). Lastly, the purported physiological mechanisms responsible for the increase in phase 1 of exercise V_E are also inconsistent, and require further investigation (Whipp 1983; Bell 2006; Kaufman 2010). An additional factor known to increase ventilation both at rest and during activity is a rise in body temperature, also known as hyperthermia. Hyperthermia is a known input for an increased ventilatory response at rest. When hyperthermia is coupled with exercise, its role in human control of breathing remains to be resolved (White 1996; Nybo and Nielsen 2001; Chu, Jay et al. 2007).

1.2. Hyperthermia

1.2.1. Hyperthermic Breathing at Rest

A potent afferent input to resting pulmonary ventilation is hyperthermia. Hyperthermia in humans is described as a rise in core temperature (T_{core}) of ~1^{o}C above normal T_{core} (Bligh and Johnson 1973). The rise in T_{core} can occur by either passive exposure to warm environmental temperatures or through increased heat generated by the working muscles during physical activity and exercise. The role of hyperthermia has been reported as early as 1905 when hyperthermic hyperpnea resulted from exposure to high air temperature in humans (Haldane 1905). Evidence of the linear relationship between elevated core temperature and pulmonary ventilation has been repeatedly reported by the literature over the last decade, providing evidence to support the existence of hyperthermia-induced hyperpnea at rest and exercise (Gaudio and Abramson 1968; MacDougall, Reddan et al. 1974; Whipp 1983; Cabanac and White 1995; Mariak, White et al. 1999; Nybo and Nielsen 2001; White 2006; Chu, Jay et al. 2007). Evidence supporting passively induced hyperthermic-induced hyperpnea at rest, were shown in 1995 by Cabanac and White (Cabanac and White 1995). Their study included a 41^{o}C bath, where volunteers were seated in until core temperature rose by ~2^{o}C. Their results illustrated the contribution of elevated core temperature to the rise in

pulmonary ventilatory response (Cabanac and White 1995). The authors showed that despite an increase of about 80 % in ventilatory flow at rest (V_I), VO_2 increased by only ~30 %. Therefore, the increase in ventilation could not have been attributed to metabolic production, but rather due entirely to the influence of the core temperature (Cabanac and White 1995).

Several explanations for hyperthermic hyperpnea have been suggested (Cunningham 1957; Gaudio and Abramson 1968; Cabanac and White 1995; White 2006). They include either a potential increased CO_2 sensitivity during hyperthermia (Gaudio and Abramson 1968), or a potentially afferent temperature signal from temperature sensitive neurons that increases the respiratory efferent signal (Cunningham 1957; Cabanac and White 1995). The explanation for the proposed increased CO_2 sensitivity was shown by Gaudio and Abramson (Gaudio and Abramson 1968). Their results showed that with an elevation of T_{core} by ~1^oC, there was a hyperventilation with Pa_{CO_2} that went down from 44 to 33 Torr, plasma bicarbonate decreased from ~25.5 to 22.3 meq/l, and venous blood pH was elevated from ~7.38 to 7.46 (Gaudio and Abramson 1968; White 2006). Collectively, these findings support that at rest the increase in ventilation can be directly attributed to an increased CO_2 sensitivity during hyperthermia (Gaudio and Abramson 1968; White 2006 ; Chu, Jay et al. 2007; Curtis, Walsh et al. 2007).

Cabanac and White (1995), suggested a potential autonomic ventilatory response mechanism, stimulated by elevated core or cerebral temperatures. The authors noted that the ventilatory responses seen in resting humans was due to an increase in V_T (Cabanac and White 1995), which is different than the phase 1 panting response in animals that is primarily driven by an increase in f_B with a concurrent reduction in V_T (Robertshaw 2006). They did show, however, that during passively induced hyperthermia in resting subjects (Cabanac and White 1995), a potential human panting response can be seen as an increase in resting pulmonary ventilation after core temperature thresholds at 38.5^oC in esophageal temperature and 38.1^oC in tympanic temperature. This response was also reported during metabolically-induced hyperthermia during exercise (Nybo and Nielsen 2001; Chu, Jay et al. 2007). The mechanism for this threshold during exercise still remains to be fully established in

humans (Chu, Jay et al. 2007). Nevertheless, as it does appear both at rest and during exercise, the response may be a vestigial panting response that contributes to human selective brain cooling (SBC). As mentioned above, this thermal hyperpnea is similar to that of second phase panting or thermal hyperpnea as is evident in many mammalian species (Cabanac 1993; Cabanac and White 1995; White 2006 ; Chu, Jay et al. 2007).

Selective brain cooling is defined as the reduction of brain temperature below arterial blood or thoracic core temperature (Mitchell 2002). For panting animals this response acts as a vital heat loss response which is also used for fluid conservation. Specialized anatomical structures known as the carotid rete (CR) are evident in many animals that demonstrate SBC. The CR helps reduce heat gain in the brain through funneling cool air flow from the upper air ways and generating vascular heat loss (Jessen 1998). More specifically, cool air flow through the upper air ways, cools the nasal cavity's venous vessels that drain their blood to the cavernous sinus where the CR resides. Here, through countercurrent heat exchange, cooled venous blood from the upper airways cools the blood in the CR. Subsequently, cooler blood in the internal carotid artery gives SBC by feeding the arterial network known as the Circle of Willis that is located in the inferior part of the brain around the hypothalamus (Jessen 1998).

Despite humans lacking a CR, a collection of several venous plexuses and lakes along the carotid artery, support the idea of an existing counter current system along the main blood delivery to the cerebral cortex, similar in action to the CR of panting animals (Cabanac 1993). The potential contribution of SBC by this mechanism in humans was supported by Mariak et al. (Mariak, White et al. 1999) who implanted two temperature sensors in the brain, one in the midline between the cribriform plate and frontal lobes, and the second in the subdural space on the vault of the cranium (Mariak, White et al. 1999). They demonstrated an intracranial temperature decrease around 0.4–0.8°C after participants voluntarily hyperventilated through the mouth and nose (Mariak, White et al. 1999). Another example by Abou-Chebl, et al. (2011), showed significant reductions in human brain temperatures by ~1°C while spraying perfluorocarbon coolant onto the upper surface of the nasal cavity.

On the contrary, Nybo, et al. 2014 (Nybo, Wanscher et al. 2014), evaluated the effects of intranasal cooling, percutaneous cooling of the carotid arteries, and nasal ventilation on cerebral temperature balance and oxygenation. They reported that intranasal cooling may lower the cerebral venous blood temperature but that the cooling effect is insufficient to be regarded as a selective brain cooling mechanism. It is important to note that their study is lacking intracranial temperature measurements, which introduces a limitation to the assessment of brain temperature balance due to nasal cooling. Also, the usage of narrow catheters to cool the upper airways with a fluid as performed by the group, may limit the cooling effect to a very small and localized surface area, which may explain the limited cooling effect shown in their results. Collectively these studies show that the influence of hyperthermia on exercise V_E and its components, and its potential contribution as a heat loss mechanism during metabolic hyperthermia still remains controversial.

1.2.2. Breathing during Exercise with Hyperthermia

Increased pulmonary ventilation during exercise is driven by an array of potential mechanisms as discussed above. Although hyperthermia is a potent ventilatory stimulator, it's difficult to distinguish its independent contribution from other chemical, neurological and mechanical mechanisms during different exercise modes and intensities (MacDougall, Reddan et al. 1974; Wasserman 1978; Whipp 1983; White 1996; Nybo and Nielsen 2001; White 2006).

During exercise, core temperature increases in proportion to increased metabolism within the working muscles, and therefore depends largely upon the severity of the exercise (Nielsen and Nielsen 1962; MacDougall, Reddan et al. 1974). Around 70-85 % of an individual maximal work rate, V_E appears to disproportionally increase relative to metabolic rate (White 1996). The reasons for this increase remain to be fully elucidated (White 1996; Nybo and Nielsen 2001). Since pulmonary ventilation is also a thermolytic response (Cabanac 2005), it has been suggested that the reason for this increase could potentially be a heat loss response rather than a metabolically-induced response (White 1996; Nybo and Nielsen 2001; Chu, Jay et al. 2007). White and Cabanac (White 1996), studied the V_E response during two graded exercise tests from

rest to maximal exhaustion, using different work rate increments. Their findings showed that despite changes in metabolic rate, the ventilatory increase or "ventilatory breakaway" (White 1996) occurred at the same core temperature during both trials, thus providing evidence that V_E is controlled by temperature input rather than being secondary to metabolic responses (White 1996).

Another observation made by Nybo and Nielsen (Nybo and Nielsen 2001), suggested that the increase in exercise ventilation may be attributed to reduced middle cerebral arterial blood velocity (MCA_V) and increased Pa_{CO_2} sensitivity during hyperthermia (Nybo and Nielsen 2001). Their results showed that reduced MCA_V corresponded to the increase in both core temperature and ventilation, as a consequence of a hyperventilation-induced drop in Pa_{CO_2} (Nybo and Nielsen 2001). Their results support the ventilatory thermolytic response as a heat loss mechanism potentially contributing to SBC in humans during exercise. Recently, Coverdale et al. (2014) showed that the instrumentation used in the past to measure changes in MCA_V, known as Trans Cranial Doppler systems (TCD), tends to underestimate cranial blood flow due to linear changes in cross sectional area in the middle cerebral artery, under conditions where sensitivity to Pa_{CO_2} increases, as seen during hyperthermia. Following Coverdale's group findings (Coverdale et al. 2014), Verbree et al (2014), showed MCA changes in diameter, are linear only after an increase of ~15 mm Hg in P_{ETCO_2} from normal resting values. Therefore, Nybo and Nielsen's ability to accurately report MCA_V values using TCD instrumentation and relate them to changes in ventilation in general and especially during hyperthermia are questionable.

