CEREBRAL AND PERIPHERAL HEMODYNAMIC RESPONSES TO INCREASED END-TIDAL CARBON DIOXIDE VOLUMES

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Justin R. Geijer

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Donald R. Dengel, Ph.D. – Major Advisor

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ABSTRACT

Though hypercapnia is a naturally occurring physiological state, it is generally accompanied by hypoxic conditions (Venkataraman et al., 2008). The convolution associated with concurrent changes in carbon dioxide and oxygen volumes offer unclear results to researchers investigating the effects of arterial gas changes (Brogan et al., 2003; Cinar et al., 2012). Researchers at the University of Toronto have developed a computer-controlled gas blender (RespirAct™, Thornhill Research, Toronto, Ontario, CA) capable of measuring and altering end-tidal gas volumes, which are indicative of arterial blood gas changes (Brogan et al., 2003; Cinar et al., 2012). Researchers have utilized this technology to investigate cerebral vascular reactivity (Kassner et al., 2010; Mandell et al., 2008; Mark et al., 2011; Prisman et al., 2008), but differing methodologies and a lack of reproducibility studies raise questions about the validity of the findings. In addition, the peripheral response to a hypercapnic, normoxic environment is not well documented. This dissertation will investigate the effects of a hypercapnic environment on the cerebral and peripheral vascular beds. We hypothesize that the vascular changes associated with a hypercapnic environment are repeatable in both the cerebral and peripheral beds. We further hypothesize that the cerebral vascular changes will occur more quickly than the peripheral changes. Lastly, we hypothesize that a comparison between hypercapnia-induced vasodilation of the brachial artery will provide a similar, but slower dilatory response than reactive hyperemia. The results of this dissertation may provide further insight into the mechanisms responsible for hypercapnia-induced vasodilation of the cerebral and peripheral blood vessels, and may provide repeatable methodologies to be utilized in future research.

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CHAPTER 1. INTRODUCTION

Increases in arterial carbon dioxide levels (P_aCO_2), known as hypercapnia, are known to induce dilatory responses in the vasculature of the brain and periphery (Harino et al., 1995; Kara et al., 2003; Len et al., 2011; Rodriguez-Roisin et al., 2009). However, the increase in carbon dioxide (CO_2) is generally accompanied by changes in arterial oxygen levels (Venkatarman et al., 2008), both of which are known to cause alterations in vascular responses. Since there are few ways to examine each independently it is difficult to understand the independent effects of CO_2 on the vascular system. Various clinical populations, such as patients with chronic obstructive pulmonary disease (Toussaint et al., 2007), cystic fibrosis (Fauroux et al., 2012; Sheikh et al., 2011; Waterhouse et al., 2009), and muscular dystrophy (Vohwinkel et al., 2011), experience hypercapnia on a daily basis, but little is known about the short or long term effects of increased CO_2 levels in healthy populations.

Recently, researchers at the University of Toronto developed a computer-controlled gas delivery system that utilizes end-tidal gas volumes as a surrogate measure of arterial blood gases (Brogan et al., 2003; Cinar et al., 2012). Not only is the device (RespirAct™, Thornhill Research, Toronto, Ontario, CA) capable of measuring end-tidal gas volumes, but also manipulating end-tidal oxygen ($P_{et}O_2$) and end-tidal carbon dioxide ($P_{et}CO_2$) volumes (Mark et al., 2011). The ability to successfully manipulate $P_{et}CO_2$ while clamping $P_{et}O_2$ allows researchers to truly investigate the effects of a hypercapnic environment. This dissertation will focus on the effects of hypercapnia on the cerebral and peripheral vascular system, as well as whether or not the effects are of a repeatable nature. The following hypotheses will be addressed:

1. Hypercapnia has a rapid, significant dilatory effect on the cerebral vascular beds, and is a repeatable measure.
2. Hypercapnia has a relatively slow, significant dilatory effect on the peripheral vascular system and is a repeatable measure.
3. Reactive hyperemia and hypercapnia have similar dilatory effects on the peripheral vascular system.

The second chapter of this dissertation offers a review of the existing literature on hypercapnia. Populations in which hypercapnia is naturally occurring will be reviewed, as will the effects of hypercapnia on the cerebral and peripheral vascular beds. Available methods for measuring the vascular beds will also be addressed.

The third chapter of this dissertation investigates hypercapnia's effects on the cerebral vascular system. The acute response to hypercapnia will be detailed, in addition to the repeatability of the measure. The blood-oxygen-level-dependent signal will be measured and contrasted with the volumetric changes to $P_{et}CO_2$.

The peripheral vascular response to an acute hypercapnic stimulus will be explained in chapter four. The dilatory response of the brachial artery to a hypercapnic environment is detailed, as well as the repeatability of the measure.

A comparison between the peripheral vascular response to reactive hyperemia and hypercapnia is the topic of chapter five. The brachial artery is again the vessel of interest. A discussion of the mechanistic differences between the two stimuli is presented.

Chapter six of this dissertation summarizes the findings of the studies discussed in previous chapters. Clinical applications of the findings and future research are outlined in this chapter.

CHAPTER 2. LITERATURE REVIEW

Introduction

Cerebral and peripheral vascular responses to alterations in blood gases are critical to maintain proper physiological function (Ainslie, et al., 2005; Fitch, et al., 1975; Suzuki, et al., 1997; Urboniene, et al., 2005; van den Bos, et al., 1979). Several diseased states (i.e., Chronic obstructive pulmonary disease (COPD), cystic fibrosis, and congestive heart failure) cause alterations in arterial blood gases (Agostoni, et al., 2008; Cutillo, et al. 1974; De Backer, 1995; Fauroux et al., 2012; Harino et al., 1995; Kara, et al. 2003; Len et al., 2011; Rodriguez-Roisin, et al. 2009; Sheikh et al., 2011; Somers, et al., 1989; Toussaint et al., 2007; Vila et al., 2007; Vohwinkel et al., 2011; Waterhouse et al., 2009; Windisch, et al., 2009). Arterial blood gases can be measured invasively (Sheikh et al., 2011), but are often measured using end-tidal gases as a surrogate (Agostoni, et al., 2008; Cutillo, et al. 1974; De Backer, 1995; Fauroux et al., 2012; Harino et al., 1995; Kara, et al. 2003; Len et al., 2011; Rodriguez-Roisin, et al. 2009; Somers, et al., 1989; Toussaint et al., 2007; Vila et al., 2007; Vohwinkel et al., 2011; Waterhouse et al., 2009; Windisch, et al., 2009). Most diseased states (i.e., COPD, cystic fibrosis, and congestive heart failure) affecting the lungs cause decreases in end-tidal oxygen tensions ($PetO_2$) and increases in end-tidal carbon dioxide tensions ($PetCO_2$) as a result of poor gas exchange between the alveoli and the arterial blood (Cutillo, et al. 1974; Rodriguez-Roisin, et al. 2009; Windisch, et al., 2009).

While research exists on the effects of altered arterial blood gases on the cardiovascular system (Harino et al., 1995; Kara, et al. 2003; Len et al., 2011; Rodriguez-

Roisin, et al. 2009; Somers, et al., 1989; Toussaint et al., 2007; Vila et al., 2007; Vohwinkel et al., 2011), few studies isolate blood gases to determine their individual effect on cerebral or peripheral vascular reactivity (Fauroux et al., 2012; Sheikh et al., 2011). A secondary confounding issue is the lack of reproducibility of blood gas alterations due to individual variation in resting end-tidal tensions. Without a repeatable measure and isolation of individual blood gases, research utilizing alteration of blood gases to stimulate changes in vascular reactivity is not repeatable or valid. Isolation of a single gas while clamping the other would allow for establishment of repeatable methods and eradication of the most prominent confounding issues in the existing research.

The following review of literature will establish a need for repeatable measures of vascular reactivity using a hypercapnic state as a stimulus. Between-day and within-day repeatability will be observed for both cerebral and peripheral vascular reactivity. Cerebral vascular reactivity will be measured using the whole brain, while peripheral vascular reactivity will focus on the brachial artery.

Hypoxia and Hypercapnia

Rowell (1986) defines hypoxia as decreased oxygen levels in the blood, lungs and tissues. The typical partial pressure of oxygen (PO_2) in the arterial blood and the lungs is approximately 100 mmHg at sea level (Sherwood, 2010). The PO_2 of the tissues at sea level is typically less than 40 mmHg (Sherwood, 2010). The difference between the

arterial blood PO_2 and the tissue PO_2 establishes a sizable diffusion gradient, allowing for oxygen diffusion between arterial blood and the tissues (Sherwood, 2010).

Hypercapnia is defined as increased levels of carbon dioxide (CO_2) in the blood (Sherwood, 2010) and often accompanies hypoxia. The typical limit defined as hypercapnia is a blood gas level of CO_2 above 45 mmHg (Sherwood, 2010). The body responds to a hypercapnic and/or the hypoxic state primarily by increasing sympathetic nerve stimulation (Ainslie et al., 2005; Rowell, 1986). Epinephrine and norepinephrine are released from sympathetic nervous stimulation, which have a profound effect systemically. In a hypercapnic state, the effects on the heart, lungs, and blood vessels are the most notable (Fitch, et al., 1975; Ainslie, et al., 2005).

The lungs are directly affected by a hypercapnic state. Sympathetic nerve stimulation causes dilation of the bronchioles through relaxation of the smooth muscle within the bronchioles (Boussat et al., 2000; Grek et al., 2010). The increased airway diameter allows for increased total airflow, which is combined with an increased respiratory rate (Pierce et al., 1966). The increased flow, combined with increased blood flow, ideally increases gas exchange between the lung and the alveoli, repairing the hypercapnic state.

Hyperncapnia leads to a more alkalytic blood pH, causing an increase in the production and excretion of bicarbonate (Batile et al., 1985; Jack et al., 2004). As the

blood becomes more alkalytic, the oxyhemoglobin dissociation curve shifts to the left (known as the Haldane effect), causing oxygen to bind more strongly to hemoglobin (Batile et al., 1985; Jack et al., 2004).

The Haldane effect causes less O_2 to be unloaded at the tissues, and thus perpetuates a hypoxic/hypercapnic environment. The increased CO_2 levels are buffered by converting CO_2 and H_2O to HCO_3^- (bicarbonate) and H^+ (Batile et al., 1985; Jack et al., 2004). The access H^+ ions cause an increase in the acidity of the blood, and leads to a shift in the oxyhemoglobin dissociation curve back to the right (Bohr Effect) (Batile et al., 1985; Jack et al., 2004).

Natural Occurrences of Hypercapnia and Hypoxia

Hypercapnia and hypoxia occur naturally in several diseased populations. These populations include chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), Kyphoscoliosis, Muscular Dystrophy, and Cystic Fibrosis (CF). Hypoxia and hypercapnia can also be observed in healthy populations during acute exposure to altitude.

Chronic obstructive pulmonary disease (COPD) is a collection of respiratory afflictions including asthma, chronic bronchitis, and emphysema that are the most

common causes of respiratory failure (Cutillo, et al. 1974; Rodriguez-Roisin, et al. 2009; Windisch, et al., 2009). COPD is characterized by the damage and destruction of the alveoli (Cutillo, et al. 1974; Rodriguez-Roisin, et al. 2009; Windisch, et al., 2009). The destruction of alveoli leaves less surface area available for gas exchange between the lungs and the arterial blood, which is a common side effect of other diseases, such as Cystic Fibrosis and Congestive Heart Failure (Fauroux et al., 2012; Sheikh et al., 2011; Sherwood, 2010; Waterhouse et al., 2009). The decreased gas exchange elevates PCO_2 of the arterial blood supply, which decreases the diffusion gradient between the blood and the tissues of the body (Windisch, et al., 2009). Decreased diffusion gradients lead to a hypercapnic state in the blood and the tissues.

Kyphoscoliosis is a genetic deformity that causes a compression of the thoracic cage, and thus decreases the ability of the lungs to fully expand and contract (Sherwood, 2010). Without the full range of motion in the thoracic cage, total lung volume is decreased and gas exchange to the blood is compromised (Sherwood, 2010). Similarly, Muscular Dystrophy is a condition in which muscle cells are unable to repair themselves, leading to weakness or destruction of the respiratory muscles, limiting range of motion in the thoracic cage and decreasing air movement through the system and gas exchange at the alveoli (Turner et al., 2010).

Methods to Induce Hypercapnia and/or Hypoxia

Researchers attempt to create hypercapnia using breathing techniques (such as bag breathing or breath holds) or altering carbon dioxide content of inhaled gas mixtures (Kastrup et al., 1998; Kastrup et al., 1999; Li et al., 1999; Stillman et al., 1995; Arend et al., 1994; Chung et al., 1999; Hosking et al., 2004; Sicard and Duong, 2005). Breath hold techniques reduce the partial pressure of oxygen in the blood stream by forcing deoxygenated red blood cells to recirculate. While the musculature is attempting to extract oxygen from the recirculating red blood cells, it is simultaneously attempting to offload CO_2 created during metabolic processes. The offloading of CO_2 to a deoxygenated red blood cell (which is already carrying CO_2 from the first circulation) leads to an increase in the arterial partial pressure of CO_2, known as hypercapnia (Kastrup et al., 1998; Kastrup et al., 1999; Li et al., 1999; Stillman et al., 1995).

