Effect of Mild Hypercapnia and Skin Temperature on Physiological Responses during Face Immersion

by

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Abstract

The studies in this thesis were to assess if face cooling and CO_2 combine in their influences on pulmonary ventilation and cardiovascular responses in humans. It was hypothesized that mild hypercapnia enhances these ventilatory and cardiovascular response to cold water face immersion. The first study resulted in significant elevations in pulmonary ventilation ($p = 0.014$), tidal volume ($p = 0.008$), inspiratory duty cycle ($p = 0.013$) and reductions in inspiratory flow ($p = 0.051$) during face immersions. The second study resulted in significant graded elevations in mean arterial blood pressure ($p < 0.001$), and reductions in the index of cerebral conductance in the middle cerebral artery (MCA) ($p = 0.045$) during face immersion. In conclusion, cold face immersion during mild hypercapnia increases ventilatory gasping and the blood pressure response while decreasing the conductance for cerebral blood flow to cerebral tissues supplied by the MCA.

Keywords: cerebral blood flow; cold; diving; face; immersion; pulmonary ventilation

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List of Acronyms

BA	Basilar artery
BA_V	Basilar artery blood velocity (cm/s)
BBB	Blood-brain barrier
BP	Blood pressure (mm Hg)
CBF	Cerebral blood flow (mL/s)
CO_2	Carbon dioxide
CPP	Cerebral perfusion pressure (mm Hg)
CSF	Cerebrospinal fluid
ETF	End-tidal forcing
F_B	Frequency of breathing (breaths/min)
H^+	Hydrogen ion
HR	Heart rate (bpm)
ICP	Intracranial pressure (mm Hg)
K^+	Potassium ion
LBNP	Lower body negative pressure
MAP	Mean arterial pressure (mm Hg)
MCA	Middle cerebral artery
MCA_V	Middle cerebral artery blood velocity (cm/s)
MRI	Magnetic Resonance Imaging
O_2	Oxygen
PaO_2	Arterial partial pressure of oxygen (mm Hg)
PCA	Posterior cerebral artery
PCA_V	Posterior cerebral artery blood velocity (cm/s)
PCO_2	Partial pressure of carbon dioxide (mm Hg)
$P_{ET}CO_2$	End tidal carbon dioxide partial pressure (mm Hg)
$P_{ET}O_2$	End tidal oxygen partial pressure (mm Hg)
pH	pH = -log [H+]
Q	Cardiac output (L/min)
SaO_2	Hemoglobin oxygen saturation (%)
$SkBV_{HAND}$	Skin blood velocity at the hand (cm/s)
SNS	Sympathetic nervous system

TCD	Transcranial Doppler
T_E	Expiratory time (s)
T_I	Inspiratory time (s)
T_I/T_{TOT}	Inspiratory duty cycle (unitless)
T_{TOT}	Total breathing cycle time (s)
VA	Vertebral artery
VA_V	Vertebral artery blood velocity (cm/s)
V_E	Pulmonary ventilation (L/min, BTPS)
V_T	Tidal volume (L, BTPS)
V_T/T_I	Mean inspiratory flow (L/s)

Glossary

Autonomic Conflict	Simultaneous activation of the sympathetic and parasympathetic nervous systems (Shattock & Tipton, 2012).
Cerebral Autoregulation	Cerebral blood flow is able to be maintained over a range of blood pressure values and is minimally altered over a range of PO_2 and PCO_2 (Ainslie & Duffin, 2009).
Cerebrovascular-CO_2 reactivity	A homeostatic mechanism that helps to maintain cerebral pH through modulations in CO_2 (Ainslie & Duffin, 2009).
Chemosensitivity	Tissues that are responsive to chemical stimuli and induce changes in breathing (Ainslie & Duffin, 2009).
Dive Response	Response to apnoeic cold water face immersion that results in bradycardia (Asmussen & Kristiansson, 1968), increased peripheral vasoconstriction (Elsner, Gooden, & Robinson, 1971), and increased MAP (Bruce & Speck, 1979).
Gasp Response	Response to head-out whole body cold water immersion that results in an initial gasp, hyperventilatory response, hypertension (Tipton, 1989) and tachycardia (Keatinge & Evans, 1961). This response is also known as the cold shock response (Jay et al., 2007).

Executive Summary

This thesis assessed the effect of cold water face immersion and mild hypercapnia on ventilatory and cardiovascular variables in two studies. The first chapter of the thesis is a literature review of the control of breathing, the dive response and the control of cerebral blood flow. The second chapter gives the study on the effect of cold water face immersion and mild hypercapnia on ventilatory responses while the third chapter gives a study that examined these effects on cardiovascular variables.

It was hypothesized that the greatest inspiratory flow and inspiratory duty cycle would be seen during 5°C water face immersion when compared to immersion in both 15 and 33°C water, and that the greatest increases in ventilation will be in a hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

It was hypothesized that the greatest increases in mean MCA_V and VA_V, will occur during the mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values. It was also hypothesized that the lowest skin blood velocity and greatest blood pressure, cerebrovascular conductance, and the greatest Q, SV and HR will occur during mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

Chapter 1.

Introduction

1.1. Literature Review of Control of Breathing, Dive Response and Control of Cerebral Blood Flow

1.1.1. Control of Breathing in Humans

Breathing in humans under normothermic conditions is primarily under control of the respiratory centres found in the medulla oblongata and the pons in the brainstem. The dorsal respiratory group, responsible for inspiration, is a group of neurons located in the nucleus of the tractus solitarius found within the medulla oblongata (Mitchell et al., 1963). The ventral respiratory group, also found in the medulla oblongata, is responsible for expiration (Mitchell et al., 1963). Ventilation is altered by a variety of inputs including those from the pneumotaxic centre also in the medulla oblongata. Partial pressures of arterial carbon dioxide ($PaCO_2$) and hydrogen ions (Haldane & Priestley, 1905) and oxygen (PaO_2) are sensed by chemosensitive tissues in the periphery. In the cerebrospinal fluid (CSF) PCO_2 and H^+ are sensed by tissues on the ventral surface of the medulla oblongata within the respiratory control center (Gessel, 1923).

Control of breathing includes a chemoreflex arc that is comprised of central and peripheral chemoreceptors, the central nervous system and the effector muscles. This system operates as a negative feedback loop where an increase in PCO_2 will increase $[H^+]$ and stimulate the chemoreceptor (Euler & Söderberg, 1952). Increased chemoreceptor activity brings about a reflex increase in alveolar ventilation as a method to eliminate CO_2. The drop in PCO_2 decreases hydrogen ion concentration $[H^+]$ and the chemoreceptor stimulus, thus ventilation returns towards pre-stimulus levels (Ainslie & Duffin, 2009). The arterial partial pressure of oxygen (PaO_2) also influences breathing, but indirectly through the peripheral chemoreceptors, and has little effect on the respiratory centres in the CNS (Heymans, 1963). This minimal effect of PO_2 on pulmonary ventilation

occurs for two reasons. First, adequate saturation of haemoglobin with oxygen, and thus adequate oxygen supply, occurs even when PaO_2 falls from 95 to as low as 60 mmHg. This is represented by a fall in haemoglobin saturation from 97 to 90% and is due to the high affinity of haemoglobin for oxygen over those pressures (Aste-Salazar & Hurtado, 1944) and since peripheral chemoreceptors respond only once PaO_2 decreases below 50 mmHg (Heymans, 1965).

The central chemoreceptors are found bilaterally beneath the ventral surface of the medulla oblongata. They are sensitive to changes in CSF $[H^+]$. The $[H^+]$ can be influenced through changes in the $PaCO_2$ (Elsner & Gooden, 1983; Issa & Remmers, 1992). Hydrogen ions cannot pass through the blood-brain barrier (BBB), however, CO_2, is able to pass through the BBB and once it does, it can react with water to form carbonic acid. This reaction (Equation 1) occurs as part of the bicarbonate buffer system where CO_2 and water form H_2CO_3 through the action of carbonic anhydrase (CA), and then H_2CO_3 dissociates into H^+ and HCO_3^- (Meldrum & Roughton, 1933).

Equation 1: $$CO_2 + H_2O \overset{CA}{\leftrightarrow} H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

Therefore, CO_2 and H^+ both are strong influences on inspiration by acting on a chemosensitive area in the brainstem.

The peripheral chemoreceptors are found bilaterally both in the carotid bodies at the bifurcation of the common carotid artery, and in the aortic bodies, in the arch of the aorta. They are sensitive to increases in $PaCO_2$, $[H^+]$, and to PaO_2 when it falls below 70 mmHg (Heymans & Heymans, 1927). Additionally, the sensitivity of the peripheral chemoreceptors to $PaCO_2$ and $[H^+]$ increases if PaO_2 is below 70 mmHg (Comroe & Schmidt, 1937). The peripheral chemoreceptors relay this information to the brainstem via the glossopharyngeal and vagus nerves to alter ventilation. Of the two of the peripheral chemoreceptor groups, the carotid bodies are dominant and they are responsible for 90 % of the total peripheral chemosensitivity in response to hypoxia while the aortic bodies are responsible for the remaining 10 % (Honda, 1985). Carotid chemoreceptors also respond to changes in $PaCO_2$ and $[H^+]$, appearing to be responsible for 20 % of the ventilatory response to arterial hypercapnia and acidosis, with the remaining 80 % mediated by the central chemosensitive tissues (Dejours, 1962).

Additional sensory information from baroreceptors, J receptors (Paintal, 1977), and irritant receptors (Knight & Holgate, 2003) found in the lung are relayed via the glossopharyngeal and vagus nerves up to the tractus solitarius into the dorsal respiratory group to influence breathing.

Notably, the baroreceptors can increase breathing rate through sensed changes in arterial pressure (Heymans & Heymans, 1927). Baroreceptors are stretch receptors that sense increased pressure in the internal carotid arteries and arch of the aorta through stretch of these vessels (Widdicombe, 2006). Increased stretch of the baroreceptors results in their firing. Impulses are sent from the carotid baroreceptors through the Hering's nerve, a branch of glossopharyngeal nerve, and from the aortic baroreceptors through the vagus nerve up to the tractus solitarius of the medulla oblongata (Widdicombe, 2006). When the respiratory centre is stimulated, this increases the nervous stimuli sent to the diaphragm and external intercostal muscles through the spinal cord motor nuclei (Widdicombe, 2006). Another reflex that occurs at the lung is the Hering-Breuer inflation reflex. An overstretched lung tissue, sensed by pulmonary stretch receptors found in smooth muscle of the airways of the lung, will inhibit inspiration. When the lung tissue is overstretched, pulmonary stretch receptors fire impulses that travel through vagus nerve to the medulla oblongata and the apneustic centre of the pons (Poon, 2004).

During inspiration, contraction of the diaphragm allows for inhalation, whereas relaxation of the diaphragm permits passive relaxation through elastic energy stored in the lungs and diaphragm. The pneumotaxic centre, found in the pons, inhibits ventilation as a method to shorten inspiratory time, which can also increase frequency of breathing since a shortening of inspiration will also shorten breath duration (Cohen, 1973).

Ambient cold has also been seen to modulate the ventilatory response to CO_2. In a study conducted by Bullard and Crise (1961) subjects breathed a gas mixture including 6% CO_2 warmed to 30°C or cooled to 5°C while the subjects sat in a climatic chamber cooled to 5°C. There appeared to be a positive multiplicative effect of cold and CO_2 inhalation on V_E, since the slope of the relationship between pulmonary ventilation expressed as a function of time during cold CO_2 breathing was much steeper than for warm CO_2 breathing. In the control condition, where the subject breathed 5°C room air in

the climatic chamber, V_E had a negligible increase (Bullard & Crise, 1961). Assuming the skin temperature cooled the same degree in both CO_2 – breathing conditions as well as during the control condition, the disproportionate increase in ventilation is likely due to the effect of differing temperatures of the inhaled air since the fraction of CO_2 in the inspired air was held constant at 6% in the warm and cold conditions. Also, mean heat production during CO_2 – breathing was much lower than when the subject breathed room air, which indicates that metabolic rate is unlikely to influence V_E while the subjects breathed CO_2. Similarly, Argacha et al. (2008) found that cooling the face with cold air until the face temperature decreased by 11°C augmented the ventilatory and heart rate response to hypoxia.

In summary, this section shows the main chemical inputs that influence breathing at rest, therefore, are $PaCO_2$, PaO_2, and $[H^+]$. Central chemosensitive areas are sensitive to variations of CO_2 and $[H^+]$, while peripheral chemoreceptors are sensitive to falls in PaO_2 below 50 mmHg and increases of CO_2 and [H+]. The carotid bodies dominate peripheral chemoreception since they are responsible for 90 % of the total peripheral chemosensitivity in response to hypoxia, whereas aortic bodies are responsible for the remaining 10 % (Honda, 1985). Carotid chemoreceptors responses to changes in $PaCO_2$ and $[H^+]$, appear to be responsible for 20 % of the ventilatory response to arterial hypercapnia and acidosis, with the remaining 80 % mediated by the central chemosensitive tissues (Dejours, 1962). More recently, complex interactions have been reported between the peripheral and central chemoreflexes (Duffin & Mateika, 2013; Teppema & Smith, 2013; Wilson & Day, 2013).

1.1.2. Apnoea and Face Immersion

Breath-hold diving is a practice that the women of Korea (Cachido ama) and Japan (ama) have participated in for thousands of years in order to collect pearls, sponges and seafood from the ocean to feed and support their families (Dujic & Breskovic, 2012). Traditionally, the Korean ama dove with only a face mask for vision, and light clothing in water temperatures that vary from 10 - 27°C (Rennie et al., 1962). They dove to depths of 10 – 15 m for an average time of 30 s per dive (Dujic & Breskovic, 2012). More recently from the mid-20th century until the present, the sport of free-diving has required divers to

descend without the aid of compressed gases to test the limits of human breath-holding capacity (Ferretti, 2001).

The means to increase the depths of these dives include the lengthening of apnoea time and through lung packing where the diver uses muscle actions of the trunk to 'pack' more air into the lungs (Dujic & Breskovic, 2012). An increase in apnoea time can also be achieved through decreased O_2 consumption (VO_2) or through a decreased stimulus to breathe. The 'diving response' (DR) is a collection of physiological changes that assists in prolonging dive time. The DR results in a bradycardia (Asmussen & Kristiansson, 1968) and an increased peripheral vasoconstriction (Elsner et al., 1971), as well as an increased mean arterial pressure (MAP) (Bruce & Speck, 1979). The subsequent prolonged apnoea can be considered a method of oxygen conservation and prevention of the aspiration of water into the lungs (Gooden, 1994). The benefit of peripheral vasoconstriction during the diving response is to reduce blood flow to the periphery and gastrointestinal system, and this shunts blood to more oxygen-sensitive tissues (Elsner & Gooden, 1983). It occurs in both non-human mammals and in humans, although humans have a reduced magnitude of the DR. It is produced with immersion of just the face into cold water (Kjeld et al., 2009), as opposed to submerging the entire body in cold water which is known as the 'gasp' response (Gooden et al., 1970). The DR appears due to the application of cold stimulus to the face, as opposed to water alone, since the DR can still be elicited with an ice bag positioned over the face (Brown et al., 2003).

The inputs that influence the DR include facial cold receptors, carotid chemoreceptors, atrial receptors, baroreceptors, pulmonary stretch receptors and carotid chemoreceptors (Foster & Sheel, 2005). These inputs all converge onto the nucleus tractus solitarius which in turn acts on different areas of the brainstem including the respiratory centre, cardioinhibitory and the vasomotor centres in the medulla oblongata. Stimulation of these centres results in apnoea, bradycardia, vasoconstriction and increased sympathetic outflow. Apnoea occurs due to stimulation of facial cold receptors whose afferent pathway is through the trigeminal nerve to nuclei in the pons (Elsner & Gooden, 1983). This results in an inhibition of the respiratory centre and therefore reduced efferent nerve impulses sent to the inspiratory muscles via the phrenic nerve (Gooden, 1994). The DR appears maximally when areas supplied by the ophthalmic branch of the trigeminal nerve (Figure 1.1) are in direct contact with cold water (Gooden et al., 1970),

when compared to cooling of the maxillary and mandibular branches (Parfrey & Sheehan, 1975).

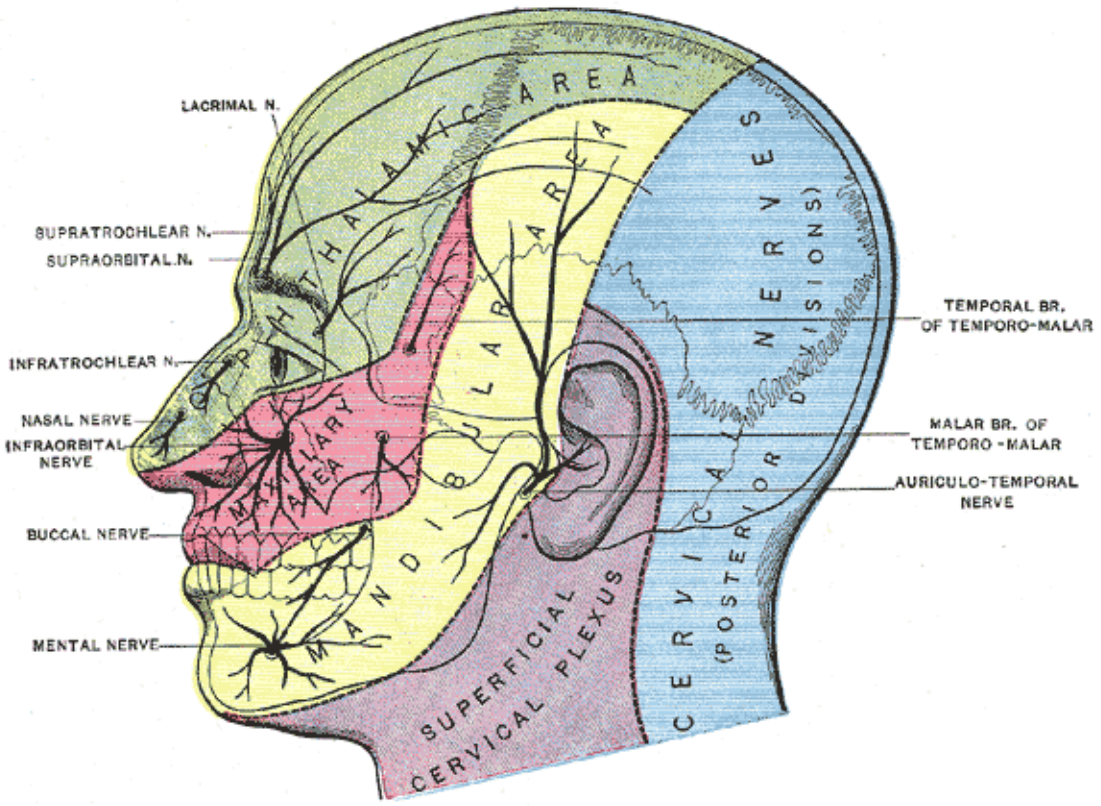

Figure 1.1. Distribution of facial areas supplied by branches of the trigeminal nerve, the fifth Cranial nerve, which include the ophthalmic, maxillary, and mandibular branches (Gray, 1918, Fig. 784).

With stimulation of facial cold receptors and inhibition of the respiratory centre there is also an activation of both the vasomotor centre and the cardioinhibitory centres in the medulla oblongata (Gooden, 1994; Mukhtar & Patrick, 1986). This results in inhibition of the cardiac pacemaker cells via the vagal innervation of the heart as part of an increase in parasympathetic activity (Finley, Bonet, & Waxman, 1979). This is accompanied by a peripheral vasoconstriction due to increased sympathetic output to the arterioles in the extremities. The increase in sympathetic output increases vascular tone which reduces vessel cross-sectional area and consequently increases resistance to flow (Gooden, 1994;

Speck & Bruce, 1978). Subsequently, blood pressure (BP) during the DR is increased (Speck & Bruce, 1978) due to the reduced vessel cross sectional area of blood vessels. Speck and Bruce (1978) found that MAP progressively increased from ~ 101 mm Hg in 35°C water to 116 mm Hg in 5°C water during face immersions with breath-holds.

Diving reflex bradycardia is influenced by P_aO_2. A greater P_aO_2 than normal attenuates the bradycardia, whereas a P_aO_2 lesser than normal accentuates it (Gooden, 1994). This suggests the influence of PaO_2 on the heart rate response during face immersion in cold water is due stimulation of the peripheral chemoreceptors (Gooden, 1994), found in the carotid and aortic bodies.

The bradycardia experienced with face immersion in water is primarily due to breath holding, but is marginally enhanced by face immersion (Brick, 1966; Gooden et al., 1970; Lindholm & Lundgren, 2009). Face immersion in 33°C water with an apnoea elicited a reduction in HR of 8.9% from resting values which was similar to the 11% reduction seen when the breath hold took place in room air (Paulev et al., 1990). Stroke volume (SV), however, increased by 3% of resting values during a breath hold which was similar to the 1% increase when the breath hold occurred in 33°C water. There was a difference in SV when the face was immersed in 10°C with a breath hold where it increased to 24% greater than the resting value (Paulev et al., 1990). The cold-induced decrease in cardiac output (Q) could be beneficial for conserving energy for the heart and brain (Stewart et al., 1998).

Apnoea time is reduced in face immersion in cold water, ≤10°C, when compared to facial immersion in 33°C water or breath-holds without immersion (Hayward et al., 1984; Jay et al., 2007; Jay & White, 2006). This indicates that a lower water temperature may not be advantageous for increasing apnoea time even if it is advantageous for stimulating bradycardia. These apnoea responses occurred to the same degree in males and females matched for pulmonary capacities (Jay & White, 2006). One potential mechanism limiting apnoea time could be attributed to respiratory drive from cold temperature-sensitive neurons. This is since after a series of breath holds with face immersion in differing water temperatures neither $P_{ET}O_2$ nor $P_{ET}CO_2$ were above values seen to break a breath-hold (Jay & White, 2006).

Overall, the DR is due a face immersion with apnoea and it results in bradycardia, increased peripheral vasoconstriction and increased MAP. It appears to be an evolutionary response in order to conserve energy as well as oxygen for the heart and brain. The DR is due to stimulation of facial cold receptors, which synapse with the trigeminal nerve to inhibit the respiratory centre in the medulla oblongata, helping to prolong apnoea. Inhibition of the respiratory centre also increases parasympathetic nerve impulses that travel through the vagus nerve to inhibit cardiac pacemaker cells. Concurrently, the respiratory center activates the adjacent vasomotor center that increases sympathetic nerve impulses sent to vascular smooth muscle in the peripheral arterioles. Collectively these responses result in bradycardia, hypertension, peripheral vasoconstriction and changes to the drive to breathe that are dependent on water temperature.

1.1.3. Cardiorespiratory Responses during Non-Apnoeic Cold Face Immersion

The DR is evident with apnoea and face immersion in cold water, but it can also be elicited, albeit to a lesser degree, with just cold face immersion. Jay et al. (2007) found that during a 0°C face immersion without apnoea, HR exhibited a two-phase response. The first phase is a tachycardia that occurs during the first 10 s of face immersion and the second phase was a bradycardia (Jay et al., 2007). Gagnon et al. (2013) also found that HR exhibited a two-phase response when the face or whole head was immersed in 17°C water. When the entire head was immersed, HR increased ~15% above resting values in the first 30 s and then dropped to ~82% of resting values 90 s into the head immersion (Gagnon et al., 2013). During face immersion alone, however, there was no initial increase in HR seen, but after 90 s of immersion decreased to ~85% of resting values (Gagnon et al., 2013). The lack of tachycardia seen in the 17°C face immersion (Gagnon et al., 2013) is likely due to the decreased cold stimulus when compared to other cold face immersion studies that employed lower water temperatures (Andersson et al., 2000; Jay et al., 2007) where lower water temperatures were used (Gagnon et al., 2013).

Jay et al. (2007) found during 60 s face immersions that V_E increased by ~162 % in the 0°C face immersion when compared to a 33°C water face immersion. Face

immersion in 0°C water stimulated an increase in peak V_E along with a significant increase in $P_{ET}O_2$ and decrease in $P_{ET}CO_2$ when compared to 33°C face immersion (Jay et al., 2007). Stewart et al. (1998) have also found that face immersion without apnoea in cold water of temperatures as high as 17°C can elicit an increase in ventilation which is primarily due to increases in tidal volume. In one volunteer's peak V_E increased by 1147 % of control values, and the average V_E increased for the 6 volunteers in the study was by 457% with face immersion in 17°C water (Stewart et al., 1998). They also found that non-apnoeic face immersion in 35 - 37°C water did not have much effect on ventilation.

Shattock and Tipton (2012) hypothesised that 'autonomic conflict' may occur during simultaneous DR and cold shock response due to activation of both sympathetic and parasympathetic nervous systems, and may result in arrhythmias. This proposed autonomic conflict may therefore occur during the crossover point for two-phase response that is seen in both V_E and HR during non-apnoeic, cold face immersion.