Despite the number of studies on the control of V_E and its components during rest and exercise, under normothermic and hyperthermic conditions, much less has been devoted to this response at underwater depths. Here, environmental differences such as increased barometric pressure (Biersner 1972) as well as changes in gas pressure and volume (Biersner 1972) are responsible for altering V_E responses.

1.3. Diving and Nitrogen Narcosis

1.3.1. Nitrogen Narcosis and Nitrous Oxide Gas

Nitrogen narcosis is a reversible alteration in consciousness that occurs while diving at depths greater than 30 m below sea level (Biersner 1972). Symptoms tend to develop insidiously, starting with lightheadedness, euphoria, impaired judgment and a false sense of security and over confidence (Grover and Grover 2014). The risks involved with this mixture of symptoms can lead to a potentially dangerous behavior that can intensify with greater depths and durations. Added symptoms that follow at deeper underwater depths are impaired concentration and memory (Grover and Grover 2014).

Various degrees of nitrogen narcosis can be simulated using different fractions of nitrous oxide (N_2O) gas in normoxic mixtures under normobaric conditions. Biersner showed in 21 US Navy divers, that 30% normoxic N_2O gas mixture was equivalent to a narcosis exposure of ~210 ft (7 ATA) below sea level while inhaling normal air (Biersner 1972). According to Biersner, at that depth the risk of fatality is profound, and is mainly associated with CNS inhibition, sometimes producing motor disturbances, defective vision, and auditory hallucinations (Biersner 1972; Grover and Grover 2014). The suggested benefits for nitrogen narcosis simulation using normoxic N_2O gas in closed, controlled environments are the reduced risks associated with these hyperbaric exposures and the elimination of decompression sickness (Biersner 1972).

1.3.2. Nitrous Oxide Gas

Nitrous oxide, commonly known as laughing gas, is a colourless, non-flammable gas, with a slightly sweet odour and taste. Predominantly its usage is in light surgical practices such as dentistry and localized anesthetic procedures. Despite its common clinical use over 150 years, its pharmacological influences are not fully understood. Suggestions concerning the potential mechanisms of N_2O include modulations of several ligand-gated ion channels (LGIC's). The inhibitory analgesic effect is presumed to be associated with moderate blocking of N-methyl-D-aspartate (NMDA) receptors, responsible for Na^+ transport and synaptic plasticity effecting mental processing such as

short-term memory and learning (Mennerick, Jevtovic-Todorovic et al. 1998; Yamakura and Harris 2000). In addition to its effects on LGIC's, N_2O may act to imitate nitric oxide (NO) by elevating NO synthase (NOS) production in the central nervous system, which may be related to its analgesic and anxiolytic properties (Emmanouil and Quock 2007). A study by Henry, et al. 2005 (Henry, Ohgami et al. 2005), examined the effects of N_2O on NOS production in the mouse brain using a gas mixture of 70 % N_2O and 30 % O_2. They showed that for mice breathing N_2O relative to air, NOS production was significantly elevated in the brain (Henry, Ohgami et al. 2005). Also, it's been reported in rats that N_2O stimulates the mesolimbic reward pathway by inducing dopamine release (Sakamoto, Nakao et al. 2006; Benturquia, Le Marec et al. 2008; Koyanagi 2008). This action is suggested to implement the euphoric effects associated with N_2O inhalation, and appears to enhance its analgesic properties as well (Sakamoto, Nakao et al. 2006; Benturquia, Le Marec et al. 2008; Koyanagi 2008).

1.3.3. N_2O Zones of Anaesthesia in Man

The anesthetic effects of N_2O in humans are classified into four zones (Fig. 1.3) of analgesia in normoxic gas mixtures (Parbrook 1967; Jastak and Donaldson 1991). Zone I characteristic of a 6-25% fraction of N_2O gas in the inhalant, gives some analgesia while the individual is maintaining full verbal contact (Fig. 1.3) (Parbrook 1967; Jastak and Donaldson 1991). Zone II is characterised by 26-45% fraction of N_2O gas in the inhalant, which gives increased psychological detachment and light sedation (Parbrook 1967; Jastak and Donaldson 1991). In Zone III, characteristic of a 46-65% fraction of N_2O gas in the inhalant, gives marked inebriation, but often with some remaining verbal contact (Parbrook 1967; Jastak and Donaldson 1991). Lastly, Zone IV is characterised by >65% fraction of N_2O gas in the inhalant which produces light general anesthesia (Parbrook 1967; Jastak and Donaldson 1991). Despite the understandings of the anesthetic effects of N_2O listed above, the effects of the N_2O on exercise breathing is yet to be fully understood.

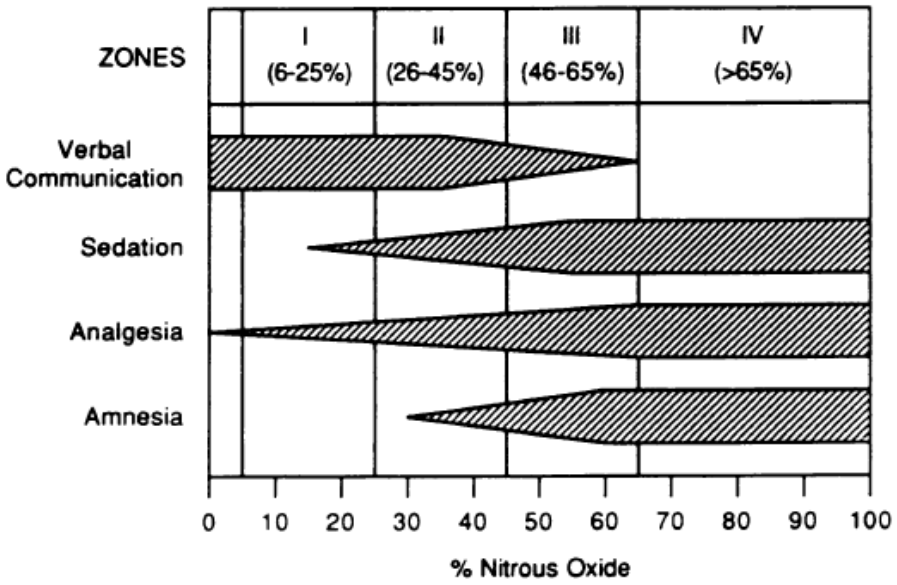

Figure 1.3. Parbrook`s zones and response to different zones of normoxic N2O. Adapted from Jastak (Jastak and Donaldson 1991).

1.3.4. Breathing During N_2O Gas Inhalation

The scientific literature has yet provided conclusive evidence on the effects of N_2O on human breathing. Ostlund and Linnarsson 1998 (Ostlund and Linnarsson 1998) and Dahan and Ward 1994 (Dahan and Ward 1994), investigated the effects of 39 % and 20 % fractions in the inspirate of normoxic N_2O, on ventilatory responses at rest. Each of these studies (Dahan and Ward 1994; Ostlund and Linnarsson 1998) reported no significant changes in breathing responses while inhaling the gas. Royston et al. 1983 (Royston, Jordan et al. 1983), examined the effects of 20 and 40 % fractions in the inspirate of normoxic N_2O during loaded and unloaded breathing. In their results they focused on first breath response and reported a significant decrease in both T_I and V_T under loaded 40 % normoxic N_2O breathing. They concluded that the decrease may be associated with reduced ability of sensing the respiratory load while under the influence of 40 % normoxic N_2O (Royston, Jordan et al. 1983). Despite these findings, Royston and his group reported no changes in breathing components during steady state breathing against resistance (Royston, Jordan et al. 1983).

1.3.5. N_2O Effects on Exercise Ventilation and Aerobic Power

Fothergill and Carlson (1996) observed the effects of N_2O narcosis on breathing during steady state exercise at 75 % of VO_2peak and reported a small but significant increase in V_E (4%; $p < 0.01$), with a concomitant decrease in P_{ETCO_2} (-2%; $P < 0.05$) (Fothergill and Carlson 1996). Ciammaichella and Mekjavic (2000) report no change at 100% of aerobic power for exercise ventilation but their results suggest a suppression of breathing during normoxic 30% N_2O breathing at this maximal exercise intensity. With regards to performance, Ciammaichella and Mekjavic (2000), showed at 100% of maximal exercise intensity a significant increase in VO_2 per body mass of 7% which was approximately 4 ml • kg • min^{-1} ($p < 0.01$) when volunteers breathed normoxic 30% N_2O. They inferred the increase in performance to a potential ergogenic effect of N_2O inhalation during the progressive decrease in hemoglobin (Hb) affinity due to the lower pH associated with the accumulation of lactic acid at higher intensities. Despite not directly implicating narcosis-induced changes in ventilation, the authors proposed that a potential effect of N_2O gas on the O_2-Fe bond in hemoglobin, ultimately reducing hemoglobin affinity to oxygen, could be potentiated with decreasing pH above the VT_1, thus improving O_2 uptake and total performance (Ciammaichella and Mekjavic 2000).

