PHYSIOLOGY OF EXTREME BREATH-HOLDING

By
Anthony R Bain
MSc, University of Ottawa, 2011

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The undersigned certify that they have read, and recommend to the College of Graduate Studies for acceptance, a thesis entitled:

Physiology of Extreme Breath-holding

submitted by Anthony Bain in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

Philip Ainslie. Health and Social Development. UBCO

Supervisor, Professor (please print name and faculty/school above the line)

Supervisory Committee Member, Professor (please print name and faculty/school in the line above)

Bill Milsom. Science / Zoology. UBC

Supervisory Committee Member, Professor (please print name and faculty/school in the line above)

University Examiner, Professor (please print name and faculty/school in the line above)

Kevin Shoemaker. Heath Sciences. University of Western Ontario

External Examiner, Professor (please print name and university in the line above)

July 5th/ 2016

(Date Submitted to Grad Studies)

Additional Committee Members include:

Neil Eves. Health and Social Development. UBCO

(please print name and faculty/school in the line above)

(please print name and faculty/school in the line above)
Abstract

The practice of competitive breath-hold (apnea) diving has provided a gateway for studying the physiologic limits of severe hypoxemia and hypercapnia beyond otherwise possible in healthy humans. In elite apnea competitors, the broad objectives of this Thesis were to, a) quantify the impact of peripheral and central chemoreception, and lung volume on the elite dry-land apnea breakpoint, and b) examine the consequences of prolonged apnea on the cerebral metabolic functioning. These objectives were achieved in four experimental studies. Study 1 explored the impact of peripheral chemoreflex silencing with low-dose dopamine. Here, compared to placebo, dopamine blunted the ventilatory response to hypercapnic-hypoxia by ~27%; however, maximal apnea duration was only increased by ~5%. At the breakpoint, arterial hypoxemia was identical with dopamine compared to placebo, indicating that the apnea termination may largely be determined by a threshold level of hypoxemia to maintain consciousness. To eliminate the influence of hypoxia, Study 2 assessed the main determinants of an apnea breakpoint following hyperoxic pre-breathing. Here, the apnea duration was related to the individual forced vital capacity, and unrelated to the central chemoreflex. Respiratory muscle fatigue and pending atelectasis likely determined the capacity of a maximal hyperoxic apnea. Study 3 quantified the cerebral metabolism during apnea. The cerebral metabolic rate of oxygen, measured from the product of cerebral blood flow and the radial artery-jugular venous oxygen content difference, was reduced by ~29% at the termination of apnea. However, there was no change in the cerebral non-oxidative metabolism, calculated from the ratio of oxygen and carbohydrate metabolism. Study 4 examined the cerebral metabolic response in three apneas eliciting separate levels of hypoxemia and hypercapnia. Apneas generating the most severe hypercapnia, irrespective of hypoxia, elicited the largest reduction in the cerebral metabolic rate of oxygen. Moreover, apneas generating the most severe hypoxia, irrespective of hypercapnia, caused a cerebral net release of lactate, suggesting astrocyte glycogenolysis. Together, the findings of this thesis provide new insight into the determinants of an extreme apnea breaking point, and the observations of hypercapnic-induced reduction in oxidative cerebral metabolism provides a tenable mechanism for cerebral protection against prolonged apnea.
Preface

The University of British Columbia Clinical Research Ethics Board approved all experimental chapters (4 through to 7) - ID: H14-00922

Some aspects of Chapter 2 have been published: Bain et al., (2015) Cerebral vascular control and metabolism in heat stress. Compr Physiol, 5(3): 1345-80. Co-authors Lars Nybo, and Philip Ainslie helped with revisions and drafting sections (not included in this Thesis) of the manuscript. Anthony Bain drafted the majority of the manuscript, was responsible for all figures / tables, interpretations, and is contact author.

The study presented in Chapter 4 has been published: Bain et al., (2015) Peripheral chemoreflex inhibition with low-dose dopamine: New insight into mechanisms of extreme apnea. Am J Physiol Regul Integr Comp Physiol, 309(9): 1162-71. Co-authors Zeljko Dujic, Ryan Hoiland, Otto Barak, Dennis Madden, Ivan Drvis, Mike Stembridge, David MacLeod, Douglas MacLeod and Philip Ainslie helped with data collection and manuscript revisions. Philip Ainslie, Ivan Drvis and Anthony Bain conceived the study idea and design. Alongside data collection and study design, Anthony Bain performed all data analysis, drafted the manuscript, and is contact author.

The study presented in Chapter 5 “Forced vital capacity, but not central chemoreflex predicts the hyperoxic breath-hold duration in elite apnea divers” is being prepared for publication as a short-communication in an academic journal yet to be determined. Co-authors are Otto Barak, Ryan Hoiland, Ivan Drvis, Damian Bailey, Zeljko Dujic, David MacLeod, & Philip Ainslie. Philip Ainslie, Zeljko Dujic, Ivan Drvis and Anthony Bain conceived the study idea and design. Alongside data collection and study design, Anthony Bain performed all data analysis, and drafted the manuscript.

Hoiland, Otto Barak, Marija Cavar, Ivan Drvis, Mike Stembridge, Douglas MacLeod, Damian Bailey, Zeljko Dujic and David MacLeod, helped with data collection and manuscript revisions. Philip Ainslie, Zeljko Dujic, Ivan Drvis and Anthony Bain conceived the study idea and design. Alongside data collection and study design, Anthony Bain performed all data analysis, drafted the manuscript, and is contact author.

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Chapter 6 was presented at the 2015 International Hypoxia Symposium in Lake Louise. A report of all presentations from the conference is published in Kayser B., (2015), High Alt Med Biol. 16(3):261-266.

Other first author academic publications from Anthony Bain, not included in this thesis, but performed while conducting the PhD dissertation under supervision of Prof Ainslie at the University of British Columbia, include:


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holders fundamentally underscores the differences in apnea breakpoint mechanisms.
## List of Symbols and Abbreviations

a – Arterial  
ATP – Adenosine triphosphate  
BBB – Blood brain barrier  
BL – Baseline  
CA – Cerebral autoregulation  
CBF – Cerebral blood flow  
CO – Cardiac output  
CDO₂ – Cerebral delivery of oxygen  
CMRO₂ – Cerebral metabolic rate of oxygen  
CPP – Cerebral perfusion pressure  
CSF – Cerebral spinal fluid  
FVC – Forced vital capacity  
FEV₁ – Forced expired volume in the first second  
gCBF – Global cerebral blood flow  
Glu – Glucose  
Glu Ext – Glucose extraction  
HCT – Hematocrit  
Hb – Hemoglobin  
HV – Apnea performed with prior hyperventilation (Chapter 7)  
HX – Apnea performed with prior 100% oxygen pre-breathing (Chapter 7)  
IBM – Involuntary breathing movements  
ICA – Internal carotid artery  
ICP – Intracranial pressure  
Lac – Lactate  
Lac Ext – Lactate extraction  
MAP – Mean arterial pressure  
MCA – Middle cerebral artery  
Mild-HH – Mild hypercapnic hypoxia (Chapter 6)  
NTS – Nucleus tractus solitarius  
NM – Apnea performed with normal air, without prior hyperventilation (Chapter 7)  
OCI – Oxidative carbohydrate index  
OGI – Oxidative glucose index  
O₂EF – Oxygen extraction fraction (in Chapter 6 denoted as O₂ Ext)  
SO₂ – Oxygen saturation  
PCA – Posterior cerebral artery  
PCO₂ – Partial pressure of carbon dioxide  
PO₂ – Partial pressure of oxygen  
PETCO₂ – Partial pressure of end-tidal carbon dioxide  
PETO₂ – Partial pressure of end-tidal oxygen  
PT – Percent time during the apnea (Chapter 6)  
Q – Blood flow (used as a prefix, e.g. QICA)
Severe-HH – Severe hypercapnic hypoxia (Chapter 6)
SNA – Sympathetic nervous activity
SV – Stroke volume
v – Venous
VA – Vertebral artery
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Chapter 1 - Introduction

“The need for oxygen is the most insistent requirement for human life... The brevity of duration of life without oxygen leaves only a narrow margin of oxygen reserve to separate life from death.” – Irving, 1934

Exemplified by Irving (1934), breathing is critical to life; it is necessary for both metabolic and acid-base homeostasis. When breathing stops, a limited capacity for oxygen storage makes humans especially vulnerable to hypoxemic conditions that compromise neuronal functioning and consciousness. The volitional human breath-hold record of 11:35 minutes is, in turn, truly remarkable.

The simple solution to prolonging a conscious breath-hold (apnea) is to prioritize oxygen rich blood flow to the brain, and use the available oxygen as efficiently and as little as possible. The Weddell seal (Leptonychotes weddellii) is an expert at this, with recorded dive times beyond an hour. In humans, however, this simple solution for oxygen conservation holds complex, interacting and, sometimes, paradoxical mechanisms.

Due to the high metabolic demand and small energy stores of the brain, extreme hypoxemia is a major challenge for cerebral functioning. For example, acute reduction in oxygen supply to the brain results in unconsciousness within 4 to 6 seconds (Smith et al., 2011), and brain death within a few minutes. Indeed, although the human brain is a mere 2-3% of body total mass, it requires ~20% of total body oxygen consumption. When faced with hypoxia, the first line of cerebral defense is an increase in the cerebral blood flow (CBF) to maintain oxygen delivery (Hoiland et al., 2016b). During a prolonged breath-hold (>5 minutes), CBF may increase by ~80 to 100% (Willie et al., 2014a) – close to the maximum reserve (Donnelly et al., 2016). The regulation of CBF is by vasomotor, chemical, metabolic, and neurogenic mechanisms, and by factors that change the prevailing perfusion pressure, vascular resistance and surrounding pressures (Willie et al., 2014b; Donnelly et al., 2016). During apnea, all of these interacting CBF regulatory
systems are initiated. For example, increases in arterial CO₂, while increasing the CBF, may reduce the oxidative metabolic rate, thereby decreasing the demand for oxygen delivery / blood flow. Meanwhile, profound hypertension and hypoxia increase the CBF (via perfusion pressure and pial artery dilation, respectively), while sympathoexcitation may increase the vascular resistance, thus reduce the CBF.

Pathological impairments in the regulation of arterial oxygen and carbon dioxide pressures, for example with acute respiratory disorders, are often fatal. The practice of commercial (e.g. pearl diving) and competitive apnea diving has provided a gateway to experimentally study the physiologic limits of severe hypoxemia (decreased arterial oxygen tension) and hypercapnia (increased arterial carbon dioxide tension) tolerance, otherwise unfeasible in healthy human volunteers. In studying the physiology of extreme volitional apnea, information on integrative reflex control mechanisms (e.g. respiratory, circulatory and metabolic) and oxygen conservation is gleaned. The ultimate reaching goal of this Thesis is to better understand the fundamental human physiology associated with apnea and extreme alterations in arterial blood gases. Understanding these complex and non-linear integrative processes associated with extreme apnea has potential to help describe unique aspects of brain survival in related pathological situations.

1.1 Objectives

The broad objectives of this Thesis are to, a) quantify the impact of peripheral and central chemoreception, and lung volume on the elite dry-land (out of water) apnea breakpoint (i.e. the re-initiation of breathing), and b) quantify the consequences of extreme apnea on the cerebral metabolic functioning. These objectives are met through four distinct experimental studies. The first two studies focus on partitioning the impact and contribution of the peripheral (carotid body) and central (medulla) chemoreception and lung function on the elite apnea breaking point. The latter two studies focus on quantifying and describing the cause and consequences of the related changes in cerebral metabolism during a prolonged apnea.
1.2 Specific aims and hypotheses

1.2.1 Study 1 (Chapter 4)

Aim: To determine if the maximal breath-hold duration in elite apnea divers is altered by the administration of low dose (2 µg/kg/min) dopamine that blunts the carotid body sensitivity to hypoxia and hypercapnia.

Hypotheses: 1) low dose dopamine infusion in elite apnea divers will decrease the ventilatory response to hypoxia, and in turn delay the onset of involuntary breathing movements during a maximal breath-hold; however, apnea duration would be unaltered; and 2) the termination of apnea in the placebo and dopamine condition would occur at identical arterial oxygen tensions, suggesting that it is a hypoxemic threshold that determines the breath-hold break point in elite apnea divers, rather than a chemoreflex drive to breathe.

1.2.2 Study 2 (Chapter 5)

Aim: To determine whether the elite maximal hyperoxic breath-hold duration is related to the individual hyperoxic-hypercapnic ventilatory response, or the forced vital capacity.

Hypothesis: The forced vital capacity, but not the hyperoxic-hypercapnic ventilatory response, will predict the duration of a maximal hyperoxic breath-hold in elite apnea divers.

1.2.3 Study 3 (Chapter 6)

Aim: To examine the oxidative and non-oxidative cerebral metabolism during a maximal breath-hold in elite apnea divers.

Hypothesis: Compared to a resting baseline, the cerebral metabolic rate of oxygen will be reduced and indices of cerebral non-oxidative metabolism will be increased near the termination of a maximal elite breath-hold.
1.2.4 Study 4 (Chapter 7)

**Aim:** 1) to quantify the cerebral metabolic rate of oxygen under three distinct apnea paradigms that yield separate levels of hypoxemia and hypercapnia; and 2) to quantify whether hypoxia has a role in astrocyte glycogenolysis during apnea, as indexed from an increased net cerebral lactate release.

**Hypotheses:** 1) the reduction in the cerebral metabolic rate of oxygen near the termination of apnea will be mediated by hypercapnia; and 2) breath-holds yielding severe hypoxia will elicit a net cerebral release of lactate.

1.3 Thesis presentation

This dissertation encompasses eight distinct chapters. Following this introductory chapter, Chapter 2 provides a literature review of the concepts covered throughout the rest of the thesis, focusing on I) the integrative mechanisms of cerebral blood flow regulation, II) normal cerebral metabolic functions; III) a brief overview of ventilatory control, and IV) the physiologic functioning specific to a volitional apnea. Chapter 3 provides an overview of the methodological techniques used in the experimental chapters. Chapter 3 will often be referred to in the subsequent experimental chapters when applicable. Chapters 4 to 7 (inclusive) contain the aforementioned four experimental studies. The final Chapter (8) provides a synopsis of the experimental chapters, and suggestions for future work are provided.
Chapter 2 – Literature Review

The purpose of this literature review is to broadly describe the complex physiological processes that underlay an extended breath-hold in humans, with focus on the integrative mechanisms that regulate the delivery and use of oxygen in the human brain. Specifically, this literature review is organized into four distinct sections; I) the integrative mechanisms of cerebral blood flow regulation; II) normal cerebral metabolic functions; III) a brief overview of ventilatory control, and; IV) the physiologic functioning and mechanism(s) specific to a volitional apnea.
Section 2.1 Integrative Mechanisms of Cerebral Blood Flow Regulation

Despite considerable anatomical knowledge acquired by seventeenth century anatomists like Wepfer and Willis [reviewed in: (Clark and O'Malley, 1996)], little was known about the brain’s circulatory control until the later half of the nineteenth century. Here, Mosso (Mosso, 1880) and others (Roy and Sherrington, 1890) conducted the first estimations of cerebral blood flow (CBF) by observing pressure changes in open brain tissue. More landmark descriptions of the mechanisms responsible for CBF control in humans did not come until the inception of more quantifiable techniques to assess CBF, most notably by Kety and Schmidt (Kety, 1948; Kety and Schmidt, 1948b), in the late 1940’s.

Because the human brain accounts for up to 20% of total body oxygen consumption, optimal cerebral functioning is dependent upon meticulous blood flow control and oxygen supply, whereby loss of consciousness occurs within 4 to 6 seconds following abrupt and complete cessation of CBF (Smith et al., 2011). Regulation of CBF is broadly achieved by vasomotor, chemical, metabolic, and neurogenic mechanisms, and by factors that change the prevailing perfusion pressure (Figure 2.1) [reviewed in (Willie et al., 2014b; Donnelly et al., 2016)]. Although normally viewed in isolation, each regulatory mechanism rarely acts without the influence of other interacting factors. For example, levels of arterial blood pressure closely dictate the CBF reactivity to changes in the partial pressure of arterial CO$_2$ (PaCO$_2$) (Harper and Glass, 1965), while changes in vascular tone with varying levels PaCO$_2$ alter the relationship between arterial pressure and CBF. Moreover, increases in PaCO$_2$ (e.g. via breathing 5% CO$_2$) rarely occur without concomitant increases in arterial blood pressure and sympathetic nervous activity (SNA) (Kety and Schmidt, 1948a; Jouett et al., 2015). Global CBF is therefore inherently determined by the interaction of all regulatory mechanisms. This fundamental interplay precludes a clear regulatory hierarchy of CBF control. Of relevance to this Thesis, changes in CBF during apnea are governed by the combination of several interacting factors, e.g., arterial blood gases, perfusion pressure, cerebral metabolism, and neurogenic control.
2.1.1 Pressure flow relationship of cerebral blood flow regulation

Cerebral perfusion pressure: Cerebral perfusion pressure (CPP) is the difference between the mean arterial pressure (MAP) and the mean cerebral vascular resistance, or intracranial pressure (ICP), e.g. CPP = MAP - ICP. When describing the pressure flow relationship of the cerebrovasculature, many studies run under the assumption that changes in MAP reflect changes in perfusion pressure. That is, ICP remains constant (see: Cerebral autoregulation). When posture is kept constant, the balance between cerebral spinal fluid (CSF) and movement of blood-brain fluid and solutes generally maintains ICP within ±1 mmHg (Mann et al., 1978); in such situations changes in MAP accurately reflect changes in CPP. However, because the brain is encapsulated in an inflexible skull, unattended expansion of extracellular fluid or tissue increases the ICP for a given CBF/cerebral blood volume. An array of pathologies and conditions acutely or chronically change the prevailing ICP, including; intracranial tumors (Rees, 2011), traumatic brain injury and hemorrhage (Sheth et al., 2013), reduced cerebral spinal fluid (CSF) absorption or increased CSF production (Lyons and Meyer, 1990), increases in intrathoracic pressure (e.g. during Valsava maneuvers) (Porth et al., 1984), and disruption of blood brain barrier (BBB) integrity (Gumerlock et al., 1994).
Cerebral autoregulation: In 1895, Bayliss and colleagues suggested that the cerebrovasculature is completely pressure passive; that is, “in all physiological conditions a rise in arterial pressure accelerates the flow of blood through the brain, and a fall slackens it” (Bayliss et al., 1895). This statement, however, negates the potential for the brain to self-buffer changes in MAP – i.e. autoregulate. Cerebral autoregulation (CA) is the paradigm created to characterize the relationship between MAP and CBF, and is used as a yardstick to describe the capability of the cerebrovasculature to buffer changes in MAP. Several methodological approaches have been employed to measure CA; unfortunately, the metric of CA can vary depending on which approach is employed to assess it (Tzeng et al., 2012), and the related experimental paradigm. Comparisons, advantages and limitations of the most common methodological approaches are reviewed in (Tzeng et al., 2012; Donnelly et al., 2016).

In contrast to a completely pressure passive cerebrovasculature proposed by Bayliss et al., it is now recognized the brain is, to some extent, able to autoregulate. The cerebral circulation has a more effective capacity at buffering slow (<0.2 Hz) compared to fast (>0.20 Hz) oscillations in MAP (Aaslid et al., 1989). When MAP oscillations are greater than 0.20 Hz, CA is largely ineffective (Zhang et al., 1998; Claassen et al., 2009; Tzeng et al., 2012), and changes in MAP pass unimpeded to the cerebrovasculature. This fundamental component of cerebrovasculature control is easily recognized upon moving quickly from the supine to the upright position [i.e., CA lags by ~10 sec and BP is not fully restored until 30 seconds after standing, yielding a marked 20-50% initial reduction in CBF with standing (Thomas et al., 2009)]. Therefore, although on a continuum, CA is generally presented as static (steady-state measures operating over minutes or hours) or dynamic (operating over seconds). The precise mechanisms of CA remain largely controversial (Tzeng et al., 2012; Ainslie and Brassard, 2014), and might vary from dynamic to static metrics, and from increases and decreases in MAP. Nonetheless, it is generally accepted that CA in general is mediated by the integration of metabolic, myogenic and neurogenic factors (Tan, 2012; Tan et al., 2013; Willie et al., 2014b). The
evidence of these factors in the regulation of human CA have been recently reviewed (Tzeng and Ainslie, 2014).

In 1959, Lassen (Lassen, 1959) produced a seminal review that incorporated seven studies and 11 separate subject groups to illustrate static CA. Each subject's CBF was plotted against their resting MAP. Lassen determined that CBF remains constant between MAP values of 60-150 mmHg, suggesting that the cerebral circulation has particularly efficacious static autoregulatory abilities. The notion of perfect cerebral autoregulation with static MAP values between 60-150 mmHg persists today, and Lassen’s autoregulatory curve continues to be cited [e.g. (Dagal and Lam, 2009; Barret et al., 2010)]. However, a wealth of data now indicates that CBF is maintained within a much narrower range, i.e. within ± 5 to 25 mmHg from resting values (Lucas et al., 2010); for review see (Willie et al., 2014b). It is likely that fixing a line through individual inter-subject data points, rather than plotting a range of intra-subject CBF/MAP relationships, and utilizing subjects with pre-existing pathology, confounded Lassen’s curve (Numan et al., 2014).

2.1.2 Neurogenic regulation of cerebral blood flow

With neurogenic end-feet and associated neurotransmitter receptors across pial vessels and penetrating arterials, the cerebrovasculature is anatomically well equipped for prominent extrinsic and intrinsic neurogenic control (Hamel, 2006). On the superficial pial vessels, projecting afferents arrive from the superior cervical, sphenopalatine, otic, and trigeminal ganglion (extrinsic nerves). The extrinsic neural component of the cerebrovasculature is therefore composed of sympathetic, parasympathetic, and sensory neural pathways. Deep to the Virchow-Robin Space, ‘intrinsic’ nerves, including those originating from sub-cortical areas, innervate the penetrating arterials. Neurogenic input from extrinsic and intrinsic pathways can either constrict or dilate the cerebrovasculature, depending on the neurotransmitter released [for review see: (Hamel, 2006)]. However, despite a relatively detailed anatomical description of the cerebral neurogenic innervation, there remains controversy and confusion of its role in cerebrovasculature
control. Ultimately, the inherent experimental burden, and in turn, a) species related differences, b) varying experimental setups, c) regional heterogeneity, and d) redundantly acting vasoactive substances, precludes a clear understanding of cerebrovascular neurogenic control in humans (ter Laan et al., 2013; Ainslie and Brassard, 2014).

**Sympathetic regulation:** The sympathetic extrinsic arm of neurogenic control (i.e. projections from the superior cervical ganglia) exhibits tonic vasoconstrictor control over the cerebrovasculature, and has importance for a range of physiological conditions. For example, the majority of human studies report increases in CBF upon blockage or ligation of the superior cervical ganglia (Linden, 1955; Umeyama et al., 1995; Ide et al., 2000; Treggiari et al., 2003; Yokoyama et al., 2004). Moreover, CA appears to be impaired with administration of sympathetic blockade (Zhang et al., 2002; Hamner et al., 2010) or α1-adrenergic receptor blockade (Ogoh et al., 2008). It is therefore reasonable to speculate that conditions increasing global SNA synchronously provoke cerebrovasculature constriction, and reduce CBF. However, experimental evidence rarely suggests such a finite relationship. The vasoactive influence of increase cerebral SNA is governed by background levels of MAP and circulating metabolites, including potent vasodilators. Indeed, the known heterogenic CBF increase to a hypertension from unilateral exercise is governed by the interaction of sympathetic innervation and metabolically active tissue, i.e., a functional sympatholysis. Notably, this heterogenic CBF response to unilateral exercise is abolished with selective alpha1-adrenergic blockade (Fernandes et al., 2016). In the absence of hypertension, however, animal studies suggest that isolated cervical ganglia stimulation yields no change in cerebrovascular tone (Mayhan et al., 1987; Cassaglia et al., 2008a, b), reviewed in (Ainslie, 2009; ter Laan et al., 2013; Willie et al., 2014b). That is, sympathetic control of the cerebral circulation seems particularly important to buffer surges in blood pressure, but probably exhibits little more than tonic activity when normo or hypotensive. The mechanism(s) for pronounced cerebral constriction to increased cerebral SNA only during hypertension is elusive, but may relate to phasic modulating dilators (e.g. nitric oxide, prostanoids etc.).
Parasympathetic regulation: Like the sympathetic arm, parasympathetic innervation also displays tonic influence on the cerebrovasculature, but instead acts as a potent vasodilator (D’Alecy and Rose, 1977; Busija and Heistad, 1981; Boysen et al., 2009). However, beyond its tonic role, and importance in certain pathologies [e.g. Alzheimer’s (Ganey et al., 1990)], the active parasympathetic contribution to physiological perturbations remains less understood. Recent evidence points to an active parasympathetic modulation of CA, at least with changes in blood pressure at a frequency above 0.04 Hz (Hamner et al., 2012). Additionally, cholinergic receptor blockade with glycopyrrolate (a muscarinic receptor blockade) has been shown to abolish the exercise-induced increase in middle cerebral artery blood velocity (MCAv) (Seifert et al., 2010). A more recent imaging study of regional CBF, however, indicates no effect of cholinergic muscarinic receptor blockade on the blood flow response to handgrip exercise and visual stimulation (Rokamp et al., 2014).

Sensory regulation: Perhaps less appreciated [excluding related research on chronic headache (Osborn, 2016)], is the potential for the sensory trigeminal ganglion to elicit changes in CBF. Indeed, the trigeminal ganglion is central to the cardiovascular adjustments associated with the mammalian diving reflex upon cold-water facial immersion (Andersen, 1966), and releases known potent vasodilators, namely substance P (Moskowitz et al., 1979). In animals, trigeminal stimulation increases CBF (Lambert et al., 1984; Goadsby and Duckworth, 1987; Salar et al., 1992; Atalay et al., 2002; Just et al., 2010). Similarly in humans, stimulation of the trigeminal ganglion by local injection of glycerol (Tran Dinh et al., 1991) or by thermocoagulation (Tran Dinh et al., 1992) yields marked increases in CBF. Only one study reported decreases in MCA velocity upon electrical stimulation of the trigeminal ganglion (Visocchi et al., 1996). The inconsistency of this study might be explained by changes in PaCO₂, velocity as an index of flow, using patients with expected pathological disorders of the trigeminal nerve, or the use of anesthesia.

Muscle afferents: Stimulation of group III (metaboreceptors) and IV (mecahonoreceptors) skeletal muscle afferents may increase the CBF. For example, blood flow restriction to
exercising muscle (e.g. using a proximally inflated blood pressure cuff) to ‘trap’ muscle metabolites and therefore accentuate the muscle metaboreceptor response, increases the CBF above control levels (Prodel et al., 2016). However, group III and IV activation also increases ventilation (Eckert and Butler, 2016); therefore, their cerebral vasodilatory effects are only observed when ventilation is controlled, or PaCO$_2$ kept eucapnic. The effects of type III/IV muscle afferents on the CBF response may have particular implications in those suffering from peripheral vascular or heart disease with heightened muscle afferent responses. These studies have yet to be conducted.

2.1.3 Metabolic regulation of cerebral blood flow

Neurovascular coupling: Local cerebral metabolism is tightly coupled to local blood flow, i.e. neurovascular coupling (Buxton and Frank, 1997). Increases in neuronal activity are detected by the surrounding glial cells at the neurovascular unit, which then generate retrograde propagating dilatory signals to the penetrating cortical arterials through gap junctions of adjacent vascular smooth muscle cells (Lagaud et al., 2002; Iadecola, 2004; Willie et al., 2014b). In turn, increases in cerebral metabolic activity are met by increases in CBF. In fact, the transient increase in CBF is beyond that required to meet the CMRO$_2$ demand. This disproportionate increase in CBF relative to CMRO$_2$ forms the basis of blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (Sharma and Sharma, 2004). In conditions of compromised CBF (e.g. hemorrhage and hypopcapnia), CMRO$_2$ is maintained by proportional increases in oxygen extraction (Lennox et al., 1935; Kety et al., 1950; Boysen, 1973; Derdeyn et al., 2002; Gjedde, 2005; Bain and Ainslie, 2014; Erdmann and Kunke, 2014; Trangmar et al., 2014). Under most physiologic conditions, the oxygen extraction fraction (O$_{EF}$) is within 30-40% (Gjedde, 2005; Ainslie et al., 2014). Theoretically, the O$_{EF}$ is determined by the oxygen conductivity of cerebral tissue, which itself is determined by the geometry of the capillaries (diffusion area and distance) and tissue metabolism for a given PO$_2$ gradient between the tissue and capillaries (Gjedde, 2005). Unlike in the skeletal muscle or the lungs, it is generally accepted that the diffusive surface area for O$_2$ remains constant in the brain – i.e. the brain does not increase capillary recruitment (Kuschinsky and Paulson,
Therefore, cerebral \( O_{EF} \) can be described as being inversely proportional to CBF when metabolism is held constant, and directly proportional to metabolism when CBF is held constant. Early experimental data indicate that \( O_{EF} \) can increase upwards of 70-80% in extreme syncopal conditions (McHenry et al., 1961). With profound hypocapnia (\( \text{PaCO}_2 \approx 20 \text{ mmHg} \)), the \( O_{EF} \) increases to \( \approx 50\% \) (unpublished findings). According to theoretical considerations, the critical reduction in CBF where increases in \( O_{EF} \) no longer suffice to maintain a constant \( \text{CMRO}_2 \) is \( \approx 50-60\% \) (Lennox et al., 1935; Gjedde, 2005). It follows that the theoretical limit of critical CBF changes for a given cerebral metabolic demand.

**Partial pressure of arterial carbon dioxide:** For each mmHg rise in \( \text{PaCO}_2 \) from eucapnia, CBF increases by 3-6\%, and for each mmHg fall in \( \text{PaCO}_2 \) from eucapnia, CBF decreases by 1-3\% (Sato et al., 2012; Willie et al., 2012). Indeed, the cerebral vasculature is highly sensitive to changes in \( \text{PaCO}_2 \). The mechanisms and location of cerebrovascular changes to arterial blood gases are described in detail elsewhere (Willie et al., 2014b). Briefly, decreases and increases in extravascular pH cause dilation and constriction of the cerebrovascular smooth muscle, respectively. Except for targeted transport and transcytosis, only the non-polar molecules, including \( \text{CO}_2 \) and \( \text{O}_2 \), readily cross the BBB. Therefore, levels of \( \text{PaCO}_2 \) will ultimately determine the level of cerebral extravascular pH following its conversion to carbonic acid with the release of hydrogen ions. Increasing pH appears to increase \( \text{Ca}^{2+} \) intracellular influx, while decreasing pH inhibits it (Lamberts et al., 1961; Lassen, 1968).

**Partial pressure of arterial oxygen:** Like \( \text{PaCO}_2 \), the partial pressure of arterial oxygen (\( \text{PaO}_2 \)) also proffers changes in CBF; albeit to a much lesser degree, and is dependent upon the level of background \( \text{PaCO}_2 \). In hypoxia, increases in CBF occur only below a threshold \( \text{PaO}_2 \) of \( \approx 50 \text{ mmHg} \) – i.e. upon descending from the flat portion of the oxygen dissociation curve. Each percentage point reduction in oxygen saturation elicits an average 0.25 to 2.5\% increase in CBF (Cohen et al., 1967; Willie et al., 2012). Although typical acting in unison, the mechanisms of cerebral dilation to hypoxia are likely mediated via the reductions in oxygen content, rather than the \( \text{PaO}_2 \) per se (Hoiland et al.,
2016b). Here, hemoglobin in the erythrocytes appears to act as the primary upstream regulator of cerebrovascular tone to hypoxia. Reviewed in (Hoiland et al., 2016b), downstream mechanisms include an adenosine triphosphate-mediated release of adenosine (Bowton et al., 1988), nitric oxide (Van Mil et al., 2002), and epoxyeicosatrienoic acids (Liu et al., 2015).