Gagnon et al. (2013) assessed the DR when the entire head, the head dorsum or just the face was submerged in 17°C water. They found that face immersion without apnoea delays the onset of the decrease in ventilatory drive when compared to face immersion with apnoea due to the cold shock response, which results in an increase in V_E during the first 20 s of immersion. They also found that whole head immersion gave a tendency for a larger increase in the initial V_E when compared to immersing the face or back of head. When the face was immersed, V_E increased ~ 90% from non-immersed rest values, and when the entire head was immersed there were similar response for V_E in the first 30 seconds of immersion. Like Stewart et al. (1998), they also found that with the face immersed, the increased seen in V_E was primarily due to increased V_T that was ~ 85% above resting values while F_B decreased by ~ 20% (Gagnon et al., 2013). After two minutes of immersion, V_T lowered to ~ 5% above rest values whereas F_B decreased to ~ 80% of rest values. In contrast, another study reported that face immersion without apnoea provides an insignificant reduction in ventilatory drive indicated by elevated alveolar PCO_2 due to breath-holding that is not reduced by face immersion (Mukhtar & Patrick, 1986). Mukhtar and Patrick (1986) also found that breathing room air when compared to breathing 5% CO_2 during face immersion in 13°C water resulted in a significant reduction ($p < 0.01$) in post-immersion V_E. Since Mukhtar and Patrick's (1986) measurements were

only taken at 1 min intervals it is probable that they missed the initial hyperventilation reported by Gagnon et al. (2013) and Jay et al. (2007).

Face immersion without apnoea elicits a weaker peripheral vasoconstriction when compared to face immersion with apnoea, and colder face immersion temperatures elicit a stronger peripheral vasoconstriction (Fagius & Sundlöf, 1986). Gagnon and colleagues (2013) found that 30 s into a face immersion without apnoea in 17°C water, skin blood velocity dropped to 70% of non-immersed rest values and after 90 s the skin blood velocity in the finger returned to baseline values. Since peripheral vasoconstriction during the DR is due to increased sympathetic output to the arterioles in the extremities (Gooden, 1994; Speck & Bruce, 1978), then this lessened effect without apnoea suggests there is less sympathetic outflow. Paulev et al. (1990) have found that face immersion without breath-holding in 33°C water elicits SV to increase in values 7% above the resting value, whereas 10°C water elicits a decrease in SV by 3% from resting values. They also found that MAP increased by 1% during face immersion without breath-holding in 33°C water and by 7% during face immersion without breath-holding in 10°C water when compared to resting values. Brown et al. (2003) found there was no change in Q when a 0°C bag was placed over the face.

In summary, ventilation and HR in response to face immersion without apnoea have a two-phase response. There is an initial increase in both V_E and HR and then a subsequent drop in both variables. It is thought the initial increase is due to the cold shock-like response, whereas the subsequent ventilatory and cardiovascular responses are accounted for by the dive response. It also appears the magnitude of these responses increases proportionally to decreases in water temperature.

1.1.4. Cardiorespiratory Responses during Non-Apnoeic, Head Out, Cold, Whole Body Immersion

The gasp response, also known as the cold shock response, differs from the DR since it originates from head-out cold water whole body immersion (Tipton, 1989). It results in an initial gasp, and a hyperventilatory response with about a 450% increase in ventilation during the first minute of immersion (Tipton, 1989). The volume of the initial gasp after whole body immersion in cold water is generally around 2-3 L (Tipton et al.,

1991). The gasp response is elicited through a large increase in afferent input from the peripheral cold receptors (Keatinge & Nadel, 1965; Mekjavić et al., 1987). The pathway of the initial gasp is not entirely known, but appears to be due to stimulation of the peripheral cold receptors that stimulate the respiratory control centre (Duffin et al., 1975).

For the gasp response there is a positive correlation between the rate of cooling of the skin temperature and the inspiratory drive in the first 100 milliseconds ($P_{0.1}$) (Burke & Mekjavic, 1991). Stated otherwise, the quicker the skin temperature falls the greater will be the initial gasp. It appears the thorax is more sensitive to surface cooling than the limbs with respect to the magnitude of the gasp response (Burke & Mekjavic, 1991; Keatinge & Nadel, 1965). Important variables to assess the initial gasp are mean inspiratory flow (V_T/T_I) that gives a measure of inspiratory drive without the effect of timing, and inspiratory duty cycle (T_I/T_{TOT}) that indicates the portion of the breath cycle when the inspiratory muscles are active (Milic-Emili & Grunstein, 1976).

Tipton et al. (1991) found that frequency of breathing (F_B) in 5, 10 and 15°C water initially increased after the first 10 s of head-out whole body immersion and then decreased after ~ 20 s of immersion. In the 5°C water immersion, F_B increased the most by ~8 breaths/min, whereas this response was dampened in both the 10° and 15°C immersions with an increase of only ~5 breaths/min. Tipton et al. (1991) showed increased in tidal volume occurs during the first minute during a whole body immersion, but during the first 20 s ventilation is mainly increased via frequency of breathing.

The gasp response also results in a tachycardia and hypertension (Keatinge & Evans, 1961; Tipton, 1989). Tipton et al. (1991) assessed how physiological responses differ in head-out sudden whole body immersions in three water temperatures 5, 10, and 15°C. The HR response at the 50 s mark in the immersion only differed in the 15°C condition, where it was lower by ~20 beats/min than the response in 5 or 10°C conditions.

The gasp response is aptly named since it results in an initial gasp, a large increase in V_E and also a tachycardia. This response is due to head-out whole body immersion as opposed to face immersion. The afferent pathway responsible for the gasp response appears to be from non-cranial skin surface cold receptors, whereas the DR results from skin surface cold receptors on the face.

1.1.5. Cerebral Artery Anatomy and Regulation of Cerebral Blood Flow

Cerebral Artery Anatomy

Cerebrovascular-CO_2 reactivity is a homeostatic mechanism that helps to maintain cerebral pH. Thus regulation of cerebral blood flow (CBF) has an important role in the stabilization of breathing which occurs due to perturbations in PCO_2 (Ainslie & Duffin, 2009). This occurs because cerebral blood pH and CO_2 influence pulmonary ventilation. Maintenance of CBF is also important to maintain an O_2 supply that will match the metabolic demand put forth by the brain. The brain has an O_2 consumption of 49 mL O_2/min but represents only 2% of total body weight, whereas the average metabolic needs for the whole body are approximately 250 mL O_2/min (Ainslie & Duffin, 2009). Since, the metabolic demand per gram of brain tissue is quite high, conservation of cerebral blood flow is imperative.

Main cerebral arteries include the middle cerebral artery (MCA) and the anterior cerebral artery (ACA). These are both terminal branches from the internal carotid arteries (Edvinsson et al., 1993). The cranial sections of the vertebral arteries unite at the caudal border of the pons to form the basilar artery (Edvinsson et al., 1993). The anterior and posterior cerebral arteries, anterior and posterior communicating arteries, and internal carotid artery combine to form the circle of Willis (Windle, 1888). The circle of Willis has various communicating branches to reduce the chance of an area of the brain losing blood supply, and it is found near the hypothalamus (Kramer, 1912). The anterior and middle cerebral arteries supply the forebrain, including structures such as the basal ganglia and thalamus. The blood from the basilar and vertebral arteries supplies the posterior cortex, midbrain and brainstem, with the pattern of blood supply occurring with midline vessels supplying medial structures, whereas lateral blood vessels supply lateral structures (Kramer, 1912). The vertebral artery supplies the caudal and upper medulla, which includes nucleus of the tractus solitarius that is responsible for inspiration (Willie et al., 2012). The MCA has a diameter of ~2.5 mm (Serrador et al., 2000) while the VA has a diameter of ~3.6 mm (Seidel et al., 1999).

Regulation of Cerebral Blood Flow

Cerebral blood flow (CBF) is a product of cerebral perfusion pressure and cerebrovascular conductance where cerebral perfusion pressure can be estimated from the difference between mean arterial pressure (MAP) and intracranial pressure. Intracranial pressure is equal to central venous pressure and the pressure in the cerebrospinal fluid (Ainslie & Duffin, 2009). In experiment circumstances, the index of cerebrovascular conductance can be used since intracranial pressure may be unobtainable and because it better reflects the importance of the response in pressure regulation than cerebrovascular resistance. The index of cerebrovascular conductance can be calculated by dividing CBV by MAP, which assumes that MAP is comparable to cerebral perfusion pressure (O'Leary, 1991). Maintenance of intracranial volume is important for the control of cerebral perfusion. In response to pH changes, small cerebral arterioles alter cerebral resistance relatively quickly to allow for changes in artery diameter in approximately 10 seconds (Edvinsson et al., 1993). Thus the change in resistance in the brain is mainly due to changes in flow controlled by varying the caliber of blood vessels in the cerebral arteriolar beds. Large cerebral arteries mainly act as conduits for blood as opposed to controlling resistance (Ainslie & Duffin, 2009), but have been recently found to modulate their diameters with larger changes in $P_{ET}CO_2$ (Coverdale et al., 2014; Willie et al., 2012). Coverdale et al. (2014) measured CBV in the MCA at baseline, hypercapnia, defined as a $P_{ET}CO_2$ increased by 10 mm Hg, and at hypocapnia, defined as a $P_{ET}CO_2$ decreased by 15 mm Hg. They also imaged the MCA using magnetic resonance imaging (MRI) to determine the cross-sectional area and cerebral blood flow velocity. They found that MCA cross-sectional area increased by a mean (SD) of 16 (7)% during hypercapnia and decreased by 8 (6)% during hypocapnia, which is equal to a MCA diameter change of 0.4%/mm Hg (Coverdale et al., 2014). In a similar study, Verbree et al. (2014) imaged the MCA using a high resolution 7 Telsa MRI system while the participants were under hypocapnia, defined as a decrease in $P_{ET}CO_2$ of 7.5 mm Hg, and during two levels of hypercapnia, defined as an increase in $P_{ET}CO_2$ of 7.5 and 15 mm Hg, respectively. They found that at when $P_{ET}CO_2$ was increased or decreased by 7.5 mm Hg there was no change in diameter witnessed, whereas the 15 mm Hg increase in $P_{ET}CO_2$ elicited a 6.8(2.9)% increase MCA diameter.

Cerebrovascular resistance is generally believed to be adjusted through cerebral autoregulation to match metabolic needs, and there are many factors that alter CBF including changes in MAP, cardiac output, metabolism, and autonomic nerve activity, and arterial PCO_2 (Ainslie & Duffin, 2009). These influences are described in the following sections.

Maintenance of CBF in response to variations in MAP is mediated by static and dynamic autoregulation responses. Static cerebral autoregulation is beneficial for gradual changes in cerebral perfusion to keep CBF constant, whereas dynamic cerebral autoregulation occurs when there are rapid changes in MAP (Paulson, Strandgaard, & Edvinsson, 1990). Cerebral blood flow is maintained over a range of MAP between 60 to 150 mm Hg (Paulson et al., 1990) but this range seems to be variable between subjects (Lassen, 1959). CBF is also influenced by Q. Ogoh and colleagues (2005) measured MCA_V during both increases and decreases Q. They were able to increase Q by infusing the subject with two doses of human albumin, and decrease Q through lower body negative pressure (LBNP) at 8 and 16 mm Hg. As Q increased from 6.5 to 8.5 L/min, MCA_V also increased from ~ 66 to 73 cm/s, or as Q decreased from 6.5 to 5.3 L/min, MCA_V also decreased from ~66 to 62 cm/s. The relationship was linear between Q and MCA_V and occurred over a relatively constant MAP and $PaCO_2$.

Metabolism also modifies CBF since the brain is intolerant to ischemia. Changes in CBF due to differing metabolites have been imaged via PET scans (Villringer & Dirnagl, 1995). Several molecules are thought to be mediating the link between metabolism and CBF including potassium and hydrogen ions, and other metabolites, as follows. Production of potassium and hydrogen ions through synaptic transmission have been shown to increase vasodilation in cerebral blood vessels (Paulson & Newman, 1987). Metabolites, particularly adenosine, which are by-products of metabolism demonstrate a sharp rise in their concentration with neuronal activity, and applying adenosine to small cerebral vessels causes vasodilation (Pelligrino et al.,1996).

CBF can also be maintained through autonomic nerve activity. Extracerebral blood vessels, those found outside the CNS, have sympathetic inputs from superior cervical, trigeminal, otic and sphenopalatine ganglion (Hamel, 2006). Vessels within the CNS,

however, are supplied with neural input from neurons and interneurons within the brain. Depending on the vasoactive mediator that is released, the cerebral vessels dilate or constrict (Hamel, 2006). The involvement of autonomic nerve activity on cerebral blood vessels appears to act when there are large increases in MAP in order to maintain cerebral blood flow (Ainslie & Brassard, 2014; ter Laan et al., 2013). Kimmerly et al. (2003) have shown that sympathetic nerve activity block, using phentolamine, results in an increase in CBV four-times greater than without when the same increase in MAP occurs. It has also been found, albeit in dogs, that elimination of the baroreceptor and chemoreceptor responses to large changes in MAP result in no autoregulation of CBF (Sagawa & Guyton, 1961).

To review, maintenance of CBF is important to keep a constant supply of oxygen and nutrients as well as removal of CO_2 and waste products from brain tissues. CBF is sensitive to changes in MAP, Q, autonomic nerve activity, and metabolism. CBF is able to be maintained over MAP ranging 60 – 150 mm Hg although increases in Q cause linear increases in MCA_V. Metabolism increases CBF (Villringer & Dirnagl, 1995) likely due to potassium and hydrogen ions (Paulson & Newman, 1987), and adenosine (Pelligrino et al.,1996). Small cerebral blood vessels respond to autonomic nerve activity and will dilate or constrict dependant on which vasoactive mediator is released (Hamel, 2006). This autonomic response generally occurs due to large changes in MAP in order to maintain CBF (Ainslie & Brassard, 2014; ter Laan et al., 2013).

1.1.6. Changes in Cerebral Blood Velocity due to Variations in Blood Gas Tensions

Cerebral blood flow acts to minimize changes in local cerebral pH when modulated by many factors including changes in $PaCO_2$. Maintenance of pH in turn acts to stabilize ventilatory stimulus at the central chemoreceptors (Xie et al., 2006). CBF is highly sensitive to changes in local PCO_2 (Chesler, 2003). Increases in PCO_2 increases CBF as a method to 'wash out' CO_2 and decrease the central chemoreceptor stimulus to breathing (Ainslie & Duffin, 2009). This high level of sensitivity allows for tight control of cerebral pH in order to maintain steady pulmonary ventilation. As such, hypercapnia can lead to vasodilation of cerebral arterioles and a subsequent increase in CBF, whereas hypocapnia

can lead to vasoconstriction and a decrease in CBF (Kety & Schmidt, 1948). The Henderson-Hasselbalch equilibrium underlies control of breathing and CBF. Increases in H^+ in CSF promotes the formation of CO_2 (Equation 1) (Joseph, 1958). Dependent on whether one hyperventilates or hypoventilates, there will be subsequent decreases or increases in cerebral $PaCO_2$, respectively. This effect also occurs with incremental steps in hypocapnia or hypercapnia, produced by changing inspired PCO_2 (Ainslie et al., 2008).

There are two potential modes of action for CO_2 to modulate CBF, which are either through myogenic and metabolic actions, although the exact mechanism is not fully understood. The myogenic mode of action starts with a drop in pH due to an increase in CO_2 that activates potassium (K^+) channels, ATP-sensitive K^+ and voltage-gated K^+ channels, in smooth muscle surrounding cerebral arterioles (Xu et al., 2001). Activation of these channels increases their opening probability of the channels and allows for hyperpolarization and eventually vasodilation of the smooth muscle surrounding the cerebral arterioles (Faraci & Sobey, 1996). For the metabolic mechanism of the control of CBF, CO_2 may cause the release of nitric oxide and prostaglandins that cause vasodilation in the cerebral arterioles (Leffler et al., 1994).

A mild hypercapnia (with an increase in PCO_2 of ~5 mmHg above resting values) impairs cerebral autoregulation of CBF (Zhang et al., 1998). Hypercapnia produced by breathing 5 – 7% CO_2, therefore, causes an increase in CBF ~75% above resting values, with a reduction in cerebrovascular resistance (Kety & Schmidt, 1948).

Sato et al. (2012) have investigated cerebral CO_2 reactivity through differences in CBF and CBV during exposure to CO_2. They tested two levels of hypercapnia, one at 3% CO_2, defined as ‘mild’, and the other at 6% CO_2, defined as ‘severe’, both with 21% O_2, balance N_2. They found that ‘severe’ hypercapnia elicited an increase in internal carotid artery blood flow of ~ 152 mL/min from a normocapnic value of 235 mL/min, and increased in vertebral artery blood flow of 41 mL/min above a flow of 112 mL/min obtained during normocapnia. They also found that the ICA and VA vessel diameters did not change during these trials. MCA_V and BA_V increased by 28 cm/s and 14 cm/s during ‘severe’ hypercapnia, when compared to normocapnic velocities. Sato et al. (2012) also found that cerebral CO_2 reactivity in the BA and VA was significantly lower than in the ICA and MCA.

This suggests that vertebro-basilar circulation responded to a lesser extent to CO_2-mediated changes that to anatomical regions supplied by the ICA and MCA. Cerebral vessel diameter, however, was unable to be measured in the MCA and BA which might have influenced their conclusions with respect to CO_2 reactivity.

Alternatively, Willie et al. (2012) have found that cerebrovascular reactivity to $PaCO_2$ is greater in ICA and VA when compared to MCA and PCA. ICA diameter was seen to change with variations in $PaCO_2$ across a range of 15 to 65 mm Hg, whereas there were no changes in VA diameter over the same $PaCO_2$ range. Vertebral artery diameter, however, was seen to change during extreme hypoxemia where SaO_2 was at 70% of resting values.

With a hypocapnia of 30 mmHg, Mardimae et al. (2012) found MCA_V decreased by ~25% of normocapnic values, and during hypercapnia of 50 mmHg, MCA_V increased by ~29%. In another study conducted by Serrador et al. (2000), MCA diameter and velocity was assessed during normocapnia, hypocapnia; their volunteers hyperventilated until $P_{ET}CO_2$ reached 25 mm Hg and hypercapnia was induced by inspired gas with 6% CO_2. They found despite changes in MCA_V there was no change in MCA diameter in hypocapnia or hypercapnia when compared to normocapnia. Because the diameter did not change, the measured CBV and calculated CBF had a high correlation ($r = 0.94$, $p < 0.001$), which supported that transcranial Doppler measures of CBV approximates CBF. More recently, Coverdale et al. (2014) have found that MCA cross-sectional area increases by 0.4% with every 1 mm Hg increase in $P_{ET}CO_2$. Verbree et al. (2014), however, propose that the relationship between CO_2 and MCA cross-sectional area is not linear and that small changes in CO_2 does not affect vessel caliber.

In animal studies (Chapman et al., 1979), where CBF was reduced by 30%, ventilatory responsiveness to CO_2 increased, which suggests that CBF affects ventilation through perfusion of the medullary chemosensitive area (Ainslie & Duffin, 2009). This implies that a decrease in CBF allowed for an accumulation of CO_2 that stimulated the chemosensitive area to increase ventilation. When CBF was reduced by 50%,however, the ventilatory response to CO_2 was attenuated, which might suggest that severe cerebral ischemia limits the sensitivity of the ventilatory chemoreflex (Ainslie & Duffin, 2009).

As stated earlier, O_2 does not stimulate the central chemoreceptors and so any effect it has on CBF will be exerted through the peripheral chemoreceptors when altering breathing (Haldane & Priestley, 1905). Arterial PO_2, however, does not have as crucial a role on the maintenance of CBF, unless PO_2 drops below 50 mmHg or acts locally (Gupta et al., 1997) . With PO_2 below 40 mmHg, it acts as a vasodilator in the cerebral vessels (Gupta et al., 1997). Changes in $PaCO_2$ at the level of the peripheral chemoreceptors on CBF is dependent on PaO_2, since peripheral chemoreceptors have an increased sensitivity to CO_2 at the during hypoxia (Nielsen & Smith, 1952). Mardimae and colleagues (2012) assessed the differences that occurred in MCA_V during normocapnic conditions, but with varying inspired partial pressures of O_2. They found that there was no significant change in MCA_V when PO_2 was as low as 45 mmHg, and only experienced an 11% change from normoxic values when PO_2 was 40 mmHg. The sensitivity of the response to hypoxia, however, increased when PCO_2 was increased to 50 mmHg (Mardimae et al., 2012).

Similarly, with hyperoxic and hypoxic rebreathing $P_{ET}CO_2$ was first decreased by ~21 mmHg through hyperventilation, which decreased MCA_V to 53% of resting values (Ainslie & Burgess, 2008). During hyperoxic rebreathing, $P_{ET}CO_2$ increased to ~19 mmHg above resting normocapnic values, which caused an increase in MCA_V by ~98 % above baseline values (Ainslie & Burgess, 2008). Similar results, were seen with hypoxic rebreathing, which would suggest that the peripheral chemoreceptors have no influence on the CBF lowering effect of decreased $P_{ET}CO_2$. Furthermore, the slope of the MCA_V – CO_2 relationship did not differ between hypoxic and hyperoxic rebreathing (Ainslie & Burgess, 2008).

In summary, CBF is modulated by both variations in cerebral PCO_2, where hypercapnia induces cerebral vasodilation and increases CBF whereas hypocapnia induces cerebral vasoconstriction and decreases CBF. There is evidence to suggest that CBF is primarily modulated by changes in CO_2 and CBF regulation take place at the level of cerebral arterioles. More recent evidence suggests the influence of CO_2 on cerebral vessels also extends to the larger vessels including the MCA. The pH drop activates potassium (K^+) channels, ATP-sensitive K^+ and voltage-gated K^+ channel, in smooth muscle surrounding cerebral arterioles which causes vasodilation (Xu et al., 2001).

1.1.7. Changes in Cerebral Artery Blood Velocity with Face Immersion and Apnoea

Cerebral artery blood velocity may be altered both via apnoea (Jiang et al., 1994), face immersion (Kjeld et al., 2009) or cold stimulus on the face (Brown et al., 2003). Maximal breath-holding at rest can elicit increases in $PaCO_2$ which results in an increase in mean MCA_V of ~178%, a decrease in blood pH (Kjeld et al., 2009), and increase end diastolic blood flow velocity in the common carotid artery by ~15% (Jiang et al., 1994). The max MCA_V was reached at the end of the breath hold at peak $PaCO_2$ (Kjeld et al., 2009). As stated earlier, an increased mean MCA_V was due to K^+ channel opening probability or increased release of vasodilatory factors that promote vasodilation.

Breath hold divers appear to have an enhanced response to breath-holding since they have a greater increase in MCA_V when compared to non-divers following apnoea. Trained divers had an increase of MCA_V of 106% when compared to their resting values (Palada et al., 2007). Non-divers had a more modest response to apnoea with an increase in MCA_V of 53% above their rest values. This study did not control for breath hold duration. Breath-hold divers were able to have a breath hold of 244 ± 50 s whereas non-divers had 109 ± 31 s (Palada et al., 2007). Another study by Valic et al (2006) found when breath-hold divers completed an apnoea that was the same length as non-divers, the divers experienced a lessened dive response. This response suggests that through training, the dive response onset becomes more gradual but ends up being a stronger response at the end of breath-hold. This is likely due to the fact that divers tend to have a larger forced vital lung capacity (Adir et al., 2005) and vital capacity (Schagatay et al., 2012). The rise in $PaCO_2$ occurs more slowly in those with larger lung volumes (Lin, 1982).

Jiang et al. (1994) found that breath-hold face immersions in either cold (20°C) or warm (35°C) water increased the maximum blood flow velocity in the left common carotid artery during systole by ~24% and ~30%, respectively when compared to rest values. Similarly, face immersion in 10°C water during 100 W cycling exercise can further the elevation in mean MCA_V caused by maximum apnoeas, where MCA_V increases from ~55 to 65 cm/s due to apnoea and this increases further to ~ 76 cm/s during cold face immersion (Kjeld et al., 2009). Brown and et al. (2003) simulated face immersion and the DR using a bag filled with ice and water, measuring 0°C in temperature, to assess the

effect of facial cooling on cerebral artery velocity without the concurrent effect of apnoea. Cold stimulus on the face caused a slight but significant ($p < 0.01$) increase in mean MCA_V relative to non-immersed values whereas a marked decrease in blood flow in the finger and increase in MAP occurred during the cold stimulus (Brown et al., 2003). These changes occurred with a ~14% increase in the index of cerebrovascular resistance, estimated by dividing MAP by MCA_V, and an increase of ~24% of total peripheral resistance when compared to rest values (Brown et al., 2003). There were also no changes in $P_{ET}CO_2$, which suggests that the increase in MCA_V was not due to CO_2 stimulation. The exact mechanism underlying the CBF response to facial cooling is not entirely resolved. Miyazawa et al. (2012) conducted an experiment where they cooled the face using 4°C water and a fan (Table 1.1). During rest as well as during exercise they asked if the increase in MCAv with face cooling could be explained by reductions in skin blood flow on the face. They found that face skin blood flow decreased during facial cooling to a greater extent during exercise compared to rest. MCA_V, however, did not increase in proportion to the decreases in skin blood flow which suggests that there are other mechanisms that alter CBF other than a diversion of blood flow from the periphery to the brain (Miyazawa et al., 2012).

Therefore, both breath-holds and face immersion in water temperatures from 0 - 10°C can give small increases of MCA_V (Table 1.1). Breath-holds appear to increase MCA_V via build-up of CO_2. Face cooling without apnoea also results in increases in MCA_V, but is not due to CO_2 stimulation. Face immersion during exercise with apnoea results in a larger increase in MCA_V when compared to apnoea alone.