Bag breathing is another technique used to increase the arterial partial pressure of CO_2. By re-breathing expired air, the subject is inhaling gas with a higher partial pressure of CO_2 than the atmosphere at sea level (Patel et al., 2001). The inhalation of a gas with a higher partial pressure of CO_2 causes an increase in arterial CO_2. This technique can also be accomplished by adding CO_2 to a bottled gas mixture (Arend et al., 1994; Chung et al., 1999; Hosking et al., 2004; Sicard and Duong, 2005). The bottled gas mixtures typically contain 5% CO_2, which is significantly higher than the atmospheric partial pressure of CO_2 (Arend et al., 1994; Chung et al., 1999; Hosking et al., 2004; Sicard and Duong, 2005).

From a research prospective, the inhalation of higher concentrations of CO_2 or cessation of breathing is rather unpredictable. The resting levels of arterial CO_2 vary quite a bit between individuals (Mark et al., 2011; Tancredi & Hoge, 2013), causing inconsistencies in the response to hypercapnia. In addition, the true effect of hypercapnia cannot be accurately measured using the aforementioned techniques because of the concurrent alterations in the partial pressure of oxygen (Kastrup et al., 1998; Kastrup et al., 1999; Li et al., 1999; Stillman et al., 1995). Bag breathing and breath holds both cause increases in arterial oxygen levels, known as hypoxia (Kastrup et al., 1998; Kastrup et al., 1999; Li et al., 1999; Stillman et al., 1995).

Recently, the development of a computer-controlled sequential rebreathing device, capable of independently controlling $P_{et}CO_2$ and $P_{et}O_2$ (RespirAct™, Thornhill Research, Inc., Toronto, Canada) has enabled researchers to isolate the effects of hypercapnia and hypoxia on CVR and BOLD signal changes (Kassner et al., 2010; Mandell et al., 2008; Mark et al., 2011; Prisman et al., 2008). The RespirAct™ has been shown to produce improved BOLD signal calibration, as well as more stable and faster CVR changes when compared with inhalation of a fixed fractional concentration of CO_2 (Mark et al., 2010; Mark et al., 2011). The precise targeting ability of the RespirAct™ allows excellent reproducibility of the signal over time and between people, whereas all other methodologies are affected by the participants' individual responses to the stimuli.

Cerebral Vascular Response to Hypercapnia

Cerebral vascular responses to increases in pressure and sheer stress occur more quickly than do peripheral responses (Ainslie, et al., 2005; Fitch, et al., 1975; Suzuki, et al., 1997; van den Bos, et al., 1979; Urboniene, et al., 2005). Willie et al. (2012) observed increases in vessel diameter and blood flow of the middle cerebral artery, internal carotid artery, and the vertebral artery under hypercapnic and hypoxic conditions. The vertebral artery showed a 50% greater reaction to hypoxic conditions than the middle cerebral and internal carotid arteries (Willie et al., 2012).

Methods to Measure Cerebral Vascular Reactivity

The increase in sympathetic outflow to the cerebral vessels in a hypercapnic environment causes vasodilation of the cerebral blood vessels (Ainslie, et al., 2005; Fitch, et al., 1975; Suzuki, et al., 1997; van den Bos, et al., 1979; Urboniene, et al., 2005). Increasing end-tidal CO_2 pressure ($PetCO_2$) in conjunction with magnetic resonance imaging (MRI) or transcranial doppler (TCD) has been previously used as a method to view the vascular reactivity of the cerebral blood vessels (Driver et al., 2010).

Magnetic resonance imaging (MRI) is used to view the blood-oxygen level dependent (BOLD) signal increase created in a hypercapnic environment (Driver et al., 2010;

Mandell et al., 2008). The BOLD signal is created when oxygenated blood enters the cerebral blood vessels. Any increase in flow will cause an increase in BOLD signal, allowing the BOLD signal to be used as a measure of cerebral vascular reactivity (CVR).

Transcranial Doppler (TCD) is used to view the velocity of blood flow through blood vessels, such as the middle cerebral artery (MCA) and the arteries comprising the circle of Willis. The measure of flow is directly related to the radius of the vessel. Any change in the radius of the vessel alters vessel resistance to the fourth power. Thus, any increase blood flow velocity associated with a change in diameter from a stimulus, such as CO_2, can be measured with the TCD. However, TCD only provides flow rate and does not depict any structural detail so that changes in diameter are not possible. In addition, dropout rates in TCD studies have been reported at 20% or more due to insufficient acoustic temporal bone window (Seidel et al., 1995). The acoustic temporal bone window is determined by race, gender, and stature; all of which can impact the ability to identify vessels using the TCD (Seidel et al., 1995).

Peripheral Vascular Response to Hypercapnia

The sympathetic stimulation seen in a hypercapnic environment not only causes a dilatory response in the cerebral vessels, but also in the peripheral vasculature (Acka, et al., 2006; Serebrovskaya, 1992; Steinback et al., 2009). However, Serebrovskaya reports that the sympathetic response to hypercapnia requires the PCO_2 to be elevated above 50

mmHg (1992). Acka et al. (2006) stated that while it remains plausible that PCO_2 could have a direct effect on the endothelium, causing a dilatory response, it is more likely that the vascular response to elevated PCO_2 levels is triggered by the sympathetic nervous system. The precise mechanism responsible for the dilatory response is as of yet, unknown.

Measurement of Peripheral Vascular Reactivity

The most common measurement of peripheral vascular reactivity is done using inflation of a pressurized cuff over the forearm to produce reactive hyperemia upon the release of the cuff. Reactive hyperemia induced by a 5-minute cuff occlusion of the forearm is performed during resting conditions (Welsch, Allen, & Geaghan, 2002). At rest, the blood vessels are constricted in part due to the action of insulin (Fujishima et al., 1995; Horgan et al., 1991; Lopez et al., 1990; Sakai et al., 1993; Suzuki et al., 1991). As insulin is released from the pancreas, it binds to the endothelium, releasing endothelin-1, and causing phosphorylation of the myosin light chain (Fujishima et al., 1995; Horgan et al., 1991; Lopez et al., 1990; Sakai et al., 1993; Suzuki et al., 1991). Inflation of the cuff at the forearm causes a decrease in the vessel diameters from the cuff down to the hand and fingers (Welsch, Allen, & Geaghan, 2002). The decreased vessel diameters allow minimal blood flow to the extremity, and lead to an increase in pressure behind the cuff (toward the shoulder) (Welsch, Allen, & Geaghan, 2002).

Upon release of the cuff, there is a dramatic increase in flow resulting in an increase in shear stress, causing the release of nitric oxide (NO) (Altura et al., 1987; Boron & Boulpaep, 2005; Christie et al., 1988; Furchgott et al., 1985; Ignarro et al., 1987; Shikano et al., 1987). NO triggers an increase in cAMP activity, and the eventual dephosphorylation of the myosin light chain (MLC) (Altura et al., 1987; Boron & Boulpaep, 2005; Christie et al., 1988; Furchgott et al., 1985; Ignarro et al., 1987; Shikano et al., 1987). Dephosphorylation of the MLC initiates the relaxation of the vessel, and an increase in vessel diameter. Because there is not an increase in metabolic need, as during exercise, there is not an increase in stroke volume over time, thus the hyperemia is only transient. The lack of a continuous high shear stress results in a decline in NO production, and the eventual return to baseline diameter (Rossitti et al., 1993; Snow et al., 2001).

Diameter of the brachial artery (and other peripheral vessels) can be viewed using B-mode ultrasound (Welsch, Allen, & Geaghan, 2002). Whether the vessel diameter is altered using reactive hyperemia from vessel occlusion or introduction of a hypercapnic state, the use of ultrasound is still viable. Images are recorded from the ultrasound to an external computer (Templeton et al., 2012; Dengel et al., 2011). Wall tracking software is then utilized to quantify the changes in vessel diameter (Templeton et al., 2012; Dengel et al., 2011).

Summary

While a great deal is known about hypercapnia in conjunction with hypoxia, due to its natural occurrences, little is known about the individual effects of hypercapnia on the

vascular system. The cerebral and peripheral vascular beds have differing sensitivities to changes in blood chemistry, and may respond very differently to a hypercapnic environment. In addition, little is known about the mechanisms responsible for vascular changes during hypercapnic conditions. Therefore, this dissertation will focus on the cerebral and peripheral vascular responses to hypercapnia, and will compare the peripheral response to an existing, standardized test.

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CHAPTER 3. REPRODUCIBILITY OF BLOOD OXYGEN LEVEL DEPENDENT SIGNAL CHANGES WITH END-TIDAL CARBON DIOXIDE ALTERATIONS

Reproducibility of Blood Oxygen Level Dependent Signal Changes with End-Tidal Carbon Dioxide Alterations

Justin R. Geijer[a], Bryon A. Mueller[b], Joseph A. Fisher[cd], Kelvin O. Lim[b], Donald R. Dengel[ef]

[a]Department of Health, Exercise and Rehabilitative Sciences, Winona State University, Winona, Minnesota, 55987

[b]Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota, 55455

[c]Department of Physiology, University of Toronto, Toronto, Ontario, Canada

[d]Department of Anaesthesiology, University of Toronto, and University Health Network, Toronto, ON, Canada

[e]School of Kinesiology, University of Minnesota, Minneapolis, Minnesota, 55455,

[f]Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, 55455,

Correspondence to: Justin Geijer, MS
358 Maxwell Hall
Winona State University
Winona, MN, 55987
Phone: 507-457-5205
Email: jgeijer@winona.edu

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Author Contributions

Conception and design of the experiments
- Justin R. Geijer, Bryon A. Mueller, Kelvin O. Lim, Donald R. Dengel

Collection, analysis and interpretation of data
- Justin R. Geijer, Bryon A. Mueller, Kelvin O. Lim, Donald R. Dengel

Drafting the article or revising it critically for important intellectual content
- Justin R. Geijer, Bryon A. Mueller, Joseph A. Fisher, Kelvin O. Lim, Donald R. Dengel

Summary

Hypercapnia has been utilized as a stimulus to elicit changes in cerebral blood flow (CBF); however, the results may be convoluted due to individual differences in carbon dioxide changes and varying blood oxygen levels. The introduction of a computer-controlled partial re-breathing method of independently controlling end-tidal carbon dioxide ($P_{et}CO_2$) and end-tidal oxygen ($P_{et}O_2$)(RespirAct™), has enabled examination of the effects of hypercapnia on blood oxygen level dependent (BOLD) MRI signal changes. Hypercapnic conditions trigger vasodilation of cerebral blood vessels, increasing CBF. The purpose of this study was to show the within- and between-day reproducibility of BOLD signal changes to a consistent isoxic alteration in $P_{et}CO_2$. Twelve participants (6 females) were recruited to participate in the study, with eleven (mean age 26.5±5.7 years) completing all testing protocols. Two scans were performed on the same day. The protocol was repeated on a separate day with minimum of 3 days between scans. A 3-Tesla MRI scanner was utilized for all scans. Following structural scans, $P_{et}CO_2$ was altered to stimulate changes in cerebral vascular reactivity (CVR). $P_{et}O_2$ was held constant. Mean BOLD signal changes of all participants between day 1 (0.395±0.043 %BOLD/mmHg) and day 2 (0.396±0.043 %BOLD/mmHg) displayed a percent mean difference of 0.10%±9.58% from day 1 to day 2. Within-day differences in CVR between scan 1 (0.397±0.044 %BOLD/mmHg) and scan 2 of day 1 (0.392±0.040 %BOLD/mmHg) were 0.45% (0.45%±7.44%). The results of our study are novel in that the reproducibility of BOLD signal changes in response to a consistent hypercapnic stimulus has not previously been observed. Establishment of reproducible methodologies for measuring BOLD signal changes while altering $P_{et}CO_2$ may allow future research to compare case and control populations.