In contrast to hypoxia, prolonged hyperoxia (e.g. PaO\(_2\) ~400 mmHg) can moderately decrease CBF (Willie et al., 2012). The mechanisms responsible are presumably due to a direct effect of hyperoxia on neurons causing mild hyperventilation (and therefore reduction in PaCO\(_2\)), in combination with free radical mediated vasoconstriction due to the inactivation of nitric oxide (Demchenko et al., 2000). Although not a universal finding (Diringer et al., 2007; Ainslie et al., 2014), hyperoxia may also cause a reduction in CBF by depressing the CMRO\(_2\). Notional mechanisms for reductions in CMRO\(_2\) are attributed to free-radical toxicity – discussed in (Xu et al., 2012).
Section 2.2 Cerebral Metabolism

Ever since its first quantitative assessment by Kety and Schmidt (Kety, 1948; Kety and Schmidt, 1948a, b), it has been well recognized that cerebral energy metabolism at rest is attained primarily (90%) by oxidative means (Brown and Ransom, 2007). Non-oxidative metabolism is thus restricted to less than 10% (Siesjo, 1978; Gjedde et al., 2002). Applying the Fick principle, the CMRO$_2$ is fundamentally derived from the product of oxygen delivery (CBF) and cerebral oxygen extraction (i.e., the PaO$_2$ to jugular PvO$_2$ difference). Given a normal resting CBF of ~750 mL, and an arterial-jugular venous oxygen content difference of ~8 ml per deciliter of blood, a typical CMRO$_2$ in humans holds at ~60 ml·min$^{-1}$. As emphasized in the previous section of this literature review, a normal resting whole-body oxygen consumption of ~300 ml·min$^{-1}$ means that ~20% of the total oxygen is consumed at the brain – a formidable achievement given that it occupies a mere 2-3% of the total body mass.

Although restricted at rest, the non-oxidative metabolism may increase substantially during exhaustive exercise, contributing to over 50% of the total cerebral metabolic activity (Volianitis et al., 2008). The non-oxidative metabolism can be estimated by the ratio of oxygen and carbohydrate consumption, also known as the oxidative carbohydrate index (OCI). Calculation of the OCI is outlined in Chapter 3 of this Thesis. The assumption of OCI is that oxidation of glucose requires one oxygen molecule per six carbon atom, while carboxylation of lactate to pyruvate acid yields only one molecule of pyruvate, compared with the two derived from the breakdown of glucose. A ratio of six indicates that all of the carbohydrates taken up by the brain were oxidized. Any reduction from six indicates the presence of non-oxidative metabolism. At rest, a typical OCI sits at five to six, and can decrease down to approximately two during exhaustive exercise (Volianitis et al., 2008).

2.2.1 Cerebral nutrient usage
The cerebral yield for ATP from macronutrient carbon atoms, whether aerobically or anaerobically, derives primarily from circulating glucose (Brown and Ransom, 2007). However, lactate (van Hall et al., 2009) and even ketone body (Owen et al., 1967) metabolism is elevated under conditions that increase their cerebral delivery. The presence of ketones in the blood is generally negligible for cerebral metabolism in the absence of disease or starvation (Owen et al., 1967). In contrast, up to ~8% of cerebral metabolism is accounted for by the oxidation of lactate even with low resting arterial concentrations (~0.9 mmol/L) (van Hall et al., 2009). During exercise, upwards of 30 to 40% of the total cerebral metabolism can be accounted for by lactate with elevated arterial concentrations of ≥15 mmol/L (Ide and Secher, 2000; Volianitis et al., 2008). In vivo animal data indicate that the oxidation of lactate may be the preferential energy substrate when both glucose and lactate are available (Wyss et al., 2011). A small amount of glycogen stored in astrocytes may also protect against hypoglycemia [e.g. during hyper-exhaustive exercise in rats (Matsui et al., 2011)] by degrading to lactate for oxidative metabolism (Brown and Ransom, 2007). Lactate release from the brain has also been observed during hypoxic conditions (Overgaard et al., 2012), suggesting that astrocyte glycogenolysis may also be triggered by factors independent of hypoglycemia. Here, a rise in noradrenaline may underpin this astrocyte glycogen mobilization (Matsui et al., 2011).

Unlike other organs, the brain does not rely on the oxidization of fatty acids (baring of course ketone bodies during prolonged starvation). The lack of brain fatty acid metabolism is notionally a consequence of three factors: 1) a rate limiting passage of fatty acids across the BBB, 2) a slow rate of ATP generation and higher demand of oxygen with fatty acids compared to glucose, and 3) an increased ratio of reactive oxygen species production to removal when fatty acids are oxidized. The unfavorable use of fatty acids in brain metabolism is reviewed in (Schonfeld and Reiser, 2013).

2.2.2 Factors affecting the cerebral metabolic rate
With considerable advances in non-invasive MRI techniques to assess the CMRO₂, there has been recent focus on quantifying factors that will change it. The following briefly describes some of the non-pharmacological modulating factors of the cerebral metabolic rate, with particular relevance to those possibly involved with apnea.

*Arterial PCO₂*: The first quantitative estimation of the CMRO₂ during changes in acid-base balance from hypercapnia and hypocapnia initially suggested little effect (Kety and Schmidt, 1948a). However, later work in animals by Seijo and colleagues (Folbergrova et al., 1975) indicated a modulatory effect, with hypocapnia increasing, and hypercapnia decreasing the CMRO₂. The majority, albeit not all [discussed in (Yablonskiy, 2011)], of most recent studies using novel MRI techniques corroborate this latter notion (Chen and Pike, 2010; Xu et al., 2011; Peng et al., 2016). For example, Chen et al., (2010) report a ~7% reduction in CMRO₂ with a ~9 mmHg increase in end-tidal CO₂; Xu et al., (2012) report a ~13% reduction in CMRO₂ with a ~7 mmHg increase in end-tidal CO₂; and most recently Peng et al., (2016) report a ~8% reduction in CMRO₂ with a ~5 mmHg increase in end-tidal CO₂. The opposite response in CMRO₂ (i.e. an increase) seems to hold true with hypocapnia (Waaben et al., 1989; Chen and Pike, 2010). In agreement with changes in CMRO₂, neural activity is consistently reduced with hypercapnia (Dulla et al., 2005; Zappe et al., 2008; Thesen et al., 2012). This is clinically recognized in epileptic patients, where hypocapnia can incite seizures [associated with known increases CMRO₂ (Wasterlai et al., 2010)], whereas hypercapnia can suppress them (Yang et al., 2014).

The hypercapnic suppression of CMRO₂ can be described by three related mechanisms. First, hypercapnia / extracellular acidosis increases adenosine concentrations which activate adenosine A1 receptors (Dulla et al., 2005) and inhibit excitatory glutamatergic neurotransmission. Second, hypercapnia / acidosis may reduce phosphofructokinase activity, evidenced from an accumulation of fructose-6 phosphate and glucose-6 phosphate in un-anesthetized rat cerebral cortexes following CO₂ inhalation (Folbergrova et al., 1975). Any reduction in PFK activity, and therefore glycolytic flux, will correspondingly reduce the CMRO₂ provided that other non-carbohydrate substrates (e.g. amino acids) are not oxidized. This is a fair assumption given that glucose and lactate
provide the primary metabolite for cerebral metabolism in the absence of ketogenesis (Brown and Ransom, 2007; Rasmussen et al., 2010). Finally, hypercapnia may depress the CMRO$_2$ via a reduction in cerebral temperature resulting from the increased CBF [(Bain et al., 2015b) and see, Temperature, below].

**Hypoxia:** Hypoxia may increase the CMRO$_2$ (Xu et al., 2012; Vestergaard et al., 2015; Peng et al., 2016), although this is not a universal finding (Kety and Schmidt, 1948a; Ainslie et al., 2014). The proposed mechanism(s) for increased CMRO$_2$ in hypoxia remain undefined, but is suggested to involve decreased mitochondrial efficiency for ATP production (Solaini et al., 2010), and perhaps also from increased circulating catecholamines acting at the neuronal level (see below). On the other hand, an increased CMRO$_2$ during fixed FiO$_2$ hypoxia may simply be a consequence of the concomitant hyperventilation-induced hypocapnia. For example when PaCO$_2$ is kept eucapnic, hypoxia has no effect on the CMRO$_2$ (Ainslie et al., 2014).

**Adrenaline:** It is well established that adrenaline increases the glycogenolytic and glycolytic flux at the muscle (Febbraio et al., 1998), and it appears that adrenaline similarly affects the brain. For example, in humans, intra-venous adrenaline infusion (~8ug/ml/min) with concurrent increases in MAP (~25 mmHg) to promote amine passage across the BBB, significantly increases the CMRO$_2$ by ~18% (King et al., 1952). Moreover, infusion of high dose adrenaline (0.08 μg·kg$^{-1}$·min$^{-1}$ for 15 min) significantly increases the percentage of non-oxidative cerebral metabolism, as determined by a shift in the OCI from 5.1 to 3.6 (Seifert et al., 2009). In both studies, adrenaline (but not-noradrenaline) generated the cerebral metabolic changes, suggesting the changes in CMRO$_2$ are mediated through a β2-adrenergic mechanism. Such a mechanism likely underscores the increased non-oxidative metabolism during exercise where plasma adrenaline may be increased >10-fold (Pott et al., 1996; Tank and Lee Wong, 2015). Administration of a non-selective β-adrenergic blockade during exercise prevents the increase in non-oxidative metabolism (Larsen et al., 2008).
Temperature: It has long been recognized that the rate of biological activity is temperature sensitive (Belehradek, 1957). Indeed, deep hypothermia is readily used clinically to depress the metabolic rate in invasive surgery (Fukui and Takanashi, 2016), a technique acquired from historical studies of the hibernating mammal [e.g., (Lust et al., 1989)]. At the turn of the 19th century, Svante Arrhenius quantified the relationship between temperature and chemical/biological reactions by creating the $Q_{10}$ temperature coefficient (Figure 2.3). The $Q_{10}$ temperature coefficient describes the rate of change in any biological or chemical system from a change in temperature of 10°C. The $Q_{10}$ temperature coefficient is calculated using the equation below:

$$Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_2-T_1)}$$

Where; R is the rate, and T is the temperature in degrees Celsius or Kelvin.
Figure 2.2. Theoretical change in CMRO$_2$ assuming a temperature coefficient (Q$_{10}$) of two, see equation in text above. Although a simple exponential curve is presented, in vivo data indicate large variability, depending on the hyperthermic or hypothermic condition (e.g. if combined with pro-inflammatory states), and whether increasing or decreasing temperature. See text below for detail.

Biological Q$_{10}$ values are tissue and species specific (South, 1958; Bennett, 1984), and the logarithmic aspect dictates that reaction rates vary from baseline temperature. It is generally cited that most biological systems, including mammalian cardiac muscle, skeletal muscle, and cerebral tissue, adhere to a Q$_{10}$ factor of ~2 to 3 (Donnelly et al., 1956; South, 1958; Bennett, 1984; MacVeigh et al., 1997; Sakoh and Gjedde, 2003) (Figure 2.2). As such, without the influence of any other factors, each degree Celsius change in cerebral temperature should yield a proportional change in metabolism by ~10 to 20%. However, during whole body or local brain heating, the CMRO$_2$ in animals is only increased by 5 to 10% per degree Celsius (Nemoto and Frankel, 1970a, b; Carlsson et al., 1976; Busija et al., 1988), and in exercising humans an increased core temperature by ~1.7°C increases CMRO$_2$ by 7-8%; values in magnitude smaller than posited by a Q$_{10}$ of 2 to 3. The Q$_{10}$ values derived during hypothermia [e.g. (Donnelly et al., 1956;
MacVeigh et al., 1997) may therefore be confounded by the use of anesthesia or sleep, or both. However, Sebert and colleagues (Sebert et al., 2003) found that the metabolic change for a given change in temperature from 37.0°C in rats is approximately three times higher with hypothermia than during the same temperature increase above 37.0°C. These findings may reflect a hysteresis in the $Q_{10}$ of mammalian tissue, depending on the specific chemical milieu for enzymatic activity (e.g. pH).
**Section 2.3 Ventilatory Control**

Breathing is conceptually controlled by simple feedforward and feedback mechanisms, with the reflex arc containing a ‘controller’, ‘effector’, and ‘sensor’ (Figure 2.3). The controller sits centrally in the brainstem, with separate neurons responsible for rhythmic inspiration and expiration. The effector includes respiratory muscles responsible for moving air in and out of the lungs. The sensor includes a multitude of afferents from mechanoreceptors, metaboreceptors, chemoreceptors, and nocireceptors, among others, that stimulates respiratory modulations based on the prevailing physiologic condition.

The following section briefly highlights components of the autonomic respiratory system that have particular relevance to a prolonged apnea, namely those involved with sensory and controller systems.
Figure 2.3. Schematic of the multiple inputs (sensors) capable of regulating the effectors (muscles responsible for airflow in the lungs) integrated at the central controller. Plus (+), minus (-) signs denote an excitatory or inhibitory effect, respectively. For simplicity, emotional modulation and higher brain center modulation of respiratory control is drawn directly to the central controller (at the medulla), however, the latter may work directly on the respiratory motor neurons (albeit unknown), and the former via the limbic system.

2.3.1 Central controller

The central respiratory controller, located within the pons and medulla of the brainstem, integrates all modulator signals coming from the sensory organs, and projects rhythmic output to the effector organs. The respiratory centers located in the pons are largely
responsible for “inspiratory-expiratory phase transition” (Eckert and Butler, 2016) and therefore breathing frequency. Respiratory rhythm generation occurs within the medulla. Of particular distinction in the medulla are the dorsal and ventral respiratory groups, with the former primarily involved with receiving respiratory sensory input, and the latter with respiratory pacemaking.

The nucleus tractus solitarius (nTS), contained within the dorsal respiratory group of the medulla, receives input from several cardiorespiratory sensory afferents, including those from the peripheral chemoreceptors via the glossopharyngeal nerve, the diaphragm via the phrenic nerve, and the heart via the vagus nerve (Eckert and Butler, 2016). The nTS then sends key output information to surrounding respiratory centers, including the retrotrope zone. In itself the retrotropezoid nucleus is a key component of the central chemoreflex (see below), but it also acts as a peripheral sensory relay by innervating respiratory pattern generators, most notably the cluster of interneurons in the ventrolateral medulla called the pre-Bötzinger complex (Bochorishvili et al., 2012). The pre-Bötzinger complex, forming part of the ventral respiratory group, holds the current title as respiratory pacemaker (Ramirez et al., 2012). Indeed, removing the pre-Bötzinger complex in rats abolishes respiratory rhythm generation, whereas thin medullary slices that isolate the pre-Bötzinger complex generate respiratory oscillations similar to those in an intact brainstem (Smith et al., 1991). Along with initiating normal (eupneic) respiratory pattern, the pre-Bötzinger complex further initiates the sigh and gasping pattern (Lieske et al., 2000). Outside of the dish and rat preparation, humans suffering from diseases with distinct breathing irregularities (e.g. multiple systems atrophy) show anatomical degeneration of the pre-Bötzinger complex (Schwarzacher et al., 2011), laying evidence for its role in human respiratory physiology.

2.3.2 Sensory modulators

At rest, cells that respond to changes in O₂, CO₂ and pH, i.e. chemoreceptors, dominate respiratory modulation. Chemoreceptors are located peripherally in the aortic and carotid bodies, and centrally in the brainstem. Experimentally, the magnitude of the ventilatory
response to changes in CO₂ (pH), and O₂ depends on the methodology employed, e.g. steady state, transient, or rebreathe (Pfoh et al., 2015). Although beyond the scope of this literature review, variations in the ventilatory slope to O₂ and CO₂ exist under numerous factors, for example, sex (Aitken et al., 1986), age (Kronenberg and Drage, 1973), duration of the exposure (Ainslie et al., 2013), and the time of day (Cummings et al., 2007). Such variation makes a normative assessment of the ventilatory response for a given change in O₂ and CO₂ (together or in isolation), nearly impossible. Nevertheless, a considerable amount of research has focused on describing the central and peripheral chemoreflex, as plasticity (whether adaptive or maladaptive) occurs in a myriad of pathologies (e.g. heart failure and sleep apnea), and environmental stimuli (e.g. exposure to high altitude).

Central chemoreception: Although oxygen-sensing cells exist in the central nervous system (Neubauer and Sunderram, 2004; Angelova et al., 2015), their functional role in human respiratory physiology remains unclear. The most recognized feature of central chemoreception is therefore CO₂ and pH sensing. Neurons sensitive to both molecular CO₂, and pH (Guyenet and Bayliss, 2015) are most abundantly located at the rostral medulla in the retrotrapazoid nucleus. In conscious rats with intact carotid bodies, inhibition of neurons in the retrotrapazoid nucleus causes a ~60% reduction in the respiratory response to CO₂ (Marina et al., 2010). Moreover, the absolute reduction in peak ventilatory response to hypercapnia in humans with bilateral carotid body resection is small (despite complete removal of the hypoxic ventilatory response – see below) (Holton and Wood, 1965). Nevertheless, the increase in ventilation from hypercapnia seems to reflect the combined input from peripheral and central chemoreception, with their commonly cited contribution at 40:60% (Smith et al., 2006), respectively.

Peripheral chemoreception: The peripheral chemoreceptors predominantly respond to changes in oxygen, carbon dioxide and hydrogen ion concentration (pH), but also to changes in temperature, catecholamines, potassium, glucose, insulin and immune-related cytokines [reviewed in (Kumar and Prabhakar, 2012)]. Oxygen sensing in particular is ascribed almost exclusively to the glomus cells of the peripheral chemoreflex. In humans,
the carotid bodies located at the bifurcations of the common carotid artery into the external and internal carotid arteries, predominate chemosensory ventilatory control compared to the aortic bodies that are located in the aortic arch. Indeed, bilateral ligation of the carotid bodies in humans abolishes, or in some cases reverses, the ventilatory response to hypoxia (Holton and Wood, 1965). A consequence of their location, the carotid bodies per unit mass are the most heavily perfused organ in the body, responsible for the fast (within seconds) changes in ventilation to alterations in blood gases. In contrast to the central chemoreceptors that may take minutes to respond to changes in arterial blood gases, the dynamic attributes of the carotid bodies makes them particularly important for ‘moment-to-moment’ alterations in respiration. This makes the peripheral chemoreceptors highly implicated in a range of respiratory disorders, most notably the pathophysiology of sleep apnea (Dempsey et al., 2010; Eckert and Butler, 2016).

Central – peripheral chemoreflex interactions: In the intact model, respiratory control from peripheral and central chemoreception never acts in unison - indeed the sites are anatomically connected. Considerable research has focused on the interaction between the central and peripheral chemoreflex, with some claiming a hypoadditive (Day and Wilson, 2009), hyperadditive (Blain et al., 2010), or simple additive effect (Cui et al., 2012). It should be noted, however, that each viewpoint is based on a completely different methodological model and species used. Ultimately, as ostensibly concluded from the recent Journal of Physiology ‘cross talk’ debate (Duffin and Mateika, 2013; Teppema and Smith, 2013; Wilson and Day, 2013), the chemoreflex interaction changes with the prevailing physiologic condition, and a single stance conclusion is misleading. For example, given the logarithmic relation between PCO₂ and pH, when acid-base balance is modified (e.g. respiratory or metabolic pathologies, or at high altitude), an otherwise 'additive' system may become 'hypo-additive' or even vice-versa (Teppema and Dahan, 2010). The central-peripheral chemoreflex interaction has recently been excellently reviewed (Wilson and Teppema, 2016)

Baroreflex – peripheral chemoreflex interaction: Related to the known interaction between the baroreceptors and chemoreceptors with converging afferents at the nTS, the
mean arterial pressure can modify peripheral chemoreflex control of ventilation (Richter and Seller, 1975; Eckberg and Orshan, 1977; Somers et al., 1991). That is, unloading the baroreflex (i.e. reducing MAP) potentiates the chemoreflex and increases ventilation, while loading the baroreflex (i.e. increasing MAP) dampens the chemoreflex and decreases ventilation (Heistad et al., 1974). This interaction is readily observed from the hyperventilatory response during graded lower body negative pressure that generates progressive reductions in central blood volume (Lewis et al., 2015).

2.3.3 Mechano and metaboreceptors

Inputs from skeletal muscles (type III and IV afferents), the diaphragm, and heart further contribute to ventilatory modulation, and become particularly important for controlling ventilation during exercise (Dempsey et al., 2014; Eckert and Butler, 2016). Of relevance to this review, pulmonary afferents seem to play a critical role in the drive to breathe during apnea, and may largely underscore the non-elite apnea breakpoint (Parkes, 2006) – for detailed discussion see Section 4 of this literature review (Physiology of volitional apnea).

2.3.4 Higher brain centers

The ability for higher brain centers (supra medullary inputs) to supersede respiratory modulation from all sensory inputs has particular relevance to a volitional apnea. This is perhaps best demonstrated by the capability for some elite-apnea competitors, even at sea level barometric pressures, to override all ventilatory sensory stimuli and hold an apnea until unconscious.
Section 2.4 Physiology of Volitional Apnea

The objective of this section is to outline the fundamental physiology associated with extreme apnea in humans. The focus is on elite breath holding (over five minutes), however, a large body of the basic concepts derives from study in naïve breath-holders. Although apnea is often performed at depths, the mechanical constraints and physiological changes associated with increased barometric pressure are not discussed here. Following a brief historical overview of apnea diving, the majority of this section focuses on the mammalian dive reflex, and factors that can change it. The remainder of this section describes the underpinning factors of an elite-apnea breakpoint, and lastly describes the adaptations or maladaptations associated with the practice of extreme apnea.

2.4.1 Historical perspective of apnea diving

Apnea diving for the harvest of pearls, sponges and shellfish has been practiced for over 2,000 years in Greece, Persia, India, Korea and Japan. Even earlier (circa 415 BC), apnea diving was performed for military purposes around the Aegean (Ferretti, 2001). Commercial apnea diving also existed in Western modern society, for example skin divers of the US Navy (Carey et al., 1956) that were deployed for underwater demolition during World War II.

The immergence of SCUBA (self-contained underwater breathing apparatus) has largely replaced the commercial need for apnea diving, but it still flourishes in Japan and Korea (Ama divers). The Ama divers have in turn received the most academic interest, with published physiological reports dating to the 1920’s [reviewed in (Ferretti, 2001; Ferretti and Costa, 2003)]. Physiological study of the Ama continue to the present day [e.g. (Tanaka et al., 2016)]. With exception of the Ama, however, apnea diving elsewhere has evolved from a commercial practice, to primarily sport, including competition apnea, and spear fishing – although some may include the latter as a commercial means.
Interest in apnea diving for sport may stem from the celebrated story of the Greek sponge fisherman, Georghios (Yorgos) Haggi Statti (Ferretti, 2001; Seedhouse, 2011). His story dates to June of 1913, when the flagship of the Italian Navy, Regina Margherita, lost its anchor in a heavy storm while docked in a small Bay of the Aegean Sea. Dishonourable to return to port with a missing anchor, resting untethered 77 m below on the ocean floor, the battleship’s Captain turned to the local sponge fisherman for help. Amongst the fisherman, Yorgos claimed to be able to descend easily to 77 m, and further touted the capability of a seven-minute breath-hold. Yorgos’s physical characteristics did not support his claim; he was a feeble man, diagnosed with pulmonary emphysema, with a resting heart rate (assessed by the Ship’s physician) of ~80 to 90 bpm. Nevertheless, to surprise of the Captain, physician and crew, Yorgos quickly completed the task of passing a rope through the anchor’s eye. For his trouble, Yorgos was awarded five-pounds of sterling, and permission to fish using dynamite, a practice that at the time was forbidden to anybody outside the Italian Navy.

If a man with apparent pulmonary emphysema can reach 77 m, what is the capacity of man in absence of pathology? Currently, the record no limits (i.e. descent by any means possible, usually by weighted sled and inflatable bag to return) apnea is 253 m. In other disciplines, the longest distance covered in a pool with a single breath is 281 m. And, as mentioned at the beginning of this literature review, the longest recognized static apnea duration is 11:35 min.

2.4.2 The mammalian dive response

The capacity for man to descend >100 m underwater without supplementary air is accomplished in large part with help from the mammalian dive response. The mammalian dive response is initiated by apnea alone, but majorly accentuated when performed under water with facial cooling (Schagatay, 2009). It is conventionally characterized by initial apnea, vagally-mediated bradycardia, and sympathetically-mediated splenic and peripheral vasoconstriction. The latter prioritizes blood flow to the brain causing the poorly perfused peripheral tissue (e.g. skeletal muscle) to turn to non-oxidative metabolic
means. That the dive response consists of integrations from both sympathetic and parasympathetic pathways underlies its phylo- and ontogenetic origin (Lemaitre et al., 2015).

**Bradycardia:** At the latter part of a prolonged breath-hold in water, depending in the related temperature, heart rate may be reduced to as low as 10 bpm in diving mammals (e.g. seals) (Ferrante and Opdyke, 1969), and as low as 20 to 30 bpm in humans (Ferrigno et al., 1997; Schagatay, 2009). In elite breath-hold divers, apnea performed in water reduces heart rate by an average ~50% (Schagatay, 2014). The teleological benefit is reduced myocardial oxygen consumption. Hoiland et al., (2016a) recently reported that administration of a cardiac specific beta1-blockade (esmolol) to accentuate bradycardia in elite divers, increased a dry maximal apnea duration by ~10% (33 sec) compared to a placebo. At the apnea breakpoint, the arterial oxygen saturations were similar, confirming that the increased apnea duration with cardiac selective beta 1-blockade was associated with reduced oxygen consumption. This study is corroborated by earlier reports of attenuated arterial oxygen desaturation in elite breath-hold divers that are able to achieve the largest bradycardia response, compared to normal untrained controls (Stewart et al., 2005). Moreover, in an apnea world competition, the lowest achieved heart rates were correlated to the best apnea times (Schagatay, 2010).

The magnitude of the bradycardia response to apnea depends largely on the extent of facial cooling and to a lesser extent level of hypoxia and arterial blood pressure. Most importantly, however, the extent of bradycardia requires the cessation of breathing (Lin et al., 1983; Gooden, 1994). Indeed, facial cooling without apnea does not produce bradycardia (Paulev, 1968; Song et al., 1969; Folinsbee, 1974), and breathing a hypoxic gas causes tachycardia (Kato et al., 1988). Ultimately, initiation of bradycardia with apnea stems from removal of the phasic tachycardia during inspiration (Lin et al., 1983; Kato et al., 1988) and removal of pulmonary stretch receptor afferents converging at the nTS – i.e. the Hering-Breuer reflex. The interacting autonomic control that allows bradycardia during apnea is complex, and the exact central pathway remains undefined in humans; however, this topic has been reviewed extensively in animal studies (Blix and
Folkow, 2011; McCulloch, 2012). Because mental stimulation prevents bradycardia during apnea (Ross and Steptoe, 1980), there may also be a supramedullary influence on the heart rate response. Indeed, reports exist of sinus arrest from simply thinking of, or, preparing for apnea (Wolf, 1978). Nevertheless, secondary to the tachycardia ‘release’ when cyclic pulmonary stretch receptors become dormant in combination with potential higher brain center control, the profound influence of facial cooling, hypoxia, and blood pressure point to three important bradycardia modulating and overlapping reflex pathways; I) trigeminal nerve activity, II) peripheral chemoreceptor activity, and III) baroreceptor activity.

I) Modulatory influence of trigeminal stimulation: Temperature and pain sensation at the face is received through the trigeminal nerve, with its nucleus extending throughout the brainstem. Descending efferent activity originate from its convergence with the motor nucleus of the vagus nerve (Schaller, 2004). Upon stimulation the trigeminal nerve initiates a well established ‘trigeminocardiac reflex’, characterized by bradycardia, hypotension, gastric hypermobility, and cerebrovascular vasodilation (Schaller et al., 2009a) – see Section 2.1. Details of the complete trigeminocardiac reflex has been recently reviewed (Lemaitre et al., 2015), but, its central role in the dive response is undoubtedly for bradycardia. The leading influence of trigeminal stimulation to the bradycardia dive response is best displayed by the different heart rate responses to apnea with varying water temperature during facial immersion Figure 2.4.

II) Modulatory influence of the peripheral chemoreceptors: It is well established that carotid body activation alone (e.g. via isolated hypoxia) causes bradycardia, described extensively by Daly and colleagues in the anesthetized dog [e.g. (De Daly and Scott, 1958; De Burgh Daly and Scott, 1962; Hashimoto et al., 1964; James and Daly Mde, 1969)], cat [e.g. (Macleod and Scott, 1964; Carmody and Scott, 1974; Daly, 1991)], and monkey (Daly et al., 1978; de Burgh Daly et al., 1978). Similar bradycardia also occurs following carotid body activation in the conscious dog (Rutherford and Vatner, 1978) and rabbit (Daly and Taton, 1979). Results from animal studies hold true in the human. For example, sodium cyanide injection into the pulmonary artery to stimulate carotid body discharge causes bradycardia (Jain et al., 1972). Moreover, those with bilateral carotid
body resection display a tachycardia response to apnea (Gross et al., 1976), and bradycardia is attenuated during apnea performed under hyperoxia conditions that suppress carotid body activity (Gross et al., 1976). In elite breath-hold divers, peripheral chemoreflex inhibition with low-dose dopamine prevents bradycardia during apnea in comparison to placebo (Bain et al., 2015a). However, whether the latter was simply consequent to the lower arterial pressure, rather than carotid body induced bradycardia per se, remains uncertain. Nevertheless, although hypoxia in the spontaneously breathing animal (and humans) causes tachycardia (Kato et al., 1988), it is clear that this is contingent upon pulmonary afferent gating, which is removed with apnea.

Cholinergic blockade with atropine or vagotomy largely removes the bradycardia response to chemoreceptor activation in the dog (De Daly and Scott, 1958; De Burgh Daly and Scott, 1962), cat (Macleod and Scott, 1964; Daly and Kirkman, 1989), and rabbit (Schmidt et al., 1985), and increased cardiac vagal activity is observed upon chemoreceptor stimulation in the dog (Jewett, 1964; Davis et al., 1977) and cat (Kunze, 1972). These findings indicate vagal activity as the primary pathway for bradycardia upon chemoreceptor stimulation. However, bradycardia is completely abolished only upon both vagotomy and sympathetic resection to the heart, at least in the dog (De Burgh Daly and Scott, 1962) and cat (Macleod and Scott, 1964). These findings indicate a small role for sympathetic inhibition, albeit results are not consistent with propranolol administration in the monkey (de Burgh Daly et al., 1978). Extensive review on the mechanisms underpinning the cardiac response to chemoreceptor stimulation is found elsewhere (Marshall, 1994).

III) Modulatory influence of baroreception: Baroreceptor modulation during a prolonged apnea is readily observed by the biphasic heart rate response in elite breath-holders (Lemaitre et al., 2015). That is, tachycardia presents at the beginning of apnea, coinciding with the transient hypotension. Upon deep inspiration before the apnea onset, a compressing effect of the inflated lungs around the heart causes stroke volume to fall by ~50% of resting values (Batinic et al., 2011), and mean arterial pressure falls by ~20%. Tachycardia is in turn initiated from arterial baroreceptor unloading in defense of critical
reductions in cardiac output, and risk of syncope. The fall in arterial blood pressure is exacerbated with glossopharyngeal insufflation (lung packing) that may generate an extra ~24% of air volume on top of normal TLC (Batinic et al., 2011). With glossopharyngeal insufflation, left and right end diastolic filling volumes are dramatically reduced, and left ventricular ejection fraction may fall to 30% (Potkin et al., 2007; Batinic et al., 2011). It is therefore not surprising that symptomatic hypotension is often observed at the onset of competitive apnea [(Ferrigno et al., 1986; Liner, 1994) and unpublished observations].