1.4. Thesis Rationale

There is limited information in the literature on the combined effects of hyperthermia and N_2O narcosis on the control of human breathing during exercise (Ciammaichella and Mekjavic 2000). Despite observations made on the individual effects of these factors showing an increased ventilatory response during hyperthermia (Jastak and Donaldson 1991; Lanphier and Camporesi 1993; Cabanac and White 1995; White 1996; Chu, Jay et al. 2007) and a potential breathing inhibition response during N_2O inhalation (Royston, Jordan et al. 1983; Dahan and Ward 1994; Fothergill and Carlson 1996; Ciammaichella and Mekjavic 2000), the combined effect of these treatments on the control of breathing during heavy exercise (>100% Wmax) remains unclear. In addition, the increased performance during maximal exercise while breathing N_2O at work rates of 100% (Ciammaichella and Mekjavic 2000), suggest a study is needed that examines the individual and combined factors of N_2O gas and body

temperature on exercise V_E. Supramaximal short duration exercise, greater than 100% of exercise intensity while inhaling N_2O (Bar-Or 1987; Ciammaichella and Mekjavic 2000), following passively induced hyperthermia (Cabanac and White 1995), may lead to a better understanding of the ventilatory response under these conditions.

In summary the literature supports hyperthermia increases human ventilation either with the rise in body temperature from metabolic heat production, or from passive environmental heat stress that increases body temperature (Haldane 1905; Jastak and Donaldson 1991; Lanphier and Camporesi 1993; Cabanac and White 1995; White 1996; Chu, Jay et al. 2007). When it comes to the effects of normoxic N_2O gas on human pulmonary ventilation, the mechanism of its influence are not fully understood and there are controversial findings on its influences on human breathing (Royston, Jordan et al. 1983; Dahan and Ward 1994; Fothergill and Carlson 1996; Ciammaichella and Mekjavic 2000). The combined effect of hyperthermia, with its influence on the control of breathing, along with the effects of N_2O gas, can yield novel and important evidence as to how the control of human breathing is affected by narcosis in different environments. To address this topic the following hypotheses have been generated.

1.5. Hypotheses

Hypothesis #1: 30% Normoxic N_2O gas inhalation will suppress and superimposed hyperthermia will increase pulmonary exercise ventilation and its components during short duration, high intensity exercise.

Hypothesis #2: 30% Normoxic N_2O gas inhalation will increase breathing timing indices and suppress ventilatory drive, while superimposed hyperthermia will shorten breathing timing indices and increase ventilatory drive during short duration, high intensity exercise.

1.6. References

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Chapter 2.

Effects of elevated core temperature and 30% normoxic nitrous oxide on human ventilation and its components during short duration, high intensity exercise

2.1. Introduction

Undersea SCUBA divers breathing compressed air between 4 and 6 Atmospheres Absolute (ATA) of pressure experience nitrogen narcosis, the effects of which can be simulated by breathing normoxic 30% nitrous oxide (N_2O) at sea level pressure (Biersner 1987). At submaximal exercise intensities small but significant increases in pulmonary ventilation are evident during normoxic N_2O breathing, but at higher work intensities (Fothergill and Carlson 1996) or in hypoxic conditions (Yacoub, Doell et al. 1976), normoxic N_2O appears to inhibit exercise ventilation, possibly due to a selective suppression of peripheral chemoreceptors (Yacoub, Doell et al. 1976). Possible suppression of exercise ventilation during work by deep-sea divers (Ciammaichella and Mekjavic 2000) is of concern since there are unexplained deep-water black-outs in this population. SCUBA diver fatalities (Vann, Freiberger et al. 2005) are often occurring during the bottom phase of the dive, with 90% of divers breathing compressed air. The largest fraction of these fatalities occurred during diving in warmer water temperatures in the southeast/southwest USA or the Caribbean throughout the summer months of June to August. Forty-eight percent of these fatalities due to loss of consciousness were at the bottom of the dive and 27 of these were for no apparent reason (Vann, Freiberger et al. 2005).

Divers conducting physical work at depth can be rendered hyperthermic with hot water from a surface supply warming system (Mekjavic, Eglin et al. 2001) or by exerting

themselves in warm seas or oceans. Potential influences on breathing in these divers include nitrogen gas under pressure (Behnke, Thomson et al. 1935), compressed air with a higher gas density (Bennet 1969), and elevated body temperatures (Cunningham and O'Riordan 1957; White 1996). The narcotic effects of compressed air at depth are thought to inhibit ventilation (Bennet 1969), whereas an elevated core temperature is known to stimulate human ventilation at rest (Haldane 1905; Cabanac and White 1995) or during exercise (Cunningham and O'Riordan 1957; White 1996; Nybo and Nielsen 2001).

The present study was conducted to assess the separate influences of passively-induced hyperthermia and narcotic gas on human ventilation during intense exercise. To induce a large initial increase in ventilation without time for an exercise-induced change in core temperature or accumulation of metabolites from exercise, a 30 s Wingate exercise test was employed in 4 exercise sessions. This exercise protocol included a transition from light to intense exercise and narcosis was induced with inhalation of normoxic 30% N_2O at ~1 ATA. As such, the combined and separate effects of hyperthermia and narcosis were assessed for their influences on a sudden increase in exercise ventilation. It was hypothesized that N_2O would inhibit and that increased core temperature would stimulate exercise ventilation in these exercise conditions.

2.2. Methodology

2.2.1. Volunteers

Seven male participants volunteered for the study. They were 23.1 ± 1.3 years of age (mean ± SD), 1.8 ± 0.03 m tall, 74.7 ± 7.4 kg in weight and had a body mass index of 23.4 ± 2.0 kg/m^2 (Table 2.1). The volunteers were physically fit as evident from their Wingate power output values when classified with a ranking method developed from Wingate tests on 485 male, Division 1 NCAA athletes (Zupan, Arata et al. 2009). Using this ranking method our volunteers peak power during the control condition (Fig. 2.1) put them above the highest ranking of 1163 W for an ‘elite’ ranking and their mean power values during the control condition (Fig. 2.1) put them within the range of 778 – 823 W for an ‘excellent’ ranking (Zupan, Arata et al. 2009). Each volunteer was made aware of

any risks associated with the protocol in this experiment. After reading a detailed outline of the study each participant signed a consent form. The sample size was determined using a power calculation. The difference worth detecting was set at 10%, with an alpha level of 0.05, a beta value of 0.8 and a standard deviation of 7% of the estimated mean scores for exercise ventilation (V_E), frequency of breathing (f_B), tidal volume (V_T), end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), and the difference between mean rest and mean Wingate for ΔV_E, Δf_B and ΔV_T. The Office of Research Ethics at Simon Fraser University approved the research.

2.2.2. Instrumentation

A calibrated breath-by-breath Vmax 229 series metabolic cart (Sensormedics, CA, USA) was employed to assess expired gases as well as ventilation and its components as described previously (Sancheti and White 2006).

Esophageal temperatures (T_{ES}) were estimated using an esophageal temperature thermocouple (size 9Fr, Mallinkroft Medical Inc., St Louis, MO, USA) inserted to the level of the left ventricle (Mekjavic, Morrison et al. 1984). Mean un-weighted skin temperatures (T_{SK}) were estimated from sites at the chest, thigh, forehead and lower back with surface copper constantan thermocouples. All thermocouples were connected to a data acquisition system (National Instruments, USA) and controlled by LabVIEW software (National Instruments, USA) on a personal computer.

The gas inhalant included either normoxic nitrous oxide (30% N_2O, 21% O_2, balance N_2, Air Liquide Canada, Inc.) or air that was supplied to the volunteer in corrugated Collins respiratory tubing from a meteorological balloon.

2.2.3. Protocol

Following instrumentation the volunteer participated in one of four 30 s Wingate trials (Bar-Or 1987) on an electrically braked, seated, cycle ergometer. Trials were performed ~1 week apart and all participants were asked to refrain from ingesting caffeine for 12 hr prior to exercise tests. Each participant inhaled either room air or 30% normoxic N_2O in a given Wingate test. The participant was normothermic in two

Wingate tests and hyperthermic in two other Wingate tests. Prior to the hyperthermic tests the participant was warmed in a 40ºC water bath. After this, the participant was quickly seated on the cycle ergometer after exiting the bath and then wrapped in blankets plus impermeable raingear to maintain an elevated T_{ES} until the start of the Wingate test.

At the start of each exercise trial the volunteer was seated on the cycle ergometer and began breathing air or N_2O through the mouthpiece for ~10 min of rest, followed by a 30 s warm-up at 40 W with a pedaling cadence between 80 to 90 rpm. The volunteer was then given a 5 second countdown at the end of warm-up cycling and then proceeded to perform a 30 s Wingate test, with instructions to pedal as hard and as fast as possible with a resistance of 0.09 kp (0.88 N)/kg.

2.2.4. Statistical Analysis

The dependent outcome variables of interest were the means of T_{ES}, T_{SK}, V_E, f_B, V_T and $P_{ET}CO_2$ as well as the difference (Δ) between mean rest and 30 s mean exercise values for ΔV_E, Δf_B and ΔV_T. An ANOVA model was employed with repeated factors of Thermal State (Normothermia and Hyperthermia) and inhaled Gas Type (AIR, normoxic N_2O). Paired t-tests were employed to compare means. Results were considered significant at an alpha level of 0.05.

2.3. Results

2.3.1. Temperature

Table 2.2 gives the mean values between the Normothermic and Hyperthermic conditions for mean T_{ES} and mean T_{SK}. For T_{ES} there was a main effect of Thermal State ($F = 30.2$, $P < 0.01$) but no main effect of Gas Type. The mean T_{ES} in the Normothermic trials was ~ 37.4 ± 0.1°C, and ~38.4 ± 0.04°C, in the Hyperthermic trials. There were no effects of Thermal State or Gas Type on mean T_{SK}. The mean T_{SK} in Normothermic trials was ~33.5 ± 0.3°C, and ~34.2 ± 0.9°C, in the Hyperthermic trials.