Introduction

Hypercapnia has been commonly utilized to elicit an increase in cerebral blood flow (CBF) (Kety and Schmidt, 1949; Ellwein et al., 2012; Federau et al., 2012; Gauthier et al., 2012; Huang et al., 2012; Scouten et al., 2008). Magnetic resonance imaging (MRI) has been utilized to investigate cerebral blood flow (CBF) changes, as well as changes in cerebral vascular reactivity (CVR). CVR refers to changes in CBF in response to vasodilatory stimuli (Ogawa et al., 1993). Previous studies have used Blood-Oxygen-Level-Dependent (BOLD) signal changes as a surrogate measure of CBF changes (Kassner et al., 2010; Spano et al., 2012; Winter et al., 2010). Resting state functional magnetic resonance imaging (R-fMRI) BOLD signal changes correlate linearly with responses related to hypercapnic tasks (i.e., breath hold), which concurrently alter the partial pressure of oxygen (Kannurpatti et al., 2012; Kannurpatti and Biswal, 2008; Scouten et al., 2008). Studies utilizing this method have not addressed the relationship between isoxic hypercapnia (measured in the arterial blood or end-tidal) and BOLD signal changes (Kannurpatti et al., 2012; Kannurpatti and Biswal, 2008; Scouten et al., 2008).

The arterial blood partial pressure of carbon dioxide (P_aCO_2) is measured through invasive means, such as arterial blood draws (Walker et al., 1990). The partial pressure of end-tidal CO_2 ($P_{et}CO_2$) can be utilized as a surrogate, non-invasive method of determining P_aCO_2, provided that participants are free from severe chest or brain trauma (Brogan et al., 2003; Cinar et al., 2012). Sequential rebreathing has been utilized to generate a $P_{et}CO_2$ that produces a mean difference of CO_2 volumes between the arterial and end-tidal measures of only -0.1 mmHg (±1.6 mmHg) (Ito et al., 2008). Sequential rebreathing also highly correlates with P_aCO_2 (r=0.99)(Willie et al., 2012). A number of methods have been used to increase $P_{et}CO_2$ in an attempt to create a hypercapnic environment, including breath holding techniques (Kastrup et al., 1998; Kastrup et al., 1999; Li et al., 1999; Stillman et al., 1995) and adding CO_2 to inspired air (Arend et al., 1994; Chung et al., 1999; Hosking et al., 2004; Sicard and Duong, 2005). These

hypercapnia-inducing methods are also associated with increases in P_aO_2 (Baddeley et al., 2000), which lead to convoluted results regarding the effects of $P_{et}CO_2$ changes on the cerebral vasculature (Venkataraman et al., 2008). Not only are the results convoluted (due to concurrent changes in P_aO_2), but also difficult to reproduce due to individual variation in resting $P_{et}CO_2$ levels, and individual ventilatory responses to changes in $P_{et}CO_2$ (Mark et al., 2011; Tancredi & Hoge, 2013).

Recently, the development of a computer-controlled sequential rebreathing device, capable of independently controlling $P_{et}CO_2$ and $P_{et}O_2$ (RespirAct™, Thornhill Research, Inc., Toronto, Canada) has enabled researchers to isolate the effects of hypercapnia and hypoxia on CVR and BOLD signal changes (Kassner et al., 2010; Mandell et al., 2008; Mark et al., 2011; Prisman et al., 2008). The RespirAct™ has been shown to produce improved BOLD signal calibration, as well as more stable and faster CVR changes when compared with inhalation of a fixed fractional concentration of CO_2 (Mark et al., 2010; Mark et al., 2011). The precise targeting ability of the RespirAct™ allows excellent reproducibility of the signal over time and between people, whereas all other methodologies are affected by the participants' individual responses to the stimuli.

The purpose of this study was to examine the reproducibility of BOLD signal changes normalized for $P_{et}CO_2$ changes under isoxic conditions. Within subject repeatability of BOLD signal changes indicate that a consistent hypercapnic, isoxic stimulus results in a consistent measure of CVR. The ability to reproduce BOLD signal changes in participants within and between days will allow for studies observing cerebral vascular changes within an individual longitudinally and between groups of people.

Methods

Subjects:

Twelve participants (6 females) (Table 1) were recruited and consented into the study in compliance with all guidelines set by the Institutional review board of the University of

Minnesota using flyers and word of mouth at the University of Minnesota. All were free of neurological disorders and drug or alcohol dependence. Participants were required to abstain from caffeine consumption 12 hours prior to participation. Participants were brought into the Laboratory of Integrative Human Physiology at the University of Minnesota for a familiarization session. One participant was unable to tolerate the increase in $P_{et}CO_2$ administered by the RespirAct as part of the protocol and withdrew from the study. The remaining eleven participants (mean age 26.5±5.7 years) completed two separate testing sessions. Each session consisted of structural scans and two separate scans utilizing the RespirAct™ to alter $P_{et}CO_2$ values.

RespirAct™:

A mask (Thornhill Research, Inc. Toronto, Canada) was fitted to the participant's face using skin tape (Tegaderm, 3M, St Paul USA) to prevent air leaks, and connected to a sequential gas delivery (SGD) breathing circuit (Slessarev et al. 2007). The RespirAct™ was utilized to manipulate and analyze $P_{et}CO_2$ and end-tidal oxygen volumes ($P_{et}O_2$) values. Baseline $P_{et}CO_2$ and $P_{et}O_2$ values were established during a seated resting state, and corrected if necessary when participants were placed into the MR scanner in a supine position.

The RespirAct™ protocol was used to target $P_{et}CO_2$ values. Participants were instructed to synchronize their breathing rate to a metronome set at 12 breaths per minute. $P_{et}O_2$ values were targeted to 100 mmHg throughout the protocol. Subject specific baseline $P_{et}CO_2$ values were initially maintained for a period of three minutes, and were then increased, over 1-2 breaths to 10 mmHg from baseline for a period of three minutes (Figure 1). $P_{et}CO_2$ values were then returned to baseline for a period of four minutes before another 10 mmHg step increase for four minutes and then returned to baseline for a period of four minutes. This was followed by a ramp protocol where the $P_{et}CO_2$ was brought to 30 mmHg and held there for 40 seconds before being linearly ramped to 51

mmHg over a period of approximately six minutes and held at 51 mmHg for 1 minute. The ramp protocol targeted a small increase in $P_{et}CO_2$ every few seconds to create a slow, linear increase in $PetCO_2$. Upon completion of the ramp, the $P_{et}CO_2$ was rapidly returned to baseline. The protocol was introduced to participants during the initial visit, which took place in the Laboratory of Integrative Human Physiology at the University of Minnesota.

All imaging was performed at the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota. Once screened for MR safety, participants were refitted with the breathing circuit and were placed in the Seimens (Erlangen, Germany) 3-Tesla Skyra Connectome MRI scanner (http://www.humanconnectome.org/about/project/MR-hardware.html). A 32 channel receive only matrix coil was used during acquisition; head motion was minimized using a foam head cushion and padding placed snugly around the subject's head. Communication with participants was via typed messages that appeared on a visual screen. Typed messages were used to provide feedback to the subjects when they were not breathing at the correct rate or to urge the subject to maintain the minimal minute ventilation needed to sustain rebreathing.

The MR protocol included short localizer scans to allow for scan prescription and AC-PC determination, a high resolution T_1 weighted scan used for tissue segmentation (TR=2530ms, TE=3.52ms, TI=1100ms, flip angle=7 degrees, voxel=1mm isotropic, grappa=2, 4 minutes), a field map scan used to correct the BOLD data for susceptibility induced geometric distortions (TR=300ms, TE=2.79ms and 5.25ms, flip angle=50 degrees, voxel=3.4x3.4x4mm, 39 seconds), and two long BOLD EPI scans (TR=2000ms, TE=30ms, flip angle = 77 degrees, voxel=3.4x3.4x4mm, 840 volumes, 28 minutes per scan) used to compute CVR.

Image processing was performed using tools from the FSL toolkit (BET, FLIRT, FEAT, FAST, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) in combination with custom tools developed in MATLAB (Natick, Massachusetts, U.S.A.). Processing of the high resolution T1 weighted imaging consisted of brain extraction using BET and a 3-compartment tissue segmentation, using FAST. Preprocessing of the BOLD imaging was conducted within FEAT, and included brain extraction, correction for magnetic field induced geometric distortion (using the separately acquired field map) and slice timing correction. No temporal or spatial smoothing was applied to the data. The extracted brain from the T1 scan was aligned to the distortion corrected mean BOLD image using FLIRT. The gray matter partial volume estimate (PVE) map from the T1 segmentation was aligned to the BOLD data using the same transform, and a gray matter mask was created in BOLD space by thresholding the aligned PVE map at a 50% partial volume estimate. The gray matter BOLD signal, which is the mean voxel intensity within the gray matter mask per time point, was then computed.

Mean cerebral vascular response (CVR) in gray matter was then computed from the measured $P_{et}CO_2$ and gray matter BOLD time courses. First, a linear detrend was applied to both the BOLD and $P_{et}CO_2$ waveforms to remove the effects of scanner drift from the BOLD time course. Next, the $P_{et}CO_2$ waveform was recalculated from the measured values at each point of exhale (approximately every 5 seconds) to represent a sample every 2 seconds using spline interpolation within MATLAB. To correct for the timing error between the measured BOLD and $P_{et}CO_2$ wave forms caused by delays in the pulmonary-cerebral response and the delay in the measurement of the end tidal gas content the $P_{et}CO_2$ wave form was temporally shifted to the point of maximum correlation with the BOLD signal. The CVR (% BOLD signal change/mmHg CO_2) was then computed in MATLAB using the robust linear least squares fit to the correlation between the two time courses.

To determine within day reproducibility, participants underwent two consecutive CVR scans on each testing day. The second scan was started approximately ten minutes after the conclusion of the first scan to allow the participants' $PetCO_2$ to return to initial baseline levels. The testing protocol was then repeated on each participant on a second day, with a minimum of three days between tests.

Statistical Analysis

Statistical analyses were performed to determine within-day and between-day reproducibility. Variation was determined by computing the percent change of BOLD signal responses to $P_{et}CO_2$ changes between trials. Correlations and paired t-tests were utilized to examine order effect. Bonferroni adjustments were made for multiple tests, and significance level was set at $p<0.05$.

Results

Between-Day Results

Mean CVR was determined by averaging between within-day scans across all participants. An excellent agreement of mean CVR values was observed between days, 0.395±0.043 %BOLD signal change/mmHg CO_2 for day 1 and 0.396±0.043 %BOLD signal change/mmHg for day 2. Bland-Altman analysis found a 0.1% change in mean CVR between days and a 9.6% standard deviation (Figure 2). Paired t-test showed no significant difference in mean CVR results between day 1 and day 2 ($p = 0.991$, CI (-0.0184, 0.0213)). CVR results between days were moderately but significantly correlated ($r = 0.708$, $p = 0.015$) (Figure 3).

Within-Day Results

Average CVR for scan 1 of day 1 was 0.397±0.044 %BOLD signal change/mmHg CO_2 and for scan 2 of day 1 was 0.392±0.040 %BOLD signal change/mmHg CO_2 (Figure

4). A Bland-Altman analysis revealed a 0.5% change in mean CVR with a 7.4% standard deviation (Figure 2). Paired t-tests showed no significant difference between scans ($p = 0.932$, CI (-0.02013, 0.01861)). Scan 1 and scan 2 of day 1 were moderately, yet significantly correlated ($r = 0.777$, $p = 0.005$) (Figure 4).

Conclusions and Discussion

This study investigated the reproducibility of a method for measuring average cerebral vascular response in gray matter using BOLD fMRI and a controlled, isoxic hypercapnic stimulus. The main finding from this study is that Bland-Altman 95% confidence interval for within- and between-day standard deviations in CVR were 15% and 20%, respectively. Knowledge of the within and between day measurement uncertainly is critical for determining the necessary sample and effect sizes for future studies. The average percent change of the response between and within day were 0.10% and 0.45%, indicating there is an insignificant bias in CVR measurement on the same day after 10 minutes or after several days. Finally, CVR was moderately correlated within subjects both within ($r=0.708$) and between ($r=0.777$) days, indicating that individuals have a reproducible CVR response and that there is significant variation within even healthy individuals to observe subject variability.

BOLD fMRI allows for the investigation of blood flow and oxygen metabolism in brain structure and various disease states (Leontiev & Buxton, 2007). However, until recently, hypercapnic tests were unable to isolate the BOLD signal response to CO_2 alteration alone due to the inability to disambiguate the effects of uncontrolled P_aO_2 changes that often accompany breath hold and fixed CO_2 concentration inhalation methods (Baddeley et al., 2000; Rhoades, 2003; Venkataraman et al., 2008). The fact that individuals have significant variation in ventilatory responses to changing CO_2 levels

provides an additional barrier to identifying the BOLD signal response to a hypercapnic stimulus (Baddeley et al., 2000; Rhoades, 2003; Venkataraman et al., 2008). The advent of the RespirAct^{TM} allows for the reproducible, isolated manipulation of end-tidal PCO_2 while end-tidal PO_2 is held constant. The precisely controlled, isoxic, hypercapnic stimulus allows BOLD signal changes to be unambiguously related to CVR changes induced by $P_{et}CO_2$ alterations, and that the CVR measurement is reproducible both within-day and between days.