As apnea progresses, peripheral vasoconstriction from the elevated sympathetic nervous activity progressively leads to hypertension, at least in humans. The magnitude of the increase in MAP during an apnea is variable, but appears similar whether performed in dry (out of water) or wet (with water immersion) conditions (Breskovic et al., 2011); but is accentuated when apnea is performed with exercise (Breskovic et al., 2011; Marongiu et al., 2015). On average, MAP is increased at the termination of a prolonged apnea (>5 min) by 35 to 55% from resting (Breskovic et al., 2011; Willie et al., 2014a; Bain et al., 2015a), resulting from both systolic and diastolic pressure elevations (Breskovic et al., 2011). Progressive baroreceptor loading may therefore cause bradycardia. However, Gooden (Gooden, 1994) reported no relationship between the increase in arterial blood pressure and heart rate during apnea with facial cooling, and bradycardia with facial cooling develops even in the absence of hypertension (Heistad et al., 1968; Finley et al., 1979). Hypertension is therefore not essential for the bradycardia during apnea with water immersion. Notably, seals do not become hypertensive during diving (Blix and Folkow, 2011), suggesting that bradycardia associated with baroreceptor loading can be considered an artefact, rather than direct effect of the dive response.

In elite breath-holders, the latter ~50% of a maximal apnea involves oscillatory suppression of involuntary breathing movements (IBMs) (Heusser et al., 2009). The hemodynamic consequence of IBMs is a transient increase in venous return and arterial pressure (Dujic et al., 2009). This in turn results in pronounced phasic bradycardia from baroreceptor unloading - see Figure 2.5.
Figure 2.5. Representable trace of the latter part of an apnea demonstrating the cardiovascular effects of involuntary breathing movements [displayed from the chest wall displacement – chest plethysmograph (pleth), bottom trace] that elicit surges in arterial blood pressure (intra-radial, second bottom trace), cerebral blood flow (middle cerebral artery velocity – MCAv, second top trace), and transient changes in heart rate (echocardiogram – ECG, top trace). Dashed vertical lines align the onset of the involuntary breathing movements.
Other modifying factors of bradycardia in the dive response: Several additional factors will alter the bradycardia response to apnea. Most recognized is the influence of age, time of day, apnea experience, and whether combined with exercise.

**Age:** Diving bradycardia decreases with age (Gooden, 1994). Possible mechanisms may be associated with reduced parasympathetic activity and/or arterial stiffening that blunts the baroreceptor control of heart rate.

**Time of day:** Peak bradycardia response to apnea is shown in the morning, and blunted responses in the afternoon and evening (Konishi *et al.*, 2016). Reductions in heart rate variability, an index for sympathetic vs. parasympathetic tone, mirrored the changes in diving bradycardia throughout progression of the day. These findings indicate that reduced parasympathetic tone explains the reduced diving bradycardia in the afternoon.

**Apnea experience:** Two weeks apnea training increases the diving bradycardia (Schagatay *et al.*, 2000). Here, rather than an active ‘training’ of the dive reflex, it was proposed that apnea training re-establishes the bradycardia reflex that has become attenuated from disuse (Schagatay *et al.*, 2000). More mechanistically, however, these findings may relate to increased conduit arterial compliance and heightened arterial baroreceptor activity from an upregulation of vasodilatory agents (Joulia *et al.*, 2002). On the other hand, two-weeks apnea training to improve the bradycardia response may point to heightened supramedullary control.

**Exercise:** Exercise proportionally reduces maximal apnea duration - related to the intensity dependent increase in metabolic demand (Lindholm and Lundgren, 2009). Not surprisingly, exercise blunts the diving bradycardia response, presumably consequent from increased muscle afferents (Fisher *et al.*, 2015), central command, and baroreceptor resetting (Raven *et al.*, 2006). For example, a reciprocal relationship between the rate of oxygen desaturation and bradycardia during apnea combined with exercise has been reported [see Fig. 3 in (Lindholm and Lundgren, 2009)].
In summary, secondary to elimination of phasic pulmonary stretch receptor activity and perhaps also influence of higher brain centers, when a prolonged apnea is performed without facial cooling, the mild bradycardia occurring at the latter stages is consequent to a combination of baroreceptor and chemoreceptor activity. The magnitude of bradycardia in this ‘dry’ condition is therefore contingent upon the magnitude of hypertension and hypoxia. Meanwhile, when apnea is performed with facial cooling, the bradycardia is dominated by trigeminal nerve activity, and shadows the effect of baroreceptor loading. In both cases (e.g. with and without facial cooling), pronounced depression of the diving bradycardia with atropine suggests the final common pathway is primarily vagally mediated (Finley et al., 1979). The bradycardia response to apnea is further modulated by a number of other factors, including age, time of day, and apnea training status.

Peripheral vasoconstriction and blood redistribution: The ultimate goal of the dive response is for oxygen conservation. A logical step is to therefore interrupt oxygen supply to metabolically active tissue able to withstand profound hypoxia, e.g., the skeletal muscle. Evidence for skeletal muscle vasoconstriction in the diving mammal is best demonstrated from the landmark experiment by Scholander in 1940 [reviewed (Blix and Folkow, 2011)]. Scholander demonstrated that cutting skeletal muscle in the facially immersed seal did not lead to bleeding until the face was removed from the water. In the human, determined using less crude methods (e.g. intra-arterial cannulation and Doppler ultrasound), skeletal muscle (and skin) vasoconstriction is also apparent upon apnea and facial immersion (Heistad et al., 1968; Shamsuzzaman et al., 2014).

Peripheral vasoconstriction during apnea and facial immersion is consequent to increased sympathetic nerve activity from the integration of neural reflexes (Shoemaker et al., 2015). In humans, muscle sympathetic nerve activity (burst frequency and amplitude) greatly increases from baseline to the end of apnea even in the absence of facial immersion, and is dependent on the duration of apnea (Heusser et al., 2009; Steinback et al., 2010c). The magnitude of the increase in muscle sympathetic nerve activity is further heightened with facial cooling (Shamsuzzaman et al., 2014), with an increase from
baseline up to 360% when apnea and facial cooling are performed together (Fagius and Sundlof, 1986).

The increased SNA during apnea distributes blood flow away from the periphery in prioritizing flow towards the brain. Figure 2.6 provides a schematic of the major regional distribution of blood flow associated with the latter stages of an apnea in humans. Of note, Figure 2.6 is based on values derived from apnea without added influence of facial cooling or hyperbaric stress. When apnea is combined with facial cooling the blood flow changes - especially at the skin, muscle and spleen - are accentuated. Although a large body of work exists on the regional blood flow distribution during apnea in aquatic mammals (e.g. seals) (Zapol et al., 1979; Blix and Folkow, 2011) some marked differences make comparisons with humans difficult. For example, the seal expresses reductions in mean arterial pressure upon diving, while during the latter stages of an apnea dive in humans, arterial pressure is increased. Moreover, seals never dive at total lung capacity. Such changes muddle comparisons for blood flow arriving centrally (i.e. to the heart and lungs) between species. On the other hand, although not experimentally observed in the human, seals express little blood flow reduction in the adrenal bed, despite marked blood flow reductions in other visceral tissue (Zapol et al., 1979; Blix and Folkow, 2011). These findings highlight that the adrenals are in continual demand of blood flow for active metabolic production of adrenaline and noreadrenaline. Both adrenaline and noradrenaline are substantially elevated in humans during apnea [by ~400% (Chapter 6)] suggesting that, like in the seal, adrenal blood flow is not compromised during apnea.
Figure 2.6. Regional blood flow distribution in humans at the latter stages of an apnea without facial cooling. The magnitude of the responses is variable with the duration of apnea, level of hypoxia, and largely enhanced, at least in the skeletal muscle and skin, when performed with water cooling (Heistad et al., 1968). The increases in brain blood flow based from [(Willie et al., 2014a; Bain et al., 2015a) – duplex ultrasound]. Reductions in heart blood flow are based on the changes in cardiac output (Heusser et al., 2009; Bain et al., 2015a). Reductions in splenetic blood flow based from ultrasound (Bakovic et al., 2003). Notably, the onset of involuntary breathing movements can partially restore the reductions in splenetic blood flow (Palada et al., 2008). The presence of splenetic contractions increases hemoglobin concentration during apnea, a protective reflex that is lost with splenectomy (Bakovic et al., 2005). Reductions in skeletal muscle blood flow based on blood flow changes in the brachial artery (Palada et al., 2007), forearm and finger (Heistad et al., 1968); reductions in skin blood flow based from changes in Doppler flux at the skin (Andersson et al., 2000).

Cerebral blood flow and oxygen delivery: Described in section 2.2 of this literature review, CBF is tightly coupled to the metabolic needs of the brain. The coupling is readily modulated by chemical/metabolic (e.g. PaO₂ and PaCO₂), and neurogenic factors, and by the prevailing perfusion pressure. Throughout an apnea, all modulating factors, including the brain’s metabolic demands (Chapters 6 and 7), are changing. Figure 2.7 (bottom panel) provides a schematic of the typical CBF response during apnea [data from
(Bain et al., 2015a). At the initial stages of the apnea, CBF is depressed below baseline values, primarily attributable to the hypocapnia, and to a lesser extent the hypotension. Thereafter the CBF increases from progressive increases in hypercapnia, hypoxia and hypertension. Transient surges in CBF are further present near the end of an apnea in elite divers, resulting from the surges in blood pressure occurring with the IBMs, see Figure 2.5 (Dujic et al., 2009). The plateauing effect of CBF near the end of apnea may indicate maximal CBF, along with increases in cerebral venous pressure (unpublished observations). Of note, it is likely that normal cerebral autoregulation (based on simple pressure and flow slopes) is generally intact during apnea (Willie et al., 2014a); however, in contrast, Cross et al., (2014) report an impaired dynamic CA using transfer function analysis (i.e. TFA – see Section 2.1.). The nuances of the physiological interpretation of transfer function analysis to reflect CA have been reported (Shoemaker, 2007; Tzeng et al., 2012; Tzeng and Ainslie, 2014).
Figure 2.7. Change in cerebral blood flow (bottom panel) and global cerebral oxygen delivery (top panel) throughout a maximal dry apnea in elite breath-hold divers. Cerebral oxygen delivery calculated from the product of arterial oxygen content and cerebral blood flow. Data from Chapter 6.

Except at the onset of a maximal apnea when the CBF is reduced below baseline levels, the global CDO$_2$ quickly recovers to values above baseline, even despite a ~40 to 50% reduction in arterial oxygen saturation (Figure 2.7, top panel) (Willie et al., 2014a). It therefore stands to reason that the global CDO$_2$ is not a determining factor in the breaking point of an apnea; however, regional impairments in the cerebral delivery of oxygen
remain unknown, but certainly possible. The global CDO₂ above metabolic needs throughout the mid sections of an apnea is likely a consequence of the cerebral vasodilatatory properties of hypercapnia, and the increased perfusion pressure (see Section 2.1).

2.4.3 Oxidative and non-oxidative metabolism

Oxidative metabolism: It is now widely accepted that the dive response causes a reduction in oxidative metabolism (Andersson et al., 2004). Indeed the magnitude of the dive response is inversely associated with the reduction in arterial O₂ saturation (Andersson and Schagatay, 1998; Lindholm et al., 1999; Andersson et al., 2002). Oxygen conservation likely occurs primarily at the muscle (from reduced blood flow and increased non-oxidative metabolism), and the myocardium (from reduced heart rate). In addition, near the end of a prolonged apnea we have recently shown that the oxidative metabolism is reduced at the cerebral tissue (see Chapter 6 and 7). The reduced CMRO₂ can largely be attributed to the hypercapnia (Chapter 7). On the other hand, seals are able to reduce their body core and brain temperature by ~2.5°C during a 20 min apnea in 4°C water (Blix et al., 2010). According to the Q₁₀ of biological tissue, this may reduce the metabolism by ~25% (See Section 2.2). Clearly, a reduction in body temperature is beneficial.

Non-oxidative metabolism: Non-oxidative metabolism at the level of the whole-body plays a leading role for maintenance of metabolic activity during prolonged dives in the seal (Hochachka and Murphy, 1979). In humans, plasma lactate concentrations may increase by ~10% with apnea alone, and up to 20% when apnea is combined with facial cooling (Andersson et al., 2004). This suggests that, like in the seal, non-oxidative metabolism is increased during apnea in humans. Mechanistically, it is likely that the increase in non-oxidative metabolism results from the known influence of catecholamines on skeletal muscle metabolism, that is, increased non-oxidative metabolism (see Section 2.2). Unlike in the skeletal muscle, there appears to be no increases in non-oxidative
metabolism at the cerebral level, at least during apnea without facial water immersion (Chapters 6 and 7).

2.4.4 What determines the elite apnea breaking point?

The underpinning mechanisms of a voluntary apnea break point (whereby breathing is re-initiated) in untrained breath-holders have been topic of several reviews (Mithoefer, 1965; Godfrey and Campbell, 1968; Lin et al., 1974; Parkes, 2006), and are summarized in Figure 2.8. The same physiological factors that determine the naïve-apnea breakpoint will be present in an elite apnea; however, the extended ability to consciously suppress the drive to breathe means that other breakpoint governing factors may appear in the elite, for example those involving consciousness.

Figure 2.8. Schematic of the major factors involved with a non-elite breakpoint. See text for detail.

In the naïve but motivated breath-holder, apnea breakpoint is predominantly governed by an integration of chemoreceptor and diaphragm afferents arriving at the central respiratory center that is continually generating a respiratory rhythm despite conscious suppression of breathing (Cooper et al., 2003; Cooper et al., 2004; Parkes, 2006). Mounting input from these afferents eventually lead to involuntary contractions of the diaphragm and inspiratory muscles (IBMs). The initiation of IBMs has been termed the
‘physiological break point’ (Lin et al., 1974), but should not be confused with the actual apnea breaking point, where breathing is re-initiated, although, the physiological breaking point and the actual apnea breaking point generally occurs together in untrained breath-holders. Strong evidence for the role of chemo afferents in the physiological breakpoint is that hyperventilation and / or breathing 100% oxygen can double apnea duration (Klocke and Rahn, 1959), while a breath-hold following hypoxic breathing can halve it (Engel et al., 1946). Evidence for diaphragmatic afferents is the ability to perform a successive maximal apnea despite breathing an asphyxiating gas mixture upon breaking the first apnea (Fowler, 1954). Moreover a performing a Mueller maneuver partially relieves dyspnea during apnea (Rigg et al., 1974). Complete muscle paralysis with tubocurarine further supports a leading role for muscle diaphragm afferents, where the authors speculated a breath-hold could be maintained until unconsciousness without ever experiencing an urge to breathe (Campbell et al., 1969). A similar study by Gandevia and colleagues (Gandevia et al., 1993), however, did not produce similar results, perhaps a function of the different neuromuscular block employed – atracurium in the latter study. When only isolated phrenic nerves are temporarily blocked, apnea time is doubled, but not prolonged until unconsciousness (Noble et al., 1971).

An easily overlooked component of respiratory control is the capacity for higher brain centers (the cortex) to override all involuntary sensory input. This becomes particularly relevant in the extreme apnea (>5 min), and fundamentally underlies the difference between the naïve (physiological) versus the ultra-elite apnea break point. That is, elite apnea divers are able to consciously suppress involuntary contractions from the respiratory muscles by maintaining a closed glottis and mouth. The latter part of the elite apnea involving IBM suppression has been coined the ‘struggle phase’, versus the ‘easy phase’, before the onset of IBMs (Figure 2.9). The ability to override sensory respiratory control, or at least peripheral chemoreflex input, is evident by the marginal impact of blunting carotid body activity with low-dose dopamine on the elite apnea duration (Bain et al., 2015a), compared to the large affect that low dose dopamine has on the naïve apnea breaking point (van de Borne et al., 1998). Many ultra-elite apnea competitors can in turn maintain apnea until unconsciousness, even when performed out of water. This
striking ability has prompted the governing body for competitive apnea competitions (Association Internationale pour le Développement de l'Apnée; AIDA) to implement strict rules disqualifying an apnea until unconsciousness. That a breath-hold until unconsciousness is a merely a ‘myth’ and ‘impossible’ (Parkes, 2006), is now challenged by the immergence of competitive apnea.

Figure 2.9. Typical trace of chest wall movements [Chest Plethysmograph (pleth)] throughout a six-minute maximal dry apnea in an elite-breath hold diver. See text for detail.

While factors determining the naïve apnea breakpoint still exist in the elite, a critical level of hypoxemia to maintain consciousness fundamentally limits the duration of an ultra-elite apnea. This critical level of hypoxemia likely resides at an oxygen saturation slightly above ~50% (Willie et al., 2014a; Bain et al., 2015a) - notably just before the theoretical limit for consciousness (Nunn, 1987). If hypoxemia fundamentally determines the elite apnea breaking point, what determines the elite apnea breaking point with prior 100% O₂ breathing? The elite ‘hyperoxic’ breath-hold may be held for over 20 minutes (see Chapter 5). At the breakpoint, arterial oxygen saturation is well above euoxia (PaO₂ ~350 mmHg). In the non-elite breath-holder, the hyperoxic breath-hold breaking point stems mainly from respiratory muscle and to a lesser extent (because of the hyperoxic-induced carotid body attenuation) chemoreflex stress (Klocke and Rahn, 1959). Likewise,
in the elite, the hyperoxic breath-hold breakpoint is likely explained almost exclusively by severe respiratory muscle stress, which is accentuated with the reduced lung volumes from the disproportionate extraction of oxygen versus release of carbon dioxide (Hong et al., 1971). Ultimately, as demonstrated by Klocke and Rahn more than 50 years ago (Klocke and Rahn, 1959), the theoretical maximum duration of a hyperoxic breath-hold is determined by the length of time before reaching lung residual volume, or atelectasis, which itself is dependent on barometric pressure, vital capacity, and consumed oxygen (metabolic rate). The maximum possible breath-holding time with hyperoxic pre-breath can thus be calculated via the equation below as determined by (Klocke and Rahn, 1959).

\[
\frac{\text{Vital Capacity (STPD)}}{\text{VO}_2(\text{STPD})}
\]

Where; \(\text{VO}_2\) = volume of oxygen consumed (function of the oxidative metabolic rate), and STPD = the standard conditions for dry temperature and pressure. See (Klocke and Rahn, 1959) for conversion of body temperature pressures (BTPS).

### 2.4.5 Adaptations and maladaptations in trained apnea divers

Physiological adaptation, or maladaptation, occurs with repeated exposure to a given stress – extreme apnea is no exception. The following section briefly describes some of the physiological adaptions and to a lesser extent the proposed maladaptations associated with the practice of apnea diving.

Respiratory adaptations: It is generally accepted that the ventilatory response to \(\text{CO}_2\) is blunted in trained apnea divers (Masuda et al., 1981; Grassi et al., 1994; Ivancev et al., 2007; Walterspacher et al., 2011) and reviewed in (Ferretti, 2001) – in only one study was it similar (Dujic et al., 2008). Though less clear, some also suggest that the ventilatory response to hypoxia is depressed (Masuda et al., 1981; Walterspacher et al., 2011), however, this is not a consistent finding (Grassi et al., 1994; Breskovic et al.,
A ventilatory adjustment to CO₂ in particular likely prolongs the onset time before IBMs, increasing the ‘easy going’ phase of a prolonged apnea.

It is also generally accepted that trained apnea divers have larger lung volumes (Ferretti and Costa, 2003). Whether this is an adaptive or simply inherent characteristic of apnea divers remains debatable. In support of an adaptive characteristic, increased lung volumes (or forced vital capacity) may in part result from practiced glosospharyngeal insufflation, and strengthened inspiratory muscles (Ferretti and Costa, 2003). For example, Schagatay reports that eleven weeks of apnea training increases vital capacity by 0.45 L (Schagatay, 2014). Moreover, it was found that vital capacity was on average 1.8 L larger in practiced apnea divers than aged and sized matched controls (Schagatay, 2014), and trained US Navy skin divers have a ~15% larger vital capacity compared to untrained controls (Carey et al., 1956). Ama divers have also been reported to have a ~15% higher vital capacity than their non-diving counterparts [original studies reviewed in (Ferretti and Costa, 2003)]. However, a more recent study of 115 Ama divers compared to 33 healthy matched controls, report no differences in vital capacity (Tanaka et al., 2016). These latter findings may relate to the distinct breathing patterns in pearl divers compared to competitive apnea divers, whereby glosospharyngeal insufflation is avoided in the Ama of today to avoid excessive hyperventilation and the risk of blackout with many successive dives (Tanaka et al., 2016). Nevertheless, the ability to pack more air in the lungs attenuates the rate of blood oxygen desaturation during apnea, prevents lung collapse in depth dives, and lessens diaphragmatic stress associated with reduced lung volumes. Together, a larger lung volume and depressed sensitivity to hypercapnia is certainly advantageous for prolonging the duration of an apnea.

Vascular adaptations: Ama divers have greater central (aortic) arterial compliance compared to age, BMI and activity level controlled counterparts living in the same community (Tanaka et al., 2016). It was speculated that the circulatory stress and increased cardiac pulsation during apnea foster improved arterial elasticity; these changes may promote a greater bradycardia response to diving via more active baroreception. Altered arterial stiffness in trained versus untrained divers, however, is not a universal
finding (Steinback et al., 2010a). Moreover, in this same study, spontaneous assessment of the cardiovagal baroreflex gain was similar in the apnea divers compared to controls. Unfortunately the method of measuring arterial stiffness (whether central or peripheral) in Steinback et al., (2010a) was not reported, nor was the experience level of the apnea divers. Nevertheless, although using the pathophysiology of sleep apnea as a model for long-standing practice of apnea diving suggests that arterial compliance and baroreceptor gain would be impaired in apnea divers (Dempsey et al., 2010), it appears that, if anything, the opposite may be true. Future cross-sectional and longitudinal study on the vascular adjustments in trained apnea divers warrants attention.

Hemodynamic adjustments: It is well accepted that hypoxic exposure from living at high altitude increases erythropoiesis. Similar adjustments may occur in practiced apnea divers. For example, resting hemoglobin is higher in elite breath-holders compared to matched controls, and erythropoietin formation is increased following repeated apneas (de Bruijn et al., 2008). The later finding may stem from splenetic contractions, which can increase hemoglobin by 10% in elite breath-hold divers. Apnea performance has been correlated with the size of the spleen (Schagatay et al., 2012), and trained breath-hold divers have larger spleens than matched controls (Schagatay, 2014). Long-term apnea training with spleen size as the outcome measure, however, has not been performed.

Maladaptations: As mentioned, the effects of cyclic hypoxia in commercial apnea divers performing over 150 dives in a single day, notionally, may mirror some of the consequential physiological changes associated with sleep-disordered breathing. However, while cerebrovascular CO$_2$ reactivity is reduced in obstructive sleep apnea patients (Prilipko et al., 2014), it is unimpaired in elite breath-hold divers (Ivancev et al., 2007). Moreover, as previously mentioned, sympathetic baroreflex gain and respiratory muscle SNA modulation is also unimpaired in trained apnea divers (Steinback et al., 2010a), suggesting that, unlike in pathophysiological sleep apnea (Dempsey et al., 2010), apnea competition does not yield autonomic dysregulation. Nevertheless, long-term neurological problems [especially following cerebral decompression sickness (Kohshi et al., 2014; Matsuo et al., 2014)] have been reported. In support of the latter, peripherally
circulating plasma neuron specific enolase (a marker for acute neuronal damage) is increased following apnea competition (Kjeld et al., 2015). It is clear that more longitudinal research is required to understand the long-term maladaptations of apnea diving.

Acute risks: Although beyond the scope of this literature review, the major acute risks associated with extreme apnea include: 1) Barotrauma, drowning and shallow water blackout during depth dives (Lindholm and Lundgren, 2009); 2) Lung injury associated with glossopharyngeal insufflation (Chung et al., 2010); 3) Cardiac arrest, with the incidence increasing when dives are performed in the winter months when the water temperature is colder (Hong et al., 1967) - suggesting profound autonomic conflict (Shattock and Tipton, 2012).]; and 4) Cerebral decompression sickness [e.g. (Kohshi et al., 2014; Matsuo et al., 2014)] and nitrogen narcosis (Lindholm and Lundgren, 2009) with repeated depth dives.
Chapter 3 – Measurement Techniques

Detailed descriptions of the experimental protocols are outlined in the individual experimental Chapters. The purpose of the following Chapter is to briefly highlight and expand on the fundamental concepts of the major measurement techniques used in this Thesis, namely; the measurement techniques for quantification of CBF; blood gases; and respiratory sensitivity to changes in blood gases.
3.1 Cerebral Blood Flow / Velocity

Measures of CBF and velocity in this Thesis were accomplished by use of Doppler ultrasound. Intra-cranial vessels encapsulated within the skull, i.e. middle cerebral artery (MCA) and posterior cerebral artery (PCA), were insonated for velocity only using trans-cranial Doppler (TCD). The extra-cranial vessels feeding into the brain, i.e. internal carotid artery (ICA) and vertebral artery (VA), were imaged for both velocity and diameter, and therefore flow, using linear array Duplex ultrasound. The following sections briefly highlight the utility and caveats of these measures.

3.1.1 Doppler Physics

Both TCD and Duplex ultrasound apply the principles of the Doppler shift, whereby the change in frequency of a reflected wave (sound wave with ultrasound) is proportional to the velocity of the reflecting object (red blood cells in blood) moving towards or away from the observer (the transducer). When blood is moving towards the transducer, the distance of the reflected waves shortens therefore increasing their frequency; when blood is moving away from the transducer, the distance between sound waves increases, and the frequency decreases. The frequency shift is thereafter converted to velocity in cm/s using a fast Fourier transform algorithm.

3.1.2 Trans-cranial Doppler

In 1982, Aaslid and colleagues were the first to demonstrate TCD as a non-invasive assessment tool to provide an index of CBF (Aaslid et al., 1982). It was shown that by using a lower ultrasonic frequency (~2 MHz) than conventional ultrasound (~10 MHz), the attenuation of sound from the skull bone and soft tissue was much less. Following this report by Aaslid and colleagues, the advancement of cerebrovascular knowledge in health and pathology, for example, stenosis, anemia, brain death, emboli, and cerebral vasospasm, has largely been shaped by use of TCD. Although the immergence of newer high resolution MRI techniques has become gold standard for static measures of the
intracranial arteries (MacDonald and Frayne, 2015), the relative ease of use, high temporal resolution, and inexpensive cost of TCD has kept it mainstay in many neurophysiological clinics and physiology laboratories. Indeed, TCD provides the only non-invasive measure of beat-by-beat velocity of intra-cerebral vessels. Figure 3.1 provides an MR angiograph image of the PCA and MCA with a typical blood velocity recording attained with TCD.

Figure 3.1. A) Magnetic resonance angiograph of a normal Circle of Willis showing the right and left internal carotid arteries (ICA) and basilar artery (BA). B) Arrows denote the M1 segment of the middle cerebral arteries (MCA), and a normal (healthy) blood velocity recording from trans-cranial Doppler. C) Arrows denote the P1 segment of the posterior cerebral arteries (PCA) and a normal (healthy) blood velocity recording from trans-cranial Doppler. Note the lower velocity in the PCA than the MCA, on average by ~30% (Willie et al., 2012). Insonation of both the MCA and PCA is through the temporal window. Figure reproduced, with © permission, from (Smith et al., 2012).
**Vessel acquisition and data collection:** Vessel search techniques, reliability and repeatability of TCD are detailed extensively in (Willie et al., 2011). Briefly, a headband fixation device (model M600 bilateral head frame, Spencer Technologies) is used to fix the probes in position. Signal quality is optimized using standardized search techniques that produced test-retest reliability of ~3% and 2% for MCAv and PCAv, respectively. Nevertheless, once fixed into place, probe positioning can be kept constant between interventions lasting less than a few hours, thereby removing test-retest reliability issues. The MCA is usually insonated through the left temporal window, at a depth 1 cm distal to the MCA-anterior cerebral artery bifurcation, and the PCA is usually insonated at the P1 segment through the right temporal window. The MCAv and PCAv are integrated into PowerLab® and LabChart® (ADInstruments) for online monitoring and offline analysis.

**Limitations of trans-cranial Doppler:** When TCD is used as a surrogate for volumetric blood flow, two main limitations apply; 1) assumption of ~0° angle insonation, and 2) assumption that the insonated vessel diameter remains constant.

1) Acquisition of true red blood cell velocity using TCD is dependent on a 0° angle of insonation. That is, true velocity is dependent on the cosine of the insonated angle. An angle difference of 60° will underestimate true velocity by 50% [\(\cos(60) = 50\)]. With TCD, the angle of insonation can only be assumed, and variations from 0° are not corrected for. Proper search and optimizing techniques are therefore instrumental to accurate measures. With respect to repeatability, choosing the identical acoustic window and insonation depth is paramount.

2) The assumption that velocity provides an accurate surrogate for volumetric blood flow (ultimately the variable of interest) is contingent upon a constant vessel diameter. Changes in diameter of MCA during manipulation of PaO\(_2\) (Wilson et al., 2011), PaCO\(_2\) (Giller et al., 1993; Coverdale et al., 2014; Verbree et al., 2014; Coverdale et al., 2015) have been reported. Figure 3.2 provides a schematic of the estimated changes in MCA diameter with alterations in PaCO\(_2\). Measures of CBF using the surrogate of velocity
must therefore be interpreted with the potential underestimation, if the vessel becomes dilated, or overestimation, if the vessel becomes constricted. Correction for diameter changes (if known) can be made using a continuity equation for fluid below:

\[
Volumetric \text{ Flow} = v_1 \times \pi \times r_1^2 = v_2 \times \pi \times r_2^2
\]

Using the equation above, assuming that velocity remains unchanged, a 2% change in diameter would yield a ~4% change in volumetric flow.

**Figure 3.2.** Change in MCA diameter over end-tidal partial pressure of CO\textsubscript{2} measured by MRI. Data from derived from (Valdueza et al., 1997; Serrador et al., 2000; Coverdale et al., 2014; Verbree et al., 2014). Original Figure adapted from (Ainslie and Hoiland, 2014)

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### 3.1.3 Duplex ultrasound of extra-cranial cerebral arteries
Peripheral Duplex ultrasound provides a measure of velocity (via the Doppler shift) and vessel diameter by capturing images in both M-mode (motion mode) and B-mode (brightness mode), respectively, thereby allowing calculation of volumetric flow (see 3.1.4. Duplex ultrasound analysis). Unlike TCD, with Duplex ultrasound, the insonation angle is known and any variation from 0° (up to, or fixed at 60°) is corrected for. Under the assumption of equal flow in the contralateral neck arteries, unilateral insonation of the ICA and VA provides a measure of global CBF at the highest possible temporal frequency. In turn, global CBF can be calculated as: (2 x ICA flow) + (2 x VA flow).

However, although similar blood flow is received through the right and left ICA (Zarrinkoob et al., 2015), the left VA on average receives more blood than the right VA [~10%; (Schoning et al., 1994; Seidel et al., 1999; Thomas et al., 2015)]. Comparison of absolute CBF between study groups should therefore consider ICA and VA flow measures on both sides. Search techniques, image optimization and technical aspects of using linear array duplex ultrasound for blood flow measures of the ICA and VA are extensively covered in (Thomas et al., 2015).

The major limitation with Duplex vascular ultrasound is its dependency on the operator. Small changes in the insonation angles (% error equivalent to the cosine of the angle change) in addition to any differences in diameter selection throughout an intervention, will dramatically affect the measure of flow. The average resting within day coefficient of variation (CoV) for the two sonographers used in this Thesis was 8.2% for the ICA, and 7.5% for the VA. In this Thesis, the right ICA was on average insonated ~2cm from the carotid bifurcation, while the left VA was insonated at the C5-C6 or C5-C4 space. The steering angle was fixed to 60-degrees among all trials, and the sample volume was placed in the center of the vessel adjusted to cover the entire vascular lumen.