2.3.2. Ventilation

Figure 2.2 gives the mean values for V_E, f_B, V_T and partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$) over the 30 s of each Wingate test in each of the 4 conditions. No main effects of Thermal State or Gas Type were evident for V_E (Fig. 2.2 A). The mean V_E was at similar rates of 102 ± 16 liters • min^{-1} in the normothermic AIR and of 100 ± 26 liters • min^{-1} in hyperthermic AIR. The lowest rate for V_E was 85.1 ± 27 liters • min^{-1} during the normothermic N_2O condition and the highest rate was of 104 ± 23 liters • min^{-1} in the hyperthermic N_2O condition. There were no main effects of Gas Type and no effects of Thermal State on f_B (Fig. 2.2 B). Frequency of breathing was highest during the normothermic AIR condition at 59 ± 12 breaths • min^{-1}. It was 52 ± 14 breaths • min^{-1} during the hyperthermic N_2O condition and was approximately 48 breaths • min^{-1} in the normothermic N_2O and hyperthermic AIR conditions. No main effects of Thermal State or Gas Type were evident for V_T (Fig. 2.2 C) and its values ranged from approximately 1.9 to 2.1 liters across the four conditions. As well, there was a main effect of Gas Type ($F = 6.6$, $p < 0.05$) but no effect of Thermal State for $P_{ET}CO_2$ (Fig. 2.2 D). Partial pressure of carbon dioxide was highest during the normothermic N_2O condition at 42 ± 14 mm Hg. It was 41 ± 13 mm Hg during the hyperthermic N_2O condition and was approximately 35 mm Hg in the normothermic AIR and hyperthermic AIR conditions.

2.3.3. ∆Ventilation

Changes in breathing components during hyperthermia at rest were taken into account by subtracting the mean Wingate breathing response from the mean pre 40°C tub immersion. This calculation yielded the change (Δ) from baseline levels at rest to mean supramaximal values during exercise. Figure 2.3 gives ΔV_E, Δf_B and ΔV_T difference between mean rest and mean Wingate test value in each of the 4 conditions. No significant main effects of Thermal State or Gas Type were evident for ΔV_E (Fig. 2.3 A), Δf_B (Fig. 2.3 B) or ΔV_T (Fig. 2.3 C). The lowest ΔV_E was 68 ± 25 liters • min^{-1} during the normothermic N_2O condition and the highest rate was 90 ± 22 liters • min^{-1} in the hyperthermic N_2O. For Δf_B the rates were approximately 31 to 45 breaths • min^{-1} across

the four conditions. For ΔV_T the values were approximately 0.7 to 1.3 liters across the four conditions.

2.3.4. Interactions of Thermal State and Gas Type

Figure 2.4 gives interactions for mean V_E and f_B and ΔV_E and Δf_B between mean rest and mean Wingate test in each of the 4 conditions. There was a main effect ($F = 8.6$, $P < 0.05$) for an interaction between Thermal State and Gas Type for V_E (Fig. 2.4 A) and this same interaction had a main effect ($F = 11.9$, $P < 0.05$) for ΔV_E as well (Fig. 2.4 B). During normoxic nitrous oxide breathing in the hyperthermic exercise condition for ΔV_E there was a significant increase and a trend for V_E for an increase in ventilation relative to the same exercise in normothermic condition. The lower ΔV_E and the trend for a lower V_E during the normothermic N_2O condition relative to the normothermic AIR condition, was explained by a corresponding significantly lower f_B between the normothermic N_2O condition and the normothermic AIR condition ($F = 9.1$, $P < 0.05$) and Δf_B ($F = 11.4$, $P < 0.05$) between the normothermic N_2O and normothermic AIR condition (Fig. 2.4 C and 2.4 D).

2.4. Discussion

The main results support a significant interaction of the effects of Gas Type and Thermal State on exercise ventilation during high intensity exercise. This interaction for exercise ventilation was explained during normoxic N_2O breathing by a significant inhibition in V_E in the change between rest to exercise under normothermic relative to hyperthermic conditions (Fig. 2.4 A, B). This decrease was due to a decrease in frequency of breathing in the Air relative to the N_2O condition (Fig. 2.4 C and D) and this was coupled with carbon dioxide retention in both N_2O exercise conditions (Fig. 2.2 D). Carbon dioxide retention is one possible cause of deep-water black-out in hypoventilating divers (Lanphier and Camporesi 1993) and fatalities in divers appear to be more prevalent in warm water conditions (Vann, Freiberger et al. 2005).

The increased CO_2 retention was evident in the normothermic condition when the $P_{ET}CO_2$ was lower at ~35 mm Hg during air breathing than that of ~42 mm Hg during

N_2O breathing (Fig. 2.2 D). It might be anticipated that hyperthermia-induced hyperventilation during N_2O breathing would have helped mitigate this CO_2 retention. Relative to air breathing, however, $P_{ET}CO_2$ was elevated during the hyperthermic N_2O as it was for normothermic N_2O exercise condition. In addition to a N_2O-induced hypoventilation, it is worth noting that the increase in $P_{ET}CO_2$ during the hyperthermic exercise with N_2O breathing may have arisen in part on account of the Q_{10} effect of hyperthermia that elevates metabolically produced CO_2. Overall, during high intensity exercise, the results support a suppression of breathing that contributes to CO_2 retention and an increased potential for hypercapnia and deep water black-out (Glatte, Motsay et al. 1967; Lambertsen 1971).

Exercise ventilation during diving appears to be inhibited by the combined effects of increased ambient pressure and nitrogen narcosis. Tetzlaff and colleagues (Tetzlaff, Neubauer et al. 1998) found a significant decrease in V_E and V_T during exercise at 4 ATA relative to exercise at 1 ATA. The current finding that N_2O decreases exercise ventilation under intense exercise is in agreement with Tetzlaff and colleague's study (Tetzlaff, Neubauer et al. 1998) as well as results of Fagraeus and colleagues (Fagraeus, Hesser et al. 1974). Fagraeus and colleagues (Fagraeus, Hesser et al. 1974) also showed a decrease in V_E during heavy exercise at 4.5 ATA breathing compressed air as compared to 1.0 ATA breathing air. The current results support that the central narcotic effect of N_2O, or possibly nitrogen gas in the CNS for undersea divers breathing air, should be considered in addition to the decrease in ventilation in that is attributed to the increased density of air in hyperbaric conditions (Spaur, Raymond et al. 1977).

Mekjavic and colleagues (1992) suggested that nitrous oxide affected neural mechanisms controlling thermoregulation through modifications of synaptic transmission and the propagation of action potentials. The mechanism through which exercise ventilation is reduced in these conditions may be similar to what has been suggested by Mekjavic and colleagues (1992), however, at the present time the neural pathways by which N_2O influences exercise ventilation are unknown. Eisele (Eisele and Smith 1972) report that inhalation of 40% N_2O under resting conditions induced slower and deeper ventilation in their volunteers since they 'felt better', but they did not include any

ventilation data in support of their observation. This suggests the effect of N_2O on the conscious control of breathing may be separate or in combination with its influence on the respiratory control center where efferent outputs changing frequency of respiration and tidal volume are believed to originate. Our results support for 30% N_2O relative to air breathing our volunteers took less frequent breaths, an effect that would be from a change in output from the respiratory control center in the medulla oblongata. Several studies suggest that f_B is entrained to pedalling frequency (Bechbache and Duffin 1977; Jasinskas, Wilson et al. 1980), and that exercise rhythm directly influence breathing frequency during moderate, steady state exercise. These findings are not in conjunction with Casaburi, et al. (Casaburi, Whipp et al. 1977), which observed the ventilatory response to sinusoidal work, and showed no evidence to support that neural afferents from the exercising limbs induced changes in the ventilatory responses, thus rendering the effects of exercise rhythm on f_B controversial.

Dejours' (Dejours 1964) Phase I of submaximal exercise ventilation is thought to be a neurally mediated and it is evident during transitions from rest to submaximal intensities of exercise. The current design was targeted to simulate a transition from a light to a high intensity effort, such as that potentially evident as active, swimming diver attempts to escape a threat or a danger. Consequently, the present results are not directly applicable to statements about influences of hyperthermia or narcosis on Dejours' Phase I of exercise ventilation (Dejours 1964), but they do suggest during quick increases in exercise intensity that exercise ventilation is suppressed with narcosis and this leads to CO_2 retention plus an increased risks of deep water black out in both normothermic and hyperthermic divers.

As shown by Nishiyasu and colleagues (Hayashi, Honda et al. 2006 ; Fujii, Honda et al. 2008; Tsuji, Honda et al. 2012) and our own group (Cabanac and White 1995; White 1996; Sancheti and White 2006; White 2006), elevated core temperature stimulates exercise ventilation, although the resolution of mechanism underlying hyperthermia-induced hyperventilation is still ongoing. This may result from a physical effect of temperature on peripheral chemoreceptors and/or central chemosensitive areas that may increase their responses to normal metabolic stimuli, such as seen for carbon dioxide with body warming (Cunningham and O'Riordan 1957; Sancheti and White 2001)

or during hyperthermia when breathing hypoxic gas at rest (Curtis, Walsh et al. 2007) or during light exercise (Chu, Jay et al. 2007). In addition, McDougall et al. (MacDougall, Reddan et al. 1974) suggested that increased H^+ concentration which occurs during hyperthermia, may act as a possible modulator of central and/or peripheral chemoreceptors, and as a result increase ventilation. Also a decreased buffering capacity for CO_2 by body fluids takes place at higher body temperatures (Stadie, Austin et al. 1925) and this may lead to a lowered pH and compensatory hyperventilation. In the N_2O breathing trials the effect of hyperthermia was to elevate exercise ventilation, relative to that during normothermic trials but this was not a great enough extent to mitigate the associated CO_2 retention (Fig. 2.2 A, C and D). Consequently it did not appear that the N_2O narcosis suppressed the hyperthermia-induced hyperventilation.