While the ability to control end-tidal gas volumes creates reproducible BOLD signal changes, there are limitations to the methodology. Analysis requires alignment of the timing between the $P_{et}CO_2$ waveform and the BOLD signal to determine the change in signal per unit of CO_2. We aligned the waveforms based upon the maximal correlation between the BOLD signal and the $P_{et}CO_2$ change. This is a reasonable approach to account for delays in gas sampling, but ignores the potential for delay or the physiological response. Further research is required to determine if a time lapse exists between $P_{et}CO_2$ changes and the BOLD signal response.

The results of our study are novel in that the reproducibility of BOLD signal changes in response to hypercapnia has not previously been observed under such controlled conditions. The ability to isolate and manipulate $P_{et}CO_2$ while controlling $P_{et}O_2$ sets the methodology apart from breath holds or fixed CO_2 concentration breathing. Establishment of reproducible methodologies for measuring BOLD signal changes while altering $P_{et}CO_2$ may allow future research to compare case and control populations. The comparisons of populations can assist researchers in creating correlations between BOLD signal changes and a myriad of conditions that may cause alterations in CVR.

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Figure 1.

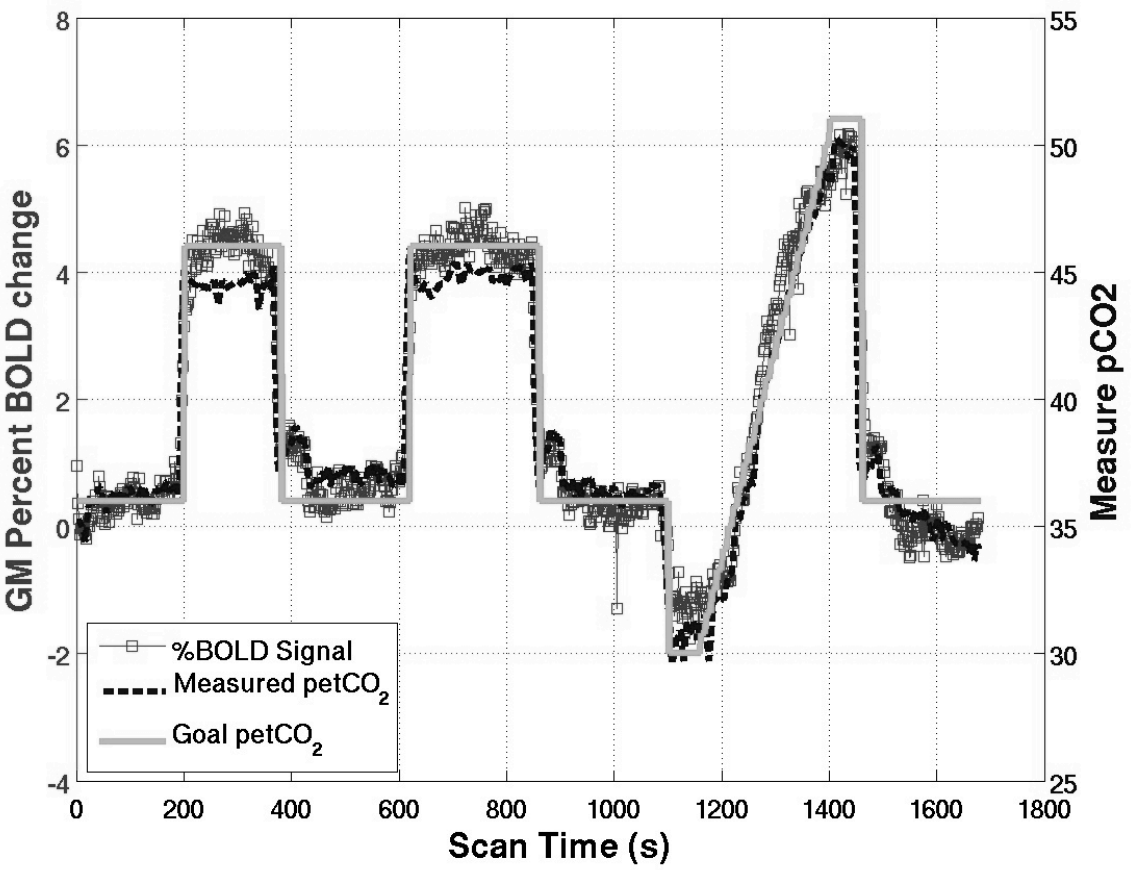
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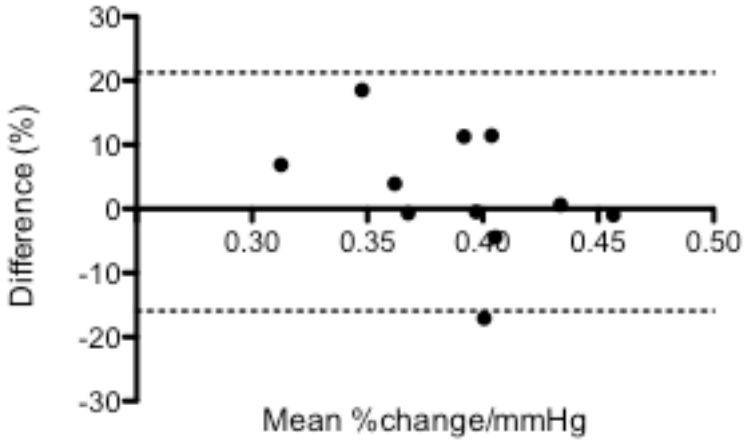
Figure 2.

Panel A.



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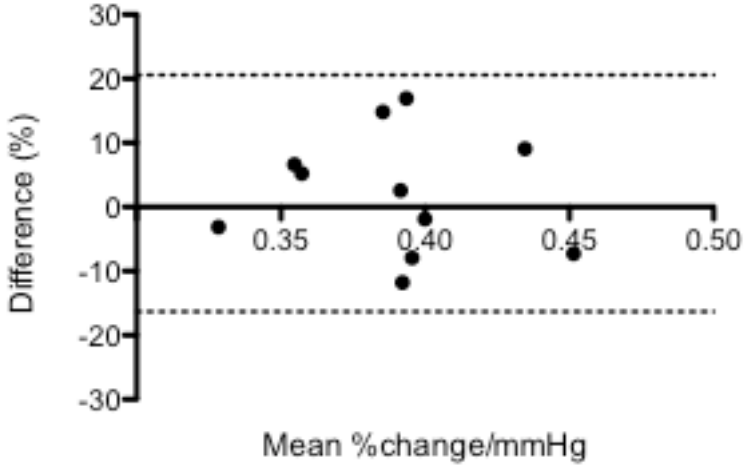

Figure 3.

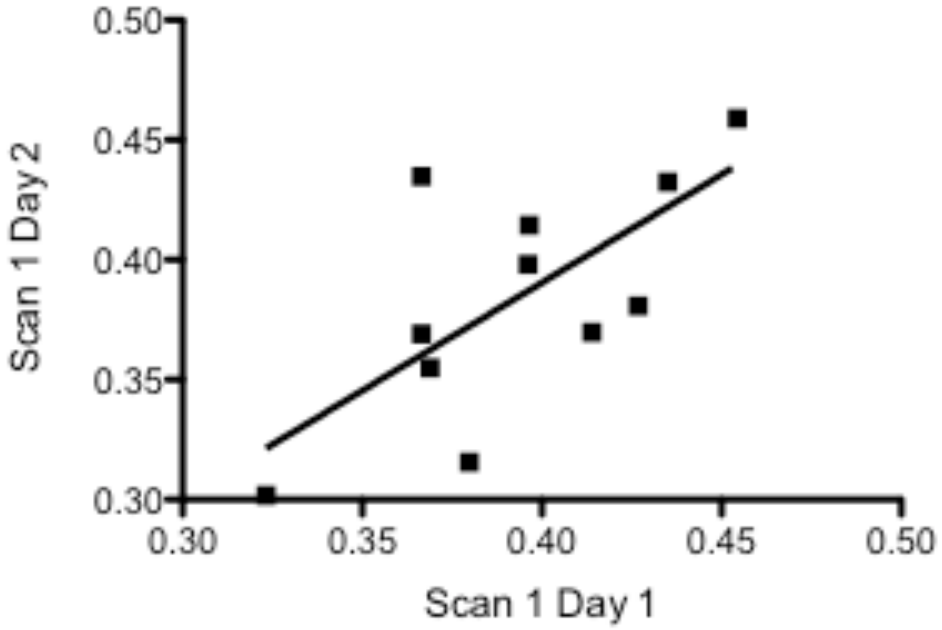

Figure 4.

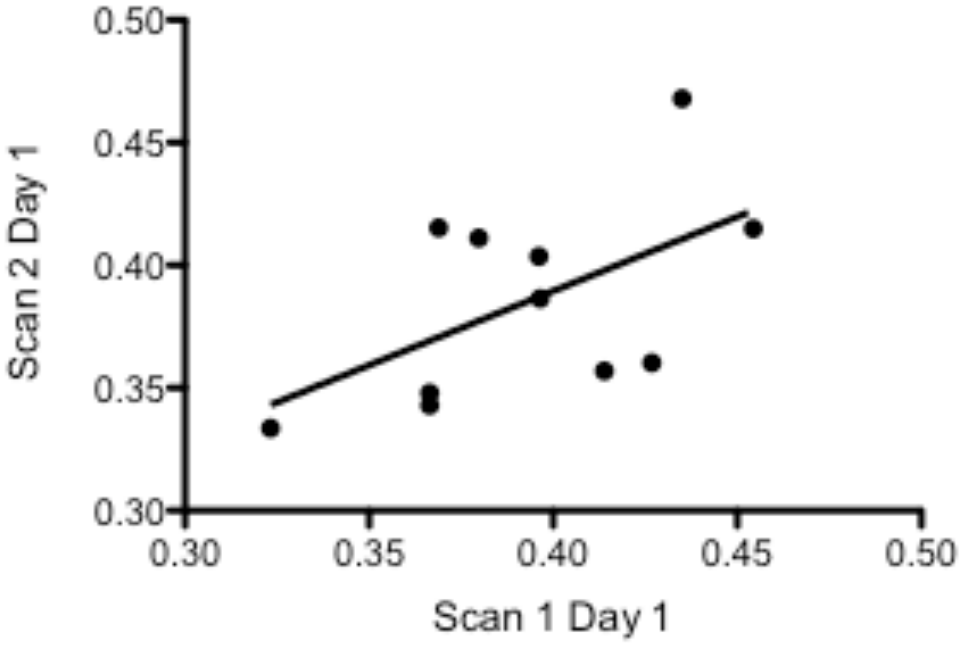

Table 1. Mean (± standard error) Descriptive Variables

Variable	Males (n=5)	Females (n=6)	*p-value*
Age (years)	30.4 ± 5.8	23.2 ± 3.1	0.047*
Height (cm)	180.3 ± 5.7	168.9 ± 7.5	0.018*
Weight (kg)	83.2 ± 5.2	65.2 ± 2.4	>0.001*
Body Mass Index (kg/m^2)	25.6 ± 2.0	22.9 ± 2.2	0.064*

Figure Legend

Figure 1. Simultaneous depiction of targeted $P_{et}CO_2$ values, measured $P_{et}CO_2$ values, and percent BOLD signal change/mmHg CO_2 in the gray matter.

Figure 2. Panel A displays a Bland-Altman plot of the between day measures of CVR. The y-axis designates the % difference between repeated measurements, while the x-axis designates the average CVR values.

Panel B displays a Bland-Altman plot of the within day measures of CVR.

Figure 3. Between-day correlations of %BOLD signal change/mmHg CO_2. ($r=0.708$, $p=0.015$)

Figure 4. Within-day correlations of %BOLD signal change/mmHg CO_2. ($r=0.777$, $p=0.005$)

CHAPTER 4. REPRODUCIBILITY OF PERIPHERAL VASCULAR CHANGES WITH ALTERATIONS IN END-TIDAL CARBON DIOXIDE

Reproducibility of Peripheral Vascular Changes with Alterations in End-Tidal Carbon Dioxide

Justin R. Geijer[a], Aaron S. Kelly[b,c], Michael A. Chernin[d], Matthew G. Stoltman[d], Donald R. Dengel[bd]

[a]Department of Health and Exercise Rehabilitation Sciences, Winona State University, Winona, Minnesota, 55987

[b]Department of Pediatrics, University of Minnesota, and University of Minnesota Masonic Children's Hospital, Minneapolis, Minnesota, 55455,

[c]Department of Medicine, University of Minnesota, Minneapolis, Minnesota, 55455

[d]School of Kinesiology, University of Minnesota, Minneapolis, Minnesota, 55455,

Correspondence to:

Donald R. Dengel, Ph.D.
University of Minnesota
1900 University Avenue S.E.
110 Cooke Hall
Minneapolis, MN 55455
Phone Number: (612) 626-9701
Fax Number: (612) 625-8867
E-mail Address: denge001@umn.edu

Summary

The purpose of this study was to examine the reproducibility of the peripheral vascular response to hypercapnia. Healthy college-aged males (n=7) and females (n=10) underwent an iso-oxic 10-mmHg increase in $P_{et}CO_2$ for 12 minutes. Brachial artery diameter changes were measured using ultrasound imaging. Two tests were completed on day one with 15 minutes of rest between tests. Tests were repeated on day two. Paired t-tests, Bland-Altman plots, and intraclass correlations (ICC) determined reproducibility. No significant differences existed in peak dilation within day (5.33±3.73% vs. 4.52±2.49%, p=0.378). ICC within day was excellent (0.851, p<0.001). Within day time-to-peak dilation was not significantly different (660.0±231.8 sec vs. 602.7±259.9 sec, p=0.379) and the ICC was fair (0.416, p=0.113). Between days peak dilation was not significantly different (4.91±3.70% vs. 5.08±5.49%, p=0.923) and ICC was excellent (0.881, p<0.001). Hypercapnia-induced brachial artery dilation is similar within- and between-days. ICC for peak dilation suggests the methodology is reproducible.