1.2. Rationale

The dive response occurs during apnoeic face immersion in cold water and results in a bradycardia (Asmussen & Kristiansson, 1968), an increased peripheral vasoconstriction (Elsner et al., 1971), and an increased MAP (Bruce & Speck, 1979). Diving response with apnoea occurs when the face is immersed in cold water or just cooling of the face (Brown et al., 2003). The gasp response, on the other hand, results from whole body immersion and is evidenced by an initial gasp, a large increase in V_E and a tachycardia (Tipton, 1989). For the gasp response, there is a positive correlation

between the rate of cooling of the skin temperature and the inspiratory pressure in the first 100 milliseconds ($P_{0.1}$) (Mekjavić et al., 1987). Face immersion without apnoea appears to have a two-phase response for HR and V_E where the first phase is similar to the gasp response and the second phase is similar to the dive response (Gagnon et al., 2013; Jay et al., 2007).

The increases seen in V_E are primarily due to an increase in V_T during non-apnoeic face immersion (Stewart et al., 1998), whereas during whole body, head-out immersions, the increases in V_E are due to increases in F_B (Tipton et al., 1991). Colder water temperatures increase both the dive and gasp responses, with exception of the HR response in the gasp response where the lowest HR was seen with 15°C water (Tipton et al., 1991).

The DR pathway is due to stimulation of facial cold receptors whose afferent pathway is through the trigeminal nerve, particularly the ophthalmic branch, to nuclei in the pons (Elsner & Gooden, 1983) which results in an inhibition of the respiratory centre (Gooden, 1994). Inhibition of the respiratory centre in the medulla oblongata also activates both the vasomotor centre and the cardioinhibitory centre (Gooden, 1994; Mukhtar & Patrick, 1986). This results in inhibition of the cardiac pacemaker cells via the vagal innervation of the heart as a result of increased parasympathetic activity (Finley et al., 1979). In addition, there is an increased sympathetic output to the peripheral blood vessels during the DR and this gives a vasoconstriction in the arterioles in the extremities. Similarly, the gasp response is elicited through a large increase in afferent drive from the peripheral cold receptors (Keatinge & Nadel, 1965; Mekjavić et al., 1987). The pathway of the initial gasp is not entirely known, but appears to be due to stimulation of the non-facial peripheral cold receptors that stimulate the respiratory control centre (Duffin et al., 1975).

Since there are differing responses between the gasp and dive response, and face immersion without apnoea has a two-phase response, there may be some sort of autonomic conflict between the simultaneous activation of both the sympathetic and parasympathetic nervous systems from the various inputs to the respiratory and cardiovascular control systems during face immersion without apnoea (Shattock & Tipton, 2012).

The control of breathing operates on a negative chemoreflex arc where an increase in $PaCO_2$ will increase $[H^+]$ and stimulate chemosensitive areas of the medulla and peripheral chemosensitive tissues (Braman, 1995). $PaCO_2$ therefore will promote an increase in pulmonary ventilation as a method to eliminate CO_2. These tissues that influence breathing and are highly sensitive to changes in CO_2 and H^+, in order to regulate cerebral pH. Therefore hypercapnia can lead to vasodilation while hypocapnia can lead to vasoconstriction of cerebral arterioles**.** Vasoconstriction of downstream arterioles results in a lowered MCA_V while vasodilation of downstream arterioles results in an elevated MCA_V (Sato et al., 2012). More recent research has indicated that MCA also vasoconstricts and dilates in response to alterations in $P_{ET}CO_2$ at a ratio of 0.4%/mm Hg (Coverdale et al., 2014). Verbree et al. (2014), however, propose that the relationship between CO_2 and MCA cross-sectional area is not linear and that small changes in CO_2 does not affect vessel caliber. CBF is also maintained through autonomic nerve activity in response to large increases in MAP (Ainslie & Brassard, 2014; ter Laan et al., 2013). Thus, an increase in $P_{ET}CO_2$, which occurs during apnoea or through breathing hypercapnic mixtures CO_2 mixtures, stimulates both ventilation and mean MCA_V**.** Furthermore, MCA_V is increased from rest values during cold water face immersion without apnoea, and is even greater during apnoea. This suggests that there are mechanisms modulating cerebral blood vessel diameter other than CO_2 which may be through sympathetic innervation (Hamel, 2006) or due to metabolites (Villringer & Dirnagl, 1995). Also since CBF is able to be maintained over a MAP ranging 60 – 150 mm Hg (Paulson et al., 1990) then changes in CBF are most likely not due to changes that occur in MAP during the dive response.

This study aims to better understand the mechanisms behind the control of breathing in humans. This goal is to determine whether an external source of CO_2 will confer the same effects as apnoea during cold water face immersion on ventilation and its components as well as MCA_V. The three water temperatures tested in this study were chosen to represent a thermoneutral temperature as well as provide an indication if there was a graded response in outcome variables due to cold exposure. The thermoneutral zone is defined as “the range of ambient temperatures at which temperature regulation is achieved only by control of sensible heat loss, i.e., without regulatory changes in metabolic heat production or evaporative heat loss” (IUPS Thermal Comission, 2001). The water

temperature that allows participants to maintain the body temperatures within the thermoneutral zone is approximately between 33 – 35.5°C (Kingma, 2012). In the current study, the 33°C water face immersion will help to understand what responses are due to face immersion and not as result of the cold stimulus. The 5 and 15°C water face immersions will help to determine whether lower water temperatures elicit a greater response in outcome variables.

Vertebral artery velocity has yet to be recorded during cold water face immersion, and this would be beneficial to know since VA supplies the caudal and upper medulla, which includes nucleus of the tractus solitarius that is responsible for inspiration (Willie et al., 2012). This study also aims to help better understand the control of CBF during face immersion. It would be expected that a two-phase response for both V_E and HR will be seen since this study involves face immersion without apnoea, where the first phase is an increase in V_E and HR and the second phase is a decrease in V_E and HR. It is expected that the largest initial increase in V_E will be during 5°C due to the increased cold stimulus on the peripheral cold receptors. It is also expected that the initial increase in V_E will be due to increases in V_T and there will be a greater inspiratory drive (V_T/T_I) during 5°C due to the initial gasp when compared to 15 or 33°C face immersions. The effects of variations of inspired PCO_2 and face temperatures on respiratory timing, as assessed by the inspiratory duty cycle (T_I/T_{TOT}), remain to be determined for volunteers during face immersion. This ratio for resting volunteers gives an index of the relative times spent in the periods of active inspiration (T_I) and passive expiration (T_E). It would also be expected that MCA_V and VA_V will experience the greatest increase during hypercapnic 5°C face immersion when compared to 15 or 33°C face immersions.

Exposure to cold and CO_2 appears to have a positive multiplicative effect on V_E, where V_E is greater during cold CO_2 breathing than breathing warm CO_2 (Bullard & Crise, 1961). Similarly, facial cooling augmented the ventilatory and heart rate response to hypoxia, which mediated by the peripheral chemoreceptors (Argacha et al., 2008).

By extension, these results suggest that together with the independent effect of face cooling on ventilation (Christensen 2004; Jay et al 2007) that CO_2 and face cooling will positively interact in their influence on V_E and its components.

1.3. Hypotheses

Study 1: Effect of Mild Hypercapnia and Skin Temperature on Pulmonary Ventilation Responses during Face Immersion

It was hypothesized that the greatest inspiratory flow and inspiratory duty cycle will be seen during 5°C water face immersion when compared to immersion in both 15 and 33°C water. It was also reasoned that there will be an enhanced effect of cold water during face immersion and mild hypercapnia on ventilation. It was hypothesized that the greatest increases in ventilation will be in a hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

Study 2: Effect of Mild Hypercapnia and Skin Temperature on Cardiovascular Responses during Face Immersion

It was suggested that there will be an enhanced effect of cold water during face immersion with mild hypercapnia on systemic cardiovascular responses and cerebrovascular blood velocities as represented by cerebral blood velocities measured in the middle cerebral and vertebral arteries. It was hypothesized that the greatest increases in mean MCA_V and VA_V, will occur during the mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values. It was also hypothesized that the lowest skin blood velocity and greatest blood pressure, cerebrovascular conductance, and the greatest Q, SV and HR will occur during mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

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1.5. Tables

Breathing Condition	Cold Exposure Type	Water Temperature (°C)	Study	Time Exposed (s)	MCA_V (cm/s)		Difference from Baseline (%)
					Baseline	Exposed	
Breathing through snorkel	Bag of Ice	0	Brown et al., 2003	60	57 (6)	62 (8)	9
	Water Mist and Fan	4	Miyazawa et al., 2012	180	53 (3)	55 (3)	4
	Face Immersion	10	Kjeld et al., 2009	30	35 (8)	45 (10)	NS
Breath-hold	Face Immersion	10	Kjeld et al., 2009	30	35 (8)	43 (15)	NS

Table 1.1. Mean (SD) MCA_V responses with face temperatures between 0 and 10°C in 3 different studies.

Chapter 2.

Study 1 - Effect of Mild Hypercapnia and Skin Temperature on Pulmonary Ventilation Responses during Non-apnoeic Face Immersion in Cold Water

2.1. Introduction

The control of breathing operates on a negative reflex arc where an increase in $PaCO_2$ will increase $[H^+]$ and stimulate chemosensitive areas of the medulla oblongata and peripheral chemosensitive tissues (Braman, 1995). Elevations in $PaCO_2$ therefore will promote an increase in pulmonary ventilation as a method to eliminate CO_2.

Carbon dioxide augments the response of pulmonary ventilation (V_E) to ambient cold (Bullard & Crise, 1961). Similarly, Argacha et al. (2008) found that cooling the face with cold air until the face temperature decreased by 11°C augmented the ventilatory response to hypoxia. This response is mediated by the peripheral chemoreceptors and suggests that there is an interaction between ambient temperature and the ventilatory response to inspired gases.

Face immersion without apnoea appears to have a two-phase response V_E where the first phase is more similar to the whole body gasp response and the second phase is more similar to the dive response (Gagnon et al., 2013; Jay et al., 2007). For face only immersion, increases in V_E are primarily due to an increase in V_T during non-apnoeic face immersion (Stewart et al., 1998), and colder water temperatures increase both the dive and face only gasp responses (Jay et al., 2007).

The ratio between T_I/T_{TOT}, also known as the duty cycle for breathing, gives an index of the time spent in the period of active inspiration (T_I) whereas V_T/T_I gives an index of inspiratory effort. The whole body gasp response has a positive correlation between the rate of cooling of the skin temperature and the inspiratory drive in the first 100 milliseconds ($P_{0.1}$) (Mekjavić et al., 1987). Stated otherwise, the quicker the skin temperature falls, the greater the initial gasp will be (Burke & Mekjavic, 1991; Keatinge & Nadel, 1965).

It is unknown whether face cooling and mild hypercapnia will have the same enhanced effect on V_E as seen when subjects breathed cooled CO_2 by Bullard and Crise (1961). The effects of variations of inspired PCO_2 and face temperatures on respiratory timing, as assessed by the inspiratory duty cycle (T_I/T_{TOT}), and inspiratory effort as assessed by V_T/T_I remain to be determined for volunteers during for face immersion in these conditions. It was hypothesized that the greatest inspiratory flow and inspiratory duty cycle will be seen during 5°C water face immersion when compared to immersion in both 15 and 33°C water. It was also hypothesized that there will be enhanced effect of cold water touching the face and mild hypercapnia on ventilation. It was hypothesized that the greatest increases in ventilation will be in a mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

2.2. Methods

2.2.1. Participants

This study used nine healthy male participants (20 – 29 years of age) who were non-smokers, and without health complications. They had a mean (SD) height of 1.81 m (0.07), weight of 78 kg (6.1), and age of 26 years (3.3) (Table 2.1). The volunteers were instructed to abstain from caffeine and strenuous exercise for 24 hours prior to the testing period. Each volunteer was given a tour of the lab and had an orientation session prior to the experiment trial to explain potential risks and the study protocol. Next the volunteer had a 24-hour reflection period, after which he signed an informed consent, filled out a medical questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q). This study has been approved by the Simon Fraser University Office of Research Ethics.

A power calculation was used to determine the sample size required to have a power of 80% and significance of 0.05 and was based on inter-subject variability. The sample size varied depending on the variable. The number of volunteers required is a minimum of 8 in order to find a significant result, if it exists, in all variables of interest. It was determined that the variables most pertinent to this study are V_E, V_T and F_B.

Jay et al. (2007) measured peak V_E during non-apnoeic face immersion in 0 and 33°C water. They found a difference of 10 L/min with a common standard deviation of 6 L/min. With these parameters, 6 participants were needed.

Gagnon et al. (2013) measured V_T and F_B during non-apnoeic cold face immersion in water measuring 17°C compared to non-immersed values. The difference in V_T between these two conditions was 0.85 L with a common standard deviation of 0.09 L, which resulted in a sample size of 6. The difference in F_B between these two conditions was 18 breaths with a common standard deviation of 2.5 breaths, resulting in needing a sample size of one in order to have a power of 80%.

2.2.2. Instrumentation

Ventilatory/Metabolic Variables

For all parts of the study, the participant laid prone on a padded table. During the immersion trials, each participant breathed through a snorkel, with a volume of 210 mL, attached to a low resistance mouthpiece that connects to the two-way mass flow sensor (Sensormedics, Yorba Linda, CA, USA). The dead space of the snorkel gave a mild hypercapnia of 42 – 43 mm Hg (Table 2.2). The participant also wore a nose clip to ensure all exhaled gases were collected through the low resistance mouthpiece. During the rest period, the participant did not have their face immersed but still breathed through the mouthpiece and snorkel. During immersion trials, the participant immersed their face in a face-bath measuring 0.42 m x 0.39 m x 0.17 m filled with ~19.6 L of water. It had water flowing to and from the bath through a water chiller (model no. 1196, VWR International, Mississauga, ON, Canada) at a rate of 3 L/min.

A breath-by-breath metabolic cart (Vmax 229c, Sensormedics, Yorba Linda, CA, USA) was used to measure pulmonary ventilation (V_E, BTPS), tidal volume (V_T), breathing frequency (F_B), inspiratory time (T_I), expiratory time (T_E), total breath time (T_{TOT}), end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), and end-tidal partial pressure of oxygen ($P_{ET}O_2$). Inspiratory flow (V_T/T_I) and inspiratory duty cycle (T_I/T_{TOT}) were calculated after the data collection.

The calibration of gas analyzers in the metabolic cart was with room air and with compressed gas tanks with mixtures of 26% O_2, balance N_2; and 16% O_2, 4% CO_2, and balance N_2. The two-way mass flow sensor was calibrated with a 3 L syringe.

Body Temperature

Skin temperature was measured at six sites using 0.3 mm diameter T-type and Copper-constantan thermocouples (Omega Eng. Inc., Stanford, CT, USA). These sites included centre of the forehead (T_{FH}), right zygomatic arch (T_{CH}), right earlobe (T_E), the mid-point of the dorsal right forearm (T_{FA}), dorsal right hand (T_{HA}), and right lower back at the level of L4/L5 vertebrae (T_{LB}). The thermocouples were attached to the skin using hypoallergenic tape (Transpore, 3M, St Paul, MN, USA). These skin temperatures were represented as an un-weighted mean immersed skin temperature (Immersed T_{SK}) which includes T_{FH} and T_{CH}, as well as an un-weighted mean non-immersed skin temperature (Non-Immersed T_{SK}) which includes the remainder of the skin temperature sites. A water bath (VWR Int, Model 1196, West Chester, Penn, USA) monitored by a platinum thermometer (Fisher Scientific, Nepean, ON, Canada) was used to calibrate the Copper-constantan thermocouples. Previous studies measuring core temperature during face immersion found that it did not change significantly, therefore core temperature was not measured in this study (Mukhtar & Patrick, 1986).

Data Acquisition

Body temperature values, as well as other physiological outcome variables were sampled at 40 Hz using LabVIEW software (Ver. 7.1, National Instruments, Austin, TX, USA) and recorded on a breath-by-breath basis. Ventilatory measures were sampled and recorded with the VMAX229c metabolic cart (Sensormedics, Yorba Linda, CA, USA).

2.2.3. Protocol

There were three face immersions in water temperatures of 5, 15, and 33°C during mild hypercapnia (Fig. 2.1). The order of the experimental trial temperatures was single-blinded and randomized using a Latin Square design. Each participant was unaware which condition they were experiencing prior to each face immersion. Rest values for all variables were collected for 5 min before and 5 min during recovery after the face

immersion while the subject laid prone on a table without their face immersed. Each face immersion lasted five minutes and a time of 10 to 35 min between face immersions was to allow the face skin temperature return to pre-immersion values.

2.2.4. Statistical Analysis

The effect of Breathing Condition (Rest, 5, 15, 33°C) was examined with a 1-factor ANOVA using the software program SPSS (Version 19, Surrey, UK) across the first 45 breaths in each level of Breathing Condition. Two CO_2 conditions were tested with normocapnia during rest without face immersion and during mild hypercapnia with face immersion in the 3 different water temperatures. The hypercapnia during face immersions was with a $P_{ET}CO_2$ ~ 3 to 4 mm Hg above the non-immersed, resting normocapnic $P_{ET}CO_2$. In addition, to explore the transient responses immediately after face immersion, t-tests were employed to compare rest to breath numbers 1 through 5. The variables that were analyzed included V_E, V_T, F_B, $P_{ET}CO2$, Immersed, Non-Immersed and Hand T_{SK}, T_I, T_E, T_{TOT}, as well as T_I/T_{TOT} and V_T/T_I.

A two-tailed, paired Student's t-test was used to compare means if there were significant main effects evident from the ANOVA model. The level of significance was set at 0.05.

2.3. Results

The effect of Breathing Condition for V_E ($F = 4.11$, $p = 0.014$), and V_T ($F = 4.63$, $p = 0.008$) was significant but this main effect was not significant for F_B ($F = 1.70$, $p = 0.19$) (Fig. 2.2). The difference in V_E occurred between the rest period and the 33°C immersion ($p = 0.034$) where V_E decreased from a mean (SD) 13.1 (1.2) L/min during rest to 11.9 (1.2) L/min during the 33°C immersion (Table 2.2 & Fig. 2.3). The difference in V_T occurred between rest and the 5°C immersion ($p = 0.046$) where V_T increased from 0.70 (0.06) L during rest to 0.92 (0.29) L during the 5°C immersion (Table 2.2 & Fig. 2.3).

The effect of Breathing Condition was significant for $P_{ET}CO_2$ ($F = 4.12$, $p = 0.014$) (Fig. 2.4). The difference in $P_{ET}CO_2$ occurred between the rest condition and the 5°C

immersion ($p = 0.016$), the 15°C immersion ($p = 0.003$), and the 33°C immersion ($p < 0.001$). The $P_{ET}CO_2$ increased from 38.9 (1.8) mm Hg in rest, to 42.0 (2.9) mm Hg in the 5°C immersion, 42.1 (3.4) mm Hg in the 15°C immersion, and 43.4 (2.8) mm Hg in the 33°C immersion (Table 2.2). There was no difference between immersed conditions for $P_{ET}CO_2$ ($p = 0.59$).

The effect of Breathing Condition was significant for T_I ($F = 2.90$, $p = 0.046$) and not significant for T_E ($F = 2.04$, $p = 0.13$) showed a trend for a change for T_{TOT} ($F = 2.30$, $p = 0.096$) (Fig. 2.5). The difference in T_I occurred between the rest condition and the 33°C immersion ($p = 0.052$) where T_I increased from 1.4 (0.1) s during rest to 1.5 (0.2) s during the 33°C immersion. The difference in T_{TOT} occurred between rest and the 15°C immersion ($p = 0.057$) where T_{TOT} decreased from 3.4 (0.4) s at rest to 3.2 (0.4) s during the 15°C immersion (Table 2.2 & Fig. 2.6).

The effect of Breathing Condition was significant for T_I/T_{TOT} ($F = 4.21$, $p = 0.013$) and for V_T/T_I ($F = 2.88$, $p = 0.051$) (Fig. 2.7). The difference in T_I/T_{TOT} occurred between the rest and 15°C immersion ($p = 0.001$) where T_I/T_{TOT} increased from 41.6 (2.6) % during rest to 44.6 (2.1) % in the 15°C immersion (Table 2.3). The difference in V_T/T_I occurred between the rest period and the 33°C immersion ($p = 0.061$) where V_T/T_I decreased from 0.53 (0.031) L/s during rest to 0.49 (0.050) L/s during the 33°C immersion (Fig. 2.8).

Post-hoc t-tests were employed to compare between rest and the first 5 breaths during face immersion over the three immersion temperatures for V_E, V_T, T_I/T_{TOT} and V_T/T_I (Table 2.4). For V_E and V_T, the first breath in all immersions were significantly ($p < 0.05$) increased when compared to rest, while breaths 2-5 were also significantly increased during the 5 and 15°C immersions (Table 2.4). On the other hand, T_I/T_{TOT} was not significantly increased until the 4th or 5th breaths. V_T/T_I was also significantly increased for the 1st breath of all immersions when compared to rest, and remained significantly increased over breaths 2-5 in the 5 and 15°C immersions, and increased in the 2nd breath during the 33°C immersion (Table 2.4).

The effect of Breathing Condition was significant for F_ICO_2 ($F = 12.44$, $p < 0.001$) (Fig. 2.9). The difference in F_ICO_2 occurred between the rest condition and the 5°C

immersion ($p < 0.001$), the 15°C immersion ($p = 0.001$), and the 33°C immersion ($p < 0.001$). F_ICO_2 increased from ~ 0.4 (0.1) % during rest to ~ 1.1 (0.3) % in 5°C immersion, to ~ 0.9 (0.3) % in the 15°C immersion, to ~ 1.0 (0.3) % in the 33°C immersion.

The effect of Breathing Condition was significant for mean Immersed T_{SK} ($F = 33.26$, $p < 0.001$) and not significant for mean Non-Immersed T_{SK} ($F = 0.59$, $p = 0.63$) and Hand T_{SK} ($F = 1.44$, $p = 0.25$) (Fig. 2.10). The difference in mean Immersed T_{SK} occurred between the rest condition and the 5°C immersion ($p < 0.001$), 15°C immersion ($p < 0.001$), and the 33°C immersion ($p = 0.008$) (Fig. 2.11). Mean Immersed T_{SK} decreased from 34.7 (0.8)°C during rest, to 19.7 (6.36)°C in the 5°C immersion, to 24.0 (4.2)°C in the 15°C immersion, to 33.9 (0.6)°C in the 33°C immersion (Table 2.2).

2.4. Discussion

The outcomes of this study help broaden our understanding of the mechanism of the face only cold water gasp-like response that was demonstrated previously (Jay et al., 2007), but it was unknown if this response came about from changes in inspiratory effort or timing of breathing. A main physiological outcome in this study occurred during lower temperature face immersions where there was a distinct stimulation of pulmonary ventilation and tidal volume (Fig. 2.2). This increase in ventilation was most pronounced in the first 5 breaths (Table 2.4) and included the greatest inspiratory flow, which is an index of the inspiratory effort, in the two lower water temperatures over these first 5 breaths (Table 2.4). Since inspiration time was relatively constant, with respect to the changes in V_T, across lower temperature face immersion conditions (Table 2.3), it appears the added inspiratory effort during the cold immersions were due to the greater amplitude of the efferent signal to the muscles of breathing, including the diaphragm, rather from their more rapid stimulation as would be evidenced by a reduced duty cycle in the lower water temperatures.

When the first five breaths were examined, V_E significantly increased during the 1st breath of immersion in all three temperatures, and the increase was the greatest in the 15°C immersion. V_E quickly decreased over breaths 2 – 5 to resting values. Tidal volume increased between rest and both the 5 and 15°C immersions, and these results are

consistent with what has been demonstrated in the literature (Christensen et al., 2005; Jay et al., 2007). As expected, there were no significant differences found in F_B (Christensen et al., 2005; Jay et al., 2007).

The greatest T_I/T_{TOT} occurred during the 5°C immersion (Fig. 2.8), and there was a two-phase response in T_I/T_{TOT}. Both of these findings are novel since these results have not been shown in the literature thus far. The largest increase in V_T/T_I occurred during the first breath of face immersion and out of the three face immersions the increase was the greatest in the 5°C immersion.

The results of this study showed that ventilation initially increased at the beginning of face immersion and then rapidly decreased, which is consistent with the literature where V_E experiences a two-phase response of hyperventilation followed by a hypoventilation (Jay et al., 2007). Jay et al. (2007) had previously found that a 60 s face immersion in 0°C elicited a ~162% increase in V_E when compared to a face immersion in 33°C water. The current study found a similar result where face immersion 5°C water elicited a ~191% increase in V_E from the non-immersed rest condition over the first 5 breaths. The 15°C immersion, however, elicited an even larger increase in V_E of ~227% of resting non-immersed values. Similarly, the increase in V_T during the 5°C immersion supported what is seen in the literature where cold water face immersion elicits a greater V_T (Gagnon et al., 2013; Stewart et al., 1998). There were no significant differences between the conditions for F_B, and this result is similar to what is seen in the literature where there is little difference in F_B when the face is immersed in cold water (Gagnon et al., 2013; Stewart et al., 1998).

A potential shortcoming of this study was the assumption that expiration during face immersion is passive. Inspiratory duty cycle works under the assumption that inspiration is active while expiration is passive, thus giving an index of time spent in active respiration. If expiration is not passive, then this ratio will not be an accurate representation of the work of breathing. In a future study, electromyography could be used over the respiratory cycle to determine when these muscles are active. Another shortcoming of this study is the lack of control conditions included. The additional control conditions would include a control where participants are mildly hypercapnic at rest and another where participants experience face immersions without the influence of CO_2. The addition of

these control conditions would help to better understand the separate influences from face immersion and CO_2. Lastly, a possible shortcoming in this study is the assumption that the 33°C water face immersion will encompass the thermoneutral zone for all participants, since the thermoneutral zone in water has been cited to range from 33 – 35.5°C (Kingma, 2012).