3.1.4. Duplex ultrasound analysis

A high-resolution video screen-capture of the m-and b-mode ultrasound image allows offline analysis of ICA and VA blood flow. See Figure 3.3 for typical duplex ultrasound
images of the VA and ICA. The video file is analyzed offline using automated edge detection software (LabView®). A criterion of simultaneous measures for luminal diameter and velocity over a minimum of 10 cardiac cycles is used to calculate flow. Edge detection software is proven reliable for determining arterial diameter (Woodman et al., 2001) and blood flow responses (Black et al., 2008), while largely removing the potential for observer bias compared to manual (calliper) measures of diameter (Thomas et al., 2015). A region of interest is selected across the vessel for diameter tracking, and the envelope of the velocity is traced (Figure 3.4). Data are averaged from a sampling frequency of 30 Hz. Volumetric blood flow is subsequently calculated from the below equation:

\[
\text{Volumetric blood flow} = (\pi (0.5 \cdot d)^2) \cdot ((1/2)(V_{\text{max}}))
\]

The first part of the equation (\(\pi (0.5 \cdot d)^2\)) calculates the cross section area of the vessel (where \(d = \text{diameter}\)), and the second part calculates the mean velocity (where \(V_{\text{max}} = \text{the envelope velocity}\)). The product of cross sectional area and velocity in turn yields volumetric flow. A schematic example of the edge detection software is depicted in Figure 3.4.
Figure 3.3. Representable duplex ultrasound image of the right vertebral artery (VA) and internal carotid artery (ICA) on a healthy female subject (29 years old). For comparative purposes, images were captured at an identical scale. Note the larger vessel diameter and higher velocity in the ICA compared to the VA. On average the ICA’s supply ~70-80% of the total CBF, while the VA’s supply the remaining ~20-30% (Willie et al., 2012).
Figure 3.4. Example of the edge detection software applied to the Duplex ultrasound images. A representable ICA is depicted here. The yellow rectangles represent the user-selected regions of interest, and the dotted lines represent the edge detection tracking by the software.
3.2 Cerebral Arterial-Venous Blood Gas and Metabolite Measures

In this Thesis, blood sampled from the radial artery and right internal jugular vein represent the cerebral arterial and venous blood, respectively. It is thus assumed that the peripheral arterial blood (radial) is representative of carotid and vertebral artery blood, and that the internal jugular veins are largely representative of global cerebral blood drainage, since they contribute >90% of the total drainage in the supine position (Wilson et al., 1972; Macmillan and Andrews, 2000). For cannulation of the internal jugular vein in this Thesis, an 8cm jugular catheter was placed under ultrasound guidance and local aesthetic (1% lidocaine), and advanced cephalad towards the jugular bulb with the resting catheter tip position estimated above the C1/C2. Negligible facial vein contamination was assured by a resting SvO₂ below 75%, and upon hearing a ‘swishing’ sound behind the ear when flushing the catheter with saline (Macmillan and Andrews, 2000). See experimental Chapters 5 and 6 for further description of the catheter placement techniques.

3.2.1 Blood gas, pH, oximetry and metabolite analysis

Arterial and venous pH, blood gases, glucose, lactate, and SO₂ were analyzed in whole blood using a commercially available cartridge-based analyzer with integrated CO-oximetry, blood gas, electrolyte and metabolite measurement capabilities; ABL90 Flex, Radiometer, Copenhagen, Denmark. The application of bedside blood gas analyzers stems from the invention of electrodes sensitive to O₂ (invented by Leland Clark in 1954), CO₂, and pH (invented by Richard Stow and John Severinghaus in the late 1950’s (Severinghaus, 2002). Blood gas analysis has since then evolved with more sophisticated analytical methodologies, for example the use of spectrophotometry. The ABL90 Flex has shown excellent measurement agreement with other gold standard (ABL800, Radiometer) analysis techniques in all variables (De Koninck et al., 2012; Poesen et al., 2013). The ABL units are therefore regularly used for point-of-care clinical decisions, and also in the research laboratory. Radiometer utilizes a spectrophotometric method for
pH, blood gases, and metabolites measurements. Glucose and lactate is first converted to gluconic acid or pyruvate, respectively, and hydrogen peroxide. The hydrogen peroxide is then oxidized and the released electrons are proportional to the concentration of glucose / lactate. The SO₂ and Hb are measured by CO-oximetry. All measurements are standardized to 37°C.
3.3 Assessment of the Chemoreflexes

The ventilatory response to changes in blood gases was quantified using the steady state method (Chapter 4), and the hyperoxic rebreathing method (Chapter 5). The utility of these separate methodologies are briefly discussed here.

3.3.1 Steady-state ventilatory responses (Chapter 4)

Steady-state methods involve fixing a constant CO$_2$ or O$_2$ stimulus for ~5 to 20 minutes thereby providing a method to assess the combined ventilatory response of both O$_2$ and CO$_2$. Using this method, Nielsen and Smith (1951) first described the combined influence of CO$_2$ and O$_2$ on the ventilatory response in humans, which was followed by Lloyd and Cunningham (1963) at the University of Oxford. The chemoreflex control of breathing to combined CO$_2$ and O$_2$ thereafter became known as the ‘Oxford’ model, or Oxford ‘fan’ due to the hyperbolic ventilatory response of steady state CO$_2$ with changing PO$_2$. The subsequent development of end-tidal forcing (Robbins et al., 1982) allowed steady-state methods to better control the chemosensory stimulus without the confounding variability of individual respiratory sensitivity that changes the individual PaO$_2$ and PaCO$_2$ for a given fixed inspired gas mixtures. End-tidal forcing therefore better standardizes the chemosensory stimulus (Duffin, 2011). Here, the P$_{ET}$CO$_2$, P$_{ET}$O$_2$, and inspiratory and expiratory tidal volumes are determined for each breath using specifically designed software, while breathing from a mouth piece connected to a pneumotachometer, gas sample line, and two-way valve with the inspirate connected to a 6L inspiratory reservoir bag. This system uses independent gas solenoid valves for O$_2$, CO$_2$, and N$_2$ and controls the volume of each gas being delivered to the inspiratory reservoir through a mixing and humidification chamber. With use of feedback information regarding P$_{ET}$CO$_2$, P$_{ET}$O$_2$, and inspiratory and expiratory tidal volume, the system prospectively targets the inspirate to bring end-tidal gases to the desired level. Gas control is fine-tuned using a feedback control and error reduction algorithm.
The primary caveat with steady-state ventilatory assessment (aside from using end-tidal gases as a surrogate for actual arterial blood gases) is that it relies on assumed linearity of the ventilatory response. In most cases, with varying CO₂, choosing a stimulus above the ventilatory threshold, but below the maximum ventilation, provides a linear response (Mohan et al., 1999). However, during steady-state hypoxia – depending on the degree of hypoxic stimuli – the ventilatory response increases quickly, peaking with one to three minutes, and then slowly declines, i.e., the so-called hypoxic ventilatory decline (Easton et al., 1986). Ventilatory responses to hypoxia are therefore generally expressed from the peak response, i.e. the hypoxic ventilator response.

3.3.2 Rebreathing (Chapter 5)

In 1892 Haldane and Smith first described rebreathing methods to assess the central chemoreflex ventilatory response [reviewed in (Duffin, 2011)]. In their first rebreathing apparatus, a hyperoxic gas was introduced into the bag, thereby ‘silencing’ the peripheral chemoreflex, and in turn isolating the central chemoreflex to CO₂. The rebreathing methods ostensibly, de-compartmentalize CO₂ gradients across the body. However, this assumes that brain CO₂ production is equivalent to whole-body CO₂ production. Furthermore, this test used as a measure to ‘isolate’ the central chemoreflex response assumes that the peripheral and central chemoreflex act independently, that is, are additive (see Chapter 2 for discussion). Lastly, in both the steady-state and rebreathing methods, end-tidal gases are used as a surrogate for arterial blood gases. In many experimental situations, however, arterial and end-tidal gases can differ. To circumnavigate this problem, the experimental chapters of this Thesis indexed the ventilatory response against arterial blood gases, not end-tidal gases.

In this Thesis, stemming from the method described by Haldane and Smith, a modified ‘Duffin’ hyperoxic-rebreathing method was employed. This approach was further modified based on recent work by (Boulet et al., 2016) that shortens the pre-hyperventilatory protocol from five to two minutes. The prior hyperventilation (to a P_{ET}CO₂ of ~20 mmHg) provides a means to assess the ventilatory recruitment threshold,
by starting the rebreathing below resting eucapnia. Exact details of the rebreathing procedure used in this Thesis are described in Chapter 5.
3.4 Cerebral Metabolic Calculations

Content of arterial (CaO$_2$) and venous (CvO$_2$) oxygen are calculated using the equations:

$$\text{CaO}_2(\text{ml.dl}^{-1}) = [\text{Hb}] \cdot 1.36 \cdot \frac{\text{SaO}_2(\%)}{100} + 0.003 \cdot \text{PaO}_2$$

$$\text{CvO}_2(\text{ml.dl}^{-1}) = [\text{Hb}] \cdot 1.36 \cdot \frac{\text{SvO}_2(\%)}{100} + 0.003 \cdot \text{PvO}_2$$

Where 1.36 is the affinity for oxygen to hemoglobin for a given arterial saturation, and 0.003 is the percentage of oxygen dissolved in the blood. Values are expressed as ml of O$_2$ per 100 ml of blood (ml.dl$^{-1}$).

Cerebral delivery of oxygen (CDO$_2$) is calculated from:

$$\text{CDO}_2(\text{ml.min}^{-1}) = \text{CaO}_2 \cdot \frac{\text{gCBF}}{100}$$

The cerebral metabolic rate of oxygen (CMRO$_2$) is calculated from:

$$\text{CMRO}_2(\text{ml.min}^{-1}) = (\text{CaO}_2 - \text{CvO}_2) \cdot \frac{\text{gCBF}}{100}$$

Cerebral oxygen, glucose and lactate extraction fraction is calculated from the arterial-venous content difference divided by the arterial value, and then multiplied by 100.

Net cerebral glucose and lactate exchange is calculated from:

$$\text{Net Gluc Exchange (mmol.min}^{-1}) = (\text{Gluc v} - \text{Gluc a}) \cdot \text{gCBF}$$
Net Lac Exchange (mmol·min\(^{-1}\)) = (Lac \text{v} - Lac \text{a}) \cdot gCBF

Where a negative value indicates a net uptake, and positive value indicates a net release. Glucose and lactate values are in mmol·ml\(^{-1}\) and gCBF is in ml·min\(^{-1}\).

The oxidative glucose index (OCI), which provides an estimation of oxidative versus non-oxidative metabolism (see (Ainslie et al., 2014)), is calculated from the equation below. The oxidative glucose index (OGI) is calculated the same way, but by omitting the addition of the halve lactate arterial-venous difference. In short, a reduction of 6 reflects the presence of non-oxidative metabolism, given that the oxidization of glucose by oxygen requires one oxygen molecule per six carbon atoms found in glucose, and carboxylation of lactate to pyruvate acid yields only one molecule of pyruvate, compared with the two derived from the breakdown of glucose. This is then converted to a percent OCI by dividing by 6 and then multiplying by 100.

\[
\text{OCI} (%) = \frac{CaO_2 - CVO_2}{(Glu_a - Glu_v) + 0.5(Lac_a - Lac_v)} \times \frac{6}{6} \times 100
\]
Chapter 4 – Experimental Study #1

‘Peripheral chemoreflex inhibition with low-dose dopamine; new insight into mechanisms of extreme apnea’

By: Anthony R. Bain, Zeljko Dujic, Ryan L. Hoiland, Otto F. Barak, Dennis Madden, Ivan Drvis, Mike Stembridge, David B. MacLeod, Douglas M. MacLeod, and Philip N. Ainslie
4.1 Overview

The purpose of this study was to determine the impact of peripheral chemoreflex inhibition with low-dose dopamine on maximal apnea time, and the related hemodynamic and cerebrovascular responses in elite apnea divers. In a randomized order, participants performed a maximal apnea while receiving either I.V. 2µg·kg⁻¹·min⁻¹ dopamine or volume matched saline (placebo). The chemoreflex and hemodynamic response to dopamine was also assessed during hypoxia (arterial O₂ tension, [PaO₂]~35mmHg) and mild hypercapnia (arterial CO₂ tension [PaCO₂]~46mmHg) that mimicked the latter parts of apnea. Outcome measures included apnea duration, arterial blood gases (radial), heart rate (HR, ECG), mean arterial pressure (MAP, intra-arterial), middle (MCAv) and posterior (PCAv) cerebral artery blood velocity (transcranial ultrasound), internal carotid (ICA) and vertebral (VA) artery blood flow (ultrasound), and the chemoreflex responses. Although dopamine depressed the ventilatory response by 27±41% (vs. placebo; p=0.01), the maximal apnea duration was increased by only 5±8% (p=0.02). The PaCO₂ and PaO₂ at apnea breakpoint were similar (p>0.05). Compared to placebo, dopamine increased HR and decreased MAP during both apnea and chemoreflex test (p all<0.05). At rest, dopamine compared to placebo dilated the ICA (3.0±4.1%, p=0.05) and VA (6.6±5.0%, p<0.01). During apnea and chemoreflex test, conductance of the cerebral vessels (ICA, VA, MCAv, PCAv) was increased with dopamine; however, flow (ICA and VA) was similar. At least in elite apnea divers, the small increase in apnea time and similar PaO₂ at breakpoint (~31mmHg) suggest the apnea breakpoint is more related to PaO₂, rather than peripheral chemoreflex drive to breathe.
4.2 Background

A voluntary breath-hold breakpoint in the untrained but motivated person normally occurs within 1 to 2 minutes, and is generally associated with an autonomic drive to breathe that provokes involuntary contractions of respiratory muscles (involuntary breathing movements, IBMs) (Lin, 1982). The IBM onset is aptly termed the ‘physiological break point’ (Parkes, 2006). In the untrained breath-holder, the IBM onset and therefore apnea duration may be doubled in duration by phrenic and glossopharyngeal nerve blockade (Noble et al., 1971; Parkes, 2006), and in turn is in large part mediated by powerful chemosensory and type III and IV diaphragmatic afferents (Parkes, 2006, 2012). In contrast, the underlying mechanism(s) of an extreme apnea breakpoint remains poorly characterized (Ferretti, 2001; Parkes, 2006, 2012; Dangmann, 2015), as it occurs well beyond the physiological break point, evidenced by the current apnea world record of 11:35 min. The apneic period beyond the physiological break point, termed the ‘struggle phase’, is hallmarked by frequent IBMs, and comprises roughly 50% of the total breath-hold duration in the elite apnea diver (Dujic et al., 2009).

The carotid and aortic bodies (peripheral chemosensors) are highly sensitive to the catecholamine dopamine. At low doses, dopamine inhibits the calcium currents in chemoreceptor glomus cells (Zapata et al., 1969; Zapata, 1975; Gonzalez et al., 1994). The suppression of peripheral chemoreflex activity with low dose dopamine infusion (<5 µg/kg/min) prolongs the physiological break point and is reflected by reductions in oxygen saturations at apnea termination in untrained volunteers (van de Borne et al., 1998). In the elite apnea diver, however, delaying the physiological break point (onset of IBMs) may have no benefit in prolonging breath-hold time. That is, the elite apnea break point may be governed by cerebral metabolic needs (Dangmann, 2015), and therefore consciousness, rather than the autonomic drive to breathe per se. For example, although lower arterial oxygen partial pressure (PaO\textsubscript{2}) values have been reported (Ernsting, 1963), loss of consciousness in humans is suggested to occur at a PaO\textsubscript{2} threshold of ~27 mmHg (Nunn, 1987; Parkes, 2006), values on average very similar to the PaO\textsubscript{2} breakpoint previously recorded in elite apnea divers; ranging from 23 to 37 mmHg, with an average
of ~30 mmHg (Willie et al., 2014a). That the elite divers indicate that prolonging their breath-hold any further (by seconds) will, and has done (e.g. during competition and training), result in unconsciousness, corroborates the notion of a threshold consciousness breakpoint; that is, the point whereby any further drop in PaO$_2$ will result in unconsciousness. Notably, this average breakpoint PaO$_2$ closely reflects the 50% oxygen hemoglobin saturation ($P50$).

With low dose dopamine, an alternate impact on the elite maximal breath-hold may be via the known cardiovascular adjustments (Missale et al., 1998; Niewinski et al., 2014). For example, low dose dopamine likely interferes with the normal hemodynamic responses associated with the mammalian dive reflex (e.g. peripheral vasoconstriction and attenuated heart rate), and prevents a rise in cerebral blood flow that maintains the cerebral oxygen delivery (Willie et al., 2014a). As such, the primary aim of the study was to determine if the maximal breath-hold duration in elite apnea divers was altered by the administration of low dose (2 µg/kg/min) dopamine. In a randomized order, elite apnea divers performed a maximal breath-hold while receiving either low dose dopamine or placebo. The cerebrovascular, hemodynamic and arterial blood gas responses were quantified throughout. The chemoreflex and hemodynamic response was further independently assessed via the ventilatory response to hypoxia with mild hypercapnia. It was hypothesized that 1) low dose dopamine infusion in elite apnea divers would decrease the ventilatory response to hypoxia, and in turn delay the onset of IBMs during a maximal breath-hold; however, apnea duration would be unaltered; and 2) the termination of apnea in the placebo and dopamine condition would occur at identical arterial oxygen tensions, suggesting that it is a threshold PaO$_2$ and therefore consciousness that determines actual break point in elite apnea divers, rather than a chemoreflex drive to breathe.
4.3 Methods

4.3.1 Participants

Thirteen elite apnea divers (1 female) were recruited from the National Croatian Apnea team. Subject specifications and training history are presented in Table 4.1. All participants were normotensive and free from any cardiovascular and respiratory disease. The experimental procedures were approved by the ethical committee of the University of Split School of Medicine, and by the Clinical Research Ethics Board of the University of British Columbia, and conformed to the standards set by the Declaration of Helsinki.

Table 4.1. Subject characteristics

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>FVC (L)</th>
<th>FEV₁ (L)</th>
<th>Years competing</th>
<th>PB Static apnea (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>26</td>
<td>187.5</td>
<td>79.8</td>
<td>7.4</td>
<td>3.5</td>
<td>394</td>
</tr>
<tr>
<td>Min</td>
<td>20</td>
<td>164</td>
<td>56.4</td>
<td>5.0</td>
<td>1.5</td>
<td>296</td>
</tr>
<tr>
<td>Max</td>
<td>48</td>
<td>194</td>
<td>106.4</td>
<td>8.6</td>
<td>14.0</td>
<td>560</td>
</tr>
</tbody>
</table>

PB Static apnea indicates the personal best record static (resting) apnea while face down in water. FVC = forced vital capacity (standard spirometry, without glossopharyngeal insufflation). FEV₁ = forced expired volume in one second.

4.3.2 Experimental Design

Experimentation for a single subject was completed on a single day, following strict adherence to pre-testing protocol, including abstinence from vigorous exercise and alcohol at least 48 hours, and from caffeine at least 12 hours before arriving to the laboratory. All testing was performed at the University of Split, School of Medicine, Department of Integrative Physiology. Upon arrival to the laboratory, a medical history and standard anthropometric and pulmonary functioning metrics were assessed. Following instrumentation, participants rested supine and randomly received either 2
μg/kg/min of dopamine, or volume matched saline (placebo) through an intravenous cathetor placed in the left arm. The apnea coach and participants were blinded to the experimental condition. The study drug (dopamine or placebo) was continually administered throughout the apnea and hypoxic breathing. To assure complete dopamine clearance (half life of 8 minutes) and recovery from the first apnea, participants were given a 75 min break before completing the second condition.

**Apnea protocol:** Following 20-min from the start of infusion, baseline measures were collected. The participants then underwent a preparatory phase that included two sub-maximal practice apneas. The first preparatory apnea was performed at the end of a normal expiration, until reaching six IBMs. Following a two minute rest, the second preparatory apnea was performed at total lung capacity, until reaching 10 IBMs. The practice apneas were performed as a well accepted method for prolonging the maximal apnea time. Following six minutes rest from the end of the second preparatory apnea, participants performed the maximal apnea. Participants were permitted to lung pack (glossopharyngeal insufflation) for the apnea. Identical preparatory phases were completed in each experimental condition (dopamine or placebo). Participants were instructed to break the maximal breath-hold before loosing consciousness. The national apnea coach was the respective coach for each participant, and was present at all times.

**Chemoreflex test:** Following ~15 min recovery from the maximal apnea, the hypoxic breathing test was performed using dynamic end-tidal forcing (Tymko et al., 2015). The gas delivery consisted of 1-minute baseline (eucapnic and normoxic) followed by 5-minutes of mild hypercapnic hypoxia. All outcome variables were collected during the last 30 seconds of the test (from minutes 4.5 to 5.0). The assigned values of PaCO₂ and PaO₂ were selected on an individual basis from the serial arterial blood gas samples taken during the maximal apnea, as the PaCO₂ recorded at 50%, and average PaO₂ value over the last 1.5 min of the first performed maximal apnea (whether placebo or drug). The average levels of PaCO₂ were 45.9±3.3 mmHg and PaO₂ were 36.1±6.3 mmHg and were kept equal during the dopamine and placebo trials (average difference of -0.4±2.0 and -
0.3±2.8 mmHg, respectively). In both conditions, the PaCO₂ was on average 5.0 mmHg higher from baseline resting conditions.

4.3.3 Measurements

*Blood gases:* Arterial PO₂, PCO₂, and O₂ saturation (SaO₂%), were sampled from a 20-gauge radial arterial catheter (Arrow, Markham, Ontario, Canada) placed in the right radial artery. The catheter was attached to an in-line waste-less sampling system (Edwards Lifesciences VAMP) and a pressure transducer located at the height of the right atrium (Edwards Lifesiences, TruWave transducer, Irvine, CA, USA). During the apnea, blood samples (2 mL) were collected at baseline, then every 30-seconds into the apnea, and immediately at apnea termination. During the hypoxic breathing test, blood samples were collected at baseline, and during the last 30 seconds of the test.

*Cardiovascular:* Heart rate (HR) was obtained from the R-R intervals measured from a three-lead ECG. Beat-to-beat arterial blood pressure was measured by finger photoplethysmography (Finometer PRO, Finapress Medical Systems, Amsterdam, Netherlands) normalized to intra-arterial pressures of the radial artery. Due to the frequency of blood sampling, intra-arterial pressure measures were available only for ~5 to 10 second data bins around each blood draw. End-apnea values for MAP and HR were acquired from an average of the last 30 seconds immediately before termination of the apnea.

*Cerebrovascular:* Cerebral blood velocity envelope of the right middle cerebral artery (MCAv) and left posterior cerebral artery (PCAv) were measured using a 2-MHz pulsed transcranial Doppler ultrasound system (Spencer Technologies, Seattle, WA). Volumetric blood flow of the right internal carotid artery (QICA) and left vertebral artery (QVA) was concomitantly measured using duplex vascular ultrasound (Terason 3000, Teratech, Burlington, MA).

Please refer to Chapter 3 for vessel location, image optimization and analysis.
Because of the involuntary breathing movements, concomitant images of ICA and VA velocity and diameter were unattainable up to the breath-hold break point in five subjects for the VA, and four subjects for the ICA. In these subjects, the vessel diameter only (not velocity) was measured during the latter parts of the breath-hold. The end breath-hold (i.e. 80% and 100%, or just 100%) VA and ICA flow was subsequently calculated from the change in PCAv and ICAv, respectively. Specifically, velocity of the ICA and VA was calculated from the velocity of the last successful ICA / VA velocity measurement multiplied by the % change in the respective MCAv / PCAv of the next stage. Peak (envelope) flow was subsequently calculated from: 0.25 * Π * (diameter²) * velocity. During the first 50% of the breath-hold, the average within subject r-squared value between the ICA velocity and MCAv was ~0.90, and ~0.95 for the VA velocity and PCAv. Nevertheless, estimations of ICA and VA flow during the latter part of the breath-hold were performed in the same subjects between conditions, to avoid potential within-subject trial differences attributed to measurement technique.

_Ventilatory data:_ The chemoreflex test was performed using end-tidal forcing (Tymko et al., 2015). The custom built end-tidal forcing system uses independent gas solenoid valves for oxygen, carbon dioxide and nitrogen for delivery of each gas. The system controls the volume of each gas delivered to the inspiratory reservoir through a mixing and humidification chamber. With use of feedback information regarding end-tidal pressures for CO₂ and O₂, and inspiratory / expiratory tidal volumes, the system prospectively targets the inspirate to bring end-tidal gas to the desired mmHg, while allowing participants to breath spontaneously. Gas control was fine-tuned using a feedback control and error reduction algorithm (LabView, Austin, TX). Respired gases were sampled at the mouth by securing a sample line into the mouthpiece attached to a calibrated online gas analyzer (model ML206, AD Instruments, Colorado Springs, CO). Respiratory flow for minute ventilation (VE), tidal volume (TV) and respiratory rate (RR) was measured at the mouth using pneumotachography (model HR 800L, HansRudolph, Shawnee, KS). Outcome variables during the hypoxic ventilatory tests were ventilatory, blood pressure, cerebrovascular (ICA, VA, MCAv and PCAv) and heart
rate responses for a given SaO$_2$.

*Involuntary breathing movements (IBM)*: The IBM onset was recorded in real time during the apnea by the apnea coach. The presence of IBMs were further verified from a chest plethysmography belt integrated into Labchart®.

*Statistical methods*: Mean values ± standard deviations (SD) are presented. To normalize the apnea data between participants, comparisons were made at 20% increments from the start to termination of the apnea. Values between baseline and 100% apnea were derived from 20-sec averages around the blood draw that best corresponded with the 20% increment (i.e. 20%, 40%, 60% and 80%). Normal distribution of variables was confirmed with the Shapiro-Wilk normality test. To compare differences in ICA, VA, MCAv, PCAv, HR, MAP, PaCO$_2$ and PaO$_2$ throughout the apnea, a 2×6 repeated measures ANOVA using the factors of condition (placebo and dopamine) and time [baseline (0%), 20%, 40%, 60%, 80% and apnea termination (100%)] was employed. Degrees of freedom were modified using the Greenhouse-Geisser correction when sphericity could not be assumed. When a significant main effect or interaction was observed post-hoc comparisons were made using Student’s paired t-tests corrected for multiple comparisons using the Holm’s sequential Bonferroni method. Post-hoc comparisons for significant interactions were made using the changes from baseline. Simple *a priori* Student’s paired t-tests were used to compare differences in apnea time, PaCO$_2$ and PaO$_2$ at the IBM onset, IBM onset time, and percent of apnea in the struggle phase (with IBMs) between placebo and dopamine. Differences in the ventilatory, PCAv, MCAv, ICA, VA, MAP and HR response to mild hypercapnic hypoxia between placebo and dopamine were also compared using a priori Student t-tests. Significance was set at a confidence level of 95% (p<0.05). Statistical measures were performed using the statistical software package IBM SPSS 20 for Mac (SPSS, Chicago, IL).
4.4 Results

4.4.1 Outlier

A statistical outlier (confirmed with a Dixon’s Q test, with >95% confidence) was found in the apnea protocol. This male subject was therefore removed from analysis for both apnea and chemoreflex tests; thus, analyzed data were based on 12 subjects. However, the outlier sets a trend (Fig. 4.1), suggesting that dopamine prolongs apnea time most in the less trained, while it may in fact reduce apnea time in the best (as determined by the placebo breath-hold time). When this participant is removed from the correlation in Figure 4.1, the $r^2$ is reduced from 0.418 ($p=0.017$) to 0.113 ($p=0.286$).

![Figure 4.1](image)

**Figure 4.1.** Relationship between the changes in apnea time with dopamine compared to placebo (y axis), over the placebo apnea time. $R^2 = 0.418$ with the outlier, and 0.113 without. Although heavily skewed by the outlier (circled), this relationship highlights the potential for low dose dopamine to in fact shorten or have no impact on apnea time in the ultra elite, where the peripheral chemoreflex drive to breathe likely has little to no role on the actual maximal breakpoint. Dopamine may attenuate the maximal apnea time in the ultra elite by preventing the reduction in HR and metabolism associated with the mammalian dive reflex. See discussion for detail.
4.4.2 Apnea

Apnea time and IBM onsets: Maximal apnea duration was increased (p=0.02) by 5±8% with dopamine compared to placebo (328±46 sec vs. 313±48 sec, respectively; Fig 4.1). The IBM onset time was also significantly (p<0.01) delayed by 29±22% with dopamine compared to placebo (185±51 sec vs. 143±37 sec, respectively; Fig. 4.2, top pane). In turn, the percent of apnea spent in the struggle phase (with IBMs) was significantly reduced with dopamine compared to placebo (44±11% vs. 54±9%; p<0.01).

Figure 4.2. Involuntary breathing movement (IBM) onset time (top), partial pressure of arterial CO₂ (PaCO₂) at the IBM onset (middle), and partial pressure of arterial O₂ (PaO₂) at the IBM onset (middle) during the maximal apnea with placebo and dopamine. The elongated dashed bar with open circles denotes mean data. Asterisks (*) denote significant difference from placebo.
Arterial blood gases: At the onset of IBMs, PaO₂ and PaCO₂ were significantly decreased (p=0.02) and increased (p<0.01), respectively, with dopamine compared to placebo (Fig 4.2, bottom and middle pane, respectively). However, there was no main effect or condition*time interaction between dopamine and placebo in PaO₂ or PaCO₂ throughout the apnea (p all >0.05) (Fig. 4.3). Of note, a more liberal statistical analysis with simple a priori single tailed t-tests of PaO₂ and PaCO₂ between dopamine and placebo at the termination of apnea, further revealed no significant differences (p=0.100 and p=0.360, respectively).

Figure 4.3. Dynamics of arterial partial pressure of oxygen (PaO₂; top panel), and carbon dioxide (PaCO₂; left panel) throughout a maximal apnea with placebo (closed circles) and dopamine (open circles).

Hemodynamics: Values for HR and MAP throughout the apneas are presented in Figure 4.4. There was a significant main effect (p<0.01) and condition*time interaction (p<0.01) for HR during the dopamine compared to placebo apneas. Post-hoc tests revealed a significant elevation in HR at each time point with dopamine compared to placebo (p all <0.05). The change (increase) in HR from baseline was significantly greater at 60% and 80% of the apnea in the dopamine compared to the placebo trial (p both <0.01). A significant main effect (p<0.01) and condition*time interaction (p<0.01) was also observed for values of MAP between placebo and dopamine apneas. Post-hoc comparisons revealed significantly lower MAP values at 20% (p=0.02), 40% (p=0.02), 60% (p<0.01), 80% (p<0.01) and 100% (p=0.02), but not at baseline (p=0.243) with
dopamine compared to placebo. The MAP change from baseline was significantly lower with dopamine at 20% (p=0.01), 40% (p=0.03), 60% (p<0.01), and 80% (p<0.01), compared to placebo.

**Figure 4.4.** Mean arterial pressure (MAP, left pane) and heart rate (HR) response throughout a maximal apnea with placebo (closed circles) and dopamine (open circles). Asterisks (*) denote a significant difference between placebo and dopamine. Daggers (†) denote a significant difference in the change from baseline between placebo and dopamine.

