Description of the dynamic responses to hypoxia: Ventilation, Cerebral Blood Flow (CBF), Blood Pressure (BP), and Heart Rate (HR)

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A thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Physiology, Faculty of Medicine, University of Toronto

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This thesis describes experiments to measure the ventilatory response to hypoxia at a constant (isocapnic) level of CO$_2$ (HVR) in 18 subjects. So as to provide a complete picture of the autonomic responses, middle cerebral artery velocity, a surrogate for cerebral blood flow (CBF), as well as finger plethysmography blood pressure (BP) were also measured. Ventilatory responses have been previously described only in terms of an acute peak followed by a decline. However, rather than a single type of response, I found four types categorized as: Decline, Double, Plateau, or No response. The Double pattern, characterized by a second peak of response was the most common, yet is described here for the first time. These patterns are also characteristic of the CBF and BP responses. Furthermore the temporal correlations between these brainstem-controlled responses are also reported here for the first time.
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List of abbreviations

AHI: apnea-hypopnea index
AHVR: high acute hypoxic ventilatory response
AMPK: adenosine monophosphate-activated protein kinase
AMS: acute mountain sickness
ARDS: acute respiratory distress syndrome
ATP: adenosine triphosphate
Av: arterio-venous
bCBR: bilateral carotid body resection
BK: calcium-activated potassium
BMI: body mass index
BP: blood pressure
CA: cerebral autoregulation
Ca**: calcium ion
CB: carotid body
CBF: cerebral blood flow
cGMP: guanosine 3’, 5’-monophosphatase
CHHS: congenital central hypoventilation syndrome
CMS: chronic mountain sickness
CNP: C-natriuretic peptide
CNS: central nervous system
CO2: carbon dioxide
COPD: chronic obstructive pulmonary disease
CPG: central pattern generator
CPP: cerebral perfusion pressure
CSA: central sleep apnea
CSF: cerebrospinal fluid
CSN: carotid sinus nerve
CVR: cerebrovascular reactivity
DA: dynamic autoregulation
DAH: developmental acclimatization to hypoxia
DBP: diastolic blood pressure
DEF: dynamic end tidal forcing
DRG: dorsal respiratory group
EAH: evolutionary adaptation to hypoxia
ECF: extracellular fluid
EEG: electroencephalogram
EPAS1: endothelial PAS domain-containing protein 1
EPSPs: excitatory postsynaptic potential
ESRD: end-stage renal disease
FGF: Fresh gas flow
FRC: functional residual capacity
GPN: glossopharyngeal nerve
H+: hydrogen ion
H2O2: peroxides
HA: high altitude
Hb: hemoglobin
HCO3-: bicarbonate
HCR: hypoxic cerebral response
HCVR: hypercapnia ventilatory responses
HD: hypoxic desensitization
HIF: hypoxia inducible factor
HO-2: hemoxygenase-2
HPV: hypertensive pulmonary vasoconstriction
HR: heart rate
HVD: Hypoxic ventilatory decline
HVR: hypoxic ventilatory response
ICP: intracranial pressure
K: potassium
MAP: mean arterial pressure
MCAv: middle cerebral artery velocity
MSNA: muscle sympathetic nerve activity
NIV: non invasive ventilation
nNOS: neuronal nitric oxide
NO: nitric oxide
NTS: nucleus tractus solitaries
O2: oxygen
OSA: obstructive sleep apnea syndrome
PACO2: alveolar partial pressure of carbon dioxide
PaCO2: arterial partial pressure of carbon dioxide
PbCO2: brain partial pressure of carbon dioxide
PcCO2: central partial pressure of carbon dioxide
PCO2: partial pressure of carbon dioxide
PDP1: pyruvate dehydrogenase phosphatase catalytic subunit 1
PDP2: pyruvate dehydrogenase phosphatase catalytic subunit 2
PETCO2: end tidal partial pressure of carbon dioxide
PETO2: end tidal partial pressure of oxygen
PHDs: sprolyl hydroxylase-domain enzymes
PKG: protein kinase G
PmvCO2: mixed venous partial pressure of carbon dioxide
Q: cardiac output
RA: RespirAct
REM: rapid eye movement
RGF: RespirAct gas flow
ROS: reactive oxygen species
RR: respiratory rate
RTN: retrotrapezoid nucleus
S: sensitivity
SBP: systolic blood pressure
SD: standard deviation
SGD: sequential gas delivery
SID: strong ion difference
SL: sea level
SNP: single nucleotide polymorphism
SpO2: pulsed oxygen saturation
SS: steady state
SV: stroke volume
T: threshold
TCD: transcranial Doppler
TRH: thyrotropin
VA: alveolar ventilation
VAH: ventilatory acclimatization to hypoxia
VCO2: carbon dioxide production
VD: dead space ventilation
$\dot{V_E}$: minute ventilation
VEGF C: vascular endothelial growth factor C
VO2: oxygen consumption
VRG: ventral respiratory group
VRT: ventilatory recruitment threshold
VT: tidal volume
WBC: white blood cell
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I. Introduction

Humans breathe at a rate of about 12 breaths per minute at rest and execute 17,280 respiratory cycles in a single day, without even noticing it. Moreover, every day, millions of people breathe under hypoxic conditions due to their environment or specific health condition. Understanding the physiological processes and adaptation to hypoxia in the respiratory system is therefore critical for proper health care.