Some benefits and limitations were evident in the experimental design. By employing normoxic N_2O inhalation during exercise our results separated the combined effects of narcosis and elevated ambient pressure that both decrease exercise ventilation in hyperbaric conditions (Fagraeus, Hesser et al. 1974; Tetzlaff, Neubauer et al. 1998). Also the pre-warming of volunteers provided a means to assess the separate effect hyperthermia on exercise ventilation during high intensity work. Possible limitations of the design include that the transition in work intensity that brought about large increases in exercise ventilation were from light to high intensity exercise. Another limitation was the potential effects of greater gas density when employing normoxic 30% N_2O, relative to air. Ambient air is ~1.225 g/cm^3, whereas N_2O gas is denser and therefore heavier at ~1.977 g/cm^3. According to Cerretelli et al. (1969), greater gas density may influence the work of breathing and limit the capacity for physical exercise to levels under anaerobic threshold. A future study could be to assess influences of normoxic N_2O breathing and hyperthermia on changes in ventilation from resting to maximal values where a more pronounced influence of these treatments might be evident, while factoring gas density with the usage of lighter gas such as helium, to compensate for potential increase in cost of breathing while using N_2O.

2.5. Conclusion

In conclusion, the results support the hypothesis that normoxic nitrous oxide breathing decreased high intensity exercise ventilation in normothermic humans and this was on account of reductions in frequency of breathing. The evidence supports that CO_2 retention and increased risk of deep water black out is likely evident for normothermic as well as hyperthermic deep sea divers.

Table 2.1. Participants' characteristics.

Subject #	Age (y)	Height (m)	Weight (kg)	BMI (kg / m^2)
1	25	1.76	71.8	23.2
2	23	1.78	65.9	20.8
3	22	1.73	66.8	22.3
4	22	1.78	86.8	27.4
5	23	1.83	80.5	24.0
6	25	1.82	76.1	23.0
7	22	1.79	74.8	23.3
Mean ± SD	**23.1 ± 1.3**	**1.8 ± 0.03**	**74.7 ± 7.4**	**23.4 ± 2.0**

Table 2.2. **Mean T_{ES} and mean T_{SK} values in normothermic and hyperthermic conditions. **p < 0.05 between normothermic and hyperthermic conditions**

Variable	Normothermia	Hyperthermia
T_{ES} (^{o}C)	37.4 ± 0.1	**38.4 ± 0.04
T_{SK} (^{o}C)	33.5 ± 0.3	34.2 ± 0.9

(mean ± SD)

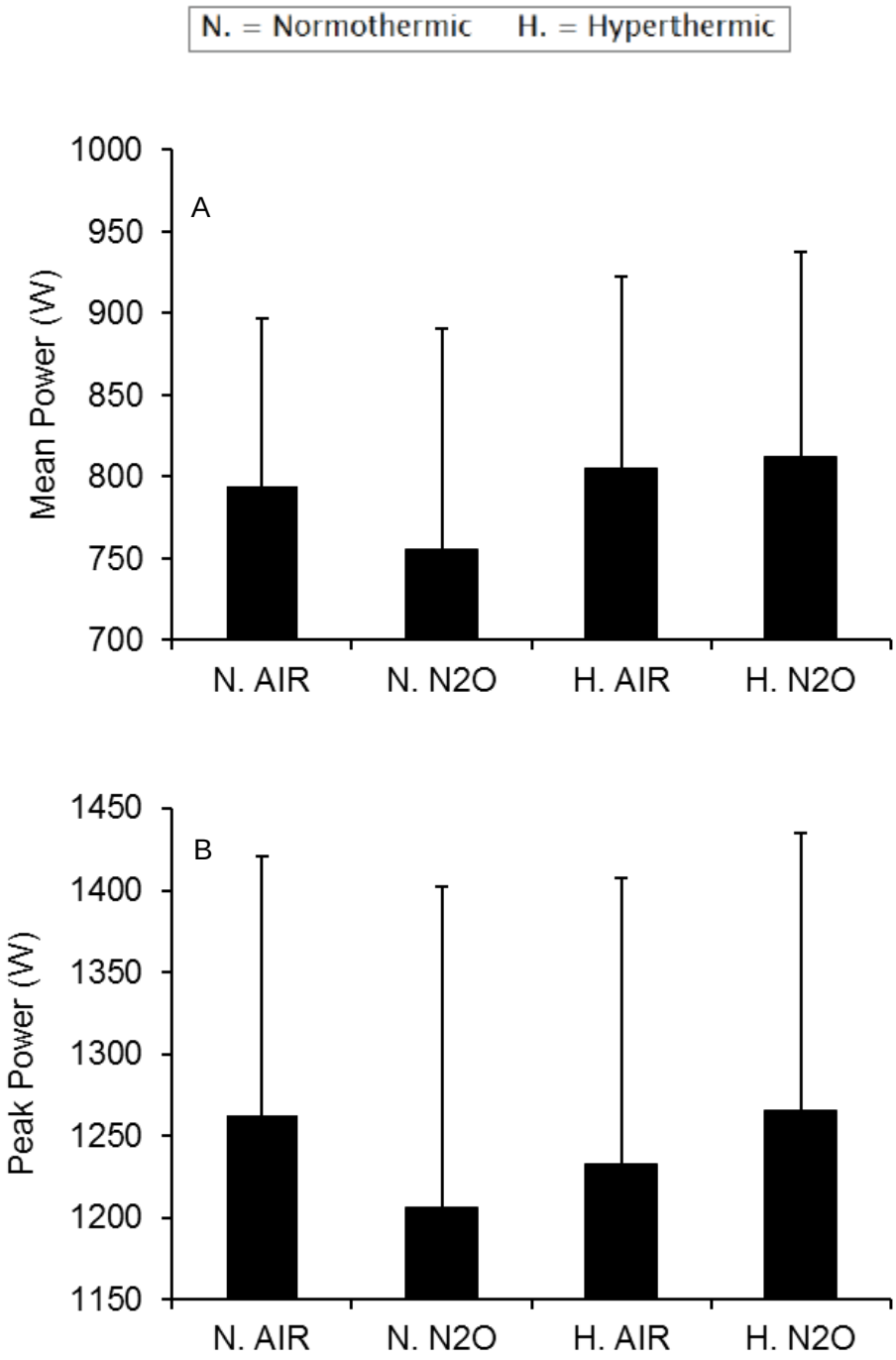

Figure 2.1. **Mean Power and Peak Power during the supramaximal Wingate exercise tests in each of the four conditions. Each vertical bar represents the mean response for 7 volunteers and the error bars present the standard deviation of the mean.**

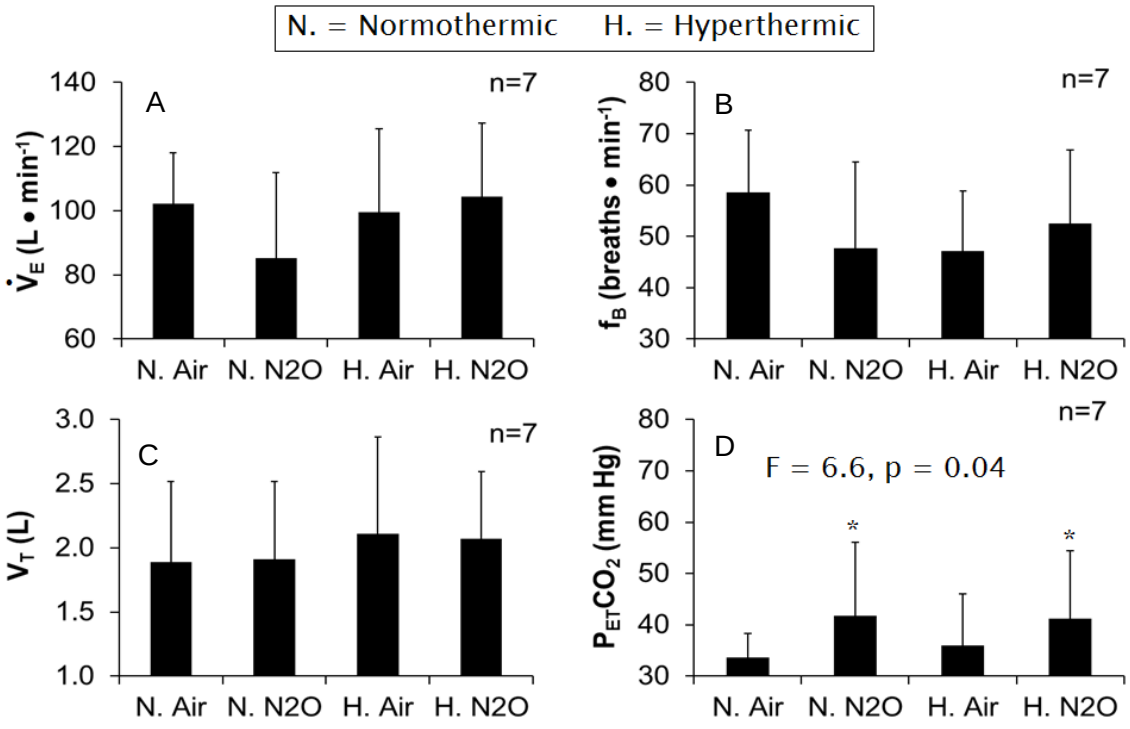

Figure 2.2. **Mean V_E, f_B, V_T, and $P_{ET}CO_2$ during the supramaximal Wingate tests in each of the four conditions. Each vertical bar represents the mean response for 7 volunteers and the error bars present the standard deviation of the mean. *P< 0.05 from N. Air.**