*Intra-cerebral hemodynamics:* Values for MCAv and PCAv throughout the apneas are presented in Figure 4.5 (bottom panes). In two participants, who did not respond to standard landmarking, compression or visual tests, reliable PCA recordings were unattainable; therefore, the sample size for PCAv was reduced to 10. There was no significant main effect of condition for MCAv (p=0.891) or PCAv (p=0.271). However, there was a significant condition*time interaction for both MCAv (p=0.01) and PCA (p=0.04). Post-hoc comparison revealed an attenuated increase from baseline in MCAv at 60% (p=0.05), and in PCAv at 20% (p=0.03), 40% (p=0.03), 60% (p<0.01) and 80% (p=0.04) of the apnea with dopamine compared to placebo. There was a significant main effect (p=0.015) and condition*time interaction (p=0.023) in MCAv conductance (i.e. MCAv/MAP) between dopamine and placebo. The post-hoc comparisons revealed a significantly elevated MCAv conductance at 80% (0.86±0.13 vs. 0.72±0.13 cm·s⁻¹·mmHg⁻¹, p<0.01) and at 100% (0.82±0.21 vs. 0.70±0.12 cm·s⁻¹·mmHg⁻¹, p=0.04) with
placebo compared to dopamine. Post-hoc testing revealed no significant differences at any time point in the change from baseline MCAv conductance between placebo and dopamine. In contrast to the MCAv conductance, there was no significant main effect (p=0.102) or condition*time interaction (p=0.193) in PCAv conductance between placebo and dopamine.

**Figure 4.5.** Cerebrovascular responses throughout a maximal apnea with placebo (closed circles) and dopamine (open circles). Starting from the top left pane; ICA = internal carotid artery blood flow; VA = vertebral artery blood flow; MCAv = middle cerebral artery velocity; PCAv = posterior cerebral artery velocity. Asterisks (*) denote a significant difference between placebo and dopamine. Daggers (†) denote a significant difference in the change from baseline between placebo and dopamine.

**Extra-cerebral hemodynamics:** There was no main effect (p=0.151) or condition*time interaction (p=0.318) in VA flow during the apnea between dopamine and placebo (Fig. 4.5). In contrast, there was a significant main effect of condition (p=0.027), but no
interaction (p=0.209) in ICA flow between dopamine and placebo (Fig 4.5). Post-hoc analysis revealed a significantly lower ICA flow at 60% of the apnea with dopamine compared to placebo (p=0.05, Fig. 4.5). There was no main effect (p=0.711) or condition*time interaction (p=0.401) in the VA conductance (i.e. VA/MAP) between dopamine and placebo. Likewise, there was no main effect (p=0.357) or condition*time interaction (p=0.334) in ICA conductance between dopamine and placebo.

### 4.4.3 Chemoreflex test

Because of the relatively low level of hypercapnia (average PaCO$_2$ of 45.9±3.3 mmHg) but extreme level of hypoxia (average PaO$_2$ of 36.1±6.3 mmHg), measures during the chemoreflex test are expressed as changes relative to absolute reduction in SaO$_2$ only. Presenting data over SaO$_2$ and not the PaO$_2$ was chosen because of the linear relationship between Ve and SaO$_2$, to account for the hypercapnia induced Bohr effect, and to compare with existing literature. As predicted, the ventilatory response to hypoxia at the 4.5 to 5.0 time bin (ΔVe/ΔSaO$_2$) was significantly depressed by 27±41% with dopamine compared to placebo (p=0.01; Fig. 4.6, left pane). Because of the known temporal Ve pattern to steady-state hypoxia, a secondary assessment of Ve at the peak response was performed. The peak Ve response occurred at 180±66 sec with dopamine, and earlier with placebo, at 140±70 sec, p=0.003. Similarly to the 4.5 to 5.0 min time bin, the magnitude of the Ve was blunted with dopamine (39.2±11.2 L/min) compared to placebo (48.7±10.5 L/min), p=0.01.
Figure 4.6. Ventilatory slope (left), mean arterial pressure slope (middle) and heart rate slope (right) to severe hypoxic ($\text{PaO}_2 = 36.1 \pm 6.3 \text{ mmHg}$) and mild hypercapnic breathing ($\text{PaCO}_2 = 45.9 \pm 3.3 \text{ mmHg}$) with placebo versus dopamine. Asterisks (*) denote significant difference from placebo.

**Hemodynamics:** The MAP ($\Delta \text{mmHg}/\Delta \text{SaO}_2$) and HR ($\Delta \text{BPM}/\Delta \text{SaO}_2$) responses to hypoxic breathing with dopamine and placebo are depicted in Figure 4.6. Dopamine significantly reduced the MAP response by $52 \pm 46\%$ compared to placebo ($p<0.01$). In contrast, dopamine significantly increased the HR response by $200 \pm 336\%$ compared to placebo ($p<0.01$). Because the HR response follows the Ve response to hypoxia, the HR at the peak Ve was further assessed. Similarly to the 4.5 to 5.0 min time bin, the peak HR response was increased with dopamine ($91.3 \pm 13.2 \text{ bpm}$) compared to placebo ($87.9 \pm 11.3 \text{ bpm}$), $p<0.01$.

**Intra-cerebral hemodynamics:** The responses in MCAv ($\Delta \text{cm}\cdot\text{s}^{-1}\cdot\Delta \text{SaO}_2^{-1}$) and PCAv ($\Delta \text{cm}\cdot\text{s}^{-1}\cdot\Delta \text{SaO}_2^{-1}$) to hypoxic breathing with dopamine and placebo are depicted in Figure 4.7. Dopamine significantly reduced the MCAv response by $24 \pm 25\%$ ($p<0.01$) and PCAv response by $26 \pm 40\%$ ($p=0.01$) compared to placebo. However, the MCAv ($p=0.02$) and PCAv conductance ($\Delta \text{cm}\cdot\text{s}^{-1}\cdot\Delta \text{mmHg}^{-1}\cdot\text{SaO}_2^{-1}$) response to hypoxia is increased with dopamine compared to placebo ($p=0.02$ and 0.03, respectively; Fig. 4.7).
Figure 4.7. Cerebrovascular response to severe hypoxic (PaO$_2$ = 36.1±6.3 mmHg) and mild hypercapnic breathing (PaCO$_2$ = 45.9±3.3 mmHg) with placebo versus dopamine. Starting from the top left pane; ICA = internal carotid artery blood flow; VA = vertebral artery blood flow; MCAv = middle cerebral artery velocity; PCAv = posterior cerebral artery velocity. Asterisks (*) denote significantly different from placebo. Although the MCAv and PCAv response to hypoxia is reduced with dopamine, this is confounded by an increased compliance of the cerebral vascular bed (middle panes). Therefore, true cerebral volumetric flow (ICA and VA) response to hypoxia is largely unaltered with dopamine.
Extra-cerebral hemodynamics: At baseline, dopamine caused a significant dilation in the ICA (3.0±4.1%, p=0.05; Fig. 4.8) and VA (6.6±5.0%, p<0.01; Fig. 4.8), compared to placebo. Although flow was unchanged, velocity was lower with dopamine compared to placebo (p both <0.05). The ICA and VA diameter and flow changes to hypoxia with dopamine compared to placebo were similar (p all>0.05; Fig 4.7). Similarly to the MCAv and PCAv conductance, dopamine significantly increased the ICA (p=0.05) and VA (p=0.01) conductance to hypoxia (Fig. 4.7).

Figure 4.8. Baseline internal carotid artery diameter (top) and vertebral artery diameter (bottom), with placebo versus dopamine. Asterisks (*) denote significantly different from placebo.
4.5 Discussion

The novel findings in this study are three-fold. First, blunting the peripheral chemoreflex by ~27% with low-dose dopamine in elite breath-holder only marginally (~5%) increased maximal breath-hold time, and the apnea breakpoint occurred at similar mmHg of PaO$_2$. The apnea breakpoint in the elite diver is therefore more determined by a threshold PaO$_2$, than it is by the peripheral chemoreflex drive to breath. Second, low-dose dopamine during an apnea and hypoxic breathing mitigated the rise in arterial blood pressure and caused a reflex increase in heart rate. And lastly, although dopamine blunted the rise in MAP for a given decrease in arterial oxygen saturation, the cerebral blood flow response was minimally altered, during both hypoxic breathing and apnea, likely owing to an increased compliance of the cerebral vascular bed.

4.5.1 Apnea breakpoint

The mechanism(s) of an apnea break point is no doubt integrative. Central to the current apnea break point hypothesis, however, is a critical integration of diaphragmatic (phrenic nerve) and to a lesser extent chemoreceptor (for PaO$_2$ and PaCO$_2$) afferents arriving at the respiratory center in the medulla [reviewed in: (Parkes, 2012)]. Central hypoxia, and oxygen sensing neurons in the central nervous system (e.g. in the thalamus, hypothalamus, pons, and medulla) are likely also involved, although experimental study in humans is limited (Neubauer and Sunderram, 2004). Of relevance to this study, lending credence to the role of chemoreception is the fact that the hypoxic ventilatory response is a significant predictor of breath-holding performance in the untrained breath-holder (Feiner et al., 1995); the ventilatory response to hypoxia (Masuda et al., 1981) and hypercapnia (Grassi et al., 1994) is generally depressed in elite apnea divers [see: (Ferretti, 2001) for review]; and breath-hold time in the untrained is approximately doubled with glossopharyngeal and vagus nerve blockade (Noble et al., 1971; Parkes, 2006).
While the current apnea break point hypothesis is dominated by autonomic input (from both chemoreception and lung afferents) (Parkes, 2006), some elite athletes often hold a maximal apnea until unconsciousness – a grounds for disqualification from competition in accordance with the governing body for international apnea competition (Association Internationale pour le Développement de L’Apnée). In the ultra elite diver, as examined in this study, it therefore seems reasonable to posit that the motivated break point is dependent on a functional limitation for consciousness, rather than from an autonomic drive to breath, *per se*. The current data indicate this breakpoint likely falls just below a threshold PaO$_2$ of approximately 30 mmHg (Willie *et al.*, 2014a) (and Fig. 4.3). The present data do not offer any interpretation on the molecular or neurological underpinnings of this threshold PaO$_2$ breakpoint, although some cerebral metabolic theories have been proposed (Dangmann, 2015).

If the apnea break point in the ultra-elite is indeed dependent on consciousness, prolonging the apnea break point in elite breath-holders must fundamentally rely on oxygen conservation. This may be achieved either by slowing oxidative metabolism (e.g. with beta-blockers; un-published observations), or by super saturating the tissue PO$_2$ before commencing an apnea (e.g. with hyperoxic pre-breathing; which can double maximal breath-hold time, with the current world record at 23:01 min). The similar arterial blood gases despite an average 15±23 second longer apnea time with dopamine compared to placebo may in turn point to a metabolic shift. That is, delaying the onset of IBMs with dopamine may have prolonged breath-hold time by mitigating the oxygen cost of inspiratory muscle contractions. Indeed, with the outlier excluded a simple linear regression between the change in IBM onset time and breakpoint yields an $r^2$ of 0.26 ($p=0.089$). With the outlier included, this relationship is stronger ($r^2$ of 0.688, $p<0.001$). Alternatively, chemoreceptor silencing with dopamine may have contributed to a psychophysical relaxation and reduction of skeletal muscle tone. A blunted PaCO$_2$ and PaCO$_2$ slope was not observed in the current study (absolute times), however, perhaps a result of the confounding elevated HR (discussed below) and marginal changes in breath-hold time.
4.5.2 Cardiovascular effects of low-dose dopamine

During a maximal breath-hold, elite apnea divers rely heavily on the mammalian dive reflex. The peripheral vessels are constricted, cerebral blood flow is maximized, and the heart rate is reduced (Butler and Jones, 1997; Heusser et al., 2009). In effect the cerebral oxygen delivery is maintained (Willie et al., 2014a), and metabolism is slowed. The mammalian dive reflex in humans is in large part owing to trigeminal nerve activation following cold-water facial immersion (Kawakami et al., 1967; Schaller, 2007). Although this effect was absent in the conditions of the current study, the dive reflex in humans is also mediated by carotid and aortic body activation via the glossopharyngeal and vagus nerve, respectively (Gross et al., 1976).

Low-dose dopamine infusion aimed to inhibit the carotid and aortic body blunted the MAP increase during the maximal apnea (Fig. 4.4), and hypoxic breathing (Fig. 4.6). A blunted MAP response in transient hypoxia with low dose dopamine infusion has been previously reported in healthy humans (Niewinski et al., 2014). The present data extends these findings by observing a compensatory increase in HR during the apnea (Fig. 4.4), and hypoxic breathing (Fig. 4.6). The HR response is in contrast to the findings by Niewinski et al., (Niewinski et al., 2014) and is likely explained by the differences in temporal hypoxic stimulation. For example, the hypoxic stimulus in Niewinski et al. (Niewinski et al., 2014) was acute and transient (10 – 45 seconds of 100% nitrogen breathing), compared to following 4.5-minute steady state exposure in the present study. The separate effect of dopamine to blunt the vasomotor, but increase the cardiac response to hypoxia is likely consequent to the direct vasodilatory properties of dopamine (see discussion below). That is, vasodilation (Fig. 4.8) likely led to a baroreflex mediated increase in HR. The cardiac baroreflex may have further been potentiated from carotid body chemoreflex inhibition (Somers et al., 1991; Ponikowski et al., 1997). For example, chemoreflex activation in the absence of ventilatory feedback increases both sympathetic and parasympathetic activity (de Burgh Daly et al., 1988). The increased HR response to dopamine may therefore have also been consequent to decreased parasympathetic activity, at least during the apnea.
4.5.3 Cerebrovascular effects of low-dose dopamine

Although its clinical use is now largely abandoned (Lauschke et al., 2006), low-dose dopamine infusion was traditionally used to improve renal blood flow via its direct D₁ receptor vasodilatory effects. Indirectly, and more systemically, low-dose dopamine also causes vasodilation via pre-junctional D₂ receptors on postganglionic sympathetic nerve terminals that inhibit release of norepinephrine (Missale et al., 1998). This latter vasodilatory effect was directly quantified by observing a 3.0±4.1% and 6.6±5.0% increase in resting ICA and VA diameter, respectively, with dopamine compared to placebo (Fig. 4.8). The lack of any considerable drop in resting MAP despite such pronounced vasodilation (although peripheral vascular resistance is unknown) was likely due to the elevated resting HR (Fig. 4.5) (i.e. MAP = cardiac output * total peripheral resistance). Nevertheless, the increased cerebral conductance - proportional to the fourth power of the vessel radius - with dopamine is clearly observed with the increased conductance of the ICA, VA, MCAv and PCAv with the hypoxic breathing (Fig. 4.7). The reduced cerebrovascular response to hypoxia with dopamine (Fig. 4.7) was therefore confounded by the increased compliance of the cerebral vascular bed, and highlights the need for concomitant diameter and velocity flow measures during experimentations prone to alter vessel diameter (Ainslie and Hoiland, 2014). In the end, the cerebral volumetric flow response in the hypoxic breathing trial, as demonstrated by ICA and VA flow, was negligibly lower with dopamine (Fig. 4.7, top panel) even despite the attenuated MAP response. On the other hand, the attenuated MAP response with dopamine compared to placebo during the apnea caused a significantly lower ICA flow at 60% (Fig. 4.5, top left panel). The decreased MAP response during the dopamine apnea was likely accentuated by the delayed onset of IBMs. Specifically, the IBMs result in sinusoidal surges in venous return (negative thoracic pressures) and consequently arterial pressure (Dujic et al., 2009).

A blunted cerebral blood flow response during the apnea will theoretically increase the drive to breathe by reducing the CO₂ washout at the central chemoreceptors (Ainslie and
Moreover, a blunted cerebral blood flow response will reduce cerebral oxygen delivery. However, cerebral oxygen delivery does not appear to be a factor in determining the elite apnea breakpoint [(Willie et al., 2014a), and unpublished observations]. In the present study, the moderate difference in ICA flow exclusively at 60% between dopamine and placebo is unlikely to have majorly affected cerebral CO$_2$ washout or the apnea breakpoint. Nevertheless, the interplay between cerebral oxygen delivery, tissue PO$_2$, tissue pH, and local metabolism (Dangmann, 2015) in determining the apnea breakpoint, and ultimately consciousness, remains to be determined.

4.5.4 Considerations

The apnea outcome of peripheral chemoreflex inhibition is selective to the unique population tested. In the untrained breath-holder, blunting the peripheral chemoreflex with low-dose dopamine may double maximal apnea and proffer a dramatically reduced peripheral oxygen saturation at the apnea breakpoint [e.g. from 78% to 63% (van de Borne et al., 1998)]. In the current study, breath-hold time was increased by only ~5% (with the outlier excluded); however, the variability of the response (Fig. 4.1) must be acknowledged. From previous studies in the untrained breath-holder (van de Borne et al., 1998), and in Fig. 4.1, it is evident that the underlying variations (aside from inherent genetic ability) seem to be influenced by level of training, which in turn may affect level of motivation. As exemplified in the outlier displayed in Fig. 4.1 (who at the time of writing is the world record holder for a dynamic apnea and apnea with oxygen pre-breathing), low-dose dopamine infusion may in fact decrease maximal breath-hold time. Here, the dopamine-induced elevation in HR combined with the decreased psychophysical relaxation and therefore potentially increased muscular tone, negatively impacted breath-hold time by increasing the metabolic rate. Indeed, in this participant the maximal apnea time was reduced by 79 seconds with dopamine compared to placebo, yet the end apnea PaO$_2$ (25.2 mmHg with placebo vs. 27.0 mmHg with dopamine) was similar. These data, again, support the notion of a threshold PaO$_2$ for the elite apnea breakpoint.
A growing body of literature now supports differential cardiovascular control between sexes (Joyner et al., 2015). In this study, the single female displayed cardiovascular responses and changes with dopamine that closely reflected the mean group responses. Specifically, in the dopamine compared to placebo apnea trial, apnea time was increased by 3.7%, while MAP was lower by 24 mmHg, and HR was increased by 4 BPM at the breakpoint. The sole female participant was therefore kept within the group analysis. Future study will be required to establish cardiovascular differences (if any) in prolonged breath-holding between the sexes.

An obvious caveat to this study is the lack of a control group. Nevertheless, the primary goal of this study was to test the impact of carotid body inhibition on prolonged apnea that yields extreme levels of arterial hypoxia and hypercapnia. As such, a separate independent control group would be irrelevant, given that control subjects would not tolerate such levels of hypoxia and hypercapnia. A second study limitation is the difficulty in completely standardizing the apnea conditions. For example, the protocol for the preparatory phase before each maximal apnea was identical between placebo and dopamine condition, however, because dopamine delayed the onset of IBMs, the preparatory phase was generally longer in the dopamine condition. Participants were instructed to perform an identical glossopharyngeal insufflation and pre-apnea breathing pattern for both trials. Although there is inherent within-subject variability in the glossopharyngeal insufflation and pre-breathing patterns between conditions, this was small given that the within-subject PaCO₂ at 30 seconds into the maximal apneas was nearly identical between conditions (difference of 0.42±2.5 mmHg).

4.5.5 Perspectives and Significance

The apnea break point in elite apnea divers seems to be determined by a threshold PaO₂ of approximately 30 mmHg, with little influence of the peripheral chemoreflex drive to breathe. Although the drive to breathe from lung afferents was not measured here, increasing apnea time in the elite diver must rely most on either a reduction in metabolism, or an increase in the oxygen availability (e.g. via increased lung volume and
hematocrit, improved splenic contraction, better O$_2$ extraction at the tissue level, increased mitochondrial bioenergetics, etc.). While highlighting the apnea break point in elite divers, this study further provides a unique insight to the cardiovascular responses to hypoxia that is modified with low-dose dopamine. Specifically, low-dose dopamine blunted the MAP, but increased the HR response to steady state hypoxia. These differences may be attributable to the vasodilatory properties of low dose-dopamine, a heightened cardiac baroreflex, and potentially reduced parasympathetic activity. Finally, low-dose dopamine caused a dilation of the cerebral arteries, however this did not affect the cerebral blood flow response (change) to 5-minutes of steady state hypoxia.
‘Forced vital capacity, but not central chemoreflex predicts the hyperoxic breath-hold duration in elite apnea divers’

By: Anthony R. Bain, Otto F. Barak, Ryan L. Hoiland, Ivan Drvis, Damian M. Bailey, Zeljko Dujic, David B. MacLeod, & Philip N. Ainslie
5.1 Overview

The mechanistic underpinning(s) of a hyperoxic apnea breakpoint in elite apnea divers has remained unexplored, although is unrelated to hypoxemia. The purpose of this study was to determine whether the elite hyperoxic apnea duration is predicted by the individual central chemoreflex (for CO₂) or forced vital capacity (FVC). Eleven elite apnea divers performed a maximal apnea with prior hyperoxic (100% oxygen) pre-breathing. For comparison, a maximal apnea was also performed without hyperoxia. The central chemoreflex was assessed by a modified hyperoxic re-breathing test (hyperoxic-hypercapnic ventilatory response: HCVR). Ventilatory responses are expressed as the L·min⁻¹ increase in ventilation (pneumotachometry) per mmHg⁻¹ increase in arterial CO₂ tension (PaCO₂) (radial artery cannulation). Pulmonary function was assessed using standard spirometry. The maximal hyperoxic apnea duration ranged from 807 to 1262 seconds (mean = 1034 seconds). The control apnea duration ranged from 267 to 431 seconds (mean = 348 seconds). Average HCVR was 2.0±1.2 L·min⁻¹·mmHg⁻¹ PaCO₂. The hyperoxic apnea duration was related to the FVC (r² = 0.45, p=0.024), but not with the HCVR (r² < 0.01). The control apnea was to a lesser extent related to the FVC (r²=0.32, p=0.072), and as expected not with the HCVR (r² < 0.01). Although the central chemoreflex has no impact on the elite hyperoxic apnea duration, lung volume can explain ~45% of the variability in apnea duration. A higher starting lung volume theoretically prolongs the apnea time before reaching residual lung volume during the hyperoxic apnea, and attenuates the rate of arterial oxygen desaturation during apnea without hyperoxia.
5.2 Background

The longest recorded volitional apnea duration without performing exercise (static apnea) is a remarkable 11:35 minutes. When apnea competitors are allowed to breath 100% oxygen prior, the apnea duration may double, with the current world record of 24:03 minutes. The underlying physiology determining the euoxic pre-breathe elite apnea breakpoint has received recent attention [e.g. (Parkes, 2006, 2012; Bain et al., 2015a)] however, the mechanistic underpinning(s) of a hyperoxic pre-breathe apnea breakpoint has remained unexplored.

The apnea breakpoint in the untrained but motivated breath-holder is governed largely from integration of pulmonary and to a lesser extent chemosensory afferents converging with the respiratory control centers in the brainstem (Parkes, 2006). Mounting input from these afferents eventual lead to involuntary respiratory muscle contractions, at which point the apnea is soon terminated (Mithoefer, 1965). On the other hand, when motivated, the maximal apnea breakpoint in elite breath holders encompasses partly separate mechanisms, which may simply rely on a critical level of oxygen tension to maintain consciousness (Bain et al., 2015a). That is, elite apnea divers are able to consciously suppress involuntary muscle respiratory contractions. That oxygen saturation plays a dominant role in determining the apnea breakpoint, beginning with euoxia pre-breathe, fails to explain the elite hyperoxic apnea breaking point – an event which occurs at an oxygen saturation of 100% (see Chapter 7).

Apnea generates hypercapnia, and for a given metabolism, the magnitude of hypercapnia is proportional to the duration of apnea. Prolonging the apnea duration with hyperoxia in turn yields more severe levels of hypercapnia compared to a shorter apnea that begins at euoxia (see Chapter 7). The elite hyperoxic breath-hold breaking point may therefore be in part determined by severe respiratory discomfort from CO₂ chemoreception and related acidotic build up. However, CO₂ chemoreception from carotid body afferents, encompassing ~40% of the ventilatory response to CO₂ (Smith et al., 2006), is attenuated from hyperoxia (Marshall, 1994). Pulmonary afferents may therefore better underlie the
hyperoxic elite-apnea breaking point. The latter becomes particularly relevant when lung volumes are reduced near residual volume from the disproportionate oxygen extraction relative to CO₂ release (Hong et al., 1971). In this case, a breath-hold prolonged any longer fundamentally runs the risk of atelectasis. Early work by Klocke and Rahn (1959) in motivated but untrained breath holding volunteers suggest that the theoretical maximal breath-hold duration with hyperoxia is simply determined by the initial lung volume and metabolic oxygen consumption, which in itself determines the apnea duration before lung squeeze.

The purpose of this study was to determine whether an elite maximal hyperoxic breath-hold duration is related to the individual hyperoxic-hypercapnic ventilatory response (HCVR), or the forced vital capacity (FVC). For comparison, a maximal breath-hold starting at euoxia was also performed. Given the estimations by Klocke and Rahn (1959), it was hypothesized that the FVC, but not the HCVR, will predict the duration of a maximal hyperoxic breath-hold in elite apnea divers. Based on the fact that larger lung volumes will attenuate the arterial oxygen desaturation during apnea without hyperoxia, it was also hypothesized that the FVC will predict the duration of a maximal breath-hold starting at euoxia.
5.3 Methods

5.3.1 Participants

This study was part of a larger study described in Chapter 7. Eleven elite breath-hold divers were recruited from the Croatian national apnea team. Participant specifications are shown in Table 5.1. All participants provided informed written consent before experimentation. The ethical committees of the University of Split School of Medicine, the University of British Columbia, and the University of South Wales approved the study procedures and experimentation. Years competing ranged from 3 to 15 years primarily in the discipline of dynamic (swimming) apnea. Three of the 11 participants were also involved with depth disciplines. Four subjects were considered world-class apnea competitors by placing top-ten within the last three years in international competition in at least one event. Participants were free from respiratory disease and known cardiovascular disease assessed by a medical history questionnaire.

Table 5.1. Subject characteristics

<table>
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<th></th>
<th>Age</th>
<th>Weight</th>
<th>Height</th>
<th>BMI</th>
<th>FVC</th>
<th>FVC%</th>
<th>FEV1</th>
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<td>180.8</td>
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<td>6.6</td>
<td>131.6</td>
<td>5.1</td>
<td>123.2</td>
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<tr>
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<td>15.0</td>
<td>11.4</td>
<td>2.2</td>
<td>1.5</td>
<td>13.7</td>
<td>1.0</td>
<td>13.2</td>
</tr>
</tbody>
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5.3.2 Experimental Design

All experimentation for a single subject was completed on a single day, at the Department of Integrative Physiology, University of Split, School of Medicine, Croatia. Participants arrived to the laboratory following abstinence from vigorous exercise, alcohol, and caffeine at least 24 hours prior. Upon arrival to the laboratory a standard spirometry pulmonary function test was performed (Quark b², Cosmed, Rome, Italy) for
measures of FVC and forced expired volume in the first second (FEV$_1$). Following the spirometry test, a 20-gauge arterial catheter (Arrow, Markham, Ontario, Canada) was placed in the right radial artery under local anesthesia (1% lidocaine) and attached to an in-line waste-less sampling system (Edwards Lifesciences VAMP). Blood samples (2 mL each) were acquired for measurement of carbon dioxide tension (PaCO$_2$) during the HCVR test analyzed immediately using a commercially available cassette based blood gas analyzer (ABL90 Flex, Radiometer, Copenhagen, Denmark). The order of trials for each subject consisted of; 1) the control apnea, 2) the hyperoxic apnea, and lastly 3) the HCVR test. Ample rest was allowed between tests, with a minimum 30 min break between. Both apneas were performed while lying supine in a bed, without water immersion or facial cooling. The hyperoxic apnea was performed after the control apnea due to the potential fatiguing factor associated with the longer hyperoxic apnea.

Euoxic ‘Control’ Apnea: A standard preparation protocol was performed before the control apnea (see Chapter 7). Briefly, preparation consisted of two sub-maximal apneas, one performed following a normal expiration, and the second at total lung capacity. Before the maximal apnea, participants hyperventilated to achieve an end-tidal CO$_2$ tension ($P_e$CO$_2$) of ~20 mmHg. During the hyperventilation respiratory gases were sampled at the mouth connected to a calibrated gas analyzer (ADInstruments). The calculated $P_e$CO$_2$ was displayed in real-time using LabChart (ADInstruments), and the rate of hyperventilation was controlled with auditory feedback from the researcher. Apnea began after a full inspiration, but without glossopharyngeal insufflation (lung packing). Lung packing was avoided to best compare the initial apnea lung volumes with the FVC, i.e. without the confounding impact of variably increased lung volumes above total lung capacity at apnea onset.

Hyperoxic Apnea: Aside from the absence of water immersion, the hyperoxic protocol was designed to best replicate procedures performed in competition (personal correspondence, Croatian National apnea coach). Before the apnea, participants breathed for 15-minutes on a two-way valve with the inflow connected to a Douglas bag containing 100% oxygen, and the expired port to the ambient air. During the last 5-
minutes of breathing 100% oxygen, participants hyperventilated to achieve an end-tidal CO\textsubscript{2} tension (P\textsubscript{ET}CO\textsubscript{2}) of ~20 mmHg, similar to the control apnea. Apnea began at the end of the 5-min hyperventilation after a full inspiration of 100% oxygen to total lung capacity, but again without glossopharyngeal insufflation.

Hyperoxic-Hypercapnic Ventilatory Response: The HCVR was performed following ~30 to 45 minutes rest of the apnea via a hyperoxic rebreathing test, based on a modified version from Boulet and colleagues (Boulet \textit{et al.}, 2016) of the ‘Duffin’ (Duffin, 2011) rebreathe test. Participants were instrumented with a nose-clip and breathed through a mouthpiece connected to a calibrated pneumotachometer, gas sample line, and three-way valve to allow switching the airflow between room air or a 9L bag filled with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. Breathing rate and tidal volumes were calculated from the integral of the respiratory flow tracing. The P\textsubscript{ET}CO\textsubscript{2} and P\textsubscript{ET}O\textsubscript{2} were continually measured from the gas sampling connected to a calibrated gas analyzer (ADInstruments). Immediately before starting the breathing test, baseline measures (end-tidal gases) were acquired during three-minutes of quiet rest. Thereafter the rebreathe test consisted of two-minutes coached hyperventilation to achieve a P\textsubscript{ET}CO\textsubscript{2} of ~20 mmHg (consistent with the apnea P\textsubscript{ET}CO\textsubscript{2} starting points). Participants were then asked to perform a forced exhalation, and were switched from breathing air to rebreathing from the 9L bag. The first three breaths from the rebreathing bag consisted of large inspired breaths to equilibrate gases with the circuit. Participants were then instructed to breathe normally without any conscious suppression of ventilation. The protocol was terminated once; 1) P\textsubscript{ET}CO\textsubscript{2} reached +20 mmHg above baseline measures, or 2) the rebreathing bag was emptied. At each +5 increment of P\textsubscript{ET}CO\textsubscript{2} above baseline eucapnea, arterial blood gases were acquired. The ventilatory response is expressed as the change in ventilation over the change in measured PaCO\textsubscript{2}.

5.3.3 Statistical analysis

Values are presented as mean ± standard deviation (SD). The HCVR is presented as a simple slope from the change in ventilation (in L/min) over the change in PaCO\textsubscript{2}. Linear
regressions were used to compare the FVC and HCVR with the apnea durations. Significance was determined at an alpha below 0.05.
5.4 Results

5.4.1 Apnea

The hyperoxic maximal apnea duration ranged from 807 to 1262 seconds, with an average of 1034 seconds. The control (euoxic pre-breathe) apnea duration ranged from 267 to 431 seconds with an average of 348 seconds.

5.4.2 Hyperoxic-Hypercapnic ventilatory response

Ventilatory slopes are presented as individual averages from the three blood gases taken at +5, +10 and +15 mmHg above resting eucapnia, because seven participants emptied the 9 L rebreathing bag before reaching +20 mmHg. Linearity of the ventilatory response was confirmed at an $r^2$ of 0.90±0.10. The ventilatory response at +5 mmHg was above the ventilatory recruitment threshold in all participants. The average HCVR was in turn 2.0±1.2 L·min$^{-1}$·mmHg$^{-1}$ PaCO$_2$.