Keywords: Hypercapnia, Ultrasound, Vasodilation

Introduction

Previously, researchers (Ainslie et al., 2005; Blair et al., 1960; Kontos et al., 1972; Vantanajal et al., 2007) have reported that alterations in arterial carbon dioxide volumes (P_aCO_2) via inhalation of varying concentrations of carbon dioxide (CO_2) result in vasodilation of the peripheral vasculature in humans. These studies, however, failed to control for concurrent changes in arterial oxygen volumes (P_aO_2), which have also been shown to elicit peripheral vasodilatory effects similar to those produced during hypercapnia (Simmons et al., 2007). To accurately describe the independent effects of P_aCO_2 on the peripheral vasculature, one needs to maintain P_aO_2.

One way to manipulate P_aCO_2 while maintaining P_aO_2 is to utilize end-tidal forcing, which is a method that independently controls end-tidal carbon dioxide ($P_{et}CO_2$) and end-tidal oxygen ($P_{et}O_2$) (Ainslie et al., 2005; Essfeld et al., 1990; Vantanajal et al., 2007). Ainslie et al. used end-tidal forcing to examine the peripheral blood flow response to hypercapnia, and reported no significant femoral blood flow changes in response to a hypercapnic, euoxic state (2005). Soto et al. (2012) used Transcranial Doppler (TCD) and ultrasound to compare blood flow velocity of the middle cerebral artery (MCA) to peripheral measures in the external carotid artery, internal carotid artery, and the vertebral artery. Soto et al. (2012) identified significantly lower blood flow responses to hypercapnia in the peripheral arteries, than in the MCA. Other studies have also used the end-tidal forcing method to alter end-tidal gases while examining cerebral vascular reactivity with magnetic resonance imaging (MRI) (Kassner et al., 2010; Mandell et al., 2008; Mark et al., 2011; Mutch et al., 2012; Prisman et al., 2008). The end-tidal forcing

method allows for concurrent control of $P_{et}CO_2$ and $P_{et}O_2$, which enables researchers to isolate the effects of end-tidal gas manipulation on the vasculature (Mandell et al., 2008; Prisman et al., 2008). End-tidal forcing has also been used in conjunction with ultrasound to examine peripheral vascular changes of the carotid arteries and femoral artery during a hypercapnic state (Ainslie et al., 2005; Soto et al., 2012). Despite this previous work, to date, the reproducibility of end-tidal forcing to examine manipulate various peripheral arterial beds has yet to be determined.

Methods

Subjects:

Thirty participants were recruited to participate in the study at the University of Minnesota. Five participants were unable to perform the breathing protocol (the participants felt uncomfortably short of breath) and withdrew from the study. The remaining 25 participants (mean age 22.9 ± 3.0 years) were free of neurological disorders and drug or alcohol dependence. Of the 25 participants, 17 completed all phases of testing. Movement artifact due to changes in respiration during end-tidal forcing resulted in poor image capture during ultrasound imaging. Due to poor image quality these scans were not used. Participants were required to abstain from caffeine consumption and exercise 12 hours prior to participation. Participants were also required to fast for six hours prior to participation to eliminate the potential effects of diet on the vasculature.

Gas Manipulation and Vascular Imaging

All testing was performed in the Laboratory of Integrative Human Physiology at the University of Minnesota. Subjects were tested in a quiet, climate-controlled room (22-23°C). Measurements of height and weight were obtained with a standard digital scale and stadiometer (Detecto, Webb City, MO, USA). Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters-squared (m^2). Participants were placed in the supine position with their left arm extended and supported. A specially designed re-breathing mask was fitted, taped to the participant's face to prevent air leaks, and connected to a sequential gas delivery breathing circuit (Thornhill Research, Inc. Toronto, Canada). A computer controlled end-tidal forcing device (RespirAct™, Thornhill Research, Inc. Toronto, Canada) was utilized to manipulate $P_{et}CO_2$ and $P_{et}O_2$ values. Baseline $P_{et}CO_2$ and $P_{et}O_2$ values were obtained with the participant resting in the supine position prior to the study.

Vascular images were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions) with an 8-15 MHz linear array probe held in place by a stereotactic device. This system is interfaced with a computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. All arterial images were triggered and captured at the end-diastolic diameter. End-diastolic diameter was determined using the R-wave from the ECG, then digitized and stored on a personal computer for later offline analysis using electronic wall-tracking software (Vascular Research Tools 5, Medical Imaging Application; LLC). All image files were averaged over a 20 second period, and peak dilation during each study was

defined as the greatest percentage change from resting baseline brachial artery diameter. The same trained reader performed digital image analysis.

An audio-based metronome (Thornhill Research, Inc. Toronto, CA) was utilized to help participants maintain the targeted breathing rate of 12 breaths per minute. $P_{et}O_2$ values were targeted to resting values throughout the protocol to ensure an iso-oxic environment was maintained. Baseline $P_{et}CO_2$ values were initially maintained for a period of two minutes, and were then increased 10 mmHg from baseline for a period of 12 minutes (Figure 1). Following the period of elevated $P_{et}CO_2$ values, $P_{et}CO_2$ was returned to baseline for a period of one minute. Ultrasound imaging of the brachial artery was initiated at the start of the gas control sequence. Following the initial end-tidal forcing protocol (test 1) the subjects rested in the supine position for 15 minutes, while breathing room air. The end-tidal forcing protocol was then duplicated (test 2). On a separate date the entire end-tidal forcing protocol was repeated. The two end-tidal forcing testing sessions was separated by a minimum of 24 hours.

Statistical Analysis

Graphpad Prism (version 6, Graphpad Software, Inc., San Diego, CA, USA) was used for all statistical analyses. Demographic data are presented as mean ± standard deviation (SD). Paired t-tests were used to compare characteristics of male and female participants. Paired t-tests were also used to compare the mean change in $P_{et}CO_2$ from baseline value to the targeted increase of 10 mmHg. Changes in $P_{et}CO_2$ were compared within days (test 1 vs. test 2) as well as between days (day 1 vs. day 2). Targeted $P_{et}O_2$

values were also compared within days and between days using independent t-tests to determine if an iso-oxic environment was maintained.

Baseline brachial artery diameter, peak brachial artery dilation, time-to-peak artery dilation, and heart rate at rest and peak dilation were all compared between test 1 and test 2 of day 1 (within day), as well as between test 1 of day 1 and test 1 of day 2 (between day) (Figure 2). Alpha level for all statistical comparisons was set to 0.05. Bland-Altman plots and intraclass correlations (ICC) were used to determine repeatability of peripheral vascular and time-to-peak measures.

Brachial artery dilation measures were averaged over 20-second intervals. Repeated measures analysis of variance was used to determine peak dilation had been achieved. Peak dilation was determined when no significant differences existed between 20-second averages of brachial artery diameter.

Results

Subject characteristics are displayed in Table 1. As expected, males (n = 7) were significantly taller (178.5±6.5 cm vs. 164.5±8.3 cm, $p<0.0001$) and heavier (85.2±17.4 kg vs. 66.4±12.4 kg, $p=0.010$) than females (n = 10). Age (24.0±3.4 vs. 22.2±2.5, $p=0.171$) and BMI (26.7±4.8 vs. 24.6±4.7, $p=0.294$) were not significantly different between males and females.

Within Day Repeatability

Within day comparisons of baseline $P_{et}CO_2$ values were not significantly different (37.2±2.5 mmHg $P_{et}CO_2$ vs. 37.3±3.3 mmHg $P_{et}CO_2$, p=0.564). Partial re-breathing produced mean within day increases in $P_{et}CO_2$ that were not significantly different (9.7±1.9 mmHg $P_{et}CO_2$ vs. 9.8±2.3 mmHg $P_{et}CO_2$, p=0.860). Comparison of $P_{et}O_2$ values within day revealed that an iso-oxic environment was maintained (103.1±10.6 mmHg $P_{et}O_2$ vs. 104.3±9.2 mmHg $P_{et}O_2$, p=0.701).

Bland-Altman plots revealed a percent mean difference in peak dilation of 16% (16%±160%)(Figure 2) between within day measurements. The mean baseline brachial artery diameter was not significantly (p=0.142) different between test 1 and test 2 measurements (5.51±1.91 mm vs. 5.29±1.99 mm)(Table 2), and was significantly (p<0.0001) correlated (r=0.938). The time-to-peak brachial artery dilation did not significantly (p=0.379) vary within day between test 1 and test 2 (660.0±231.7 sec vs. 602.7±259.9 sec). ICC for within day time-to-peak dilation was in the fair range (0.416, p=0.113). There was no significant (p=0.37) difference in peak brachial artery dilation between test 1 and test 2 measurements within day 1 (5.33±3.73% vs. 4.52±2.49%). ICC between test 1 and test 2 peak brachial artery dilation measures (0.851) indicates that within day measurements are repeatable (p<0.001)(Table 2).

Resting heart rate (HR) was not significantly (p=0.105) different between test 1 and test 2 (68±14 bpm vs. 65±12 bpm,). Additionally, the HR at peak brachial artery dilation was not significantly (p=0.119) different between test 1 (75±13 bpm) and test 2 (74±13 bpm). HR increased significantly from rest to peak dilation on day 1 during both

test 1 (68±14 bpm to 76±14 bpm, p=0.0003) and test 2 (65±12 bpm to 74±13 bpm, p=0.0001).

Between Day Repeatability

Comparison of $P_{et}O_2$ values between day 1 and day 2 revealed that an iso-oxic environment was maintained (102.9±8.3 mmHg $P_{et}O_2$ vs. 103.7±9.4 mmHg $P_{et}O_2$, p=0.811). Within day comparisons of baseline $P_{et}CO_2$ values were not significantly different (37.9±2.3 mmHg $P_{et}CO_2$ vs. 37.1±3.0 mmHg $P_{et}CO_2$, p=0.688). Partial re-breathing produced mean within day increases in $P_{et}CO_2$ that were not significantly different (9.6±2.1 mmHg $P_{et}CO_2$ vs. 9.7±2.0 mmHg $P_{et}CO_2$, p=0.883).

Baseline diameter measures were not significantly different between days (4.84±1.51 mm vs. 5.38±1.62 mm, p=0.133)(Table 3). Bland-Altman plots revealed a mean difference in peak dilation measures of 32% (32%±203%) between days (Figure 2). Peak dilation measures were not significantly (p=0.923) different between days (4.91±3.70% vs. 5.08±5.49%). ICC for peak dilation measures between days was excellent (0.881, p<0.001). Similar to peak brachial artery dilation, time-to-peak dilation measures were also not significantly (p=0.729) different between days (623.5±248.9 sec vs. 597.7±258.9 sec). However, ICC of time-to-peak dilation between days was in the poor range (0.202, p=0.314)(Table 3).

Resting HR between days was significantly (p=0.041) different (68±14 bpm vs. 63±11 bpm,). While resting HR differed between days, the difference between resting HR and HR during peak dilation was not significantly different (8±1 vs. 10±1, p=0.219).

Heart rate at peak brachial artery dilation was not significantly ($p=0.09$) difference between days (76±14 bpm vs. 73±16 bpm).

Discussion

While researchers have previously shown that the end-tidal forcing method is capable of producing reproducible alterations in end-tidal gas volumes (Mark et al., 2011), the reproducibility of the peripheral vascular effects of hypercapnia (10 mmHg above resting values) have not been examined. In the present study, we observed that the peripheral vascular dilatory response to an iso-oxic, hypercapnic environment produced reproducible results in terms of dilatory measures, though the time course of the dilatory response was not well correlated. To our knowledge, this is the first study to examine the reproducibility of alterations in peripheral vascular response due to an iso-oxic, hypercapnic environment.