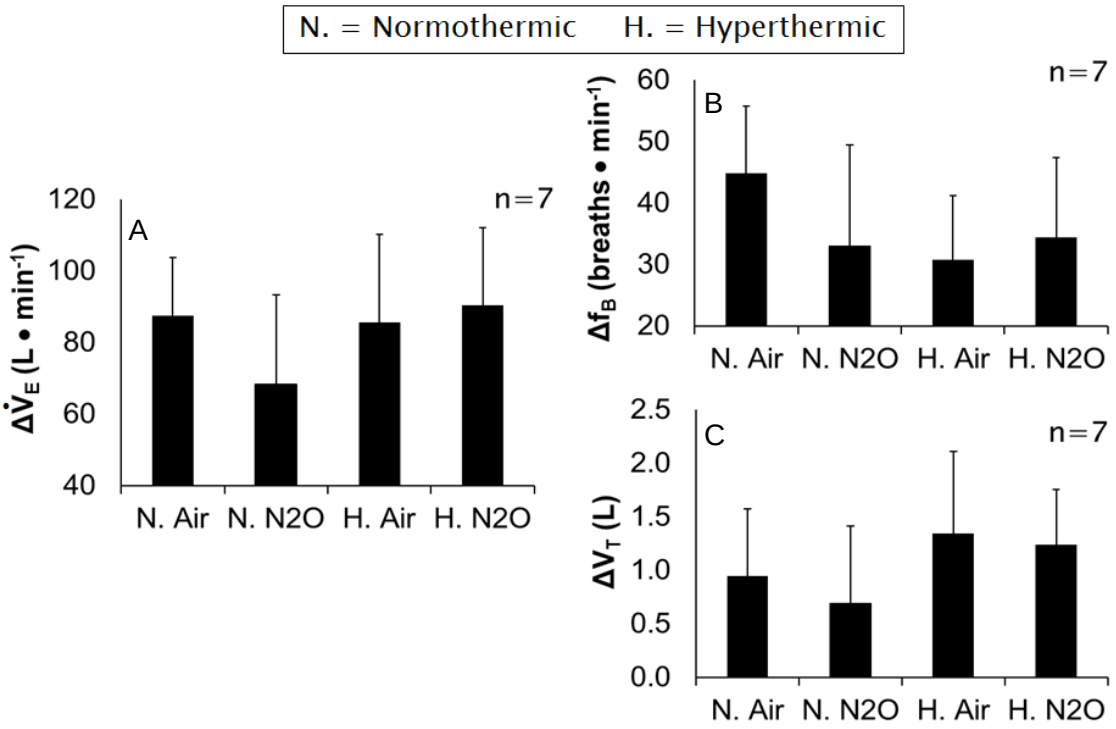

Figure 2.3. Changes in exercise ventilation (ΔV_E), frequency of breathing (Δf_B) and tidal volume (ΔV_T). Each vertical bar represents the mean response for 7 volunteers and the error bars give the standard deviation of the mean.

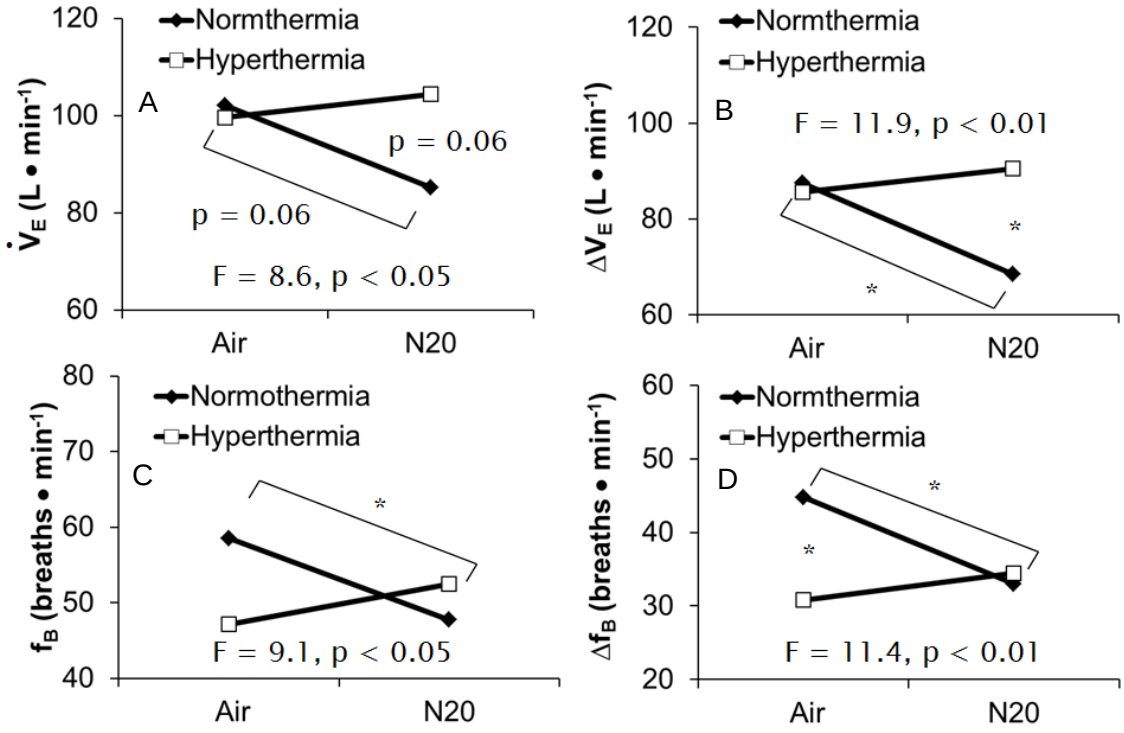

Figure 2.4. **Thermal State by Gas Type Interaction plots for V_E and f_B as well as for ΔV_E, and Δf_B between mean rest and during supramaximal Wingate exercise. *P< 0.05**

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Chapter 3.

Effects of hyperthermia and 30% normoxic nitrous oxide on timing and ventilatory drive of human ventilation during short duration, high intensity exercise

3.1. Introduction

Beyond the known anesthetic effects of normoxic nitrous oxide (N_2O) in humans (Jastak and Donaldson 1991), exposure to ~30% fractions of the gas has been found to simulate nitrogen narcosis as seen in undersea SCUBA divers breathing compressed air between 4 and 6 Atmospheres Absolute (ATA) of pressure (Biersner 1987). During inhalation of similar fractions of normoxic N_2O in resting humans (Royston, Jordan et al. 1983), a shortening of inspiratory time (T_I) relative to air breathing is evident, and as a result an increase in ventilatory drive (V_T/T_I) occurs. At submaximal exercise intensities a small but significant increase in pulmonary ventilation (V_E) is evident during normoxic N_2O breathing (Fothergill and Carlson 1996). The increase in V_E during steady state submaximal exercise tests implies that the efferent signal sent from the CNS to the respiratory muscles is greater under N_2O inhalation. This increase in efferent signaling was displayed by Fothergill's group in 1996 (Fothergill and Carlson 1996), while exposing 13 male Navy divers to 23% normoxic N_2O gas, while riding at 75% of their individual peak work rates. It has been reported by Fothergill's group that the result of the increase in pulmonary ventilation was a result of an increase in T_I, which increased the inspiratory duty cycle (T_I/T_{TOT}) of the divers as well.

The changes in T_I or T_I/T_{TOT} shown by Forthergill's group, associated with normoxic N_2O during submaximal exercises are not the same as those changes under hypoxic conditions (Yacoub, Doell et al. 1976) or during higher work intensities (Ciammaichella and Mekjavic 2000). Under these conditions, normoxic N_2O appears to

inhibit breathing, possibly due to a selective suppression of peripheral chemoreceptors (Yacoub, Doell et al. 1976). Suppression of maximal or supramaximal exercise ventilation under N_2O inhalation can be used to simulate deep-sea diving (Ciammaichella and Mekjavic 2000) and potentially answer unexplained deep-water black-outs. SCUBA diver fatalities (Vann, Freiberger et al. 2005) occur most often during the bottom phase of the dive, with 90% of divers breathing compressed air. The largest fraction of these fatalities occurred during diving in warmer water temperatures in the southeast/southwest USA or the Caribbean throughout the summer months of June to August. Forty-eight percent of these fatalities due to loss of consciousness were at the bottom of the dive and 27 cases of these 48% were for no apparent reason (Vann, Freiberger et al. 2005).

The present study was conducted to assess the separate influences of passively-induced hyperthermia and narcotic gas on timing components and ventilatory drive in humans during short, duration high intensity exercise. To induce a large initial increase in breathing without time for an exercise-induced change in core temperature or accumulation of metabolites from exercise, a 30 s Wingate exercise test was employed in 4 exercise sessions. This exercise protocol included a transition from light to intense exercise and narcosis was induced with inhalation of normoxic 30% N_2O at ~1 ATA. As such, the combined and separate effects of hyperthermia and narcosis were assessed for their influences on a sudden increase in exercise ventilation and its timing components and drive. It was hypothesized that N_2O would reduce and that increased core temperature would increase timing components and ventilatory drive of exercise ventilation in these exercise conditions.

