HYPOXIC RESPONSES IN RESTING HYPERTHERMIC HUMANS

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Abstract

This thesis investigated the interaction between steady state hypoxia and passive hyperthermia on human ventilation and the influence of the $P_{ET}CO_2$ on this interaction. On one of two days males twice breathed 12% oxygen for 20 min while either normothermic or hyperthermic with $P_{ET}CO_2$ clamped ~1 mm Hg above resting (iHVR). On the other day the same tests were performed except $P_{ET}CO_2$ was uncontrolled (pHVR). Hyperthermia increased euoxic ventilation compared to normothermia ($p \leq 0.001$). During iHVR ventilation increased more during hyperthermia than normothermia ($p=0.002$), but not during pHVR ($p=0.98$). Heart rates at conclusion of pHVR compared to iHVR were greater for normothermia ($p=0.05$) and hyperthermia ($p=0.004$). Therefore during pHVR the decreasing $P_{ET}CO_2$ levels blunt the ventilatory response to hypoxia more than hyperthermia augments it as seen during iHVR. Also pHVR increases cardiovascular responses to hypoxia more than iHVR. An end tidal forcing system was developed to control $P_{ET}CO_2$.

(147 words)

KEYWORDS:

Hyperthermia, hypoxia, altitude, hvr, cardiovascular, ventilatory, rest

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"Have a wonderful week, a great month and a pretty not bad year."

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Abbreviations and Acronyms

The following abbreviations and units have been used throughout this thesis, in addition to those abbreviations commonly accepted.

ATP ..... adenosine triphosphate
AVP ..... arginine vasopressin
Ca^{2+} ..... calcium ion
CA ..... carbonic anhydrase
cal ..... calorie
CBF ..... Cerebral blood flow
CBV ..... Central blood volume
CNS ..... central nervous system
CO_2 ..... carbon dioxide gas
COPD..chronic obstructive pulmonary disease
CSF ..... cerebral spinal fluid
DRG ..... dorsal respiratory group
ETF ..... end-tidal forcing
f_R ..... respiratory frequency
g ..... grams
h ..... hour
H^+ ..... hydrogen ion
H_2O ..... water
H_2CO_3 ..... carbonic acid
HCO_3^- ..... bicarbonate ion
HR ..... heart rate
HCVR ..... hypercapnic ventilatory response
HVD ..... hypoxic ventilatory decline
HVR ..... hypoxic ventilatory response
IA ..... Immersed rest in Air
IH ..... Immersed iHVR test
iHVR ..... isocapnic HVR
ISS ..... Initial Steady State
I_{rest} ..... body insulation at rest
K^+ ..... potassium ions
kg ..... kilogram
kPa ..... kilopascal
L ..... liter
$\dot{M}$ ..... rate of metabolic heat production
m ..... meter
min ..... minute
mm ..... millimeter
mm Hg ..... millimeters of mercury
n ..... number of participants
N_2 ..... nitrogen gas
Na^+ ..... sodium ion
NE ..... Nor-epinephrine
O_2 ..... oxygen gas
p ..... alpha value
PI ..... exercise ventilation phase one
PII ..... exercise ventilation phase two
PIII ..... exercise ventilation phase three
PIV ..... exercise ventilation phase four
P_ACO_2 ..... alveolar CO_2 pressure
P_AO_2 ..... alveolar O_2 pressure
P_aCO_2 ..... arterial CO_2 pressure
P_aO_2 ..... arterial O_2pressure
$P_{ET}CO_2$ ..... end-tidal CO_2 pressure
$P_{ET}O_2$ ..... end-tidal O_2pressure
pHVR ..... poikilocapnic HVR
pH_a ..... arterial pH
P_IO_2 ..... inspired oxygen pressure
pmol ..... pico moles
r ..... correlation coefficient
RAR's ..... rapidly adapting receptors
$\dot{S}$ ..... rate of body heat stores
s ..... second
S_aO_2 ..... % saturation of arterial hemoglobin
SAR's ..... slow adapting receptors
SEM ..... standard error of the mean
SkBF ..... skin blood flow
SNS ..... sympathetic nervous system
SD ..... standard deviation
T_c ..... core body temperature
T_{es} ..... esophageal temperature
T_{re} ..... rectal temperature
T_{sk} ..... skin temperature

T_{tp} ..... tympanic temperature
T_w ..... water temperature
UA ..... Unimmersed rest in Air
UCP ..... uncoupling protein
$\dot{V}_E$ ..... ventilation rate
VRG ..... ventral respiratory group
VMS ..... ventral medullary surface
$\dot{V}CO_2$ ..... rate of CO_2 production
$\dot{V}O_2$ ..... rate of O_2 consumption
$\dot{V}O_{2max}$ ..... maximal rate of O_2 consumption
V_T ..... tidal volume
W ..... Watts

CHAPTER 1

Thesis Overview

The purpose of this short chapter is its is to be used as a guide for reading this thesis.

Initially the wider topics of thermoregulation and control of ventilation are broadly discussed in Chapter 2. Hypoxia is then identified as an area of interest for its influence on ventilation, and the methodologies and factors influencing the human responses to hypoxia are discussed. As both hypoxia and body temperature affect ventilatory responses to exercise, the phases of ventilation during exercise are described, followed by a brief review of several hypotheses on the control of ventilation during exercise. As body temperature increases during exercise and this increase can affect ventilatory responses, these responses and then their possible mechanisms are expressed. Linking back to the earlier discussion on hypoxia, the potential interaction between a raised body temperature and human responses to hypoxia is identified as a relatively unexplored area of the literature. As such the research objectives are developed in the review of literature summary. Following these objectives are the research hypotheses with testable questions that will address the issues raised.

The following two chapters are divided into separate studies which address the hypotheses and objectives of this thesis. Chapter 3 investigates the independent and combined effects of passive hyperthermia and isocapnic hypoxia on ventilatory responses. Chapter 4 extends the knowledge gained with isocapnic hypoxia and

hyperthermia to investigate how carbon dioxide tensions influence the interaction between hyperthermic and hypoxic ventilatory and cardiovascular responses.

Chapter 5 explains the development of a dynamic end-tidal forcing which instead of using a continuous flow system utilizes a breath-by-breath gas bolus injection to force the end-tidal partial pressures of CO_2 towards their desired value.

The thesis is concluded in Chapter 6 which specifically responds to the research hypotheses and testable questions. The Appendix A that follows contains definitions for some of the terms used in this thesis, while appendix B describes pilot work used in the derivation of the research hypotheses.

Throughout the thesis reference citation numbers are applicable to that chapter or appendix only, and concordantly there is a separate reference section at the end of each chapter or appendix.

CHAPTER 2

Literature Review

The primary goal for a single cell or the whole organism is to function optimally in its environment. This goal is carried out independently or mutually by many cellular or whole body regulatory systems and/or whole body behaviors. Such behaviors include drinking, feeding and resting, while some systems are the cardiovascular, thermoregulatory and ventilatory systems. Independently the human thermoregulatory system functions to maintain body temperature and the ventilatory system to help and meet metabolic demands during rest and exercise. These two systems also interact as changes in body temperature affect responses of the ventilatory system, and these responses may in turn affect thermoregulation. The purpose of this review is to investigate the interaction between the ventilatory and thermoregulatory systems, with specific emphasis on control of ventilation during increases in body temperature.

2.1 Temperature regulation

Animals, insects and bacteria all attempt to maintain their body temperatures within a certain range using behavioral and/or physiological responses (135). Behavioral responses include the following: bacteria forming a dense band at 34°C in a 17 to 39°C thermal gradient (88), reptiles basking in the sun or burrowing in the ground to change thermal status (37; 142), and mammals huddling together, taking warm showers or baths, or using physical means to change the room temperature in order to remain thermoneutral (135). Thermophysiological responses include vasomotion, shivering, adaptive

thermogenesis, sweating, fluid-regulatory changes and panting. Due to evolution these responses vary between organisms, however the focus of this review will be on human responses.

Originally it was understood that mammals and humans regulated their body temperature using a set-point approach much like a heaters' thermostat (23; 65). Contention arose about whether this was so when Mekjavic et al. (1991), demonstrated the presence of a thermoneutral zone, between where sweating stopped (esophageal temperature (T_{es}) 37.42 ± 0.29 (SD)°C) and shivering began (T_{es} 36.84 ± 0.38 (SD)°C) in 9 male subjects cooling down after exercised immersed in water (28°C) from a mild hyperthermic to a mild hypothermic state (98). Since then more studies have demonstrated the presence of a thermoneutral zone (3; 55; 180) and this hypothesis is gaining acceptance (151). The presence of a thermoneutral zone is also evident in many other species (14).

In humans, numerous physiological responses are largely under autonomic control and the main, but not the sole, controlling center for temperature regulation is the hypothalamus. Human thermoregulatory mechanisms should be considered multiple rather than unitary (135). The hypothalamus detects changes in the body's thermal state directly from changes in blood temperature and indirectly by integrating inputs from the peripherally located skin and muscle thermosensors (61). The hypothalamus is the key controller of body warmth with its anterior portion primarily activating responses for heat dissipation and its posterior portion primarily for heat production and conservation (135; 147).

2.1.1 Physiological responses to cold exposure

Insulative

Heat is transferred between humans and their environment via either convection, conduction, radiation, and/or evaporation. When the heat gradient favors body heat loss, the human body has three mechanisms of defense. Firstly, an insulative response when stimulation of cold sensors in the skin produces vasoconstriction whereby blood is redirected from the cooler periphery to the warmer core. Skin blood flow (SkBF) can change from 250 mL · min^{-1} in a thermoneutral state down to almost 0 mL · min^{-1} under extreme cold stress while resting (76). With further cooling at rest, skin, subcutaneous fat (27) and poorly perfused muscle from the decreased peripheral blood flow (120; 157) become the body's insulating instruments to prevent further heat loss.

Shivering

The second response is active thermogenesis through an increased metabolic rate and the associated increase in heat production. The onset of shivering to increase metabolic heat production occurs at a T_{es} of 36.84 ± 0.38°C (98). This threshold however does vary, six males and one female of average fitness had a mean T_{es} threshold of 36.2 ± 0.3°C (81), seven male submariners (33 ± 3 yr) mean T_{es} threshold was 36.01°C (29), while 10 male military personal (22.8 ± 1.4 yr) mean T_{es} threshold was 35.8 ± 0.2°C (29). For these studies differences in skin temperature (T_{sk}) may have affected the shivering onset thresholds as T_{sk} is one variable which has a large influence on shivering onset. Experiments on goats in which core or T_{sk} remained clamped while the other was altered demonstrated that both T_{sk} and T_{es} provide multiplicative and additive inputs to metabolic heat production through shivering (74; 109). The shivering started at a higher T_{es} when

the T_{sk} was lower and conversely when T_{sk} was higher shivering started at a lower T_{es}. Increased age and body fat content decrease the time to onset of shivering, while a greater cold acclimatization status and ability to draw on non-shivering thermogenesis increase the time to and core temperature level at the onset of shivering (26; 54; 83; 151).

Adaptive thermogenesis

Adaptive or non-shivering thermogenesis is a more complex form of metabolic heat production, which at a very basic level increases basal metabolic rate. This process is primarily mediated by hormones, specifically the thyroid hormones and norepinephrine (NE). Individually, the thyroid hormones stimulate some metabolic processes (e.g., Na^+/K^+-ATPase, Ca^{2+} cycling in muscle) that generate heat (146). The main molecules responsible for adaptive thermogenesis are uncoupling proteins (UCP) (51). The thyroid hormones interact peripherally with the sympathetic nervous systems (SNS) neurotransmitter, NE, to promote the synthesis of UCP. Basically, UCP substitute themselves for ATP synthetase, thereby uncoupling oxidative re-phosphorylation, leading to the dissipation of heat from all the energy produced in the respiratory chain (184). The main site for this is brown adipose tissue and the uncoupling protein-1 (26). There are however, at least five UCP whose individual functions are still under investigation (51).

Adaptive thermogenesis in brown adipose tissue is a well documented metabolic response to cold stress in infants and several animal species. In adult humans, the relative functional capacity of adaptive thermogenesis is diminished due to their increased volume relative to surface area. Our basal metabolic rate can provide much of the adaptive thermogenesis required for warmth with our current lifestyles based around sufficient clothing and indoor living (26). Although, as the need arises adult human's can

increase their use of adaptive thermogenesis through increases in basal metabolic rate, as evident in winter swimmers (162). However, more consideration is now given to adult human's being able to increase uncoupling protein-1 concentrations in white adipose tissue (116), or possibly regain brown adipose tissue use as other primates can do (60) and therefore acclimate and/or acclimatize to the cold (26).

2.1.2 Physiological responses to heat exposure

As with cold environments, human's also have the ability to adapt to heat-stressful environments by lowering the thresholds for our acute thermoregulatory responses to heat stress (4; 118; 182). Body core temperature increases are detected by the anterior hypothalamus, thermosensitive neurons in the central neurons system (CNS) and deep muscle thermosensitive neurons. Such increases can be from passive or active exposure to environments where the heat gradients between the skin and air and/or water favor heat gain. Increases in the rate of heat storage occurs through two main forms: an increase in the rate of heat absorbed from the environment, through increased rates of conduction, convection or radiation; and the elevated rate of conversion of chemical energy to thermal energy that is coupled to increased movement, or alternatively the Q_{10} effect, both causing an increase in metabolic processes (14).

Skin Blood Flow

If extra heat is stored in the body there are two main responses to remove it, a vasomotor and a sweating response. Cutaneous vasodilation is increased by active stimulation of cutaneous blood vessels via release of acetylcholine and other unknown neurotransmitter(s) to raise SkBF. Blocking of SNS activity does not remove the active vasodilation response meaning part of the response is due to co-transmitters (79). A

simultaneous SNS adrenergic vasoconstriction of the viscera and areas of the body with low metabolic demands also occurs in order to maintain mean arterial pressure (112). During passive exposure to intense dry heat (~100°C inside a sauna), SkBF increases from 0.5 to 7.0 $L \cdot min^{-1}$ (161). For every 1°C rise in core body temperature, SkBF was noted to increase by ~3 $L \cdot min^{-1}$ (161). Increases in skin blood flow also occur during exercise in temperate and heat stressful environments (75; 80). When five males biked for 20 min at 25°C and 35 % relative humidity (RH), an increase in T_{es} of between 0.2 to 0.3°C from resting was required to increase forearm SkBF (150). Whereas, eight men either cycling for 20 to 30 min at 100 to 150 W in water perfused suits to maintain T_{sk} at 38 to 38.5°C, required an increase in T_{es} from resting of between 0.28 to 0.71°C to increase forearm SkBF (77). Therefore a greater thermal load on the body through increased physical activity and/or passive exposure to heat-stressful environments will increase SkBF to aid in thermoregulation, however this may not always be enough to maintain body temperature.

Eccrine Sweating

In heat stressful environments the skin to air thermal gradient often favors heat gain. To combat this gradient the body uses the evaporation of eccrine sweat from the skin to place the thermal gradient again in favor of heat loss. Evaporation of sweat cools the skin, which in turn cools the blood passing near the skin. Increased SNS output activates the cholinergic sympathetic nerve fibers which innervate eccrine sweat glands causing them to secrete large amounts of sweat that resembles a hypotonic saline (NaCl) solution (111). Stephenson et al. (1984), showed an increase in T_{es} of between 0.05 to 0.10°C from resting, initiated an increase in sweat output on the chest. Johnson & Park

(1981), demonstrated a rise in the T_{es} from rest to 0.28 to 0.50°C was required to increase forearm sweat rate. Both these studies illustrate that there is a threshold for the changes in both SkBF and sweat rate in exercise and rest. Again this value varies and a major factor influencing the core temperature (T_c) threshold is the T_{sk} level. Clamping of T_{sk} at various levels and varying T_c in goats (109) and humans (7) demonstrated that a lower T_{sk} resulted in a higher T_c threshold for the onset of resting evaporative heat loss. The size of the change in T_c needed to increase sweating can be reduced through a greater heat acclimation/acclimatization status, physical fitness and hydration status while females and people with a greater age and fat mass require a larger rise in T_c (119; 137). Also the time of day affects the onset threshold for both sweating and increased SkBF, with the lowest sweating and SkBF thresholds (T_{es} 36.6°C, 36.5°C respectively) at 0400 h and the highest (T_{es} 37.3°C, 37.2°C) at 1600 h (150). Finally, due to changes in central venous pressure an upright body position during resting heat exposure raises the T_{es} threshold, relative to supine rest, for increased SkBF by 0.39 ± 0.07 °C ($p > 0.01$) (77).

Fluid regulation

The effect of increasing both sweat rate and skin blood flow, while trying to maintain muscle blood flow, is that the body must now work to also manage and maintain its fluid reserves. The loss of water through sweat increases the plasma osmolarity. This increase is detected by osmolarity sensitive neurons located throughout the brain (96) and also peripherally (16) and causes osmoregulatory inhibition of thermoregulation, thereby decreasing the body's evaporative heat loss capacity (114). To try and maintain fluid balance and thermoregulation, the body releases arginine vasopressin (AVP) from the posterior pituitary gland and aldosterone from the adrenal cortex (94). These hormones

act to increase renal water and Na^+ absorption respectively, and aldosterone also aids in generalized vasoconstriction via the rennin-angiotensin-aldosterone system (82). Fluid consumption also aids in maintaining fluid balance and attenuates hormone release (17). These hormonal and behavioral responses prolong the body's thermoregulatory response to heat stress.

Metabolite responses

Due to the heat-induced stress placed on the body's fluid reserves, anaerobic metabolism increases (183). Reduced blood flow to the viscera decreases lactate uptake by the liver and thereby increases lactate concentrations in the blood (166). Combined with the increased activity of the SNS and increased levels of cortisol, anaerobic metabolism increases further from catecholamine release (52). These changes cause an increase in blood and muscle lactate concentrations during one and a half hours cycling at 52 to 59 % $\dot{V}O_{2max}$ in 35°C air (33). Blood serum potassium (K^+) levels also change during heat stress. The change varies however depending on the circumstances of the exposure. Drinking water for fluid replacement during exercise heat stress can cause hypokalemia, whereas intense exercise in heat-stressful environments without adequate fluid replacement can cause hyperkalemia. In general, passive heat exposure and/or moderate exercise in heat stressful environments causes little change in blood serum K^+ levels as the increased efflux of intracellular K^+ can be accommodated for and an ionic equilibrium is maintained (53).

Thermal tachypnea

Finally, a more controversial heat loss mechanism for humans is a vestigial panting or thermal tachypnea. Thermal tachypnea is a rapid respiratory frequency, with

an increased minute respiratory rate and a decreased tidal volume (1), or more simply the taking of short, fast, shallow breaths. Many hyperthermic animals use panting for thermoregulation. Coupled with salivation in the mouth, secretions in the nose and vasodilation of the tongue, panting is an effective heat loss mechanism (130). The hypothesis of humans utilizing a form of panting for cooling, especially for selectively cooling the brain, remains unresolved. Ventilation is elevated with increasing body temperature (64). Some studies have proposed this increase in ventilation also cools the brain (25) via increased convection with dilated nasal mucosa (178) and the cooling of venous blood leaving the brain (24). Seven participants in Cabanac & White, 1995, showed ~0.3°C lower tympanic temperatures (T_{tp}) than T_{es} when heated using a 41°C water bath for ~30 min. Direct measures of brain temperatures in four participants showed a decrease in brain temperature measured in the cribriform plate (almost directly above the nasal cavity) during voluntary hyperventilation after passive heating. However, no differences were observed between the subdural space, T_{ty} and T_{es} (92). Others dispute the notion of selective brain cooling for numerous reasons which are outlined and rebutted by Cabanac, (1993). The purpose of this review is not to resolve the selective brain cooling dispute, but to emphasize that the thermoregulatory and ventilatory systems appear to interact as increases in body temperature raise ventilation, which may influence cranial thermoregulation.

2.2 Regulation of ventilation

Body temperature has only a secondary influence on ventilation during rest. The controlling mechanisms of ventilation at rest, like thermoregulation, involve a complex interaction of peripheral and central sensors, an integrating center and an effector system

to carry out commands. The key medium used to regulate ventilation at rest in eucapnic and euoxic individuals is blood chemistry. Chemosensors monitor changes in the partial pressure of arterial oxygen (P_aO_2, at rest ~100 mm Hg) and carbon dioxide (P_aCO_2, at rest ~40 mm Hg) and the arterial pH (pH_a, at rest ~7.4) (10). Peripheral chemoreceptors sense variations of all of these while central chemosensitive areas sense changes in pH or CO_2.

2.2.1 Ventilatory sensors

The sensors for control of ventilation fall into four broad categories: 1) peripheral arterial chemoreceptors, 2) central chemosensitive areas 3) intrapulmonary receptors' and 4) chest wall and muscle mechanoreceptors.

2.2.1.1 Peripheral arterial chemoreceptors

There are two areas for peripheral chemoreception, the aortic and the carotid bodies. The carotid bodies are primarily (90 %) in control of our responses to hypoxia. The carotid bodies are located at the junction of the internal and external carotid arteries and have a very high blood supply (~2 $L \cdot min^{-1} \cdot 100\ g^{-1}$) (28). They respond only to changes in P_aO_2 and not to changes in the percentage of oxygen bound to arterial hemoglobin (S_aO_2). This was demonstrated in goats when carbon monoxide was used to acutely decease blood oxygen saturation while maintaining P_aO_2. This resulted in no ventilatory changes from increased carotid body activity (134).

Decreases in P_aO_2 increase peripheral chemoreceptor activity and is one stimulus that can increase ventilation, others are changes in P_aCO_2 or pH_a. The intensity of the response to decreased P_aO_2 varies in a non-linear manner with the severity of the stimulus. Peripheral chemoreceptors in human's are solely responsible for sensing changes in P_aO_2 as their inhibition with dopamine removed the hypoxic ventilatory

response (HVR) in 20 humans (39). The carotid bodies are the primary sensors; humans with bilateral carotid body resection show a very attenuated response to hypoxia. The remaining response (10 %) is thought to be due to output from the aortic bodies (69).

The carotid bodies are also the primary responders to isocapnic acidosis and responsible for ~30 % of the ventilatory response to hypercapnia (10; 69). Isocapnic, iso-oxic acidosis in the cat showed a two to three fold increase in carotid body activity (13). Thirty-one hypercapnic trials on dogs with intact and then denervated peripheral chemosensors demonstrated one fast acting peripheral (4 s) and two slower acting (20 and 118 s) central chemoreceptor responses (8). The continued ventilatory response to hypercapnia and acidosis in bilateral carotid body resected human's confirms that there are central chemosensitive areas which affect the control of ventilation through sensing changes in CO_2 or pH (69).

2.2.1.2 Central chemosensitive areas

The central chemosensitive areas for ventilation are located in the brainstem. The main locations are the ventral medullary surface (VMS), the nucleus tractus solitarius (located deeper) and the locus ceruleus (located rostrally) (20). The VMS, as a sensory site, was discovered when perfusions of mock cerebral spinal fluid (CSF) with either dissolved hydrogen ions (H^+) or CO_2 were infused into the brains of cats and mongrel dogs and an increase in nerve activity was produced. Activity changes were compared to inhaled CO_2 responses to gauge the relative physiological importance of stimulated areas (100). Further investigations of the chemosensitive areas in the VMS have recently focused on the retrotrapezoid nucleus as the most likely candidate (108). Their exact

location however, remains unclear and clarification is needed to allow the interaction of these areas with CO_2 and pH to be fully understood.

The response of the central chemosensitive areas is relatively well understood as they respond primarily to acute deviations of $[H^+]$ in CSF and the medullary interstitial fluid. An increase will cause both the frequency and intensity of central receptor output to rise, culminating in increased ventilation (47). Changes in the medullary interstitial fluid and subsequent ventilatory responses take longer (minutes) compared to the CSF (seconds) as the interstitium possesses a greater buffering capacity.

2.2.1.3 Intrapulmonary sensors

Intrapulmonary sensors can provide breath-by-breath, long-term and independent control of breathing. Below is a brief, general description of their function as found in physiology textbooks and/or respiratory review articles. There are three types, the slowly adapting receptors (SARs), the rapidly adapting receptors (RARs) and the juxtacapillary receptors. The SARs are located amongst the smooth muscle of the airways and are primarily responsible two reactions. Firstly, prolonging inspiration time when lung inflation is impeded (due to decreased lung compliance or airway obstruction) thereby promoting adequate tidal volume (V_T). Secondly SAR's do the reverse when expiration is impeded, increasing expiration time and allowing a normal end-tidal lung volume to be obtained. The RARs function independently of the breathing cycle. In human's they are irritant receptors located in airway epithelial cells. In animals, these receptors also respond to increased airway resistance with a cough, sneeze or sigh. The juxtacapillary receptors are located near the capillaries in the alveolar walls and their exact function remains unclear but may be related to the rapid shallow breathing and bronchorestriction

in asthma. The combined effect of this group of sensors blunts the ventilatory responses to hypoxia and hypercapnia in dogs and may be related to the abnormal breathing patterns associated with asthma in humans (9; 28).

2.2.1.4 Chest wall and muscle mechanoreceptors

There are two focal groups of receptors here, muscle spindles and golgi tendon organs. Again this is a brief, general description of their function as found in physiology textbooks and/or respiratory review articles The muscle spindles are located in muscle fibers and respond to changes in muscle length. They are activated by muscle stretching and respond with muscle contraction. These receptors may be important for the timing of breathing and the increase in ventilation with exercise. Tendon organs in the muscle respond to the force of contraction of muscles and act to help co-ordinate muscle activity during breathing by inhibiting inspiration. Tendon organs in the joints also help co-ordinate muscle activity during the breathing cycle by sensing changes in the magnitude of chest wall movement. These sensors help signal the end of breathing during exercise via the Hering-Breuer inflation reflex. A tidal volume greater that 1 L activates stretch recpetors which singal the brain stem to terminate inspiration. All the information from all these receptors is sent to the central respiratory controller to initiate, monitor and end each and all breathing cycles (9; 28).

2.2.2 Central respiratory control

The control of every breath is not performed in one area of the brain but many. The integrating centers or central respiratory controllers are found in three areas of the brainstem and in the cerebral cortex. The brainstem controllers are responsible for involuntary breathing thereby maintaining most of our daily respiratory needs. The first

area is the pneumotaxic center in the pons. This area regulates inspiratory duration and may be influenced by hypoxia and hypercapnia. The apneustic center is the second area and is located in the lower pons. It serves to regulate inspiratory cut-off, as severing of this area in animals produces apneustic breathing; an increase in inspiratory time with a short expiratory phase (124). The final area, the medullary center is divided into the dorsal respiratory group (DRG) and ventral respiratory group (VRG). The DRG is the processing center for the sensory information from various areas of the body including, chemoreceptors, lung receptors and possibly proprioceptors. The DRG is the output center for normal rhythmic inspiratory drive. The VRG primarily controls respiration through the respiratory effector muscles and increases its output to these muscles when more forceful expiration is needed such as during exercise (11). The general stages of the involuntary breathing cycle are the removal of inhibitory impulses to the DRG and subsequent increases in DRG output to motorneurons. The output is in a ramp-like manner to the threshold required for adequate ventilation as determined by the inputs to the DRG. When these signals are terminated, inspiration is inhibited and expiration begins. The inclusion of the VRG as mentioned depends on the force needed for expiration. For voluntary-related control of ventilation the cerebral cortex can bypass the central control mechanisms to achieve behaviors such as breath-holding, speech and singing. The cerebral cortex can also exert an influence on ventilation through the limbic system during emotions such as rage, fear and grief (46).

2.2.3 Effector system

The skeletal muscule system produces the final ventilatory response, a breath, to all the inputs and outputs from the sensors and controllers. The system also provides

inputs to the controllers over the size of every breath. These muscles, collectively known as the respiratory muscles, are connected via descending pathways from the DRG and VRG (11). These pathways help maintain inhibition of expiratory muscles during inspiration and vice versa. The major muscle is the diaphragm, where during quiet breathing it is responsible for 75 % of inspiration, while resting expiration is passive. To increase either inspiration or expiration, such as required during exercise, the intercostal, abdominal, sternocleidomastoid and other muscles can be recruited (28).

2.3 Overview of control of resting ventilation

The responses of the ventilatory system to certain stimuli is the product of the inputs from the ventilatory sensors, the processing and outputs of the central integrating center and the resulting movements of the effector system. The stimuli are very extensive, the ones discussed here relate to CO_2, pH and O_2.

2.3.1 Responses to carbon dioxide

The ability of the central chemosensitive areas to constantly monitor and respond to changes in P_aCO_2 is due to deviations in the pH of CSF. These changes occur following CO_2 hydration to carbonic acid (H_2CO_3) and subsequent dissociation into H^+ and bicarbonate ions (HCO_3^-), as seen in the Henderson-Hasselbach equation (1), below.

$$CO_2 + H_2O \overset{CA}{\leftrightharpoons} H_2CO_3 \leftrightharpoons H^+ + HCO_3^- \quad \text{(Henderson-Hasselbach equation)} \ldots\ldots (1)$$

Decreases in ventilation without changes in metabolism raise the P_aCO_2 level pushing the equation to the right and raising $[H^+]$. Ventilation is subsequently increased to push the equation back to the left (47). Carbonic anhydrase (CA) is an essential catalyst in this reaction, without it the reaction would take much longer to occur in aqueous solutions

such as blood The relation between P_aCO_2 (mm Hg) and ventilation ($L \cdot min^{-1}$) during normoxia is positive and linear once a threshold of 39 mm Hg is reached (47), where increases in P_aCO_2 increase ventilation and decreases in P_aCO_2 reduce ventilation. The Read rebreathing method is the most common technique used to demonstrate the slope of this relationship (127).

Duffin & McAvoy, 1988 used a modified Read rebreathing method and isolated the peripheral and central chemoreflex response and thresholds for to CO_2 in 8 participants. By maintaining the partial pressure of end-tidal O_2 ($P_{ET}O_2$) at 75 mm Hg, changes in ventilation were assumed to be a chemoreflex response to the increased P_aCO_2, as inferred by the end-tidal partial pressure of CO_2 ($P_{ET}CO_2$). There was a large amount of inter-individual variation in the thresholds but the mean (SD) peripheral and central thresholds were a $P_{ET}CO_2$ of 39.40 (0.66) mm Hg and 45.51 (0.81) mm Hg, respectively. In a later study, the sensitivity of peripheral chemoreceptors and central chemosensitive areas was reported to be 2.7 (1.2) $L \cdot min^{-1} \cdot mm\ Hg^{-1}$ and 5.0 (1.6) $L \cdot min^{-1} \cdot mm\ Hg^{-1}$ (mean (SE)), respectively (103). The inter-individual variation in these values is not as large as for the HVR, but is caused by similar circumstances such as fitness, which decreases your response to hypercapnia (140), and heat, which increases your response (110). All individuals however, exhibit an increased sensitivity to CO_2 during hypoxia and a decreased sensitivity during hyperoxia (47; 113).

2.3.2 Responses to pH

As mentioned above, the initial response to metabolic changes in pH is mediated by the peripheral arterial chemoreceptors and then by the central chemosensitive areas in the medulla (10). At any level of P_aO_2, a decrease in pH_a will cause an increase in

ventilation and an increase in pH_a will decrease ventilation (13). In the central chemosensitive areas, acute or chronic metabolic changes in pH_a will not have such an immediate or sizeable affect on central chemoreceptor output as acute hypercapnia. This is due to the blood brain barrier being relatively impermeable to H^+ and highly permeable to CO_2. If CO_2 levels remain constant the H^+ take longer (minutes to hours) to cross the barrier and elicit their effect (100). During chronic acidosis or hypercapnia, renal compensation increases the bloods buffering capacity by retaining HCO_3^-. The extra HCO_3^- gradually diffuses across the blood brain barrier into the CSF and helps buffer the increased levels of H^+ and thereby reduces the body's ventilatory response to increased levels of arterial H^+ or P_aCO_2 (99).

Also mentioned previously, there is a delay in the response to hypercapnia of dogs with carotid body denervation (8). This delay is also seen in humans (69). The inability of the central nervous system to rapidly respond to isocapnic acidosis, and the delay in the response to hypercapnia are the basis of the notion that peripheral chemoreceptors are responsible for breath to breath variations in ventilation while central chemoreceptors provide longer term (minute to minute variation) control (10).

2.3.3 Responses to oxygen

A decrease in P_aO_2 causes an increase in ventilation. The threshold at which human's increase their ventilation in response to hypoxia is a P_aO_2, as inferred by the $P_{ET}O_2$, of $\leq$ 70 mm Hg (28). There is, however, a lot of inter-individual variance in the HVR. A large range of ventilatory sensitivities, from 1.09 to 4.90 $L \cdot min^{-1} \cdot \%\ S_aO_2^{-1} \cdot$ that were elicited by falls in S_aO_2 were reported in 11 normal participants during eucapnic rebreathing (129). Maintenance of eucapnia during HVR tests removes the attenuation of

ventilation seen during the resultant hypocapnia. Eucapnia during an HVR test also prevents the augmentation of ventilation that would be seen during hypercapnia (35; 110; 126). However, resting P_aCO_2 levels are influenced by any deviation from a normal breathing pattern that may be due to stress or even by diet (149; 186), also different methods and other factors are also known to interact and influence the human HVR.

2.4 Methodologies for measuring the hypoxic ventilatory response

The human ventilatory response to isocapnic hypoxia has three stages. The first phase (acute) involves a rapid increase in ventilation and lasts a few seconds (117). The second phase lasts minutes and involves a gradual decrease in ventilation while the hypoxic stimulus remains constant, this phase is termed hypoxic ventilatory decline (HVD) (117). The final phase involves a progressive rise in ventilation and has a time constant of hours (70). There are many different methods for elucidating different aspects of the HVR. Presently there is a movement to try and establish a common method to be used by all investigators so as to allow uniformity in measuring the ventilatory response to hypoxia. The current method advised for a sea level HVR is a 20 min steady state test under both poikilocapnic and isocapnic conditions (separated by at least 1 h) with a F_IO_2 of 12 % (143; 149). The difficulty with choosing an appropriate method for measuring the HVR is which aspect of the HVR is of interest. The steady state method allows measurement of the whole body response to hypoxia, while progressive methods allow investigators to measure whole body sensitivity to hypoxia and transient methods are assumed to measure the response of the peripheral chemoreceptors to hypoxia.

2.4.1 Steady-state methods

A steady state method was amongst the first manipulations used by investigators to record the HVR and involved breathing a single hypoxic gas mixture for a fixed amount of time of ~5 to 15 min. The difference between ventilation at baseline levels and ventilation at the end of the hypoxic period represented the HVR (123). For eucapnia or isocapnia to be maintained either the gas mixture had a preset level of CO_2 in it, or the CO_2 was added manually. These methods were limited because the amount of time spent breathing the gas mixture meant HVD occurred and reduced the calculated HVR (185). This method also does not allow investigators to record the sensitivity of the HVR and compare it after an experimental stimulus. One test that provides many levels of hypoxia is the progressive HVR test developed by Weil and colleagues (171).

2.4.2 Progressive HVR methods

Weil and colleagues used a real-time $P_{ET}O_2$ plot to add N_2 manually and a real-time $P_{ET}CO_2$ plot to manually add CO_2 into an open-ended circuit to produce progressive eucapnic hypoxia. When ventilation ($\dot{V}_E$, $L \cdot min^{-1}$) is plotted as a function of P_AO_2 (mm Hg) a hyperbolic curve is seen (171). The hypoxic threshold is measured by the first substantial and continual increase in $\dot{V}_E$ and size of the HVR is measured by the shape of the hyperbolic curve defined as unitless parameter A, where a greater A value indicates a greater HVR. This method is susceptible to inflated intra-individual variations as the hypoxic stimulus received each time may vary slightly depending on the rate at which N_2 is added (185). A modification of this technique by some studies involved using a rebreathing circuit to progressively lower the P_IO_2 and an absorbing circuit to control $P_{ET}CO_2$ (128). Both these methods allow investigators to record the sensitivity to hypoxia

but can be influenced by the acute phase of the HVR. The ventilatory response seen at a given level of hypoxia is likely to overestimate the true response as having a continually dropping P_aO_2 places the chemoreceptors in the dynamic phase of their response, rather than the steady state phase. The dynamic stage response may be larger than the true acute stage, which represents a fine-tuned and appropriate response to the level of simulation. Hence the continual reduction in P_aO_2 may cause a greater response than if a step-wise method was used to decrease P_aO_2.