The initial gasp after whole body immersion is elicited through a large increase in afferent drive from the peripheral cold receptors on the face (Keatinge & Nadel, 1965; Mekjavić et al., 1987). The pathway of the initial gasp is not entirely known, but appears to be due to stimulation of the peripheral cold receptors that stimulate the respiratory control centre (Duffin et al., 1975). For face immersion during apnoea, the afferent pathway is through the trigeminal nerve, particularly the ophthalmic branch, to nuclei in the pons (Elsner & Gooden, 1983). This results in an inhibition of the respiratory centre and therefore reduced efferent nerve impulses are sent to the inspiratory muscles (Gooden, 1994). There is a positive correlation between the rate of cooling of the skin temperature and the initial gasp (Burke & Mekjavic, 1991; Datta & Tipton, 2006).

Conclusion

It was hypothesized that the greatest inspiratory flow and inspiratory duty cycle will be evident during 5°C water face immersion when compared to immersion in both 15 and 33°C water. Over the first 45 breaths of the immersions there was no significant difference in inspiratory flow between rest and the 5°C water face immersion. Over these first 45 breaths there was an effect of cold immersion on the inspiratory duty cycle with the greatest inspiratory response occurring during the 15°C water face immersion. Over an analysis of the first 5 breaths of the face immersions, it was found that the greatest inspiratory flow occurred during the first breath of face immersion across all water temperatures and these responses, as well as most of breaths 2,3 4 and 5, were significantly elevated relative to the resting values. It was also hypothesized that there will be an enhanced effect of cold water touching the face during mild hypercapnia on ventilation. It was expected that the greatest increases in ventilation will be in a hypercapnic face immersion in 5°C water when compared to resting values. Over the first 45 breaths face immersions there was no significant difference found between rest and

the 5°C water face immersion on V_E. Pulmonary ventilation, however, was increased during the first five breaths of face immersion and this increase was greater in the 15°C face immersion relative to the 5 and 33°C face immersions.

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2.6. Tables

Volunteer Code	Weight (kg)	Height (m)	Age (y)
1	71.0	1.9	28
2	72.3	1.8	33
3	70.0	1.7	27
4	75.7	1.8	26
5	83.0	1.9	22
6	79.4	1.8	26
7	79.0	1.8	22
8	85.0	1.8	27
9	86.4	1.9	26
Mean (SD)	78.0 (6.1)	1.8 (0.1)	26.3 (3.3)

Table 2.1. **Summary of volunteers' weight (kg), height (m), and age (y) used in this study, as well as mean (SD) values.**

	Breathing Condition			
	Rest	**5°C**	**15°C**	**33°C**
V_E (L/min)	13.08 (1.23)	13.93 (2.21)	14.09 (0.92)	11.92* (1.20)
V_T (L)	0.69 (0.06)	0.92* (0.29)	0.72 (0.09)	0.69 (0.04)
			NS	
$P_{ET}CO_2$ (mm Hg)	38.93 (1.83)	42.02* (2.94)	42.13† (3.39)	43.36‡ (2.76)
Immersed T_{SK} (°C)	34.73 (0.82)	19.72‡ (6.36)	23.97‡ (4.23)	33.86† (0.59)

Table 2.2. **Mean (SD) values for ventilation (V_E), tidal volume (V_T), end-tidal CO_2 ($P_{ET}CO_2$), and mean immersed skin temperature (T_{SK}) during the normocapnic pre-face immersion resting period, and over the first 45 breaths in 5, 15, and 33°C face immersions during mild hypercapnia. t-test comparisons are between Rest and the immersion conditions. Significance: * = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$.**

	Breathing Condition			
	Rest	5°C	15°C	33°C
T_I (s)	1.41 (0.11)	2.02# (0.97)	1.43 (0.19)	1.50* (0.18)
T_{TOT} (s)	3.42 (0.39)	4.36 (1.83)	3.23* (0.43)	3.53# (0.46)
T_I/T_{TOT} (%)	41. 61 (2.64)	45.31# (3.24)	44.57† (2.13)	42.81 (1.43)
V_T/T_I (L/s)	0.53 (0.03)	0.56 (0.08)	0.54 (0.02)	0.49# (0.05)

Table 2.3. **Mean (SD) values for inspired (T_I) and total (T_{TOT}) breathing time, inspiratory duty cycle (T_I/T_{TOT}), and inspiratory flow (V_T/T_I) during the normocapnic pre-face immersion resting period, and over the first 45 breaths in 5, 15, and 33°C face immersions during mild hypercapnia. t-test comparisons are between Rest and the immersion conditions. Significance: # = 0.055 – 0.10; * = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$.**

	Rest	Face Immersion Temperature (°C)	Breath Number 1	2	3	4	5
V_E (L/min)	12.09 (1.05)	5	34.62† (14.07)	26.02‡ (7.35)	19.57† (5.01)	18.75# (9.09)	16.58* (5.12)
		15	48.66* (37.51)	29.75‡ (6.46)	22.16† (6.55)	19.35* (7.00)	17.88* (5.73)
		33	32.33* (24.90)	19.82 (13.56)	17.76* (6.98)	15.04 (5.13)	12.11 (3.28)
V_T (L)	0.67 (0.06)	5	2.17† (0.95)	1.41† (0.48)	1.19† (0.37)	1.08† (0.36)	1.02† (0.24)
		15	1.75‡ (0.44)	1.44‡ (0.27)	1.15‡ (0.30)	1.05† (0.29)	0.86* (0.23)
		33	1.93† (0.82)	1.20 (1.18)	0.95# (0.45)	0.92* (0.29)	0.77 (0.21)
T_I/T_{TOT} (%)	41.21 (1.94)	5	39.44 (12.14)	37.02 (15.11)	39.95 (13.91)	34.71 (15.15)	47.33# (8.25)
		15	36.89 (6.83)	43.44 (6.69)	43.00 (4.69)	43.13 (3.31)	46.78* (6.10)
		33	34.00# (10.32)	45.75# (6.71)	42.86 (5.77)	45.22* (3.53)	42.11 (6.41)
V_T/T_I (L/s)	0.52 (1.05)	5	1.75* (1.50)	0.92† (0.32)	0.80 (0.44)	0.77* (0.27)	0.67* (0.15)
		15	1.70‡ (0.67)	1.14‡ (0.25)	0.89† (0.30)	0.70# (0.24)	0.64* (0.16)
		33	1.52* (0.92)	0.81* (0.32)	0.69 (0.29)	0.49 (0.17)	0.52 (0.12)

Table 2.4. **Mean (SD) values during the normocapnic pre-face immersion resting period, and the first 5 breaths during face immersions in 5, 15, and 33°C water with mild hypercapnia for ventilation (V_E), tidal volume (V_T), inspiratory duty cycle (T_I/T_{TOT}) and inspiratory flow (V_T/T_I). t-test comparisons are between Rest and the immersion conditions. Significance: # = p: 0.055 – 0.10; * = p < 0.05; † = p < 0.01; ‡ = p < 0.001.**

2.7. Figures

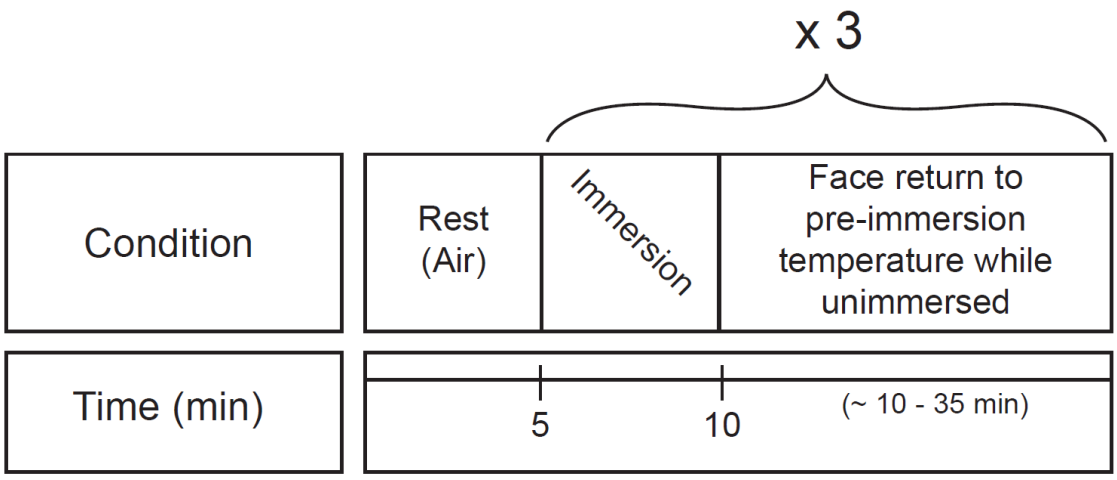

Figure 2.1. **Protocol employed in this study consisting of a 5 min normocapnic rest period, followed by a 5 min immersion during mild hypercapnia and ~10 – 35 min recovery period when the participant was not immersed to allow the face to return to the pre-immersed temperature. The immersion and recovery period was repeated three times.**

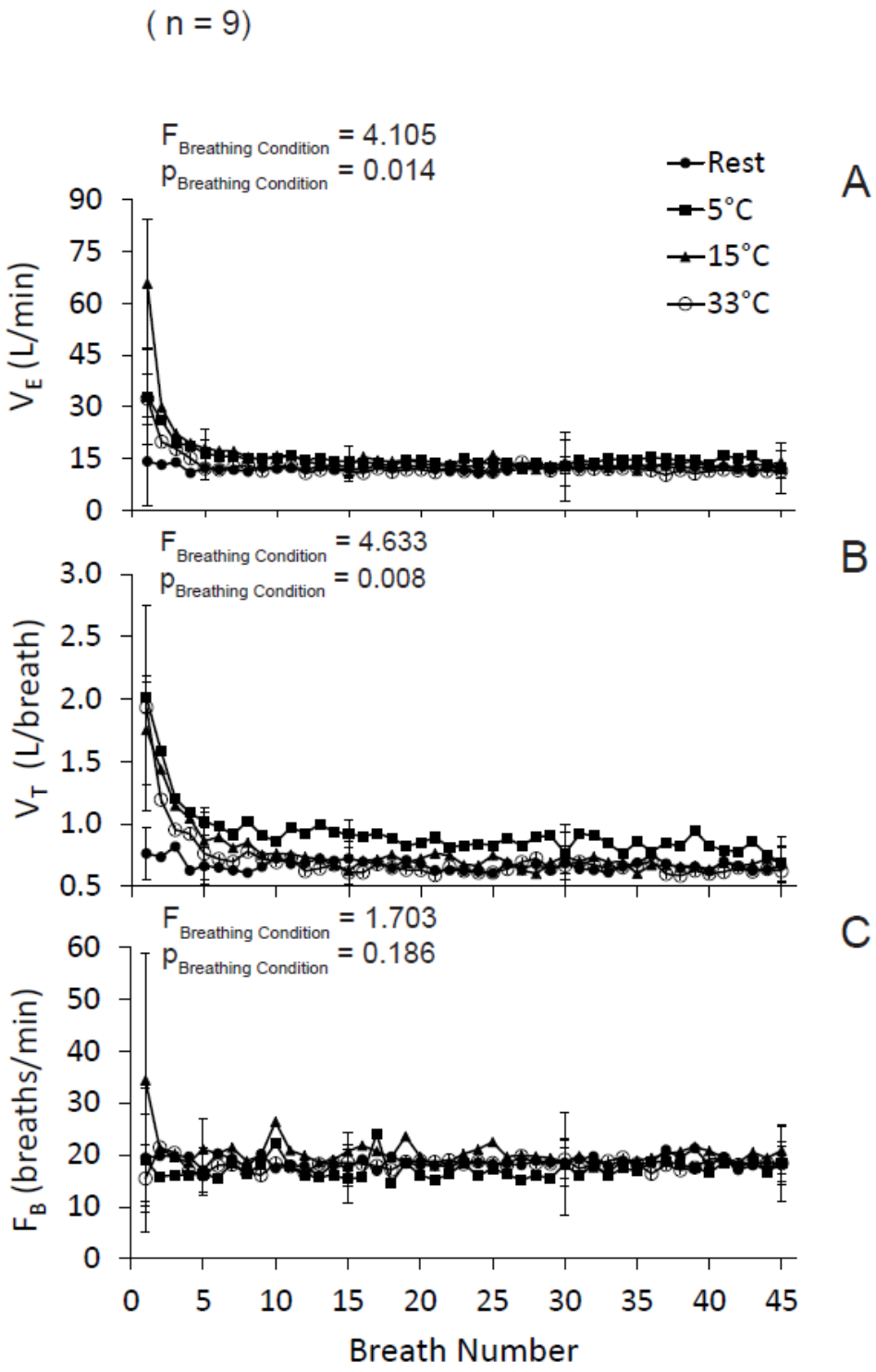

Figure 2.2. **Ventilation (V_E), Tidal Volume (V_T), and Frequency of Breathing (F_B) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

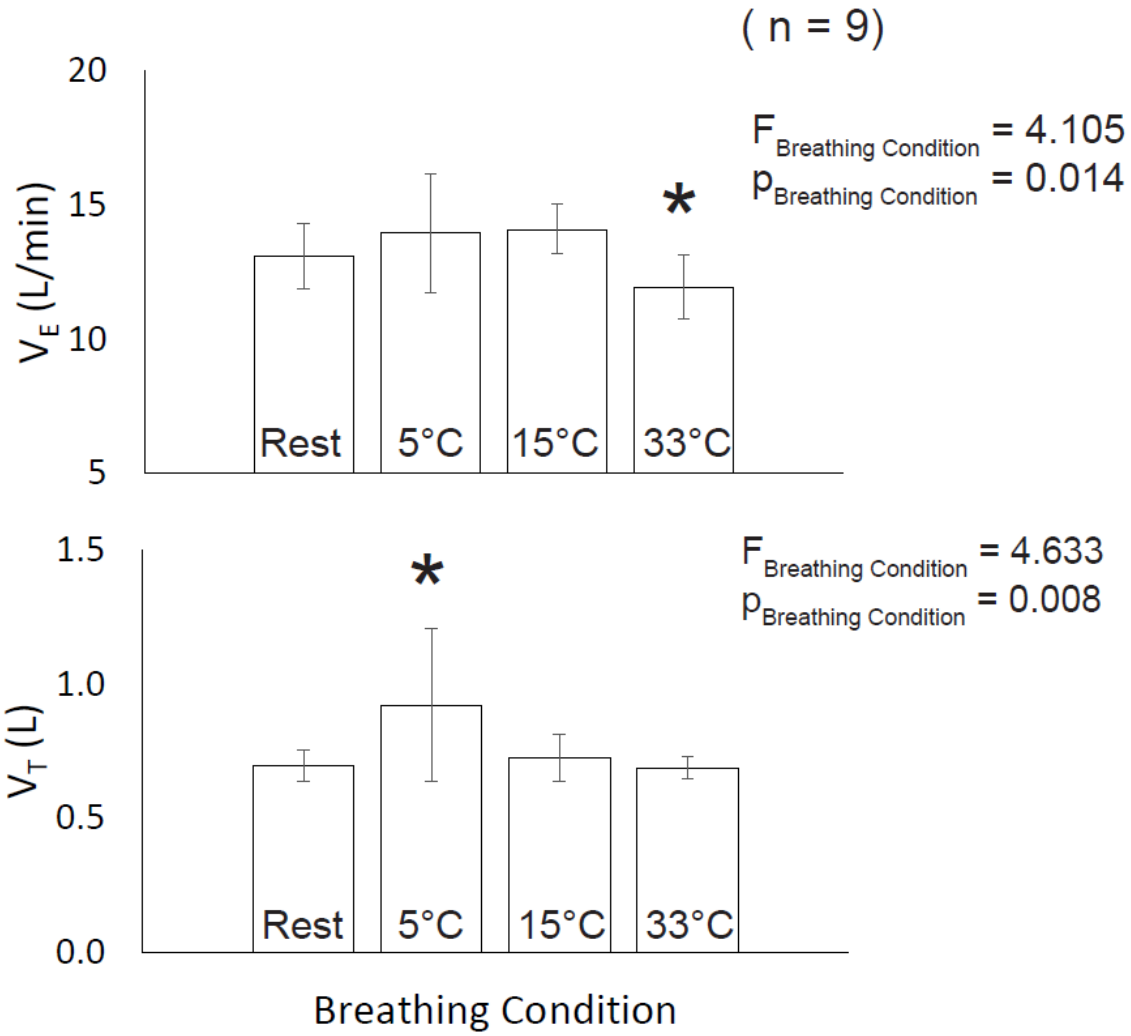

Figure 2.3. **Mean responses over the first 45 breaths of the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C for Ventilation (V_E) and Tidal Volume (V_T). Error bars represent the standard deviation. Significance: * = $p < 0.05$.**

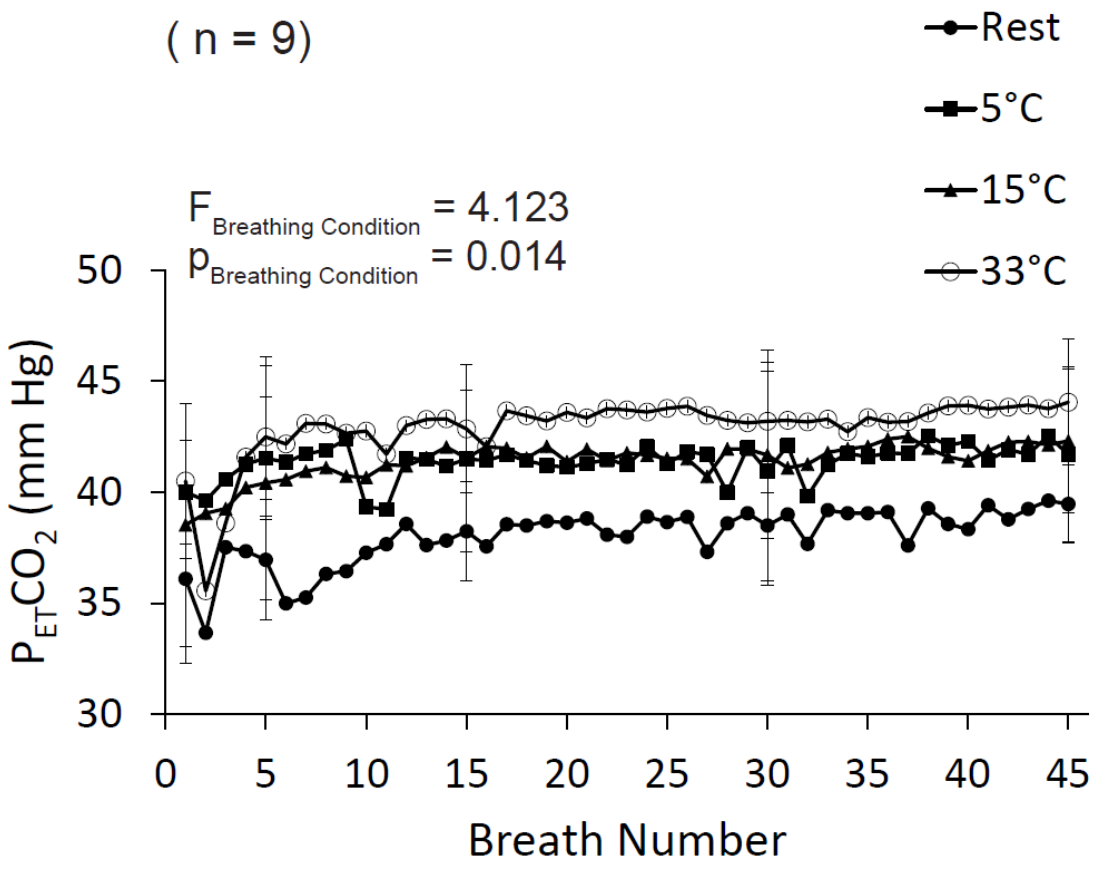

Figure 2.4. **Partial pressures of end tidal CO_2 ($P_{ET}CO_2$) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

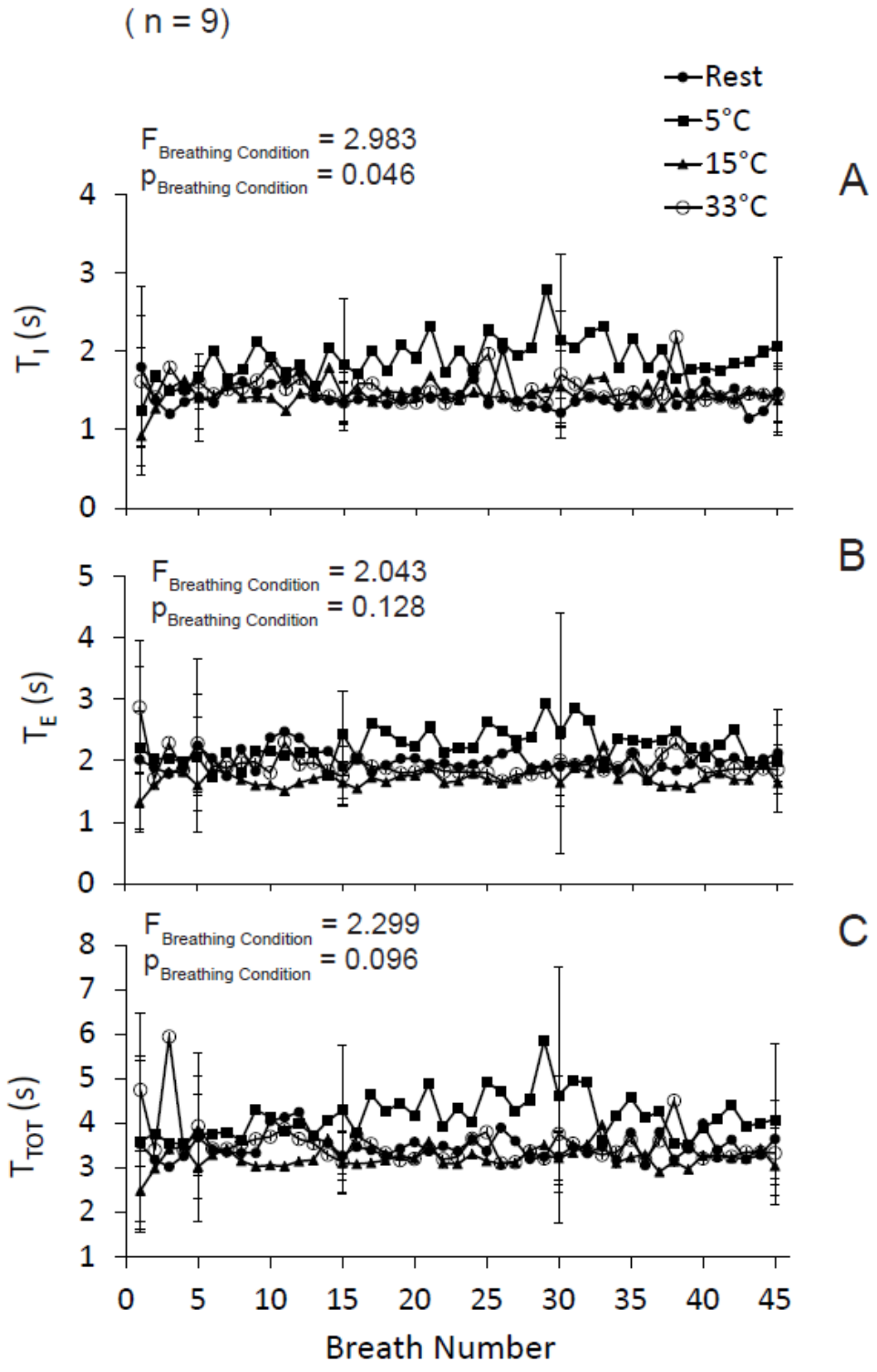

Figure 2.5. **Inspiration time (T_I), expiration time (T_E) and total breath time (T_{TOT}) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