Correlations with apnea duration: There was a significant correlation between the hyperoxic maximal apnea duration and the FVC (Figure 5.1, panel A). Likewise, the control apnea duration was related to the FVC, but to a lesser extent, and did not strictly reach significance (Figure 5.1, panel C). There was no correlation between the both apnea durations and the HCVR (Figure 5.1, panel B and D).
Figure 5.1. Comparison of apnea duration with the forced vital capacity (FVC) – left panels, and the hyperoxic-hypercapnic ventilatory response (HCVR) – right panels. Panels A and B display the hyperoxic apnea, panels C and D display the control apnea.
5.5 Discussion

The primary purpose of this study was to determine whether the breaking point of a maximal hyperoxic apnea in elite breath-hold divers is related to the hyperoxic HCVR or the anatomical FVC. Although the central the chemororeflex is the primary source of chemoreception during a hyperoxic breath-hold, it is clearly shown here that the individual HCVR is not related to the maximal duration. Rather, it is the FVC that can explain ~45% of the variation in the hyperoxic apnea duration.

At the end of the hyperoxic apnea, most participants reported respiratory muscle (intercostal) pain, likely stemming from maintaining the breath-hold throughout the involuntary breathing movements at low lung volumes approaching functional residual capacity. While a hyperoxic breath-hold held any longer fundamentally runs the risk of atelectasis, severe respiratory muscle discomfort – and fatigue – must underpin its breaking point. Even with euoxic apnea, it has been reported that increased respiratory (diaphragm, inspiratory rib cage, and expiratory abdominal muscles) approach ‘fatiguing’ levels at the breaking point in elite apnea divers (Cross et al., 2013). Indeed, the control apnea (with euoxic pre-breathe) was related to the FVC, albeit to a lesser extent than the hyperoxia apnea. Here, the larger lung volumes, for a given metabolism, would attenuate the rate of arterial oxygen desaturation, while potentially also attenuating respiratory muscle fatigue.

The findings of this study are in clear agreement with the estimations from Klocke and Rahn (1959). Based on data in naïve breath-holders, these authors elegantly proposed that the theoretical limit of a hyperoxic breath-hold duration is dependent on barometric pressure, vital capacity, and consumed oxygen (metabolic rate). These three variables ultimately determine the time before lung volumes are diminished to their residual volume. Given that each apnea was performed at the same barometric pressure, it stands to reason that the remaining ~55% of the variability in apnea duration can in part be explained by differences in whole-body metabolic rate, in combination with the differences in motivation and pain tolerance.
The average FVC of ~132% above predicted for age and sex (Table 5.1) reflects the respiratory adaptation and/or disposition of trained apnea-divers (Ferretti and Costa, 2003). For instance, Ama divers have a ~15% higher vital capacity than their non-diving counterparts [original studies reviewed in (Ferretti and Costa, 2003)], and trained US Navy skin divers have a ~15% larger vital capacity compared to untrained controls (Carey et al., 1956). Forced vital capacity is also reported to be ~1.8 L larger in competitive apnea divers than aged and sized matched controls (Schagatay, 2014), and moreover, an eleven week apnea training program in prior naïve breath-holders increases vital capacity by ~0.45 L (Schagatay, 2014). A further adaptation in trained apnea divers is a depressed ventilatory response to CO₂ (Masuda et al., 1981; Grassi et al., 1994; Ivancev et al., 2007; Walterspacher et al., 2011). It therefore stands to reason that, unlike in the trained breath-holders, the central chemoreflex may, to some extent, predict the hyperoxic breath-hold duration in the naïve breath-holder.

It is well accepted that lung volume, irrespective of chemical stimuli, influences the drive to breathe during apnea (Mithoefer et al., 1953; Whitelaw et al., 1987). That is, the drive to breathe is greater during apneas performed at lower lung volumes than at volumes near total lung capacity. Indeed, apneas performed at high compared to low lung volumes delay the onset and decrease the frequency of respiratory muscle contractions (Whitelaw et al., 1987). Mechanistically, activated pulmonary stretch receptors send inhibitory afferents to the respiratory control center (Feldman et al., 1976). It is also noteworthy that higher lung volumes increase the surface area of the alveolar capillary membranes (Rose et al., 1979). An increased diffusive capacity at higher lung volumes may theoretically increase the ‘euoxic’ breath-hold duration by accentuating arterial CO₂ clearance and O₂ alveolar extraction.

Ultimately, when apnea is performed with euoxic pre-breathing, an increased lung volume allows greater oxygen storage that attenuates the rate of oxygen desaturation. When apnea is performed with hyperoxic air pre-breathing, the increased lung volume prolongs the time before reaching residual lung volumes. In both scenarios, an increased
lung volume maintains respiratory inhibition from pulmonary stretch afferents. This study in particular highlights the impact of lung volumes on the maximal apnea duration that is independent of chemoreception.
Chapter 6 – Experimental Study #3

‘Cerebral oxidative metabolism is decreased with extreme apnea in humans; impact of hypercapnia’

By: Anthony R. Bain, Philip N. Ainslie, Ryan L. Hoiland, Otto F. Barak, Marija Cavar, Ivan Drvis, Mike Stembridge, Douglas M. MacLeod, Damian M. Bailey, Zeljko Dujic, & David B. MacLeod
6.1 Overview

Prolonged apnea in humans is reflected in progressive hypoxemia and hypercapnia. Here, the cerebral metabolic responses under extreme hypoxia and hypercapnia associated with prolonged apnea are described. It was hypothesized that the cerebral metabolic rate for oxygen (CMRO$_2$) will be reduced near the termination of apnea, attributed in part to the hypercapnia. Fourteen elite apnea-divers performed a maximal apnea (range: 3:36 to 7:26 minutes) under dry laboratory-conditions. In a subset study with the same divers, the impact of hypercapnia on cerebral metabolism was determined using varying levels of hypercapnic breathing, in the background of similar hypoxia. In both studies the CMRO$_2$ was calculated from the product of cerebral blood flow (ultrasound) and the radial artery–internal jugular venous oxygen content difference. Non-oxidative cerebral metabolism was calculated from the ratio of oxygen and carbohydrate (lactate and glucose) metabolism. The CMRO$_2$ was reduced by ~29% (P<0.01, Cohen’s d = 1.18) near the termination of apnea when compared to baseline, but non-oxidative metabolism remained unaltered. In the subset study, in similar backgrounds of hypoxia (arterial O$_2$ tension: ~38.4 mmHg), severe hypercapnia (arterial CO$_2$ tension: ~58.7 mmHg), but not mild-hypercapnia (arterial CO$_2$ tension: ~46.3 mmHg), depressed the CMRO$_2$ (~17%, P=0.04, Cohen’s d = 0.87). Similarly to the apnea, there was no change in the non-oxidative metabolism. These data indicate that hypercapnia can in part explain the reduction in CMRO$_2$ near apnea breakpoint. This hypercapnic-induced oxygen conservation may protect the brain against severe hypoxemia associated with prolonged apnea.
6.2 Background

Though it accounts for only 2% of total body mass, the human brain utilizes a disproportionate 20% of the body’s basal oxygen consumption. The relatively high cerebral metabolic rate of oxygen (CMRO$_2$) is required to support a high rate of adenosine tri-phosphate (ATP) production and neuronal activity (Brown and Ransom, 2007). Constant oxygen supply is therefore obligatory for the maintenance of normal brain function. With respect to cerebral energy demands for oxygen, the current world record apnea duration in humans of 11:35 minutes is truly remarkable.

In terms of oxygen conservation, decreasing the cerebral oxidative metabolism and increasing non-oxidative metabolism is of teleological benefit. Not surprisingly a reduction in the CMRO$_2$ during deep hypothermia is readily utilized in medicine (e.g. during bypass surgery (Fukui and Takanashi, 2016)) and can in part explain extreme survival following prolonged anoxic (>15 min) cold-water immersion (Young et al., 1980; Antretter et al., 1994). It appears, however, that moderate hypoxia alone does not provide the stimulus of oxygen conservation. For example, no appreciable changes in the CMRO$_2$ have been reported during isocapnic or poikilocapnic hypoxic breathing (PaO$_2$ of ~40 – 55 mmHg) (Kety and Schmidt, 1948a; Cohen et al., 1967; Bailey et al., 2009; Overgaard et al., 2012; Ainslie et al., 2014). In fact, using recent magnetic imaging techniques, some studies report significant elevations in CMRO$_2$ by 5-10% with acute poikilocapnic hypoxia (Xu et al., 2012; Vestergaard et al., 2015). Recent and available data further indicate no increases in cerebral non-oxidative metabolism (estimated via the oxidative carbohydrate index, OCI) with isocapnic hypoxic breathing (Ainslie et al., 2014).

An increase in CMRO$_2$ with acute hypoxia shown by some studies (Vestergaard et al., 2015), but not all (Ainslie et al., 2014), may relate to the background PaCO$_2$, or pH. In one study (Ainslie et al., 2014), subjects were kept at eucapnia, whereas in another (Vestergaard et al., 2015) the hypoxia-induced increase in ventilation reduced PaCO$_2$ by
an average 10 mmHg (absolute 31 mmHg). A large body of both human and animal data indicates changing PaCO\textsubscript{2} may impact CMRO\textsubscript{2}, with hypocapnia increasing, and hypercapnia decreasing it (see (Yablonskiy, 2011) for synopsis). Indeed, synaptic transmission (Dulla \textit{et al.}, 2005; Thesen \textit{et al.}, 2012) and phosphofructokinase (PFK) activity (Folbergrova \textit{et al.}, 1975) are each dependent on pH. Because a prolonged breath-hold yields both extreme levels of hypoxia and hypercapnia (Willie \textit{et al.}, 2014a; Bain \textit{et al.}, 2015a), it stands to reason that, in contrast to hypoxia alone, the CMRO\textsubscript{2} may be reduced during apnea. Additionally, in contrast to hypoxic breathing (Ainslie \textit{et al.}, 2014), a greater increase in sympathetic nerve activity during apnea, despite the same chemoreflex stimuli (Steinback \textit{et al.}, 2010b), may promote a shift towards non-oxidative metabolic pathways. For example, infusion of high dose adrenaline (0.08 \textmu g \cdot kg\textsuperscript{-1} \cdot min\textsuperscript{-1} for 15 min) in humans significantly increases the percentage of non-oxidative cerebral metabolism (Seifert \textit{et al.}, 2009), and adrenaline appears to underscore the increased non-oxidative metabolism during exercise (Larsen \textit{et al.}, 2008). A shift towards non-oxidative cerebral metabolism, and a decrease in the CMRO\textsubscript{2}, may in turn help describe how some elite breath-hold divers may hold their breath for over 10 minutes.

Despite obvious implications in neuropathology, describing the cerebral metabolic profile under severe apneic stress in humans has remained a methodological and ethical challenge. Elite breath-hold divers provide a unique population to test the limits of hypoxemia tolerance beyond possible in otherwise healthy humans. As such, the primary purpose of this study was to examine the oxidative and non-oxidative cerebral metabolism during a prolonged dry-land apnea in elite human breath-hold competitors. In a subset study, mechanistic information was garnered by quantifying the impact of hypercapnia / pH on cerebral metabolism in the background of hypoxia. It was hypothesized that 1) compared to a resting baseline, CMRO\textsubscript{2} will be reduced and indices of cerebral non-oxidative metabolism will be increased during a prolonged apnea, and 2) the reductions in CMRO\textsubscript{2} will in part be a function of a hypercapnic-mechanism.
6.3 Methods

6.3.1 Participants

Fourteen competitive and elite breath-hold divers (2 female; age 29.5 ± 7.3 years; BMI 23.5 ± 2.5 kg/m\(^2\)) were recruited from the Croatian national apnea team, and provided informed written consent for participation. All participants were actively competing, and had been practicing competitive breath hold diving for 1.5 to 14.0 years (mean 5.2 ± 3.7 years). The majority of subjects were competitive in the discipline of dynamic apnea (maximal swimming distance underwater). Some were also involved in various depth disciplines (with regular exposure to high pressure). Seven of the subjects were world-class free diving competitors, having placed top-ten within the last three years in international competition in at least one event. Three subjects had recently set new official world records. All subjects were normotensive and free from cardiovascular and respiratory disease. The ethical committees of the University of Split School of Medicine, the University of British Columbia, and the University of South Wales approved the experimental procedures.

6.3.2 Experimental Design

Experimentation was completed on a single day, following strict adherence to pre-testing protocol, including abstinence from vigorous exercise and alcohol at least 48 hours, and from caffeine at least 12 hours before arriving to the laboratory. Information on previous caffeine use was not obtain. All testing was performed at the University of Split, School of Medicine, Department of Integrative Physiology. Upon arrival, a medical history and standard anthropometric and pulmonary functioning metrics were assessed. After, a 20-gauge arterial catheter (Arrow, Markham, Ontario, Canada) was placed in the right radial artery, and a central venous catheter (Edwards PediaSat Oximetry Catheter) was placed in the right internal jugular vein and directed cephalad to the jugular bulb. Using the identical technique and performed by the same anesthesiologist, correct placement of the jugular bulb catheter has been verified by lateral skull X-ray (Ainslie et al., 2014).
Arterial and jugular cannulations were completed under ultrasound guidance with local anesthesia (1% lidocaine), and under sterile conditions. Following cannulation, the catheters were attached to an in-line waste-less sampling system and a pressure transducer located at the height of the right atrium (Edwards Lifesciences VAMP), and TruWave transducer (Irvine, CA, USA). Subjects were then instrumented with the remaining measurements (see measurements).

Apnea: Baseline measures were acquired following a minimum of 30 minutes supine rest, and prior to preparatory apneas. After baseline measures were acquired, each participant completed two preparatory (non-experimental) apneas. The first preparatory apnea was performed after a normal end-expiration until seven involuntary breathing movements were attained. Two minutes later the second preparatory apnea was performed at total lung capacity until ten involuntary breathing movements were attained. These preparatory apneas were performed to generate the longest possible time for the experimental maximal apnea, chosen by the national Croatian apnea team coach (co-author, Ivan Drvis) based on a best-suited standardized preparatory phase for all subjects. Following the preparatory phase, subjects rested for six minutes before commencing the maximal apnea. Subjects were allowed to lung pack (glossopharyngeal insufflation) prior to the experimental apnea, based on individual preference to attain the longest apnea possible. Of note, the individual magnitude of glossopharyngeal insufflation will have profound effects on the initial hemodynamic changes at the apnea onset (Batinic et al., 2011). The magnitude of glossopharyngeal insufflation was not measured. Data were collected throughout the maximal breath-hold until breathing was resumed. Arterial and jugular venous blood draws were attained prior to the preparatory apneas (baseline), every 30 seconds throughout the maximal apnea, and immediately upon termination (100% of apnea).

Subset Study - end-tidal forcing: Following the maximal apnea, participants’ rested supine for a minimum of 30 minutes before starting the hypercapnic-hypoxic breathing subset trials. The mild-hypercapnic hypoxic (mild-HH) and severe-hypercapnic hypoxia (severe-HH) breathing was performed using a custom built end-tidal (P_{ET}) forcing
system. Subjects were equipped with a mouthpiece and nose clip, and were instructed to breathe normally. Gases were sampled at the mouthpiece and analyzed by a calibrated gas analyzer (ML206 ADInstruments, Colorado Springs, CO, USA). Respiratory flows were measured by pneumotachography (HR 800L, Hans Rudolph, Shawnee, KS, USA). Custom written software (Labview, Austen, TX, USA) determined the breath-by-breath tidal volumes and end-tidal partial pressures of oxygen and carbon dioxide ($P_{ETO_2}$ and $P_{ETO_2}$). The end-tidal forcing system prospectively delivered inspired gases to clamp $P_{ETO_2}$ and $P_{ETCO_2}$ at desired input levels. Independently controlled solenoid valves delivered the desired volumes of O$_2$, CO$_2$, and N$_2$ as determined by an error reduction algorithm incorporating $P_{ETO_2}$, $P_{ETCO_2}$, and inspiratory and expiratory tidal volume from the last breath. Levels of desired $P_{ETO_2}$ for both mild-HH and severe-HH were individualized to the average PaO$_2$ measured during the last minute and a half of the maximal breath-hold. Levels of $P_{ETCO_2}$ during the mild-HH trial were individualized to the PaCO$_2$ achieved at ~50% of the maximal apnea. Levels of $P_{ETCO_2}$ during the severe-HH trial were targeted for +10 mmHg from the mild-HH. Each condition (mild-HH and severe-HH) lasted 5 minutes, and the severe-HH trial was performed immediately following mild-HH, while continually maintaining hypoxia. Arterial and jugular venous blood draws were taken following 4.5 min in each stage.

6.3.3 Measurements

**Blood gases, oximetry and metabolites:** Measurements of arterial and jugular venous PO$_2$, PCO$_2$, O$_2$ saturation (SO$_2$%), glucose (Glu) and lactate (La) were analyzed immediately following each draw using a commercially available cassette based analyzer (ABL90 Flex, Radiometer, Copenhagen, Denmark). Each measure required <2 mL of blood. Arterial (radial) and jugular venous draws were taken at the same time.

**Catecholamines:** Blood samples (7 mL) were collected into tubes containing ethylenediaminetetraacetic acid (K-EDTA) and centrifuged at 600g for 10 min at 4°C. Plasma (2 mL sample volume) was transferred into cryovial tubes and immediately snap-frozen under liquid nitrogen (N$_2$, Cryopak CP100, Taylor-Wharton, Theodore, AL, USA)
and then stored at -80°C prior to analysis. Analysis was performed within 1 month of receipt of the sample. All chemicals including reagents and standards were of the highest available purity from Sigma-Aldrich® (UK). Plasma concentrations of adrenaline, noradrenaline and dopamine were measured by reverse-phase ion pair high performance liquid chromatography [Gilson ASTED.XL (Anachem)] with electrochemical detection [ESA Coulochem II (ESA Analytical)] (Bouloux et al., 1985). The CV was 5% for noradrenaline at 4.8 nmol/L and 8% for adrenaline at 1.0 nmol/L.

**Cardiovascular:** Heart rate (HR) was obtained from the R-R intervals measured from a three-lead ECG. Beat-to-beat arterial blood pressure was measured by finger photoplethysmography (Finometer PRO, Finapress Medical Systems, Amsterdam, Netherlands) normalized to manual cuff measurements of the brachial artery. Intra-radial arterial pressure and jugular venous pressure were also recorded, however, due to the frequency of blood sampling, measures were available for ~5 to 10 second bins around each blood draw. Online calculations of stroke volume were obtained using the three-element nonlinear arterial model of the arterial blood pressure waveform (Finometer). Cardiac output was then derived from the product of stroke volume and HR.

**Cerebrovascular:** Cerebral blood velocity of the right middle cerebral artery (MCAv) and left posterior cerebral artery (PCAv) were measured using a 2-MHz pulsed transcranial Doppler ultrasound system (Spencer Technologies, Seattle, WA). Volumetric blood flow of the right internal carotid artery (QICA) and left vertebral artery (QVA) was concomitantly measured using duplex vascular ultrasound (Terason 3000, Teratech, Burlington, MA).

Please refer to Chapter 3 for vessel location, image optimization and analysis.

**Calculations:**

Under the assumption of symmetrical blood flow of contralateral ICA and VA arteries, global CBF (gCBF) was calculated from:
\[
gCBf(\text{ml. min}^{-1}) = (\text{QICA} \cdot 2) + (\text{QVA} \cdot 2)
\]

Due to the onset of involuntary breathing movements (IBMs) during the latter ~60% of the breath-hold (struggle phase), which encompasses large movements of the chest wall and movement of the sternocleidomastoid muscles, the CBF following the onset of IBMs was calculated as described in Chapter 4. This technique is was also used in (Hoiland et al., 2016a).

Please refer to Chapter 3 for calculations of the CMRO\(_2\) and OCI.

### 6.3.4 Statistical analysis

Mean values ± standard deviations (SD) are presented. Baseline measures were acquired during quite rest prior to the preparatory apneas (~15 minutes before the maximal apnea), and one minute before the end-tidal forcing trials. Baseline measures were averaged over one minute around the baseline blood draw. Mean values for MAP (Finometer corrected with intra-arterial) HR, and gCBF during the apnea were averaged over 20 seconds around the blood draws.

Statistical analysis for both apnea and the end-tidal forcing was performed using one-way repeated measures ANOVA. When a main effect was observed, pre-planned paired comparisons were performed to baseline only using two-tailed Student t-tests. A Bonferroni adjustment was applied to correct for multiple comparisons (six stages of the breath hold, and three stages of end-tidal forcing). When significant, Cohen’s d (d) was calculated for effect size of the primary outcome variable (CMRO\(_2\)).
6.4 Results

6.4.1 Apnea

Maximal apnea times ranged from 3:36 to 7:26 min with an average of 5:14 min.

Blood gases, oximetry and metabolites: Arterial and jugular venous blood gas, oximetry, pH, and metabolite data are presented in Table 6.1. To illustrate the near normalization of arterial with venous blood at the end of the apnea, arterial and jugular venous PO$_2$ from baseline to percent time 100 of the apnea are presented in Figure 6.1. As expected there was a main effect for all blood gas variables (p all <0.05). Significant post-hoc comparisons to baseline are denoted in Table 6.1.

Table 6.1. Arterial (radial) and cerebral venous (internal jugular bulb) measures of the partial pressure of carbon dioxide (PCO$_2$), oxygen saturation (SO$_2$), Hematocrit (Hct), Hemoglobin (tHb), Glucose (Glu), Lactate (Lac), and pH at baseline and during 20% increments of the maximal apnea. * Denotes significant difference from baseline

<table>
<thead>
<tr>
<th></th>
<th>PO$_2$ (mmHg)</th>
<th>SO$_2$ (%)</th>
<th>Hct (%)</th>
<th>tHb (mmol/L)</th>
<th>Glu (mmol/L)</th>
<th>Lac (mmol/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>40.9±3.3</td>
<td>97.8±0.9</td>
<td>44.4±3.1</td>
<td>14.5±1.0</td>
<td>5.6±0.8</td>
<td>0.8±0.3</td>
<td>7.41±0.02</td>
</tr>
<tr>
<td>Venous</td>
<td>51.8±3.6</td>
<td>62.1±5.1</td>
<td>44.1±2.6</td>
<td>14.4±0.9</td>
<td>4.9±0.8</td>
<td>0.8±0.3</td>
<td>7.36±0.02</td>
</tr>
<tr>
<td>20% Arterial</td>
<td>32.4±5.1*</td>
<td>99.0±0.4*</td>
<td>44.3±3.2</td>
<td>14.4±1.0</td>
<td>5.3±0.6</td>
<td>0.9±0.2</td>
<td>7.48±0.04*</td>
</tr>
<tr>
<td>Venous</td>
<td>47.6±3.5*</td>
<td>50.5±8.1*</td>
<td>45.3±3.4</td>
<td>14.8±1.1</td>
<td>4.6±0.6</td>
<td>0.9±0.2</td>
<td>7.40±0.02*</td>
</tr>
<tr>
<td>40% Arterial</td>
<td>39.6±4.3</td>
<td>96.9±1.9</td>
<td>44.8±4.2</td>
<td>14.6±1.4</td>
<td>5.3±0.6</td>
<td>0.9±0.2</td>
<td>7.42±0.02</td>
</tr>
<tr>
<td>Venous</td>
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<td>61.2±6.5</td>
<td>45.5±3.6</td>
<td>14.9±1.2</td>
<td>4.8±0.6</td>
<td>0.9±0.3</td>
<td>7.38±0.02</td>
</tr>
<tr>
<td>60% Arterial</td>
<td>46.1±5.0*</td>
<td>89.2±5.1*</td>
<td>45.4±4.4</td>
<td>14.8±1.5</td>
<td>5.3±0.7</td>
<td>1.0±0.3</td>
<td>7.38±0.03*</td>
</tr>
<tr>
<td>Venous</td>
<td>52.5±3.4</td>
<td>63.7±6.0</td>
<td>45.7±3.8</td>
<td>14.9±1.2</td>
<td>4.9±0.6</td>
<td>1.0±0.3</td>
<td>7.36±0.02</td>
</tr>
<tr>
<td>80% Arterial</td>
<td>49.7±4.7*</td>
<td>80.6±7.8*</td>
<td>45.5±3.7*</td>
<td>14.8±1.2*</td>
<td>5.5±0.6</td>
<td>1.0±0.3</td>
<td>7.36±0.02*</td>
</tr>
<tr>
<td>Venous</td>
<td>54.9±3.7</td>
<td>59.5±6.0</td>
<td>45.7±3.2*</td>
<td>14.9±1.0*</td>
<td>5.1±0.6</td>
<td>1.0±0.3</td>
<td>7.34±0.02*</td>
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<tr>
<td>100% Arterial</td>
<td>53.4±4.8*</td>
<td>60.9±14.0*</td>
<td>45.8±3.4*</td>
<td>14.9±1.1*</td>
<td>5.4±0.7</td>
<td>1.1±0.3*</td>
<td>7.34±0.02*</td>
</tr>
<tr>
<td>Venous</td>
<td>57.8±4.7*</td>
<td>45.6±13.0*</td>
<td>46.0±3.7*</td>
<td>15.0±1.2*</td>
<td>5.1±0.6</td>
<td>1.2±0.4*</td>
<td>7.32±0.02*</td>
</tr>
</tbody>
</table>
Figure 6.1. Mean ± standard deviation of absolute arterial (PaO$_2$) and internal jugular venous (PvO$_2$) partial pressure of oxygen throughout the duration of the maximal apnea.

**Hemodynamics:** Heart rate (HR), mean arterial pressure (MAP), stroke volume (SV), cardiac output (CO), and global cerebral blood flow (gCBF) are presented in Table 6.2. There was a main effect of for all variables ($p$ all $<0.05$). Despite a reduction in CO from baseline, the MAP was significantly elevated throughout the entire apnea ($p$ all $<0.05$), indicating an elevated total peripheral resistance. The reductions in indices of SV from percent time 20 to 80 of the apnea ($p$ all $<0.05$) are attributable to the glossopharyngeal insufflation prior to the start of the apnea. As expected, gCBF was reduced at the onset of the apnea, and then was progressively elevated by $\sim 70\%$ at percent time 100 of the apnea. All significant post-hoc comparisons to baseline are denoted in Table 6.2.
Table 6.2. Heart rate (HR), stroke volume (SV), cardiac output (CO), mean arterial pressure (MAP), and the global cerebral blood flow (gCBF) at baseline and during 20% increments of the maximal apnea. * Denotes significant difference from baseline

<table>
<thead>
<tr>
<th></th>
<th>HR (b/min)</th>
<th>SV (ml)</th>
<th>CO (L/min)</th>
<th>MAP (mmHg)</th>
<th>gCBF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>64±11</td>
<td>108±21</td>
<td>6.78±1.65</td>
<td>88±8</td>
<td>610±153</td>
</tr>
<tr>
<td>20%</td>
<td>80±17*</td>
<td>58±16*</td>
<td>4.52±1.47*</td>
<td>92±10</td>
<td>469±140*</td>
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<td>40%</td>
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<td>613±196</td>
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<tr>
<td>60%</td>
<td>66±16</td>
<td>81±15*</td>
<td>5.11±1.46*</td>
<td>115±21*</td>
<td>828±267*</td>
</tr>
<tr>
<td>80%</td>
<td>59±14</td>
<td>95±18</td>
<td>5.41±1.52*</td>
<td>131±21*</td>
<td>991±325*</td>
</tr>
<tr>
<td>100%</td>
<td>56±15</td>
<td>96±33</td>
<td>5.17±1.91*</td>
<td>139±23*</td>
<td>1041±318*</td>
</tr>
</tbody>
</table>

Cerebral metabolic dynamics and oxygen delivery: Cerebral delivery of oxygen (CDO₂), oxygen extraction (O₂ Ext), lactate extraction (Lac Ext), glucose extraction (Glu Ext), OCI and, CMRO₂ are presented in Table 6.3. Individual CMRO₂ at baseline and percent time 100 of the apnea are presented in Figure 6.2. There was a main effect of CDO₂ (p<0.01), O₂ Ext (p<0.01), Glu Ext (p<0.01), OCI (p=0.02), and CMRO₂ (p<0.01). Compared to baseline, the CMRO₂ was reduced by ~29% at percent time 100 of the apnea (P<0.01, d=1.18), but similar at all other time points (p all >0.05). The O₂ Ext was increased at percent time 20 of the apnea, and decreased at the latter half of the apnea (p all <0.05). The CDO₂ fell below baseline values at percent time 20 of the apnea (p<0.01), above baseline at percent time 60 and 80 of the apnea (p both <0.01) and was similar to baseline at percent time 100 of the apnea. All significant post-hoc comparisons to baseline are denoted in Table 6.3. Although there was a main effect of OCI, no post-hoc comparisons were different. Values for OGI were identical to the OCI. There was no correlation between the absolute or change in PaCO₂ or PaO₂ with the change in CMRO₂ from baseline to percent time 100 of the apnea.
**Figure 6.2.** Individual data of the absolute cerebral metabolic rate of oxygen (CMRO$_2$) before the apnea (Baseline) and at apnea termination (100% Apnea). Dashed line with open circles denotes mean data. Compared to baseline, the CMRO$_2$ was reduced by ~29% at percent time 100 of the apnea (P<0.01, d=1.18).

**Table 6.3.** Cerebral oxygen delivery (CDO$_2$), oxygen extraction fraction (O$_2$ Ext), lactate extraction (Lac Ext), glucose extraction (Glu Ext), oxidative carbohydrate index (OCI), and metabolic rate of oxygen (CMRO$_2$) at baseline and during 20% increments of the maximal apnea. * Denotes significant difference from baseline.
**Catecholamines:** The radial arterial plasma adrenaline and noradrenaline increased by ~380% (670±380 to 2012±1020 pmol/L) and ~483% (1893±673 to 8260±4375 pmol/L) from baseline to percent time 100 of the apnea, respectively (p both <0.01). Likewise, the jugular venous plasma adrenaline and noradrenaline increased by ~258% (584±258 to 1762±801 pmol/L) and ~385% (1860±577 to 8559±3707 pmol/L) (p both <0.01). The arterial-venous adrenaline and noradrenaline differences were not different from baseline to percent time 100 of the apnea (p both >0.05).

**6.4.2 Subset Study: Variable Hypercapnic Breathing with Similar Hypoxia**

Three participants were unable to complete the end-tidal forcing trial due to ventilatory volumes during the severe-hypercapnic hypoxia (severe-HH) that exceeded the capacity of the end-tidal forcing system (i.e. >100 L/min). The sample size for the hypoxic breathing trial was in turn reduced from 14 to 11 subjects.