2.4.3 Transient methods

Investigators sometimes wish to measure the transient response to hypoxia as this is thought to represent the response of the peripheral chemoreceptors (49). The transient methods measure the ventilation of the first 1 to 3 breaths after the introduction of a hypoxic stimulus. The other HVR methods use a longer time frame for their tests during which hypoxia depresses the central nervous system and lowers cerebral blood flow, both of which cause the ventilatory response to hypoxia to decline (2; 49). Therefore, the response measured for these tests represents the whole body response to hypoxia and not the peripheral one as hypothesized to be shown by the transient methods.

2.4.4 Dynamic end-tidal forcing methods

This method uses electric valves and computer programs to control the $P_{ET}O_2$ at a set level by varying the P_IO_2. This method allows the measurement of both the whole body response and whole body sensitivity to hypoxia. Eucapnia or isocapnia are also maintained using the computer program and electrical valves (131; 132). Whole body hypoxic responses during both poikilocapnic and isocapnic conditions (separated by at

least 1 h) as recently recommended as the universal method to measure the sea level HVR (143; 149) can be more accurately recorded by removing the human error associated with using pre-mixed gases and manually titrating CO_2 into the inspirate. The major drawback to the end-tidal forcing system is the expensive, timely and highly complicated setup required.

Progressive methods for measuring the whole body sensitivity to hypoxia may be more accurately implemented using a dynamic end-tidal forcing approach. Utilizing a step-wise method where participants are exposed to step changes in S_aO_2 allows for the accurate determination of multiple steady states. This method does not provide continuous data but still allows whole body sensitivity to hypoxia to be expressed by the shape parameter A. The main issue with this progressive dynamic end-tidal forcing technique is to decide the optimum time to provide each stage of hypoxic stimulus, so as to avoid both the dynamic and depressed HVR responses. Between 50 to 90 s stages are used with the mean data from the last 20 s used to represent the HVR at that stage. Both Mou et al. (1995) and Ainslie & Poulin (2004) have used the end-tidal forcing method to measure the HVR during evenly spaced ascending and/or descending levels of % S_aO_2. They concluded that the last 20 s of either a 50 s stage (106) or a 90 s stage (2) represents an HVR that was neither dynamic nor depressed as both ascending and descending protocols produced the same ventilatory responses. The dynamic end-tidal forcing method can also be used to measure peripheral chemoreceptor sensitivity. On the whole, the major drawback to using the dynamic end-tidal forcing method is the complex experimental setup that is required (185). While choosing an appropriate methodology for

measuring a human HVR is important, there are many intra- and inter-individual differences.

2.5 Factors influencing humans hypoxic ventilatory response

Humans response to hypoxia varies greatly both within and across individuals. A study on 40 males and four females using a eucapnic rebreathing HVR method produced a mean (SD) shape parameter A of 185.9 (84.9), where the range was from 59 to 400 (68). Sex has not been reported as a factor as a comparison of 10 men and 10 women using a 20 min steady state isocapnic HVR method reported that after 2 min and after 20 min, the mean (SD) male minute ventilation was 131 (6.1) % and 115 (5.0) % greater than at baseline respectively while females minute ventilation was 136 (7.7) % and 116 (6.6) % greater than at baseline respectively (133). However, both long and short-term factors do influence the human HVR.

2.5.1 Environmental and age related factors

During the first week at altitude an un-acclimatized person will show an elevated HVR (136), however, natives to altitude show an attenuated HVR (144; 170), although this is not present in children born at altitude (21). This lead to the hypothesis that where you are born does not influence your HVR but the time spent at altitude and hence the time of your exposure to hypoxia does influence your HVR (21; 170). Some people however, do not acclimate well to high altitudes and these people were found to have an attenuated HVR. In contrast, Schoene, (1982) demonstrated that 14 high altitude climbers, who had climbed to 7470 m or higher, had a significantly greater HVR than 10 world or nationally ranked middle-distance runners (A-value means (SE) were, 158.9

(29.9) vs. 49.3 (7.1), respectively, $p < 0.001$). The 10 control subjects' mean (SE) A-shape parameters in this study were between these values at 109.1 (21.0). Another study showed 13 highly trained athletes to have a 35 % smaller A shape parameter than 10 non-athletes ($p < 0.05$) (22). When participants in another study underwent five weeks endurance training there was no change in their HVR, although the degree of training was not as great as the years typically completed by true endurance athletes (87). Age also attenuates the HVR (85). Eight healthy elderly men (64 to 73 yr) had a mean (SEM) HVR 51 (6) % lower than eight young men (22 to 30 yr) ($p < 0.001$). Also their heart rate responses were lower, as the mean (SEM) percentage increase in heart rate, relative to resting heart rate, produced by the HVR was 12 (2) % in the elderly men and 34 (5) % in the young men ($p < 0.005$) (85). Therefore, prolonged exposure to hypoxia, high levels of endurance training and age appear to attenuate one's HVR, while mountain climbers who can tolerate high altitudes appear to have an enhanced HVR as opposed to those who do not acclimate well to altitude.

2.5.2 Genetic factors

Observations that familial members of medical patients who exhibit hypoventilation and hypoxemia also showed attenuated HVR led to the suggestion of a linkage between genetic influences and HVR responses (72). Studies comparing the HVR of patients with chronic obstructive pulmonary disease (COPD) and chronic hypoventilation and the HVR of their immediate family's demonstrated that all had a lowered HVR (104; 107), as did endurance athletes and their immediate family (141). The influence of genetics on the HVR has been demonstrated using inbred strains of mice. Mice with a phenotype for rapid, shallow breathing patterns (C57BL/6J)

demonstrated a greater response to hypercapnic hypoxia than the mice with a phenotype for slow, deep breathing patterns (C3H/HeJ) (155; 156). More recently spontaneously hypertensive mice produced a greater and more variable HVR than the control Fischer 344 mice, mean (SEM) A shape parameters of 108.0 (24.1) and 13.8 (6.6), $p < 0.002$, respectively (173). Weil et al. (1998), also concluded that these responses were the primary affect of genotype and not a secondary effect of the hypertension. In humans, studies involving mono- and dizygotic twins also showed a strong relationship in HVR for monozygotic twins (169). In one study on 12 sets on mono- and dizygotic twins, the monozygotic twins had a significant correlation ($r = 0.76$, $p < 0.01$) in their HVR responses whereas the dizygotic show no significant correlation (r-not stated, $p > 0.05$) (34). Therefore 58 % of the variation in the human HVR could possibly be explained by genetic influences, leaving 42 % of the variation affected by other factors. In conclusion, while environmental factors do influence the human HVR, these factors may be initially influenced by a genetic factor with people self-selecting themselves to certain lifestyles and/or sports.

2.5.3 Variability of the human hypoxic ventilatory response

The genetic and/or lifestyle influences are long-term influences on the human HVR. Zhang and Robbins, (2000) compared the variability of the isocapnic HVR within a day and across weeks. Ten participants were tested six times and each session was separated by at least one week. For each session one of the three different methods was used to measure the isocapnic HVR. The three methods were a square-wave protocol, a progressive rebreathing protocol and an incremental step protocol. They concluded that the HVR varied significantly more ($p < 0.001$) between days than during the same day.

There was also a significant difference ($p < 0.01$) in the HVR for the three methods. Mean (SD) HVR's for the square wave, incremental step and rebreathing protocols were 1.02 (0.48), 1.15 (0.55), and 0.93 (0.60) $L \cdot min^{-1} \cdot \%S_aO_2^{-1}$ respectively. However, the coefficients of variation for the three protocols were 20, 23, and 36 % respectively and these were not significantly different. They concluded that while each method was adequate for measuring the human HVR, the two versions of the end-tidal forcing methods (square wave and incremental step protocols) were more accurate if one were able to access or setup such a technique. Also that there is a large day-to-day variation in the human HVR for all protocols and that the origins of this variation remains unknown (185). This study however did not take into account the effect of previous feeding habits before testing. An augmented HVR has been reported after a carbohydrate (129 % of control, $p < 0.001$) or protein (89 % of control, $p < 0.001$) meal (186), while a depressed response, 42 % of control ($p < 0.05$), has been reported during starvation (44). These changes may be linked to both changes in metabolic rate and P_aCO_2 (186).

2.5.4 Influence of carbon dioxide

Responses to hypoxia vary according to the P_aCO_2. A decrease in P_aCO_2, such as seen during a poikilocapnic HVR, reduces the human HVR. Rapanos & Duffin, (1997) performed rebreathing tests on nine males and showed that ventilation did not increase in response to hypoxia until a threshold of $P_{ET}CO_2$ 39 ± 2.7 (SD) mm Hg despite a $P_{ET}O_2$ of 37 ± 4.1 (SD) mm Hg. Small increases in P_aCO_2 on the other hand, such as seen during an isocapnic HVR, increases a human HVR in both an additive and multiplicative manner (129). Rebuck & Woodley, (1975) used predetermined levels of CO_2 to measure the progressive HVR on 11 male subjects using rebreathing and found the greater the level of

hypercapnia the greater the ventilatory response to hypoxia. This study was fundamental in instigating the notion on what level of carbon dioxide to use when doing HVR tests. Isocapnic levels produce good results but are believed to inflate the size of the HVR (129). Eucapnic tests maintain resting levels of CO_2 but changes in ventilatory responses may be a function of differing resting P_aCO_2 levels, due to changes in environment, diet or anxiety levels, rather than changes in chemosensitivity (186). Finally, poikilocapnic tests while portraying the true response to hypoxia, such as at altitude, produce a very small and sometimes non-existent response making measurement very difficult (129). Therefore, different physiological circumstances do influence the human HVR through varying the sensitivities of peripheral receptors, or by the increased activity and/or sensitivity to humoral and/or neural factors. Such physiological circumstances occur during exercise and/or exposure to heat-stressful environments.

2.6 Regulation of ventilation during exercise

Exercise can produce the largest sustained increased in ventilation. Resting ventilation is ~6 to 10 $L \cdot min^{-1}$ and during exercise it can increase to over 170 $L \cdot min^{-1}$. The need for such an increase in ventilation makes logical sense as more O_2 is required for working muscles and subsequently more CO_2 needs to be removed. What remains unknown however, is how ventilation is regulated during exercise as baseline values for pH_a, P_aCO_2 and P_aO_2 stay the same at moderate exercise intensities, yet there can be up to a 20-fold increase in ventilation at sub-anaerobic levels of exertion (19). During prolonged steady-state exercise ventilation begins to drift upwards regardless of changes in pH_a, P_aCO_2 and P_aO_2 or work rate (41). Also, environmental conditions such as

increased altitude and/or ambient temperature raise exercise and resting ventilation (21; 64). Finally, the phase of exercise affects ventilation (40).

Dejours, (1964), outlined the first three phases involved in exercise ventilation. The first phase (PI) is at the onset of exercise and involves the immediate increase in ventilation before any humoral or metabolic effect on ventilation is possible. This increase may also occur before exercise in anticipation of the upcoming work (43). Phase two (PII) occurs ~15 to 30 s after the initiation of exercise and is a slower, exponential rise in ventilation, O_2 uptake and CO_2 production. Phase three (PIII) is the steady state of exercise and ventilation where gas exchange meets metabolic demand and pH_a, P_aCO_2 and P_aO_2 are similar to a resting level. This phase normally occurs 3 to 4 min after initiation of exercise at a constant work rate. Phase III continues until a change of exercise work rate or environmental settings or until the end of exercise (40). During prolonged exercise there is also an upward drift in ventilation for which the exact cause is as yet still unknown (41; 93). Phase four (PIV) is a continual increase in ventilation until exhaustion and is at an exercise level above anaerobic threshold or the first ventilatory threshold (148; 168). Anaerobic metabolism is now used predominantly to provide energy to the working muscles and lactate is produced. The level of lactate production is now greater than clearance and lactate accumulates in the blood. Ventilation increases more quickly than carbon dioxide production thereby inducing hypocapnia. Phase four does not occur unless anaerobic metabolism also takes place. Cessation of exercise and recovery signals an abrupt decrease in ventilation and then an exponential decay, much like PI and PII at the start of exercise (102; 177).

2.6.1 Hypotheses on control of ventilation during exercise

The primary cause of and controller for these phases (collectively termed, "exercise hypernea") has received much attention. Many theories have been used to explain the human ventilatory control system during exercise. The hypotheses generally relate to either humoral feedback from the peripheral chemoreceptors and central chemosensitive areas or to a feed forward mechanism from central and/or peripheral neurogenic pathways (101; 163). Below is a very brief description of some such theories drawn from key review and/or foundation papers relating to these theories.

A model by Wasserman et al. (1977) links the increased ventilation with increased CO_2 in the lungs by coupling alveolar ventilation and CO_2 flow to the lungs in order to maintain arterial isocapnia during exercise. Many of the experiments directly measuring this model have involved experiments on dogs and artificial increases in cardiac output and thereby increased CO_2 flow into the lungs regardless of changes in metabolic rate (167; 174). The absence of changes in P_aCO_2 during prolonged exercise (93) and delay in the PI response after prior exercise (73) have been used to dispute this theory. Also, no CO_2 flow receptors have been identified in the pulmonary circulation as of yet.

Another proposal is an increase in mechanoreceptors and efferent motor output explains the increase in ventilation during PI and then the use of these outputs to augment chemoreceptors response during PII and PIII. Evidence for this is the maintenance of increased ventilation with carotid body resection (69), the increased ventilation with passive exercise (66), how this increase in ventilation is attenuated when the limb afferent pathways are blocked (152) and how stimulation of the hypothalamus increases ventilation (50). The physiological settings of these experiments are thought to be

misrepresentative of the real physiological settings in exercise and that while it plays a part in exercise hypernea, especially during PI, it is not the primary controller (43).

A neural-humoral model is also suggested where the afferent muscle signals augment the response of the peripheral chemoreceptors, not the central chemosensitive areas. Augmentation of the body's HVR has been shown to be greater (56; 172) and faster (164) at the onset of exercise in comparison to the augmentation of the hypercapnic ventilatory response (HCVR). The HVR response can also be enhanced as an increased response was demonstrated after adaptation to a hypoxic stimulus (78). This response is again thought not to be the primary response as carotid resection removes the HVR (69).

Other models exist which are not the primary controllers but exert some influence on ventilatory control during exercise hypernea. Increased potassium (121) and catecholamine concentrations in the blood augment the chemoreceptor response (38), and in particular the peripheral chemoreceptor response to hypoxia (6; 121). During PIV, the compensatory hyperventilation from the metabolic acidosis is primarily mediated by the peripheral chemoreceptors while the subsequent decrease in P_aCO_2 diminishes the response of the central chemosensitive areas to the lowered pH_a (145; 175).

Much debate still surrounds which, if any of these models is responsible for exercise hypernea. The humoral component cannot be the sole controller as people with carotid body resections (69) or congenital chemosensor diseases (145) still show increased ventilation with exercise up to PIII, but not PIV. Factors that influence HVR and HCVR during exercise can also influence these responses at rest. Aerobic fitness may not influence ventilatory responses (90) but the genetics of individuals with different fitness levels can. Both sedentary and fit individuals' HVR are positively correlated to

other family members HVR values and therefore aerobic fitness, through genetics, is suggested to influence ventilatory responses to exercise (58; 140). Also native environment or acclimation and the exercising environment affect ventilation responses. Altitude affects the sensitivity of chemoreceptors to oxygen, but also allows individual's to adapt to changes in P_IO_2 (21; 78; 140). Another factor increasing both exercising and resting HVR and HCVR is exposure to heat stressful environments (64; 86). Rises in T_c can also occur during exercise, and have been shown to increase ventilation more than exercise in temperate environments (115; 122). Part of the increased ventilation is due to an increase in metabolic rate, however, this cannot account for all the increase in ventilation (25). Therefore, how this body temperature induced increase in ventilation occurs is still the subject of debate. A change in the output from peripheral chemoreceptors or the hypothalamus may be a cause as illustrated in hyperthermic cats (89; 153). Another and as yet relatively unexplored cause of heat-induced hypernea is the effect of an increased T_c on the bodies response to changes in P_aO_2.

2.7 Ventilatory responses to heat stress

Ventilation increases in response to heat stress from passive exposure to hot-humid environments (59; 64) or hot water (86), from active exposure (36) and to pyrogen-induced fever (63). These increases are despite relatively small changes in $\dot{V}O_2$. Cabanac & White (1995) heated seven college students in a bath (41°C) for up to 30 min. They recorded an increase in ventilation from 10.0 ± 0.9 to 19.3 ± 3.0 $L \cdot min^{-1}$, a rise in T_{es} from 36.6 ± 0.1 to 39.0 ± 2°C, but $\dot{V}O_2$ gave an increase from 0.27 to 0.35 $L \cdot min^{-1}$. The change in $\dot{V}O_2$ (30 %) approximates that predicted by the Q_{10} effect, but is not appropriate for the 80 % increase in ventilation. Therefore the increased ventilation

during hyperthermia is not due solely to changes in metabolic demands but may represent a thermoregulatory role and/or a change in chemosensor sensitivity/activity.

2.7.1 Core temperatures and changes in ventilation

The general findings related to a level for the heat-mediated increase in ventilation have been that at a critical T_c, where ventilation increases regardless of changes in metabolic rate (25). The T_c for ventilatory changes varies, during passive heating. A change in T_c of $\geq$ 1.5°C was required to induce heat-mediated increases in ventilation (138; 139), while an exact T_{es} of 38.5°C has also been reported as the critical level (25). During exercise, the level for increased ventilation was reported as a T_{es} of 37.76°C (179), while the level for a change in breathing pattern but not ventilation rate was ~38°C (122). Others have reported that the level and/or magnitude of the ventilatory response depended on the initial T_c and the rate of T_c rise during both passive (86) and active (179) exposures. Still others have reported no change in ventilation with passive and active increases of body temperature between 0.5 – 0.9°C (42; 67; 93; 176). These latter studies however, may not have reached a high enough T_c during passive heating (42). Also, in Martin et al. (1981), ventilation was not measured during the passive heating stage and only during the exercise that followed and therefore the effect of the heating phase is unknown (93) while in Henry & Bainton (1974), increases in ventilation were recorded but failed to reach statistical significance (67).

2.7.2 Magnitude of ventilation responses

The magnitude of ventilation changes from passive heating is varied. A 1.5°C rise in rectal temperature (T_{re}) increased ventilation 5.3 $L \cdot min^{-1}$ during heating using an

environmental chamber (158). Also using an environmental chamber, a 1.81 to 2.70°C rise in tympanic temperature produced a 49 % increase in ventilation with a 19 % increase in O_2 consumption and a 16 % increase in CO_2 production (139). These changes were ascribed to increases in the basal metabolic rate associated with increased breathing and sweating. When heated using a flying-suit, a 1.3°C increase in T_{es} increased ventilation 15.3 $L \cdot min^{-1}$ (123). Using bathing in water to heat participants, Landis et al. (1925), increased ventilation to a maximum of 34.4 $L \cdot min^{-1}$ at a T_{re} of 40.3°C which represented a rate of rise of 5.4°C$\cdot hr^{-1}$ (86). While Cabanac & White (1995), increased ventilation from 10.0 ± 0.9 (SD) to 19.3 ± 3.0 (SD) $L \cdot min^{-1}$ with a rate of rise of 5.8°C$\cdot hr^{-1}$ (25).

The rate of increase in ventilation also varies, possibly due to the rate of rise in T_c (86; 179). The range for the rate of increase in ventilation is 0.9 to 3.9 $L \cdot min^{-1} \cdot °C^{-1}$ during passive exposure (25; 158) and 8.3 $L \cdot min^{-1} \cdot °C^{-1}$ during 54 min active exposure (115).

2.7.3 Changes in breathing pattern

The findings for changes in breathing pattern associated with the heat-mediated ventilatory increase have been just as mixed as those for heat-induced increases in ventilation. Some studies report an increase in respiratory frequency (f_R) with no change or a decrease in tidal volume (V_T) during passive (5; 123; 158; 160) or active exposure (93). Other studies have shown a decreased f_R and increased V_T during passive body warming (59; 138; 139), or an increased f_R and V_T during active body warming (122). A look at ventilation patterns over the entire heat exposure also reveals different patterns, one study reported an increase in V_T at the start followed by a decrease in V_T and increased f_R and then the slow rise of both together (86). A more recent study reports an

increase in both V_T and f_R, then a decrease in f_R and slow rise in V_T and then eventually a rise in both (25). The increase in ventilation at the start of a heat exposure has often been attributed as psychophysical response to entering the hot environment or the effect of rapidly altering peripheral stimuli (42; 138). Using participants experienced in heat experiments and who demonstrated no signs of discomfort from the testing procedure, however, still produced the an increase in f_R (158). While Saxton, (1975) who reported to have controlled the effect of the sudden change in temperature reported no change in f_R. Another hypothesis is that the response to heat must be an increase in V_T as an increase in f_R is the response seen to hypoxia or hypercapnia (59). Therefore, the change in ventilatory pattern with exposure to heat has not yet been resolved.

2.8 Mechanisms of heat-induced hypernea

With a sufficient rise in T_c, ventilation increases while metabolic rate remains relatively unchanged. The cause of the heat-induced hypernea could be mediated by increases in central output from the hypothalamus or brainstem, through increases in peripheral output via increased skin temperature receptors output, by increases in central or peripheral chemoreceptor output or sensitivity, through changes in blood metabolites, or maybe a thermoregulatory mechanism.

2.8.1 Central controller mechanisms

Tabatabai (1972b), demonstrated that direct heating of the respiratory neurons in the medulla oblongata from (mean (SD)) 36.92 (0.90°C) to 40.63 (1.65°C) using radio frequency or thermodes decreased minute ventilation (both f_R and V_T) in 12 cats (154). This response was reversed on cooling and there were no changes in rectal temperature,

however, hypoxic or hypercapnic stimuli diminished the heat response. Ventilation also increased when 20 decerebrate cats' carotid bodies were cooled from 37°C to 28°C (153). Below 28°C ventilation decreases, these experiments demonstrate that medullary neurons and carotid bodies are temperature sensitive. The decrease in ventilation during heating may have been due to increased blood flow to the heated area and therefore an increased P_aO_2 and decreased P_aCO_2 could have accounted for the reported decrease in ventilation (154). However, heating and cooling of the superficial ventral surface of the medulla in cats influenced phrenic nerve output and ventilation along a continuous graded temperature-response curve, with enhancement above and depression below normal body temperature (30). Changes in CO_2 tension had an additive effect on this temperature response curve (30). Heating of the pre-optic and anterior areas of the hypothalamus in cats activated the panting response, peripheral vasodilation and increased sweat rate, demonstrating a thermoregulatory response (89). When 5 cats were exposed to both hypoxia and increased T_c, the T_c at which thermal tachypnea began was lowered (15). When the same cats were chronically carotid body denervated and exposed to the same conditions the same response was seen implying that the interaction between heat and hypoxia was centrally rather than peripherally mediated (15).

Central controller output responses to heat are very difficult to record in humans. A centrally or peripherally mediated response to heat was reported when ventilatory responses (first two breaths) during phase one of exercise were generally larger when participants (n = 3) were hyperthermic (T_c 1.4°C above normal) compared to normothermic (159). The results were not conclusive but imply a heat-induced increase in the neural response of PI ventilation during exercise. The increase is likely to occur

though increased output by central controllers (e.g. hypothalamus and/or medulla) as peripherally heating the legs of dogs did not affect ventilation (105). Finally, the psychophysical response to the increased discomfort at higher T_c can not be discounted as a possible cause of the increased ventilation (42; 115; 138).

2.8.2 Chemoreceptor mechanisms

A direct effect of temperature on the chemoreceptors could be the cause of heat-induced hypernea. However, it is just as difficult to directly measure chemoreceptor output in response to heat stress as direct measures on central controller output. Direct heating of the respiratory centers and chemosensitive areas in the brain of a cat decreased ventilation (154), whereas direct heating of a cats carotid body increased ventilation (97). McQueen & Eyzaguirre, (1974) performed 16 trials on nine cats where the carotid bodies were heated or cooled. Heating (to 42°C) caused a 100 % rise in carotid body activity, while cooling (to 20°C) lowered carotid body activity. These responses also occurred during cyclic or ramped heating. Using these data the mean (SEM) temperature coefficient (Q_{10}) for the carotid bodies as chemoreceptors was calculated to be 2.96 (0.18). While in vitro studies in the carotid body response to heat produced a Q_{10} of 75 (57). Bernthal & Weeks (1939), heated only the carotid bodies in 18 trials on dogs via heating the blood perfusing them, and showed up to a 32.6 % increase in ventilation from rest with a 45°C blood temperature (12). Conversely cooling the blood perfusing the carotid bodies to 15°C induced a 34 % decrease in ventilation from rest (12). Responses for all three of these studies returned to control values upon the return of the carotid bodies to control temperatures. Therefore, the increase in carotid body temperature during hyperthermia may be a peripheral cause of heat-induced hypernea.

As a result of these experiments on cats, it is known that there is an increased output from the ventral surface of the medulla (30), pre-optic hypothalamus (89) and carotid bodies (97) during heating. This evidence, however, does not resolve the issue of how the hypothalamus and carotid bodies exert their response on ventilation in humans. The heating of the hypothalamus may have an additive effect on output from the respiratory control center or medullary chemosensitive areas, or it may increase the sensitivity of the medullary chemosensitive areas. The same may be true for the direct heating of the carotid bodies. Another possibility is that the heat-induced hypernea may be caused by both the carotid bodies and the hypothalamus and may be both additive or multiplicative. The main focus areas to investigate this would be the controllers of ventilation; the chemoreceptors, intrapulmonary receptors and mechanoreceptors. The mechanoreceptors may have an additive response to the heat as hyperthermia increased ventilation during phase one of exercise (159). The effect of heat on human intrapulmonary receptors is likely to be small as their main influence is on inspiratory and expiratory times when lung inflation or deflation is impeded (28).

2.8.3 Changes in blood metabolites

Blood lactate concentration increases during passive heat stress with an elevated stable T_c (122) or until volitional exhaustion from the heat (59). Also blood lactate increases during exercise in the heat relative to exercise in normothermic conditions (33). However, none of these studies concluded that a change in blood $[H^+]$ was responsible for any change in ventilation. Respiratory alkalosis is another cause of increased lactate via reduced lactate utilization (45). The increased $[H^+]$ is probably not a direct stimulus for heat-induced hypernea as this occurs during resting thermoneutral respiratory alkalosis

(71). During a HVR test, the maintaining of isocapnia would aid in the prevention of respiratory alkalosis and thereby help prevent changes in $[H^+]$ which would influence the HVR.

Changes in serum K^+ levels also are not great enough during mild hyperthermia, T_c ~38.5°C, over a short time period, < 1 hour, (53; 84) to affect ventilatory responses to either hyperthermia (84) or hypoxia (165). Even in light exercise and hyperthermia, where increases in $[H^+]$ and K^+ are greater, these findings are supported by work in this laboratory where there were no significant changes in blood lactate ($p = 0.395$) or serum K^+ ($p = 0.352$) levels during a hyperthermic (T_{es} 38.5°C) and hypoxic (F_IO_2 = 12 %) stimulus, whereas ventilation increased significantly ($p = 0.004$) (32).

Plasma NE levels increase during exercise and significantly ($p < 0.001$) more so during exercise in the heat, while mild resting heat exposure does not increase NE levels (18). Brenner et al. (1997) measured NE levels while 11 males either cycled (30 min @ ~50 % $\dot{V}O_{2max}$) or rested (3 h seated) in a thermoneutral (23°C) or heated (40°C, 30 % RH) climatic chamber. Mean (SD) seated plasma NE levels were 1548 (505) $pmol \cdot L^{-1}$ in the thermoneutral environment and 1527 (605) $pmol \cdot L^{-1}$ in heated environment which increased T_{rec} 0.7°C. A 45 min passive exposure in a hot tub (41°C) did not significantly increase NE levels. Increases in plasma NE during heat stress are not attributed directly to the changes in T_c but indirectly via decreases in plasma volume and increases in T_{sk} and SkBF (125). Despite the non-significant increase in plasma NE levels, ventilation still increased significantly ($p < 0.05$). The changes in ventilation during exercise are not thought to be largely influenced by the significant increases in plasma NE as changes in ventilation are rapid compared to the changes in plasma NE (95). Also infusion of NE

into monkeys (91) and sheep (62; 181) during resting heat exposures does not further increase ventilation. Therefore the increase in plasma NE levels during a short, mild passive heat exposure are much smaller compared to those seen during exercise, and so the increased ventilation during passive heat exposure cannot be explained by changes in plasma NE levels.

2.9 Heat-induced hypernea and responses to hypoxia

The ventilatory responses to oxygen are mediated primarily by the carotid bodies (90 %) and partially by the aortic bodies (10 %) (69). The peripheral chemoreceptors response to hypoxia in normothermia is to increase ventilation. Animals respond to hypoxia during heat stress by showing a greater increase in ventilation to a given hypoxic stimulus. Increased ventilation through a decreased hypoxic threshold has been recorded in hyperthermic iguanas (48) and cats (15).

2.9.1 Whole body responses to hypoxia and hyperthermia

The human HVR during hyperthermia has received little attention. One study passively heated five participants using a heated flying suit to a T_{re} 1.4°C above resting level (123). Participants then breathed either normoxic hypercapnic gas mixtures where the inspired level of CO_2 varied from 0 to 6 Torr, or the same four concentrations of CO_2 but at two differing inspired levels of hypoxia, 55 and 45 Torr. The normoxic hypercapnic CO_2 mixtures were breathed for seven minutes, immediately followed by the hypoxic hypercapnic mixtures, which were each breathed for two minutes one after the other. Means of the last 20 breaths for each stage were used to represent changes in ventilation. This meant a total of 44 minutes of quiet breathing during which the gas

mixture was varied 12 times. During normothermia ventilation increased from baseline by 48 % and 104 % for the P_IO_2 of 55 and 45 Torr, respectively. During hyperthermia ventilation increased by 74 % and 131 % from baseline for the P_IO_2 of 55 and 45 Torr respectively. The change due to hypoxia was speculated to be mediated by an increase of carotid body sensitivity, while the interaction between hypoxia and hypercapnia was thought to be an augmented hypoxic response at the peripheral level combined with an increased central chemosensor output. The authors commented on the high variability of results, which means that while this study provided a platform for knowledge on heat-induced responses to iso- and hypercapnic hypoxia, the basic responses remained to be established.

Recent work in this laboratory involved 11 males exercising on an underwater cycle ergo-meter (at a mean ± SD $\dot{V}O_2$ of 0.87 $L \cdot min^{-1}$ ± 0.07) for 30 min in a hot tub (~38°C), while breathing either air or a hypoxic gas mixture (12 % O_2 and balance N_2) for 10 min each. Isocapnia was achieved by the manual addition of CO_2 to the breathing apparatus. Changes in ventilatory variables were measured as the difference between variables at the start and the end of a euoxic or hypoxic period. During hyperthermia mean (SD) ventilation increased by 2.0 (2.1) $L \cdot min^{-1}$ ($p = 0.01$) while breathing air, and by 10.2 (9.0) $L \cdot min^{-1}$ ($p = 0.004$) while breathing the hypoxic gas mixture relative to hypoxic normothermia. This demonstrates that the increase in ventilation while breathing air was dependent on T_{es} and that the eucapnic hypoxic response was augmented at a T_{es} of 38.5°C. Therefore, there was a core temperature and hypoxia interaction effect on ventilation during exercise (31; 32). This study again provides some data as to the interaction of heat and hypoxia, but used exercising participants and did not report the

early (2 to 5 min) ventilatory responses. Using the recently recommended HVR method (143) may allow a more in-depth assessment of the whole body response to hypoxia under both poikilocapnic and isocapnic conditions. Such a study would also allow for easier comparisons with studies from other investigators using the same, and now recommended method.

2.9.2 Whole body sensitivity to hypoxia and hyperthermia

Only one study has investigated the whole body sensitivity to hypoxia using the progressive HVR method developed by Weil and colleagues in the 1970's. Natalino et al. (1977) passively heated six fasted participants to produce a rise in T_{re} of either 0.7 or 1.4°C. Participants performed progressive HVR tests in both hyperthermic conditions and during normothermia. As hyperthermia produces hyperventilation and, therefore, hypocapnia, the HVR was measured using both the hyperthermic eucapnic and normothermic eucapnic levels. There was a significant increase in participants sensitivity to hypoxia during the normothermic eucapnia and this sensitivity was greater at a higher T_{re}. The mean (SEM) A-value for the normothermic control was 113 (8.8), for a T_{re} 0.7°C above resting it was 189 (21.8) and for a T_{re} 1.5°C above resting it was 240 (34). The response to hypoxia during hyperthermic eucapnia was not significantly different than during the normothermic response ($p = 0.1$). There were very large individual differences and a high SEM for both normothermic and hyperthermic HVR tests. This may be due to large inter-individual variability associated with the progressive HVR test. This was very well controlled and provides a very good insight to the interaction of hyperthermia and sensitivity of the whole body to hypoxia. Using current methods and knowledge a step-

wise end-tidal forcing test may help to reduce some of the variability and better elucidate the true response.

2.10 Summary

Both the thermoregulatory and ventilatory systems work to help the body function optimally in its environment and in doing so these systems interact though it is as yet not fully understood how. Ventilation increases with increasing body temperature during both passive (64) and active exposure (36; 179) to heat stressful environments. During passive heat exposures ventilation increases disproportionally to both P_aCO_2, which decreases, and to $\dot{V}O_2$ which only increases by 30 %, whereas ventilation increases by 80 % (25). A decrease in P_aCO_2 should diminish ventilation while a 30 % increase in oxygen consumption should not require an 80 % increase in ventilation. These contradictory changes in ventilation may be the result of an additive response from the medulla or other central controllers, or may be a multiplicative response of the chemoreceptors. Many studies have investigated these options with varying results. While only three studies have looked at the HVR during hyperthermia. Two studies used a steady-state HVR method (32; 123) and a third the progressive HVR method (110).

An exhaustive literature review did not uncover any studies that have assessed the human poikilocapnic or isocapnic response to hyperthermic hypoxia, relative to normothermic hypoxia, for 20 min and reported ventilation at the 5^{th} and 20^{th} min, the size of HVD and cardiovascular responses. The study by Chu et al. (2005), was conducted in this laboratory and provided an accurate response but has some potential shortcomings. The study used a 10 min steady state HVR method and exercising participants. With new knowledge of the recommended steady-state HVR methods and

protocol this thesis would therefore measure the human HVR while hyperthermic for the first time using the 20 min steady state HVR method under both poikilocapnic and isocapnic conditions. The poikilocapnic HVR (pHVR) would be used to simulate ventilatory responses at altitude while the isocapnic HVR (iHVR) would be used to measure the whole body response to hypoxia.

2.11 Research hypothesis

1. We hypothesize that the human whole body cardio-respiratory response to steady-state isocapnic hypoxia is greater in passively heated hyperthermic than in normothermic humans.

2. We hypothesize that the human whole body cardio-respiratory response to steady-state simulated altitude or poikilocapnic hypoxia is greater in passively heated hyperthermic than in normothermic humans.

3. We hypothesize that the effect of carbon dioxide tension on the whole body human cardio-respiratory response to steady-steady hypoxia is greater in passively heated hyperthermic than in normothermic humans.

2.12 Testable questions

1. Is human ventilation ($\dot{V}_E$, $L \cdot min^{-1}$) at rest greater in passively heated hyperthermic than in normothermic humans?