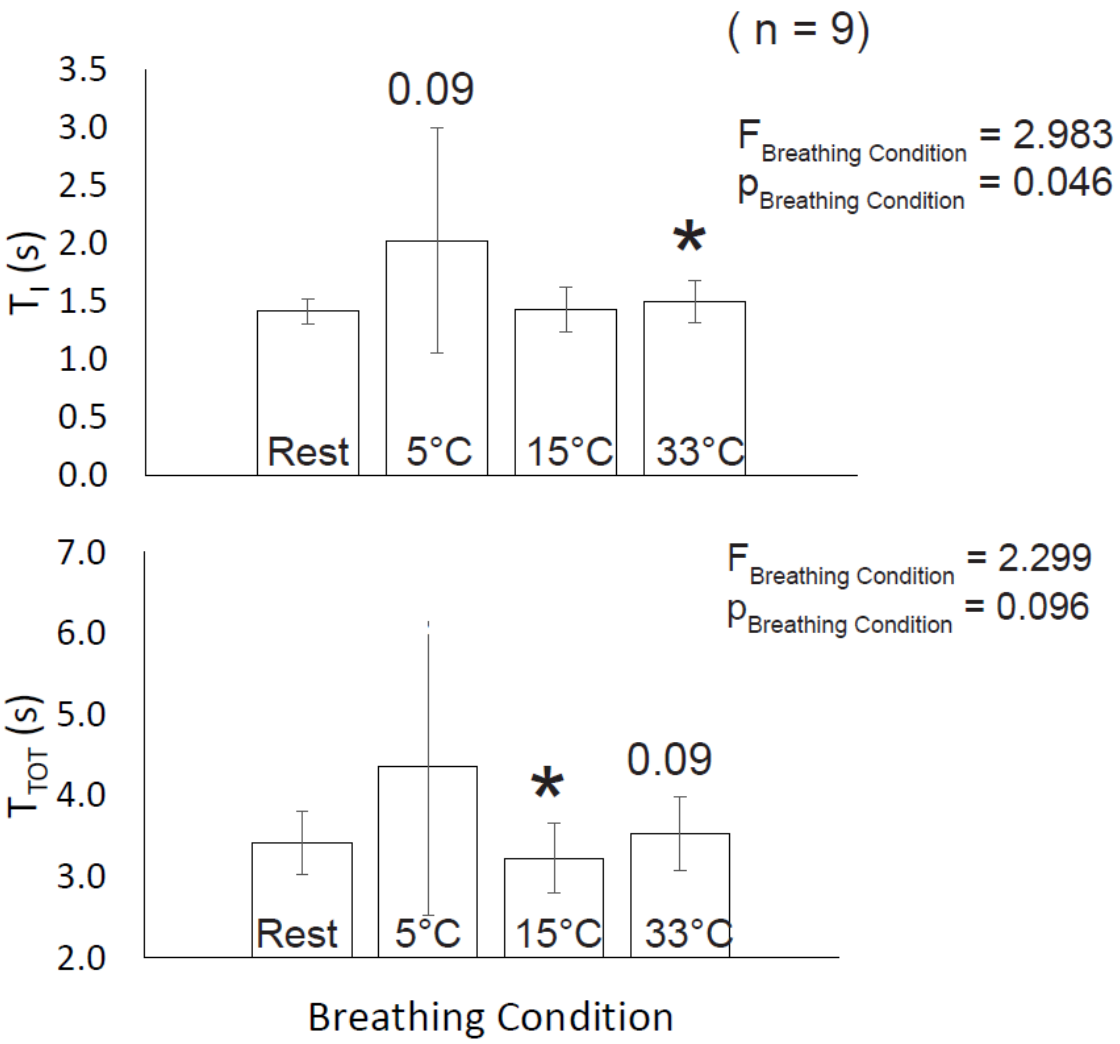

Figure 2.6. **Mean responses over the first 45 breaths of the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C for Inspiration time (T_I) and total breath time (T_{TOT}). Error bars represent the standard deviation. Significance: * = $p < 0.05$.**

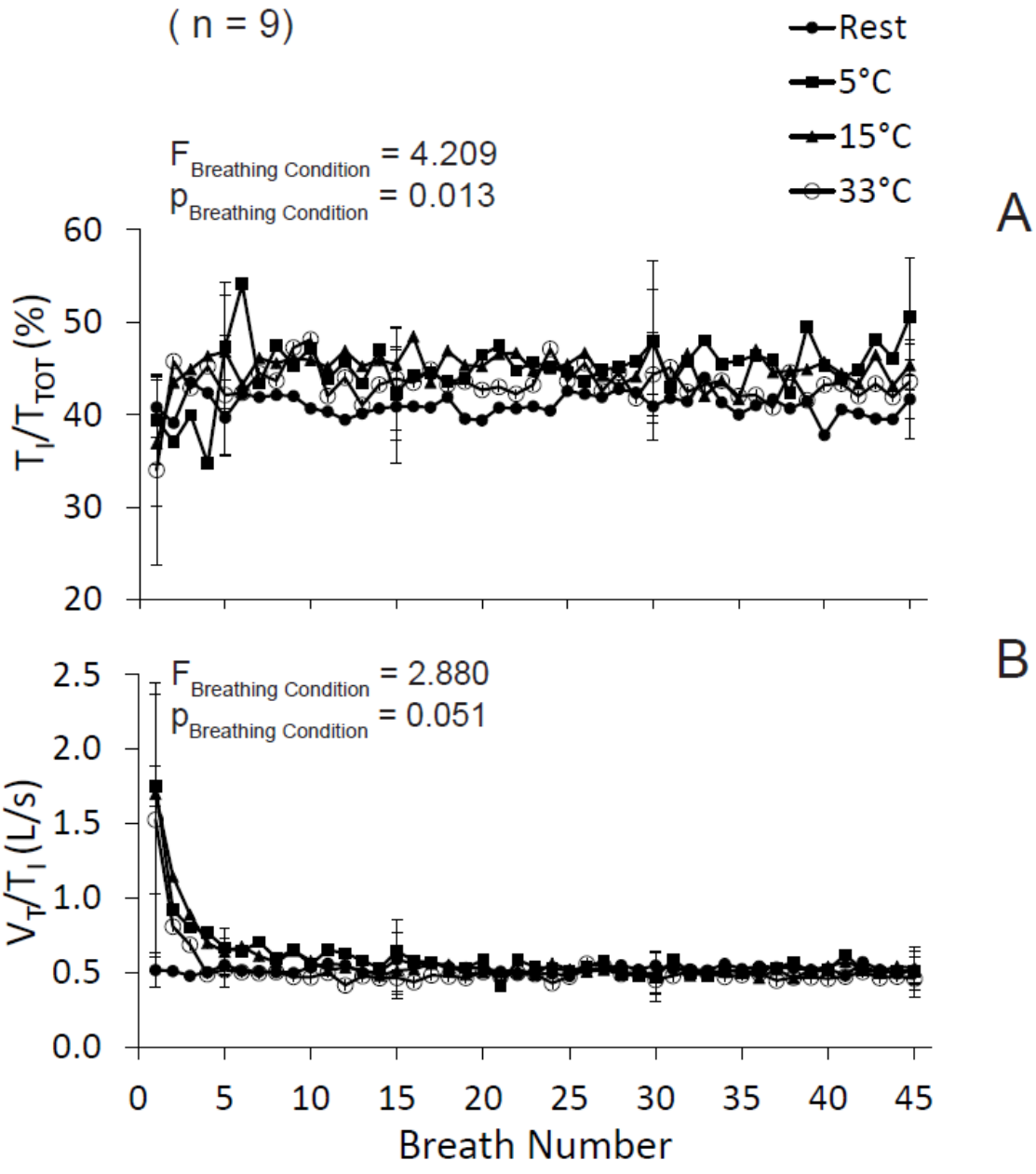

Figure 2.7. **Inspiratory duty cycle (T_I/T_{TOT}) and mean inspiratory flow (V_T/T_I) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

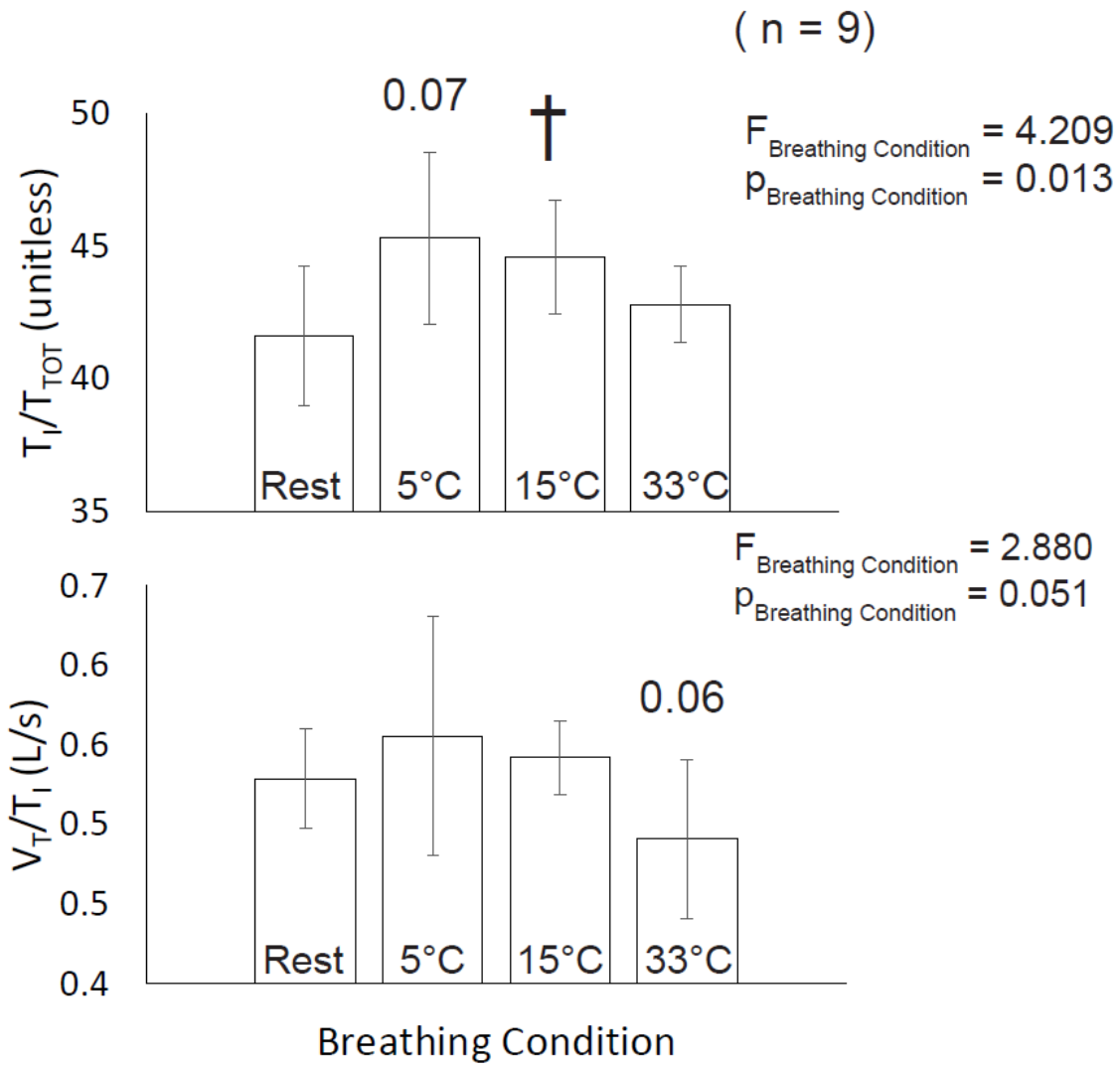

Figure 2.8. **Mean responses over the first 45 breaths of the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C for Inspiratory duty cycle (T_I/T_{TOT}) and mean inspiratory flow (V_T/T_I). Error bars represent the standard deviation. Significance: † = $p < 0.01$.**

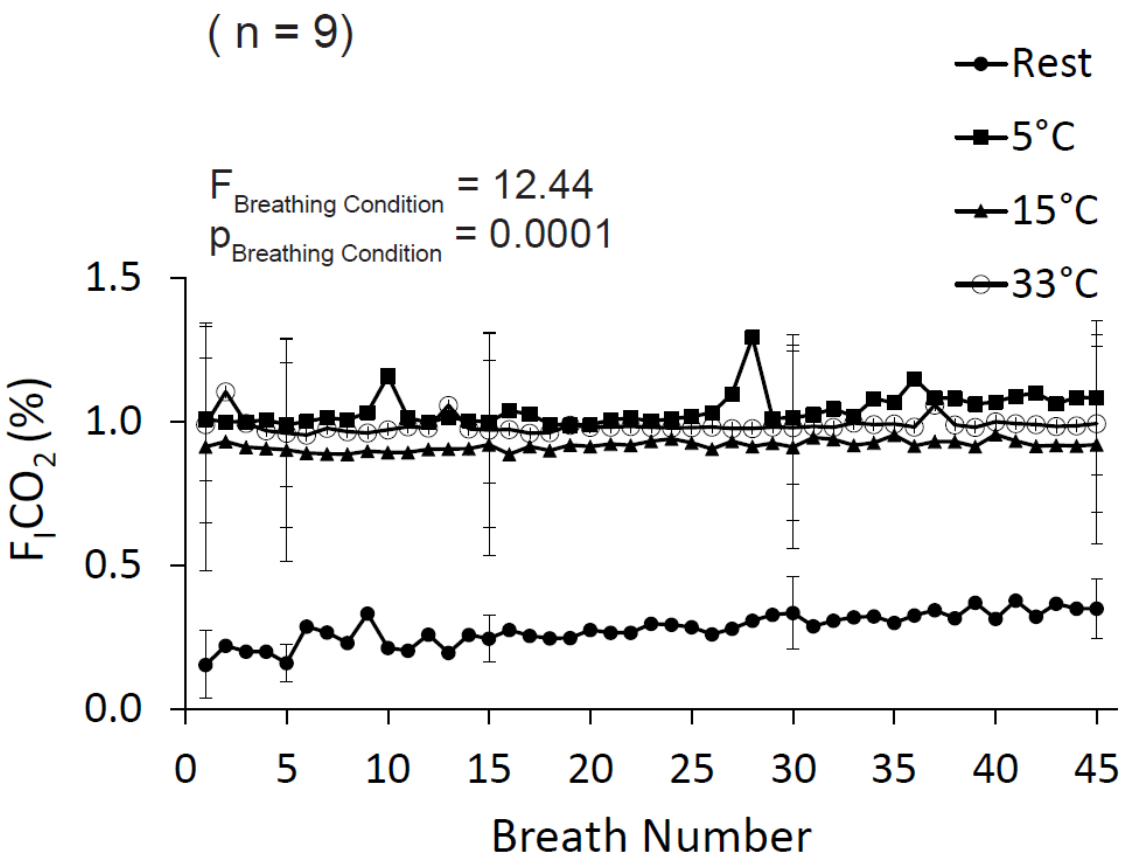

Figure 2.9. **Fraction of inspired CO_2 (F_ICO_2) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

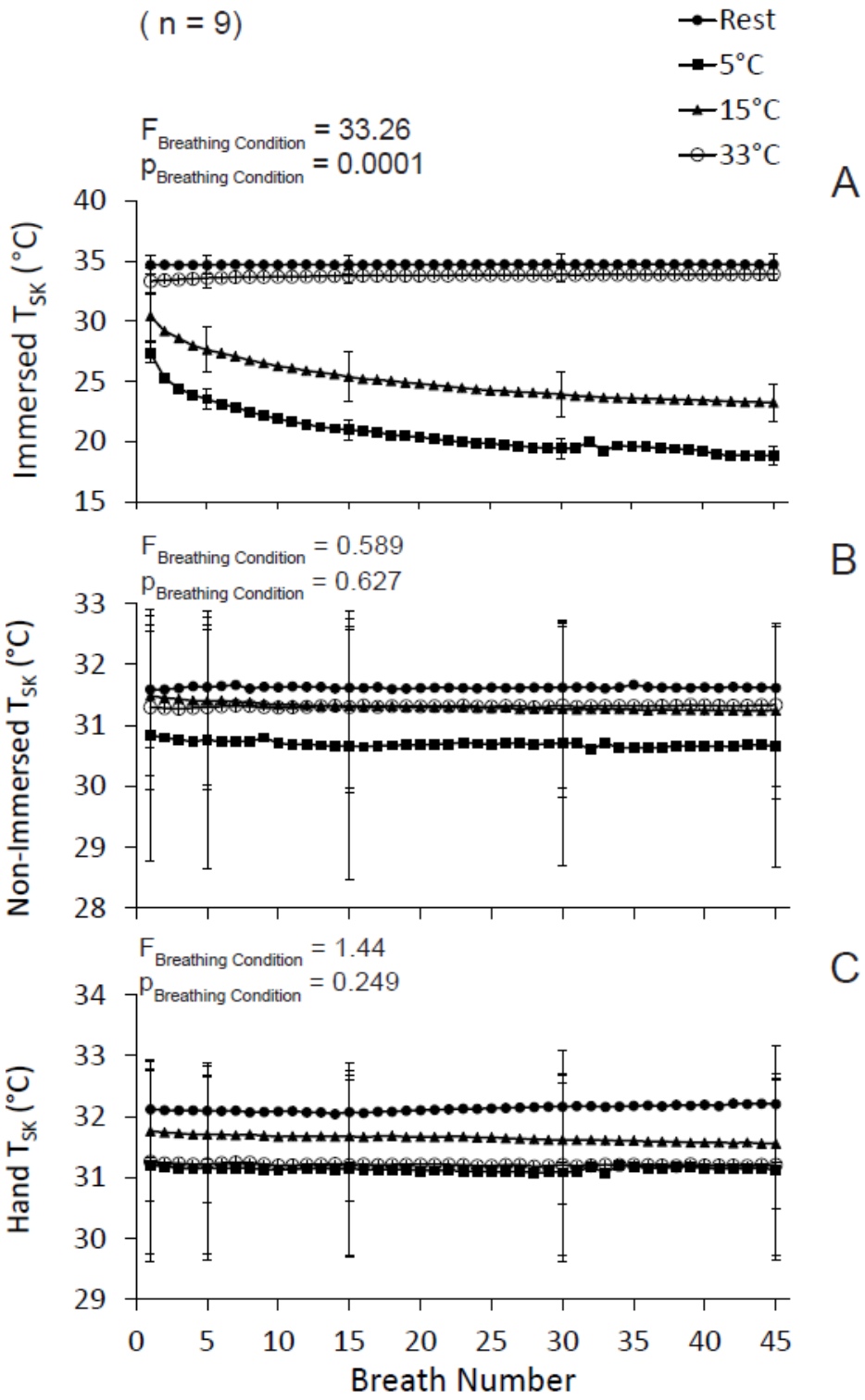

Figure 2.10. Mean Immersed, Non-Immersed and Hand Skin Temperature (T_{SK}) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.

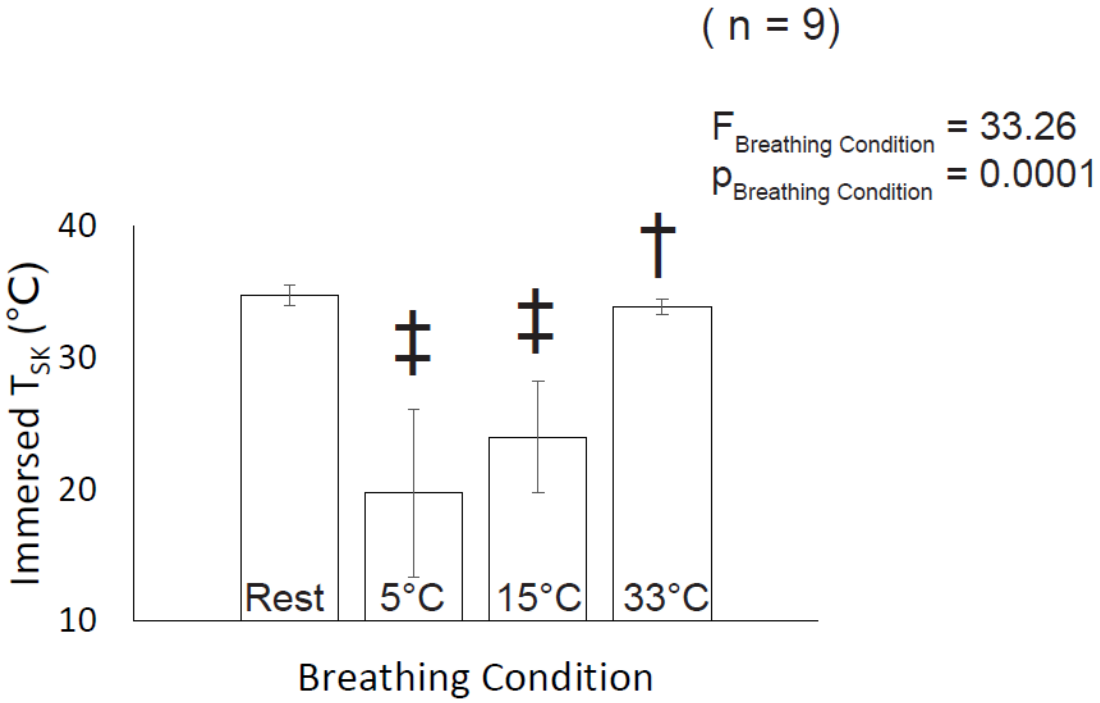

Figure 2.11. **Mean responses over the first 45 breaths of the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C for Mean Immersed Skin Temperature (T_{SK}). Error bars represent the standard deviation. Significance: * = † = p < 0.01; ‡ = p < 0.001.**

Chapter 3.

Study 2 - Effect of Mild Hypercapnia and Skin Temperature on Cardiovascular Responses during Non-apnoeic Face Immersion in Cold Water

3.1. Introduction

This study aims to determine the how mild hypercapnia and cold water face immersion influence cardiovascular response including blood pressure and heart rate, and how these cardiovascular responses influence cerebrovascular responses.

Increases in $PaCO_2$ and decreases in pH are sensed by peripheral and central chemosensitive tissues and these bring on a compensatory hyperventilation to help regulate pH and PCO_2. In addition, hypercapnia can lead to vasodilation while hypocapnia can lead to vasoconstriction of cerebral arterioles**.** Vasoconstriction of downstream arterioles results in a lowered MCA_V while vasodilation of downstream arterioles results in an elevated MCA_V (Sato et al., 2012)**.** Thus, an increase in $P_{ET}CO_2$, which occurs during apnoea or through breathing more CO_2, stimulates both pulmonary ventilation and mean MCA_V**.**

The dive response occurs when the face is immersed in cold water and results in a bradycardia (Asmussen & Kristiansson, 1968), an increased peripheral vasoconstriction (Elsner et al., 1971), and an increased MAP (Bruce & Speck, 1979). Typically low water temperatures increase the magnitude of component physiological responses of both the dive and gasp responses, with exception of the HR response in the gasp response where the lowest HR was seen with 15°C water (Tipton et al., 1991). This suggests that there are mechanisms modulating MCA_V other than CO_2 which may be through sympathetic innervation (Hamel, 2006) or due to metabolites (Villringer & Dirnagl, 1995). Middle cerebral artery velocity can also increase due to cool objects touching the face without face immersion (Brown et al., 2003).

Vertebral artery velocity has yet to be recorded during cold water face immersion, and this would be beneficial to know since the VA supplies the caudal and upper medulla, which includes nucleus of the tractus solitarius that is responsible for inspiration (Willie et al., 2012). It was expected that a two-phase response for HR will be seen since this study involves face immersion without apnoea, where the first phase is an increase in HR and the second phase is a decrease in HR (Jay et al., 2007). It was suggested based on the evidence in the literature (Bullard & Crise, 1961) that there might be an enhanced effect of cold water touching the face during mild hypercapnia on whole body cardiovascular and cerebrovascular responses as represented by cerebral blood velocities measured in the middle cerebral and vertebral arteries. It was hypothesized that the greatest increases in mean MCA_V and VA_V, will be in a mildly hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values. It was also hypothesized that the lowest skin blood velocity and greatest blood pressure, cerebrovascular conductance, Q, SV and HR will occur during mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

3.2. Methods

3.2.1. Participants

Nine male participants 20 – 29 years of age who were healthy, non-smokers volunteered for the study. They had a mean (SD) height of 1.81 m (0.07), weight of 78 kg (6.1), and age of 26 years (3.3) (Table 2.1). The volunteers were instructed to abstain from caffeine and strenuous exercise for 24 hours prior to the testing period. Each volunteer was given a tour of the lab and had an orientation session prior to the experiment trial to explain potential risks and the study protocol. Next the volunteer had a 24-hour reflection period, after which he signed an informed consent, filled out a medical questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q). This study has been approved by the Simon Fraser University Office of Research Ethics.

A power calculation was used to determine the sample size required to have a power of 80% and significance of 0.05 and examined inter-subject variability. The sample size varied depending on the variable. The number of volunteers required was a minimum

of 8 in order to find a significant result, if it exists, in all variables of interest. It was determined that the variables most pertinent to this study are HR, MAP, MCA_V, VA_V.

Speck and Bruce (1978) accessed both HR and MAP during face immersion in water temperatures 5, 15, 25, and 35°C. They found a difference of 15 bpm in HR between face immersion 5 and 35°C water, with a common standard deviation of 9.5 bpm. This resulted in six participants needed in order to have a power of 80%. They also found a difference in MAP of 25 mm Hg between the control and 35°C face immersion, with a common standard deviation of 17.5 mm Hg, resulting in eight participants needed.

Brown et al. (2003) analyzed the difference in MCA_V at rest and when a bag of ice was placed on the face. They were able to detect a difference of 10 cm/s with a common standard deviation of 6.5 cm/s. With these parameters, seven participants would be needed. In order to detect a difference of 15 cm/s, only three participants would be needed.

3.2.2. Instrumentation

For all parts of the study, the participant laid prone on a padded table. During face immersions, the participant breathed through a snorkel, with a volume of 210 mL, attached to a low resistance mouthpiece that connects to the two-way mass flow sensor (Sensormedics, Yorba Linda, CA, USA). The dead space of the snorkel gave a mild hypercapnia of 42 – 43 mm Hg (Table 2.2). The participant also wore a nose clip to ensure all exhaled gases were collected from the low resistance mouthpiece. During the rest period, the v participant did not have their face immersed but still breathed through the mouthpiece and snorkel.

During immersion trials, the participant had their face immersed in a face-bath measuring 0.42 m x 0.39 m x 0.17 m filled with ~19.6 L of water. It had water flowing to and from the bath through a water chiller (model no. 1196, VWR International, Mississauga, ON, Canada) at a rate of 3 L/min.