The control of breathing is regulated by the respiratory chemoreflexes, which respond to two of the gases that we breathe: carbon dioxide (CO₂) and oxygen (O₂). More directly, ventilation responds to the blood hydrogen ion concentration [H⁺]. Additionally, the control of breathing is regulated by an individual’s state (wakefulness), their acid-base balance, and their cerebral blood flow (CBF). The evaluation of chemoreflex control of breathing and their role in the control of breathing has been extensively studied and will be briefly reviewed in this thesis.

The assessment of the ventilatory response to hypoxia was first investigated in 1975 when Weiskopf et al. (Weiskoff & Gabel, 1975) showed not only the increase in ventilation in response to hypoxia, but also a subsequent ventilatory depression, later called hypoxic ventilatory decline (HVD). The method of hypoxic exposure affects the pattern of hypoxic ventilatory response (HVR, see chapter on HVR) and HVD.

At the moment, no standardized method is used to measure the HVR, leading to studies with inconsistent conclusions. Since a standard methodology is lacking, comparisons cannot be made between studies or subjects. Two leaders in the field have highlighted this problem: JW Severinghaus and J Duffin. In 2005 a forum was held at the International Hypoxia Symposium in Lake Louise, AB, where experts discussed proposals on how to approach this
problem. However no consensus could be reached. In 2007, Duffin published a review on the measurements of HVR and proposed a standardized method that is more extensive than any current method and allows comparisons between subjects and within the same subject under different conditions (Duffin, 2007). I have conducted a study evaluating Duffin’s methodology. I have evaluated the effect of isocapnic hypercapnia on the steady state ventilatory response to hypoxia (HVR). During these experiments, CBF and hemodynamic parameters (heart rate (HR) and blood pressure (BP) were measured in conjunction with ventilation.

This investigation is therefore both integrative and extensive by including not only the ventilatory response but also the cerebral and the hemodynamic responses. These comprehensive measurements should provide a more complete picture of mechanisms underlying HVR.

As an introduction to this thesis I will present a brief discussion of the physiology of ventilation and of cerebral blood flow. The ventilation chapter will describe the chemoreflexes and their interaction, to provide an understanding of the control of breathing. It will also describe the literature about the main focus of this work “the hypoxic ventilatory response”. The cerebral blood flow chapter will describe the adaptation of brain blood flow to carbon dioxide and hypoxia.
I Introduction: ventilation

1. Respiratory Chemoreflexes

1.1. Central Chemoreflex

In 1982, HH Loeschcke posed critical questions about ventilatory drive: "What is the adequate stimulus, where are the receptors, what is the mechanism of the stimulation and what are the conditions in the surrounding of the receptor?" He concluded by suggesting that the extracellular pH in the brain is the main chemical signal that determines ventilation. This pH is dependent on the tissue PCO$_2$ and acid-base variables. More precisely we do know that central chemoreceptors respond to [H$^+$] of their local environment and are better known as CO$_2$ receptors because central [H$^+$] is directly dependent on the PCO$_2$. However acid-base factors such as strong ion difference (SID), phosphate, and albumin, influence the relationship between H$^+$ and PCO$_2$ (see acid-base section).

1.1.1. Anatomical location

The respiratory neurons controlling breathing are located bilaterally in the medulla oblongata. There are two main groups of respiratory neurons – the ventral respiratory group (VRG) and the dorsal respiratory group (DRG). The VRG contains the retrofacial nucleus, the nucleus ambiguus, the nucleus retroambigualis, and the most important, the retrotrapezoid nucleus (RTN) (Okada 2002). The VRG also contains the Botzinger complex, which is the only group of neurons in the VRG shown to be able to inhibit inspiratory cells in the DRG, as well as some phrenic motorneurons. The DRG contains the nucleus of the tractus solitarius (NTS) and the locus coerules. The central chemoreceptors location is at present disputed. They may be scattered within the brain tissue (Nattie & Li, 2009), within the RTN (Guyenet et al., 2009), within the Raphé (Corcoran et al., 2009), and within the
locus ceruleus (Putnam et al., 2004) or at the ventral surface of the medulla such as there is not one spot called the central chemoreceptor. It is a functional unit made up of many different types of neurons, which interact each others. Central chemoreceptors and their neurons are still an area of active research. Researchers still dispute as to which specific group of neurons is “The” chemoreceptor.