2. Is the whole body cardio-respiratory response to a 20 min isocapnic HVR test greater in passively heated hyperthermic than in normothermic humans as measured by: the change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot \% S_aO_2^{-1}$) at the 5^{th} and 20^{th} min, the size of hypoxic ventilatory decline (%), ventilation ($L \cdot min^{-1}$) and its components, and the cardiovascular parameters of heart rate ($beats \cdot min^{-1}$) and arterial hemoglobin saturation (S_aO_2)?

3. Is the whole body cardio-respiratory response to a 20 min poikilocapnic HVR test greater in passively heated hyperthermic than in normothermic humans as measured by: the change in $P_{ET}CO_2$ per percent change in S_aO_2 ($mm\ Hg \cdot \% S_aO_2^{-1}$) at the 5^{th} and 20^{th} min, the change in ventilation per mm Hg change in $P_{ET}CO_2$ ($L \cdot min^{-1} \cdot mm\ Hg\ P_{ET}CO_2^{-1}$) at 5^{th} and 20^{th} min, ventilation ($L \cdot min^{-1}$) and its components, and the cardiovascular parameters of heart rate ($beats \cdot min^{-1}$) and arterial hemoglobin saturation (S_aO_2)?

4. Is the effect of carbon dioxide tension on the whole body cardio-respiratory response to hypoxia greater in passively heated hyperthermic than in normothermic humans as measured by: the change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot \% S_aO_2^{-1}$) at the 5^{th} and 20^{th} min, ventilation ($L \cdot min^{-1}$) and its components, and the cardiovascular parameters of heart rate ($beats \cdot min^{-1}$) and arterial hemoglobin saturation (S_aO_2)?

5. Can a breath-by-breath end-tidal forcing system be developed to control the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$, mm Hg) within ± 2 mm Hg from the desired level using gas bolus injections instead of a continuous flow method?

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CHAPTER 3

Study 1

Influence of passive mild hyperthermia on human ventilation during rest and isocapnic hypoxia.

3.1 Abstract

This study compared the ventilatory response to a 20 min isocapnic hypoxia in passively heated hyperthermic versus normothermic males. Eight males were seated in mean (SD) 35.8 (0.1)°C or 38.6 (0.1)°C water where they breathed a 12% O_2, balance N_2 gas while $P_{ET}CO_2$ was kept 0.99 (0.67) mm Hg above normothermic resting levels. Trials were one hour apart with the first at a normothermic, 36.31 (0.02)°C and the second at a hyperthermic esophageal temperature of, 37.82 (0.02). The 2.8 L·min^{-1} or 26% ($p = 0.01$) increase in $\dot{V}_E$ during hyperthermia coincided with a 0.068 L·min^{-1} or 29% ($p = 0.01$) increase in O_2 consumption. Hypoxia caused arterial oxygen saturation drop to ~89%. Ventilation during normothermic rest in water was 10.12 (1.07) L·min^{-1} and increased by 4.1 L·min^{-1} ($p = 0.01$) during hypoxia. The $\dot{V}_E$ during hyperthermic rest in water, 13.58 (2.58) L·min^{-1} increased by 7.21 L·min^{-1} ($p = 0.002$) during hypoxia and was 3.1 L·min^{-1} ($p = 0.01$) more than during normothermia. Hyperthermia did not ($p = 0.60$) but hypoxia did ($p \leq 0.001$) increase tidal volume and the increase during hyperthermic hypoxia was greater than during normothermic hypoxia (0.1 L, $p = 0.03$). An unexpected finding was the absence of hypoxic ventilatory decline for all trials and this is attributed to the effect of water immersion. Therefore the increased $\dot{V}_E$ from normothermia to hyperthermia was due to increased metabolic demand, while the increase in $\dot{V}_E$ during hyperthermic hypoxia was 76% greater than for normothermic hypoxia.

(247 words)

3.2 Introduction

Ventilation ($\dot{V}_E$) is increased by both hypoxia and hyperthermia. The hypoxic ventilatory response (HVR) is mediated primarily by the carotid bodies (90 %) and partially by the aortic bodies (10 %) (25). Ventilation has been shown to increase with an increase in core temperature >1.5°C during both passive (18) and active warming (11; 68). No change in $\dot{V}_E$ has been also been reported with passive and active increases of body temperature between 0.5 and 0.9°C (12; 22; 34; 67). Combining hypoxia and hyperthermia in both animals and humans has shown that these two stimuli also positively interact in their effect on $\dot{V}_E$ (10; 35; 38). The mechanism(s) underlying this interaction in humans is unresolved.

Experiments on cats showed that there is increased neural output from the hypothalamus (32) and carotid bodies (35) during direct heating. As well, heating the pre-optic and anterior areas of the hypothalamus elicited thermoregulatory responses (32) while heating of the intermediate superficial area of the medulla at clamped local partial pressures of CO_2 induced elevated phrenic nerve output (8). In addition, heating of the carotid bodies to 42°C caused a 100 % rise in carotid body activity producing an *in vivo* temperature coefficient (Q_{10}) for the carotid bodies of 2.96 (15). However, *in vitro* studies of the carotid body's response to heat produced a Q_{10} of 75 (15). The increased activity for both these studies returned to control values upon the return of the carotid bodies to control temperatures. Bernthal & Weeks (1939), heated only the carotid bodies in dogs via heating the blood perfusing them, and showed up to a 32.6 % increase in $\dot{V}_E$ with a 45°C blood temperature (6). Therefore the central and peripheral chemosensitive tissues responsible for detecting changes in blood O_2, CO_2 and pH may be influenced by changes

in temperature. This sensitivity to temperature may help explain the interaction between hypoxia and hyperthermia seen in humans (10; 38; 46).

Three studies have reported a positive interaction between hypoxic and hyperthermic stimuli during a hyperthermic human hypoxic ventilatory response (HVR) test; two used steady-state HVR methods (10; 46) and the third the progressive HVR method (38). These studies however, all had mitigating factors. These mitigating factors included potential residual effects of the preceding inspirate (46), dynamic responses of the chemoreceptors (38) and exercise (10). Also, these studies did not report how the interaction of the hypoxic and hyperthermic stimuli affected cardiovascular responses.

We know that hyperthermia alone can increase $\dot{V}_E$ during hyperthermic hypoxia in humans is greater than during normothermic hypoxia for both steady-state exercise or during dynamically induced hypoxia. The size or existence of this interaction between hyperthermia and hypoxia under steady-state and resting conditions remains unknown for its effects on both ventilatory and cardiovascular responses. This study investigated the human HVR response using the recently recommended 20 min steady-state HVR protocol (58). It was hypothesized that $\dot{V}_E$ would be greater during hyperthermic rest in water compared to normothermic rest in water; and that the ventilatory and cardiovascular responses to whole body steady-state isocapnic hypoxia, would be greater in passively heated hyperthermic versus normothermic humans.

3.3 Methods

The study was conducted in the Laboratory for Exercise and Environmental Physiology at the School of Kinesiology, Simon Fraser University. Ethical approval was granted by the Office of Research Ethics at Simon Fraser University before commencement of this study.

3.3.1 Participants

Participants were male volunteers from the Simon Fraser University population aged between 22 to 30 years of age. Their individual characteristics are presented in Table 3.1. Participants were non-smokers, non-asthmatics and refrained from caffeine, alcohol, and heavy exercise 24 hours prior to the study. Prior to experimentation the participants were informed of the potential risks associated with the protocol and after a 24 h reflection period gave their written, informed consent to participate in the experiment. Following signing of their informed consent all participants attended a preliminary testing session where they were familiarized with the experimental protocol and instrumentation. During the familiarization session the participants underwent a 10 min isocapnic hypoxic exposure where they inspired a hypoxic gas mixture with an F_IO_2 of 12 %, balance N_2 with CO_2 clamped at resting levels. Participants were tested in the morning after an overnight fast.

3.3.2 Instrumentation

The participant was seated to the level of the clavicles in either normothermic or hyperthermic water (Table 3.1) and breathed air or hypoxic gas mixtures from a 300 L Tissot spirometer.

Ventilation

During each test the participant wore a nose clip and was fitted with a mouthpiece connected to a two-way flow sensor housing, which was connected to a 2-way non-rebreathing valve (NRB 2700, Hans Rudolph Inc, Kansas Cit, MO, USA). This was connected with a 250 cm length of 3.8 cm inside diameter corrugated Collins tubing to the Tissot spirometer. Breath-by-breath gas samples were drawn from the inspired and expired air to a Sensormedics Vmax 229c metabolic cart (Sensormedics, Yorba Linda, CA, USA) at a rate of 250 $mL \cdot min^{-1}$. Carbon dioxide partial pressure was measured using non-dispersive infrared spectroscopy and oxygen content was measured using a paramagnetic sensor. The CO_2 and O_2 sensors were calibrated before each test using gases of known concentrations (room air, 26 % O_2 balance N_2, and 4 % CO_2 and 16 % O_2). The flow sensor was calibrated using a 3 L standardized volume syringe (Sensormedics, Yorba Linda, CA, USA).

To mix the different gas concentrations, room air was used to fill the Tissot before 100 % N_2 was titrated into the Tissot to attain the desired O_2 concentration as continuously measured by the metabolic cart. A fan in the Tissot mixed and humidified the gases within its bell and once the desired O_2 concentration was reached the air was left to mix for at least a further 5 min. Air was then flushed from the pipes of the Tissot spirometer to ensure only the mixed gas rather than 100 % N_2 was inspired by the participant when they were switched to the hypoxic gas mixture. The mean (SD) inspired fraction of oxygen (F_IO_2) for all the normothermic trials was 11.99 (0.25) % and for all the hyperthermic trials was 11.76 (0.07) % (Figure 3.1B).

Cardiovascular system

Heart rate (HR) and arterial hemoglobin saturation (S_aO_2) were sampled continuously using a pulse oximeter (Masimo Radical, Irvine, CA, USA) attached to the participants' left ear lobe.

Thermal responses

Core temperature was sampled with a nasopharyngeal paediatric size esophageal temperature thermistor (Mon-a-therm, Mallinckrodt Medical Inc., St Louis, USA). The tip of the probe was positioned in the esophagus at the T8/T9 level based on the equation derived by Mekjavic & Rempel (1990), for standing height: L (cm) = 0.228 x (standing height) - 0.194 (36). Esophageal temperature was maintained at a normothermic or hyperthermic level by submersing the subject in a water-filled tub that was maintained at appropriate temperatures (Table 3.1). The mean (SD) increase in T_{es} from 36.31 (0.02)°C in the normothermic, to 37.82 (0.02)°C in the hyperthermic isocapnic HVR (iHVR) test (Figure 3.1A), was 1.51 (0.09)°C with individual increases not differing significantly from 1.5°C ($t = 0.42$, $df = 7$, $p = 0.68$). The mean increase in T_{es} from normothermic rest in air to hyperthermic rest in water for the hyperthermic condition was 1.15 (0.11)°C (Table 3.3). Calibrations of esophageal thermistor probes was completed in a regulated temperature hot water bath (Appendix C).

An analog signal from the flow sensor for each breath from the metabolic cart was used to trigger breath-by-breath data collection for T_{es}, HR and S_aO_2 on a data acquisition system (SCXI-1000, National Instruments, Austin, USA) which was controlled by a LabVIEW software program (National Instruments, Austin, USA, version 7.0) on a PC.

Water temperature calculations

During the familiarization session skinfold measurements were taken at 10 different sites as described by Rennie et al. (1962) with the Harpenden skinfold calliper (British Indicators, St. Albans, UK). The skinfold values were adjusted to remove the effect of 10 skin thicknesses by subtracting 40 mm off the sum of skinfolds which was then used to determine the participant's subcutaneous fat thickness (49). The subcutaneous fat thickness values for each participant was used to predict their overall body insulation at rest (I_{rest}) by a regression equation derived from the Chu and White study (10).

$$I_{rest} = 0.001 \bullet SS + 0.0357 \quad \text{.....Equation 3.1}$$

Where SS is the corrected sum of skinfolds value. Post-hoc calculations of the actual I_{rest} value were performed to investigate the accurateness of the regression equation for predicting I_{rest} (Table 3.1). The predicted I_{rest} values were used to estimate the water temperature (T_w) for both the hyperthermic and normothermic sessions by using a re-arrangement of Park and colleagues body insulation equation (43):

$$T_w = T_{es} - (I_{rest} \bullet (0.92\,\dot{M} \pm \dot{S})) \quad \text{.....Equation 3.2}$$

In Equation 3.1 T_w is the desired water temperature for the testing session, T_{es} is the desired core temperature as measured by the esophageal probe, I_{rest} is the overall body insulation during rest, $\dot{M}$ is the metabolic heat production, $\dot{S}$ is the loss or gain of body heat stores ($\Delta T_{es} \cdot 0.83 \cdot 0.6 \cdot$ body weight, kcal $\cdot m^{-2} \cdot h^{-1}$) and 0.92 is an estimate of respiratory heat loss at rest. Metabolic heat production at rest while sitting is 50 kcal $\cdot m^{-2} \cdot h^{-1}$ and for every 1°C rise in core body temperature metabolic heat production increases by 13 % (44).

3.3.3 Protocol

Following instrumentation the participant underwent a normothermic 20-min iHVR trial that was followed 1 h later by a hyperthermic iHVR trial. The participant listened to relaxing music through ear-phones throughout the duration of each iHVR trial.

Each test began with a 20 min rest out of the water tub, of which data were collected for the last 10 min before entering the water tub. Next while in the tub the participant had to have a stable T_{es} (± 0.1°C) and $\dot{V}_E$ (± 2 $L \cdot min^{-1}$) for 10 min for both trials before being switched to the hypoxic gas mixture from the Tissot spirometer. During this 10 min immersion period the mean $P_{ET}CO_2$ over the last min was used to represent resting $P_{ET}CO_2$. Subsequently participants breathed the 12 % O_2, balance N_2 gas mixture for 20 min while $P_{ET}CO_2$ was maintained 0.99 (0.66) mm Hg above resting values by the manual addition of 100 % CO_2 into the two-way non-rebreathing valve. The difference in $P_{ET}CO_2$ between temperature conditions during each iHVR trial was not significantly different from 0 ($t = 0.30$, $df = 7$, $p = 0.77$) with a mean (SD) Normothermic value of 40.90 (1.78) and a mean Hyperthermic value of 40.82 (1.66) mm Hg (Figure 3.1C). Once the trial was completed the participant sat quietly for 1 min to ensure S_aO_2 levels returned to resting levels.

During the one-hour break between the 2 trials, each participant exited the tub, drink water *ad libitum* and was encouraged to wear clothing to ensure that T_{es} did not drop below resting levels.

The 2nd trial also started with a 20 min rest period in air, with data collected for the last 10 min, before re-entering the water tub, which had been changed to the calculated T_w which was used as a starting T_w for the warming period (Table 3.1). A ~25

min warming period was completed and after attaining the desired T_{es}, a stable T_{es} (± 0.1°C) and $\dot{V}_E$ (± 2 L·min^{-1}) for 10 min was required before beginning the second HVR trial. Performing the normothermic and hyperthermic trials on the same day is designed to remove the significant effect ($p < 0.001$) of day-to-day variation on the HVR (51). Therefore any differences between the normothermic and hyperthermic HVR responses should be the result of a physiological response and not because of day-to-day variability. Also, normothermic trials were performed first as the residual effects of hyperthermia if performed first would be much greater than normothermia.

3.3.4 Statistical analysis

The dependent variable of the iHVR was the mean change in ventilation per mean percent change in S_aO_2 (L·min^{-1}· % $S_aO_2^{-1}$) from rest to the 5th min ($iHVR_5$) and from rest to the 20th min ($iHVR_{20}$) as recommended (58; 59). These variables were also used to calculate the amount of isocapnic hypoxic ventilatory decline (HVD) using the following recommended equation (58):

$$HVD\ \% = 100 \bullet [1 - (iHVR_{20} / iHVR_5)] \quad \text{..... Equation 3.3}$$

The $iHVR_5$ and $iHVR_{20}$ values were analyzed using a 2 x 2 analysis of co-variance (ANCOVA) with factors of Time (5th and 20th min) and Temperature (Normothermia and Hyperthermia). The dependent variable was the absolute residuals when $\dot{V}_E$ was expressed as a function of its covariate S_aO_2. A 2 x 2 repeated measures analysis of variance (RM - ANOVA) was also used with the same Factors of Time (5th and 20th min) and Temperature (Normothermia and Hyperthermia). The dependent variables for this analysis were the iHVR values and $\dot{V}_E$. A correlated t-test was used to compare across Temperature changes in HVD %.

Further analysis of ventilatory and cardiovascular parameters for rest phases and the iHVR trials was performed using a 3 x 2 RM - ANOVA with factors of Test Phase (Unimmersed Air (UA), Immersed Air (IA) and Immersed iHVR test (IH)) and Temperature (Normothermia and Hyperthermia). The three levels for Test Phase were the mean values from the last 5 min of the phases shown in Figure 3.2. Repeated preplanned orthogonal contrasts were performed between levels of the Test Phase factor. Correlated t-tests were used to compare within and across Temperature changes, with directional changes using a single-tailed p-value and non-directional changes a two-tailed p-value. The dependent variables for these analyses were, $\dot{V}_E$, tidal volume (V_T), respiratory frequency (f_R), fraction of inspired CO_2 (F_ICO_2), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$), O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), HR and S_aO_2.

A simple linear regression was performed on the percent changes in $\dot{V}O_2$ and $\dot{V}_E$ from Normothermia to Hyperthermia during the iHVR trial. Single sample t-tests were used to verify a 1.5°C mean increase in T_{es} from Normothermia to Hyperthermia during the iHVR and to verify that there was no difference in $P_{ET}CO_2$ levels between normothermia and hyperthermia trials. Also a single sample t-test was used to compare the T_w employed and that predicted using Equation 3.2. The level of significance was set at an $\alpha < 0.05$ and the alpha level was adjusted using the Bonferroni correction so as to maintain the rate of Type I error at 5 % ($p < 0.05n^{-1}$; where n = number of comparisons). All data are reported as the mean (SD) unless otherwise stated.

3.4 Results

All participants completed all aspects of this study. Mean T_w was 35.8 (0.5)°C for Normothermia and 38.6 (0.2)°C for Hyperthermia (Table 3.1). Both these temperatures differed significantly from those predicted using Equation 3.2 ($t = 12.44$, $df = 7$, $p < 0.001$ and $t = 2.52$, $df = 7$, $p = 0.02$, respectively). There was a significant increase in $P_{ET}CO_2$ from UA to IA for Normothermia (Figure 3.1C). The increase in $P_{ET}CO_2$ levels from the IA to Entire iHVR was not significant due to Bonferroni corrections to an alpha level of $\alpha = 0.01$ for both Normothermia ($t = -2.89$, $df = 7$, $p = 0.012$) and Hyperthermia ($t = -2.30$, $df = 7$, $p = 0.028$). The test phases used for analysis and the time course responses of $\dot{V}_E$, F_IO_2, $P_{ET}CO_2$, S_aO_2 and T_{es} for a typical participant are illustrated in Figure 3.2.

The ANCOVA on the absolute residuals when $\dot{V}_E$ was expressed as a function of the covariate % S_aO_2 showed no effect of Time ($F = 0.13$, $df = 1$, $p = 0.73$) or Temperature ($F = 0.03$, $df = 1$, $p = 0.88$) and no Temperature x Test Phase interaction ($F = 0.11$, $df = 1$, $p = 0.92$). The responses as measured using $iHVR_5$ and $iHVR_{20}$ values were 0.61 (0.64) and 0.82 (1.24) $L \cdot min^{-1} \cdot \% S_aO_2^{-1}$ for Normothermia and 0.82 (0.74) and 1.00 (0.84) $L \cdot min^{-1} \cdot \% S_aO_2^{-1}$ for Hyperthermia respectively. A RM - ANOVA on these $iHVR_5$ and $iHVR_{20}$ variables also showed no main effects of Time ($F = 0.97$, $df = 1$, $p = 0.36$) or Temperature ($F = 1.36$, $df = 1$, $p = 0.29$) and no Temperature x Test Phase interaction ($F = 0.05$, $df = 1$, $p = 0.95$).

However, for $\dot{V}_E$ during the 5^{th} and 20^{th} min of the iHVR trial (Table 3.2), there was a significant effect of Temperature ($F = 46.51$, $df = 1$, $p < 0.001$), a trend for an effect of Time ($F = 3.23$, $df = 1$, $p = 0.12$) and no Temperature x Test Phase interaction (F

= 1.12, df = 1, p = 0.33). However, the $\dot{V}_E$ response may not have peaked until the 6th min therefore the $\dot{V}_E$ data using the 6th min instead of the 5th min was analyzed but still showed no effect of time during the HVR (F = 1.37, df = 1, p = 0.28). The size of HVD % (Equation 3.3) using the 6th and 20th min during the iHVR trial was highly variable between participants, 2.6 (64.6) % for Normothermia and -24.5 (62.7) % for Hyperthermia and these two values were not different from each other (t = 0.93, df = 7, p = 0.38). Therefore it was concluded that there was no HVD during iHVR trials for both Temperature conditions and so the last 5 min of each iHVR trial was used to represent the steady-state response to isocapnic hypoxia.

For $\dot{V}_E$ responses there were main effects of Test Phase (F = 18.73, df = 2, p = 0.001) and Temperature (F = 32.64, df = 1, p = 0.001) and there was a significant Temperature x Test Phase interaction (F = 32.28, df = 2, p < 0.001) (Figure 3.3A). Ventilation during UA was not different between Temperature conditions (t = -1.32, df = 7, p = 0.23). Between UA and IA, $\dot{V}_E$ increased significantly for Hyperthermia (Figure 3.3A) but not for Normothermia (t = -0.02, df = 7, p = 0.99). The increased $\dot{V}_E$ from UA to IA during the Hyperthermic trial was accompanied by proportionate increases in O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) (Table 3.3, Figure 3.5A). There was a significant increase in $\dot{V}_E$ from the IA to IH phase for both Normothermia and Hyperthermia (Figure 3.3A). Also, the increase in $\dot{V}_E$ from the IA to IH was significantly greater during Hyperthermia compared to Normothermia (Figure 3.4A). There was also a significant (p = 0.01) positive linear correlation between the percent increase in $\dot{V}O_2$ and $\dot{V}_E$ from the Normothermic to Hyperthermic iHVR trial (Figure 3.5B). Individual ventilatory responses are given in Table 3.4.

There was a main effect of Test phase on V_T ($F = 32.26$, $df = 2$, $p < 0.001$), where there was a significant increase in V_T from IA to IH ($F = 38.51$, $df = 1$, $p < 0.001$) but not UA to IA ($F = 0.88$, $df = 1$, $p = 0.38$), but there was no main effect of Temperature on V_T ($F = 1.62$, $df = 1$, $p = 0.24$) (Figure 3.3B). Moving from IA to IH increased V_T from 0.69 (0.09) L to 0.94 (0.18) L, or 37 %, during normothermia and 0.69 (0.11) L to 1.05 (0.15) L, or 53 %, during hyperthermia (Figure 3.3B). There was also a significant Temperature x Test Phase interaction ($F = 7.55$, $df = 2$, $p = 0.01$) where the increase in V_T from IA to IH was significantly greater during Hyperthermia compared to Normothermia (Figure 3.4B).

Changes in f_R (Figure 3.3C) were the opposite to V_T as there was no main effect of Test Phase ($F = 1.49$, $df = 2$, $p = 0.26$), a main effect of Temperature ($F = 37.28$, $df = 1$, $p < 0.001$) and a Temperature x Test Phase interaction ($F = 5.10$, $df = 2$, $p = 0.02$) (Figure 3.4C). In the hyperthermic trial there was a significant increase of 25 % in f_R from a UA level of 16.8 (2.9) breaths·min^{-1} to an IA level of 21.0 (4.3) breaths·min^{-1} while no changes in f_R were evident in the normothermic condition (Figure 3.3C). This increase in f_R from UA to IA for Hyperthermia was greater compared to Normothermia (Figure 3.4C) but there was no additional increase in f_R from IA to IH during the Hyperthermic trial ($t = 0.14$, $df = 7$, $p = 0.89$).

For F_ICO_2 (Figure 3.6A) there was also a main effect of both Test Phase ($F = 156.98$, $df = 2$, $p < 0.001$), Temperature ($F = 60.17$, $df = 1$, $p < 0.001$) and a significant Temperature x Test Phase interaction ($F = 22.97$, $df = 2$, $p < 0.001$). There was a significantly greater F_ICO_2 during IH compared to the IA for both Temperature conditions (Figure 3.6A). However, when investigating the interaction between Test

Phase and Temperature the increase in F_ICO_2 from IA to IH was 0.57 (0.23) % larger ($F = 16.97$, $df = 1$, $p = 0.004$) during Hyperthermia than during Normothermia (Figure 3.7A).

There was a main effect on Test Phase ($F = 325.51$, $df = 2$, $p < 0.001$) and Temperature ($F = 164.24$, $df = 1$, $p < 0.001$) on HR and also a Temperature x Test Phase interaction ($F = 159.48$, $df = 2$, $p < 0.001$). Heart rate was not different between Temperature conditions during UA ($t = -0.37$, $df = 7$, $p = 0.72$) (Figure 3.6B). During Normothermia HR increased from 58 (11) beats·min^{-1} during IA to 67 (10) beats·min^{-1} during IH, while during Hyperthermia HR increased from 91 (11) beats·min^{-1} during IA to 103 (11) beats·min^{-I} during IH. The Test Phase x Temperature interaction was explained by no change in HR from UA to IA during Normothermia but a significant increase in HR for Hyperthermia (Figure 3.6B, 3.7B). The increase in HR from the IA to IH was not different between the two temperature conditions ($F = 0.61$, $df = 1$, $p = 0.46$) (Figure 3.7B).

Finally S_aO_2 was ~98 to 96 % during IA regardless of temperature and then decreased significantly from IA to IH by 8.7 (2.9) % during Normothermia and 8.5 (1.8) % during Hyperthermia (Figure 3.6C). There was a main effect of Temperature ($F = 17.34$, $df = 1$, $p = 0.004$) and a Temperature x Test Phase interaction ($F = 4.98$, $df = 2$, $p = 0.02$) for S_aO_2 where Hyperthermia showed a significant decrease in S_aO_2 from the UA to IA compared to Normothermia (Figure 3.7C). However the size of the decrease in S_aO_2 from the IA to IH was not significantly different between Temperature conditions ($F = 0.07$, $df = 1$, $p = 0.80$).

3.5 Discussion

This study confirmed our hypothesis that the ventilatory responses to whole body steady-state isocapnic hypoxia, are greater in passively heated hyperthermic versus normothermic humans. Although, the cardiovascular responses measured were not greater during the hyperthermic hypoxia compared to normothermic hypoxia. The increase in $\dot{V}_E$ from both UA to IA and from IA to IH during hyperthermia was greater than the increases during normothermia (Figure 3.4A). Another interesting and unexpected finding was the absence of any hypoxic ventilatory decline (HVD) during both normothermia and hyperthermia, $\dot{V}_E$ reached a relative plateau after ~6 min and then either did not decrease or tended to slowly rise throughout the entire 20 min hypoxic exposure (Table 3.2, Figure 3.2).

The greater HVR during hyperthermia, as opposed to normothermia, confirms the findings of other studies (10; 38; 46). What makes this study unique is that for the first time it shows the size of this increase in $\dot{V}_E$ during hypoxic hyperthermia when other mitigating factors such as exercise, multiple steady-states of hypoxia, or the dynamic phase of the HVR response have been removed. The increase in $\dot{V}_E$ from IA to IH during hyperthermia was 7.2 $L \cdot min^{-1}$ or 53 % (Figure 3.3A), this increase was significantly greater than the 4.1 $L \cdot min^{-1}$ or 40 % increase (Figure 3.3A) during normothermia (Figure 3.4A). Therefore, according to our data, the human ventilation response to a 20 min isocapnic hypoxic exposure with an F_IO_2 of 12 % is 76 % greater in humans whose T_{es} is elevated by 1.5°C from normothermic rest in water.

Hypoxic ventilatory decline (HVD)

The finding of no HVD in any of the participants in any trials was not only unexpected but had only been documented once before in bilateral carotid body resected humans (26) and makes this discovery both novel and difficult to interpret. The initial increase in ventilation is known to be due to peripheral chemoreceptor excitation as reviewed by Honda (1995), while the secondary depression or HVD, remains a controversial topic (27). Immersion in water is the most likely difference in our methods that could explain the absence of the HVD. Immersion in water causes an increase in central blood volume (CBV) and concomitant increase in cardiac output via increase stroke volume, but no change in mean arterial pressure as a decrease in sympathetic tone causes vasodilation reducing total peripheral resistance (33). One possible cause of HVD to be excluded due to immersion is an increase in cerebral blood flow (CBF) from the increased cardiac output caused by immersion. An increased CBF is a proposed (4) and as yet unproven (39) cause of HVD, so an increased cardiac output would be expected to enhance HVD not remove it. Another possibility that can potentially be ruled out is an increase in external pressure on the carotid baroreceptors affecting the HVR response. Participants necks were not immersed in water suggesting there would be little change in the hydrostatic pressure on the carotid bodies. Also, pressure stimulation of carotid baroreceptors has been shown not to influence the ventilatory response to 5 000 m simulated altitude (5).

Two possible causes of the absence of HVD due to water immersion are the decreased sympathetic tone and increased CBV. Hypoxia causes an increase in sympathetic tone (40), and a decrease in this tone may be a cause of HVD. Since immersion decreases sympathetic tone, this may remove HVD by preventing the initial

overshoot in the HVR. Also the increased CBV with immersion, would increase arterial pressure and raise baroreflex activity. Increased baroreflex activity has been shown to attenuate ventilatory, (20) vascular (21) and sympathetic muscle activity responses during hypoxia (19). Our results show a trend for a continued rise in $\dot{V}_E$ (Table 3.2), however, our $\dot{V}_E$ levels appear much lower during normothermic hypoxia than other similar studies. Our peak $\dot{V}_E$ was 14.4 (3.4) $L \cdot min^{-1}$ during normothermic hypoxia (Table 3.2), whereas other studies involving similar isocapnic hypoxic exposures during rest report $\dot{V}_E$ peaks of 51.6 (9.7) $L \cdot min^{-1}$ (59), 54.4 $L \cdot min^{-1}$ (1), ~38 $L \cdot min^{-1}$ (46), ~23 $L \cdot min^{-1}$ (37) and 18.9 (2.7, SEM) (19). Therefore maybe our in the current study there is a lack of overshoot in the $\dot{V}_E$ response to hypoxia.

Another hypothesis to explain the lack of overshoot is the initial large increase in $\dot{V}_E$ and V_T during hypoxia is to increase the ventilation-perfusion ratio (48). This increase in $\dot{V}_E$ also stretches the alveolar sacs, increasing the ventilation perfusion ratio and surfactant secretion (14) and so $\dot{V}_E$ subsequently decreases as a better matched ventilation perfusion ratio develops. If this is so, this may help explain why exercise (41; 42) and head-up-tilt (23) lower the HVR. The raised $\dot{V}_E$, CBV and surfactant secretion during these conditions better matches the ventilation perfusion ratio via greater alveolar ventilation, and therefore $\dot{V}_E$ does not need to increase as much as during seated rest. Immersion in water would do the same via increased CBV (33). The greater blood volume in the thorax due to the increased CBV, would mean when $\dot{V}_E$ increases there is instantly better matching of $\dot{V}_E$ and perfusion. Therefore $\dot{V}_E$ does not need to initially overshoot recruit more alveoli. Negating this hypothesis is the assumption that water

immersion decreases the ventilation perfusion ratio during euoxic rest via a decreased functional residual capacity (47). However, the hypothesis above requires an increased $\dot{V}_E$ to better match perfusion. As mentioned the absence of any HVD was unexpected and a completely novel finding. Performing iHVR tests immersed in water then in air and measuring the post HVR ventilatory response to investigate any undershoot in $\dot{V}_E$ would help to elucidate the cause of this phenomena. However, as mentioned this is a secondary albeit very interesting finding of this study and so such data are unavailable.

Thermal effects on ventilation

While an increase in $\dot{V}_E$ (26 %) during hyperthermic rest in water was evident, this was accompanied by an increased $\dot{V}O_2$ (29 %) and $\dot{V}CO_2$ (29 %) (Table 3.3, Figure 3.5A). Therefore the increased $\dot{V}_E$ seen during the hyperthermic IA time level appears to be largely due to the increased energy cost of thermoregulation, breathing and the additional metabolic cost due to the Q_{10} effect. This was unexpected as previous work has shown that the $\dot{V}_E$ increases during passive hyperthermia are above and beyond that required to meet increased metabolic demands (7; 16; 18; 31). A possible explanation is that our increase is T_{es} may not have been large enough. No change in $\dot{V}_E$ has been previously reported with passive and active increases of body temperature between 0.5 and 0.9°C (12; 22; 34; 67). Also, during continual passive heating, an exact T_{es} of 38.5°C (7), or an absolute change in T_c of ≥1.5°C (55; 56) have been reported to be required to induce hyperthermic hyperventilation for resting humans. While our increase in T_{es} during the hyperthermic IH time level test compared to normothermic IH time level was 1.51 (0.09)°C, the maximum T_{es} achieved during IA was 37.76 (0.16)°C. Due to the

small ~0.2°C drop in T_{es} during normothermic IA, most likely caused by the increased venous return due to the increased hydrostatic pressure, the increase in T_{es} from UA to IA for the Hyperthermic condition was ~1.2°C (Table 3.3). Therefore, while this is clinical hyperthermia, it may not have been a large enough increase to produce the previously reported and expected hyperthermic hyperventilation. The level for a change in breathing pattern but not $\dot{V}_E$ rate has been reported as ~38°C (45) and therefore our T_{es} may have been large enough to induce changes in breathing pattern (45). Finally, other passive heating studies reporting hyperthermic hyperventilation (7; 16; 18; 31; 55; 56) had a continually increasing body temperature, whereas this study used a steady-state hyperthermic T_{es}.

Breathing pattern

The changes in breathing pattern due to the hypoxic and hyperthermic stimuli were clearly separate with the responses to hypoxia being mediated by changes in V_T, and responses to hyperthermia being mediated by changes if f_R (Figure 3.3B & C). Much debate has surrounded the effect of increased body temperature on breathing patterns. This study agrees with the majority of studies which show with hyperthermia there is an increase in f_R with little or no change in V_T (2; 46; 60; 61). The 25 % increase in f_R due to the raised T_{es} in IA was in contrast to the absence of change in f_R from UA to IA for normothermia (Figure 3.4B). This differs to other studies which have shown a decreased f_R and increased V_T during passive body warming (16; 55; 56). The hyperthermia induced f_R and hypoxia induced V_T increases for this study (Figure 3.3 B and C) directly oppose the view that the response to hyperthermia is an increase in V_T as an increase in f_R is the response seen to a hypoxic or hypercapnic stimulus (16). This discrepancy may be due to

the extreme and prolonged nature of the heat exposure in the Gaudio and Abramson study, while they may be referring to prolonged hypoxic exposures (> 2 h) in other studies where f_R eventually increases while V_T decreases (3). If the increased $\dot{V}_E$ during hyperthermia is to serve a thermoregulatory mechanism, increasing f_R would seem to be the most efficient method. Increasing f_R would raise the amount of dead space ventilation per minute, this would be more effective at promoting heat loss than increasing V_T which would not change the amount of dead space ventilation per minute.