Cardiovascular Variables

Systolic (BP_{SYS}) and diastolic blood pressure (BP_{DIA}) were measured by Finometer blood pressure monitor (Finapres Medical Systems B.V., Amsterdam, The Netherlands). Mean arterial blood pressure (MAP) was calculated as follows: MAP = (1/3 x BP_{SYS}) + (2/3 x BP_{DIA}).

A pulse oximeter (Masimo Radical, Irvine, CA, USA) was used to measure heart rate and arteriolar hemoglobin oxygen saturation.

A transcranial Doppler ultrasound system (Spencer Technologies, Washington, USA) with the Doppler probes secured with a headgear device (Spencer Technologies, Nicolet Instruments, Madison, USA) was used to measure cerebral blood velocity in the MCA and VA. The headband was modified to hold the probe for VA. MCA blood velocity (MCA_V) was insonated through the anterior transcranial window pointing posteriorly, with a positive velocity indicating that blood flow was toward the Doppler probe. A test to ensure the vessel insonated is MCA is to observe a decrease in velocity when the ipsilateral carotid artery is compressed (Baumgartner, 2006). The MCA was insonated at a depth of 50 mm, but can be imaged as shallow as 25 mm (Willie et al., 2011) and expected values for MCA_V are a mean (SD) of 62 (12) cm/s (Aaslid et al., 1982). The VA was insonated through the foramen magnum pointing towards the brainstem, with a negative velocity indicating that blood flow was away from the Doppler probe (Baumgartner, 2006). The VA was insonated at a depth of 80 - 115 mm (Willie et al., 2011), and expected values for VA_V were a mean (SD) of 41 (10) cm/s (Ringelstein et al., 1990).

A laser-Doppler flowmeter (LDF) 5-in-1 needle probe (MP12-V2, Moor Instruments Ltd, UK) was employed to measure skin red blood cell velocity of the right hand ($SkBV_{HAND}$). The LDF probe was calibrated using a polystyrene microspheres flux standard solution (moorLAB, MP12-V2, Moor Instruments Ltd, Devon, UK). The particles in the solution undergo Brownian motion at a set value, given that temperature is constant, which allows the calibration of the probe.

Data Acquisition

Body temperature values, as well as other physiological outcome variables were sampled at 40 Hz using LabVIEW software (Ver. 7.1, National Instruments, Austin, TX, USA) and recorded on a breath-by-breath basis. Ventilatory measures were sampled and recorded with the VMAX229c metabolic cart (Sensormedics, Yorba Linda, CA, USA).

3.2.3. Protocol

There were three conditions of face immersion in water temperatures of 5, 15, and 33°C in mild hypercapnia (Fig. 2.1). The order of the experimental trial temperatures were single-blinded and randomized using a Latin Square design. Each participant was unaware which condition they were experiencing prior to each face immersion. Rest values for all variables were collected for 5 min before and 5 min during recovery after the face immersion while the subject laid prone on a table without their face immersed. Each face immersion lasted five minutes and a time of 10 to 35 min between face immersions was to allow the face skin temperature return to pre-immersion values.

3.2.4. Statistical Analysis

The effect of Breathing Condition (Rest, 5, 15, 33°C) was analyzed using a 1-factor ANOVA using the software program SPSS (Version 19, Surrey, UK) for the mean values across the first 300 s in each level of Breathing Condition. Two CO_2 conditions were tested with normocapnia during rest without face immersion and during mild hypercapnia with face immersion in the 3 different water temperatures. The hypercapnia during face immersions was with a $P_{ET}CO_2$ ~ 3 to 4 mm Hg above the normocapnic $P_{ET}CO_2$. In addition, to explore the transient responses immediately after face immersion, t-tests were employed to compare between rest and time periods 5, 75, and 155 s into immersions in 5, 15 and 33°C water. The variables that were analyzed included BP_{SYS}, BP_{DIA}, MAP, MCA_V, VA_V, CVC_I MCA, CVC_I VA,

Reproducibility of each subject's MCA and VA TCD measurements were assessed from resting data collected from the two testing days and analyzed using Bland and Altman (1986) test of reproducibility. The first set of MCA and VA data was collected during an

orientation session prior to the test day. A two-tailed, paired Student's t-test was used to compare means if there were significant effects from the ANOVA model. The level of significance was set at 0.05.

3.3. Results

The effect of Breathing Condition was significant for BP_{SYS} ($F = 16.97$, $p < 0.001$), BP_{DIA} ($F = 3.08$, $p = 0.041$), and BP_{MAP} ($F = 12.29$, $p < 0.001$) (Fig. 3.1). The difference in BP_{SYS} occurred between rest and the 5°C immersion ($p < 0.001$), the 15°C immersion ($p = 0.005$), and the 33°C immersion ($p < 0.001$). BP_{SYS} increased from 115.9 (13.1) mm Hg during rest to 149.9 (6.3) mm Hg in the 5°C immersion, 137.5 (7.7) mm Hg in the 15°C immersion, and 128.7 (13.0) mm Hg in the 33°C immersion (Table 3.1 & Fig. 3.2). The difference in BP_{DIA} occurred between rest and the 5°C ($p = 0.003$) and 15°C immersions ($p = 0.045$). BP_{DIA} increased from 76.0 (11.7) mm Hg during rest to 89.7 (7.4) mm Hg in the 5°C immersion, and 82.6 (5.6) mm Hg in the 15°C immersion. The difference in MAP occurred between rest and all levels of Water Temperature ($p < 0.001$). MAP increased from 89.1 (8.3) mm Hg during rest to 109.7 (6.4) mm Hg in the 5°C immersion, 100.9 (5.2) mm Hg in the 15°C immersion, and 97.7 (8.8) mm Hg in the 33°C immersion.

The effect of Breathing Condition was not significant ($p > 0.05$) for MCA_V ($F = 0.342$) nor VA_V ($F = 0.244$) (Fig. 3.3).

The effect of Breathing Condition was significant for CVC_I MCA ($F = 3.01$, $p = 0.045$) but not significant for CVC_I VA ($F = 1.57$, $p = 0.216$) (Fig. 3.4). The difference in CVC_I MCA occurred between rest and the 5°C immersion ($p < 0.001$), the 15°C immersion ($p = 0.001$), and the 33°C immersion ($p = 0.003$). CVC_I MCA decreased from 0.66 (0.14) cm/s/mm Hg during rest to 0.51 (0.11) cm/s/mm Hg in the 5°C immersion, 0.54 (0.07) cm/s/mm Hg in the 15°C immersion, and 0.58 (0.13) cm/s/mm Hg in the 33°C immersion (Table 3.1 & Fig. 3.5)).

The effect of Breathing Condition was not significant for $skBV_{HAND}$ ($F = 1.08$, $p = 0.372$) (Fig. 3.6). A t-test was performed comparing rest to all of the face immersions for

$skBV_{HAND}$ and resulted in a trend toward significance (p = 0.083), where the immersed values were 65% of the non-immersed resting values.

The effect of Breathing Condition was not significant (p > 0.05) for Q (F = 0.075, p = 0.97), SV (F = 0.169, p = 0.91) and HR (F = 0.106, p = 0.96) (Fig. 3.7).

Post-hoc t-tests were employed to compare between rest and 5, 75 and 155 s into immersion over the three immersion temperatures for Q, SV and HR (Table 3.2). In all immersions, Q was significantly elevated 5 s into immersion when compared to rest. Stroke volume was significantly increased 5 s into immersion in the 15°C immersion, and tended towards significance in both the 5 and 33°C immersions. Heart rate was significantly elevated 5 s into all immersions (Table 3.2).

Bland and Altman

The Bland and Altman analysis compared the rest values collected for MCA_V and VA_V prior to the face immersions. For MCA_V there was a t-value of 1.0 (p = 0.32) and Shapiro-Wilks value of 0.95 (p = 0.69). The results for VA_V resulted in a t-value of 0.66 (p = 0.53) and Shapiro-Wilks value of 0.91 (p = 0.27) (Fig. 3.8).

The p-value for both t-tests for MVA_V and VA_V were above 0.05 which indicates that there is no difference between the conditions. The p-value for both Shapiro-Wilks tests were also both above 0.05, which indicates that the values are normally distributed.

3.4. Discussion

A main physiological finding in this study was the clear inverse relationship between water temperature and systemic blood pressures in these non-apnoeic face immersion conditions (Fig. 3.2). Similarly, there was an opposite trend in CVC_I MCA where there was an apparent stepwise decrease when the face was immersed in lower water temperatures (Fig. 3.5). This novel evidence supports that during the gasp-like response that occurs during face-only immersion in cold water (Jay et al., 2007), there is a reduction in perfusion of the cerebral tissues supplied by the MCA (Fig. 3.4) whereas the tissues supplied by the VA did not appear to influenced by the water temperature. It appears the

inspiratory effort of breathing after face immersion (Chapter 2) is increased and this hyperventilation is evident with a constant perfusion of the respiratory control centers that are on the ventral surface of the medulla oblongata and supplied by the VA. Concurrently, the cerebral cortex appears to have a reduced perfusion suggesting conscious control of breathing may be impaired in these cold face immersion conditions.

An additional novel result is both the MCA and VA cerebral blood velocity responses were maintained despite graded blood pressure responses. This supports that the SNS brought about a cerebral vasoconstriction to protect the brain against the graded blood pressures responses at the lower temperature face immersions (Ainslie & Brassard, 2014; ter Laan et al., 2013). A secondary finding is the responses in CVC_I MCA and CVC_I VA were accompanied by initial elevations of Q, HR and SV but these transient changes within the first minute of face immersion (Fig. 3.7) were not met with changes in either cerebral blood velocities (Fig. 3.3) or conductance (Fig. 3.4). This supports a maintenance of cerebral perfusion, during this transient period of face immersion.

Q, SV and HR were also analysed including time points, 5, 75 and 155 s into immersion. Cardiac output was significantly greater 5 s into immersion when compared to rest in all three temperatures. It rose to 172% in the 5°C, 193% in the 15°C and 160% in the 33°C of resting values. Q tended to return back to baseline values 75 s into the immersion time, suggesting that Q experiences a two-phase response. Likewise, SV also significantly increased 5 s into the face immersions, in all temperatures, and tended to return to resting values 75 s into immersion. Notably, SV rose to 149% of baseline values 5 s into the 15°C immersion. HR also exhibited a two-phase response where it increased 5 s into immersion and then either returned to baseline values in the 33°C immersion or decreased beyond baseline in the 5 and 15°C immersions. This response was particularly evident in the 5 and 15°C immersions where HR rose to ~131% of resting values 5 s into immersion and then decreased to ~95% of resting values 75 s into immersion (Table 3.2). As mentioned above, despite these increases cerebral blood velocities and the indices of conductance for MCA and VA were maintained and it follows this was most likely due to a SNS activity that brought about a cerebral vasoconstriction (Ainslie & Brassard, 2014; ter Laan et al., 2013).

One of the main findings of this study was the graded blood pressure response to cold face immersion. Paulev et al. (1990) have found that MAP increased by 1% of resting values during face immersion in 33°C water, and by 7% of resting values during face immersion in 10°C water. A similar result, albeit during breath holds, was previously found by Speck and Bruce (1978) where MAP progressively increased from ~ 101 mm Hg in 35°C water to 116 mm Hg in 5°C water during face immersions. These findings help to explain the mechanisms underlying the control of breathing since it now appears that with or without apnea, during face immersion in cold water, that there is hypertensive response that is inversely proportional to water or face temperature. Consequently, this progressive increase in blood pressure appears to be a function of the face temperature since it occurs with breathing movements (Fig. 3.1) as it does during apnea without breathing movements (Speck & Bruce, 1978).

This study also found that there was a decrease in CVC_I MCA when the face was immersed in progressively lower water temperatures. This result, however, was not entirely in line with what has previously been shown in the literature. Brown et al. (2003) demonstrated that CVC_I MCA increased by 14% of resting values with 0°C cold stimulus touching the face. The current result (Fig. 3.4) was due to the large increase in MAP without a significant increase in MCA_V, which resulted in a decrease in CVC_I MCA. An explanation for this difference might be due to the differing conditions employed by Brown and colleagues (2003) who employed lower temperature of 0°C ice water in plastic bags touching the forehead bilaterally. Presumably less forehead surface was stimulated by their procedure than by the full face immersion in the current study. This would lower the input to ophthalmic branch of the trigeminal that is thought to influence the DR and, possibly, the currently reported cold-shock like response. Future studies will need to be directed at resolving these apparent differences.

There were no significant differences found in MCA_V, which is similar to what is currently in the literature for the brain blood velocity response to cold face immersion (Table 1.1). Kjeld et al. (2009) saw no change in MCA_V when the face was immersed in 10°C water without an apnoea. In contrast, Brown et al. (2003) found, as described above, that a 0°C bag placed bilaterally on the forehead caused a small but significant increase

in MCA_V when compared to resting values. This current study did not find a significant result for VA_V and it is the first study to report this measure when the face is immersed.

In this current study, there was no effect of cold water immersion on $skBV_{HAND}$, albeit a trend toward significance when all three immersion conditions were grouped and compared to the rest condition (Fig. 3.6). These results are in line with what has been shown in the literature where peripheral vasoconstriction occurs with cold water face immersion with apnoea (Elsner et al., 1971) albeit to a lesser extent without apnoea (Fagius & Sundlöf, 1986). Gagnon and colleagues (2013) found that 30 s into a face immersion without apnoea in 17°C water, skin blood velocity dropped to 70% of non-immersed rest values and after 90 s the skin blood flow in the finger returned to baseline values.

Each of Q, SV, HR (Fig. 3.5) exhibited a two-phase response where the first phase is an increase above baseline and the second phase is a return to resting values or lower values as was the case for HR. There are varied results in the literature with respect to Q, SV and HR responses with cooling of the face, with or without immersion. Brown et al. (2003), saw no change in Q, when a 0°C bag was placed bilaterally on the forehead. Paulev et al. (1990) have found that non-apnoeic face immersion in 33°C water elicits SV to increase to values 107% of resting value, whereas 10°C water elicits a decrease in SV to 7% of the resting values. Heart rate has been shown to decrease to ~15% of resting values 90 s after the face was immersed in 17°C water (Gagnon et al., 2013). The current study did not see a true dive reflex where HR decreased significantly below resting values during cold face immersion, but this may be due to the lack of apnoea which has been known to lessen the effect of the DR (Fagius & Sundlöf, 1986).

One of the potential shortcomings of this study is the lack of a control condition. The additional control conditions would include a control where participants are mildly hypercapnic at rest and another where participants experience face immersions without the influence of CO_2. The addition of these control conditions would help to better resolve the separate influences from face immersion and CO_2 on these cardiovascular responses. Another potential shortcoming of this study is the assumption that the 33°C water face

immersion will encompass the thermoneutral zone for all participants, since the thermoneutral zone in water has been cited to range from 33 – 35.5°C (Kingma, 2012).

In a future study, it would be interesting to test whether respiratory movements account for the increases seen in Q, SV, and HR as opposed to the cold stimulus on the face. It is possible that the two-phase response seen in Q, SV, and HR in the current study were due to inspiratory movements that resulted in an increase in venous return and subsequently Q (Convertino et al., 2011). In order to test this hypothesis, a protocol where the participants are instructed to breath at a set pulmonary ventilation rate while Q, SV and HR are being recorded would help to address this question.

Face immersion in cold water simulates the peripheral cold receptors. The afferent pathway is through the trigeminal nerve, particularly the ophthalmic branch, to nuclei in the pons (Elsner & Gooden, 1983). This results in peripheral vasoconstriction due to increased sympathetic output to the arterioles in the extremities. The increase in sympathetic output increases vascular tone which reduces vessel cross-sectional area, which consequently increases resistance to flow (Gooden, 1994; Speck & Bruce, 1978). Subsequently, blood pressure (BP) during the DR is increased (Speck & Bruce, 1978) due to the reduced vessel cross sectional area of peripheral blood vessels. It would be expected that lower temperature face immersions provide a larger cold stimulus, and thus cause the blood pressure responses in this study (Fig 3.1) as a consequence of a greater peripheral tone of the blood vessels.

Simulation of the facial cold receptors with apnoeic face immersion also results in bradycardia, and decreased Q through the vagal innervation of the heart (Asmussen & Kristiansson, 1968; Lindholm & Lundgren, 2009). It has been speculated that the cold-induced decrease in cardiac output (Q) and redistribution of blood flow could be beneficial for supplying oxygen and energy for the heart and brain (Stewart et al., 1998). It is unknown what causes the two-phase response in non-apnoeic face immersion, but it is thought the initial increase is due to the cold shock-like response (Jay et al., 2007), whereas the subsequent ventilatory and cardiovascular responses appear to be accounted for by the dive response.

As given above, the stability in the response to cold water immersion by MCA_V and VA_V might be due to a SNS vasoconstriction of cerebral arterioles. This has been described as a cerebral autoregulation since CBF has been seen to be maintained over a range of MAP from 60 to 150 mm Hg (Paulson et al., 1990). It has been found, however, that mild hypercapnia (with an increase in PCO_2 of ~5 mmHg above resting values) impairs cerebral autoregulation of CBF (Zhang et al., 1998). Recent research has found that MCA cross-sectional area is altered at a ratio of 0.4%/ mm Hg of $P_{ET}CO_2$ within physiological ranges (Coverdale et al., 2014), although Verbree et al. (2014) have noted that the relationship between MCA cross-sectional area and CO_2 is not linear and that small changes in CO_2 do not affect vessel caliber. During face immersion in this current study (Table 2.1) there was an average increase in $P_{ET}CO_2$ of ~3.5 mm Hg above resting, which would translate to an increase of 1.4% in the MCA cross-sectional area. The increase in cross-sectional area might have dampened the response measured by the TCD in the MCA to face immersion. In comparison to past studies, Brown et al. (2003) did not have an isocapnic condition and this puts their results into question since the MCA diameter and MCA_V could have varied between volunteers. A cold-induced decrease in vessel cross-sectional area might help explain the decrease seen in CVC_I MCA, since there was a lack of significant change in MCA_V. Since, MAP increased, and CVC_I is MCA_V divided by MAP, CVC_I MCA appeared to decrease assuming face temperature had no influence on the MCA diameter. To resolve these questions further research is needed including effects of mild hypercapnia and face temperatures on MCA and VA diameters.

There is a lessened peripheral vasoconstriction without apnoea during face immersion due to less sympathetic outflow (Gooden, 1994; Speck & Bruce, 1978). This might explain why there was only a trend towards an increase in $skBF_{HAND}$ over all immersions when compared to rest in this study.

Conclusion

It was hypothesized that there would be an enhanced effect of cold water touching the face during mild hypercapnia on systemic vascular responses and cerebrovascular responses as represented by cerebral blood velocities measured in the middle cerebral and vertebral arteries. It was also hypothesized that the lowest skin blood velocity and

greatest blood pressure, cerebrovascular conductance, Q, SV and HR would occur during mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values. Over the 300 s of face immersion there was no effect of cold water touching the face on MCA_V or VA_V, however, CVC_I MCA gave a stepwise decrease when the face was immersed in progressively lower water temperatures. Cardiac output, HR and SV were found to have two-phase response. Q, HR, SV initially increased and then progressively decreased over ~ 1 min of face immersion in each water temperature. For $skBV_{HAND}$ a trend toward significance ($p = 0.083$) was evident when all immersed values were grouped and this gave a $skBV_{HAND}$ that was 65% of the non-immersed resting values. Systolic, diastolic and mean arterial pressures were inversely proportional to water temperature and consequently for the middle cerebral artery this gave lower cerebrovascular conductance for the lower water temperatures.

3.5. References

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3.6. Tables

	Breathing Condition			
	Rest	**5°C**	**15°C**	**33°C**
BP_{SYS} (mm Hg)	115.85 (13.07)	149.92‡ (6.26)	137.54† (7.73)	128.67‡ (12.98)
BP_{DIA} (mm Hg)	75.97 (11.73)	89.67† (7.41)	82.61* (5.64)	82.16 (11.94)
MAP (mm Hg)	75.97 (11.73)	89.67‡ (7.41)	82.61‡ (5.64)	82.16‡ (11.94)
CVC_I MCA (cm/s/mm Hg)	0.66 (0.14)	0.51‡ (0.11)	0.54† (0.07)	0.58† (0.13)
$skBV_{HAND}$ (cm/s)	0.38 (0.30)	0.22 (0.15)	0.25 (0.16)	0.26 (0.14)

Table 3.1. **Mean (SD) values for systolic (BP_{SYS}), diastolic (BP_{DIA}), and mean arterial (MAP) blood pressure, index of cerebral conductance in the middle cerebral artery (CVC_I MCA) and skin blood flow of the hand ($skBV_{HAND}$) during the normocapnic pre-face immersion resting period, and in the 5, 15, and 33°C face immersions during mild hypercapnia. The rest period and immersion periods lasted 300 s. t-test comparisons are between Rest and the immersion conditions. Significance: * = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$.**

	Breathing Condition									
	Rest	5°C			15°C			33°C		
		5 s	75 s	155 s	5 s	75 s	155 s	5 s	75 s	155 s
Q (L/min)	3.20 (1.19)	5.49† (1.68)	3.05 (0.88)	3.31 (1.06)	6.18† (1.53)	3.15 (0.83)	3.29 (0.82)	5.14* (1.69)	3.23 (0.94)	3.58 (1.19)
SV (mL/beat)	50.31 (18.73)	67.86# (19.74)	52.43 (11.85)	54.91 (14.01)	75.11* (14.77)	52.73 (10.17)	53.42 (11.56)	66.97# (16.96)	52.72 (12.76)	56.68 (15.49)
HR (bpm)	62.07 (7.17)	81.51‡ (10.24)	57.94* (8.39)	59.78# (8.01)	81.77‡ (8.43)	59.38# (7.42)	61.16 (6.61)	73.94† (9.43)	60.98 (6.96)	62.59 (8.25)

Table 3.2. **Mean (SD) values for cardiac output (Q), stroke volume (SV) and heart rate (HR) during the normocapnic pre-face immersion resting period, and during 5, 75 and 155 s into face immersions in 5, 15, and 33°C water during mild hypercapnia. t-test comparisons are between Rest and the immersion conditions. Significance: # = p: 0.055 – 0.10; * = p < 0.05; † = p < 0.01; ‡ = p < 0.001.**

3.7. Figures

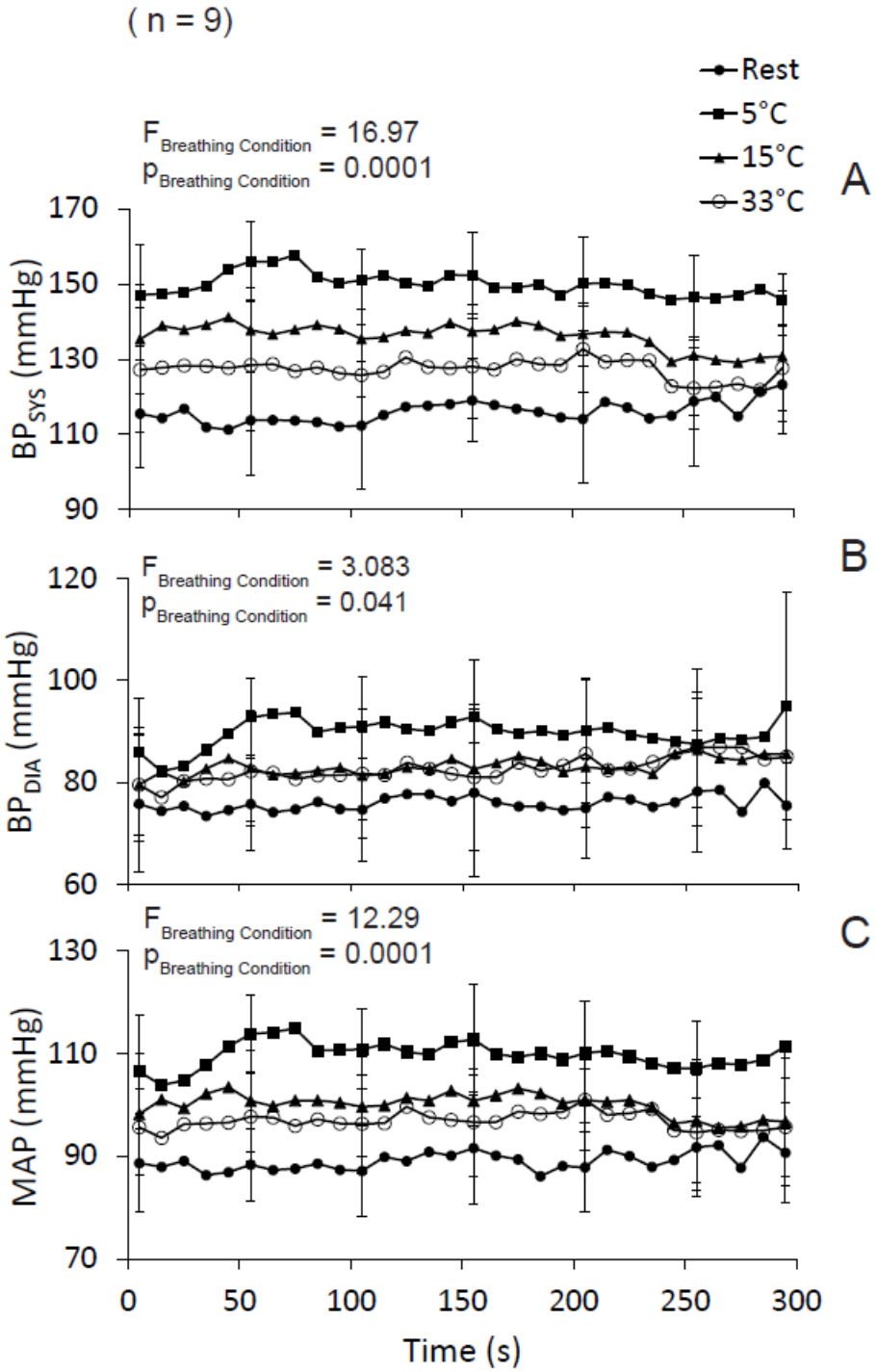

Figure 3.1. **Systolic (BP_{SYS}), diastolic (BP_{DIA}) and mean arterial (MAP) blood pressure during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