3.2. Methodology

3.2.1. Volunteers

Seven male participants volunteered for the study. They were 23.9 ± 1.8 years of age (mean ±SD), 1.8 ± 0.1 m tall, 76.3 ± 9.0 kg in weight and had a body mass index of 23.8 ± 2.1 kg / m^2. Individual characteristics are displayed in table 3.1. The volunteers were physically fit as evident from their Wingate power output values when classified

with a ranking method developed from Wingate tests on 485 male, Division 1 NCAA athletes (Zupan, Arata et al. 2009). Using this ranking method our volunteers peak power during the control condition (Fig. 3.1) put them above the highest ranking of 1163 W for an 'elite' ranking and their mean power values during the control condition (Fig. 3.1) put them within the range of 778 – 823 W for an 'excellent' ranking (Zupan, Arata et al. 2009). Each volunteer was made aware of any risks associated with the protocol in this experiment. After reading a detailed outline of the study each participant signed a consent form. The sample size was determined using a power calculation. The difference worth detecting was set at 10%, with an alpha level of 0.05, a beta value of 0.8 and a standard deviation of 7% of the estimated mean scores for the following timing components inspiratory time (T_I), expiratory time (T_E), total respiratory time (T_{TOT}) inspiratory duty cycle (T_I/T_{TOT}) and for estimating ventilatory drive, mean inspiratory flow (V_T/T_I). The Office of Research Ethics at Simon Fraser University approved this research.

3.2.2. Instrumentation

A calibrated breath-by-breath Vmax 229 series metabolic cart (Sensormedics, CA, USA) was employed to assess expired gases as well as ventilation and its components as described previously (Sancheti and White 2006).

Esophageal temperatures (T_{ES}) were estimated using an esophageal temperature thermocouple (size 9Fr, Mallinkroft Medical Inc., St Louis, MO, USA) inserted to the level of the left ventricle (Mekjavic, Morrison et al. 1984). Mean un-weighted skin temperatures (T_{SK}) were estimated from sites at the chest, thigh, forehead and lower back with surface copper constantan thermocouples. All thermocouples were connected to a data acquisition system (National Instruments, USA) and controlled by LabVIEW software (National Instruments, USA) on a personal computer.

The gas inhalant included either normoxic nitrous oxide (30% N_2O, 21% O_2, balance N_2, Air Liquide Canada, Inc.) or Air that were supplied to the volunteer in corrugated Collins respiratory tubing from a meteorological balloon.

3.2.3. Protocol

Following instrumentation the volunteer participated in one of four 30 s Wingate trials (Bar-Or 1987) on an electrically braked, seated, cycle ergometer. Trials were performed ~1 week apart and all participants were asked to refrain from ingesting caffeine for 12 hr prior to exercise tests. Each participant inhaled either room air or 30% normoxic N_2O in a given Wingate test. The participant was normothermic in two Wingate tests and hyperthermic in two other Wingate tests. Prior to the hyperthermic tests the participant was warmed in a 40ºC water bath and started exercise within ~10 min after exiting the bath. After this, the participant was quickly seated on the cycle ergometer after exiting the bath and then wrapped in blankets plus impermeable raingear to maintain an elevated T_{ES} until the start of the Wingate test.

At the start of each exercise trial the volunteer was seated on the cycle ergometer and began breathing air or N_2O through the mouthpiece for ~10 min of rest, followed by a 30 s warm-up at 40 W with a pedaling cadence between 80 to 90 rpm. The volunteer was then given a 5 second countdown at the end of warm-up cycling and then proceeded to perform a 30 s Wingate test, with instructions to pedal as hard and as fast as possible with a resistance of 0.09 kp (0.88 N)/kg.

3.2.4. Statistical Analysis

The dependent outcome variables of interest were the means of T_{ES}, T_{SK}, T_I, T_E, T_{TOT}, T_I/T_{TOT} and V_T/T_I. An ANOVA model was employed with repeated factors of Thermal State (Normothermia and Hyperthermia) and inhaled Gas Type (AIR, normoxic N_2O). Paired t-tests were employed to compare means. Results were considered significant at an alpha level of 0.05.

3.3. Results

3.3.1. Temperature

Table 3.2 gives the mean values between the Normothermic and Hyperthermic conditions for mean T_{ES} and mean T_{SK}. For T_{ES} there was a main effect of Thermal State ($F = 135.6$, $p < 0.01$) but no main effect of Gas Type. The mean T_{ES} in the Normothermic trials was ~ 37.2 ± 0.01°C, and ~38.7 ± 0.1°C ($p<0.05$), in the Hyperthermic trials. There were no effects of Thermal State or Gas Type on mean T_{SK}. The mean T_{SK} in Normothermic trials was ~33.3 ± 0.6°C, and ~33.5 ± 1.6°C, in the Hyperthermic trials.

3.3.2. Timing Components

Figure 3.2 gives the mean values for T_I, T_E and T_{TOT} over the 30 s of each Wingate test in each of the 4 conditions. No main effects of Thermal State or Gas Type nor interactions of Thermal State by Gas Type were evident for T_I, T_E and T_{TOT} (Fig. 3.2 A, B, C). The mean T_I was at similar rates of 0.6 – 0.7 s across the 4 conditions. The mean T_E was at similar rates of 0.5 – 0.6 s across the 4 conditions. The mean T_{TOT} was at similar rates of 1.1 – 1.3 s across the 4 conditions.

3.3.3. Inspiratory Duty Cycle and Ventilatory Drive

Figure 3.3 gives the mean values for T_I/T_{TOT} and V_T/T_I over the 30 s of each Wingate test in each of the 4 conditions. No main effects of Thermal State or Gas Type nor interactions of Thermal State by Gas Type were evident for T_I/T_{TOT} and V_T/T_I (Fig. 3.3 A, B). The mean T_I/T_{TOT} was at similar rates of 3.3 – 3.9 across the 4 conditions. The mean V_T/T_I was at similar rates of ~0.5 $L \bullet s^{-1}$ across the 4 conditions.

3.4. Discussion

The main results indicate no significant change in timing components between normothermia and hyperthermia and between Air and N_2O inhalation during

supramaximal exercise. These results are in agreement with the results reported by Fothergill's group (Fothergill and Carlson 1996), who showed no change in breathing timing components nor in ventilatory drive during exercise at 75% of maximal VO_2 on a bicycle ergometer, while inhaling normoxic 23% N_2O in normothermic conditions. Nevertheless, the group did report an increase in V_E of approximately 4% ($p < 0.05$) at rest and exercise, which occurred as a result of a rise in V_T ($p < 0.02$). These findings are not in agreement with the results found in study 1 (Fig. 2.2 C), however, the group also indicated that after a Post hoc analysis was employed between gas mixture and work rate for V_T, it was found that the increase in V_T under the narcotic conditions was more pronounced at rest than during exercise, providing no attention to the possible changes in breathing responses that may occur during supramaximal intensities.

In contrast to Fothergill's group, Royston et al (Royston, Jordan et al. 1983), was not in agreement with the results shown by this study, as they displayed a reduction in T_I and an increase in V_T/T_I during normoxic 40% nitrous oxide inhalation at rest. Expiratory time, as observed by Royston's group, was similar to our results (Fig. 3.2 B), showing no change between Air and N_2O. Although no change in breathing timing components was found and despite controversy between Roysten's finding with regards to T_I and V_T/T_I responses at rest, the suppression in ventilatory components displayed in study 1, are not associated with changes in the mechanics of breathing and neuromuscular activation from the phrenic nerve to the respiratory muscles. Since no change in the active portion (T_I) of the respiratory cycle was evident, it can be suggested that the changes reported in the literature in ventilation under N_2O inhalation (Mekjavic 1992; Royston, Jordan et al. 1983) or nitrogen narcosis (Fagraeus, Hesser et al. 1974; Tetzlaff, Neubauer et al. 1998) can be associated with narcotic effect of N_2O or possibly nitrogen gas during diving on central command, with no change to respiratory muscular efferent signaling or activation.

As previously reported by several groups (Cabanac and White 1995; White 1996; Sancheti and White 2006; White 2006), elevated core temperature stimulates exercise ventilation, although the changes of breathing timing components and ventilatory drive underlying hyperthermia-induced hyperventilation are still ongoing. With accordance to these reports, the effect of hyperthermia in this study was hypothesized to elevate exercise ventilation through an increase in the rate of breathing relative to normothermic

breathing, shortening timing components while increasing ventilatory drive. This, however, was not displayed in our study by any of the timing or respiratory effort during the Wingate tests across the different conditions.

Some benefits were evident in the experimental design. By employing normoxic N_2O inhalation during exercise our results gave the effects of narcosis independent of the elevated ambient pressure that decreases exercise ventilation in hyperbaric conditions (Fagraeus, Hesser et al. 1974; Tetzlaff, Neubauer et al. 1998). Also the pre-warming of volunteers provided a means to assess the separate effect hyperthermia on exercise ventilation during high intensity work. A limitation of the study is the unanticipated large variability displayed the outcome variables, which may have resulted in part from a small sample size of n=7. Despite previously calculated power calculations, the variability in results shown by this study suggest a larger sample size should be used in future studies.

3.5. Conclusion

In conclusion, evidence supports a rejection of the research hypothesis that normoxic nitrous oxide breathing would decrease and hyperthermia would increase timing components and ventilatory drive during high intensity in humans. This was on account of no change in timing components and ventilatory drive between the 4 different conditions.

Table 3.1. Participants' characteristics.