As V_T was not different during the hyperthermic IA time level (Figure 3.3B & 3.4B), it is concluded that raising T_{es} by 1.5°C using passive heating did not influence V_T for our participants. The increased V_T from IA to IH during hyperthermia was 0.1 L, 38 % greater than during normothermia (Figure 3.4B). This finding is in agreement with other normothermic steady-state HVR work using both males and females (13; 52). To the best of our knowledge only one other study has looked at the interaction of hyperthermia and hypoxia on V_T and f_R responses for humans and their findings are in agreement with ours (69). White and colleagues using a 10 min hypoxic (F_IO_2 12 %) exposure during either normothermic or hyperthermic exercise in water also showed an increased f_R due to hyperthermia alone and an increased V_T due to the hypoxic stimulus alone. The current study however also demonstrated an interaction where the increase in V_T due to hypoxia was significantly greater during hyperthermia as opposed to normothermia. Therefore, according to our data, $\dot{V}_E$ responses to short-term hyperthermia appear to be mediated by changes in f_R, while $\dot{V}_E$ responses to short-term hypoxia appear to be mediated by changes in V_T.

Previous investigations on the human responses to both hypoxia and hyperthermia have suggested the increased sensitivity to hypoxia when hyperthermic was due to an increased peripheral sensitivity, which was most likely mediated by the carotid bodies (10; 38; 46). Direct heating of the carotid bodies of dogs (6) increased $\dot{V}_E$ while direct heating of the carotid bodies of cats (35) increased carotid sinus nerve activity. These studies illustrate that the carotid bodies are temperature sensitive. In the current study increased T_{es} alone had no effect on V_T, but hypoxia increased V_T and this increase was greater during hyperthermia compared to normothermia (Figure 3.4B). This suggests the mechanism for the V_T responses to hypoxia was affected directly by the hyperthermia as V_T did not change during the $\dot{V}_E$ increase with hyperthermia. As the response to hypoxia is peripherally sensed (25) our results agree with previous conclusions that the greater $\dot{V}_E$ response to hypoxia during hyperthermia is peripherally sensed.

Another possibility is that the increased T_{es} indirectly caused the enhanced peripheral sensitivity to hypoxia via the increased metabolism by unknown mechanism(s). Hypermetabolism due to carbohydrate and protein feeding increased the A-shape parameter of a progressive HVR when compared to a placebo meal (70). The size of the increase in metabolism postprandial showed a significant positive linear correlation to the size of the increase in the A-shape parameter. A significant positive linear correlation during the iHVR was also seen in this study (Figure 3.5B) where as the size of the increase in $\dot{V}O_2$ from normothermia to hyperthermia during the iHVR test became greater, so did the size of the increase in $\dot{V}_E$. However, $\dot{V}_E$ increased disproportionally more than $\dot{V}O_2$ (Figure 3.5B) meaning that increased oxygen demand alone can not explain the larger $\dot{V}_E$ response to hypoxia during hyperthermia. Therefore,

the enhanced peripheral sensitivity to hypoxia during hyperthermia may be caused by the increased metabolism due to the increased T_{es}, rather than the increased T_{es} per se.

Cardiovascular responses

Further evidence that the increased ventilatory sensitivity to hypoxia while hyperthermic was due to increased peripheral sensitivity comes from the HR data. During the UA time level HR was the same between temperature conditions (Figure 3.6B) and the increase in HR by 35 beats·min^{-1} or 61 % (Figure 3.6B) due to hyperthermia in the IA was significantly greater than normothermia which remained relatively unchanged (Figure 3.7B). This is expected because HR increases as body temperature rises due to the increased skin blood flow as a part of thermoregulation, this response is commonly accepted to be mediated by central controllers such as the hypothalamus (17; 53). Furthermore, when exposed to hypoxia under the normothermic condition HR rose by 10 beats·min^{-1} and by 11 beats·min^{-1} under the hyperthermic condition (Figure 3.6B) and these changes were not significantly different (Figure 3.7B). Therefore, like the f_R response, the HR response to hypoxia and hyperthermia was additive, suggesting that the controller responsible for the increased $\dot{V}_E$ sensitivity to hypoxia did not also influence HR responses. This conclusion agrees with Kobayashi and colleagues (29) who reported no interaction between ventilatory and cardiovascular responses during a 20 min iHVR test. They concluded that the controllers for these systems were not closely linked. This is consistent with the enhanced peripheral sensitivity to hypoxia during hyperthermia hypotheses as the tachycardia seen during hypoxia is centrally mediated via the medullary vagal control center (50), while hypoxia on the other hand is peripherally

sensed (25). None of the other studies on hyperthermia and the human iHVR reported HR data and so this appears to be a novel finding.

The responses of S_aO_2 in this study confirm a consistent stimulus for both Temperature conditions. Although the different T_{es} seen during IA caused the hyperthermic S_aO_2 to drop and become significantly lower, 2.6 (1.6) %, than the normothermic condition (Figure 3.7C). An increased T_{es} would shift the oxyhemoglobin dissociation curve to the right lowering S_aO_2 at a given P_aO_2, although during normoxic rest this shift would have little effect. Such a change in normoxic hyperthermic S_aO_2 has been previously demonstrated and is thought to be caused by peripheral arterio-venous shunting caused by the increased skin blood flow (57; 62). This shunting means that some venous blood during arterial pulsations may be measured by the pulse oximeter (57). The different Temperature conditions during the IA time level would not have affected the ventilatory response as this is a peripheral measure of arterial oxygen saturation and esophageal measures of arterial oxygen saturation have shown this shunt does not occur centrally (62). A decreased skin blood flow during IH may have reduced the peripheral arterio-venous shunting effect (65). However, the rightward shift of the oxyhemoglobin dissociation curve due to the increase T_{es} would now have a greater effect on S_aO_2 hence why during the Hypoxic phase the hyperthermic S_aO_2 still read lower than the normothermic condition 2.4 (2.7) %. The size of the decrease in S_aO_2 from the IA to IH time level was not significantly different between Temperature conditions (Figure 3.7C) and therefore the hypoxic stimulus should still be the same and not be the case of the greater increase in $\dot{V}_E$ during hyperthermic hypoxia.

Recommended measures

To detect changes during the HVR trials and between Temperature conditions the recommended iHVR measures of $iHVR_5$ and $iHVR_{20}$ were employed (58). Using an ANCOVA and a RM - ANOVA there was no main effects of Time or Temperature and no interaction. Power calculations were done to determine a suitable sample size based on the RM - ANOVA (Appendix D). These calculations predicted a power of 1.0 for both the Temperature and Time conditions (Appendix D), using data from pilot work (Appendix B) and other studies using the same HVR method (59). However, the power for the Temperature effect was calculated using $\dot{V}_E$ data and using our current $\dot{V}_E$ data we confirm these power calculations with a power of 0.94 on the interaction effect between Time and Temperature. Failure to detect a Time or Temperature effect using the $iHVR_5$ and $iHVR_{20}$ values is most likely due to the absence of HVD during all trials and the generally lower ventilation response to hypoxia in our study. For example, our $iHVR_5$ value was 0.61 (0.64) $L \cdot min^{-1} \cdot \% S_aO_2^{-1}$ while Steinback et al. (2005) reported 2.37 (0.61) $L \cdot min^{-1} \cdot \% S_aO_2^{-1}$ (Appendix D).

The hyperthermic hypoxic $\dot{V}_E$ response also appears reduced as Petersen & Vejby-Christensen (1977), reported a resting euoxic hyperthermic (T_{re} 1.4°C above resting) $\dot{V}_E$ of ~38 $L \cdot min^{-1}$, which increased to ~55 $L \cdot min^{-1}$ during hyperthermic hypoxia. Our corresponding hyperthermic euoxic value was 13.58 (2.58) $L \cdot min^{-1}$ and 20.79 (3.73) $L \cdot min^{-1}$ for hyperthermic hypoxia. In our study we heated participants using water immersion, Petersen & Vejby-Christensen (1977), used a heated flying suit (46).

Clamping end-tidal carbon dioxide

Clamping of the $P_{ET}CO_2$ at 0.99 (0.66) mm Hg above normothermic resting levels allowed this study to investigate the whole body response to hypoxia with the effects of changing $P_{ET}CO_2$ removed. In doing so another variable, F_ICO_2, can be used to analyze the size of the hyperventilation induced during hypoxia. Figure 3.6A, indicates significantly more CO_2 during IH compared to IA was needed to maintain isocapnia during both Temperature conditions confirming the hyperventilation seen during the hypoxic stimulus. The greater increase in $\dot{V}_E$ under the hyperthermic compared to the normothermic condition is also confirmed as the F_ICO_2 required to maintain isocapnia during IH was significantly greater during hyperthermia compared to normothermia (Figure 3.7A).

Effect of immersion in water

Immersion in normothermic water in this study only affected $P_{ET}CO_2$ levels. Ventilation, V_T, f_R, $\dot{V}O_2$, HR and S_aO_2 levels all remained essentially the same between the UA and IA time levels. Increases in $P_{ET}CO_2$ levels with immersion in water are suggested to be due to the decreases in the ventilation perfusion ratio. This decrease is caused by a reduction in the functional residual capacity from the pressure exerted on the chest by the water, this shifts the diaphragm upwards and consequently reduces alveolar ventilation and raising $P_{ET}CO_2$ (47). No change in the $P_{ET}CO_2$ may also occur if the end expiratory volume is maintained via a lowered closing volume of the lung. This prevents any decrease in alveolar ventilation (9; 47). This however was not the case in the current study as all participants demonstrated an increase in $P_{ET}CO_2$.

Core temperature regulation

This study successfully achieved and maintained a normothermic and hyperthermic (1.51 (0.09)°C above normothermic) T_{es} during the apropriate time levels of this experiment. This was partially achieved using the re-arrangement of Park and colleagues body insulation equation (Equation 3.2). The equation allowed a good estimate of an suitable T_w, however, for both Temperature conditions the equation predicted a T_w lower than the final T_w used during the experiment (Table 3.1). The partial failure of the equation for this study maybe due to this study's experimental conditions not suiting the parameters under which previous I_{rest} values were calculated and under which this equation should be used. The I_{rest} values and equation were designed to calculate the lowest T_w that a resting individual could tolerate for 3 h, not to maintain T_{es} constant for 3 h. This means that the equation would be expected to predict a higher I_{rest} and lower T_w at rest, than that needed to maintain a constant T_{es} as it was not designed to maintain T_{es} but to maintain normothermia. This conclusion is substantiated firstly by the *post-hoc* calculation of the actual I_{rest} values for the current study (Table 3.1). These were slightly lower than those predicted using the re-arrangement of Park and colleagues body insulation equation (Equation 3.2). Secondly in the Park and colleagues paper, although there was no shivering induced after 3 h of thermoneutral water immersion, T_{re} dropped by ~0.7°C, 0.5°C of this drop within the first hour. Therefore the equation can predict a T_w close to that required to maintain a specific T_{es}, but under resting conditions this value needs some fine tuning.

Variability in the response to hypoxia

As expected there was great inter-individual variability in participants response to hypoxia (Table 3.3). This is a well documented phenomenon and to date no definitive

cause has been identified, but the time of day, consumption of food, inspired CO_2 content, altitude acclimation status, age and genetic background are all known to affect individuals HVR (24; 28; 30; 51; 54; 63; 64; 66). As this study used a repeated measures design and performed all tests at the same time of day and while in the fasted state, we attempted to control for these factors as much as possible. However, there is a significant ($p < 0.001$) intra-individual variability which exists in the day to day HVR responses (51). For this reason both the normothermic and hyperthermic trials were performed on the same day as within-day variability is lower than between day variability (51) and therefore any differences between the normothermic and hyperthermic HVR responses should be the result of a physiological response and not because of day-to-day variability. Performing the normothermic then hyperthermic trials was designed to remove any possible residual effects of hyperthermia if performed first. Of interest is that almost all the low and medium responders to isocapnic hypoxia (except participant # 4) in this study had a greater absolute increase in their $\dot{V}_E$ response during the hyperthermic relative to the normothermic condition. Therefore by increasing their T_{es} their $\dot{V}_E$ response increased further implying that body temperature increased their sensitivity to hypoxia.

Conclusions

The major findings of this study are that when other mitigating factors are removed, the ventilatory response to sustained hypoxia during passive hyperthermia is greater (76 %) than during passive normothermia. The increased $\dot{V}_E$ due to the hypoxic stimulus was mediated solely through changes in V_T. These changes in V_T were also greater in the hyperthermic condition which supports the notion that the increased hypoxic sensitivity is peripherally mediated via increased body temperature or increased

metabolism due to increased T_{es}. While the increased ventilation during hyperthermic air breathing was not disproportionate to the increase in metabolic demands, there was a temperature-induced increase in f_R. Heart rate responses were not greater during the hyperthermic compared to normothermic hypoxic condition as we hypothesized they would be. This again implies the effect of body temperature on ventilatory responses to hypoxia was mediated at the peripheral level. Finally a generally lower HVR response and the complete absence of HVD in all trials was a major and unexpected outcome. We hypothesize this is due to the immersion in water for all tests, and may be caused the by decrease sympathetic tone removing the initial overshoot or the greater CBV enhancing the ventilation perfusion matching at the onset of the hypoxic stimulus.

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The biggest debt in this study is owed to my participants, who, with their time and commitment made this entire study possible. Also to Dr. Matt White and Dr. Michael Walsh, your help, guidance and insights have been invaluable throughout this project. Thank-you both for influencing this study in the way you have.

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3.9 Tables

Table 3.1 Participants physical characteristics and their predicted (Pred) and actual (Act) overall body insulations at rest (I_{rest}) and water temperatures (T_w) for the Normothermic (Normo) and Hyperthermic (Hyper) temperature conditions.

					I_{rest} ($°C/kcal \cdot m^{-2} \cdot h^{-1}$)		T_w (°C)			
							Normo		Hyper	
Participant	Age (yr)	Weight (kg)	Height (cm)	SS (mm)	Pred	Act	Pred	Act	Pred	Act
1	27	81.3	180	79.2	0.115	0.013	31.3	35.7	38.8	38.4
2	24	75	193	38	0.074	0.009	33.0	35.9	37.9	38.6
3	22	82	191	52.7	0.088	0.019	32.5	35.6	38.7	38.8
4	24	75	183	46.9	0.083	0.019	32.4	35.3	37.7	38.6
5	24	79.5	183	17.4	0.053	-0.007	34.0	36.5	38.2	38.5
6	23	73	178	28.7	0.064	0.000	33.4	36.2	37.8	38.2
7	30	80	178	95.7	0.131	0.025	30.5	35.0	38.6	38.9
8	29	73	193	51.2	0.087	0.012	32.9	36.1	38.3	38.8
Mean (SD)	**25.4 (2.9)**	**77.4 (3.7)**	**184.9 (6.5)**	**51.2 (25.7)**	**0.087 (0.026)**	**0.011 (0.011)**	**32.5 (1.1)**	**35.8 * (0.5)**	**38.3 (0.4)**	**38.6 * (0.2)**

sum of skinfolds (SS); Predicted $I_{rest} = 0.001 \cdot SS + 0.0357$; equation derived using a regression on data from (Chu & White, 2005). Actual I_{rest} calculated post-hoc using Park et al. (1984), Equation $I_{rest} = (T_{re} - T_w)/ (0.92 \dot{M} \pm \dot{S})$. (*significantly different from predicted T_w $p \leq 0.02$).

Table 3.2 Mean (SD) ventilation ($\dot{V}_E$, $L \cdot min^{-1}$) over the 5th, 6th, 10th, 15th and 20th min of the isocapnic HVR test.

	5th min	*6th min*	*10th min*	*15th min*	*20th min*
Normothermic	13.65 (3.13)	14.09 (2.54)	13.77 (2.67)	14.17 (2.82)	14.35 (3.40)
Hyperthermic	19.51 (3.81)**	19.93 (4.00)**	20.48 (3.28)**	20.47 (4.33)**	20.92 (4.22)**

(**significantly different from normothermia at $p < 0.01$).

Table 3.3 Mean (SD) ventilation ($\dot{V}_E$, $L \cdot min^{-1}$), oxygen consumption ($\dot{V}O_2$, $L \cdot min^{-1}$), carbon dioxide production ($\dot{V}CO_2$, $L \cdot min^{-1}$) and esophageal temperatures (T_{es}, °C) during air breathing for the last 5 min Unimmersed rest in Air (UA) and Immersed rest in Air (IA) during both Normothermia (Normo) and Hyperthermia (Hyper) Temperature conditions.

	$\dot{V}_E$		$\dot{V}O_2$		$\dot{V}CO_2$		T_{es}	
	UA	IA	UA	IA	UA	IA	UA	IA
Normo	10.10	10.12	0.230	0.245	0.196	0.213	36.51	36.34
	(2.65)	(1.07)	(0.046)	(0.021)	(0.043)	(0.007)	(0.22)	(0.20)
Hyper	10.80	13.58	0.236	0.304	0.201	0.260	36.62	37.76
	(2.47)	(2.58)**	(0.051)	(0.046)**	(0.052)	(0.038)**	(0.22)	(0.16)**

(**significantly different from Normothermia, at $p < 0.01$).

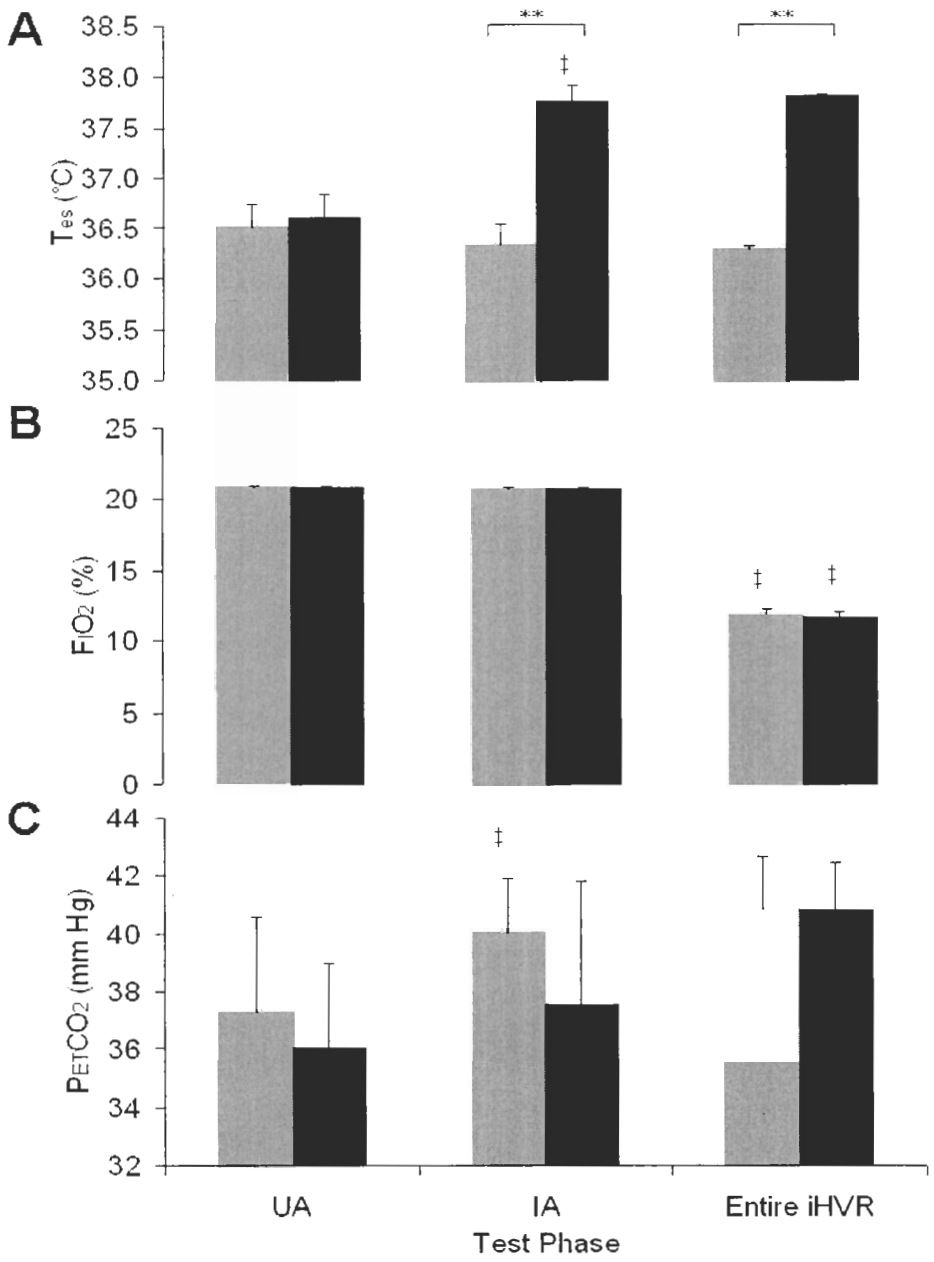
Table 3.4 Absolute change ($L \cdot min^{-1}$) and percent change (%) in ventilation ($\dot{V}_E$) from immersed rest in air (IA) to immersed isocapnic hypoxia (IH) for all participants and their subsequent responder status

Participant	Responder Status	Normothermic		Hyperthermic	
		Absolute change	% change	Absolute change	% change
1	High responder	6.40	69.57	6.31	46.74
2	Low responder	-0.06	-0.55	2.53	16.59
3	High responder	10.46	113.94	14.81	124.87
4	Med-responder	2.67	27.78	3.10	17.15
5	High responder	6.02	55.43	8.29	55.60
6	Med-responder	3.00	28.54	9.12	65.33
7	Low responder	1.14	12.93	3.74	35.12
8	Med-responder	2.97	25.06	9.77	93.85
Mean (SD)		**4.08 (3.48)**	**41.59 (36.72)**	**7.21 (4.15)**	**56.91 (37.50)**

Changes are from the final 5 min $\dot{V}_E$ during immersed air (IA) to final 5 min $\dot{V}_E$ during immersed isocapnic hypoxia (IH). Response status is judged on size of response during normothermia and was categorized as follows, High responder % increase $\geq$ 40 %, Medium (Med) responder % increase 20-39 %, Low responder % increase < 20 %.

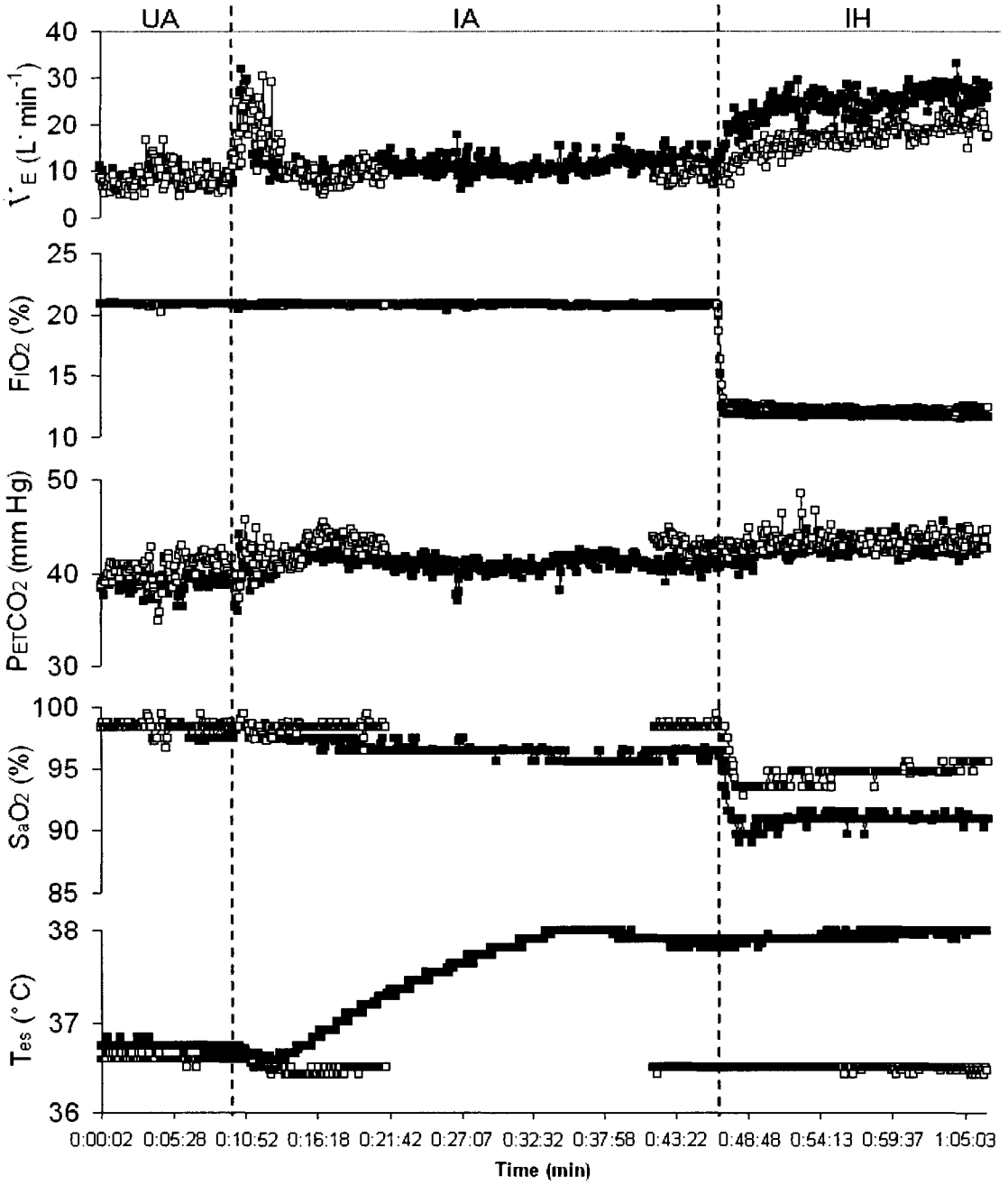
3.10 Figures

Figure 3.1 Mean (SD) esophageal temperature (T_{es}), inspired oxygen content (F_IO_2) and end-tidal CO_2 ($P_{ET}CO_2$) for the last 5 min of Unimmersed rest in Air (UA) and Immersed rest in Air (IA), and for the entire 20 min of the iHVR trial (Entire iHVR).



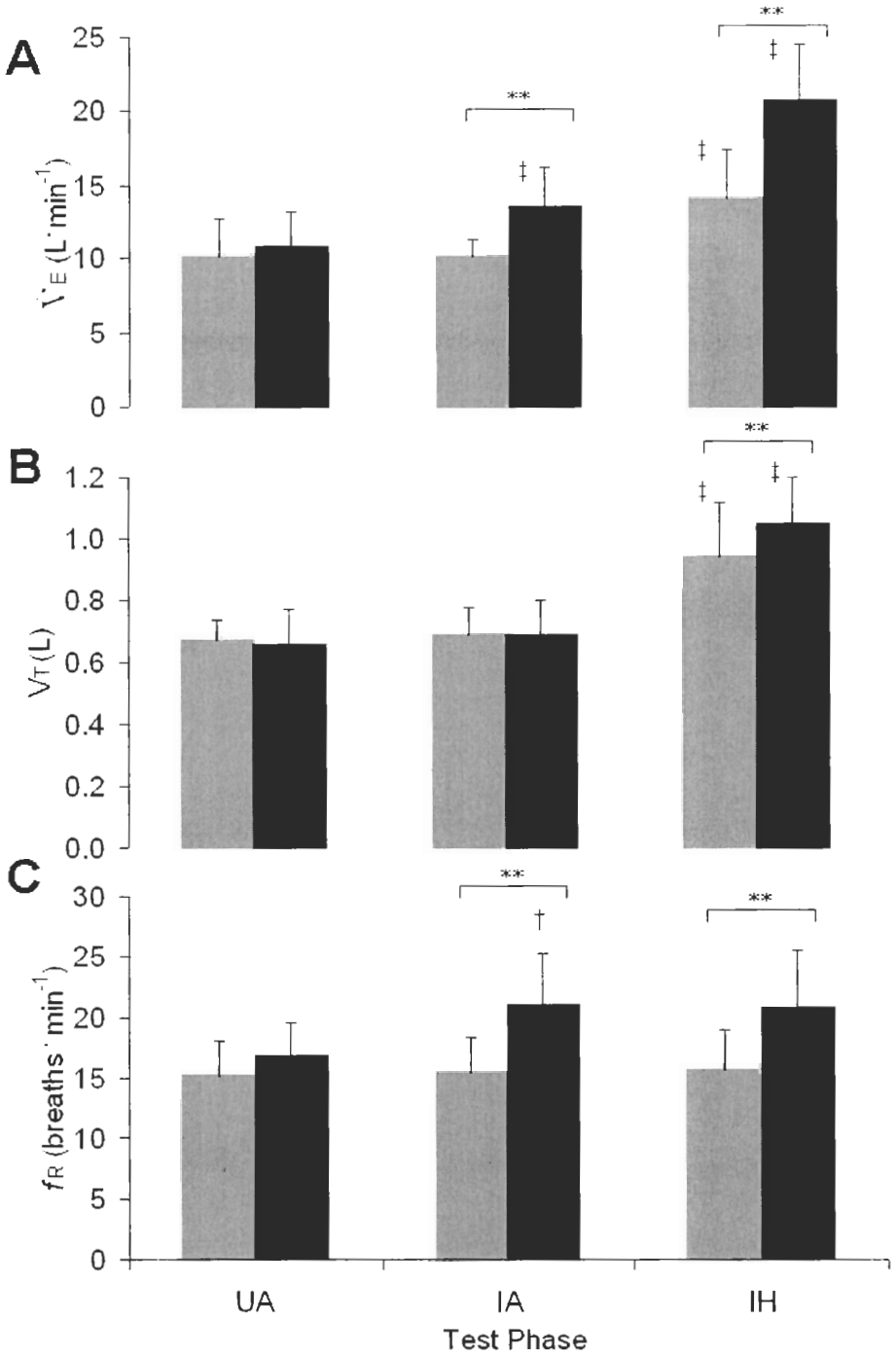
Values are means (SD) for 8 participants. □ normothermic condition; ■ hyperthermic condition. (**significantly different at $p < 0.01$, † significantly different from previous test phase $p < 0.05$, ‡ significantly different from previous test phase $p < 0.01$). Only significant differences are shown. $P_{ET}CO_2$ levels were clamped only during the Entire iHVR phase 0.99 (0.66) mm Hg above normothermic IA levels. For clarity only significant differences are shown.

Figure 3.2 Time course of ventilation ($\dot{V}_E$), fraction of inspired oxygen (F_IO_2), end-tidal CO_2 ($P_{ET}CO_2$), arterial hemoglobin saturation (S_aO_2) and esophageal temperature (T_{es}) for a typical participant during the entire iHVR trial for both Temperature conditions.



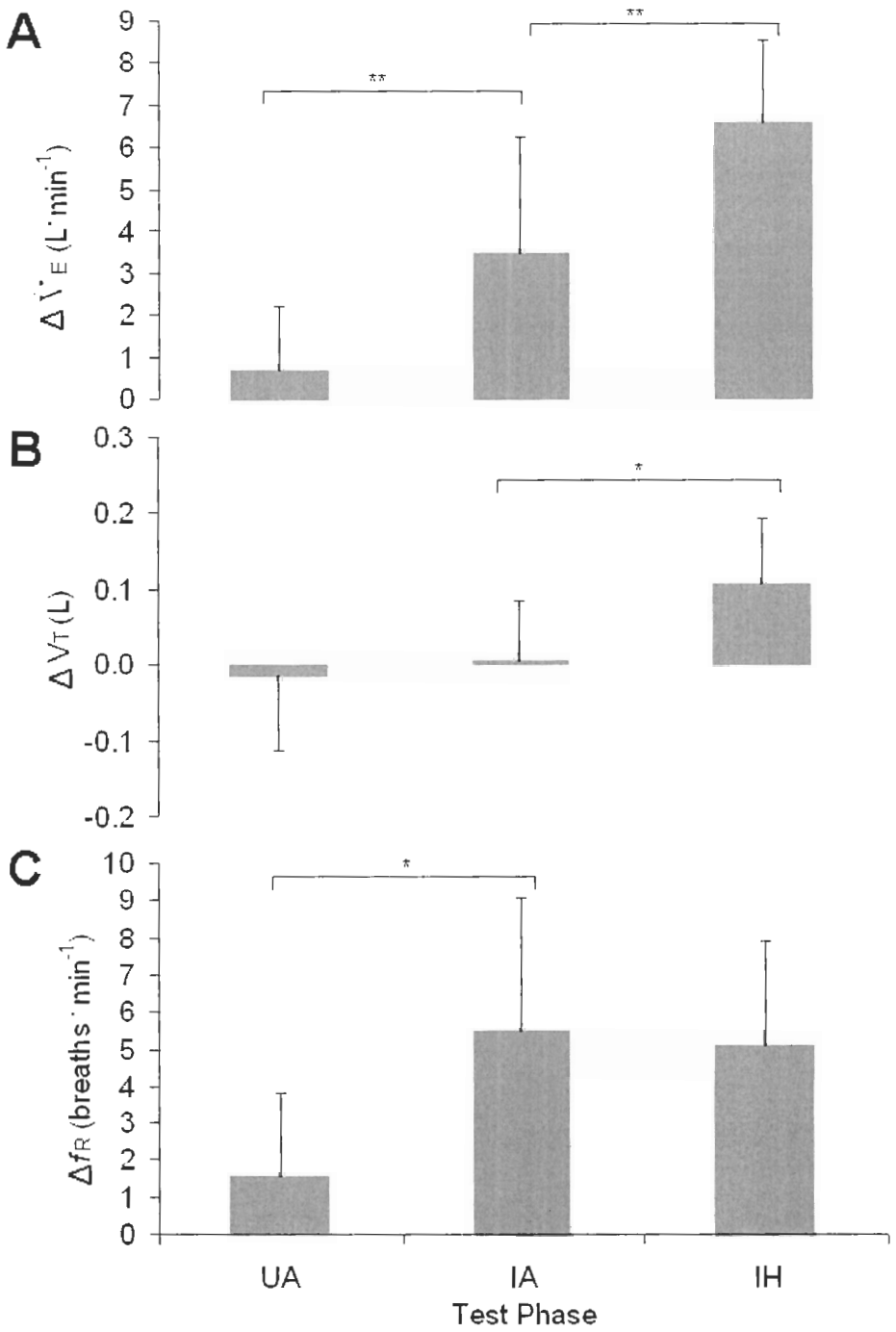
□ Normothermic condition, ■ Hyperthermic condition. Responses are for participant #3. Each data point represents a 5 s mean, this was done to standardize the timing points for both Temperature conditions and the gap during Normothermia was used to standardize timing points after the heating period during the Hyperthermic trial. UA represents 10 min rest phase out of water, IA represents the rest phase in water and IH represents the 20 min isocapnic hypoxic exposure. Means of the last 5 min of each test phase were used for most data analysis.

Figure 3.3 Ventilation ($\dot{V}_E$), tidal volume (V_T) and breathing frequency (f_R) during Unimmersed rest in Air (UA), Immersed rest in Air (IA) and the Immersed iHVR test (IH) for both Temperature conditions.



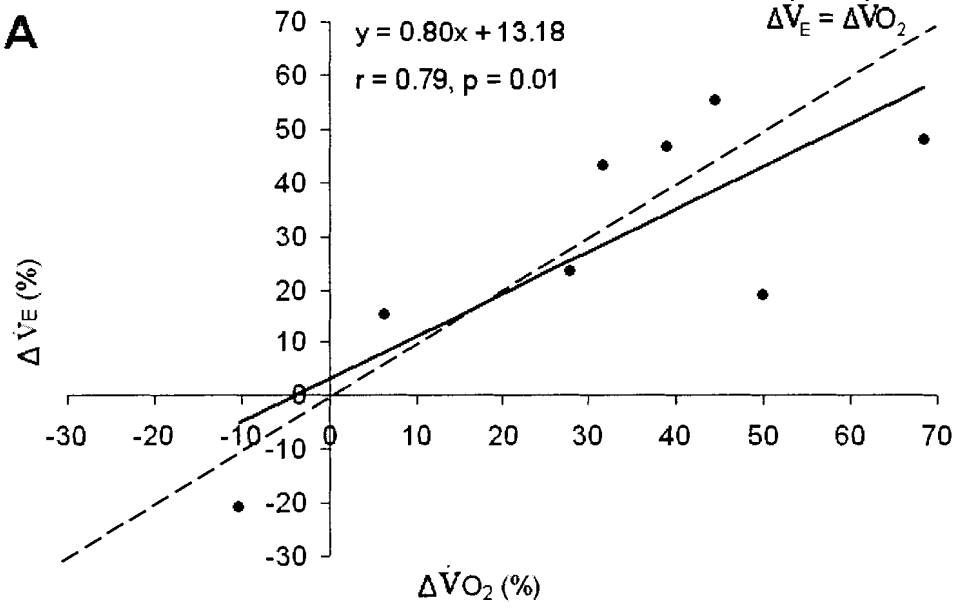
Values are means (SD) for 8 participants. ▨normothermic condition; ■hyperthermic condition. (**significantly different at $p < 0.01$, † significantly different from previous test phase $p < 0.05$, ‡ significantly different from previous test phase $p < 0.01$). For clarity only significant differences are shown.