*Blood gases, oximetry and metabolites:* Arterial and jugular venous blood gas, oximetry, pH, and metabolite data are presented in Table 6.4. By design, there was an increase in PaCO₂, and reduction in PaO₂, SaO₂ and pH in both mild-HH and severe-HH from baseline. There was no main effect of glucose or lactate (p both >0.05).
Table 6.4. Arterial (radial) and cerebral venous (internal jugular bulb) measures of the partial pressure for oxygen (PO\textsubscript{2}) and carbon dioxide (PCO\textsubscript{2}), oxygen saturation (SO\textsubscript{2}), hematocrit (Hct), hemoglobin (tHb), glucose (Glu), lactate (Lac) and pH at baseline and during similar hypoxic breathing with mild and severe levels of hypercapnia. * Denotes significant difference from baseline

<table>
<thead>
<tr>
<th></th>
<th>PO\textsubscript{2} (mmHg)</th>
<th>PCO\textsubscript{2} (mmHg)</th>
<th>SO\textsubscript{2} (%)</th>
<th>Hct (%)</th>
<th>tHb (mmol/l)</th>
<th>Glu (mmol/l)</th>
<th>Lac (mmol/l)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>91.6±13.7</td>
<td>38.6±5.1</td>
<td>98±1</td>
<td>43.9±3.7</td>
<td>14.3±1.2</td>
<td>5.4±0.5</td>
<td>0.9±0.3</td>
<td>7.43±0.05</td>
</tr>
<tr>
<td>Venous</td>
<td>29.5±4.1</td>
<td>50.1±4.2</td>
<td>60±5</td>
<td>44.6±4.1</td>
<td>14.6±1.3</td>
<td>4.9±0.6</td>
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<tr>
<td>Mild-HH</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>38.9±7.8*</td>
<td>46.3±3.2*</td>
<td>75±8*</td>
<td>44.7±3.8*</td>
<td>14.6±1.2*</td>
<td>5.3±0.5</td>
<td>0.9±0.3</td>
<td>7.38±0.03*</td>
</tr>
<tr>
<td>Venous</td>
<td>28.0±3.4</td>
<td>52.6±2.6*</td>
<td>54±7*</td>
<td>44.6±3.4</td>
<td>14.6±1.1</td>
<td>5.0±0.5</td>
<td>0.9±0.3</td>
<td>7.35±0.02*</td>
</tr>
<tr>
<td>Severe-HH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>38.0±8.9*</td>
<td>58.7±4.3*</td>
<td>68±12*</td>
<td>45.3±3.4*</td>
<td>14.8±1.1*</td>
<td>5.3±0.4</td>
<td>0.8±0.3</td>
<td>7.30±0.02*</td>
</tr>
<tr>
<td>Venous</td>
<td>30.8±5.5</td>
<td>62.3±4.1*</td>
<td>55±11*</td>
<td>45.2±3.6</td>
<td>14.8±1.2</td>
<td>5.2±0.4</td>
<td>0.9±0.3</td>
<td>7.29±0.02*</td>
</tr>
</tbody>
</table>

Hemodynamics: Absolute HR, MAP, SV, CO, and gCBF are presented in Table 6.5. Except for SV, all measures in both conditions were elevated from baseline (p all <0.05).

Table 6.5. Heart rate (HR), stroke volume (SV), cardiac output (CO), mean arterial pressure (MAP), and the global cerebral blood flow (gCBF) during baseline and hypoxic breathing with mild and severe levels of hypercapnia. * Denotes significant difference from baseline

<table>
<thead>
<tr>
<th></th>
<th>HR (mmHg)</th>
<th>SV (ml)</th>
<th>CO (l/min)</th>
<th>MAP (mmHg)</th>
<th>gCBF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>59±18</td>
<td>102±18</td>
<td>7±1</td>
<td>89±6</td>
<td>593±151</td>
</tr>
<tr>
<td>Mild-HH</td>
<td>88±14*</td>
<td>104±17</td>
<td>9±2*</td>
<td>102±11*</td>
<td>983±204*</td>
</tr>
<tr>
<td>Severe-HH</td>
<td>94±16*</td>
<td>107±15</td>
<td>10±3*</td>
<td>113±18*</td>
<td>1340±343*</td>
</tr>
</tbody>
</table>

Cerebral metabolism and oxygen delivery: Absolute CDO\textsubscript{2}, O\textsubscript{2} Ext, Glu Ext, Lac Ext, CMRO\textsubscript{2}, and OCI are presented in Table 6.6. Individual CMRO\textsubscript{2} are presented in Figure 6.3. There was a main effect of CMRO\textsubscript{2} (p<0.01). Compared to baseline, post-hoc comparisons revealed no change in CMRO\textsubscript{2} during mild-HH (P>0.05); but a ~17%
reduction in the severe-HH condition (P=0.04, d=0.87). The OCI was unchanged from baseline in both conditions. Values for OGI were identical to the OCI. As expected in both mild-HH and severe-HH the O₂ Ext was reduced from baseline, and the CDO₂ was elevated (p all <0.05).

**Table 6.6.** Blood flow in the right internal carotid artery (QICA) and left vertebral artery (QVA), flow velocity in the middle (MCA) and posterior (PCA) cerebral artery, total cerebral delivery of oxygen (CDO₂) and total cerebral metabolic rate of oxygen (CMRO₂) during baseline and hypoxic breathing with mild and severe levels of hypercapnia. * Denotes significant difference from baseline

<table>
<thead>
<tr>
<th></th>
<th>CDO₂ (ml/min)</th>
<th>O₂ Ext (%)</th>
<th>Lac Ext (%)</th>
<th>Glu Ext (%)</th>
<th>OCI (%)</th>
<th>CMRO₂ (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>117±32</td>
<td>38±7</td>
<td>-5±6</td>
<td>10±5</td>
<td>88±16</td>
<td>44±13</td>
</tr>
<tr>
<td>Mild-HH</td>
<td>149±32*</td>
<td>28±5*</td>
<td>2±8</td>
<td>7±3</td>
<td>80±18</td>
<td>41±11</td>
</tr>
<tr>
<td>Severe-HH</td>
<td>186±53*</td>
<td>19±4*</td>
<td>-5±15</td>
<td>3±3*</td>
<td>80±26</td>
<td>34±11*</td>
</tr>
</tbody>
</table>
Figure 6.3. Individual data of the absolute cerebral metabolic rate of oxygen (CMRO$_2$) at baseline, during mild-hypercapnic hypoxia (mild-HH), and severe-hypercapnic hypoxia (severe-HH). Hypoxia was on average a partial pressure of arterial oxygen of $\sim$38.5 mmHg for both mild-HH and severe-HH. Hypercapnia was on average set at an arterial partial pressure of carbon dioxide of $\sim$46.3 mmHg and $\sim$58.7 mmHg in the mild-HH and severe-HH conditions respectively. Dashed line with open circles denotes mean data. There was a $\sim$17% reduction in the severe-HH condition ($P=0.04$, $d=0.87$) compared to baseline.
6.5 Discussion

This study reports the first known measures of cerebral metabolism in humans during a prolonged dry apnea lasting on average greater than five minutes. A marked reduction in CMRO$_2$ was observed immediately prior to the termination of the apnea (~29%, $d=1.18$), compared to baseline measures. In contrast, there was no change at any time point in the indirect estimation of non-oxidative metabolism, despite a ~380% increase in arterial adrenaline concentrations from baseline to the end of apnea. With manipulation of end-tidal gases, mild hypercapnic hypoxia had little influence on CMRO$_2$; however, the same level of hypoxia but with severe hypercapnia reduced the CMRO$_2$ by ~17% ($d=0.87$). These findings are interpreted to indicate that the increased levels of hypercapnia and reduced pH, in part, explain the reduction in CMRO$_2$ near the termination of apnea.

6.5.1 Impact of hypercapnia on the CMRO$_2$

The notion of a reduced CMRO$_2$ resulting from hypercapnia is not novel, but has remained debatable (Yablonskiy, 2011). With obvious implications in general medicine and anesthesia (e.g. (Waaben et al., 1989)), the impact of PaCO$_2$ on CMRO$_2$ deserves attention. Unfortunately, results in human studies with similar MRI-based experimental designs and in the absence of anesthesia have remained inconsistent. For example, Jain et al., (2011) report no change in CMRO$_2$ with a ~8 mmHg increase in end-tidal CO$_2$; Chen et al., (2010) report a ~7% reduction in CMRO$_2$ with a ~9 mmHg increase in end-tidal CO$_2$; and Xu et al., (2011) report a ~13% reduction in CMRO$_2$ with a ~7 mmHg increase in end-tidal CO$_2$. In relation, an increase in CMRO$_2$ has been shown during high altitude (Smith et al., 2013), suspected to be consequent of the hyperventilatory-induced hypocapnia and therefore brain alkalosis. This finding is corroborated by the fact that an increase in CMRO$_2$ following six hours of normobaric hypoxia is removed with acetazolamide (Wang et al., 2015).

Using arterial and cerebral venous sampling, the present study provides evidence for reductions in CMRO$_2$ with hypercapnia, at least in the presence of hypoxia. The pH-
dependent activity of phosphofructokinase (the enzyme responsible for the phosphorylation of fructose 6-phosphate in glycolysis) provides mechanistic support for reductions in CMRO$_2$ with hypercapnia. Indeed, an accumulation of glucose 6-phosphate and fructose 6-phosphate is shown in rats exposed to acute hypercapnia, which cannot be explained by glycogen breakdown (Folbergrova et al., 1975). Additionally, hypercapnia has consistently been shown to depress cortical activity, suspected to result from acidotic-induced adenosine receptor modulation (Dulla et al., 2005; Zappe et al., 2008; Thesen et al., 2012). Ultimately, the bulk of literature now collectively supports a PaCO$_2$ modulation of CMRO$_2$. The end-tidal forcing data in the present study may indicate this modulation is dose dependent, whereby a threshold change in PaCO$_2$ is required.

6.5.2 Non-oxidative metabolism and adrenaline

During apnea, cessation of ventilation, chemosensory stimuli, and baroreflex inhibition significantly increase muscle sympathetic nerve activity (burst frequency, magnitude and recruitment) (Heusser et al., 2010; Steinback et al., 2010c), and therefore catecholamine release. Ascribed to the known effect of catecholamines on muscle metabolism (Tank and Lee Wong, 2015), an increase in whole-body anaerobic metabolism is often held integral to the mammalian dive reflex (Blix and Folkow, 2011). Whether the cerebral tissue significantly partakes in the non-oxidative metabolism is unclear, but it is generally accepted that like in the muscle, adrenaline (but not noradrenaline) increases the cerebral non-oxidative metabolism (Seifert et al., 2009). The ~380% increase in arterial adrenaline from baseline to the termination of apnea therefore offers potential for an increased cerebral non-oxidative metabolism (Seifert et al., 2009). However, there was no reduction in the indirect estimation of non-oxidative carbohydrate use (OCI) at any time point during the apnea compared to baseline. In fact non-oxidative metabolism trended above 100% from percentage time 20 to 60 of the apnea. An oxidative carbohydrate index above 100% may indicate the presence of carbon oxidation from sources other than arterial glucose or lactate. Based on animal studies, these data may in turn indicate an allosteric glycogen mobilization at the astrocytes (Saez et al., 2014).
Under the same metabolic paradigm whereby adrenaline increases non-oxidative metabolism, adrenaline should also increase the oxidative metabolism (Tank and Lee Wong, 2015). For example, intra-venous adrenaline infusion (~8ug/ml/min), with concurrent increases in MAP to assure amine passage across the blood-brain barrier, significantly increases the CMRO$_2$ in humans (King et al., 1952). However, when adrenaline concentrations are such that the glycogenolytic and glycolytic flux is increased to impact the CMRO$_2$, circulating blood glucose would be elevated in adjunct (Seifert et al., 2009; Tank and Lee Wong, 2015). Therefore, the slightly reduced arterial glucose, reduced CMRO$_2$, and unchanged oxidative carbohydrate index collectively suggests that the ~380% increase in adrenaline was insufficient to elicit noticeable metabolic changes – perhaps not surprising given that when non-oxidative metabolism does increase, plasma adrenaline may be increased >10-fold, e.g., during exhaustive exercise (Pott et al., 1996; Tank and Lee Wong, 2015). Of note, anaerobic-tolerant animals adapt to hypoxia by slowing the oxidative metabolism, rather than increasing the non-oxidative metabolism (Hochachka et al., 1996). The findings herein reveal that a similar metabolic outcome is initiated in humans during prolonged apnea.

### 6.5.3 Other potential modulating factors of the cerebral metabolic response

Although the impact of PaCO$_2$ on CMRO$_2$ is given hierarchy, other factors, including oxygen-conserving reflexes attending the dive-reflex, cannot be dismissed. Indeed it is conceivable that the CMRO$_2$ may be reduced to a greater extent had the apnea been performed in water, with activation of the trigeminal nerve and therefore a stronger oxygen-conserving / dive reflex (Schaller et al., 2009b; Lemaitre et al., 2015). However, it appears that the activation of the trigeminal nerve is more responsible for blood redistribution (from the periphery to the brain), and bradycardia, without directly influencing the CMRO$_2$ (Reis et al., 1997; Schaller et al., 2009b; Lemaitre et al., 2015). Reductions in CMRO$_2$ associated with oxygen-conserving reflexes may relate more to mechanisms observed in rapid ischemic preconditioning (Gidday, 2006; Schaller et al., 2009b). Here, neural inhibitory factors, for example adenosine and ATP sensitive potassium channels, are likely responsible, as evidenced in the anoxic tolerant turtle.
(Perez-Pinzon et al., 1993). It is difficult to speculate on these potential oxygen-conserving mechanisms in the present study, however, given that the average level of acidosis was greater in the severe-hypercapnic hypoxia trial than it was at the termination of apnea (arterial pH: 7.30 vs. 7.34, respectively), oxygen-conserving reflexes independent of acidosis are likely to have also contributed to the reduction in CMRO$_2$ during apnea. It should also be emphasized that the metabolic changes associated with PaCO$_2$ described herein are in the background of hypoxia. Nevertheless, although acidosis decreased the oxygen saturation for the same PaO$_2$ during severe-hypercapnic hypoxia compared to mild-hypercapnic hypoxia (via the Bohr effect, Table 6.5), it is unlikely that this would have influenced the CMRO$_2$. Indeed, it has been demonstrated using the same technique that similar eucapnic oxygen saturations (~70%) of 15-minute duration does not alter the CMRO$_2$ (Ainslie et al., 2014). And, if anything, the further oxygen desaturation would have increased the CMRO$_2$, rather than decrease it [e.g. (Xu et al., 2012; Vestergaard et al., 2015)]. Finally, the gCBF was higher in the severe-HH condition compared to the mild-HH condition, therefore it may be suggested that reduced temperature [via increased tissue to blood heat transfer (Bain et al., 2015b)] or a potential oxygen diffusion limitation can in part explain the reduction in CMRO$_2$. A diffusion limitation, however, is unlikely given that cerebral O$_2$ extraction is well maintained even during exercise in both normoxic and hypoxic conditions (Smith et al., 2014). A diffusion limitation is also unlikely based on the mathematical cerebral blood flow / metabolism relationship by Gjedde (2005).

Surprisingly, at the termination of apnea, neither the PaO$_2$ nor the PaCO$_2$ was independently correlated with the reduction in CMRO$_2$. This may point to the individual variability in the CMRO$_2$ response to extreme blood gas changes, an interacting facet of hypoxia and hypercapnia, or the impact of other poorly defined brain oxygen-conserving reflexes in humans (Schaller et al., 2009b).

### 6.5.4 Summary
In summary this study presents a clear reduction in CMRO\textsubscript{2} immediately prior to the termination of a prolonged apnea, attributable in part to hypercapnia. In contrast, there appears to be no change in non-oxidative metabolism, despite a 3 to 4-fold increase in arterial adrenaline concentrations. Presumably this is the first study to examine the cerebral metabolism in healthy humans during prolonged apnea yielding some of the most extreme levels of hypoxia and hypercapnia reported to date. It is suggested that hypercapnia promotes brain oxygen-conservation, and therefore provides a protective mechanism against severe hypoxia relating to apnea.
‘Hypercapnia is essential to reduce the cerebral oxidative metabolism during extreme apnea in humans’

By: Anthony R. Bain, Otto F. Barak, Ryan L. Hoiland, Ivan Drvis, Damian M. Bailey, Zeljko Dujic, David B. MacLeod, & Philip N. Ainslie
7.1 Overview

The cerebral metabolic rate of oxygen (CMRO$_2$) is reduced near the end of a prolonged (>5min) apnea. Although this oxygen-conserving response may protect the brain from severe hypoxia (SaO$_2$~50%), the mechanisms are poorly defined. It was hypothesized that the reduction in CMRO$_2$ is mediated by hypercapnia. Elite apnea competitors (n=13) completed three maximal apneas that generated separate levels of hypoxemia and hypercapnia; a) normal conditions (NM), yielding severe hypercapnia and hypoxemia, b) prior hyperventilation (HV), yielding severe hypoxemia only, and c) prior 100% oxygen breathing (HX), yielding the greatest level of hypercapnia, but in the absence of hypoxemia. The CMRO$_2$ was calculated from the product of cerebral blood flow (ultrasound) and the radial artery-jugular venous oxygen content difference (cannulation). Secondary measures included net cerebral glucose and lactate exchange and non-oxidative metabolism. The reduction in CMRO$_2$ was largest in the HX condition (-44±15%, p<0.001) that yielded the most extreme level of hypercapnia (PaCO$_2$=58±5mmHg), but with maintained oxygen saturation. The CMRO$_2$ was reduced by 24±27% in NM (p=0.050), but unchanged in the HV apnea where hypercapnia was absent. With all apneas combined, the reductions in CMRO$_2$ were related ($r^2$~0.34) to the hypercapnia and pH (p<0.001). There was an increased net cerebral lactate release at the end of apnea in the HV (p=0.035) and NM (non-significant) condition, but not in the HX apnea. These novel data support hypercapnia/pH as a key mechanism mediating reductions in CMRO$_2$ during apnea, whereas hypoxemia is required to facilitate net lactate release from the brain.
7.2 Background

With its exceptionally high-energy demand and almost exclusive reliance on oxidative metabolism, the human brain is particularly vulnerable to reductions in oxygen availability. During prolonged apnea, when oxygen becomes limited, oxygen-conserving reflexes are paramount for prolonging cell survival, and consciousness. The mammalian dive response is hallmarked by bradycardia, reduced blood perfusion to non-vital organs (e.g. skeletal muscle), and reductions in whole-body oxidative metabolism (Butler and Jones, 1997). The ability to slow oxidative metabolism in turn delays the time before reaching critical levels of hypoxemia, and prolongs the apnea breaking point to breathe in elite divers (Bain et al., 2015a; Hoiland et al., 2016a). Indeed, the remarkable human apnea times of over 10 minutes (officially recognized world record; 11:35 min) most certainly stem from effective oxygen conservation.

For the hypoxemic vulnerable brain, a logical protective mechanism of inadequate oxygen supply would be to reduce the metabolic rate. Yet, hypoxia alone may in fact increase, rather than decrease the cerebral metabolic rate of oxygen (CMRO$_2$) (Xu et al., 2012; Vestergaard et al., 2015). On the other hand, during apnea that generates both extreme levels of hypoxia (PaO$_2$ ~30 mmHg) and hypercapnia (PaCO$_2$ ~55 mmHg), it has recently been demonstrated that the CMRO$_2$ is reduced by ~29% (Chapter 5). In the same study, breathing during severe-hypercapnic hypoxia (PaCO$_2$~58.7 mmHg; PaO$_2$~38.9 mmHg), but not mild-hypercapnic hypoxia (PaCO$_2$~46.3 mmHg; PaO$_2$~38.0 mmHg), also reduced the CMRO$_2$ (by ~17%). It was therefore speculated that hypercapnia might determine the CMRO$_2$ reduction during apnea. A hypercapnic reduction in CMRO$_2$ is notionally mediated from the decreased extracellular pH, which reduces phosphofructokinase activity (Folbergrova et al., 1975), and increases adenosine modulation of synaptic transmission (Dulla et al., 2005). However, the associated hemodynamic and autonomic differences with apnea compared to breathing – even with similar arterial blood gases (Steinback et al., 2010b; Willie et al., 2014a) – make it difficult to isolate the CMRO$_2$ reduction during extreme apnea to hypercapnia. For example, the mammalian dive reflex attending apnea may support a further metabolic
reduction independent of hypercapnia (Lemaitre et al., 2015). In addition, in the previous study (Chapter 6) it was impossible to discern the metabolic impact of hypercapnia independent to that of severe hypoxia. As recently reported (Peng et al., 2016), the attending hypoxia may well offset some of the reduction in CMRO$_2$ attributed to hypercapnia.

The primary purpose of this study was to quantify the CMRO$_2$ under three distinct apnea paradigms that yield separate levels of hypoxemia and hypercapnia / acidosis. Based on Overgaard et al., (2012), it was also sought to quantify whether hypoxia has a role in astrocyte glycogenolysis during apnea, as evidenced from an increased net cerebral lactate release. In elite apnea divers, maximal apneas were performed under; a) normal conditions (NM) that yield extreme levels of both hypoxia and hypercapnia; b) prior hyperventilation (HV), yielding severe hypoxemia but limiting hypercapnia, and; c) prior hyperoxic hyperventilation (HX), thus removing the impact of hypoxia, but generating the most severe hypercapnia. It was hypothesized that the reduction in CMRO$_2$ near the termination of apnea will be mediated by hypercapnia. It was secondly hypothesized that the hypoxic apneas (HV and NM), but not the hyperoxic apnea (HX), will elicit a net cerebral release of lactate.
7.3 Methods

7.3.1 Participants

Thirteen active competitive and elite breath-hold divers (3 female; age 31 ± 8 years; BMI 23.0 ± 2.1 kg/m²) were recruited from the Croatian national apnea team. All participants provided informed written consent before experimentation. The ethical committees of the University of Split School of Medicine, the University of British Columbia, and the University of South Wales approved the study procedures and experimentation. Years competing ranged from 2 to 15 years primarily in the discipline of dynamic (underwater lapse in a pool) and static (resting while face down in water) apnea. Four of the participants were also involved with depth disciplines. Six of the subjects were world-class apnea competitors, having placed top-ten within the last three years in international competition in at least one event. One subject had recently set a new official world record in dynamic apnea. All subjects were assessed by standard anthropometric and pulmonary functioning metrics, and a medical history questionnaire. Participants were free from any known respiratory and cardiovascular diseases.

7.3.2 Experimental Design

All experimentation for a single subject was completed on a single day, at the Department of Integrative Physiology, University of Split, School of Medicine. Participants arrived to the laboratory following abstinence from vigorous exercise, alcohol, and caffeine at least 24 hours prior. Upon arrival to the laboratory and following initial screening, a 20-gauge arterial catheter (Arrow, Markham, Ontario, Canada) was placed in the right radial artery, and a central venous catheter (Edwards PediaSat Oximetry Catheter) was placed in the right internal jugular vein and advanced towards the jugular bulb. Cannulation was completed under local anesthesia (1% lidocaine) with ultrasound guidance. Facial vein contamination was ruled out by assuring that all jugular venous SO₂ recordings were below 75%. The arterial catheter was attached to an in-line waste-less sampling setup (Edwards Lifesciences VAMP) attached to a pressure
transducer that was placed at the height of the right atrium (TruWave transducer). Following cannulation subjects were further instrumented with ECG and transcranial Doppler (see Measures).

The experimental procedure comprised of three maximal apneas (see experimental procedure schematic, Figure 7.1). Each apnea protocol was separated by a minimum of 20 minutes rest before commencing the preparatory phase of the next respective apnea [see (Bain et al., 2015a) and (Hoiland et al., 2016a) for description of the preparatory phase]. The presentation of the hyperventilation apnea (HV) and the normal apnea (NM) was counter-balanced, but due to the potentially long lasting physiological effects of hyperoxia in combination with the fatiguing factor of a prolonged hyperoxic apnea (up to 21 min), the hyperoxic apnea (HX) was always performed last. For the HX protocol, subjects hyperventilated for 15 minutes from a Douglas bag that was continually filled with 100% O₂. Hyperventilation for the HV (3 min) and HX (15 min) protocol was paced with auditory feedback to achieve an end-tidal PCO₂ of approximately 20 mmHg. End-tidal gases were sampled at the mouth and integrated into a calibrated gas analyzer (ADI instruments). The 15 minutes of 100% oxygen hyperventilation is used during hyperoxic apnea competitions, and was based on a stimulus that yields the longest possible apnea time, and therefore greatest increase in PaCO₂ at the end of the apnea. The apnea coach was present at all times to assure complete motivation during the maximal apneas.
Figure 7.1. Timeline Schematic of the experimental design. Maximal apneas were performed with a) no prior hyperventilation (NM), b) 3 minutes of hyperventilation (HV), and, c) 15 minutes of hyperventilation on 100% oxygen (HX). Compared time points between apneas included baseline (BL), apnea onset (Onset), the first involuntary breathing movement (IBM), and immediately at apnea end (End). The ‘a – v draw’ denotes when radial artery and jugular venous draws were taken. The order of the NM and HV apneas were randomized, but the HX apnea was always performed last. See text for further details.

7.3.3 Measures

Involuntary breathing movements: The onset of involuntary breathing movements (IBMs) was visually assessed by the apnea coach, and verified by a plethysmography belt placed around the chest, integrated into LabChart® for offline analysis. Measures analyzed at the onset of IBMs represent approximately the half waypoint of the respective apnea.

Blood gases, oximetry and metabolites: At each of the four time points, approximately 2 ml of blood was procured from the radial artery and jugular vein into a heparinized syringe. Whole blood was immediately analyzed for PO₂, PCO₂, O₂ saturation (SO₂%), glucose (Glu), lactate (La), hemoglobin (Hg), and pH, using a commercially available cassette based analyzer (ABL90 Flex, Radiometer, Copenhagen, Denmark).
Cardiovascular: Heart rate (HR) was obtained from the R-R intervals measured from a three-lead ECG. Mean arterial blood pressure (MAP) was measured with the pressure transducer connected to the radial catheter. Because the pressure trace is lost during blood sampling, values for MAP were taken 15 seconds immediately before each blood draw. Heart rate and pressure measures were integrated into PowerLab® and LabChart® software (ADInstruments) for online monitoring, and saved for offline analysis.

Cerebrovascular: Cerebral blood velocity of the middle cerebral artery (MCAv) and posterior cerebral artery (PCAv) were measured using a 2-MHz pulsed transcranial Doppler ultrasound system (Spencer Technologies, Seattle, WA). Blood flow in the right internal carotid artery (ICA) and left vertebral artery (VA) was concomitantly measured using duplex vascular ultrasound (Terason 3000, Teratech, Burlington, MA).

Please refer to Chapter 3 for vessel location, image optimization and analysis.

Calculations:

Under the assumption of symmetrical blood flow of contralateral ICA and VA arteries, global CBF (gCBF) was calculated from:

\[
gCBf(\text{ml.min}^{-1}) = (QICA \cdot 2) + (QVA \cdot 2)
\]

Due to the onset of involuntary breathing movements (IBMs) during the latter ~60% of the breath-hold (struggle phase), which encompasses large movements of the chest wall and movement of the sternocleidomastoid muscles, the CBF following the onset of IBMs was calculated as described in Chapter 4. This technique is was also used in (Hoiland et al., 2016a).
Please refer to Chapter 3 for calculations of the CMRO$_2$, OCI and net cerebral glucose and lactate exchange.

### 7.3.4 Statistical analysis

Values are presented as mean values ± standard deviations (SD), except in Figures 7.2, 7.3 and 7.5, where means ± 95% confidence intervals are shown. Baseline measures were acquired during quite rest prior to the preparatory apneas (~10 minutes before the NM and HV apnea, and ~20 min before the HX apnea) of each respective condition. Measures were averaged over 20 seconds around the blood draws, except for arterial blood pressure that was averaged over 15 seconds immediately before each blood draw (see measurements).

Statistical analysis was performed using a two-way repeated measures analysis of variance (ANOVA) using the factors of condition (3; NM, HV, HX) and time (4; BL, Onset, IBM, End). The Huynh-Feldt correction was applied when sphericity was not met. When appropriate, post-hoc analysis was performed using a two-tailed Student’s t-test. When a significant condition*time interaction was observed, the delta from baseline only was compared between conditions (nine comparisons). When a significant main effect of time was observed, post-hoc comparisons were made to baseline only (three comparisons per condition). Correction for multiple comparisons was made using a Bonferroni adjustment. Correlation analysis was performed using a simple linear regression. Cohen’s d (d) was calculated for effect size of the primary outcome variable (CMRO$_2$). Significance was determined at an alpha of 0.05.
7.4 Results

Data from two participants were omitted due to technical difficulties with procuring blood samples. Analyzed sample size was in turn based on n=11. Apnea times ranged from 3:37 to 6:49 min (mean = 5:10 min) during the NM trial; 3:50 to 7:11 min (mean = 5:38 min) during the HV trial, and 9:39 to 21:02 min (mean = 16:11 min) during the HX trial.

7.4.1 Cerebral metabolism

Individual values for CMRO$_2$ are presented in Figure 7.2. Mean values, statistical main effects, and within trial post-hoc time effects for CMRO$_2$, OCI, CDO$_2$, O$_2$ extraction, net glucose uptake, and net lactate release are presented in Table 7.1. The end apnea CMRO$_2$ compared to within-trial baseline was reduced by 24±27% during the NM trial (p=0.050, d=1.25), unchanged during the HV trial (-6±20%, p=0.776), and reduced by 44±15% (p<0.000, d=3.05) during the HX trial. The reduction in CMRO$_2$ from baseline to end apnea during the HX trial was greater compared to the HV trial (p=0.001, d=2.24), but similar to the NM trial (p=0.237). The reduction in CMRO$_2$ from baseline to end apnea was not statistically different between the NM and HV trial (p=0.119; see Figure 7.3). The reduction in CMRO$_2$ amongst all apneas significantly correlated with the end apnea PaCO$_2$ ($r^2=0.3192$) and similarly arterial pH ($r^2=0.3536$; see Figure 7.4).
Figure 7.2. Individual data of the cerebral metabolic rate of oxygen (CMRO$_2$) at baseline (BL), the onset of apnea (Onset), the first involuntary breathing movement (IBM), and immediately at apnea end (End), for the normal apnea (NM; top panel), apnea following 5 min of hyperventilation (HV; middle panel), and apnea following 15 min of 100% O$_2$ hyperventilation (HX; bottom panel). Compared to baseline, there was a ~24% reduction in CMRO$_2$ at the end of the NM apnea, no change at the end of the HV apnea, and a ~44% reduction at the end of the HX apnea. Error bars denote 95% confidence intervals.
Table 7.1. Mean ± SD of the cerebral metabolic rate of oxygen (CMRO₂), oxidative carbohydrate index (OCI), O₂ extraction fraction, and the net cerebral glucose uptake and lactate release, at baseline (BL), apnea onset, first involuntary breathing movement (IBM), and the end of a normal apnea (NM), apnea with prior hyperventilation (HV), and apnea with prior 100% oxygen hyperventilation (HX).

<table>
<thead>
<tr>
<th>Condition</th>
<th>CMRO₂ (ml/min⁻¹)</th>
<th>OCI (%)</th>
<th>O₂ Extraction (%)</th>
<th>Net Glu Uptake (mmol/min⁻¹)</th>
<th>Net Lac Release (mmol/min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>50±8</td>
<td>101±22</td>
<td>43±7</td>
<td>0.38±0.08</td>
<td>0.02±0.04</td>
</tr>
<tr>
<td>HV</td>
<td>47±9</td>
<td>95±11</td>
<td>49±8</td>
<td>0.35±0.15</td>
<td>0.02±0.03</td>
</tr>
<tr>
<td>HX</td>
<td>52±7</td>
<td>122±65</td>
<td>50±17</td>
<td>0.38±0.13</td>
<td>0.02±0.06</td>
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<th>3 X 4 ANOVA</th>
<th>Condition</th>
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<td>BL</td>
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(*) Denotes significant change from baseline within trial. See text for individual differences where a main effect of condition and interaction is observed.
Figure 7.3. Percent change in the cerebral metabolic rate of oxygen (CMRO\textsubscript{2}) from baseline to the end the normal apnea (NM), apnea following 5 min of hyperventilation (HV), and apnea following 15 min of 100% O\textsubscript{2} hyperventilation (HX). The reduction in CMRO\textsubscript{2} was significantly larger in the HX compared to the HV apnea. Error bars denote 95% confidence intervals.