To date, most research involving hypercapnia and its effects on blood vessel function have focused on cerebral arteries and the determination of cerebral vascular reactivity (CVR) as measured with MRI (Kassner et al., 2010; Mandell et al., 2008; Mark et al., 2011; Mutch et al., 2012; Prisman et al., 2008). These studies have demonstrated a positive correlation between CVR and $P_{et}CO_2$ levels. While plenty of research (Kassner et al., 2010; Mandell et al., 2008; Mark et al., 2011; Mutch et al., 2012; Prisman et al., 2008) has involved the correlation between the cerebral blood flow changes and end-tidal carbon dioxide levels, there is only limited research examining peripheral blood flow changes in response to hypercapnia.

Palazzo et al. (Palazzo et al., 2012) and Vantanajal et al. (Vantajal et al., 2007) examined the effect of hypercapnia on vascular beds, and reported a lack of correlation between peripheral and cerebral beds in response to hypercapnia. Palazzo et al. (2012) used ultrasound imaging during flow-mediated dilation (FMD) to observe brachial artery diameter and flow changes, while also using Transcranial Doppler (TCD) to observe cerebral vascular reactivity (CVR) in response to alterations in end-tidal carbon dioxide levels. While TCD provides useful information regarding blood flow alterations, it does not identify vessel diameter changes in response to a stimulus. Therefore the authors (Palazzo et al., 2012) were only able to compare blood flow changes induced by two different stimuli (reactive hyperemia and hypercapnia) of the varying vascular beds. The current study observed diameter changes of the brachial artery in response to a steady hypercapnic, iso-oxic state.

Breath hold was used to alter end-tidal carbon dioxide levels during two separate tests. CVR and FMD results were not well correlated, which may be due to the ineffectiveness of breath hold to create a steady state in end-tidal or arterial gas measures. While Palazzo et al. (2012) used two separate methods to alter $P_{et}CO_2$ in their population, both fail to control for alterations in $P_{et}O_2$, which may impact the dilatory response of the vasculature. Additionally, Palazzo et al. (2012) used a 7% CO_2 gas mixture to alter end-tidal CO_2 volumes, which does not guarantee end-tidal CO_2 to achieve a steady state. The current study was able to maintain steady state for both end-tidal CO_2 and end-tidal O_2 volumes.

Vantanajal et al. (2007) also examined MCA flow with TCD and brachial artery flow via ultrasound imaging during a hypercapnic state. However, Vantanajal et al. (2007) used the more reproducible partial re-breathing method to induce the hypercapnic state. While the authors reported statistical comparisons of baseline end-tidal gas data, they did not analyze the reproducibility of the gas data during the interventions (Vantanajal et al., 2007). Once again, cerebral and peripheral responses to a hypercapnic state were not well correlated, as the cerebral response was much faster and of a higher magnitude. The results from these studies would suggest that there are differences in the sensitivity and/or response to hypercapnic stimuli in the cerebral and peripheral vascular beds.

In conclusion, the results of this study demonstrate that the dilatory response of the brachial artery to a hypercapnic state is repeatable, though the time course of the response was not repeatable. The variability in the time course data may be due to a learning effect, as participants became more comfortable with the sensations associated with a hypercapnic state. The significantly lower resting HR between day 1 and day 2 supports this theory, and is indicative of a lower sympathetic response at the onset of testing.

While Vantanajal et al. (2007) observed peripheral vascular changes in response to a hypercapnic state, the study did not focus on the reproducibility of the vascular measures. Additionally, neither Vantanajal et al. (2007) nor Palazzo et al. (2012) ensured $P_{et}CO_2$ was maintained at steady state, which is critical when attempting to isolate the true vascular response to a hypercapnic state. Though hypercapnia is a naturally

occurring physiological state, it is generally accompanied by hypoxic conditions (Venkataraman et al., 2008). The convolution associated with concurrent changes in carbon dioxide and oxygen volumes offer unclear results to researchers investigating the effects of arterial gas changes (Brogan et al., 2003; Cinar et al., 2012).

Based upon the results of the current study, the use of hypercapnia to create a dilatory response in the brachial artery yields reproducible dilatory results. Given the relatively slow (approximately 600 seconds) time-to-peak dilation and the complexity of the protocol and testing apparatus when compared to other methodologies, such as flow-mediated dilation, the clinical application of such a test is rather impractical. However, the longer response time may indicate that different mechanisms are triggering the vasomotor response to a hypercapnic state.

Acknowledgements

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This work was reviewed and approved by the University of Minnesota Institutional Review Board.

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Table 1. Mean (± standard deviation) participant characteristics

	All Participants (n=25)	Males (n=10)	Females (n=15)	P-value
Age (yrs)	22.9±2.9	24.0±3.4	22.2±2.5	0.171
Height (cm)	170.1±10.6	178.5±6.5	164.5±8.3	0.0001*
Weight (kg)	73.9±18.1	85.2±17.4	66.4±12.4	0.010*
BMI (kg/m^2)	25.4±4.7	26.7±4.8	24.6±4.7	0.294

* - Indicates significant difference ($p<0.05$) between genders
BMI – body mass index

Table 2. Within Day Statistics

	Test 1 Mean (±SD)	Test 2 Mean (±SD)	T-Test	Intraclass Correlation
Baseline Diameter (mm)	5.51±1.91	5.29±1.99	0.142	
Peak Dilation (%)	5.33±3.73	4.52±2.49	0.370	0.851*
Time-to-Peak (sec)	660.0±231.7	602.7±259.9	0.379	0.416

* - Indicates statistical significance ($p<0.05$)

Table 3. Between Day Statistics

	Day 1 Mean (±SD)	Day 2 Mean (±SD)	T-Test	Intraclass Correlation
Baseline Diameter (mm)	4.84±1.51	5.38±1.62	0.133	
Peak Dilation (%)	4.91±3.70	5.08±5.49	0.923	0.881*
Time-to-Peak (sec)	623.5±248.9	597.7±258.9	0.729	0.202

* - Indicates statistical significance ($p<0.05$)

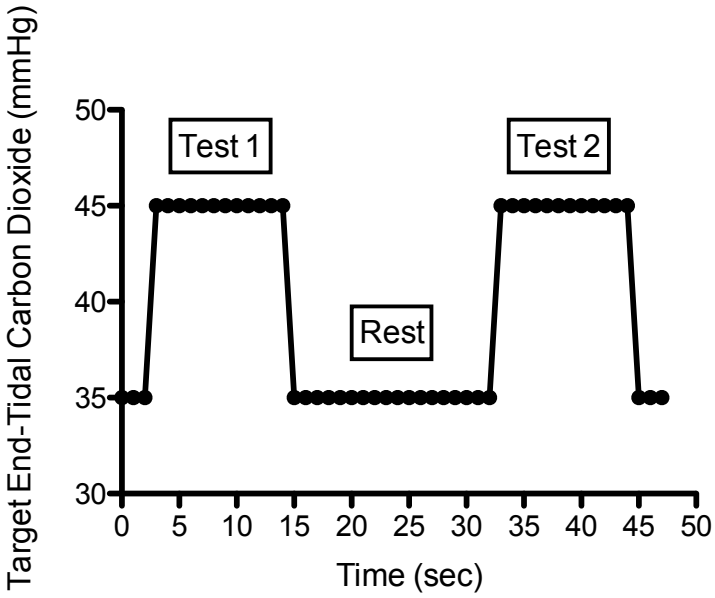
Target End-Tidal Carbon Dioxide (mmHg)
50
45
40
35
30
Test 1
Test 2
Rest
0
5
10
15
20
25
30
35
40
45
50
Time (sec)

Figure 1.

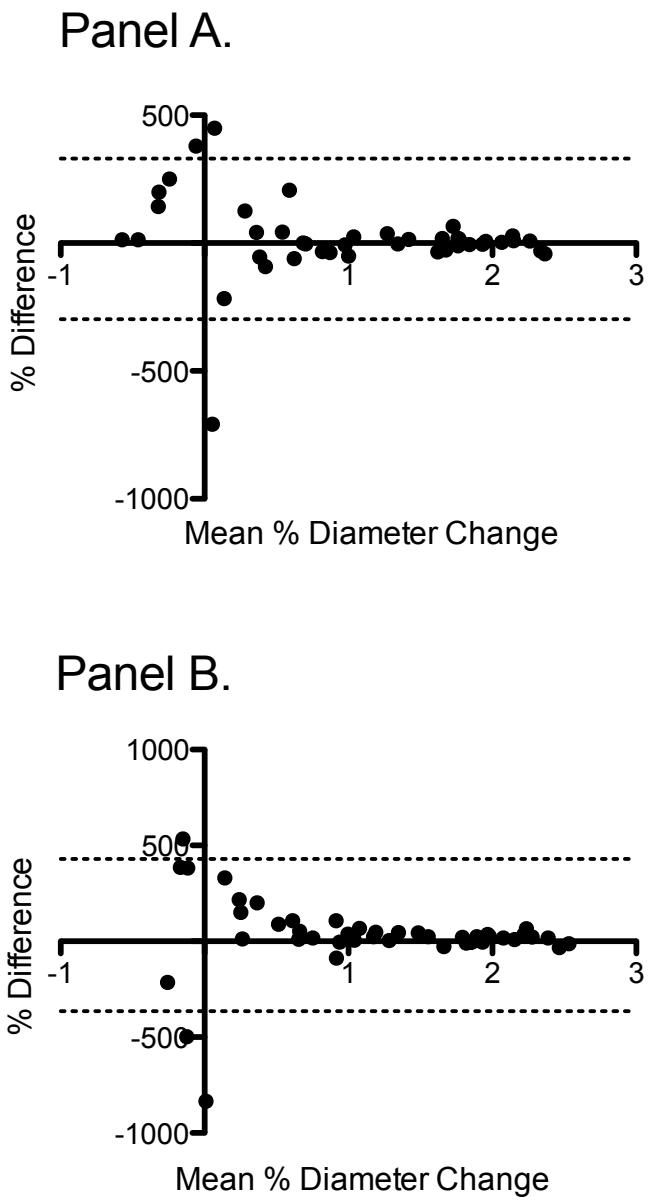
Panel A.
500
-500
-1000
% Difference
-1
1
2
3
Mean % Diameter Change
Panel B.
1000
500
-500
-1000
% Difference
-1
1
2
3
Mean % Diameter Change

Figure 2.

Figure Legend

Figure 1. Time course of targeted end-tidal carbon dioxide volumes for test 1 and test 2

Figure 2. Bland-Altman plots of the percent dilatory responses to carbon dioxide administration both within day (Panel A.) and between days (Panel B.)

CHAPTER 5. COMPARISON OF BRACHIAL DILATORY RESPONSES TO HYPERCAPNIA AND REACTIVE HYPEREMIA

Comparison of Brachial Dilatory Responses to Hypercapnia and Reactive Hyperemia

Justin R. Geijer[a], Aaron S. Kelly[b], Michael A. Chernin[c], Matthew G. Stoltman[c], Donald R. Dengel[c]

[a]Department of Health, Exercise, and Rehabilitation Sciences, Winona State University, Winona, Minnesota, 55987

[b]Departments of Pediatrics and Medicine, University of Minnesota Medical School, and University of Minnesota Masonic Children's Hospital, Minneapolis, MN 55455

[c]School of Kinesiology, University of Minnesota, Minneapolis, Minnesota, 55455,

Correspondence to:

Donald R. Dengel, Ph.D.
University of Minnesota
1900 University Avenue S.E.
110 Cooke Hall
Minneapolis, MN 55455
Phone Number: (612) 626-9701
Fax Number: (612) 625-8867
E-mail Address:denge001@umn.edu

Word count: 1573

Summary

Purpose. Flow-mediated dilation (FMD) relies on reactive hyperemia to stimulate the endothelium to release nitric oxide, causing smooth muscle relaxation. Hypercapnia also produces vasodilation, which is thought to be nitric oxide-independent. The purpose of this study was to compare and contrast the effects of hypercapnia and reactive hyperemia as stimuli for brachial artery dilation.

Methods and Results. On separate days, twenty-five participants underwent vasodilation studies via reactive hyperemia or hypercapnia (i.e., 10 mmHg increase in end-tidal carbon dioxide [$P_{et}CO_2$)]). During both studies changes in brachial artery diameter were recorded using continuous ultrasound imaging. Heart rate (HR) was measured throughout both tests. Baseline HR (63±11 beats/min vs. 68±14 beats/min, p=0.0027) and brachial artery diameter (4.57±1.51 mm vs. 5.28±1.86 mm, p =0.022) were significantly different between FMD and hypercapnia, respectively. HR (65±11 beats/min vs. 76±14 beats/min, $p < 0.0001$), peak vessel dilation (8.68±4.50% vs. 5.28±1.86%, p=0.002), and time to peak dilation (90.8±120.1 sec vs. 658.3±226.6sec, $p<0.0001$) were all significantly different between FMD and hypercapnia, respectively.