Subject #	Age (y)	Height (m)	Weight (kg)	BMI (kg / m^2)
1	23	1.78	65.9	20.8
2	26	1.86	87.7	25.4
3	22	1.73	66.8	22.3
4	22	1.78	86.8	27.4
5	26	1.73	70.5	23.5
6	23	1.83	80.5	24.0
7	25	1.82	76.1	23.0
Mean ± SD	**23.9 ± 1.8**	**1.8 ± 0.05**	**76.3 ± 9.0**	**23.8 ± 2.1**

Table 3.2. **Mean T_{ES} and mean T_{SK} values between normothermic and hyperthermic conditions. **p < 0.05 between normothermic and hyperthermic conditions**

Variable	Normothermia	Hyperthermia
T_{ES} (^{o}C)	37.2 ± 0.01	**38.7 ± 0.1
T_{SK} (^{o}C)	33.3 ± 0.6	33.5 ± 1.6

(mean ± SD)

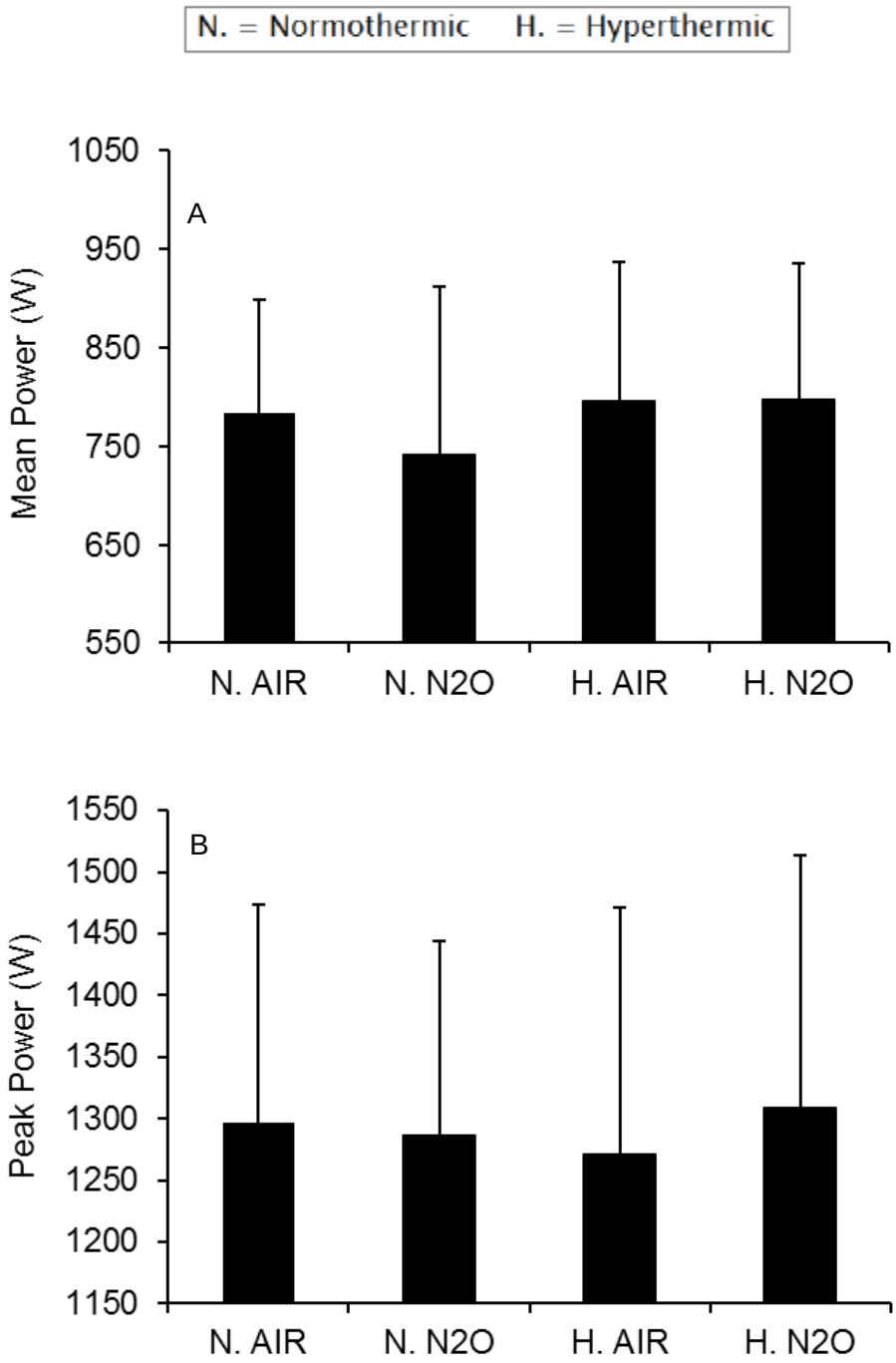

Figure 3.1. **Mean Power and Peak Power during the supramaximal Wingate exercise tests in each of the four conditions. Each vertical bar represents the mean response for 7 volunteers and the error bars present the standard deviation of the mean.**

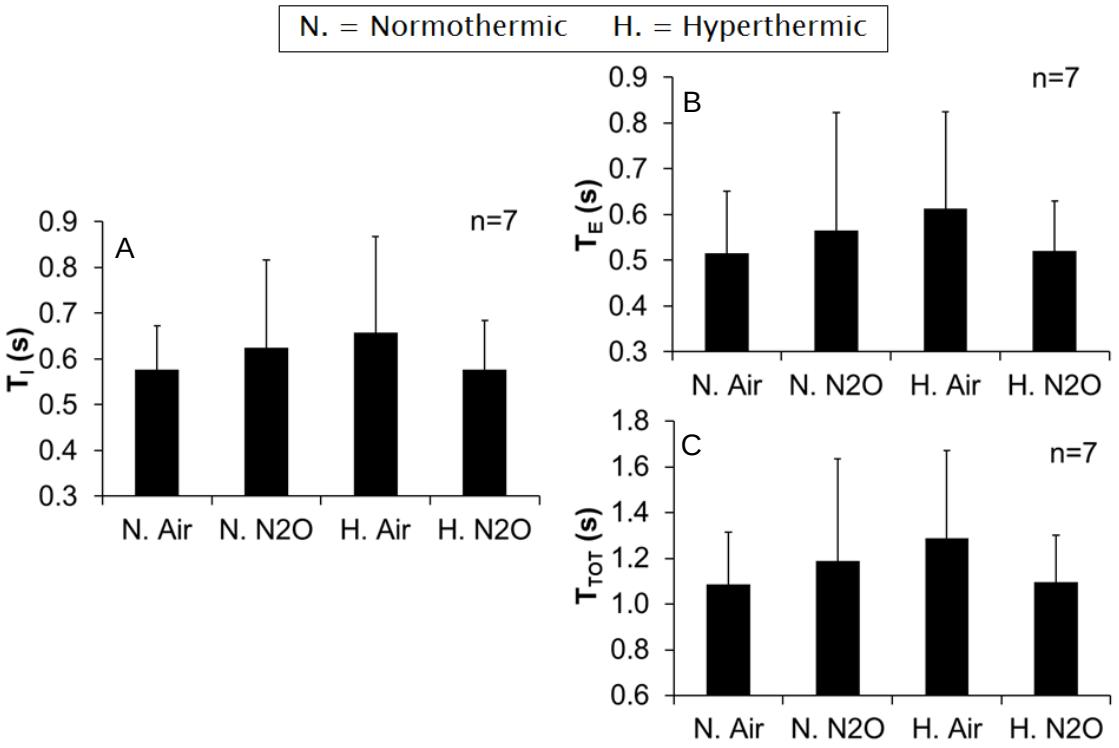

Figure 3.2. **Mean T_I, T_E and T_{TOT} during the supramaximal Wingate exercise tests in each of the four conditions. Each vertical bar represents the mean response for 7 volunteers and the error bars present the standard deviation of the mean.**

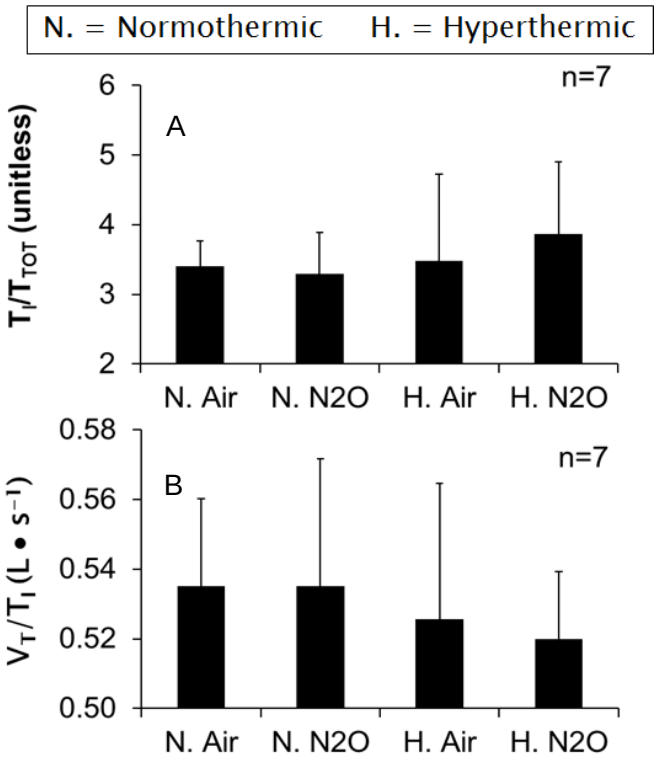

Figure 3.3. **Mean T_I/T_{TOT} and V_T/T_I during the supramaximal Wingate exercise tests in each of the four conditions. Each vertical bar represents the mean response for 7 volunteers and the error bars present the standard deviation of the mean.**

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