Figure 3.4 Differences in ventilation ($\Delta \dot{V}_E$), tidal volume (ΔV_T) and breathing frequency (Δf_R) from normothermic trial to hyperthermic trial during Unimmersed rest in Air (UA), Immersed rest in Air (IA) and the Immersed iHVR test (IH).

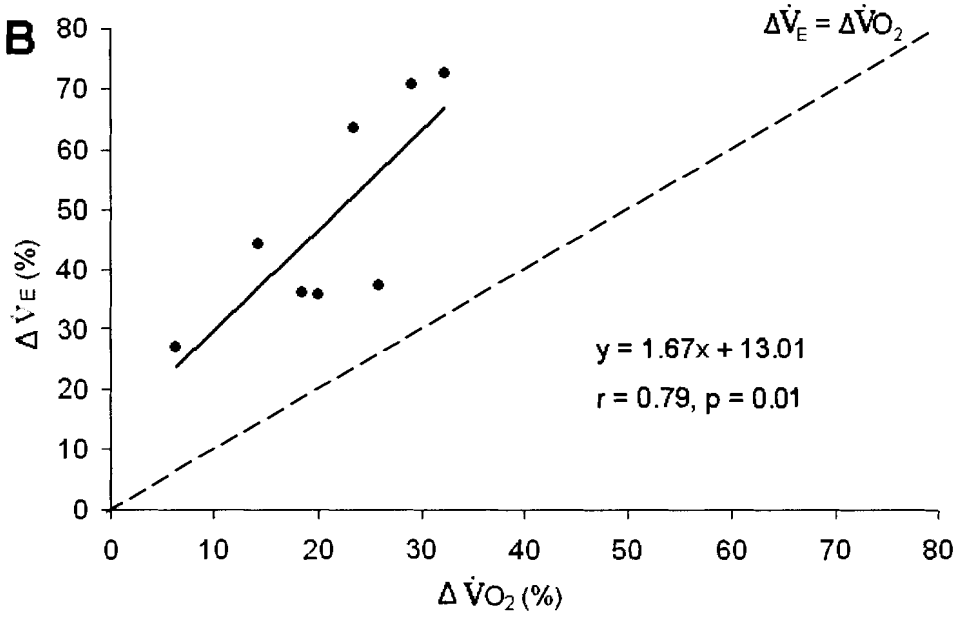


Values represent mean (SD) change from the Normothermic to Hyperthermic condition for 8 participants. (*significantly different from previous test level, $p < 0.05$, ** significantly different from previous test level, $p < 0.01$). For clarity only significant differences are shown.

Figure 3.5 The relationship between percent changes in metabolic rate ($\Delta \dot{V}O_2$) and ventilation ($\Delta \dot{V}_E$) from UA to IA test phases for the hyperthermic trial (A), and from Normothermic to Hyperthermic trials during IH (B).

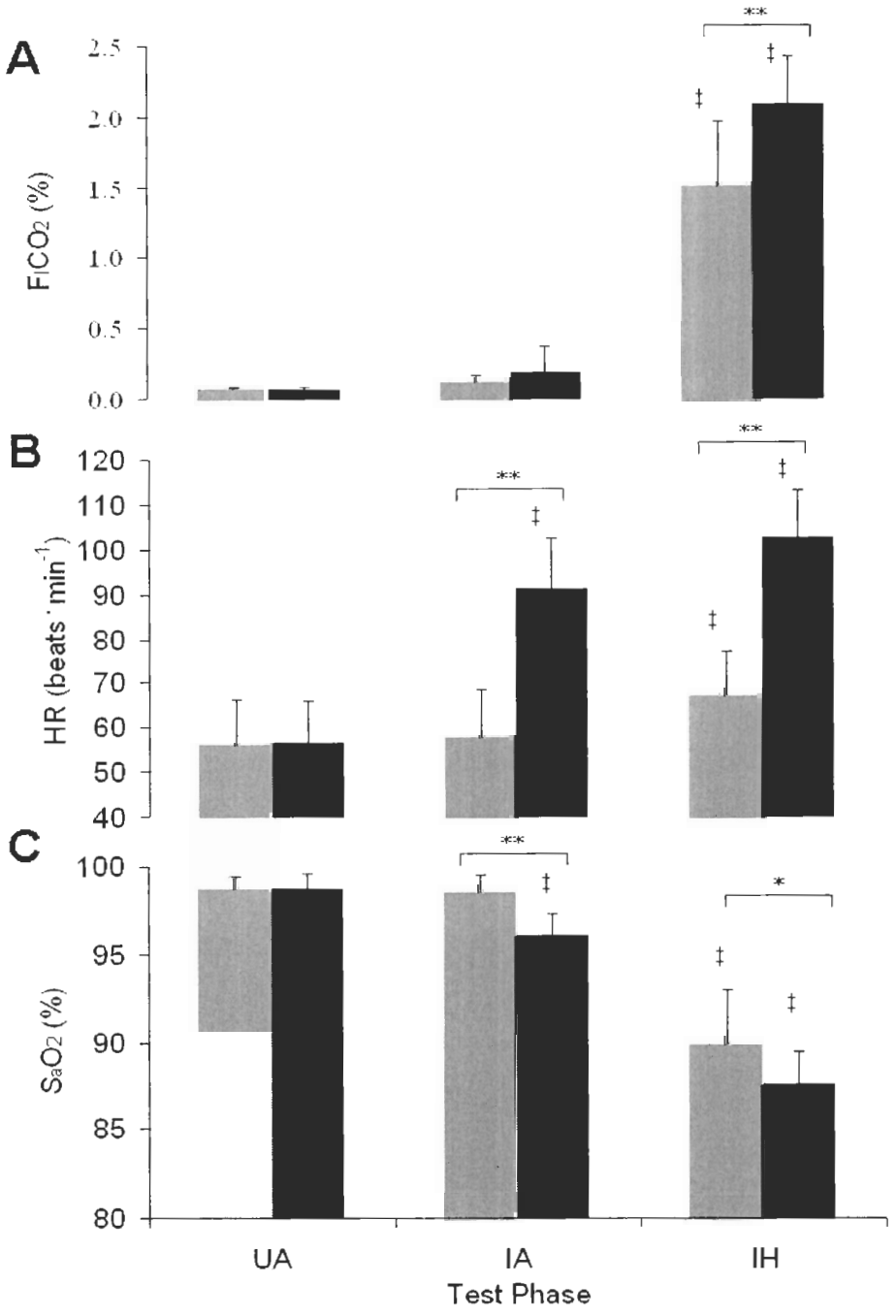


Values in panel A are the percent changes in $\dot{V}O_2$ and $\dot{V}_E$ from UA to IA test phases for the Hyperthermic trial. The figure demonstrates that the increase in ventilation due to hyperthermia was proportionate to the increase in metabolic rate. Line of identity ($\Delta \dot{V}_E = \Delta \dot{V}O_2$) is given by the dotted line.



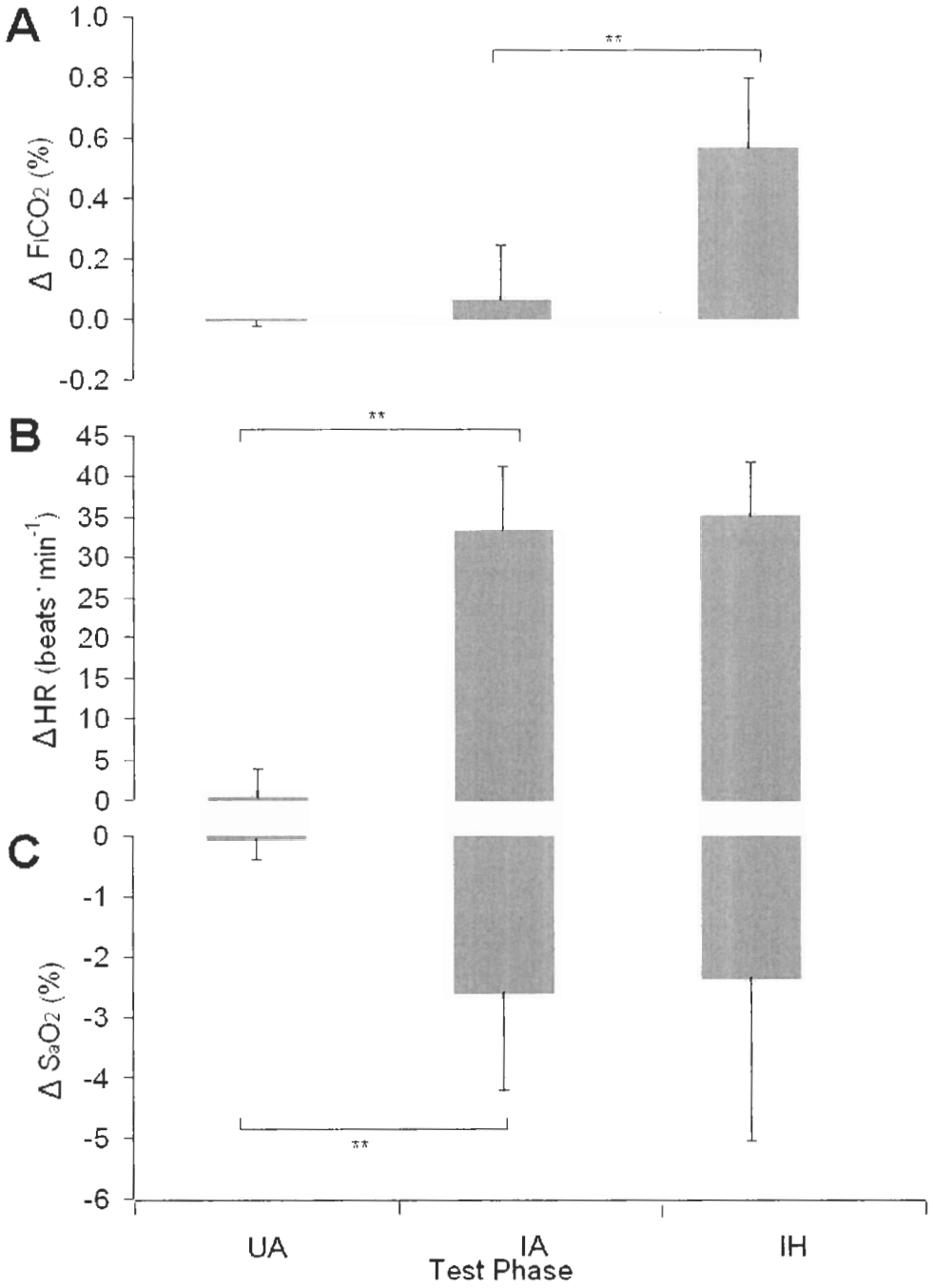
Values in panel B are the percent changes in $\dot{V}O_2$ and $\dot{V}_E$ from Normothermia to Hyperthermia during the IH test phase. The figure demonstrates that the increase in ventilation during isocapnic hypoxia was disproportionally greater than the increase in metabolic rate. Line of identity ($\Delta \dot{V}_E = \Delta \dot{V}O_2$) is given by the dotted line.

Figure 3.6 Inspired fraction of carbon dioxide (F_ICO_2) so as to maintain isocapnia, heart rate (HR) and arterial hemoglobin saturation (S_aO_2) during Unimmersed rest in Air (UA), Immersed rest in Air (IA) and the Immersed iHVR test (IH) for both Temperature conditions for each Time Phase and Temperature.



Values are means (SD) for 8 participants. ⊡Normothermic condition; ■ Hyperthermic condition. (*significantly different at $p < 0.05$, **significantly different at $p < 0.01$, ‡ significantly different from previous test phase, $p < 0.01$). For clarity only significant differences are shown.

Figure 3.7 Differences in inspired fraction of carbon dioxide (ΔF_ICO_2) so as to maintain isocapnia, heart rate (ΔHR) and arterial hemoglobin saturation (ΔS_aO_2) from normothermia to hyperthermia during Unimmersed rest in Air (UA), Immersed rest in Air (IA) and the Immersed iHVR test (IH).



Values represent mean (SD) change from the Normothermic to Hyperthermic condition for 8 participants. (**significantly different from previous test level, $p < 0.01$). For clarity only significant differences are shown.

CHAPTER 4

Study 2

Influence of passive hyperthermia on human ventilation during hypoxia.

4.1 Abstract

The purpose of this study was to investigate the effect of hyperthermia on cardio-respiratory responses to hypoxia. On two separate days 7 males twice underwent two 20 min head out immersions in either a thermoneutral mean (SD) 35.8 (0.2)°C or a warm 38.5 (0.1)°C water bath while breathing a pre-mixed 12 % O_2, balance N_2 hypoxic gas. On one day the $P_{ET}CO_2$ was controlled at an isocapnic level (iHVR), 0.98 (0.71) mm Hg above resting, on the other it was uncontrolled (pHVR). Each day the first test was at a normothermic esophageal temperature (T_{es}) 36.34 (0.03)°C and the second at a hyperthermic T_{es} 37.84 (0.02)°C. During the iHVR the rise in ventilation ($\dot{V}_E$) from ~13 $L \cdot min^{-1}$ was greater during hyperthermia than normothermia, 7.26 (4.83) and 4.29 (2.65) $L \cdot min^{-1}$ respectively ($p = 0.002$), but not during the pHVR 1.68 (2.21) and 1.69 (1.58) $L \cdot min^{-1}$ respectively ($p = 0.98$). Possibly due to the smaller $\dot{V}_E$ response during the pHVR trials, heart rates at the end of the pHVR compared to the end of the iHVR were 6 (8) $beats \cdot min^{-1}$ ($p = 0.05$) greater for normothermia from at resting value of ~59 $beats \cdot min^{-1}$ and 5 (3) $beats \cdot min^{-1}$ ($p = 0.004$) greater for hyperthermia from at resting value of ~89 $beats \cdot min^{-1}$. Also from resting values of ~98 %, arterial hemoglobin saturation levels at the end of the pHVR compared to the end of the iHVR were 11.6 (7.8) % ($p = 0.004$) and 12.4 (4.0) % ($p \geq 0.001$) lower for normothermia and hyperthermia respectively. Therefore, in the poikilocapnic condition decreased $P_{ET}CO_2$ appeared to blunt the HVR more than a 1.5°C increase in T_{es} augmented it during iHVR, while the pHVR increases cardiovascular responses to hypoxia compared to the iHVR.

(287 words)

4.2 Introduction

The magnitude of the hypoxic ventilatory response (HVR) varies according to the end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) (6; 16; 19; 20). Decreasing $P_{ET}CO_2$ levels, such as during a poikilocapnic HVR test (pHVR), blunt or lower the size of the HVR relative to eucapnic conditions. Using rebreathing tests, Rapanos & Duffin (1997), showed that ventilation ($\dot{V}_E$) did not increase in response to hypoxia until a $P_{ET}CO_2$ threshold of 39 ± 2.7 (SD) mm Hg, despite $P_{ET}O_2$ dropping as low as 37 ± 4.1 (SD) mm Hg. Small increases in $P_{ET}CO_2$ on the other hand, such as seen during the isocapnic HVR test (iHVR), increase the HVR. Rebuck & Woodley (1975), used predetermined levels of CO_2 to measure the progressive HVR using rebreathing and found the greater the level of hypercapnia the greater the ventilatory response to hypoxia.

A raised core body temperature (T_c), also enhances the ventilatory response during an iHVR test as demonstrated in Chapter 3 and by others (5; 17). The effect of poikilocapnic hypoxia on the thermoregulatory system has received attention with regards to changes in control of skin blood flow, endocrine function and sweating (13; 14; 24). Natalino, et al. (1977), investigated the effect of a clamped hypocapnic $P_{ET}CO_2$ during a hyperthermic progressive HVR test. The hypocapnia was induced by the increased $\dot{V}_E$ during hyperthermia and the size of this response was compared to the normothermic eucapnic response. They reported there was a trend ($p = 0.01$) for the HVR response to still be larger during hyperthermia compared to normothermia when hypocapnic. What has not been studied before is the effect of an increased esophageal temperature (T_{es}) on the cardio-respiratory responses to steady state poikilocapnic hypoxia or normobaric hypoxia.

At altitude both a reduced HVR and raised T_c are symptoms of acute mountain sickness (AMS) and/or high altitude pulmonary edema (HAPE) (11; 15). If the trend for a greater $\dot{V}_E$ response to isocapnic hypoxia when T_c is raised is the same during poikilocapnic hypoxia then this is in disagreement with the symptoms for AMS and HAPE. However, the pHVR causes a large decrease in $P_{ET}CO_2$ which is a strong blunter of the HVR. It is possible that a reduced $P_{ET}CO_2$ may also reduce the effect an increased T_c has on the HVR. Therefore it remains unknown whether T_c continues to enhance the steady state HVR when the $P_{ET}CO_2$ is lowered, such as during simulated altitude. The purpose of this study was to investigate the effect of hyperthermia on ventilatory and cardiovascular responses to the steady state pHVR test plus to compare these responses to the steady state iHVR test.

4.3 Methods

The methods used in this chapter were the same as in Chapter 3 except for the following:

4.3.1 Participants

All participants completed all aspects of this study, however participant number seven was left out of all analyses as he was an outlier. The difference between his hyperthermic and normothermic $P_{ET}CO_2$ for the entire pHVR was -7.75 mm Hg, while the group mean (SD) was -2.82 (0.88) mm Hg. This response is a key measures in this study his response is 5.60 standard deviations outside the group mean. The difference between his hyperthermic and normothermic tidal volume (V_T) for the entire pHVR was 0.43 L, while the group mean (SD) was 0.08 (0.17) L. Furthermore, the difference between his hyperthermic and normothermic $\dot{V}_E$, $P_{ET}CO_2$, V_T, arterial hemoglobin saturation (S_aO_2) responses for the 20^{th} min of the pHVR were 2.40, 9.15, 2.35 and 2.24 standard deviations respectively outside the group mean differences between hyperthermic and normothermic responses.

4.3.2 Instrumentation

Instrumentation was the same for all tests as reported in Chapter 3.

4.3.3 Protocol

Participants underwent two 20-min pHVR tests separated by one hour on one day and two iHVR tests separated by one hour on a separate day, the order of tests was split equally among participants and all tests were started at the same time of day. On each day

the first test was at a thermoneutral water temperature (T_w) of 35.8 (0.2)°C so as to clamp T_{es} at a resting level 36.34 (0.03)°C and the second trial at a hyperthermic T_w of 38.5 (0.1)°C so as to clamp T_{es} 1.5°C above resting at 37.84 (0.02)°C. Individual increases in T_{es} from Normothermia to Hyperthermia for both iHVR and pHVR tests were not significantly different from 1.5°C ($t = 0.06$, $df = 13$, $p = 0.96$) and the mean increase in T_{es} from Normothermia to Hyperthermia for all participants for all trials 1.50 (0.08)°C. A criteria for starting each experiment was that the participant must have a core temperature within ± 0.2°C of the first testing session. The $P_{ET}CO_2$ was 40.84 (0.16) mm Hg during the normothermic iHVR trial and 40.51 (0.62) mm Hg during the hyperthermic iHVR trial, this represented a mean overall rise from normothermic rest of 0.98 (0.71) mm Hg. During the pHVR trials $P_{ET}CO_2$ levels were not controlled. The F_IO_2 for pHVR normothermic trials was 11.99 (0.23) % and 12.04 (0.22) % for the hyperthermic pHVR trials while for the normothermic iHVR trials F_IO_2 was 11.96 (0.29) % and 11.74 (0.24) % for the hyperthermic iHVR trials.

The pHVR was used to measure the whole body response to simulated altitude as developed and recently recommended as a universal method (27; 28). The recommended main measure of the pHVR is the mean change in $P_{ET}CO_2$ per mean percent change in S_aO_2 (mm Hg · % $S_aO_2^{-1}$) from rest to the 5th minute ($pHVR_5$) and from rest to the 20th minute ($pHVR_{20}$). Another novel measure was used to quantify the pHVR response using the mean change in $\dot{V}_E$ per mean mm Hg change in $P_{ET}CO_2$ (L·min^{-1} · mm Hg) from rest to the 5th minute ($pHVR2_5$) and from rest to the 20th minute ($pHVR2_{20}$).

4.3.4 Statistical analysis

The dependent variables employed were $\dot{V}_E$, $P_{ET}CO_2$, V_T, respiratory frequency (f_R), heart rate (HR), S_aO_2, $pHVR_5$, $pHVR_{20}$, $pHVR2_5$ and $pHVR2_{20}$. The $pHVR_5$, $pHVR_{20}$, $pHVR2_5$ and $pHVR2_{20}$ values were analyzed using a 2 x 2 analysis of co-variance (ANCOVA) with factors of Time and Temperature. Each factor had two levels: for Time, 5th and 20th min and for Temperature, Normothermia and Hyperthermia. For the $pHVR_5$ and $pHVR_{20}$ variables the dependent variable was the absolute residuals for $P_{ET}CO_2$ vs. S_aO_2. For the $pHVR2_5$ and $pHVR2_{20}$ variables the dependent variable was the absolute residuals for $\dot{V}_E$ vs. $P_{ET}CO_2$. A 2 x 2 repeated measures analysis of variance (RM - ANOVA) was also used with the same Factors of Time (5th and 20th min) and Temperature (Normothermia and Hyperthermia). The dependent variables for this analysis were the pHVR and pHVR2 values.

To further assess differences in ventilatory parameters, HR and S_aO_2 during the pHVR test a 3 x 2 repeated measures analysis of variance (RM - ANOVA) was used with factors of Time (Levels: Immersed Air, Initial Steady State/5th min and the 20th min) and Temperature (Normothermia and Hyperthermia). For the Time factor Immersed rest in Air (IA) was the mean during the last 5 min of the 10 min steady state rest in water period before the start of the pHVR test. For the Initial Steady State (ISS) or 5th min value if a clear breakpoint from the initial rise or decline was obvious during min 1 to min 7 of the pHVR test then the mean over this minute was used, if no discernable change or a continued decrease was seen then the mean during the 5th min was used. The mean of 20th min and final minute of the pHVR test was employed for the third level of the Time factor. The two levels for Temperature were the Normothermic and Hyperthermic T_{es}

conditions. Repeated and Simple pre-planned orthogonal contrasts were performed on the Time factor to investigate differences between the IA and ISS/5th min, ISS/5th min and 20th min and the 20th min and IA phases. Corrections were made to the degrees of freedom and p values using the Greenhouse-Geisser correction when epsilon values were ≤ 0.7)

Correlated t-tests were used to compare within and across Temperature changes in ventilatory parameters, HR and S_aO_2 for the pHVR and within-temperature changes between the pHVR and iHVR tests for $\dot{V}_E$, V_T, HR, S_aO_2 using the three time levels IA, ISS/5th and 20th min described above. When assessing the size of a directional change the correlated t-tests, used a single-tailed p-value, while non-directional changes used a two-tailed p-value. A single sample t-test was used to verify a 1.5°C mean increase in T_{es} from Normothermia to Hyperthermia. The level of significance was set at an $\alpha < 0.05$ and all data are presented as mean (SD) unless otherwise stated. All analyses were performed using the statistical software package SPSS 13.0 for Windows (SPSS Inc. Chicago, IL, USA).

4.4 Results

The test phases used for pHVR analysis and the time course responses of $\dot{V}_E$, T_{es}, $P_{ET}CO_2$, S_aO_2 and F_IO_2 for a typical participant during the pHVR are illustrated in Figure 4.1. The ANCOVA on the absolute residuals when $P_{ET}CO_2$ was expressed as a function of the covariate % S_aO_2 showed no effect of Time ($F = 0.01$, $df = 1$, $p = 0.93$) or Temperature ($F = 0.001$, $df = 1$, $p = 0.98$). The responses as measured using $pHVR_5$ and $pHVR_{20}$ values were 0.23 (0.09) and 0.19 (0.11) mm Hg · % $S_aO_2^{-1}$ for Normothermia and 0.28 (0.10) and 0.20 (0.03) mm Hg · % $S_aO_2^{-1}$ for Hyperthermia. A RM - ANOVA on these $pHVR_5$ and $pHVR_{20}$ variables also showed no main effects of Time ($F = 4.72$, $df = 1$, $p = 0.07$) or Temperature ($F = 1.71$, $df = 1$, $p = 0.24$). The ANCOVA on the absolute residuals when $\dot{V}_E$ was expressed as a function of the covariate $P_{ET}CO_2$ showed no effect of Time ($F = 0.04$, $df = 1$, $p = 0.95$) or Temperature ($F = 0.02$, $df = 1$, $p = 0.90$). The responses as measured using $pHVR2_5$ and $pHVR2_{20}$ values were 2.41 (2.83) and 1.3 (1.61) L·min^{-1}· mm Hg $P_{ET}CO_2^{-1}$ for Normothermia and 5.32 (10.17) and 2.84 (6.58) L·min^{-1}· mm Hg $P_{ET}CO_2^{-1}$ for Hyperthermia. A RM - ANOVA on these $pHVR2_5$ and $pHVR2_{20}$ variables also showed no main effects of Time ($F = 0.39$, $df = 1$, $p = 0.56$) or Temperature ($F = 2.95$, $df = 1$, $p = 0.14$).

There was a main effect of Temperature for $\dot{V}_E$ during the pHVR ($F = 28.24$, $df = 1$, $p = 0.002$), where $\dot{V}_E$ was greater during Hyperthermia than Normothermia and a trend for a Time effect ($F = 2.55$, $df = 2$, $p = 0.12$). There was no increase in $\dot{V}_E$ from ~13 L·min^{-1} between IA and the 5th min of the pHVR for both Normothermia ($t = -1.30$, $df = 6$, $p = 0.12$) and Hyperthermia ($t = -1.33$, $df = 6$, $p = 0.12$). The ISS values for $\dot{V}_E$ during

the pHVR trials occurred during the 2nd min for Normothermia and the 3rd min for Hyperthermia (Figure 4.2A and 4.3A) and were 13.69 (2.41) and 16.47 (3.45) $L \cdot min^{-1}$ respectively. When comparing the ISS values to IA there were similar sized significant increases in $\dot{V}_E$ of ~1.69 $L \cdot min^{-1}$ for both Temperature conditions during the pHVR (Figure 4.3A). At all three levels of the Time condition during the iHVR and pHVR trials, $\dot{V}_E$ was significantly greater during Hyperthermia than in Normothermia (Figure 4.3A). Also during the pHVR trials, $\dot{V}_E$ still did not change significantly from the ISS to the 20th minute for both Normothermia ($t = 1.08$, $df = 6$, $p = 0.32$) and Hyperthermia ($t = 1.15$, $df = 6$, $p = 0.30$). There was no Time x Temperature interaction for the $\dot{V}_E$ values during the pHVR ($F = 0.01$, $df = 2$, $p = 0.99$) (Table 4.2). Ventilation during the ISS level of the Time condition was greater during the hyperthermic iHVR than the hyperthermic pHVR test, while the $\dot{V}_E$ level during the 20th min was greater during the iHVR than the pHVR for both Temperature conditions (Table 4.3).

During the pHVR trials, $P_{ET}CO_2$ began decreasing with the onset of hypoxia and reached a plateau after approximately 10 min (Figure 4.2B). Means during the pHVR 5th min of 38.09 (2.91) and 35.25 (3.46) mm Hg for Normothermia and Hyperthermia respectively were used for the analysis. At all three levels of the Time condition $P_{ET}CO_2$ was ~3 mm Hg lower during Hyperthermia than Normothermia (Figure 4.5A). From IA to the 5th min and from the 5th min to the 20th min $P_{ET}CO_2$ decreased for both Normothermia and Hyperthermia (Figure 4.5A). There was a trend for a Time x Temperature interaction for $P_{ET}CO_2$ during the pHVR ($F = 3.48$, $df = 2$, $p = 0.06$). This trend was due to the decrease in $P_{ET}CO_2$ from IA to the 5th min being greater for Hyperthermia compared to Normothermia, as there was no difference between

Temperature conditions in the size of the decrease in $P_{ET}CO_2$ from the 5th min to the 20th min ($F = 0.25$, $df = 1$, $p = 0.64$) (Table 4.2).

The ISS for V_T during the pHVR test occurred during the 3rd min for Normothermia (0.91 (0.16) L) and 4th min for Hyperthermia (0.98 (0.14) L). During the pHVR test there was an increase of ~ 0.20 L in V_T from IA to ISS for both Temperature conditions (Figure 4.3B). There was no Temperature main effect on V_T during the pHVR ($F = 1.41$, $df = 1$, $p = 0.28$). By the end of the pHVR 20th min V_T was relatively unchanged with respect to the ISS values, and was still significantly greater than IA values for Normothermia and Hyperthermia (Figure 4.2C and 4.3B). There was no Time x Temperature interaction for V_T during the pHVR ($F = 0.36$, $df = 1.13$, $p = 0.60$) (Table 4.2). The iHVR test had a greater V_T for the ISS level of the Time condition compared to the pHVR test for both Temperature conditions (Table 4.3).

For analysis of f_R, means during the pHVR 5th min of 15.82 (2.08) and 18.34 (4.19) breaths·min^{-1} for Normothermia and Hyperthermia respectively were used (Figure 4.4A and 4.5B). Hyperthermia gave a greater f_R than Normothermia during IA and 5th min levels of the Time phase (Figure 4.5B). By the end of the 20th min there was no difference in f_R between Hyperthermia and Normothermia ($t = -1.07$, $df = 6$, $p = 0.33$) (Figure 4.5B). There was a decrease in f_R from IA to 5th min during Normothermia (Figure 4.5B) and there was a trend for this decrease during Hyperthermia ($t = 1.48$, $df =$ 6, p 0.10) (Figure 4.5B). There was no Time x Temperature interaction for f_R during the pHVR ($F = 0.02$, $df = 1.28$, $p = 0.94$) (Table 4.2).

There was an initial steep decline which lasted ~5 min, then a continued gradual decline in S_aO_2 during the 20 min poikilocapnic hypoxic exposure (Figure 4.4B). Means

during the pHVR 5th min of 84.40 (2.81) and 82.75 (3.52) % for Normothermia and Hyperthermia respectively were used for the analysis (Figure 4.3C). For Normothermic and Hyperthermic conditions S_aO_2 decreased significantly during the pHVR from both IA to the 5th min and from the 5th min to the 20th min (Figure 4.3C). Although there was no Time x Temperature interaction for S_aO_2 ($F = 0.40$, $df = 1.12$, $p = 0.57$) the size of the decrease in S_aO_2 from IA to the 5th min tended to be larger during Hyperthermia compared to Normothermia ($F = 5.45$, $df = 1$, $p = 0.06$) (Table 4.2). The levels of S_aO_2 during the ISS and 20th min levels of the Time condition were greater during the iHVR test than the pHVR test for both Temperature conditions (Table 4.3).

A similar time course of responses during the pHVR test can be seen with HR as for S_aO_2. For both the pHVR and iHVR tests, HR was greater during Hyperthermia than the Normothermia (Figure 4.3D). For the pHVR test, the ISS for HR were 69 (13) beats·min^{-1} during the 5th min for Normothermia and 103 (11) beats·min^{-1} during the 6th min for Hyperthermia (Figure 4.3D and 4.4C). From IA to ISS during the pHVR, HR increased for both Temperature conditions and tended to progressively increase from ISS to the 20th min HR during Normothermia ($t = -1.65$, $df = 6$, $p = 0.08$) and Hyperthermia ($t = -1.39$, $df = 6$, $p = 0.11$) (Figure 4.3D). There was a trend for a Time x Temperature interaction for the pHVR test HR ($F = 2.52$, $df = 2$, $p = 0.12$) and this was due to the increase in HR from IA to ISS being greater during Hyperthermia compared to the Normothermia (Table 4.2). The HR during the ISS level of the Time condition was less during the iHVR test than the pHVR test for Hyperthermia, while the HR by the 20th min level of the Time condition was less during the iHVR test than the pHVR test for both Temperature conditions (Table 4.3).

4.5 Discussion

The purpose of this study was to investigate the effect of hyperthermia on ventilatory and cardiovascular responses to the steady state pHVR test, and to compare these responses to the steady state iHVR test. While $\dot{V}_E$ was greater and the $P_{ET}CO_2$ was lower during the hypoxic stimulus, the size of the ventilatory responses to the hypoxic stimulus was generally the same regardless of T_{es}. Cardiovascular changes from IA to ISS tended to be greater during hyperthermia than normothermia, however as the $P_{ET}CO_2$ decreased from ISS to the 20th min, the effect of an increased T_{es} on the cardiovascular responses to poikilocapnic hypoxia became less. These findings are in contrast to the responses seen in Chapter 3 and other studies on the isocapnic response to hypoxia (iHVR) and hyperthermia which show that hyperthermia increases the size of humans response to hypoxia (5; 17). During a progressive HVR test a slight blunting of the HVR during clamped lowered levels of $P_{ET}CO_2$ despite an increased T_c has been reported before (17). Clamping the $P_{ET}CO_2$ at eucapnic levels during hyperthermic rest (31.6 (5.4) mm Hg) instead of eucapnic levels during normothermic rest (38.6 (2.2) mm Hg), meant the HVR only tended ($p = 0.10$) to be greater during the hyperthermia compared to normothermia (17).

Hyperthermia has been shown to augment the $\dot{V}_E$ response to isocapnic hypoxia (5; 17; 29), while decreasing $P_{ET}CO_2$ levels are known to attenuate the $\dot{V}_E$ response to hypoxia (6; 19). During hyperthermia compared to normothermia $\dot{V}_E$ was greater during all Time levels (Figure 4.2A, Figure 4.3A). However, with the introduction of the hypoxic stimulus, the small additional increase of ~1.7 $L \cdot min^{-1}$ in $\dot{V}_E$ was the same for both Temperature conditions and changes throughout the test were relatively the same

between temperatures as well (Figure 4.3A & Table 4.2). Therefore this study appears unique in demonstrating that during a 20 min steady-state poikilocapnic hypoxia exposure, decreases in $P_{ET}CO_2$ appear to blunt the HVR more than a 1.5°C increase in T_{es} augments it, as seen during a 20 min steady-state isocapnic hypoxia exposure.

Ventilatory responses

Due to the poikilocapnic hypoxic stimulus, the ISS $\dot{V}_E$ level during both Temperature conditions was significantly greater than $\dot{V}_E$ level during IA (Figure 4.3A), while at the other comparison time points during the pHVR test, the 5th and 20th min, this was not so. During the pHVR, the small increase in $\dot{V}_E$ for both Temperature conditions did however produce a more measurable decrease in $P_{ET}CO_2$ levels, which is a better measure of ventilatory responses the pHVR test (Figure 4.2B) (27). The difference in $P_{ET}CO_2$ levels seen between temperature conditions appears to be due to the hyperventilation caused by the increased T_{es} (4; 8). The greater decrease in the $P_{ET}CO_2$ from IA to the 5th min during hyperthermia compared to normothermia implies a greater $\dot{V}_E$ response to the hypoxia, such as seen during isocapnia (Figure 4.3B and 4.5A). In contrast there was no difference in the size of the hyperthermic and normothermic decreases in $P_{ET}CO_2$ levels from the 5th min to the 20th min. Also when the 20th min $P_{ET}CO_2$ values were compared to IA values there was no difference in the size of the response between Temperature conditions (Figure 4.5A). This implies that initially the raised T_{es} may have increased the sensitivity to the hypoxic stimulus but as $P_{ET}CO_2$ levels dropped, the heighten sensitivity was diminished to the point where a raised T_{es} no longer influenced the size of the response.