Conclusions. The dynamics by which reactive hyperemia and hypercapnia stimulate vasodilation appear to differ. Hypercapnia produces a smaller and slower vasodilatory effect than FMD. Further research is necessary to better understand the mechanisms of vasodilation under hypercapnic conditions.

Key words: Carbon Dioxide, Flow Mediated Dilation, Vascular, Peripheral

Introduction

Flow-mediated dilation (FMD) is a non-invasive measure of the vasodilatory effect of reactive hyperemia (Welsch et al., 2002). Endogenous vasodilators, such as nitric oxide (NO), are widely believed to be responsible for the vasodilatory response during reactive hyperemia (Gurevicius et al., 1995). Hypercapnia also relies on endogenous vasodilators to create a dilatory response (Fabricius et al., 1996; Gurevicius et al., 1995; Komjati et al., 2001; Toda et al., 1996). However, Fabricius et al. (1996) reported that hypercapnic-induced increases in cerebral blood flow occurred in the absence of nitric oxide synthase (NOS). The findings of Fabricius et al. (1996) indicate that other endogenous vasodilators besides NO may be responsible for the dilatory effect of hypercapnia.

The exact mechanism(s) of vasodilation during hypercapnic exposure is largely unknown, particularly due to the fact that it is difficult to examine the independent effects of hypercapnia on the vasculature due to the concurrent changes in oxygen levels that often occur during hypercapnia (Blair et al., 1960; Kontos et al., 1972; Wingo et al., 2008). Recently, a computer-controlled gas blender capable of manipulating and clamping end-tidal gas volumes has been developed (RespirAct™, Thornhill Research, Toronto, Ontario). Researchers have previously utilized computer-controlled gas blending to manipulate end-tidal gas volumes to alter cerebral blood flow during magnetic resonance imaging (Kassner et al., 2010., Mandell et al., 2008; Mark et al., 2011; Mutch et al., 2012; Prisman et al., 2008) as well as Transcranial Doppler imaging (Battisti-Charbonnay et al., 2011).

Since hypercapnia-related vasodilation may occur independent of NO, the brachial artery may exhibit different dilatory dynamics (i.e., peak arterial dilation, time to peak dilation, etc.) compared to that observed in response to reactive hyperemia. The purpose of this study was to examine the effects of hypercapnia vs. reactive hyperemia on the dilatory dynamics of the brachial artery.

Methods

Subjects:

Thirty participants were recruited to participate in the study using flyers and word of mouth at the University of Minnesota. Of the 30 participants recruited, five were unable to perform the breathing protocol at the prescreening visit and withdrew from the study. The remaining participants (mean age 22.9 ± 2.9 years) were free of neurological disorders and drug or alcohol dependence. The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board and all participants gave written informed consent. The procedures followed in this study were in accordance with institutional review board and HIPAA guidelines.

Flow Mediated Dilation

Caffeine consumption and exercise were prohibited 12 hours prior to participation. All participants were first weighed and had their height measured using a digital scale and stadiometer (Detecto PD300MHR, Webb City, MO, USA). Participants were then asked to lie in a supine position with their left arm extended and supported. A pressurized cuff

(D.E. Hokanson, Inc., Bellevue, WA) was fitted to the left forearm, approximately five centimeters (cm) distal to the antecubital space. Electrocardiogram (ECG) electrodes were placed to monitor participant heart rate, as well as to trigger the wall-tracking software used in conjunction with the ultrasound (Vascular Research Tools 5, Medical Imaging Application; LLC). The cuff was then inflated to approximately 220 mmHg for a period of five minutes. Twenty seconds prior to the release of the cuff, an ultrasound recording of the brachial artery was initiated. Images were recorded for a total of 320 seconds.

Vascular images were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions) with an 8-15 MHz linear array probe held in place by a stereotactic device. This system is interfaced with a standard personal computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. All arterial images were gated and captured at the end-diastolic diameter. End-diastolic diameter was determined using the R-wave from the ECG, then digitized and stored on a personal computer for later offline analysis using electronic wall-tracking software. All image files were averaged over a 20 second period, and peak dilation during each study was defined as the greatest percentage change from resting baseline brachial artery diameter. The same trained reader performed digital image analysis.

RespirAct™

A re-breathing mask (Thornhill Research, Inc. Toronto, Canada) was fitted and taped to the participant's face to prevent air leaks, and connected to a sequential gas delivery

breathing circuit. A computer controlled gas delivery device (RespirAct™, Thornhill Research, Inc. Toronto, Canada) was utilized to provide constant measurement of $P_{et}CO_2$ and $P_{et}O_2$ values allowing for manipulation of these measures during the actual study. Baseline $P_{et}CO_2$ and $P_{et}O_2$ values were established during a seated resting state, and corrected if necessary during a supine resting position.

An audio-based metronome (Thornhill Research, Toronto, CA) was utilized to help participants maintain the targeted breathing rate of 12 breaths per minute. $P_{et}O_2$ values were targeted to resting values throughout the protocol. Baseline $P_{et}CO_2$ values were initially maintained for a period of two minutes, and were then increased 10 mmHg from baseline for a period of 12 minutes (Figure 1). Following the period of elevated $P_{et}CO_2$ values, $P_{et}CO_2$ was returned to baseline for a period of one minute.

Statistical Analysis

All results are expressed as mean ± SEM. Paired t-tests and Pearson's correlations were utilized to compare the two methodologies. Comparisons were made for baseline brachial artery diameter, resting heart rate peak brachial artery dilation, time to peak dilation, and heart rate during peak brachial artery dilation. Peak brachial artery dilation was corrected for baseline diameter. Due to differences between baseline diameters of the two tests, we examined the change from baseline between the two test conditions. Alpha levels of 0.05 were utilized for determination of statistical significance. Statistical analyses were performed with GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

Physical characteristics of the participants can be found in Table 1. Mean baseline brachial artery diameter was significantly different between reactive hyperemia and hypercapnia (4.57±1.51 vs. 5.28±1.86 mm, p=0.022); however, baseline brachial artery diameter during the two conditions was significantly correlated (r=0.581, p=0.001) with each other. Mean heart rate at the initiation of reactive hyperemia and hypercapnia was significantly different (62±11 vs. 68±14 beats/min, p=0.0027).

The absolute change in brachial artery diameter following reactive hyperemia (0.40±0.29 mm) was significantly (p=0.012) greater than the change in brachial artery diameter following hypercapnia (0.26±0.19 mm). The significant absolute changes are reflected in peak vessel dilation measures, which were significantly different between reactive hyperemia and hypercapnia (8.68±4.50% vs. 5.28±1.86%, p=0.002). Additionally, peak dilation between the two trials was not significantly correlated (r=0.279, p=0.093). The peak heart rate was significantly lower during reactive hyperemia compared to hypercapnia (65±11 vs. 75±13 beats/min Hg, p<0.0001). The change in heart rate from baseline to peak brachial artery dilation was significantly lower during reactive hyperemia compared to hypercapnia (3±1 vs. 7±1 beats/min). Heart rate changes were not significantly correlated (r=0.169, p=0.430). The time to peak dilation was significantly shorter in reactive hyperemia than hypercapnia (90.8±120.1 sec vs. 658.3±226.6 sec, p<0.0001), and the time to peak dilation during these two conditions was not significantly correlated (r=-0.106, p=0.311). A sample of the dilatory response to reactive hyperemia and hypercapnia can be seen in Figure 2.

Discussion

The results of this study demonstrate that hypercapnia induces a brachial artery dilation response of smaller magnitude than reactive hyperemia and that the time course of response by the brachial artery to this stimuli is different. The brachial dilatory response to hypercapnia was relatively slow compared to the rapid response of the brachial artery responding to reactive hyperemia. This slower response may indicate that other vasodilators, such as prostaglandins and endothelium-derived hyperpolarizing factor (Wang et al., 2003; Dautzenberg & Just, 2013), may be involved in hypercapnia-induced brachial artery dilation, which may possibly explain the blunted dilatory response to hypercapnia. These other vasodilators have a much slower effect on dilating the artery when compared to NO, which is the primary vasodilator during reactive hyperemia (Dautzenberg & Just, 2013).

Resting and peak heart rates were both significantly higher during reactive hypercapnia, but this can likely be attributed to the sympathetic nervous system response to increased CO_2 in the inhaled gas. Even though participants were previously acclimatized to the mask and the breathing conditions and rested in the supine position for 20 minutes prior to testing, the elevated $P_{et}CO_2$ may have induced anxiety in some of the participants. In addition, hypercapnia stimulates a physiologic increase in heart rate to increase cardiac output (Gurevicius et al., 1995; Kontos et al., 1972). The higher HR during peak dilation during hypercapnia is indicative of increased sympathetic nervous activation, which may be involved to some extent in the brachial dilatory response

(Gurevicius et al., 1995; Kontos et al., 1972). The conduit vessels of the periphery accommodate the increased cardiac output during sympathetic activation by inducing smooth muscle relaxation (Fabricius et al., 1996; Kontos et al., 1972), which could explain the larger resting diameter during hypercapnia.

The results of this study demonstrate that the dynamics of peripheral artery dilation induced by reactive hyperemia compared to hypercapnia are different. This difference in response may be due in part to the possible differences in the primary vasodilating factors associated with these stimuli. Further research is needed to better understand the mechanisms responsible for the dilatory response to hypercapnia. Measurement of individual metabolites known to trigger vasodilation, including NO, prostaglandins, and other endogenous vasodilators, during hypercapnia can provide greater insight into the mechanisms responsible for hypercapnia-induced vasodilation and aid in determining whether this technique may be useful as a measure of arterial health.

Acknowledgements

This work was supported in part by a University of Minnesota Grant-in-Aid (D.R.D.)

The authors have no conflicts of interest.

References

Table 1. Mean (± standard deviation) participant characteristics

	All Participants (n=25)	Males (n=10)	Females (n=15)	P-value
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BMI (kg/m^2)	25.4±4.7	26.7±4.8	24.6±4.7	0.294

BMI – body mass index

Figure 1.

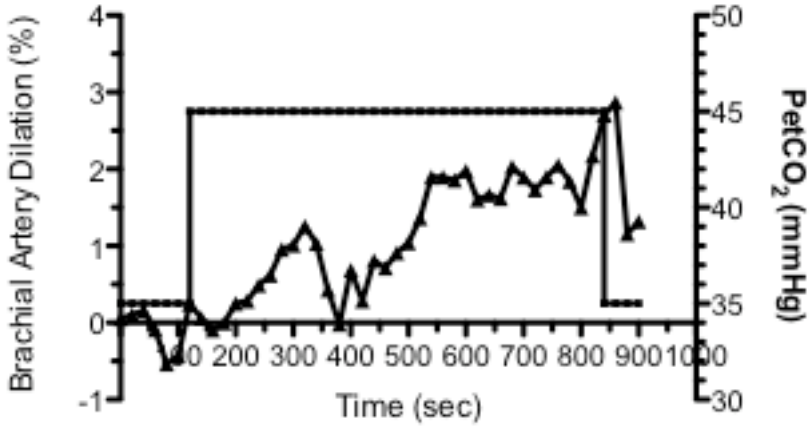
Brachial Artery Dilation (%)
4
3
2
1
0
-1
0 200 300 400 500 600 700 800 900 1000
Time (sec)
50
45
40
35
30
PetCO2 (mmHg)

Figure 2.