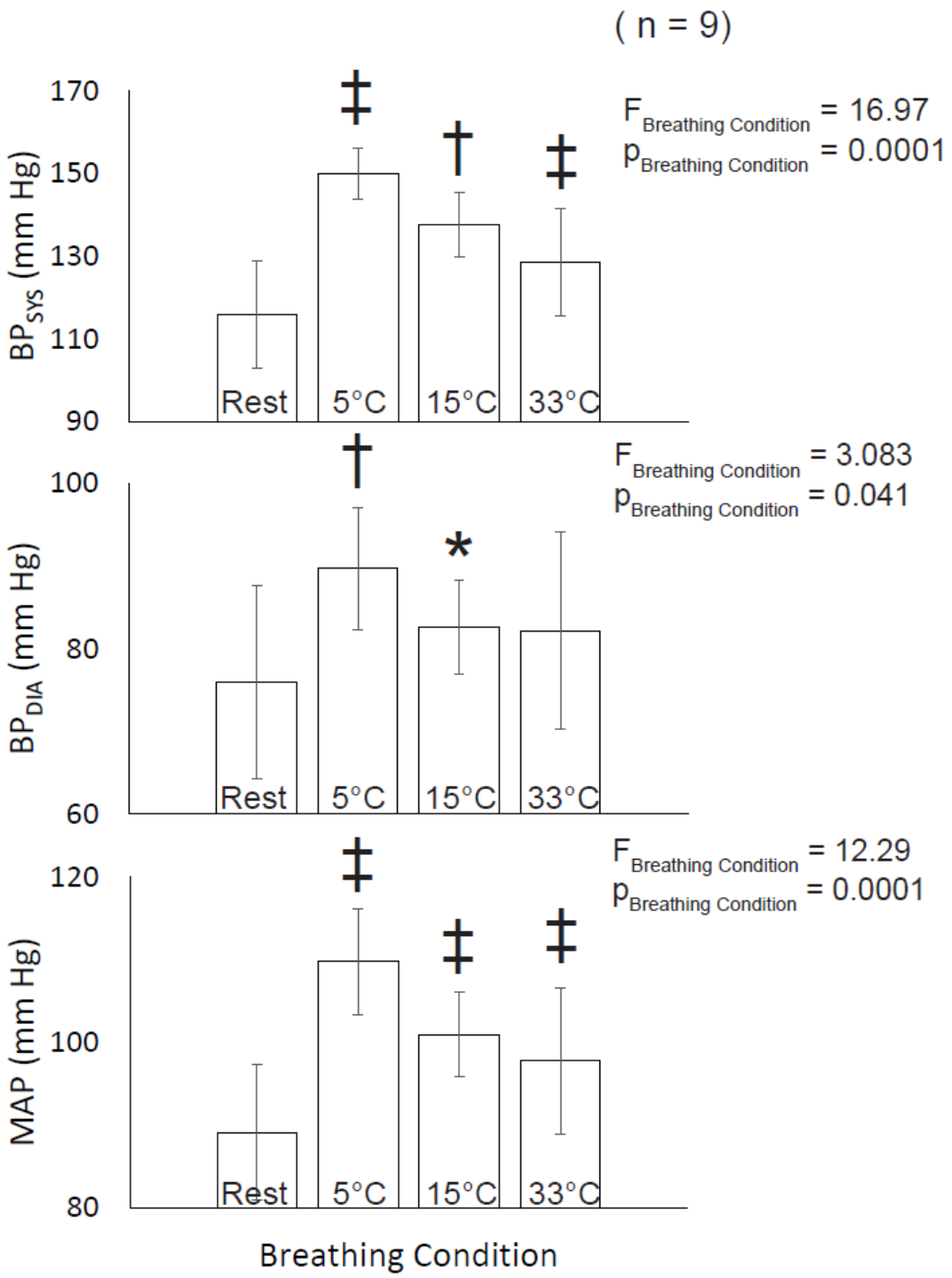

Figure 3.2. **Mean responses over the first 45 breaths of the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C for Systolic (BP_{SYS}), diastolic (BP_{DIA}) and mean arterial (MAP) blood pressure. Error bars represent the standard deviation. Significance: * = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$.**

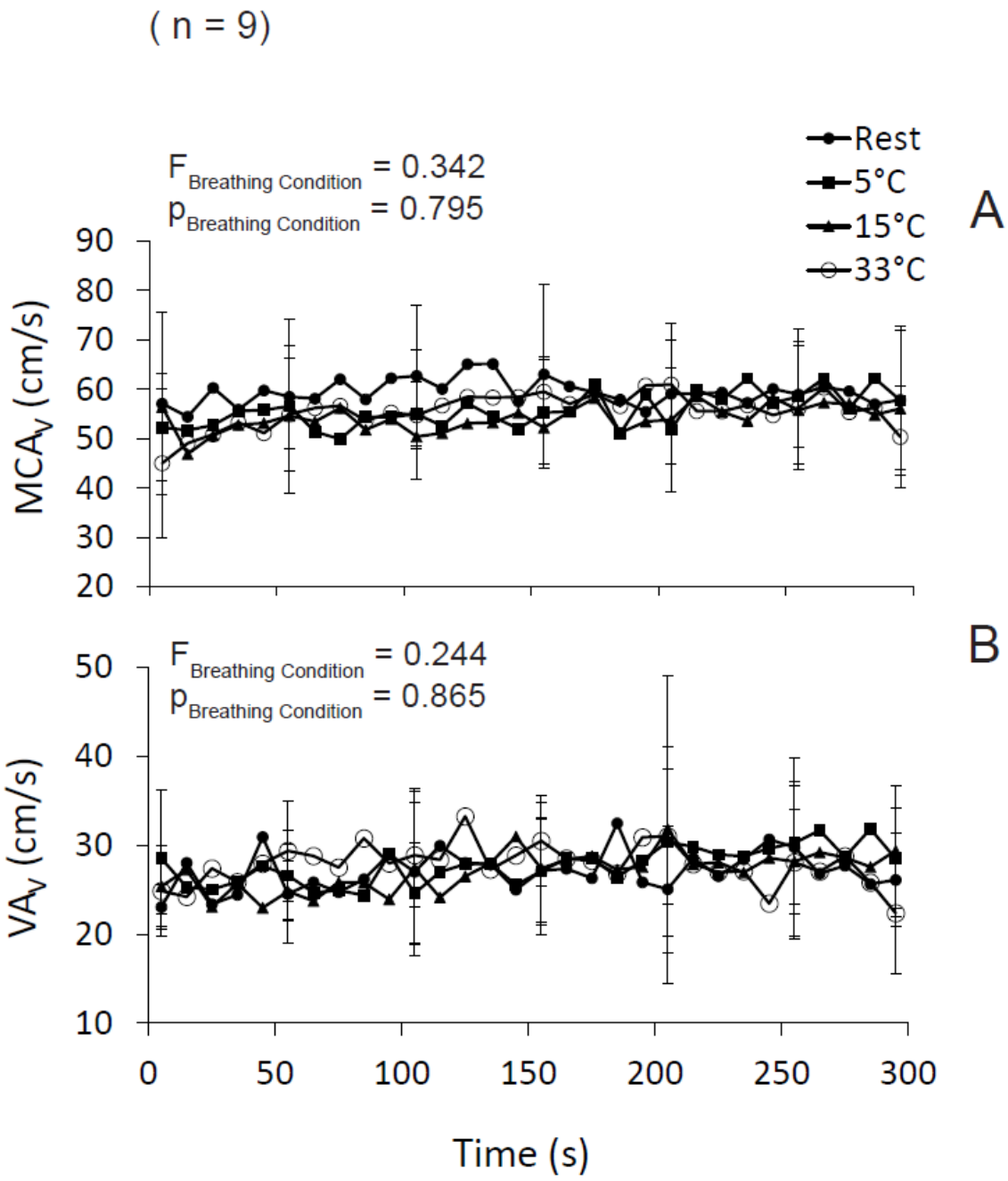

Figure 3.3. **Middle cerebral artery (MCA_V) and vertebral artery (VA_V) blood velocity during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

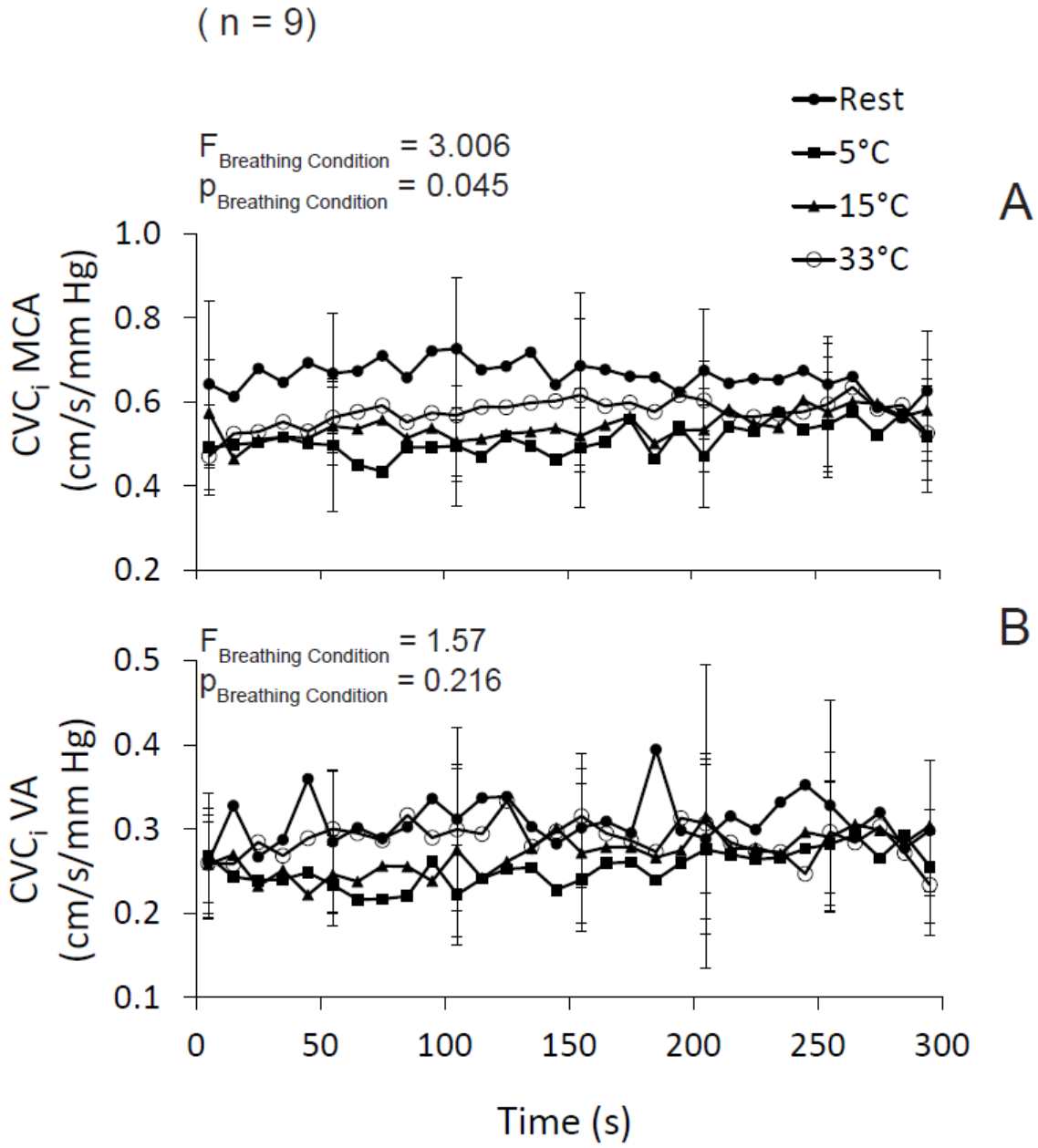

Figure 3.4. **Index of Cerebrovascular Conductance (CVC_i) in the middle cerebral artery (MCA) and vertebral artery (VA) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

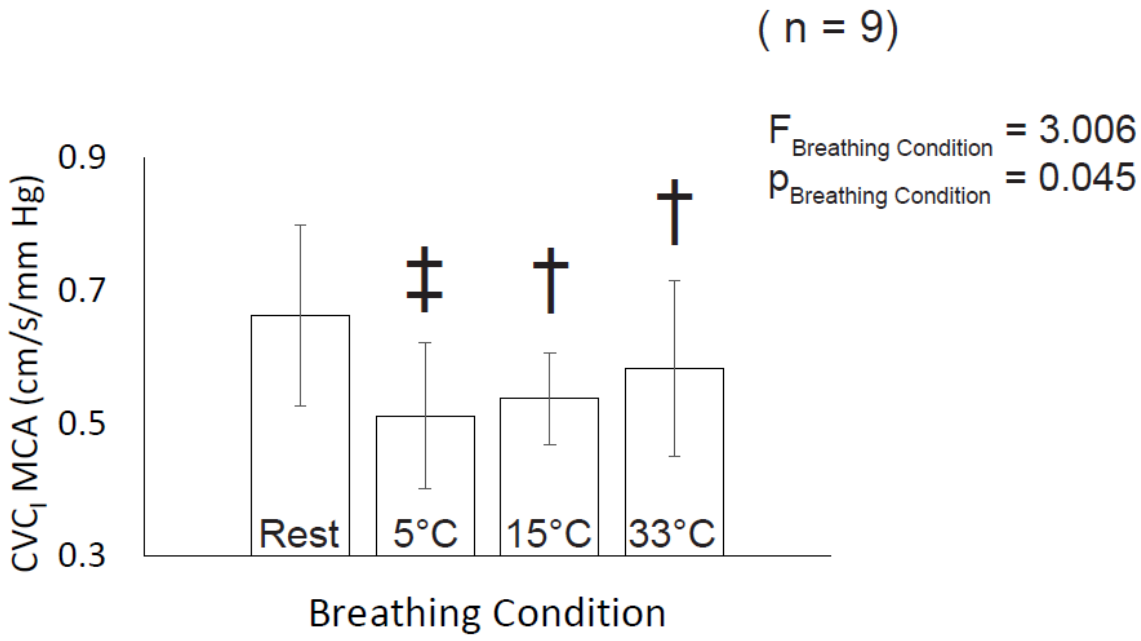

Figure 3.5. **Mean responses over the first 45 breaths of the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C for Index of Cerebrovascular Conductance (CVC_i) in the middle cerebral artery (MCA). Error bars represent the standard deviation. Significance: * = † = $p < 0.01$; ‡ = $p < 0.001$.**

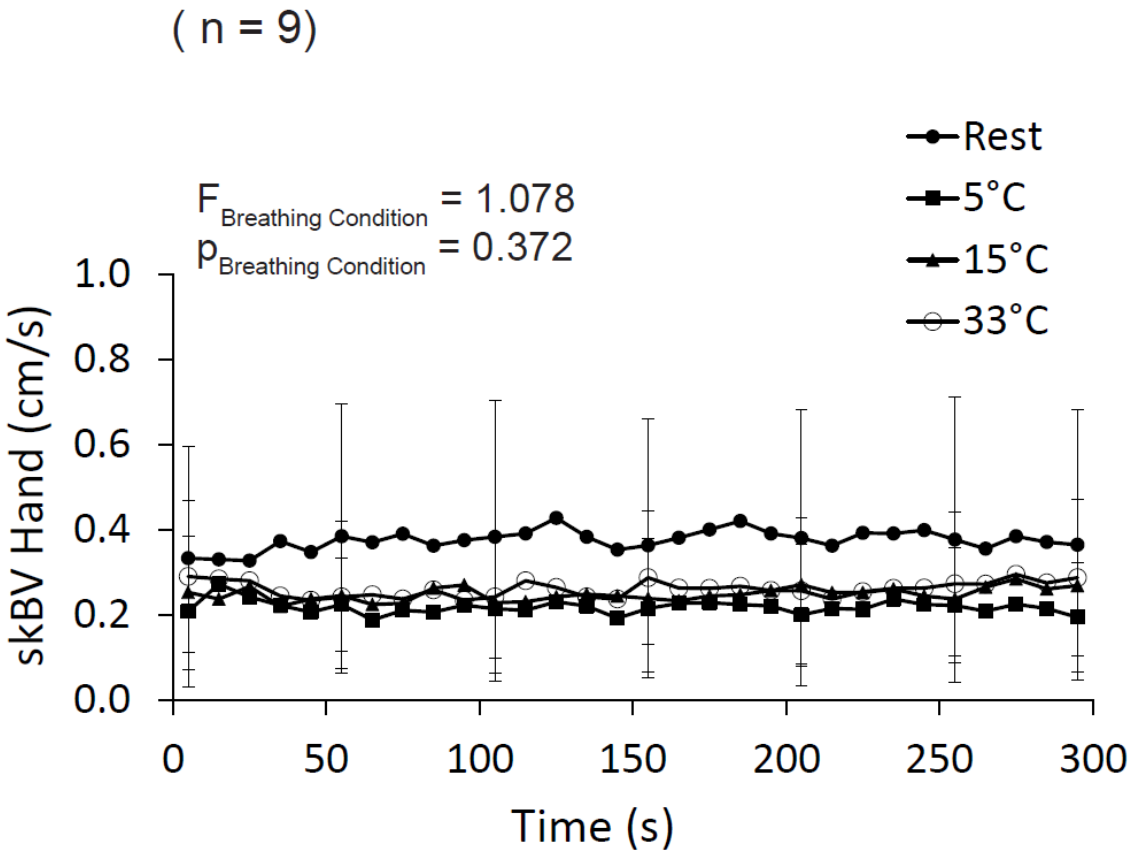

Figure 3.6. **Red blood cell velocity in the hand ($skBV_{HAND}$) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

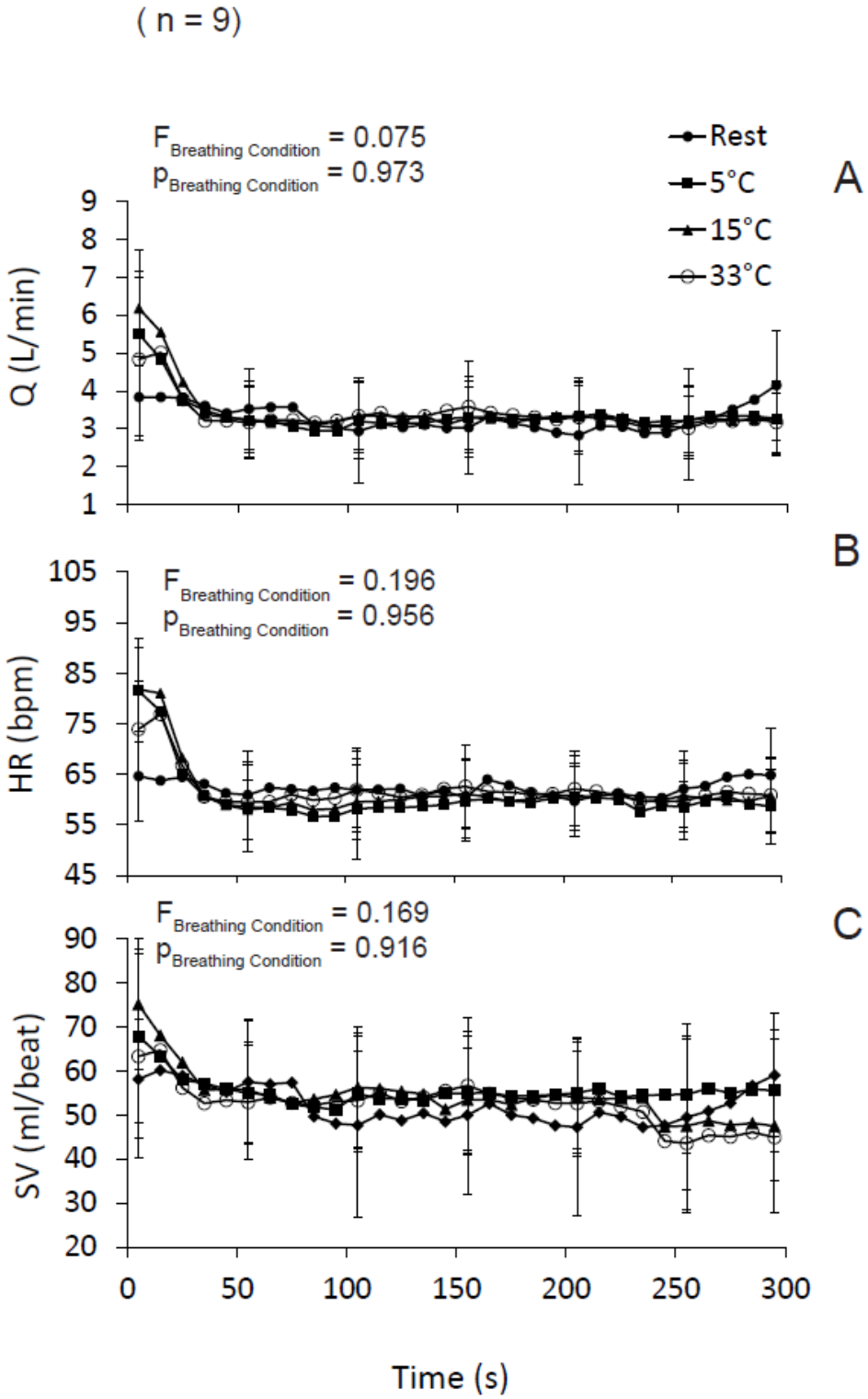

Figure 3.7. **Cardiac output (Q), heart rate (HR), and stroke volume (SV) during the rest period prior to immersion as well as during face immersion without breath hold in 5, 15 and 33°C. Error bars represent the standard deviation.**

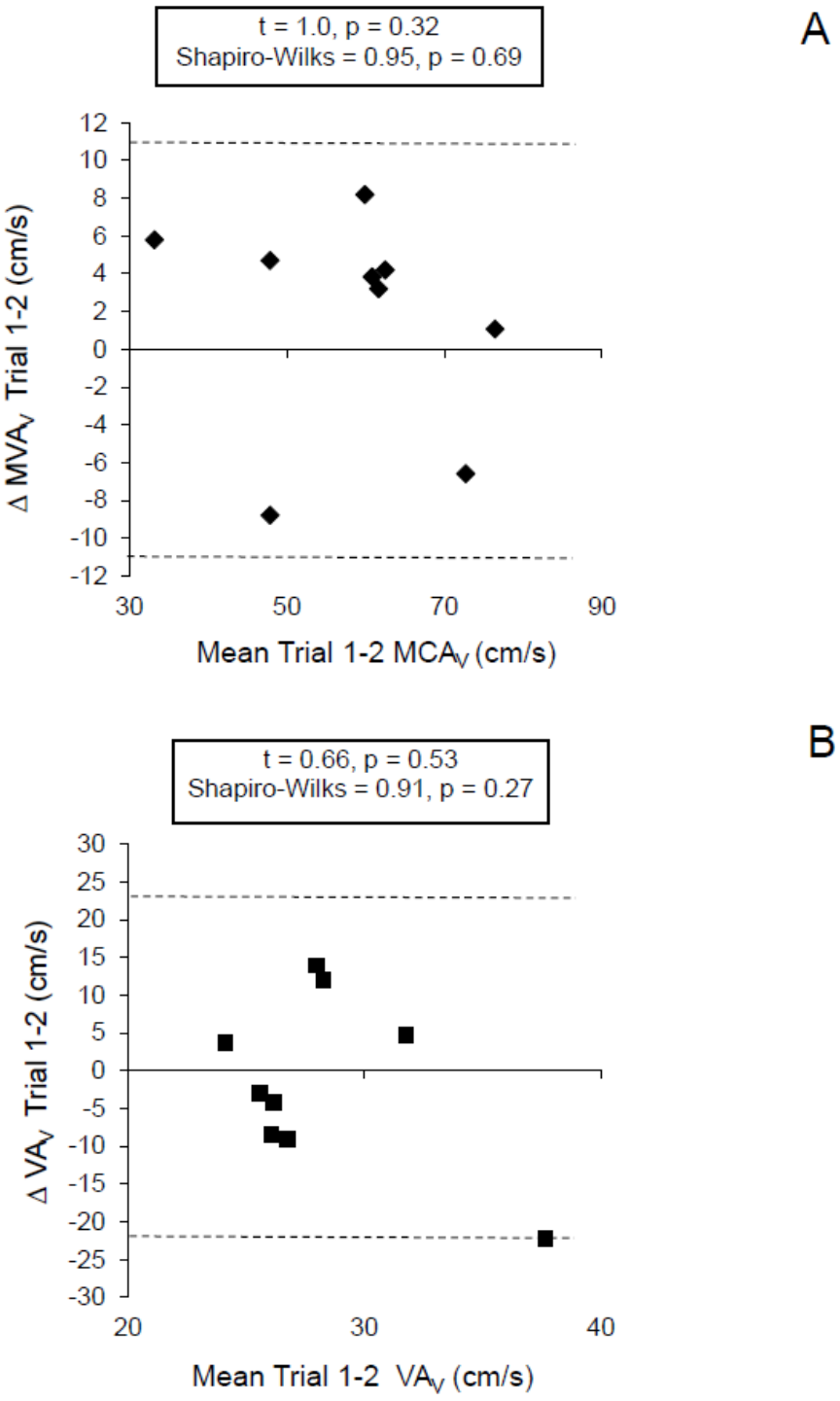

Figure 3.8. **Bland and Altman comparison of middle cerebral artery (MCA$_V$) and vertebral artery (VA$_V$) velocity at rest over two data collection days. Dotted lines are two standard deviations above and below the mean.**

Chapter 4. Response to Hypotheses

Study 1: Effect of Mild Hypercapnia and Skin Temperature on Pulmonary Ventilation Responses during Face Immersion

H1: It was hypothesized that the greatest inspiratory flow and inspiratory duty cycle will be seen during 5°C water face immersion when compared to immersion in both 15 and 33°C water.