Figure 7.4. Linear regression of the percent change in the cerebral metabolic rate of oxygen (CMRO\textsubscript{2}) from baseline to the end of apnea over the end apnea partial pressure of arterial CO\textsubscript{2} (PaCO\textsubscript{2}), (left panel), and arterial pH (right panel). Regression analysis includes apneas from all three conditions.
There were no significant main effects of OCI, or net glucose uptake across the brain; however there was a significant interaction (p=0.006) in net lactate release across the brain. Post-hoc tests revealed no significant differences in the change in lactate release between conditions, but the change from baseline to end apnea trended to be larger in the HV trial compared to the HX trial (p=0.060). A main effect of time for net cerebral lactate release did not strictly reach significance (p=0.052). Nevertheless, allowing post hoc comparisons at p=0.05, there was an increased cerebral lactate release at the end of apnea compared to baseline in the HV trial (p=0.035). The cerebral lactate release in the NM trial followed similar (albeit non-significant) change patterns as the HV trial, but there was no change in net cerebral lactate release in the HX trial (Table 7.1). Similarly to the previous study (Chapter 6), there were only very modest changes in arterial lactate from baseline to the end of apnea in all three conditions (NM: +0.1±0.2 mmol·l\(^{-1}\), HV: +0.3±0.2 mmol·l\(^{-1}\), HX: +0.1±0.2 mmol·l\(^{-1}\)).

As expected, there was a significant main effect of condition, time, and condition*time interaction in O\(_2\) extraction (see Table 7.1 for time effects). There were no post-hoc condition effects for O\(_2\) extraction between NM and HV. In contrast, the O\(_2\) extraction was lower in the HX trial compared to the HV and NM trial at the IBM onset and at the end of apnea (p all < 0.050).

### 7.4.2 Blood gases and pH

Arterial oxygen saturation (top panel) and PCO\(_2\) (bottom panel) are depicted in Figure 7.5. By design, there was a significant main effect of condition, time and condition*time interaction in both SaO\(_2\) and PaCO\(_2\) (p all < 0.001). There were no post-hoc condition differences at baseline for both SaO\(_2\) and PaCO\(_2\). Other than baseline, the SaO\(_2\) during the HX trial was elevated at all time points compared to both the HV and NM trials (p all < 0.001). Although the SaO\(_2\) was lower at the IBM during the HV trial (78±9%) compared to the NM trial (90±5%) (p<0.001), there was no difference in SaO\(_2\) at the end of apnea (p=0.229) between NM and HV trials. At the end of apnea, the PaCO\(_2\) during the HX trial was higher compared to the HV (p<0.001) and NM trial (p=0.019), and was higher in the
NM trial compared to the HV trial (p<0.001). At the onset, the PaCO$_2$ was lower in the HX trial compared to both the NM (p<0.001) and HV (p=0.008) trials, and was lower in the HV compared to the NM trial (p<0.001). At the IBM, the PaCO$_2$ was similar between the HX and NM trial (p=0.363), but reduced in the HV compared to HV and NM trials (p both <0.05). As expected, the pH inversely followed the changes in PaCO$_2$ and (most importantly) was reduced by a greater extent at the end of apnea in the HX trial (7.28±0.04) compared to the NM trial (7.36±0.02) and HV (7.41±0.03) trial (p both < 0.001). The reduction in pH was larger in the NM trial compared to the HV trial (p <0.001).
**Figure 7.5.** Arterial partial pressure of O\(_2\) (PaO\(_2\); top panel), arterial oxygen saturation (SaO\(_2\); middle panel), and the partial pressure of arterial CO\(_2\) (PaCO\(_2\); bottom panel) for all three apneas at baseline (BL), the apnea onset (Onset), the first involuntary breathing movement (IBM), and immediately at the termination of apnea (End). Error bars denote 95% confidence intervals. By design, there was a significant main effect of condition, time, and time*condition for all variables. There were no differences at baseline for any variable between conditions. See text for exact post hoc statistical analysis.
7.4.3 Cardiovascular, cerebral blood flow, and cerebral O₂ delivery

Cardiovascular (HR and MAP), gCBF and CDO₂ mean values, statistical main effects, and within trial post-hoc time effects are shown in Table 7.2. In all cardiovascular and cerebrovascular variables, there were no post-hoc condition effects at baseline. Although there was no main effect of condition for gCBF, the reduction from baseline at the onset was lower in the NM trial compared to the HV trial and HX trial (p both < 0.001). No other between condition differences were observed in gCBF. As expected the CDO₂ was significantly elevated in the HX trial at the IBM and end of the apnea compared to the HV and NM trials (p both < 0.001). The CDO₂ was also reduced at the onset in the HV trial compared to the NM trial (p=0.020). At the onset and at the end of apnea, MAP was lower in the HX compared to the NM trial (p=0.015 and 0.028, respectively). The HR was higher at the onset in the HV (p<0.001) and the HX (p=0.005) trial, compared to the NM trial, and was higher at the IBM during the NM trial compared to the HX trial (p=0.049). No other between condition differences in HR or MAP were observed.
Table 7.2. Mean ± SD of the global cerebral blood flow (gCBF), cerebral oxygen delivery (CDO₂), mean arterial pressure (MAP), and heart rate (HR) at baseline (BL), apnea onset, first involuntary breathing movement (IBM), and the end of a normal apnea (NM), apnea with prior hyperventilation (HV), and apnea with prior 100% oxygen hyperventilation (HX).

(...content continues with table and explanatory text)...
7.5 Discussion

This study reconfirms findings from the previous Chapter (Chapter 6) of a reduced CMRO\textsubscript{2} at the termination of a prolonged apnea that yields severe levels of hypoxemia (\(\text{PaO}_2 \sim 30\) mmHg) and hypercapnia (\(\text{PaCO}_2 \sim 55\) mmHg). These findings are extended by establishing that during apnea the hypercapnia and resultant acidosis is obligatory for reducing the CMRO\textsubscript{2}. This is reflected by the largest reduction in CMRO\textsubscript{2} during the hyperoxic apnea (HX) that generated the most severe hypercapnia (\(\text{PaCO}_2 \sim 60\) mmHg), without the confounding influence of hypoxia; whereas the apnea generating no hypercapnia but sever hypoxia (HV) yielded no changes in CMRO\textsubscript{2}. Moreover, with all three apneas combined, the end apnea \(\text{PaCO}_2\) and arterial pH significantly correlated with the reduction in CMRO\textsubscript{2} (\(r^2 = 0.3192\) and 0.3536, respectively, Figure 7.4). A secondary finding was a net cerebral lactate release at the end of apnea compared to baseline in the HV apnea (\(p=0.035\)) and to a lesser extent (non-significant) in the NM apnea – this lactate release occurred without any changes in the oxidative carbohydrate index (OCI). In contrast, there was no increase in net cerebral lactate release during the HX trial (main time*condition interaction effect \(p = 0.006\)). These subset findings indicate a role for hypoxia in net lactate release from the human brain.

7.5.1 Influence of hypercapnia on the CMRO\textsubscript{2}

The majority of literature, including findings from this study, now supports a role for hypercapnia / pH on the CMRO\textsubscript{2} in humans [e.g. (Chen and Pike, 2010; Xu et al., 2011)] – discrepant findings are discussed in (Yablonskiy, 2011). Depressed cortical activity is also shown with \(\text{CO}_2\) inhalation (Dulla et al., 2005; Zappe et al., 2008; Thesen et al., 2012), lending support for reductions in CMRO\textsubscript{2}. The hypercapnic suppression of CMRO\textsubscript{2} is described by three related mechanisms. First, hypercapnia / extracellular acidosis increases adenosine concentrations which activate adenosine A\textsubscript{1} receptors (Dulla et al., 2005) and inhibit excitatory glutamatergic neurotransmission. Second, hypercapnia / acidosis may reduce phosphofructokinase activity, evidenced from an accumulation of fructose-6 phosphate and glucose-6 phosphate in un-anesthetized rat cerebral cortexes.
following CO₂ inhalation (Folbergrova et al., 1975). Any reduction in PFK activity, and therefore glycolytic flux, will correspondingly reduce the CMRO₂ provided that other non-carbohydrate substrates (e.g. amino acids) are not oxidized. This is a fair assumption given that glucose and lactate irrefutably provide the primary metabolite for cerebral metabolism in humans (Brown and Ransom, 2007; Rasmussen et al., 2010), barring ketone body use during extended hypoglycemia (Morris, 2005). Finally, hypercapnia may depress the CMRO₂ via a reduction in cerebral temperature resulting from the increased CBF. A temperature effect, however, is likely small. For example, assuming that the increased CBF caused a ~0.25°C drop in global brain temperature (Hayward and Baker, 1968), a liberal Q10 temperature coefficient of 3 [see (Bain et al., 2015b) for review] would theoretically equate to a metabolic reduction of only ~5%. In summary, although it is difficult to delineate any one mechanism in the present data, it is likely that increased adenosine, inhibited PFK activity, and to a lesser extent a reduced cerebral temperature from elevated CBF each contributed to the reduction in CMRO₂ from hypercapnia.

7.5.2 Influence of hypoxia on the CMRO₂

Hypoxia may increase the CMRO₂ (Xu et al., 2012; Vestergaard et al., 2015; Peng et al., 2016) and in turn counter some of the metabolic depression from hypercapnia during apnea. Using MRI, Peng et al., (Peng et al., 2016) recently quantified the opposing facet of hypoxia and hypercapnia on the CMRO₂ in humans. Here, Peng et al., reported that hypercapnia (5% CO₂ inhalation) reduced the CMRO₂ by ~8%, hypoxia (13% O₂, balanced with N₂) increased the CMRO₂ by ~17%, but the combination of the two (5% CO₂ and 13% O₂, balanced with N₂) had no effect on the CMRO₂. It was thus concluded that the divergent metabolic influences of hypercapnia and hypoxia equated to a net oxidative metabolic balance. The ostensible mechanism(s) for increased CMRO₂ in hypoxia remain undefined, but is suggested to involve decreased mitochondrial efficiency for ATP production (Solaini et al., 2010), and perhaps also from increased sympathetic activity acting at the neuronal level. On the other hand, an increased CMRO₂ during fixed FiO₂ hypoxia may simply be a consequence of the concomitant hyperventilation-induced
hypocapnia. For example when PaCO$_2$ is kept eucapnic, hypoxia has no effect on the CMRO$_2$ (Ainslie et al., 2014).

With respect to this study, the largest decrease in CMRO$_2$ during the hyperoxic apnea may theoretically manifest in part from the absence of hypoxia, rather than simply from the largest level of hypercapnia. Nevertheless, as evidenced from the previous Chapter (Chapter 6) and the NM apnea, it is clear that the impact of hypercapnia, at least in the setting of prolonged apnea, outweighs any influence from hypoxia on the CMRO$_2$. Moreover, there was no increase in CMRO$_2$ at the end of the HV apnea, despite profound hypoxia (PaO$_2$ ~31 mmHg) with minimal hypercapnia (PaCO$_2$ ~43 mmHg). It is therefore suggested that the impact of hypoxia on CMRO$_2$, if any, is negligible.

7.5.3 Influence of hyperoxia on the CMRO$_2$

Like hypoxia, hyperoxia has also been suggested to impact the CMRO$_2$ (Rockswold et al., 2010; Xu et al., 2012), although in the opposite direction – i.e. to decrease it. However, this is not a universal finding, with others showing no change (Diringer et al., 2007; Ainslie et al., 2014). Aside from differences in measurement technique, it is difficult to speculate on the discrepancies between studies. In the present study, 15 min of hyperoxic breathing did not depress the CMRO$_2$, although it did become more variable (see Onset on Figure 7.2, right panel). The variability may stem from an interaction between the 15 min of hypocapnia and hyperoxia. With obvious implications in critical care [e.g. (Llitjos et al., 2016) for review] it is clear that more research is required to determine the impact of hyperoxia on cerebral metabolism.

7.5.4 Cerebral lactate release, and non-oxidative metabolism

There was a strong trend for a main effect of time (p=0.052) and a significant (p=0.006) time*condition interaction in net cerebral lactate release. Here, net lactate release was increased in the HV apnea and to a lesser extent in the NM apnea, but not in the HX apnea (Table 7.1). These findings suggest that the hypoxia elicited a net cerebral lactate
release. Such findings are broadly comparable to Overgaard et al., (2012) who reported that cerebral lactate release was increased from ~0.05 to 0.09 mmol·min⁻¹ with resting normoxia compared to hypoxia (PaO₂ ~35 mmHg).

The net lactate release from the brain suggests a role of astrocyte glycogenolysis (Brown and Ransom, 2007; Dienel and Cruz, 2015). That is, glycogen contained within the astrocytes, acting as the principle fuel storage of the brain, is converted to lactate as potential oxidative fuel for the neurons (Brown, 2004; Dienel and Cruz, 2015). Overgaard et al., attribute the astrocyte glycogenolysis in hypoxic exercise to a coupling of non-oxidative and oxidative metabolism. In contrast, the present data indicate that the net lactate release was independent of the CMRO₂ and non-oxidative metabolism, as determined by the oxidative carbohydrate index. Herein, it is likely that astrocyte glycogenolysis was initiated from noreadrenaline (Benington and Heller, 1995; Matsui et al., 2011), which has been shown to increase by ~500% during apneas that generate extreme hypoxia (Chapter 6).

7.5.5 Cerebral oxygen-conserving reflexes associated with the dive reflex?

The cerebral metabolic changes reported herein must be interpreted within the context of a dry (out of water) apnea. As discussed in the previous Chapter (Chapter 6), it is possible that trigeminal nerve activation with cold-water immersion prompts cerebral oxygen-conserving reflexes beyond those demonstrated from the hypercapnia – i.e. from the trigeminocardiac reflex (Lemaitre et al., 2015). It is long accepted that trigeminal nerve activation is a potent autonomic conduit of the mammalian dive reflex, in large part responsible for the bradycardia (Lemaitre et al., 2015). This undoubtedly promotes oxygen conservation at the level of whole-body metabolism (Hoiland et al., 2016a); however, direct evidence for the trigeminocardiac reflex and reductions in CMRO₂ is lacking. Rather, one function of the trigeminocardiac reflex is to increase the CBF without altering CMRO₂ [for review see (Schaller, 2004)]. The disproportionately rapid delivery of oxygen and glucose to the brain relative to its metabolic rate from trigeminal nerve activation may provide a protective means to assure adequate cerebral fuel delivery
(Lemaitre et al., 2015). Although the present data shows an elevated cerebral oxygen delivery near the midpoints of a typical prolonged apnea in this study, consistent with previous studies [(Willie et al., 2014a) and Chapter 6], it is probably consequence of the hypercapnia. Not surprisingly, when hypercapnia does not develop, i.e. during the HV apnea, the CDO$_2$ is never elevated above baseline. Nevertheless, despite lack of current evidence for a reduction in CMRO$_2$ attributable to a dive / oxygen conserving reflex, a logical future study is to test the cerebral metabolic reduction to apnea with and without facial and/or whole-body water immersion.

7.5.6 Implications and summary

Describing the role of extreme acidosis and hypoxia, alone and in combination, on the cerebral metabolic functioning have implications in a myriad of clinical settings including sepsis, cardiorespiratory disease, and neurological disorders. Indeed, it is clinically well recognized that hypocapnia can incite seizures in epileptic patients, and hypercapnia can suppress them (Yang et al., 2014). In relation to extreme apnea, one subject developed seizure like symptoms (unresponsive with mild convulsions) lasting approximately 10 seconds at the termination of the HV apnea, but not the NM or HX apnea. In this subject the CMRO$_2$ was notably elevated by 15% at the termination of the HV apnea, compared to baseline (see Figure 7.2). Although this subject was only mildly hypocapnic when the convulsions occurred at the end of the apnea (PaCO$_2$ = 36.5 mmHg vs. 43.8 mmHg at baseline), it may be that hypercapnia in the NM apnea prevented the hypoxic induced seizure. In this case the hypercapnic depression of metabolic activity is entirely protective. In apnea competition, profound hyperventilation (PaCO$_2$ at ~20 mmHg) is usually avoided to prevent shallow water blackout, notionally attributed to the reduction in breathing sensation and eventual onset of critical hypoxemia, especially upon ascent. In the ultra-elite (n=6 in the present study), profound hyperventilation also proffers no benefit to maximal apnea time with facial immersion; in fact it will often decrease it (personal correspondence with the Croatian national apnea coach). The changes in CMRO$_2$ ascribed to hypercapnia may help describe both the occurrence of
shallow water blackout, and the reduction in ultra-elite maximal apnea time following hyperventilation.

In summary, these data provides novel evidence for a reduction in CMRO$_2$ at the termination of a prolonged apnea in humans that can be ascribed primarily to hypercapnia / acidosis. The metabolic depressing influence of hypercapnia outweighs any increase in CMRO$_2$ that is potentially evoked from hypoxia. Moreover, it is shown that severe apnea induced hypoxia may mobilize glycogen stores in the astrocytes to be broken into lactate for neuronal fuel. Together, these findings have implications for the better understanding of brain survival during periods of not only voluntary apnea but also during loss of consciousness, and the myriad of clinical situations where arterial acidosis occurs.
Chapter 8 – Conclusion

Prolonged volitional breath-holding has been performed for over 2000 years for harvest of food and commodities from the ocean floor [reviewed in: (Ferretti, 2001)]. Over the past half-century prolonged breath-holding has evolved from a primarily hunter-gatherer technique to an internationally recognized sport (Dujic and Breskovic, 2012). The powerful and integrative physiological stress of a prolonged breath-hold, however, has remained unchanged, inclusive of pronounced sympathetic and parasympathetic excitation, transient hypertension, hypoxemia, and hypercapnia (Heusser et al., 2009; Breskovic et al., 2011). While examining the integrated physiologic response to prolonged apnea, the broad objectives of this Thesis were to examine the underpinning mechanisms that make an elite breath-holder terminate an apnea, and to describe the cerebral metabolic functioning during apnea. Discussion of the primary findings from the four experimental chapters in this Thesis, and suggestions for areas of future work are described below.
8.1 Synopsis of Chapter 4

The first experimental study of this thesis (Chapter 4) determined the impact of peripheral chemoreflex inhibition with low-dose dopamine on the maximal breath-hold duration. The primary novel finding was that, although blunting the peripheral chemoreflex with low-dose dopamine caused a ~27% reduction in the ventilatory response to hypercapnic hypoxia compared to placebo, the maximal breath hold duration was only marginally (~5%) increased. These data indicate that afferents from the peripheral chemoreflex have minimal impact on the elite apnea breakpoint. At the apnea breakpoint, the PaO$_2$ (~31 mmHg) and SaO$_2$ (~58 %) were identical between placebo and dopamine, suggesting that a ‘threshold’ oxygen saturation may underlie when the elite break their breath-hold. It is likely that this threshold oxygen saturation encroaches P50 – the theoretical limit for consciousness (Nunn, 1987).

Several aspects from Chapter 4 are noteworthy for further discussion. First, the impact of peripheral chemoreflex inhibition on apnea duration is contingent upon the level of apnea training. In the untrained breath-holder, blunting the peripheral chemoreflex with low-dose dopamine may double maximal apnea and proffer a dramatically reduced peripheral oxygen saturation at the apnea breakpoint [e.g. from 78% to 63% (van de Borne et al., 1998)]. Conversely, in the ultra-elite, blunting carotid body afferents may negatively impact the maximal apnea duration – exemplified in Figure 4.1. The latter, i.e. a negative impact on breath hold time, likely stems from the dopamine-induced increase in heart rate that prevents the bradycardia induced oxygen conservation. For example, as illustrated in Figure 4.1, in one ultra-elite participant (coincidentally the current world champion in dynamic apnea) the maximal apnea time was reduced by 79 seconds with dopamine compared to placebo; however, in this individual, the end apnea PaO$_2$ (25.2 mmHg with placebo vs. 27.0 mmHg with dopamine) was similar. In this subject, the increased heart rate potentially combined with the decreased psychophysical relaxation and increased muscular tone, negatively impacted the breath-hold time by increasing the metabolic rate. The elevated heart rate during apnea with low dose dopamine compared to placebo (Figure 4.4) was likely consequent to 1) a dopamine-induced vasodilation and therefore
baroreflex mediated increase in heart rate and/or 2) carotid body chemoreflex inhibition (Somers et al., 1991; Ponikowski et al., 1997), whereby carotid body stimulation in the absence of ventilation causes bradycardia [see Chapter 2 and (Marshall, 1994)].

8.2 Synopsis of Chapter 5

The purpose of Chapter 5 was to determine whether the breaking point of a maximal hyperoxic apnea in elite breath-hold divers is related to the hypoxic-hyperoxic ventilatory response (HCVR) and/or the anatomical forced vital capacity (FVC). Study of the hyperoxic breath-hold provided a means to assess the central chemoreflex, independent from the peripheral chemoreflex. It was found that the individual HCVR was unrelated to the duration of the maximal apnea, whereas the FVC explained ~45% of the statistical variation in the hyperoxic apnea duration. Likewise, when a control apnea was performed with euoxic pre-breathing the duration was also not related to the HCVR; however, albeit to a lesser, strictly non-significant extent (p=0.07), the apnea duration was related to the FVC. Although encompassing separate mechanisms, it is clear that lung volumes play an integral role in determining the elite apnea duration. It therefore comes to no surprise that trained apnea divers have larger lung volumes than untrained controls (see Chapter 2). Whether larger lung volumes is adaptive, or an inherent disposition remains speculative; however, in support of an adaptive response, as little as 11-weeks apnea training can increase the forced vital capacity by 0.45 L (Schagatay, 2014), and trained navy divers have a ~15% larger forced vital capacity (Carey et al., 1956) than untrained controls.

The findings from Chapter 5 shed light on two important mechanistic attributes of a prolonged breath-hold. First, it became evident that severe respiratory muscle discomfort – and fatigue - must underpin the hyperoxic apnea duration whereas a breath-hold held any longer fundamentally runs the risk of atelectasis. These findings support the hypothesis derived from Klocke and Rahn (1959) who proposed that the theoretical limit of a hyperoxic breath-hold duration is dependent on barometric pressure, vital capacity, and consumed oxygen (metabolic rate); these three factors ultimately determine the time before lung volumes are diminished to their residual volume. Second, during a ‘normal’
apnea performed with euoxic air pre-breathing, an increased lung volume allows greater oxygen storage thereby attenuating the rate of oxygen desaturation.

8.3 Synopsis of Chapter 6

The purpose of Chapter 6 was to describe the cerebral metabolic rate during a prolonged apnea in humans. The novel finding herein was a marked reduction in the cerebral metabolic rate of oxygen (CMRO$_2$) by $\sim$29% compared to baseline measures immediately prior to the termination of the apnea. Irrespective of these changes in CMRO$_2$, there was no change in the indirect estimation of non-oxidative metabolism (via the oxidative glucose index). The latter finding was somewhat surprising given that circulating blood adrenaline was markedly increased by $\sim$380% from the beginning to the end of apnea, and previous reports using intravenous adrenaline infusion indicate that circulating adrenaline increases the cerebral non-oxidative metabolic rate (Seifert et al., 2009). In turn, it is likely that the $\sim$380% increase in circulating blood adrenaline was insufficient to proffer any noticeable cerebral non-oxidative changes. It is also possible that potential changes in non-oxidative metabolism were masked by the profound influence of other regulatory factors exclusive to apnea, for example hypercapnia.

In a subset study of Chapter 6, mechanistic insight was gleaned by measuring the CMRO$_2$ during breathing with varying levels of hypercapnia, at the same level of hypoxia (PaO$_2$ $\sim$38 mmHg). Here, it was found that mild hypercapnia (PaCO$_2$ $\sim$46 mmHg) had little influence on CMRO$_2$; however, the same level of hypoxia but with severe hypercapnia ($\sim$59 mmHg) reduced the CMRO$_2$ by $\sim$17%. It was thus concluded that the increased levels of hypercapnia and reduced pH, in part, explain the reduction in CMRO$_2$ near the termination of apnea. The caveat to this Chapter, however, was that the CMRO$_2$ was never assessed in the absence of hypoxia, or exclusively during apnea; this question was therefore addressed next in Chapter 7.

8.4 Synopsis of Chapter 7
The primary purpose of this Chapter was to quantify the CMRO$_2$ under three distinct apnea paradigms that yield separate levels of hypoxemia and hypercapnia. The second purpose was to quantify whether hypoxia has a role in astrocyte glycogenolysis during apnea, as indexed via an increased net cerebral lactate release. This Chapter reconfirmed the two main findings from Chapter 6 – a reduced CMRO$_2$ at the termination of a prolonged apnea that yields severe levels of hypoxemia (PaO$_2$ ~ 30 mmHg) and hypercapnia (PaCO$_2$ ~ 55 mmHg), and no change in the non-oxidative cerebral metabolism. These findings were extended in this Chapter by establishing that, during apnea, the hypercapnia is obligatory for reducing the CMRO$_2$. Support for this notion was reflected in that the largest reduction in CMRO$_2$ during the hyperoxic apnea occurred in the presence of the most severe hypercapnia (PaCO$_2$ ~ 60 mmHg), without the confounding influence of hypoxia; whereas the apnea generating no hypercapnia but severe hypoxia yielded no changes in CMRO$_2$. Moreover, with all three apneas combined, the end apnea PaCO$_2$ significantly correlated with the reduction in CMRO$_2$ ($r^2 = 0.32$). The secondary finding was a net cerebral lactate release at the end of apnea compared to baseline in the apneas yielding severe hypoxemia, thereby potentially indicating a role for hypoxia in initiating cerebral glycogenolysis at the level of the astrocytes.

8.5 What determines the elite apnea breaking point?

Prior to the work of this Thesis, the mechanisms that underlie the elite-apnea breaking point have remained vague, with most research citing studies in the non-elite breath-holder (Parkes, 2006). In part using the findings from this Thesis, a schematic demonstrating the mechanisms of an elite apnea breaking point is shown in Figure 8.1. With the fundamental difference of elite apnea divers being able to consciously suppress the urge to breathe compared to untrained breath holders, what is made particularly clear from Chapters 3 and 4 is that chemoreception from both the peripheral and central chemoreflex has little impact on the elite-apnea breaking point. Ultimately, because many elite apnea competitors are able to hold a breath-hold until unconsciousness, it appears that a threshold level of hypoxemia determines when the elite and motivated apnea is
terminated and breathing is restored. A large initial lung volume and slowed metabolic rate will therefore attenuate the rate of oxygen desaturation, and thus determine the overall duration of the apnea. Of note, the global cerebral delivery of oxygen is well maintained throughout a maximal apnea. This suggests a direct link with PO₂ upon consciousness, rather than cerebral oxygen content, perhaps by way of alterations in neuronal potassium channel activity (Jiang and Haddad, 1994). However, although global cerebral oxygen delivery is maintained, it is unknown whether regional brain areas are limited by oxygen supply.

When apnea is performed in hyperoxic conditions, lung volumes become the major determining cause of when the apnea is terminated. Although respiratory muscle fatigue and discomfort is certainly a determining factor, it stands to reason that the hyperoxic apnea record (as of February 28th, 2016, a remarkable 24:03 minutes – accredited via Guinness) may be further prolonged with appropriate titration of an inert gas (nitrogen or helium) to the hyperoxic pre-breathe, to prevent the lung squeeze attributed to the disproportionate oxygen extraction relative to CO₂ release. However, the safety of this practice deserves consideration.
Figure 8.1. Schematic of the major factors involved with an elite apnea breakpoint. Unlike in the naïve breath holder, chemoreception seems to have little impact on the elite apnea breaking point. When apnea is performed with prior hyperoxic pre-breathe, respiratory muscle fatigue and pending lung collapse determine the apnea breaking point. During a euoxic apnea, while chemoreception and respiratory muscle fatigue contribute to the urge to breathe, a level of hypoxemia before loss of consciousness determines the ultra-elite apnea breaking point. The trained conscious suppression of the urge to breathe in elite compared to naive breath-holders fundamentally underscores the differences in apnea breakpoint mechanisms.

8.6 Implications and further areas for future study

8.6.1 Apnea in water

The findings in this Thesis are exclusive to a prolonged static apnea in air, without water immersion/facial cooling and therefore the addition of a heightened dive response. In addition to a heightened bradycardia and peripheral vasoconstrictor response when apnea is performed in water (dependent upon the temperature of the water, see Chapter 2), selective cerebral oxygen conserving reflexes may exist from profound trigeminal nerve activation [e.g. (Schaller, 2007), and see Chapter 2]. The cerebral metabolic responses to apnea with facial cooling and/or water immersion deserve further research.
8.6.2 Regional cerebral oxygen delivery and metabolism

A caveat of the experimental studies of this Thesis is the measures of global cerebral functioning / metabolism, as the cerebral metabolic responses and oxygen delivery during extreme apnea likely have regional disparity. Understanding the regional changes in cerebral oxygen delivery / metabolism may help better describe the breakpoint mechanisms of extreme apnea. For example, although global cerebral oxygen delivery appears maintained at or above baseline values throughout a prolonged apnea [Chapter 6 and (Willie et al., 2014a)], there may be regional impairments, particularly at the level of the brainstem that is potentially far more hypoxic than the arterial PO₂ or other areas of the brain [(Marina et al., 2015), and reviewed in (Ainslie et al., 2016)]. Indeed, the finding of released lactate from the brain at the end of hypoxic breath-holds (Chapter 7) may signify brainstem impairments in oxygen delivery (see below). Future studies employing high resolution MRI to partition regional brainstem / cortex blood flow and metabolism during extreme apnea are warranted.

8.6.3 Cerebral glycogenolysis in humans

Although the amount of glycogen in the human brain is unknown, in rats, glycogen levels in the brain are estimated to be 10-12 mol/g of brain tissue, stored predominantly in the astrocytes (Dienel and Cruz, 2015). A hallmark of activated astrocyte glycogenolysis is a net release of lactate from the human brain (Overgaard et al., 2012; Dienel and Cruz, 2015). In corroboration with the work by Overgaard and colleagues (2012), a secondary finding of Chapter 5 suggests that hypoxia may stimulate astrocyte glycogenolysis in humans. Lactate released from the brain may signify severe brainstem hypoxia, and contribute to the increased blood pressure / sympathoexcitation at the latter stages of apnea (Marina et al., 2015). Metabolically, the implications of astrocyte glycogenolysis for human cerebral functioning in a state of hypoxia remain speculative; however, lactate released from astrocytes is perhaps unbenefficial in that it requires oxygen to be metabolized. Interestingly, in the hooded seal (Cystophora cristata) a genetic adaptation compared to terrestrial mammals (e.g. the ferret and mouse) (Hoff et al., 2016), and
humans (Bittar et al., 1996), allows the astrocytes to take the oxidative burden of converting lactate to pyruvate, thereby leaving the neurons to work anaerobically. That is, the hooded seal has a ‘reversed’ astrocyte-neuron lactate shuttle, whereby the astrocytes release pyruvate rather than lactate (Hoff et al., 2016).

8.6.4 A protective mechanism of hypercapnia in severe apneic hypoxemia?

In Chapter 4, and more conclusively in Chapter 5, it is demonstrated that hypercapnia reduces the CMRO$_2$. Three related mechanisms for a hypercapnic depression of the CMRO$_2$ are proposed herein – as described in detail in Chapters 2, 6 and 7. First, hypercapnia / extracellular acidosis increases adenosine concentrations which activate adenosine A1 receptors (Dulla et al., 2005) and inhibit excitatory glutamatergic neurotransmission. Second, hypercapnia/acidosis may reduce phosphofructokinase activity, evidenced from an accumulation of fructose-6 phosphate and glucose-6 phosphate in un-anesthetized rat cerebral cortices following CO$_2$ inhalation (Folbergrova et al., 1975). Finally, hypercapnia may depress the CMRO$_2$ via a reduction in cerebral temperature resulting from the increased CBF (Bain et al., 2015b). It is clear that each of these mechanisms in isolation (e.g. via adenosine receptor blockade) deserve further research. The mechanistic association between the known anesthetic properties of high blood CO$_2$ concentration (e.g. as employed for animal euthanasia) and the CMRO$_2$ also deserves attention. A potential protective mechanism of acidosis in severe hypoxemia may have direct implications in cerebral survival during, for example, respiratory failure, organ transplant and bypass surgery.
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