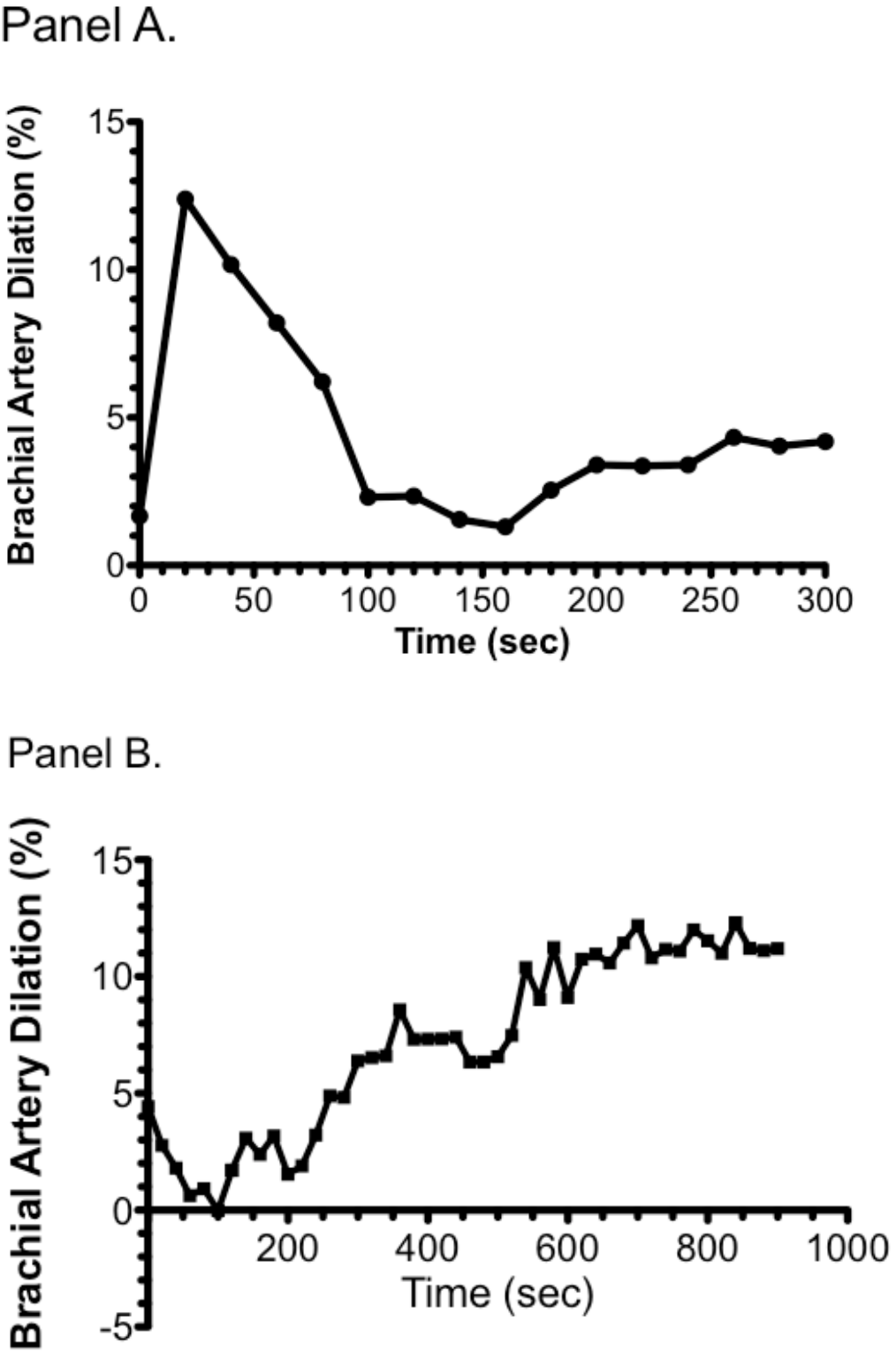
Panel A.
Brachial Artery Dilation (%)
15
10
5
0
0
50
100
150
200
250
300
Time (sec)
Panel B.
Brachial Artery Dilation (%)
15
10
5
0
-5
200
400
600
800
1000
Time (sec)

Figure 3.

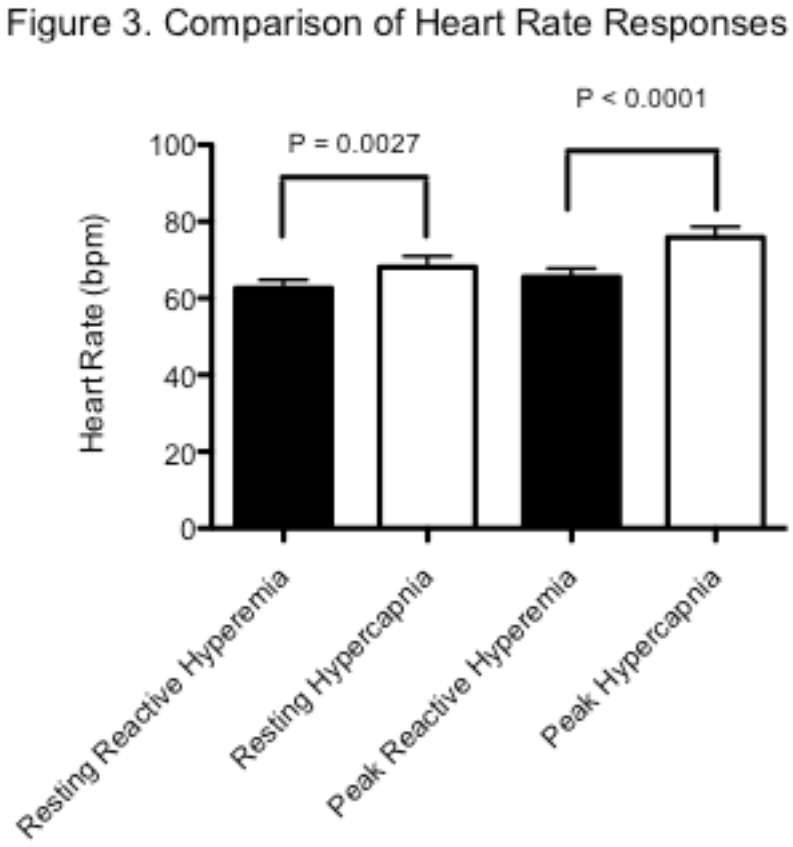
Figure 3. Comparison of Heart Rate Responses
P < 0.0001
P = 0.0027
100
80
60
40
20
0
Heart Rate (bpm)
Resting Reactive Hyperemia
Resting Hypercapnia
Peak Reactive Hyperemia
Peak Hypercapnia

Figure 4.

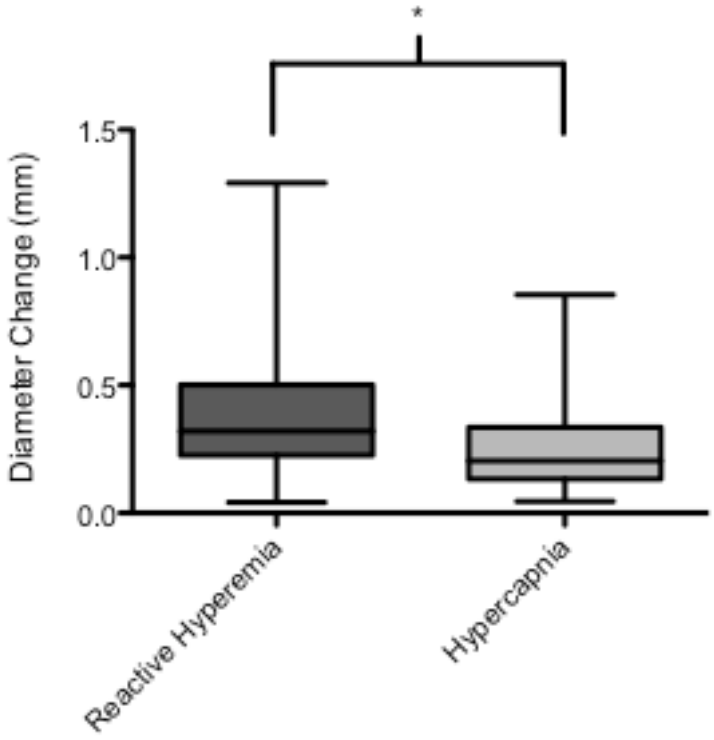
*
1.5
1.0
0.5
0.0
Diameter Change (mm)
Reactive Hyperemia
Hypercapnia

Figure Legend.

Figure 1. Targeted $P_{et}CO_2$ values (■) and a sample brachial artery dilatory response (●)

Figure 2. Sample brachial artery vasodilatory response to reactive hyperemia (Panel A) and hypercapnia (Panel B).

Figure 3. Resting and peak heart rate responses to reactive hyperemia and hypercapnia

Figure 4. Comparison of peak brachial artery diameter normalized for baseline diameter. Reactive hyperemia created a significantly greater dilatory response (0.404±0.294 mm) than did hypercapnia (0.262±0.187 mm)(*p=0.012).

CHAPTER 6. CONCLUSION

Overall Research Implications

Hypercapnia and the associated acidosis have been identified as a catalyst for cell signaling (Oliver et al., 2012). However, the difficulties in isolating hypercapnia from hypoxia limit the ability to draw conclusions on hypercapnia's effects. The advent of the RespirAct™ allows researchers to isolate the effects of hypercapnia on the physiological systems of the body. In addition, repeatable methodologies utilizing this technology in conjunction with MRI will allow for a greater understanding of the mechanisms responsible for cerebral vascular changes.

While using hypercapnia to measures peripheral vascular function did not prove to be repeatable, the differences between cerebral and peripheral vascular responses provide further evidence to support the difference in chemical sensitivity of the cerebral vessels. The peripheral vascular beds are responsive to hypercapnic conditions, but further research is needed to identify the exact mechanisms responsible for the dilatory response. In addition, methodological alterations are necessary for establishment of repeatable measurements of the peripheral vascular response to hypercapnia.

Summary of Chapter Three

Chapter three described the cerebral vascular response to controlled hypercapnia. The cerebral vascular reactivity changes were closely tied to alterations in end-tidal carbon dioxide, whether steady state or dynamic changes in volume. Additionally, the changes observed were of a repeatable nature. The repeatability of the results provides a methodology that can be utilized for future research.

The analysis utilized in this study requires alignment of the timing between the $P_{et}CO_2$ waveform and the BOLD signal to determine the change in signal per unit of CO_2. We aligned the waveforms based upon the maximal correlation between the BOLD signal and the $P_{et}CO_2$ change. This is a reasonable approach to account for delays in gas sampling, but ignores the potential for delay or the physiological response, making the analysis a limitation of the study. Further research is required to determine if a time lapse exists between $P_{et}CO_2$ changes and the BOLD signal response.

Summary of Chapter Four

Chapter four examined the peripheral response to a hypercapnic environment. The vasodilatory response to hypercapnia was of a gradual nature, taking several minutes to reach peak dilation. It is feasible that the gradual nature of the dilatory response provides ample time for other, less potent dilatory factors (i.e., prostaglandins) to act upon the vessel. The relatively small dilatory response (compared to flow-mediated dilation or nitroglycerin induced dilation) may be indicative of these less potent factors.

A potential limitation of this study is the time elapsed between tests (15 minutes). During analysis of brachial artery vasodilation from occlusion-induced reactive hyperemia, 15 minutes is an accepted time between repeated tests (Pyke et al., 2011), and was the duration utilized during this study. However, the baseline diameters between within-day tests were not significantly different, indicating that the vessel had returned to baseline diameter prior to the initiation of the second test.

While the dilatory response to hypercapnia was repeatable, the time to peak dilation of the vessel was not, raising questions of how to improve the methodology. A possible learning effect may have impacted the repeatability of the time course, as resting heart rate response was lower during the second day of testing. Longer exposure to hypercapnia and a better understanding of the mechanisms responsible for the dilatory response may lead to more repeatable time course results. However, the dilatory response itself is, indeed, repeatable.

Summary of Chapter Five

The comparison between existing methods for measuring peripheral vascular health (reactive hyperemia) and hypercapnia were made in chapter five. Reactive hyperemia yielded a much more abrupt dilation of the vessel. Hypercapnia produced a blunted response in comparison, and required almost five times the duration to reach peak. The mechanisms responsible for dilation of the brachial artery during these stimuli appear to differ, causing the difference in peak dilation and time to peak dilation.

Measurement of changes in blood pH levels could potentially provide greater insight into the vascular impact of hypercapnia. Previous studies investigating hypercapnia in animal subjects identified blood pH as a factor capable of impacting the arterial dilatory response. Blood measures were not investigated in this study, and stands as a limitation.

Clinical Significance of Presented Research

The research presented in this dissertation establishes a repeatable method for examining cerebral vascular reactivity in response to a controlled hypercapnic

environment. This establishment allows for comparisons to be made both between populations, and within an individual between time periods. Comparisons such as these allow researchers to investigate the cerebral vascular effects of various maladies, as well as investigate the effectiveness of cerebral vascular procedures.

The noticeable difference in the time to peak vasodilatory responses between the cerebral and peripheral vascular beds indicate that there may be a difference in CO_2 sensitivity between the beds. This information may be useful in investigation of populations that are consistently exposed to hypercapnic conditions (cystic fibrosis and COPD). The long-term effects of these maladies would seemingly affect the cerebral vascular beds more drastically and/or sooner than the peripheral beds.

The mechanisms stimulating vasodilation during reactive hyperemia and hypercapnia seem to differ. While further research is necessary to deduce what these differences are, these differences may lead to a greater understanding of peripheral endothelial and smooth muscle function.

Suggestions for Future Research

The repeatable cerebral responses to hypercapnia observed in this study allows for comparisons of healthy and case populations. Other case populations that may be of interest are those suffering from Alzheimer's disease and Parkinson's syndrome, due to the overwhelming impact of those diseases on brain function.

Further research is also needed to better understand the mechanisms responsible for the peripheral dilatory response to hypercapnia. Measurement of individual metabolites

known to trigger vasodilation, including NO, prostaglandins, and other endothelial-derived relaxing factors, during hypercapnia can provide greater insight into the anatomy and mechanisms responsible for hypercapnia-induced vasodilation.

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