A1: Over the first 45 breaths there was no significant difference between rest and the 5°C water face immersion for inspiratory flow, and the greatest duty cycle occurred during the 15°C water face immersion. Over an analysis of the first 5 breaths of the face immersions, it was found that the greatest inspiratory flow occurred during the first breath of face immersion across all water temperatures and these responses, as well as most of breaths 2,3 4 and 5, were significantly elevated relative to the resting values.

H2: It was hypothesized that the greatest increases in ventilation will be in a hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

A2: Over the first 45 breaths there was no significant difference between rest and the 5°C water face immersion for ventilation. Pulmonary ventilation, however, was increased during the first five breaths of face immersion and this increase was greatest in the 15°C face immersion.

Study 2: Effect of Mild Hypercapnia and Skin Temperature on Cardiovascular Responses during Face Immersion

H1: It was hypothesized that the greatest increases in mean MCA_V and VA_V, will occur during the mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

A1: There was no effect of water temperature on MCA_V and VA_V, however, CVC_I MCA gave a stepwise decrease when the face was immersed in progressively lower water temperatures.

H2: It was also hypothesized that the lowest skin blood velocity and greatest blood pressure, cerebrovascular conductance, and the greatest Q, SV and HR will occur during mild hypercapnic face immersion in 5°C water when compared to non-immersed normocapnic resting values.

A2: The greatest blood pressure did occur during the 5°C when compared to non-immersed resting values. There was no effect of water temperature on Q, HR and SV over the 300 s immersions, but these variables were analysed from 5 – 155 s into immersion and there was a two-phase response with an initial increase followed by a return to resting, non-immersed values.

Appendix A

Effect of Snorkel Length on Ventilatory Variables

Introduction

Breathing and $P_{ET}CO_2$ were assessed with and without a snorkel attached to the flow sensor to ensure the length of the snorkel did not influence $P_{ET}CO_2$.

Methods

One male participant that was 1.83 m in height, weighed 93 kg, and was 21 years of age laid supine on a bed with their face down. He breathed normally for 5 min with the snorkel and 5 min without the snorkel attached to the flow sensor housing. The volume of the flow sensor housing was 171 mL. The snorkel together with the flow sensor housing had a volume of 210 mL.

Results

Snorkel volume was plotted on a graph comparing snorkel volume and $P_{ET}CO_2$ (Julia P.H. Christensen, 2004). The volume of the flow sensor is 171 mL whereas the volume including the snorkel is 210 mL. The participant (Vol1) breathing without the snorkel (Vol1-171) was very close to previously collected data (Figure 5.1). When the participant was breathing through the snorkel (Vol1-210), the datum was 0.1 kPa (0.75 mm Hg) different from the trend line for the previously collected data.

Figures

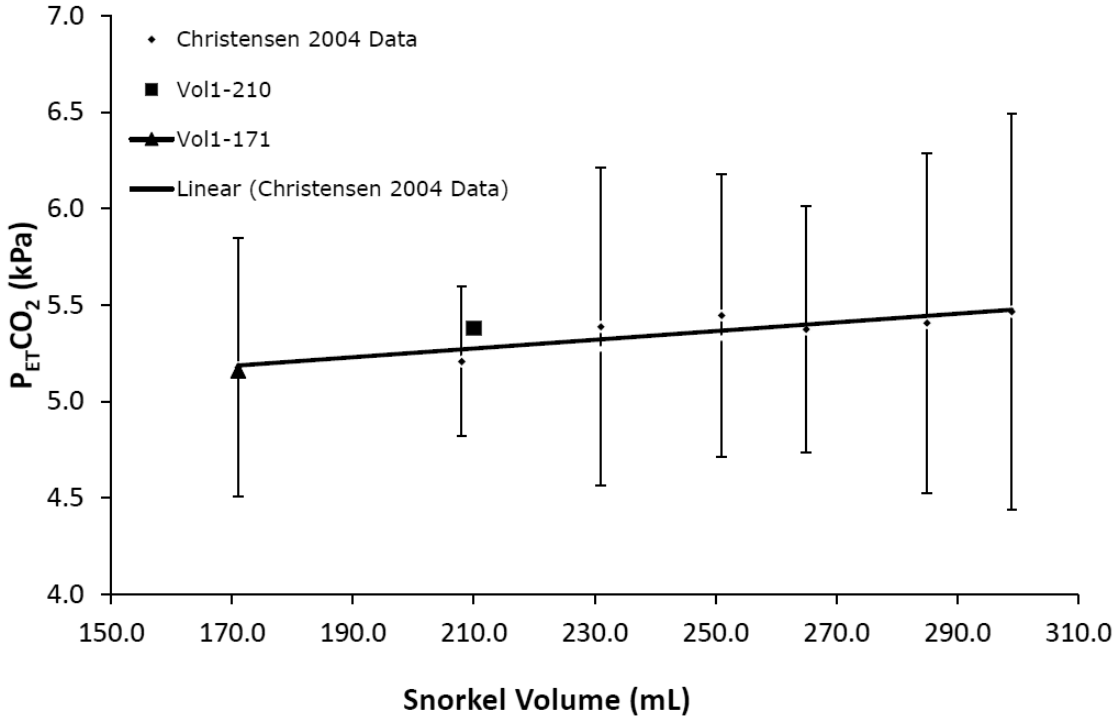

Figure A.1. Pilot Study 1: Partial Pressure of end-tidal carbon dioxide given as function of snorkel volume for volunteers in the prone position (Christensen, 2004) with data from 1 volunteer (Vol1) plotted on top.

Appendix B

Efficacy of Sommer Circuit

Introduction

This pilot was to test that PETCO2 can be clamped to baseline values during varying levels of hyperventilation using the Sommer method (Sommer et al., 1998).

Methods

Four participants (three males, one female) were tested in this pilot study. They had a mean height of 1.81 (0.72) m, a mean weight of 82.1 (10.6) kg, and a mean age of 23.5 (2.1) years. Ventilation, $P_{ET}CO_2$, F_ICO_2, F_IO_2, and V_T were analyzed using a repeated measures ANOVA. There were two CO_2 conditions, poikilocapnia and eucapnia in which four volunteers were tested. During poikilocapnia, volunteers breathed room air through the mouthpiece and flow sensor. For both trials, the volunteer was instructed to breathe as normally as possible for a 5 min rest period. The volunteer was then instructed to ventilate at a rate of 20, 30 and 40 L/min for 1 min each. Each volunteer was coached to increase his rate and depth of breathing in order to maintain the prescribed steady rate of ventilation. There were rest periods between each hyperventilation to allow $P_{ET}CO_2$ to stabilize to rest values. At the end of the trial the volunteer breathed normally for another 5 min.

Prior to the eucapnic trial, the flow rate for the air gas tank was set to equal the volunteer's pulmonary ventilation at rest. The cracking pressure on the diving regulator was set to ensure there was not excess resistance in order for the regulator to crack when the volunteer ventilated at higher rates than at rest.

Ventilatory responses made during these trials included V_E, $P_{ET}CO_2$, F_ICO_2, F_IO_2, and V_T.

Results

For the main effect of Pulmonary Ventilation Rate, V_E was became progressively greater at the higher levels of this factor ($F = 149.67$, $p < 0.0001$) for both the Clamp and No Clamp trials (Figure 4.5A). $P_{ET}CO_2$ was significantly ($F = 24.39$, $p < 0.0001$) lower across all levels of Pulmonary Ventilation Rate and became progressively lower at the higher levels of this factor of Pulmonary Ventilation Rate for the No Clamp trial (Figure 5.2B). F_ICO_2 was significantly ($F = 13.09$, $p < 0.0001$) greater in the Clamp relative to the non-Clamp condition for Pulmonary Ventilation Rate. As expected, tidal volume was significantly ($F = 7.75$, $p = 0.003$) greater as the volunteer hyperventilated more since they were instructed to increase V_T in order to increase V_E. Tidal volume increased ($F = 7.75$, $p = 0.003$) from 1.01 L/breath at rest, to 1.22 L/breath, 1.72 L/breath, 2.00 L/breath during 20 L/min, 30 L/min and 40 L/min Pulmonary Ventilation Rates, respectively.

For a main effect of Clamping, V_E was not significantly different ($F = 6.01$, $p = 0.092$), and $P_{ET}CO_2$ was significantly ($F = 21.22$, $p = 0.019$) lower in the No Clamp condition when compared to the Clamp condition. Fraction of inspired O_2 was significantly ($F = 181.07$, p

< 0.0001) higher in the No Clamp condition when compared to the Clamp condition (Figure 5.3B) for the main effect of clamping, whereas F_ICO_2 was not significant (F=3.39, p = 0.16).

The interaction between Clamping x Pulmonary Ventilation Rate yielded significant results for $P_{ET}CO_2$ (F = 21.84, p < 0.0001), F_ICO_2 (F = 11.89, p < 0.0001), F_IO_2 (F = 3.34, p = 0.047).

Figures

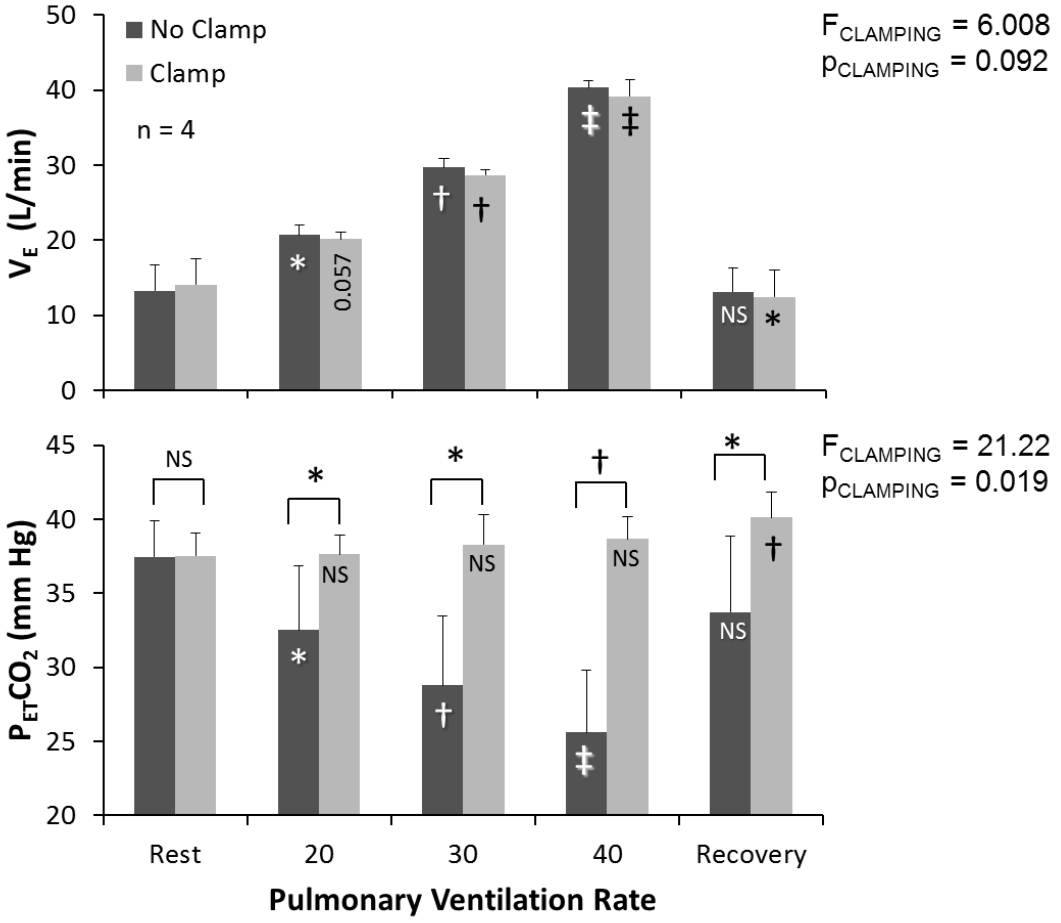

Figure B.1. Pilot Study 2: Ventilation (A) and partial pressure of CO_2 (B) during rest and varying levels of Pulmonary Ventilation Rate (V_E) with and without CO_2 – clamping. Recovery is 5 min of rest collected after the 40 L/min Pulmonary Ventilation Rate. Significance markers on bars are compared to rest within the same condition. Significance: NS = p > 0.05; * = p < 0.05; † = p < 0.01; ‡ = p < 0.001.

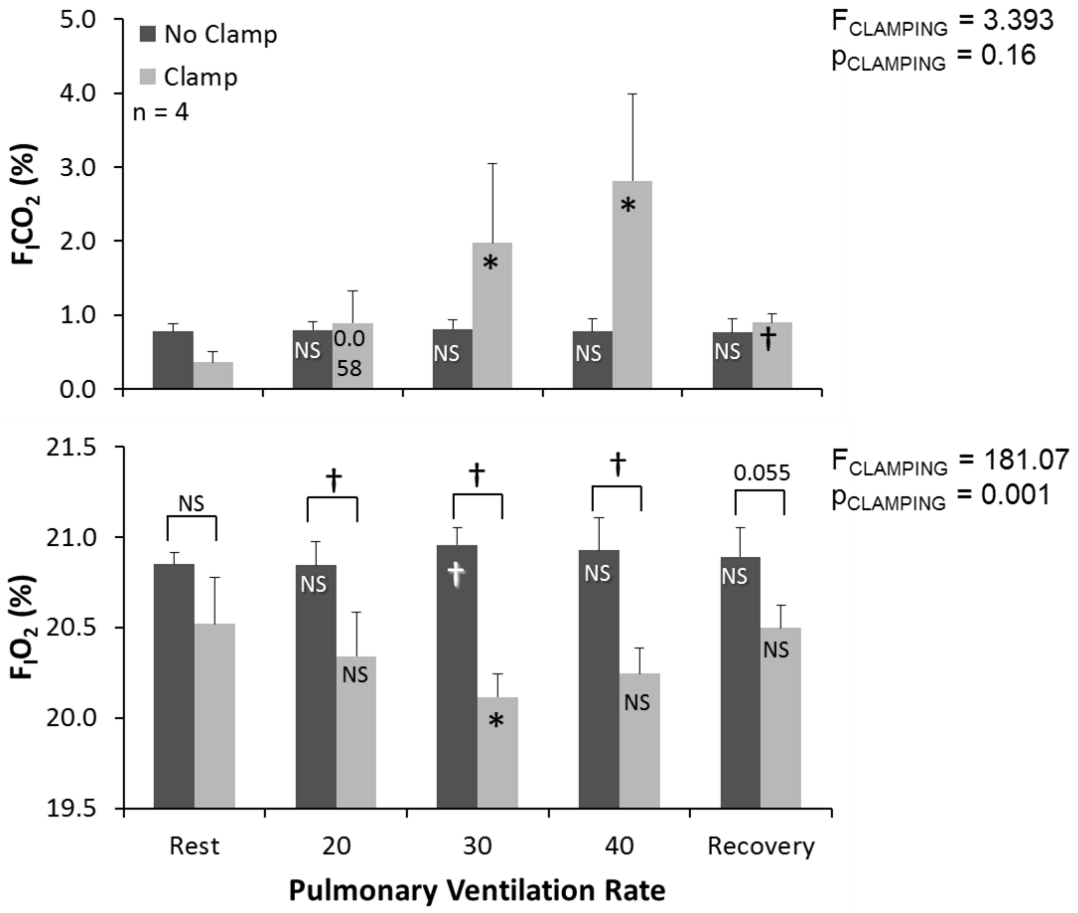

Figure B.2. ***Pilot Study 2: Fraction of inspired CO_2 (A) and O_2 (B) during rest and varying levels of Pulmonary Ventilation Rate (V_E) with and without CO_2 – clamping. Recovery is 5 min of rest collected after the 40 L/min Pulmonary Ventilation Rate. Significance markers on bars are compared to rest within the same condition. Significance: NS = p > 0.05; * = p < 0.05; † =p < 0.01.***

Appendix C

Effect of Repeated Face Immersions

Introduction

The effect of repeated face immersions was tested to assess if repeated immersions influenced the main outcome variables.

Methods

Four male participants were tested and had a mean height of 1.85 (0.24) m, mean weight of 82.9 (9.3) kg and mean age of 24.3 (2.8) years. The test day protocol included five successive face immersions in 5°C water all in poikilocapnia. Each of the immersions lasted 5 min and was under poikilocapnic conditions. The time between immersions was enough to allow the face temperature to return to resting values. The variables that were collected included V_E, $P_{ET}CO_2$, HR, MCA_V, BP_{SYS}, BP_{DIA}, MAP, mean immersed and non-immersed T_{SK}.

Acclimation data were analyzed using 1-factor ANOVA with the factor Immersion Number to determine difference across immersions 1 through 5. The level of significance was set to 0.05.

Results

Ventilation, $P_{ET}CO_2$, HR, MCA_V, BP_{SYS}, BP_{DIA}, MAP, mean immersed and non-immersed T_{SK} were analyzed using a 1-factor ANOVA for the factor Immersion Number. There was no significant ($p > 0.05$) effect of Immersion Number on V_E, $P_{ET}CO_2$, HR, MCA_V, BP_{SYS}, or non-immersed T_{SK}.

There was a significant effect of Immersion Number on Diastolic BP ($F = 3.15$, $p = 0.046$) and this difference was between immersion 1 and 3 where immersion 3 increased by 16.6 ($p = 0.034$) mm Hg (Figure 5.4A). MAP showed a trend toward significance for Immersion Number ($F = 2.52$, $p = 0.085$) and this difference was between immersions 1 and 3 where immersion 3 increased by 15.5 mm Hg ($p = 0.08$) (Figure 5.4B). There was also significant effect of Immersion Number on Immersed T_{SK} ($F = 6.48$, $p = 0.004$). Each of immersions 2 – 5 were significantly decreased when compared to immersion 1 (Figure 5.5). Immersion 2 immersed T_{SK} that decreased by 2.88°C ($p = 0.052$), immersion 3 decreased by 3.43°C ($p = 0.017$), immersion 4 decreased by 4.01°C ($p = 0.005$), and immersion 5 decreased by 4.28°C ($p = 0.003$) when compared to immersion 1, respectively.

Figures

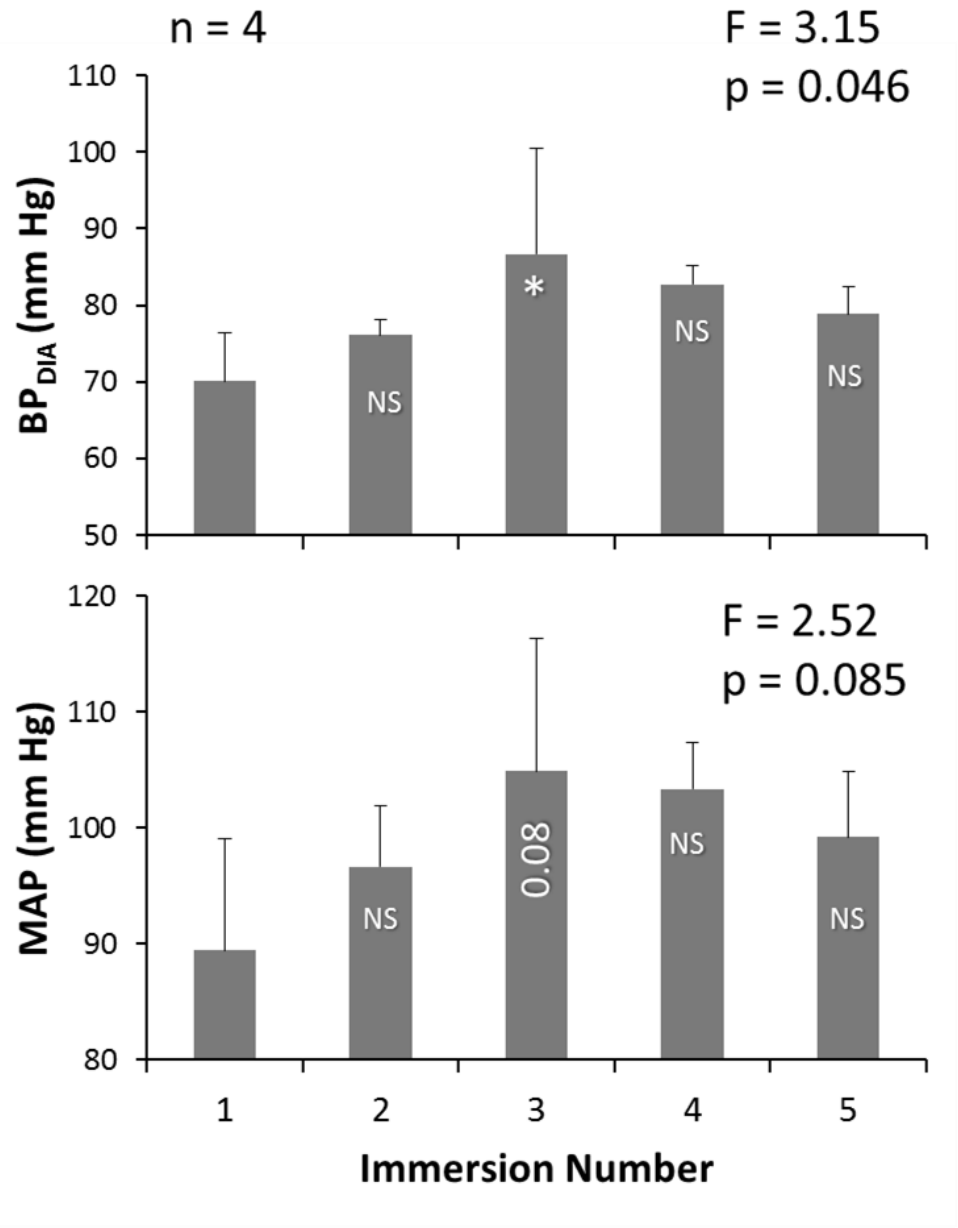

Figure C.1. ***Pilot Study 3: Diastolic blood pressure (A) and mean arterial blood pressure (B) during consecutive face immersions in 5˚C water. Immersions 2 – 5 each are compared to immersion 1. Significance: NS = p > 0.05; * = p < 0.05. F and p values are for the factor Immersion Number.***

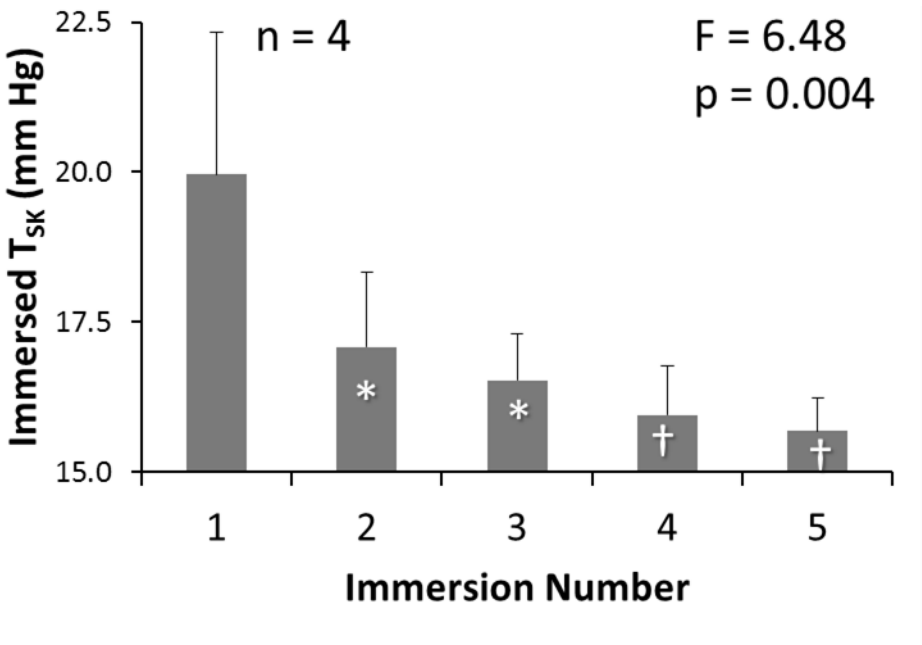

Figure C.2. **Pilot Study 3: Mean immersed skin temperature during consecutive face immersions in 5˚C water. Immersions 2 - 5 each are compared to immersion 1. Significance: NS = $p > 0.05$; * = $p < 0.05$; † = $p < 0.01$.**