The lowering of $P_{ET}CO_2$ levels by 5 to 10 mm Hg using voluntary hyperventilation before undergoing a rebreathing test to instigate progressive hypoxia has been shown to remove hypoxic responses (6). In our study we only saw a 3 to 4 mm Hg decrease in $P_{ET}CO_2$ levels by the end of the 20 min test so while not enough to completely remove the HVR, it may have been enough to remove the enhancement of the HVR brought on during isocapnic hyperthermia. Rapanos and Duffin on the other hand reported that $\dot{V}_E$ only increased during a hypoxic rebreathing test after $P_{ET}CO_2$ levels had risen above a peripheral-chemoreflex threshold of 39 (2.7) mm Hg (19). This threshold would explain the initial enhanced sensitivity to hypoxia during hyperthermia and subsequent blunting of this enhanced sensitivity by the 20th min. An increased T_{es} is thought to either increase peripheral sensitivity to hypoxia (17; 29), or lower the peripheral hypoxic threshold meaning a smaller decrease in P_aO_2 is required to increase $\dot{V}_E$ (1). The increased T_{es} may also lower the peripheral hypoxic threshold for a $P_{ET}CO_2$ influence on $\dot{V}_E$ before the response to hypoxia is blunted as a consequence of a lower $P_{ET}CO_2$. It follows that as $P_{ET}CO_2$ levels were initially lower during hyperthermia, there was a subsequent attenuation and lack of enhanced $\dot{V}_E$ sensitivity to hypoxia once the $P_{ET}CO_2$ had diminished below its threshold for its influence on ventilation.

Natalino, et al. (1977), in contrast clamped $P_{ET}CO_2$ at a hyperthermic eucapnic level of 31.6 (5.4) mm Hg, ~7 mm Hg below normothermic levels when they performed a progressive HVR(17). This decrease, although clamped, is a larger decrease than the 3 to 4 mm Hg decrease seen in this study and in contrast to our findings there was still a trend ($p = 0.01$) for the HVR to be larger during hyperthermia compared to normothermia. This may be due to the progressive decrease in S_aO_2 levels with the progressive HVR. This

decrease means the initial phase of the HVR would be measured, and according to our data, the heightened sensitivity to hypoxia during hyperthermia maybe maintained during this early period of the HVR test. Another possibility is due to the prolonged lowering of $P_{ET}CO_2$ levels during the hyperthermic exposure (30 min) (17), there was a resetting of the peripheral CO_2 threshold for a hypoxic response. Clamping $P_{ET}CO_2$ at or near this new peripheral CO_2 threshold therefore, may have maintained some of the temperature induced enhancement of HVR.

Breathing pattern

The increase in $\dot{V}_E$ during hypoxia was mediated by an increased V_T and not f_R (Figure 4.2C and 4.4A). There was an increase in V_T from IA to ISS for both Temperature conditions which was slightly attenuated by the 20th min of the pHVR, whereas f_R tended to decrease with the hypoxic exposure (Figure 4.4A). Like $\dot{V}_E$ the small hypoxia-induced increase in V_T during the pHVR was not completely inhibited by the decreased $P_{ET}CO_2$. This V_T increase during pHVR was attenuated as this increase was not as big as during the iHVR (Table 4.3). Also the size of the increase during pHVR was not different between temperature conditions so the effect of a raised T_{es} was again inhibited by a lower $P_{ET}CO_2$ (Table 4.2). Using collective data from previous studies Bender et al. (1987), were able to clearly demonstrate that the initial (min to hours) increases in $\dot{V}_E$ during progressive and steady state isocapnic hypoxia were mediated by an increased V_T with little or no change in f_R (2). Relative to isocapnic hypoxia the size of V_T and $\dot{V}_E$ increases are attenuated during poikilocapnic hypoxia or simulated altitude while f_R tends to decrease. The current study is the first to demonstrate that hyperthermia does not influence the size of the poikilocapnic hypoxia-induced increases in $\dot{V}_E$, V_T and f_R when

compared to normothermia. This is opposed to the isocapnic hypoxia-induced increase in $\dot{V}_E$ and V_T which are greater during hyperthermia compared to normothermia. We believe this may be due to $P_{ET}CO_2$ levels dropping below the peripheral CO_2 chemoreflex threshold (19), blunting the augmented peripheral sensitivity to hypoxia during hyperthermia.

Cardiovascular responses

There was a greater increase in HR during the pHVR test compared to the iHVR test at both Time levels of the hyperthermic and by the 20th min of the normothermic trials (Table 4.3). This does not support the view that both ventilatory and cardiovascular responses to hypoxia during hyperthermia are blunted once the $P_{ET}CO_2$ drops below the peripheral hypoxic threshold. During hypoxia there is a need for the body to respond in order to maintain adequate O_2 supply for the level of metabolism (3). In this study, the rate of O_2 supply is inferred through changes in S_aO_2. Increasing $\dot{V}_E$ is the most efficient mechanism to maintain alveolar ventilation and hence S_aO_2 to meet metabolic demands (21; 30). The drop in S_aO_2 for poikilocapnic hypoxia is greater than that during isocapnic hypoxia (Table 4.3) at both hypoxic time levels. Some of this may be due to decreases in arterial CO_2 levels, this increases the pH of the blood which decreases hemoglobin's affinity for O_2. This is called the Bohr effect. However this does not explain the ~12 % difference in S_aO_2 levels between the pHVR and iHVR test. Also as seen in Chapter 3, the initial drop in S_aO_2 from IA to the 5th min in this study tended to be larger during hyperthermia than normothermia (Table 4.2). Hyperthermia would be expected to increase the Bohr effect by causing a rightward shift of the oxy-hemoglobin dissociation curve. These differences in S_aO_2 levels between both iHVR and pHVR tests and between

Temperature conditions may show that HR changes were due to the attenuated $\dot{V}_E$ response rather than the decreased peripheral sensitivity to hypoxia itself.

As the increase in $\dot{V}_E$ due to hypoxia was blunted during the pHVR test, HR was increased instead to boost cardiac output. Boosting cardiac output helped maintain the ventilation perfusion ratio thereby better maintaining adequate S_aO_2 and O_2 delivery (22), and hence reduced the size of the decrease in S_aO_2. In agreement with this hypothesis, poikilocapnic vs. isocapnic progressive hypoxia during normothermia has previously been shown to produce a 61 % vs. 35 % increase in HR, a relatively greater decrease in S_aO_2 and only a 3-fold vs. 5-fold increase in $\dot{V}_E$ (12). These results of HR increases instead of $\dot{V}_E$ increases during poikilocapnic hypoxia are also supported by our normothermic and hyperthermic S_aO_2 data (Figure 4.3C and Figure 4.4B). As energy demands during the pHVR were greater during hyperthermia, 0.43 (0.06) $L \cdot min^{-1}$, than normothermia 0.37 (0.05) $L \cdot min^{-1}$, a larger increase in HR is to be expected as more O_2 needs to be delivered, and this is supported by the trend for a larger initial drop in S_aO_2 from IA to the 5^{th} min during hyperthermia relative to normothermia (Table 4.2). During the normothermic pHVR test HR was greater than during the iHVR test only at the 20^{th} min Time level (Table 4.3), this was also when S_aO_2 was at its lowest and therefore the hypoxic stimulus theoretically at its greatest (Figure 4.4B).

To maintain S_aO_2 during hypoxia, increasing HR is not as efficient as increasing $\dot{V}_E$ (10), and so this may be why S_aO_2 drops more during the pHVR test than the iHVR test (Table 4.3) (12). If decreased $P_{ET}CO_2$ levels removed or reduced the peripheral chemosensitivity to hypoxia, then the greater HR response may still be centrally sensed as suggested in Chapter 3. Decreases in cerebral oxygenation during hypoxia are greater

under poikilocapnic vs. isocapnic conditions (12). Secondary to changes in CO_2 and H^+ levels, central chemosensitive areas may sense the hypoxic stress and increase their output to increase $\dot{V}_E$ in an attempt to maintain adequate S_aO_2 levels (18). Alternatively the HR response may still be controlled by peripheral chemosensors and is not implemented when $\dot{V}_E$ changes can be utilized instead. Therefore HR increases may be greater during poikilocapnic hypoxia compared to isocapnic hypoxia because of the greater decrease in S_aO_2. However, during hyperthermia O_2 demand is greater due to the energy demands of thermoregulation (25). The size of the decrease in S_aO_2 during hyperthermia tended to be greater than normothermia (Table 4.2) and so this may be why HR was stimulated to increase more during the hyperthermic trial (12). Future studies on poikilocapnic and isocapnic hypoxic exposures could consider the effect of voluntary hyperventilation and hypoventilation on cardiovascular responses in order to better understand the cardiovascular responses discussed here.

Recommended and novel measures

The recommended pHVR measures of $pHVR_5$ and $pHVR_{20}$ (27) were employed to detect changes during the pHVR trials and between Temperature conditions. Another novel variable was employed, pHVR2 which used the mean change in $\dot{V}_E$ per mean mm Hg change in $P_{ET}CO_2$ ($L \cdot min^{-1} \cdot$ mm Hg) with the goal of helping explain the ventilatory response to poikilocapnic hypoxia. Using an ANCOVA and a RM - ANOVA there was no main effects of Time or Temperature and no Time x Temperature interaction for both the recommended pHVR values and the pHVR2 values. Power calculations (Appendix D) based on the RM - ANOVA from pilot work (Appendix B) and other studies using the same HVR method (28) established that 8 participants was a suitable sample and allowed

for the possible drop out of one participant. However, it appears that water immersion reduced the ventilatory response to isocapnic and poikilocapnic hypoxia for both Temperature conditions (Appendix D). Therefore, as there was no decline in $\dot{V}_E$ or pHVR values during the 20 min hypoxic exposure, a time effect would not be expected to be detected. Also as there was no difference between the size of the $\dot{V}_E$ or pHVR increase from IA to IH for both Temperature conditions a Temperature effect is not expected to be detected. Therefore the recommended and novel measures appear to agree with the conclusions drawn above which used non-ratio data. However, differences in the absolute values between our data and others means that not only should these measures be interpreted carefully, but they also require further testing for before being confirmed as valid and reliable measures.

Practical implications

These findings help shed light on the occurrence and influence of AMS, HAPE and HACE during simulated altitude. We have shown that an increased T_c would not be expected to help enhance the HVR during altitude exposure. If a raised T_{es} enhanced the pHVR as it did the iHVR then this could reduce the occurrence of AMS, HACE and HAPE by better maintain ventilation at altitude, but the lower $P_{ET}CO_2$ levels appear to blunt any enhancement caused by an increased T_{es}. Therefore attempts to increase T_c to help alleviate AMS, HACE and HAPE would not appear to be a prudent approach. Increasing T_c may worsen the conditions, possibly by further reducing the already lowered S_aO_2 (23; 26). What may be of use and warrants further investigation is the sporadic breathing of low levels of CO_2 to raise F_ICO_2 and thereby $P_{ET}CO_2$ and remove the blunting of the HVR. This could increase $\dot{V}_E$ improving resting S_aO_2 levels which

may reduce the risk/incidence of altitude sickness symptoms as the lower the resting S_aO_2 levels, often the greater the severity of AMS, HAPE or HACE symptoms (23; 26). This approach however must be balanced with the report that breathing 3.77 % CO_2 during a 5 day hypobaric hypoxic exposure had an adverse effect on maintaining P_aO_2 levels, as it prevented the early increase in hematocrit and increased the arterial hypoxemia (7; 9). However, the intermittent use of CO_2 to prevent or alleviate AMS, HAPE and HACE has, to the best of our knowledge, not been studied.

Conclusions

The purpose of this study was to investigate the effect of hyperthermia on ventilatory and cardiovascular responses to poikilocapnic hypoxia, and to compare these responses to isocapnic hypoxia. The size of the ventilatory responses to the hypoxic stimulus were not influenced by increases in core body temperature. This finding may be due to the CO_2 tension dropping below a peripheral chemoreflex threshold and is in contrast to the enhanced ventilatory responses to isocapnic hypoxia during hyperthermia. Possibly due to the lack of a ventilatory response, cardiovascular responses were greater during poikilocapnic hypoxia than during isocapnic hypoxia due to the apparent need to maintain adequate oxygen levels in arterial blood. Therefore this study appears unique in demonstrating that during a 20 min steady-state poikilocapnic hypoxia exposure, decreases in $P_{ET}CO_2$ appear to blunt the HVR more than a 1.5°C increase in T_{es} augments it, as seen during a 20 min steady-state isocapnic hypoxia exposure.

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4.9 Tables

Table 4.1 Physical characteristics participants and esophageal temperature (T_{es}) for each Temperature condition of the pHVR and iHVR tests.

Participant	Age (yr)	Weight (kg)	Height (cm)	Normothermic T_{es} (°C)		Hyperthermic T_{es} (°C)	
				pHVR	iHVR	pHVR	iHVR
1	27	81.3	180	36.30	36.32	37.83	37.74
2	24	75.0	193	36.28	36.33	37.88	37.91
3	22	82.0	191	36.45	36.50	37.99	37.94
4	24	75.0	183	36.17	36.15	37.66	37.63
5	24	79.5	183	36.28	36.17	37.61	37.66
6	23	73.0	178	36.27	36.19	37.85	37.83
7	29	73.0	193	36.75	36.66	38.21	38.09
Mean (SD)	**25 (2)**	**77.0 (3.9)**	**186 (6)**	**36.36 (0.19)**	**36.33 (0.19)**	**37.86 (0.20)****	**37.83 (0.17)****

(**significantly different from normothermia at $p < 0.01$).

Table 4.2 To illustrate Time x Temperature interactions, the differences in ventilation ($\Delta \dot{V}_E$, $L \cdot min^{-1}$), partial pressure of end-tidal CO_2 ($\Delta P_{ET}CO_2$, mm Hg), tidal volume (ΔV_T, L), breathing frequency (Δf_R, $breaths \cdot min^{-1}$), arterial hemoglobin saturation (ΔS_aO_2, %) and heart rate (ΔHR, $beats \cdot min^{-1}$) from normothermia to hyperthermia during Immersed rest in Air, the Initial Steady State (ISS)/5th min, and the 20th min phases of the pHVR test are shown(n = 7).

	Immersed Air	ISS/5th min	20th min
$\Delta \dot{V}_E$ ($L \cdot min^{-1}$)	2.79 (1.05)	2.78 (1.80)	2.88 (1.95)
$\Delta P_{ET}CO_2$ (mm Hg)	-2.48 (1.09)	-2.81 (0.72)†	-2.61 (1.03)
ΔV_T (L)	0.07 (0.13)	0.07 (0.15)	0.09 (0.27)
Δf_R ($breaths \cdot min^{-1}$)	2.90 (3.00)	2.52 (2.91)	2.84 (7.03)
ΔS_aO_2 (%)	-2.3 (1.4)	-4.6 (3.7)**NS**$^{(0.06)}$	-3.4 (8.6)
ΔHR ($beats \cdot min^{-1}$)	29 (6)	35 (7)‡	33 (10)

(† significantly different from previous test level, $p < 0.05$, ‡ significantly different from previous test level, $p < 0.01$, **NS**$^{(0.06)}$ not significantly different from previous test level $p = 0.06$).

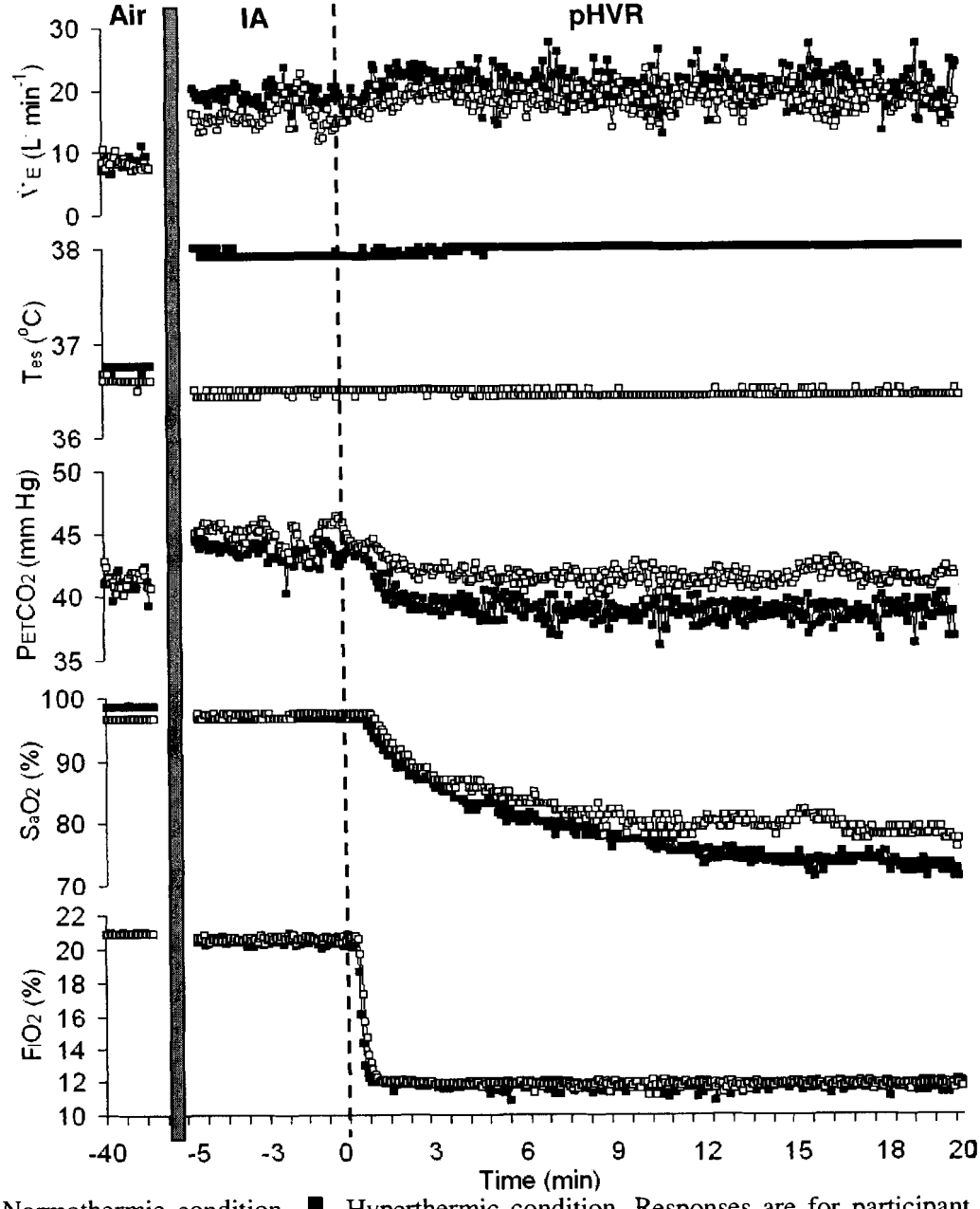
Table 4.3 To illustrate the size of the differences between iHVR and pHVR for ventilation ($\Delta \dot{V}_E$, $L \cdot min^{-1}$), tidal volume (ΔV_T, L), arterial hemoglobin saturation (ΔS_aO_2, %) and heart rate (ΔHR, $beats \cdot min^{-1}$), the difference from the iHVR to pHVR trials during the Immersed rest in Air, the Initial Steady State (ISS)/5th min, and the 20th min phases for both normothermia (Normo) and hyperthermia (Hyper) are shown (n = 7).

		Immersed Air	ISS/5th min	20th min
$\Delta \dot{V}_E$ ($L \cdot min^{-1}$)	Normo	-1.69 (2.72)	0.91 (3.04)	2.04 (2.05) *
	Hyper	-0.79 (4.25)	4.31 (2.70) **	6.23 (2.88) **
ΔV_T (L)	Normo	-0.01 (0.16)	0.10 (0.13) *	0.10 (0.21)
	Hyper	-0.09 (0.15)	0.12 (0.10) **	0.11 (0.22)
ΔS_aO_2 (%)	Normo	-0.08 (1.41)	3.94 (3.88)*	11.57 (7.81)**
	Hyper	-0.40 (1.02)	5.23 (3.66)**	12.44 (4.03)**
ΔHR ($beats \cdot min^{-1}$)	Normo	-3 (6)	-4 (6)	-6 (8)*
	Hyper	-0 (4)	-7 (5)**	-5 (3)**

(*significant difference between isocapnic and poikilocapnic trials, $p < 0.05$, **significant difference between isocapnic and poikilocapnic trials $p < 0.01$).

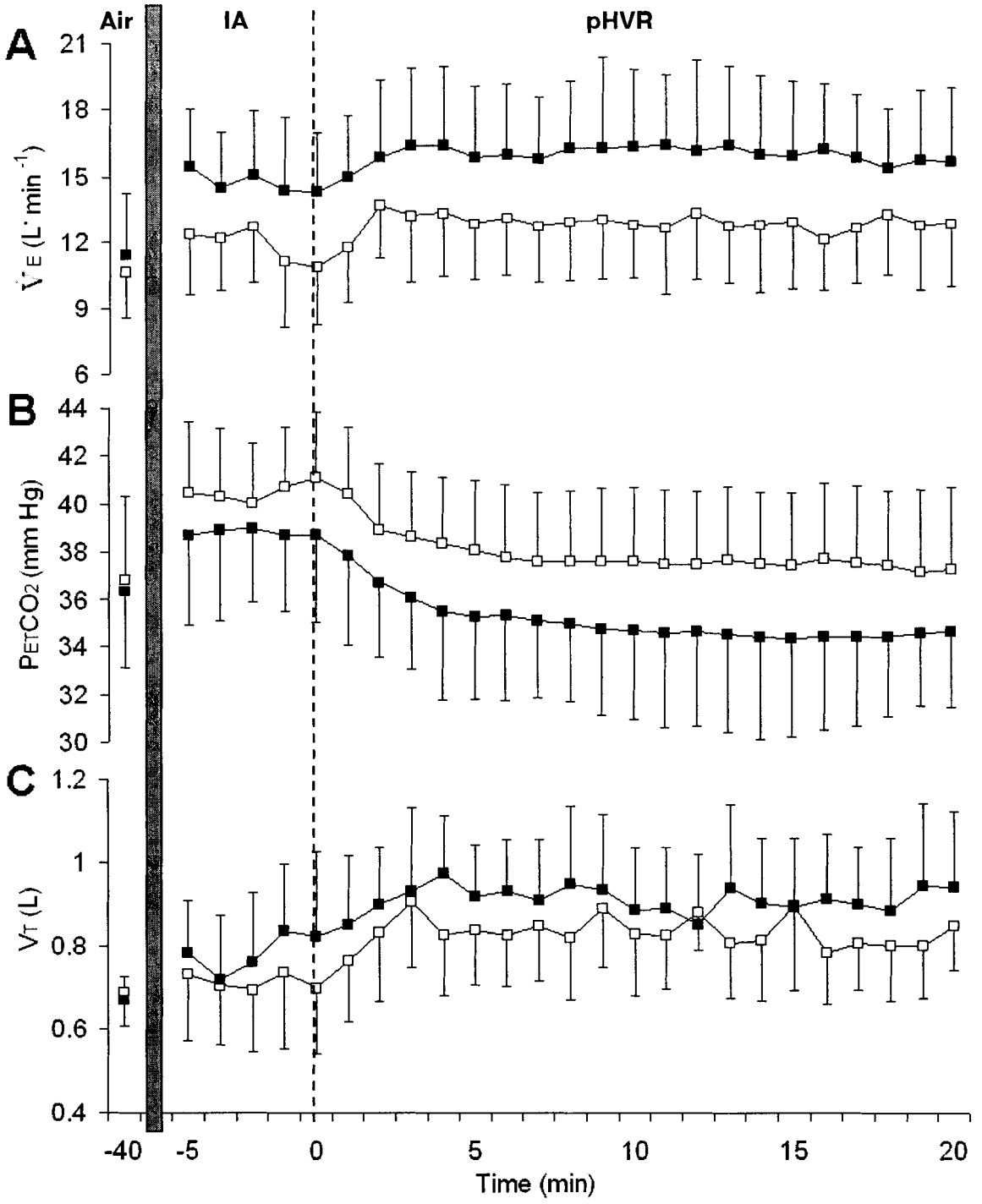
4.10 Figures

Figure 4.1 Time course of ventilation ($\dot{V}_E$), temperature (T_{es}), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$), arterial hemoglobin saturation (S_aO_2), fraction of inspired oxygen (F_IO_2) and for a typical participant during the normothermic and hyperthermic pHVR.



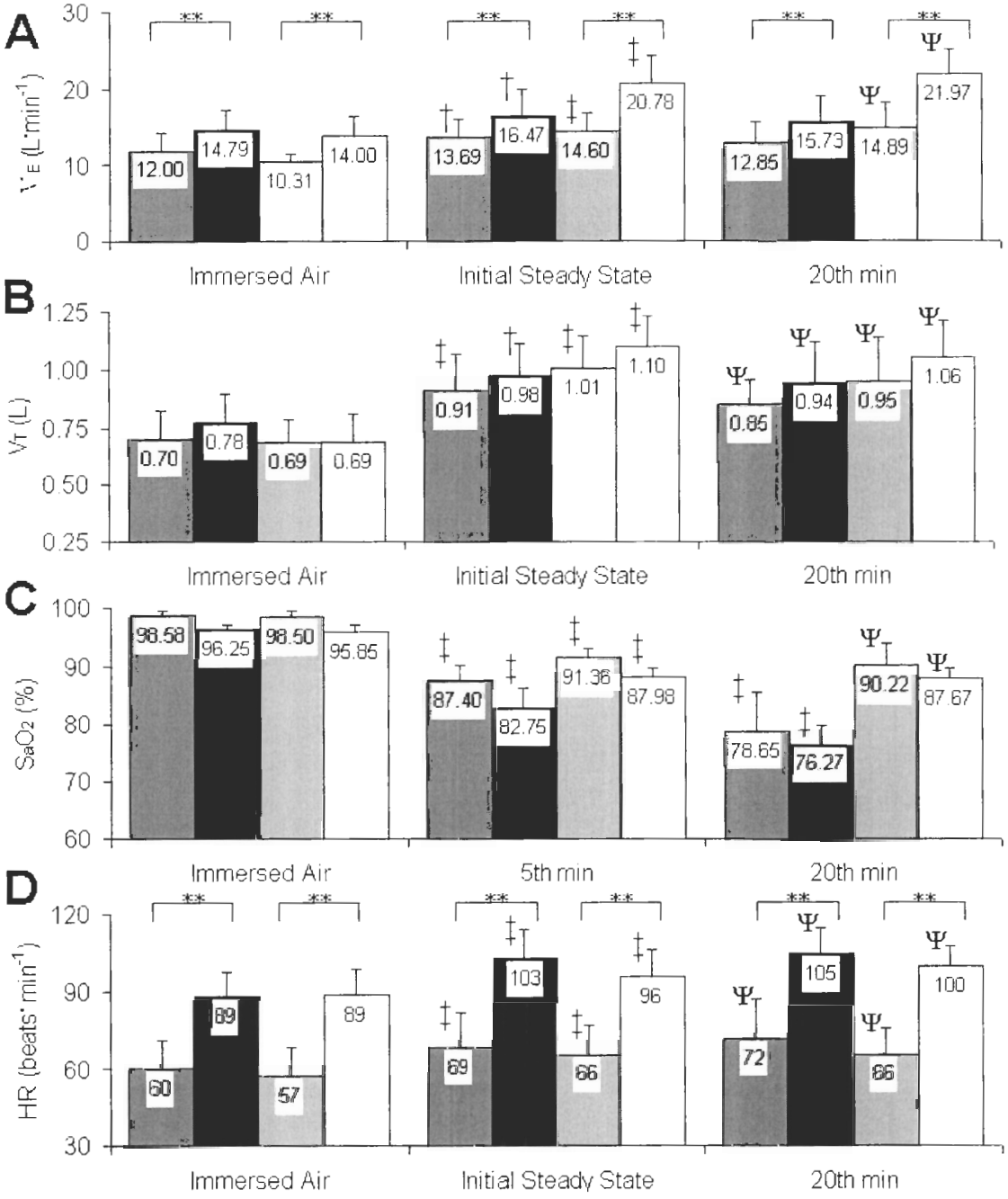
□ Normothermic condition, ■ Hyperthermic condition. Responses are for participant #3. Air represents the last 2 min rest phase before Immersed rest in Air (IA), IA represents the last 5 min of the 15 to 45 min rest phase in water. Means of the last 5 min of Immersed Rest and mean of the min of the Peak or 5th min were used for most of the data analysis. Each data point represents a 4 s mean, this was done to standardize the timing points for both Temperature conditions.

Figure 4.2 Mean (SD) ventilation ($\dot{V}_E$), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) and tidal volume (V_T) responses for the 10 min rest period in air, and minute-by-minute for Immersed rest in Air (IA) and the entire pHVR (n = 7).



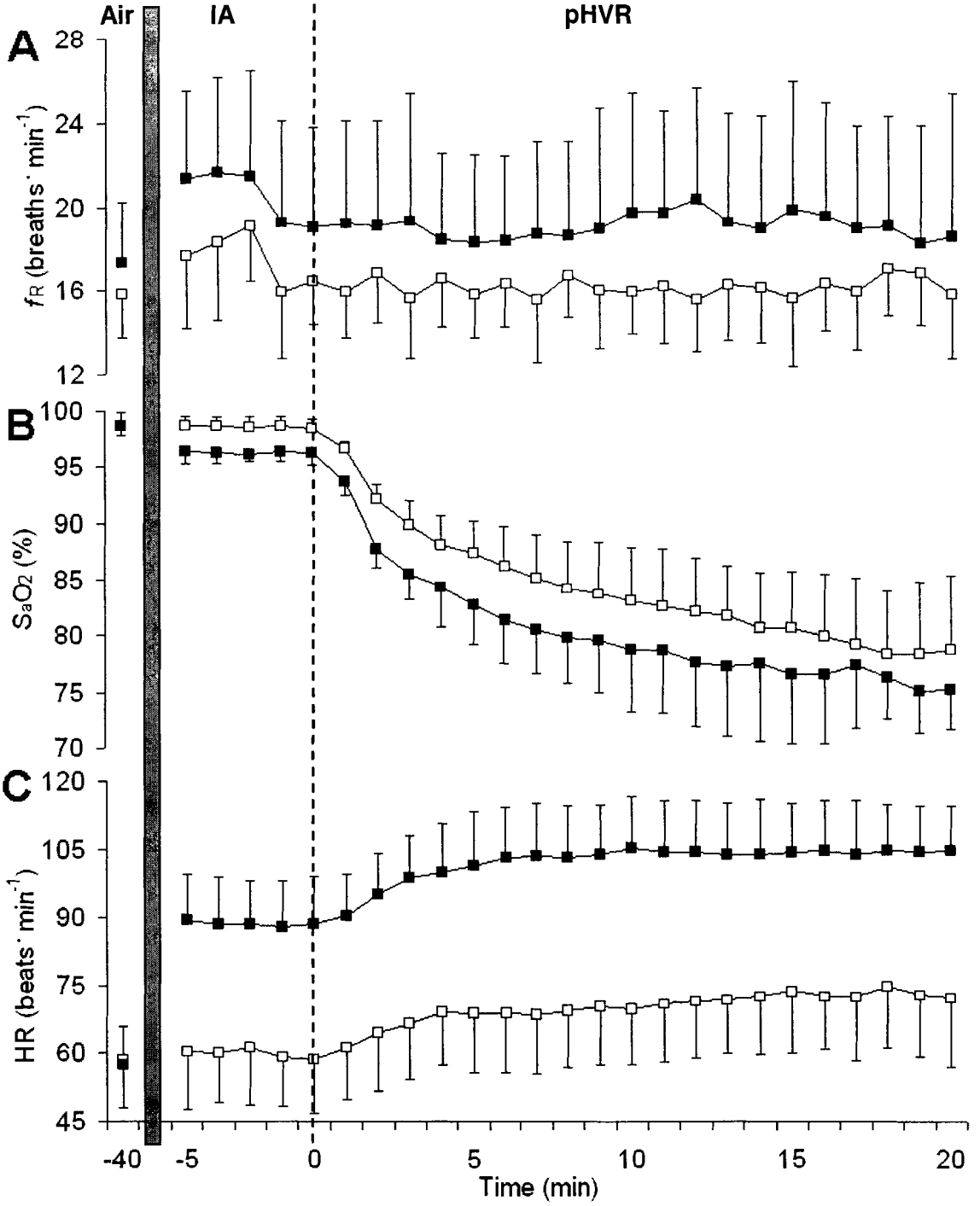
Values are means (SD) for 7 participants □ Normothermic condition, ■ Hyperthermic condition Air represents the mean (SD) 10 min normothermic rest phase in Air, Immersed rest in Air (IA) represents minute-by-minute means (SD) over last 5 min of the 15 to 45 min rest phase in water.

Figure 4.3 Mean (SD) ventilation ($\dot{V}_E$), tidal volume (V_T), heart rate (HR) and arterial hemoglobin saturation (S_aO_2) during Immersed rest in Air, the ISS/5th min, and the 20th min of both the normothermic and hyperthermic pHVR and iHVR tests (n = 7).



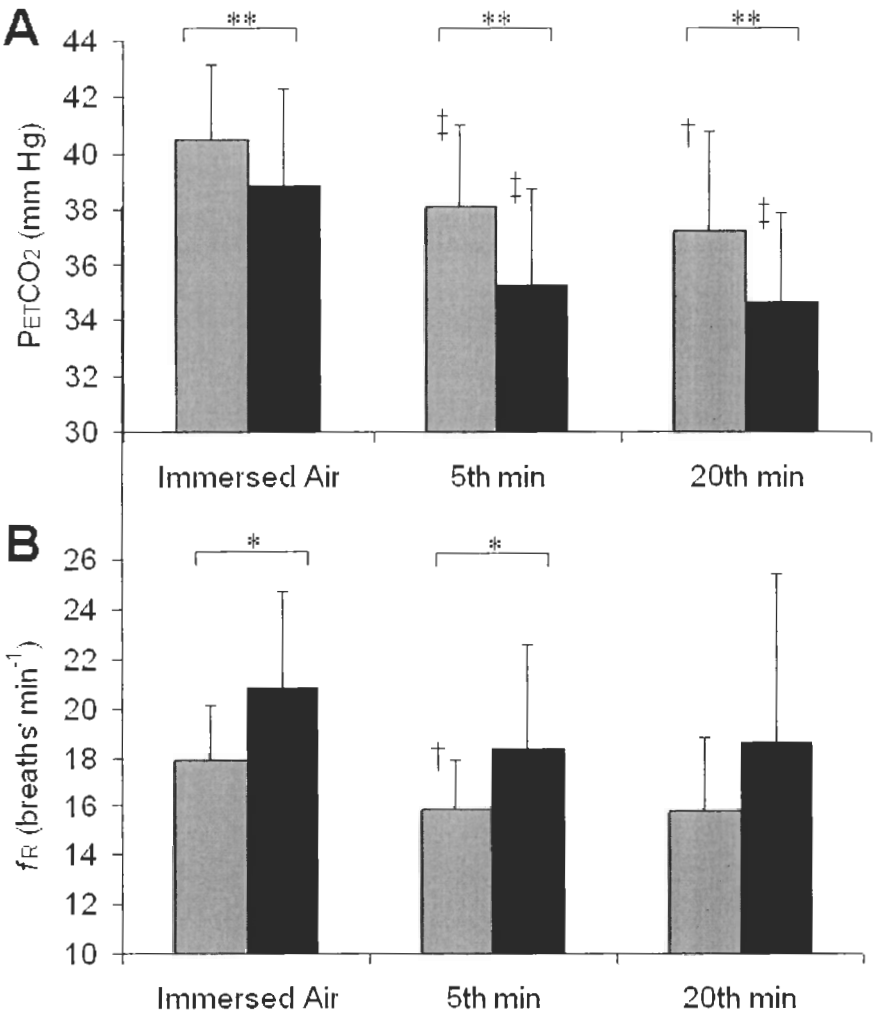
□ Normothermic pHVR ■ Hyperthermic pHVR □ Normothermic iHVR, □ Hyperthermic iHVR (**significantly different at $p < 0.01$ vs. normothermic condition, † significantly different from previous test level, $p < 0.05$, ‡ significantly different from previous test level $p < 0.01$, Ψ significantly different from Immersed Air level $p < 0.01$). For clarity only significant differences are shown.

Figure 4.4 Mean (SD) breathing frequency (f_R) arterial hemoglobin saturation (S_aO_2) and heart rate (HR) responses for the 10 min rest period in air, and minute-by-minute for Immersed rest in Air (IA) and the entire pHVR (n = 7)



Values are means (SD) for 7 participants □Normothermic condition, ■ Hyperthermic condition Air represents the mean (SD) 10 min normothermic rest phase in Air, Immersed rest in Air (IA) represents minute-by-minute means (SD) over last 5 min of the 10 to 30 min rest phase in water.

Figure 4.5 Partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) and breathing frequency (f_R) for both normothermic and hyperthermia Temperature conditions and the Immersed rest in Air, 5th min and 20th min levels of the Time condition and during the pHVR (n = 7).



Values are means (SD) for 7 participants. ▨ normothermic condition; ■ hyperthermic condition. (*significantly different at $p < 0.05$, **significantly different at $p < 0.01$, † significantly different from previous test phase $p < 0.05$, ‡ significantly different from previous test phase $p < 0.01$). For clarity only significant differences are shown.

CHAPTER 5

Study 3

Development of an end-tidal forcing system using a breath by breath gas bolus instead of continuous gas flow.

5.1 Abstract

The purpose of this study was to develop a computer controlled gas mixing system which changes inspired levels of CO_2 on a breath-by-breath basis to control the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) at desired levels. Such a system is known as a dynamic end-tidal forcing (ETF) system. The system injects a gas bolus which matches the previous volume of each breath. The $P_{ET}CO_2$ levels are controlled by changing the CO_2 concentration in each gas bolus to force the measured $P_{ET}CO_2$ towards the desired $P_{ET}CO_2$. Five participants breath compressed air (21 % O_2 balance N_2) from the system (rest) before having their $P_{ET}CO_2$ levels controlled at 45, then 50, 55 and then 45 mm Hg for 5 min at each time level. The mean (SD) clamped $P_{ET}CO_2$ for the final four min of the rest, 45, 50, 55 and final 45 mm Hg time levels were 40.50 (0.60), 46.20 (1.12), 50.50 (0.56), 55.82 (0.58) and 45.60 (0.39) respectively. The mean (SD) differences between the measured and desired $P_{ET}CO_2$ was 0.78 (0.74) with a 95 % confidence interval of 0.59 to 0.97 mm Hg. Linear regression analysis of the desired versus measured $P_{ET}CO_2$ demonstrated a significant positive linear relationship ($r = 0.987$, $p \leq 0.001$) with a slope of 0.97 and an intercept of 2.3 mm Hg. These results demonstrate that for each time level during end-tidal CO_2 control 95 % of the data is with two mm Hg. More importantly 95 % of the differences between the measured and desired $P_{ET}CO_2$ were within both 2 standard deviations of the mean and within two mm Hg of the desired $P_{ET}CO_2$. Therefore the dynamic ETF system we have developed can control $P_{ET}CO_2$ levels within two mm Hg of the level desired level.

(290 Words)

5.2 Introduction

The end-tidal forcing system (ETF) system uses electric valves and computer programs to control the $P_{ET}CO_2$ and/or $P_{ET}O_2$ at a set level by varying the F_ICO_2 and/or F_IO_2. This method allows the measurement of all three responses to hypoxia, the whole body response, whole body sensitivity and peripheral sensitivity during either eucapnia, isocapnia or poikilocapnia (8; 9). Whole body sensitivity during both poikilocapnic and isocapnic conditions (separated by at least 1 h) as recently recommended as the universal method to measure the sea level HVR (10; 11) can be more accurately done using the ETF system than previous steady state methods. Progressive methods for measuring the whole body sensitivity to hypoxia may be more accurately implemented using a dynamic ETF system approach. Utilizing a step-wise method where participants are exposed to step changes in S_aO_2 allows for the accurate determination of multiple steady states. The dynamic ETF system can also be used to measure peripheral chemoreceptor sensitivity. On the whole, the major drawback to using the dynamic ETF method is the complex experimental setup that is required (12).

To remove the human error present in $P_{ET}CO_2$ maintenance for both the Chu and White (3) and this current study (both performed in this laboratory) the purpose of the study was to develop a dynamic ETF system that can control $P_{ET}CO_2$ levels on breath-by-breath basis. We hypothesized that the developed dynamic ETF system would maintain $P_{ET}CO_2$ levels within ± 2 mm Hg from the level desired using gas bolus injections instead of a continuous flow method.

5.3 Methods

5.3.1 Participants

Participants were five volunteers from the Simon Fraser University population aged between 19 to 50 years old. Participants were non-smokers and non-asthmatics. Ethical approval was granted by the Office of Research Ethics at Simon Fraser University before commencement of this study.

5.3.2 Instrumentation

The participant breathed from a custom made, air tight mixing box and gas humidifier (Figure 5.1), into which set gas mixtures were delivered. The compositions of these gas mixtures was controlled by a computer program that receives information from the participants expired gas on a breath-by-breath basis (Figure 5.2).

During each test the participant wore a nose clip, was fitted with a mouthpiece and connected to a two-way flow sensor housing, which was connected to a 2-way non-rebreathing valve (NRB 2700, Hans Rudolph Inc, Kansas Cit, MO, USA). This was connected with 3.8 cm inside diameter corrugated Collins tubing (length, 10 cm) to the custom made, air tight mixing box and gas humidifier (Figure 5.1). Attached to the box was a 5-litre re-breathing bag (Anesthesia Assoc., Inc., San Marcos, CA), which acted as an air reservoir. Breath-by-breath gas samples were drawn from the inspired and expired air to a Sensormedics Vmax 229c metabolic cart (Sensormedics, Yorba Linda, CA, USA) at a rate of 250 $mL \cdot min^{-1}$. Carbon dioxide partial pressure was measured using non-dispersive infrared spectroscopy and oxygen content was measured using a paramagnetic sensor. The CO_2 and O_2 sensors were calibrated before each test using gases of known

concentrations (room air, 26 % O_2 balance N_2 and 4 % CO_2 and 16 % O_2). The flow sensor was calibrated using a 3 L standardized volume syringe (Sensormedics, Yorba Linda, CA, USA).

5.3.3 Gas supply

Gas was delivered to the mixing box via 3/8" PVC tubing which connects to three lengths of 1/8" PVC tubing. Two of these lengths connect to two solenoid valves (Model: EC-3M-12, Clippard, Cincinnati, USA). Both solenoid valves were attached via the PVC tubing to two-stage regulators, one regulator with pressure set to 18 PSI for breathing grade 100 % carbon dioxide (CGA 320 Prostar, Praxair, Vancouver, Canada), the other regulator with pressure set to 40 PSI for breathing grade compressed air (21 % O_2, balance N_2) (CGA 540 Prostar, Praxair, Vancouver, Canada). The solenoid valve for the air tank had a booster attached (Model: EVB-2, Clippard, Cincinnati, USA) which increased air-flow by up to seven-fold. Gas was humidified by being bubbled through warm water in one compartment of the mixing box. The bubbling action also mixed the air, which was then be breathed by the participant.

5.3.4 Control system

The entire system was controlled using the data acquisition system, the specifically written LabVIEW software and a PC. This software has two functions, firstly to collect and record input signals from the various measurement devices and secondly to use digital output signals to control the valves. The input signals always include the real-time flow, % CO_2 and % O_2 signals from the metabolic cart and can also include, data from any combination of temperature thermistors and/or thermocouples, pulse oximeter

(Masimo Radical, Irvine, CA, USA), moorLAB flow sensor (moor instruments Ltd, Devon, UK) and humidity sensors (RH-201°C, Omega Engineering Inc., Great Britain). There are two digital output signals that provide individual control of both valves. These channels passed their signals through an adaptor box which amplified the voltage from 5 V to 12 V.

The control of the inspired gas mixture is done using the LabVIEW software on a PC. There are currently two control options, Control 0 where air volume is, but neither $P_{ET}CO_2$ or $P_{ET}O_2$ are controlled and Control 1 where only $P_{ET}CO_2$ is controlled at the desired level. At the time of submitting this thesis work was continuing of this ETF system to develop Control 2 where only $P_{ET}O_2$ is controlled at the desired level and Control 3 where both $P_{ET}CO_2$ or $P_{ET}O_2$ are controlled at the desired levels. The input signals from the metabolic cart work as feedback for the control of the valves on a breath-by-breath basis. The system begins with a prediction of the gas composition required to reach the desired end-tidal partial pressure. The system must start with the participants expiration, a bolus of gas which matches the volume of gas expired is then injected into the mixing box with the predicted inspiratory gas concentration. The LabVIEW program then calculates the next expiration's volume (L, BTPS), $P_{ET}CO_2$ (mm Hg) and $P_{ET}O_2$ (mm Hg) values using the real time flow rate, % CO_2 and % O_2 from the Vmax computer. The LabVIEW program uses the volume value to calculate the volume of the next bolus of gas, while the measured $P_{ET}CO_2$ value is used as feedback to adjust the gas concentration of the gas bolus. The LabVIEW program adjusts this gas mixture to force the end-tidal gas tension towards the desired value. Each time the desired $P_{ET}CO_2$ level is changed the system predicts the required inspiratory gas bolus concentration for that

$P_{ET}CO_2$ level and maintains it for set number of breaths, which is chosen by the experimenter. After these breaths the system continues to use the measured $P_{ET}CO_2$ values as feedback to adjust the gas concentration of the next gas bolus mixture to force the end-tidal gas tensions towards new desired value. For example, if the measured $P_{ET}CO_2$ level is below the desired $P_{ET}CO_2$ level, the CO_2 valve is opened for a proportionately longer time period, compared to the previous breath, relative to the time period the air value is open. This means the composition of gas delivered to the mixing box will have a slightly higher CO_2 content than during the previous breath in order to obtain the desired $P_{ET}CO_2$. Once the desired end-tidal values are reached, the same gas concentration for each differently sized gas bolus is used until the measured values differ from the desired values. All ventilatory data and other desired variables are recorded on a breath by breath basis and written to a Microsoft Excel spreadsheet (Microsoft Corporation, USA).

5.3.5 Protocol

Each participant underwent one 25 min test while seated and breathing quietly. The participant was instrumented as outlined above and listened to relaxing music through ear-phones throughout the duration of the test. The test involved the participant breathing the compressed air (21 % O_2, balance N_2) air for five min off the dynamic ETF system at Control 0. After five 5 min, participants were switched to Control 1. Here the participant's $P_{ET}CO_2$ was controlled at 45, 50, 55 and then 45 mm Hg successively for five min each. Ventilatory data were recorded on a breath-by-breath basis for the entire duration of the test.

5.3.6 Statistical analysis

Means for each 15 s of the last four min of each stage for each participant were used standardize the breath data into similar time epochs. Also means (SD), 95 % confidence intervals and the minimum and maximum values were calculated for the last four min of each stage and for the difference between the measured and desired $P_{ET}CO_2$ levels. Graphical analysis plotting the mean (SD) measured $P_{ET}CO_2$ (mm Hg) and ventilation ($\dot{V}_E$) for all participants for each 15 s time epoch, along with the desired $P_{ET}CO_2$. Ninety-five percent of the time, the mean $P_{ET}CO_2$ and the mean differences should be within two mm Hg of the desired $P_{ET}CO_2$ for the method to be physiologically accurate according previous literature (1; 4; 6; 7). Statistically it is recommended that 95 % of the mean difference between the measured and desired $P_{ET}CO_2$ levels are within two standard deviations of the mean (2). Linear regression analysis was performed to ensure correlation of desired and measured measurements. The level of significance was set at an alpha level of $\alpha < 0.05$, all analyses were performed using the statistical software package SPSS 13.0 for Windows (SPSS Inc. Chicago, IL, USA) and all data are mean (SD) unless otherwise stated.

5.4 Results

Descriptive statistics of the measured $P_{ET}CO_2$ values for each stage can be seen in Table 5.1, while the typical response of a participant can be seen in Figure 5.3. Generally due to the initial overshoot in measured $P_{ET}CO_2$ changes, means for each stage are slightly higher than the desired level. However at each stage over 95 % of all data was within both two standard deviations of the mean and two mm Hg of the desired $P_{ET}CO_2$ as recommended to be physiologically accurate according previous literature (1; 4; 6; 7). Figure 5.4 illustrates how the mean measured $P_{ET}CO_2$ for each 15 s epoch was generally within the two mm Hg limits of the desired $P_{ET}CO_2$ and also shows $\dot{V}_E$ responses to the $P_{ET}CO_2$ changes. The mean (SD) differences between the measured and desired $P_{ET}CO_2$ was 0.78 (0.74) mm Hg with the lower and upper bound of the 95 % confidence interval being 0.59 and 0.97 mm Hg respectively. Linear regression analysis of the desired versus measured $P_{ET}CO_2$ demonstrated a significant positive linear relationship ($r = 0.987$, $p \leq 0.001$) (Figure 5.5) where as the desired $P_{ET}CO_2$ rose so did the measured $P_{ET}CO_2$ with a slope of 0.97 and an intercept of 2.3 mm Hg. This means that 97 % of the changes in $P_{ET}CO_2$ were due to the effects of the ETF system.

5.5 Discussion

The purpose of the study was to develop a dynamic ETF system that can control $P_{ET}CO_2$ levels on a breath-by-breath basis using gas bolus injections instead of a continuous flow method. This study has confirmed our hypothesis and shown that the dynamic ETF system we have developed can control $P_{ET}CO_2$ levels not only within 2 mm Hg of the level desired, but also within ± two standard deviations of the mean (Figure 5.4, Table 5.1). Furthermore, the system can control multiple levels of desired $P_{ET}CO_2$ in both an ascending and descending manner. This system is unique as it forces changes in end-tidal values by changing the gas concentration of the injected bolus of humidified gas which is matched to the volume of gas expired, which allows for the breath-by-breath control of $P_{ET}CO_2$. Other dynamic ETF systems use a continuous flow of dry gas with a continuously changing gas mixture to attain breath-by-breath control.

The results in Table 5.1 demonstrate that while the measured $P_{ET}CO_2$ means are slightly higher than the desired $P_{ET}CO_2$ level the variability and range of the measured $P_{ET}CO_2$ levels is low and similar to that seen during rest. The slightly higher measured $P_{ET}CO_2$ mean compared to desired $P_{ET}CO_2$ is due to the mechanical limitations of the system. This means the ETF system is unable to have a finer resolution of control. However, the 95 % confidence intervals in Table 5.1 and the mean differences between measured and desired $P_{ET}CO_2$ and their 95 % confidence intervals demonstrate that 95 % of the time, the ETF system is controlling $P_{ET}CO_2$ levels within a 1 mm Hg range. As discussed below, this range is even smaller when greater ventilation rates are seen (Figure 5.3 and 5.4). The measured $P_{ET}CO_2$ standard deviation values (Table 5.1) for this ETF system are comparable and often better than the standard deviation values reported with

other ETF systems. Using their ETF systems the following standard deviation values have been reported 1.9 mm Hg (1), 1.1 to 2 mm Hg (5) and 1.0 (11), while a five min mean (SD) error rate of 0.25 (0.57) has been reported (4) and Rebuck et al., (1973) reported $P_{ET}CO_2$ values were controlled within ± 2 mm Hg (7). Therefore it appears that our ETF system more than adequately controls $P_{ET}CO_2$ at the desired level.

The decision to develop this ETF system using a breath-by-breath humidified bolus gas injection instead of the continuous flow approached used by other systems slightly reduces the speed and accuracy of the system, but it reduces participant stress and increases cost efficiency and portability. As the seen in Figures 5.3 and 5.4 there is still a lag from changing the control or desired $P_{ET}CO_2$ level to the attainment of a new steady level of $P_{ET}CO_2$, this is especially true at lower $\dot{V}_E$ rates. The larger time than seen in other systems (1; 11) is likely due to 2 factors. The large amount of dead space in our mixing box (~3 L, Figure 5.1) and the use of the breath-by-breath bolus' instead of continuous flow. Lessening the amount of dead space in the mixing box, or removal of the mixing box and bubbling the gas bolus through water and then straight into a rebreathing bag would reduce the time lag from gas bolus injection to inspiration. This would also ensure the gas mixture inspired was more like the one predicted by the ETF system and not altered as much by previous bolus injections. Utilizing continuous flow and not humidifying the gas would help reduce the lag, dead space and amount of gas mixture alteration even more while possibly allowing for smaller changes in gas mixture concentration to be made. However, the disadvantages of a continuous flow system is the much larger amount of gas required, and the less portable nature of the system. This system has a much more efficient use of gas and hence costs less to run. Also as smaller

gas bottles may be used, the system can easily be moved from a climate chamber to an exercise lab or to beside a water tub as the most cumbersome piece of this system is the metabolic cart (Figure 5.2). Further development of this system while minimizing the amount of dead space could also utilize separate O_2, CO_2 and flow sensors for the real time changes in these variables, this would to remove the need for the metabolic cart.

This study changed $P_{ET}CO_2$ levels, while breathing normoxic gas. The lowest desired level of 45 mm Hg at the beginning was also the most difficult to maintain accurately. At this level $\dot{V}_E$ was also at its lowest and therefore there were fewer breaths per minute and lower alveolar ventilation rates to allow faster changes in $P_{ET}CO_2$ levels (Figure 5.3 and 5.4). During the last three stages of 50, 55 and 44 mm Hg, $\dot{V}_E$ was consistently higher and this caused the $P_{ET}CO_2$ levels to rise or fall faster, and the overshoot or undershoot to be shorter after a change in the desired $P_{ET}CO_2$ level. This is due to both the greater breathing frequency, increasing the number of breaths the system has to manipulate $P_{ET}CO_2$ levels per minute, and the larger tidal volumes increasing alveolar ventilation and thereby the effectiveness of each change in gas mixture. This bodes well for using the ETF system under conditions which increase $\dot{V}_E$, such as exercise, hot or cold stresses and hypoxia.

Future directions

At the time of submission of this thesis work was continuing of this ETF system development to include the ability to force $P_{ET}O_2$ to a desired level and to be able to force both $P_{ET}CO_2$ and $P_{ET}O_2$ to desired levels simultaneously. While making these changes to the LabVIEW program which controls the ETF system, design changes are also being made to reduce the dead space in the system. As mentioned, due to the humidification

and use of a gas bolus, the mixing box currently has too much dead space which increases the lag time from changes in desired $P_{ET}CO_2$ levels and attainment of a new steady state. Reduction of this dead space rather than removal of the humidification process and the use of a continuous gas flow approach is preferred in the continued development of this ETF system, as this maintains the cost efficiency and portability of the system.

Conclusions

This study successfully developed a dynamic ETF system that can control $P_{ET}CO_2$ levels within ± 2 mm Hg of the level desired level by changing the gas concentration of an injected bolus of humidified gas which is matched to the volume of gas expired. The greater the $\dot{V}_E$ rate while on the ETF system, the faster the desired $P_{ET}CO_2$ levels can be reached and the better they can be maintained. Work is continuing to improve the speed and accuracy of $P_{ET}CO_2$ control and to incorporate $P_{ET}O_2$ control.

5.6 Acknowledgements

The biggest debt in this study is owed to Dr. Mike Walsh, your expert guidance, unwaviering support and LabVIEW skills made this system and project as good as it was. Also my participants, who, with their time and commitment made this study possible. Finally to Dr. Matt White, your help, guidance and insights have been invaluable throughout this project. Thank-you all for influencing this study in the way you have.

5.7 Grants

This work was supported by grants from Natural Science and Engineering Research Council of Canada (NSERC) and the Canadian Foundation for Innovation (CFI).

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5.9 Tables

Table 5.1 Descriptive statistics showing mean, one standard deviation (1 SD), two standard deviations (2 SD), lower and upper 95 % confidence intervals (95 % C.I.), minimum (Min) and maximum (Max) $P_{ET}CO_2$ values during the final 4 min of the 5 min rest period and each of the four 5 min time levels with 3 different desired $P_{ET}CO_2$ values for 5 participants. All values are in mm Hg.

Desired	Mean	1 SD	2 SD	95 % C.I.		Min	Max
$P_{ET}CO_2$ (mm Hg)	$P_{ET}CO_2$ (mm Hg)			Lower	Upper	$P_{ET}CO_2$ (mm Hg)	$P_{ET}CO_2$ (mm Hg)
Rest	40.50	0.60	1.20	40.20	40.79	39.08	41.63
45	46.20	1.12	2.24	45.61	46.80	43.23	47.34
50	50.50	0.56	1.12	50.20	50.80	49.87	51.79
55	55.82	0.58	1.16	55.51	56.13	55.07	57.35
45	45.60	0.39	0.78	45.39	45.81	44.79	46.37

5.10 Figures

Figure 5.1 Side-on view of the custom made 15 cm x 15 cm x 22 cm (4.95 L) mixing box and gas humidifier.

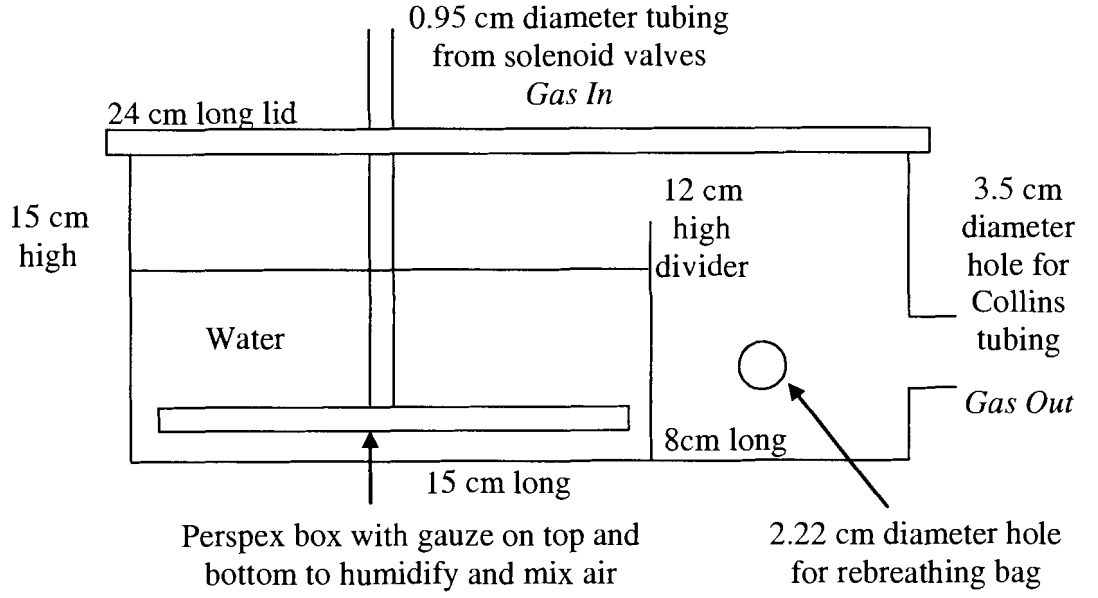


Figure 5.2 Schematic of the end-tidal forcing system set-up. The system flows in a clockwise direction from the participant.

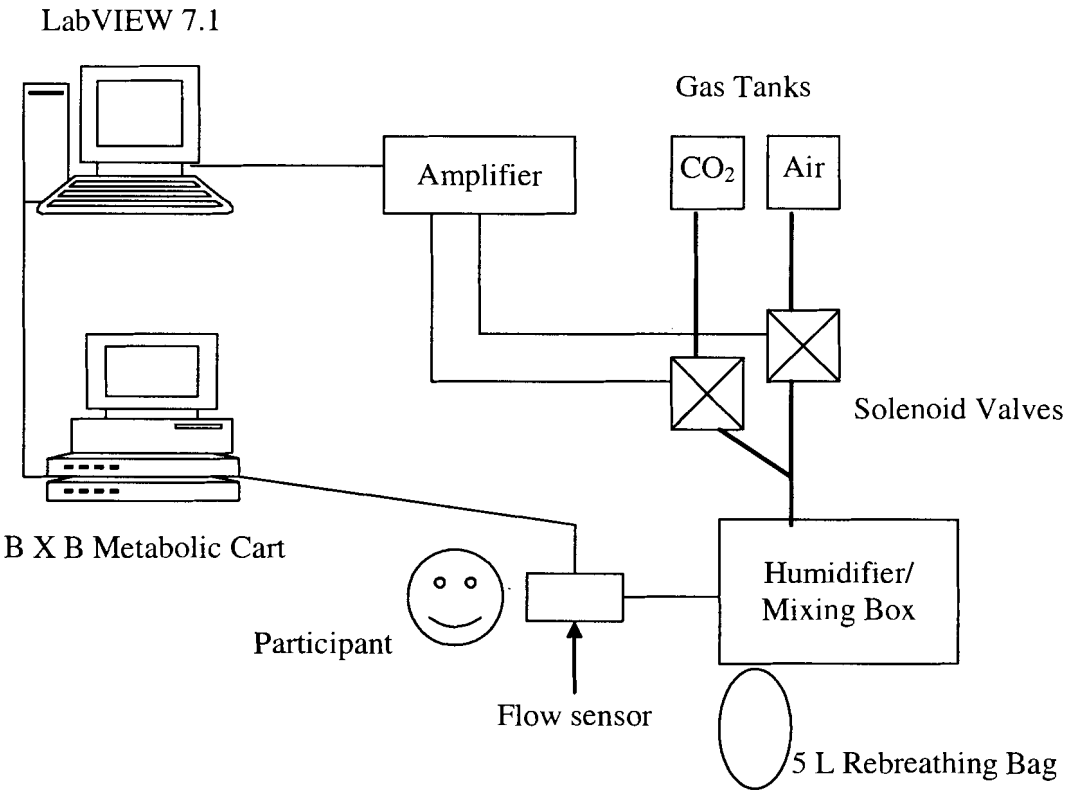


Figure 5.3 Typical changes in measured end-tidal CO_2 ($P_{ET}CO_2$, mm Hg) levels and ventilation ($\dot{V}_E$, $L \cdot min^{-1}$) during the end-tidal forcing system control.

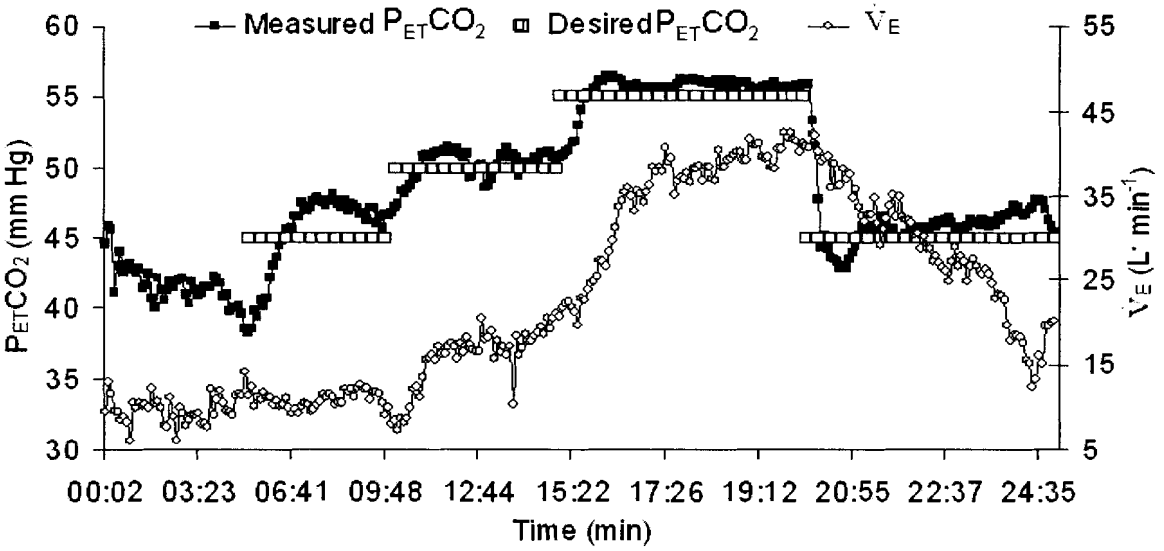
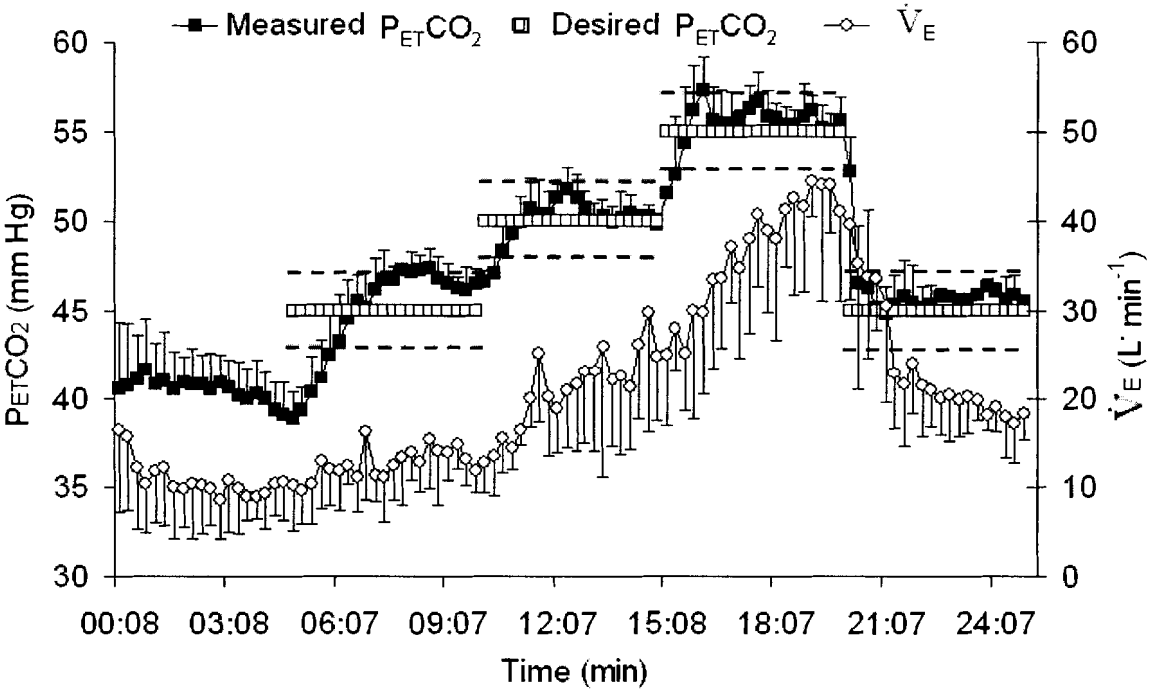
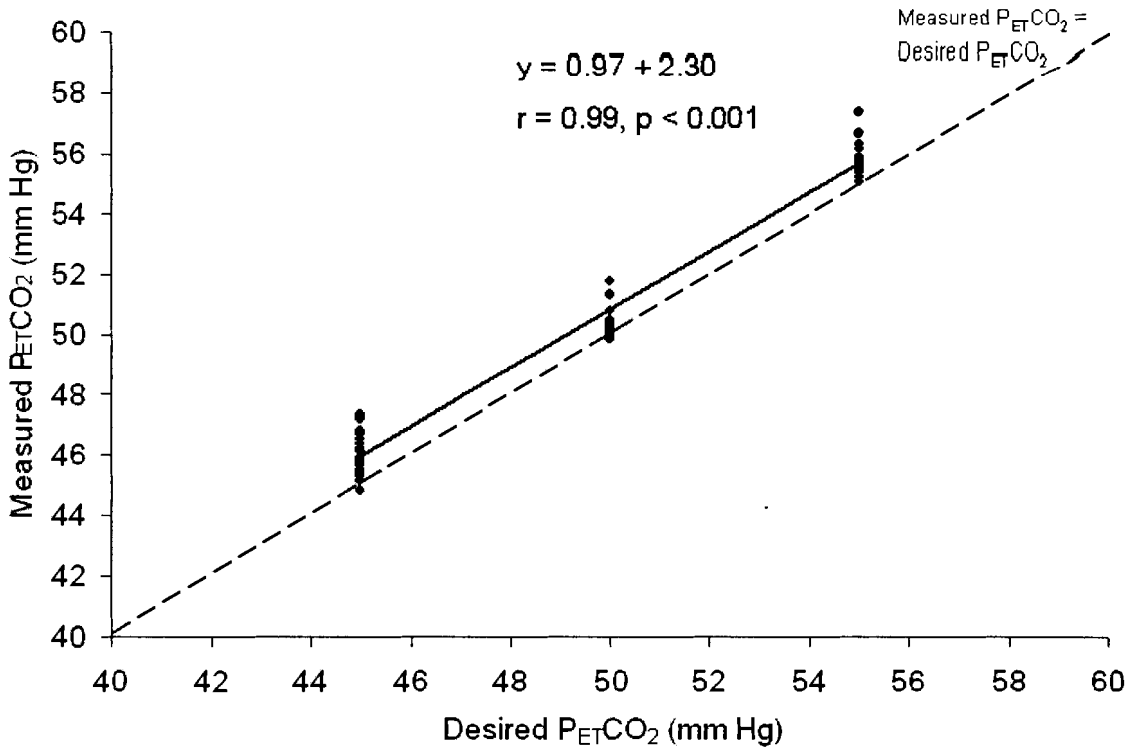


Figure 5.4 Mean (SD) measured end-tidal CO_2 ($P_{ET}CO_2$, mm Hg) and ventilation ($\dot{V}_E$, $L \cdot min^{-1}$) for each 15 sec epoch, as well as desired end-tidal CO_2 while on the end-tidal forcing system with 2 mm Hg error lines for five participants at rest.



The dotted line represents the value 2 mm Hg above or below the desired $P_{ET}CO_2$

Figure 5.5 Measured $P_{ET}CO_2$ levels against desired $P_{ET}CO_2$ levels while on the end-tidal forcing system for 5 males at rest.



Line of identity (Measured $P_{ET}CO_2$ = Desired $P_{ET}CO_2$) is given by the dotted line.

CHAPTER 6

Thesis Summary

6.1 Hypothesis

1. **We hypothesized that the human whole body cardio-respiratory response to isocapnic hypoxia would be greater in passively heated hyperthermic than in normothermic humans.**

This hypothesis is accepted as ventilatory responses to sustained isocapnic hypoxia during passive hyperthermia were greater compared to normothermia. However, the cardiovascular responses measured were not greater during the hyperthermic hypoxia compared to normothermic hypoxia.

2. **We hypothesized that the human whole body cardio-respiratory response to simulated altitude (poikilocapnic hypoxia) would be greater in passively heated hyperthermic than in normothermic humans.**

This hypothesis is rejected as there was no difference in the size of the ventilatory response to sustained poikilocapnic hypoxia during hyperthermia compared to normothermia. The cardiovascular responses measured tended to be greater at the onset of the poikilocapnic hypoxic stimulus during hyperthermia compared to normothermia, however as the $P_{ET}CO_2$ decreased during the hypoxic exposure the effect of hyperthermia on the cardiovascular responses became less.

3. We hypothesized that the effect of carbon dioxide tension on the whole body human cardio-respiratory response to hypoxia would be greater in passively heated hyperthermic than in normothermic humans.

This hypothesis was validated as hyperthermia increased the size of the ventilatory response to isocapnic hypoxia compared each of normothermic isocapnic, normothermic, poikilocapnic and hyperthermic poikilocapnic hypoxia. The cardiovascular responses measured however, tended to be greater during hyperthermic poikilocapnic hypoxia compared each of normothermic isocapnic, normothermic poikilocapnic hypoxia, hyperthermic isocapnic hypoxia.

6.2 Testable questions

1. Is human ventilation ($\dot{V}_E$, $L \cdot min^{-1}$) at rest greater in passively heated hyperthermic than in normothermic humans?

Ventilation was 26 % greater during hyperthermic rest in water compared to normothermic rest in water. The increase in ventilation was mediated solely by an increased breathing frequency, not by an increased tidal volume.

2. Is the whole body cardio-respiratory response to a 20 min isocapnic HVR test greater in passively heated hyperthermic than in normothermic humans as measured by: the change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot \% S_aO_2^{-1}$) at the 5th and 20th min, the size of hypoxic ventilatory decline (%), ventilation ($L \cdot min^{-1}$) and its components, and the cardiovascular parameters of heart rate ($beats \cdot min^{-1}$) and arterial hemoglobin saturation (S_aO_2)?

Using the recommended measure of change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot \% S_aO_2^{-1}$) at the 5th and 20th min there was no significant Time x

Temperature interaction on the isocapnic HVR response. Using non-ratio data there was a significant Time x Temperature interaction for where the size of the ventilatory response to isocapnic hypoxia was greater during hyperthermia compared to normothermia, but not the size of the cardiovascular responses. The cardiovascular Time x Temperature interaction was due to the effect of hyperthermia while breathing air, there was no effect while breathing the isocapnic hypoxic gas. There was no hypoxic ventilatory decline during the 20 min isocapnic hypoxic exposure.

3. Is the whole body cardio-respiratory response to a 20 min poikilocapnic HVR test greater in passively heated hyperthermic than in normothermic humans as measured by: the change in $P_{ET}CO_2$ per percent change in S_aO_2 (mm Hg· % $S_aO_2^{-1}$) at the 5th and 20th min, the change in ventilation per mm Hg change in $P_{ET}CO_2$ (L·min^{-1}· mm Hg $P_{ET}CO_2^{-1}$) at 5th and 20th min, ventilation (L·min^{-1}) and its components, and the cardiovascular parameters of heart rate (beats·min^{-1}) and arterial hemoglobin saturation (S_aO_2)?

There was no Time x Temperature interaction during the pHVR using both the recommended measure of change in $P_{ET}CO_2$ per percent change in S_aO_2 (mm Hg· % $S_aO_2^{-1}$) and the novel measure of change in ventilation per mm Hg change in $P_{ET}CO_2$ (L·min^{-1}· mm Hg $P_{ET}CO_2^{-1}$) each at the 5th and 20th min. Using the ventilatory non-ratio data there was still no Time x Temperature interaction during the pHVR as the size of the ventilatory response to hypoxia was the same for both normothermia and hyperthermia. However, using the cardiovascular non-ratio data there was a trend for a Time x Temperature interaction during the pHVR where the size of the cardiovascular response to hypoxia initially tended to be greater for hyperthermia compared to normothermia.

4. Is the effect of carbon dioxide tension on the whole body cardio-respiratory response to hypoxia greater in passively heated hyperthermic than in normothermic humans as measured by: the change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot \% S_aO_2^{-1}$) at the 5^{th} and 20^{th} min, ventilation ($L \cdot min^{-1}$) and its components, and the cardiovascular parameters of heart rate ($beats \cdot min^{-1}$) and arterial hemoglobin saturation (S_aO_2)?

Using the recommended measure of change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot \% S_aO_2^{-1}$) at the 5^{th} and 20^{th} min there was no Gas x Temperature interaction meaning that the responses to hyperthermia were the same regardless of CO_2 tension.

Using the ventilatory non-ratio data, hyperthermia significantly augmented the ventilatory response to isocapnic, but not poikilocapnic hypoxia when compared to normothermia. Conversely, using the cardiovascular non-ratio data, hyperthermia significantly augmented the cardiovascular response to poikilocapnic, but not isocapnic hypoxia when compared to normothermia.

5. Can a breath-by-breath end-tidal forcing system be developed to control the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$, mm Hg) within ± 2 mm Hg from the desired level using gas bolus injections instead of a continuous flow method?

A breath-by-breath end-tidal forcing system was successfully developed that controlled the end-tidal partial pressure of CO_2 within ± 2 mm Hg of the level desired level by changing the gas concentration of an injected bolus of humidified gas and not using a continuous flow method.

6.3 Thesis summary

The main findings of this thesis are that hyperthermia increased humans ventilatory responses to isocapnic hypoxia but not poikilocapnic hypoxia. Therefore this study appears unique in demonstrating that during a 20 min steady-state poikilocapnic hypoxia exposure, decreases in $P_{ET}CO_2$ appear to blunt the HVR more than a 1.5°C increase in T_{es} augments it, as seen during a 20 min steady-state isocapnic hypoxia exposure. It seems that when the ventilatory response to hypoxia is blunted due to the lowered CO_2 tension, the cardiovascular system attempts to compensate for this by increasing heart rate as heart rate responses were greater during poikilocapnic hypoxia compared to isocapnic hypoxia. This may be due to metaboreceptors sensing the decrease in oxygen supply and attempting to increase this supply by increasing vasodilation which in turn would increase heart rate via a baroreceptor-mediated mechanism. Alternatively, the lowered CO_2 tension during poikilocapnic hypoxia would lower cerebral blood flow and hence cerebral oxygen supply, this may also cause the recently proposed central oxygen sensitive areas to increase heart rate in order in increase oxygen supply.

The medical relevance of such a finding has implications for both mountaineers at altitude and respiratory disease patients (e.g. chronic obstructive pulmonary disease) living in hot climates. Both these populations are exposed to situations where increased body temperature during poikilocapnic hypoxia could be life threatening. In mountaineers the poikilocapnic hypoxia is due to the increased altitude. A rise in body temperature due to exercise and/or AMS may cause a further drop in arterial hemoglobin saturation which would increase pulmonary artery pressure and may increase the likelihood of pulmonary edema occurring. A similar situation may develop in breathing

disease patients living in hot climates where poikilocapnic hypoxia is brought on due to periods of hypoventilation induced hypoxemia. The increased ambient temperature in summer may cause a rise in body temperature which again may lower arterial hemoglobin saturation which would increase pulmonary artery pressure and possibly central blood pressure, though this remains to be tested. Especially in older patients, this increase in pulmonary and/or central blood pressure could lead to cardio-pulmonary failure and death. Especially in respiratory disease patients, education about these risks and how to prevent unnecessary increases in body temperature may be helpful in reducing the risk of cardio-pulmonary failure.

In relating these problems back to my original notion of a thermal influence as a possible cause of exercise hyperpnea, it appears that a raised thermal status influences humans responses to hypoxia in a positive manner though it is as yet unknown what the cause is and via what mechanism(s). A possible cause and mechanism is that the increased response to hypoxia during hyperthermia is peripherally mediated by greater carotid body sensitivity to hypoxia, which would mean a greater neural output to central respiratory centers via the glossopharyngeal nerve. Another possibility is that the signal received by the central control centers, most likely initially in the nucleus tractus solitarii, is amplified during hyperthermia. The cut-off threshold for inspiratory activity in the dorsal respiratory group may then be increased meaning a greater rate of ventilation would be achieved through a greater tidal volume. Greater tidal volume changes during hyperthermic compared to normothermic isocapnic hypoxia were seen in this thesis. Both the carotid bodies and central respiratory control centers have exhibited increased activity in response to direct heat stimulation from thermodes and the blood perfusing.

Measurements of phrenic and carotid nerve activity during whole body hyperthermic hypoxia in animal models may be a study that could help elucidate the mechanism of this temperature and hypoxia interaction.

From our results though it does appear that if a raised body temperature increases the response to hypoxia then it is likely that the raised body temperature during exercise can partly explain the occurrence of exercise hyperpnea. Studies investigating whether this temperature effect is only above a certain core body temperature, or whether the increased hypoxic sensitivity increases in a positive linear manner with core body temperature, would help determine how much temperature influences control of ventilation during exercise. A temperature threshold would suggest this effect is only during heavier types of exercise which induce larger changes in core body temperature. However, a positive linear relationship between increased temperature and increased hypoxic sensitivity would suggest a larger thermal influence on exercise hypernea, as all changes in core body temperature during all intensities of exercise would in theory influence ventilatory control. A problem when attempting to ascertain whether this positive linear relationship exists is that for all increases in body temperature there will be an increase in metabolic rate. It is possible that the increased core body temperature indirectly causes the enhanced sensitivity to hypoxia via increased metabolic rate by unknown mechanism(s). Changes in metabolic rate have been shown to parallel changes in hypoxic and hypercapnic sensitivity. While it is reasonably uncomplicated to discern the effect of an increased metabolic rate with core body temperature held constant, the opposite appears much more difficult. Therefore, finding a solution to this problem would

need to be made before the possibility of a positive linear relationship could be clearly shown.

The greater ventilation during hyperthermic than during normothermic immersed rest in air appears to be mediated by an increased metabolic rate. This is in contrast to other studies which have shown a disproportionate increase in ventilation compared to metabolic rate. This finding may be due to the ~1.2°C rise in body temperature being insufficient to cause the hyperthermic hyperventilation, or because ventilation was measured while body temperature was steady, whereas other studies measured ventilation while body temperature was steadily rising, and to an greater absolute level. This increase in body temperature was is large enough to cause a frequency mediated change in breathing pattern.

Finally this study successfully developed a breath-by-breath dynamic end-tidal forcing system which controls the $P_{ET}CO_2$ using gas bolus injections instead of continuous flow. The greater the $\dot{V}_E$ rate while on the end-tidal forcing system, the faster the desired $P_{ET}CO_2$ levels were reached and the better they were maintained. Work is continuing to improve the speed and accuracy of $P_{ET}CO_2$ control and to incorporate $P_{ET}O_2$ control.

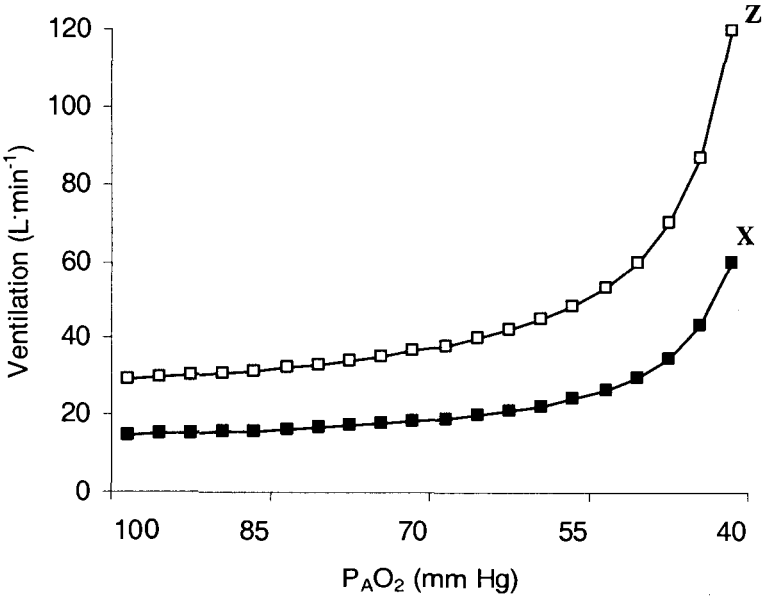
Appendices

Appendix A - Definitions

A-shape parameter

When graphing ventilation ($L \cdot min^{-1}$) vs P_AO_2 (mm Hg) the data points approximate a hyperbolic curve and therefore hypoxic responses are calculated by fitting those data points to the equation: $\dot{V}_E = \dot{V}_0 + A / (P_AO_2 - 32)$, where $\dot{V}_E$ is minute ventilation in $L \cdot min^{-1}$, BTPS, $\dot{V}_0$ is the asymptote for ventilation obtained by extrapolation, P_AO_2 is the alveolar PO_2 in mm Hg, 32 respresents the P_AO_2 at which the slope of the $\dot{V}_E$-P_AO_2 curve approaches infinity and the parameter A (unitless) describes the shape of the curve such that the greater the hypoxic drive, the steeper the curve and the higher the A value, as shown below (8).

Figure A1. Progressive hypoxic ventilatory response showing two lines where line X has an A value of 200 and $\dot{V}_0$ value of 20 and Z an A value of 100 and $\dot{V}_0$ value of 10.



Adaptive/non-shivering thermogenesis

The increase in heat production in response to acute cold exposure. The principal effector organ is brown adipose tissue (1).

Eccrine sweat glands

The more common sweat gland, which opens onto bare skin all over the body and produces a perspiration that's composed mostly of water and salt. These glands are controlled by the autonomic nervous system control center in the hypothalamus (7).

Eucapnic/eucapnia

Maintenance of arterial carbon dioxide tension at normal/resting levels (4).

Exercise hyperpnea

Caused by exercise a breathing pattern that is deeper and more rapid than is normal at rest (2).

Hyperpnea

Breathing that is deeper and more rapid than is normal at rest (2).

Hyperventilation

An increased alveolar ventilation relative to metabolic carbon dioxide production, so that alveolar carbon dioxide pressure decreases to below normal (2).

Hypoventilation

A reduced alveolar ventilation relative to metabolic carbon dioxide production, so that alveolar carbon dioxide pressure increases above normal (2).

Hypoxia

Decreased oxygen partial pressure (compared to normoxia) (9).

Hypoxic ventilatory decline

The decay in ventilation during a constant hypoxic stimulus, usually occurring after 5 min (5).

Isocapnic/isocapnia

Maintenance of arterial carbon dioxide tension at 1 to 2 torr and/or mm Hg above normal/resting levels (6).

Iso-oxic/iso-oxia

Maintenance of arterial oxygen tension at pre-specified levels (4).

Normoxic/normoxia

Normal oxygen partial pressure at a defined location and under defined conditions (9).

Temperature coefficient (Q_{10})

The ratio of the rate of a physiological process at a particular temperature to the rate at a temperature 10°C lower, when the logarithm of the rate is an approximately linear function of temperature (1). For most physiological reactions the value is close to 2, meaning for every 10°C rise in temperature, oxygen consumption and thus metabolic demand doubles.

Hoff's rule describes this relationship: $Q_{10} = R_t / R_{t-10}$

Q_{10} = temperature coefficient.
R_t = rate at any given body temperature T.
R_{t-10} = rate of body temperature at T - 10°C.

Sensitivity

The degree of an output and/or response to a certain input and/or stimulus, to increase sensitivity would mean a greater output/response to the same level of input/stimulus (3).

Thermal tachypnea

A rapid respiratory frequency accompanied by an increase in respiratory minute volume and, commonly, a decreased tidal volume, in response to a thermoregulatory need to dissipate heat (1).

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Appendix B - Pilot study

Ventilatory response to three levels of hypoxia during normothermia and hyperthermia.

Aim

An elevated core body temperature (T_c) may influence both exercise hypernea and thermal tachypnea by increasing the body's response to decreases in the arterial partial pressure of oxygen (P_aO_2). The purpose of this pilot study was to investigate the effect on ventilation of three steady states of hypoxia in passively heated hyperthermic vs. normothermic humans.

Methods and Results

Five college-aged males (25 ± 3 years, weight 78 ± 5 kg, height of 181 ± 8 cm) rested seated either in a temperate room or immersed to the shoulders in warm water (mean (SD) T_{water} 38.56 (0.58)°C, T_{es} 38.66 (0.52)°C). After a five-minute resting period breathing room air participants breathed gas mixtures of 14 % then 13 % then 12 % O_2, (balance N_2), from meteorological balloons for three minutes per gas mixture. Inhaled gas mixtures were prepared using air, 100 % N_2, a Tissot spirometer and metabolic cart. End-tidal CO_2 ($P_{ET}CO_2$) was maintained at a eucapnic (mean (SD) 38.83 (4.67) mm Hg) during all the hypoxic exposures by manually titrating 100 % CO_2 into the inspirate. Analysis of variables was performed graphically for each stage in its entirety for participant B (Figure B1) and using the mean (SD) of 40 s of data after the equilibration of end-tidal O_2 ($P_{ET}O_2$) for each stage for all participants (Figure B2). A table of mean (SD) responses for all subjects for during the last minute of the 5-min resting period and each subsequent 3-min period at the three different gas concentrations was also compiled.

Figure B2 demonstrates that during hyperthermia not only is ventilation greater but the ventilatory response to hypoxia is also greater than the increase in ventilation due to hyperthermia alone. Ventilation ($\dot{V}_E$) during hyperthermia was 0.85 $L \cdot min^{-1}$ greater than normothermia at rest and 1.78, 1.31 and 2.05 $L \cdot min^{-1}$ greater while breathing the 14, 13 and 12 % O_2 gas mixtures respectively. Figure B1 shows that F_IO_2 was very similar for both trials, while ventilation and $P_{ET}O_2$ were greater and $P_{ET}CO_2$ was lower at all stages for hyperthermia. Table B1 shows that the trends seen for participant B in Figure B2 were also evident in the means for all subjects for each stage.

Discussion

This study showed that ventilation was greater in hyperthermic humans relative to normothermic humans at all levels of hypoxia and that the increased ventilation during hypoxia was greater than the increase due to hyperthermia alone. The increased ventilation during hyperthermia also occurred despite a slightly higher $P_{ET}O_2$ and slightly lower $P_{ET}CO_2$ during the hyperthermic tests, both of which would attenuate the hypoxic ventilatory response. The results are very promising for the hypothesis that the human whole body response to hypoxia greater in passively heated hyperthermic vs. normothermic humans.

However, the protocol and apparatus used in this study was vulnerable to human error producing slightly different stimuli for each participant and for each condition. The crux of testing the above hypothesis lies in attaining a constant and accurate stimulus for all individuals across all testing sessions while also minimizing human error. A solution to this is to use an end-tidal forcing technique that uses electric valves and a computer program to control the $P_{ET}O_2$ and $P_{ET}CO_2$ at predetermined levels by varying the F_IO_2

and F_ICO_2. This method would not only allow the measurement of the whole body response to hypoxia but allow future testing of whole body sensitivity and peripheral sensitivity to hypoxia during either eucapnic, isocapnic or poikilocapnic conditions.

Other modifications to this study would be to perform both normothermic and hyperthermic tests immersed in water and to take T_{es} readings during all tests and to better maintain T_{es} at 38.5°C during hyperthermia. Also the methods should be modified to assess the whole body response to one set level of hypoxia using the recently recommended universal method for testing humans hypoxic ventilatory response (2), rather than multiple levels of hypoxia as this represents whole body sensitivity to hypoxia and has been studied previously (1).

In conclusion, this study has demonstrated that it is possible to study the ventilatory response to whole body hypoxia in passively heated hyperthermic vs. normothermic humans and that there may be a difference between the two that is not attributable to the increased ventilation during hyperthermia alone. This study has also provided data to aid in power calculations for the major study. However, a better method for producing the hypoxic stimulus needs to be developed in order to attain more accurate and reliable results.

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Table B1 Mean (SD) fraction of inspired oxygen (F_IO_2), ventilation ($\dot{V}_E$), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$), partial pressure of end-tidal O_2 ($P_{ET}O_2$), and heart rate (HR) for all participants (n = 3) during the last minute of a 5-min resting period and each subsequent 3-min period at 3 different gas concentrations while either normothermic (Norm) or hyperthermic (Hyper) (T_{es} 38.66 (0.52)°C).

	Rest		14 % O_2, balance N_2		13 % O_2, balance N_2		12 % O_2, balance N_2	
	Norm	Hyper	Norm	Hyper	Norm	Hyper	Norm	Hyper
F_IO_2 (%)	20.99 (0.08)	20.89 (0.13)	14.22 (0.26)	14.45 (0.52)	12.96 (0.17)	13.28 (0.33)	12.24 (0.36)	12.34 (0.51)
$\dot{V}_E$ ($L \cdot min^{-1}$)	7.83 (1.34)	11.32 (3.45)	9.03 (2.10)	13.24 (3.38)	9.25 (2.30)	15.34 (4.69)	9.53 (2.17)	16.29 (4.20)
$P_{ET}CO_2$ (mm Hg)	40.33 (2.15)	38.72 (6.43)	39.88 (2.04)	37.70 (6.12)	39.56 (1.79)	38.33 (6.00)	39.19 (1.75)	37.92 (5.82)
$P_{ET}O_2$ (mm Hg)	97.38 (4.32)	101.90 (8.51)	62.11 (3.06)	65.9 (4.18)	53.91 (4.10)	59.47 (4.42)	48.82 (3.70)	57.72 (4.72)
HR (bpm)	55 (5)	87 (7)	59 (6)	93 (8)	61 (6)	97 (9)	63 (7)	99 (10)

Figure B1 Breath-by-breath ventilatory variables for participant B during the last minute of a 5-min resting period and each subsequent 3-min period at 3 different gas concentrations while either normothermic or hyperthermic.

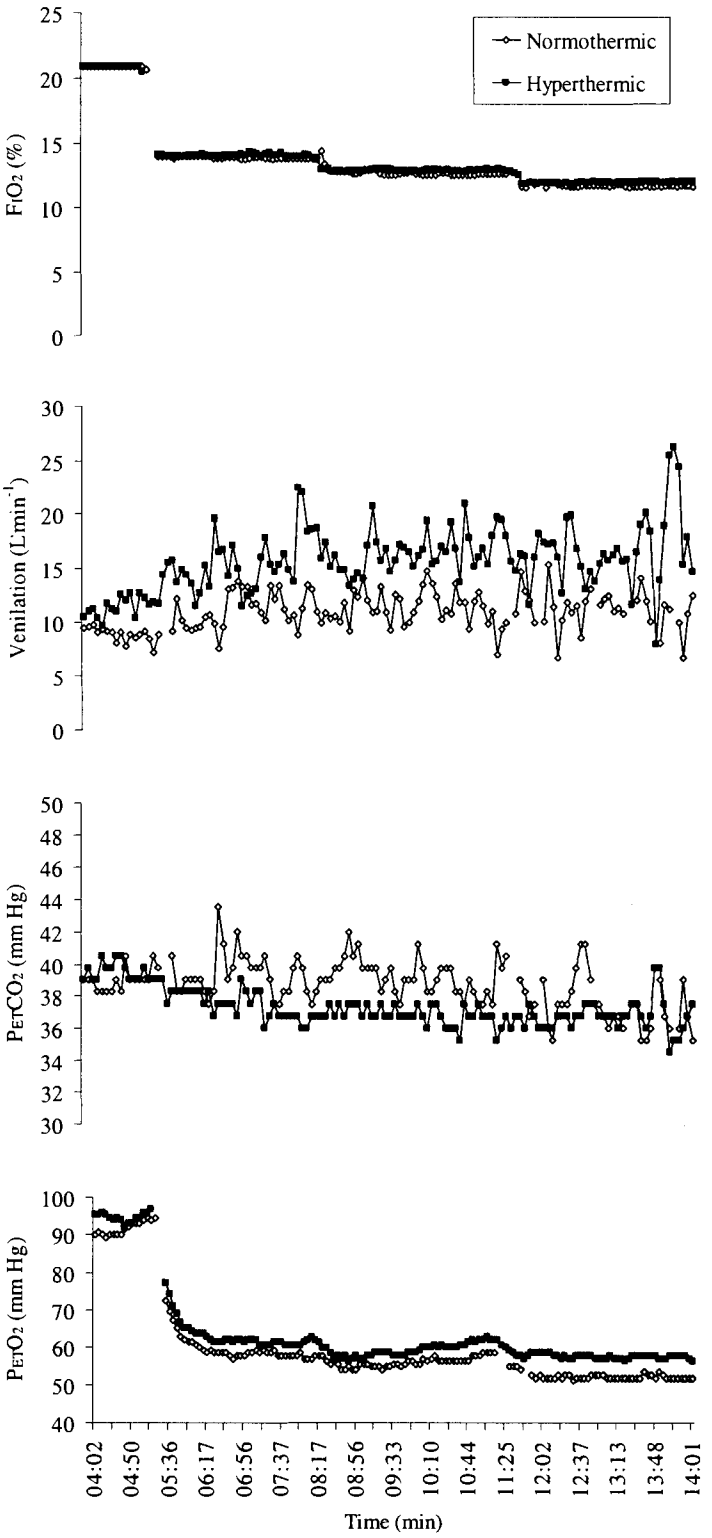
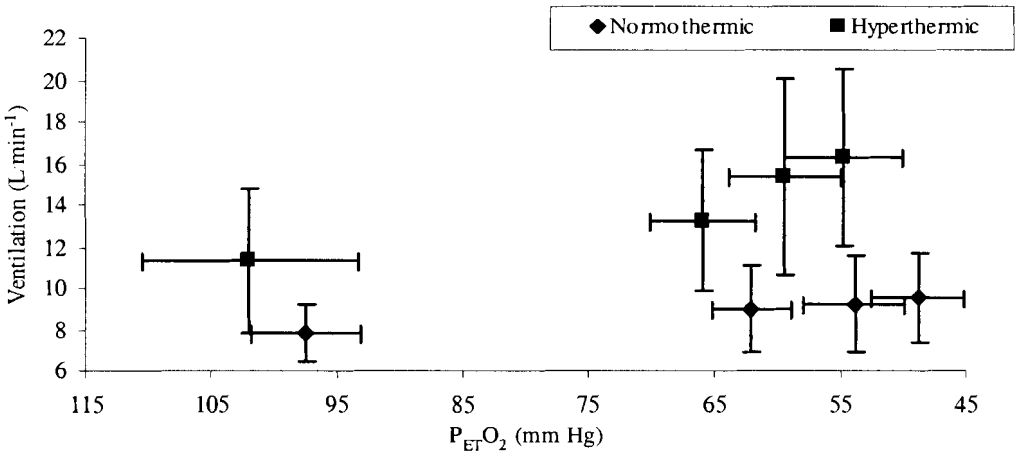


Figure B2 Mean (SD) end-tidal partial pressure of oxygen ($P_{ET}O_2$) and subsequent level of resting ventilation for 5 normothermic or hyperthermic (T_{es} 38.66 (0.52)°C) males.

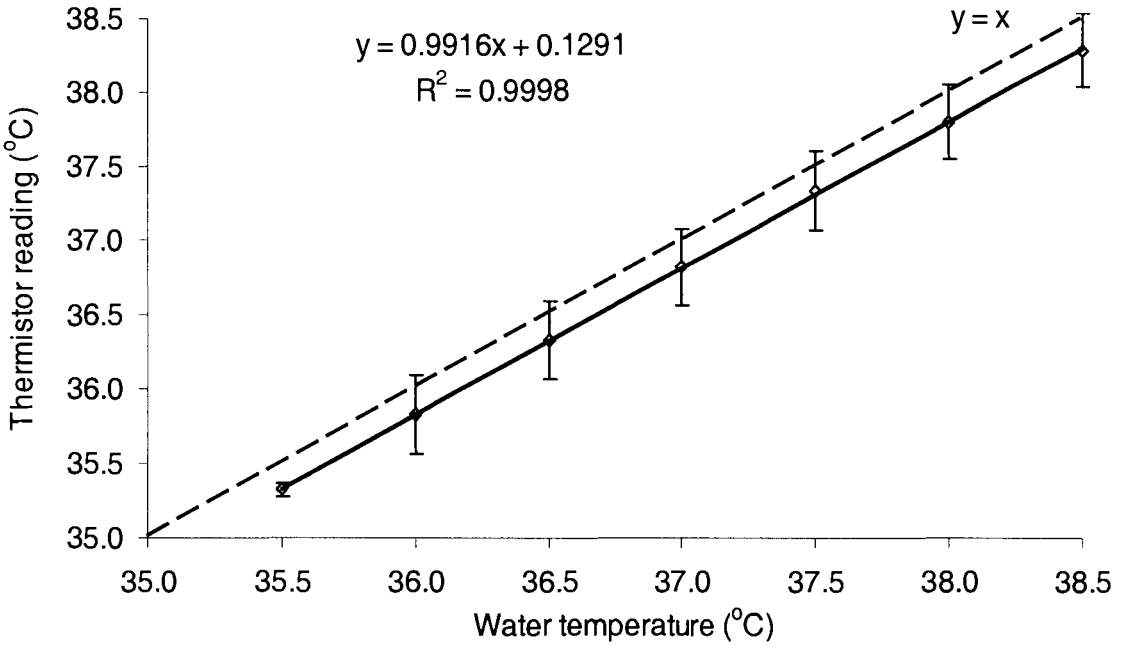


Appendix C - Esophageal thermistor calibrations

Table C1. Temperature readings (°C) from each participants esophageal thermistor, when placed in a circulating water bath at seven different water temperatures.

		Participant number & associated thermistor reading (°C)							
		1	2	3	4	5	6	7	8
	35.5	35.25	35.33	35.33	35.25	35.33	35.33	35.42	35.33
	36	35.75	35.83	35.83	35.75	35.83	35.83	35.92	35.83
Water	36.5	36.25	36.34	36.34	36.25	36.34	36.34	36.42	36.34
Temp	37	36.75	36.84	36.84	36.75	36.84	36.84	36.92	36.84
(°C)	37.5	37.28	37.37	37.37	37.28	37.37	37.37	37.37	37.28
	38	37.73	37.82	37.82	37.73	37.82	37.82	37.91	37.82
	38.5	38.20	38.30	38.30	38.20	38.30	38.30	38.40	38.30

Figure C1. Mean (SD) temperature readings (°C) from all participants esophageal thermistors, when placed in a circulating water bath at seven different water temperatures. Line of identity (y = x) is given by the dotted line.



Appendix D - Power calculations

Eight participants were deemed sufficient after a pre-study power analysis of comparisons for a detectable difference between the hyperthermic and normothermic responses to the poikilocapnic HVR (pHVR) or isocapnic HVR (iHVR) was done to justify the sample size selection. All power calculations were done using a correlated t-Test model. This involved the use of the GPower statistical program (3). The effect size was calculated using the f value:

$$f = \frac{\text{mean}_A - \text{mean}_B}{SD_{AB}} \quad \text{..... Equation C1}$$

Where mean_A and mean_B are the means of the two sample populations you intend to investigate and SD_{AB} is the pooled standard deviation of the two sample populations and is calculated using the following formula:

$$SD_{AB} = \sqrt{SD_A^2 + SD_B^2 - 2 \bullet r \bullet SD_A \bullet SD_B} \quad \text{..... Equation C2}$$

Where SD_A and SD_B are the individual standard deviations of populations A and B respectively, and r is the correlation coefficient between populations A and B (3). The effect size, sample population (n) and degrees of freedom (n - 1) and alpha level (α) are then used to calculate power for a correlated t-Test under the conditions specified.

Data from Natalino et al. (1997), which quantified the A-shape parameter changes in 7 participants during a progressive isocapnic HVR while participants were either normothermic or hyperthermic (T_{re} 1.5°C greater than resting) was used in the calculation (5), as the current study will be the first study to look at the 20 min steady state HVR during passive hyperthermia. The mean (SD) A-shape parameters while normothermic and hyperthermic were 113 (21.5) and 240 (83.3) respectively (5). The r-value for the correlation for these values was 0.73. Using the GPower computer program (3), a sample

size of n = 8, at $\alpha = 0.05$, the power for replicating this finding was 1.0. Also, using pilot data from Appendix B, the mean (SD) changes in ventilation from air to 12 % F_IO_2 while either normothermic or hyperthermic were 1.7 (1.3) and 5.0 (1.9) $L \cdot min^{-1}$ respectively (Appendix B). The r-value for the correlation for these values was 0.33. Using the GPower computer program (3), a sample size of n = 8, at $\alpha = 0.05$, the power for replicating this finding was also 1.0. Therefore a sample size of 8 was deemed adequate for detecting temperature induced changes in iHVR and pHVR trials.

Power analysis of comparisons for a detectable difference between the 5th and 20th min values of the iHVR and the pHVR was done to justify sample size selection. Data from the Steinback et al. (2005), study which investigated the 5th and 20th min iHVR values during a 20 min iHVR trial and the pHVR values during a 20 min pHVR trial for seven participants (7). For the iHVR the mean (SD) change in ventilation per percent change in S_aO_2 ($L \cdot min^{-1} \cdot$ % $S_aO_2^{-1}$) from rest to the 5th and rest to the 20th min values were 2.37 (0.61) and 0.78 (0.27) $L \cdot min^{-1} \cdot$ % $S_aO_2^{-1}$ respectively and the r-value for these data was 0.78 (7). Using the GPower computer program (3), a sample size of n = 8 at $\alpha =$ 0.05 the power for replicating this finding was 1.0. For the pHVR the mean (SD) change in $P_{ET}CO_2$ per percent change in S_aO_2 (mm Hg $\cdot$ % $S_aO_2^{-1}$) from rest to the 5th and rest to the 20th min values were 0.33 (0.05) and 0.28 (0.08) $L \cdot min^{-1} \cdot$ % $S_aO_2^{-1}$ respectively and the r-value for these data was 0.97 (7). Using the GPower computer program (3), a sample size of n = 8 at $\alpha = 0.05$ the power for replicating this finding was 1.0. Therefore a sample size of 8 was deemed adequate for detecting changes in the 5th and 20th min measures of iHVR and pHVR.

Appendix D References

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Table D1. Summary of iHVR, pHVR and ventilation ($\dot{V}_E$) values from studies using similar protocols or at similar levels of isocapnic hypoxia.

Studies	Our data		Steinback et al. (2005) (7)		Ainslie & Poulin, (2004) (1)		Mou et al. (1995) (4)	Easton et al. (1988) (2)	Petersen et al. (1997) (6)
Conditions	ISO	POI	ISO	POI	ISO	POI	$P_{ET}O_2$ ~55 mm Hg (Our ~55mm Hg)	S_aO_2 ~80 % (Our ~90 %)	S_aO_2 ~ 80 %
$iHVR_5$	0.61	0.05	2.37	0.24	1.95	0.61			
($L \cdot min^{-1} \cdot \%^{-1}$)	(0.64)	(0.02)	(0.61)	(0.06)	(1.11)	(0.51)			
$iHVR_{20}$	0.82	0.04	0.78	0.08					
($L \cdot min^{-1} \cdot \%^{-1}$)	(1.24)	(0.16)	(0.27)	(0.02)					
$pHVR_5$		0.23		0.33		0.27			
(mm Hg·%S_aO_2)		(0.09)		(0.05)		(0.30)			
$pHVR_{20}$		0.19		0.28					
(mm Hg·%S_aO_2)		(0.11)		(0.08)					
$\dot{V}_E$ rest	10.2	12.0	15.8 (1)	12.0	13.4	10.5	~13.5	~7.5	~20
($L \cdot min^{-1}$)	(1.1)	(2.3)		(1.2)	(3.1)	(3.2)			
$\dot{V}_E$ 5 min/ peak	13.7	12.8	51.6	15.9	54.4	16.7	~23	~12	~38
($L \cdot min^{-1}$)	(3.1)	(2.5)	(9.7)	(1.5)	(No SD)	(No SD)			
$\dot{V}_E$ 10 min	13.8	12.8	34.5	15.4					
($L \cdot min^{-1}$)	(2.7)	(2.4)	(4.0)	(1.4)					
$\dot{V}_E$ rest 15 min	14.2	12.9	30.6	14.5					
($L \cdot min^{-1}$)	(2.8)	(3.0)	(4.6)	(1.3)					
$\dot{V}_E$ rest 20 min	14.4	12.9	27.2	14.4				~10	
($L \cdot min^{-1}$)	(3.4)	(2.8)	(4.0)	(13)					

'Our' refers to the comparative value for our study. Ainslie & Poulin (2004) (1), peak value is the value after 90 s of hypoxic exposure, the Mou et al. (1995), study (4) is the peak value after only 50 s. While for the Easton et al. (1988), study (2), the absolute values are lower than this studies, the relative increases are still twice that of our data