Two important aspects have to be kept in mind regarding central chemoreception. First, the blood-brain barrier prevents polar molecules including \([H^+]\) from reaching the central chemoreceptors. However, \(CO_2\) can diffuse easily across the barrier. Hence, central \([H^+]\) can differ from arterial \([H^+]\). Second, the stimulus to the central chemoreceptor is the \([H^+]\) at the medullary brain tissue level, which is determined by the brain level of \(PCO_2\) and other interdependent acid-base factors (SID, phosphate, and albumin). Medullary tissue \(PCO_2\) itself is determined by three factors: arterial \(PCO_2\), the \(CO_2\) production of the medullary tissue and the medullary blood flow. Central chemoreceptor \(PCO_2\) varies directly with \(PaCO_2\) and inversely with medullary blood flow for any constant brain metabolic state (Duffin, 2010).

1.1.2. Mechanism of action

The central chemoreceptor cells’ chemosensitivity (intrinsic sensitivity to pH) and their anatomical connection enable the regulation of the respiration. \(CO_2\) sensitivity for the central chemoreflex control system has specific and narrow limits, which functions to keep \([H^+]\) close to normal values (Nattie et al., 1991). By contrast, the control system for \(PO_2\) is less precise. Two main theories exist about how central chemoreceptors work (Guyenet et al., 2005): the distributed chemosensitivity and the specialized chemoreceptor theories. The distributed chemosensitivity theory supports the notion that neurons present in many sites in the brain stem are sensitive to pH (Nattie & Prabhakar, 2001) and that the
chemosensitivity results from the cumulative effects of pH on large numbers of central rhythm and pattern generator (CPG) neurons and many of their modulatory inputs. This theory is based on 3 observations. First, in vitro studies have shown that some degree of pH sensitivity is very common in brainstem neurons. Second, in vivo studies have shown that acidification of the brainstem area stimulates breathing. Third, pH-modulated ion channels are widely distributed in the brainstem. Indeed, firing rate record of many neurons on slice preparation have been shown as chemosensitive. However, their response to pH was modest and of relatively uniform magnitude (Putnam et al., 2004). This uniformity of magnitude led think that chemosensitivity os widespread. However, these mild responses could be interpreted as non specific and the real chemoreceptors (when found) would display a higher pH-sensitivity than that which is observed. This conclusion is supported by in vitro evidence that neurons from the retrotrapezoid nucleus (RTN) are much more strongly activated by pH than the other neurons of the brainstem (see below(Mulkey et al., 2004)).

The second theory of chemosensitivity, the specialized chemoreceptor theory, is older and favored by Guyenet (Guyenet et al., 2009), and uses three points as evidence. First, central chemoreceptors exist within the retrotrapezoid nucleus (RTN: non serotonergic system) and in the medullary Raphé (serotonergic system). Second, the ventral surface of the medulla is physiologically important for the control of respiration. And third, there is a limited pattern of Fos expression (protein playing a role in signal transduction, cell proliferation and differentiation), in animals exposed to CO₂. Indeed, the central chemoreceptors could be located close to the ventral medullary surface, for example, the RTN. This theory states that the CPG is in fact weakly or not at all pH responsive. Indeed the central chemoreflex is composed of specialized acid-sensitive neurons, distinct from the CPG, which drive the network synaptically. Loeschcke stated this in 1982 (Loeschcke, 1982). Furthermore, it is
becoming more and more evident that the RTN plays a major role in central chemoreception (Guyenet et al., 2008). However, many cells may be involved in CO₂ sensitivity, and many neurons in the brain may be chemosensitive. These observations are reported first with respect to the reactivity of brainstem’s neurons to acid in vitro and second to the activation of respiration by perfusion of the different area of the brainstem with CO₂ (in vitro perfused rat brainstems exposed to CO₂) (NTS, Raphé, and ventrolateral medulla) (Sato et al., 1992; Okada et al., 2002)

1.1.3. Serotonergic neurons

Serotonergic neurons (5HT) from the Raphé are located close to large medullary arteries and are sensitive to and activated by CO₂ and pH, and the Raphé drives the VRG neurons. Moreover, there is a mechanism for respiratory network stimulation, such that serotonergic Raphé neurons have a strong ability to control ventilation. 5-HT neurons of the medullary Raphé are stimulated by hypercapnia in vivo, and their disruption results in a blunted hypercapnic ventilatory response. They might also mediate the non-respiratory effects of acidosis, such as the hypercapnic arousal response. In addition to their response to acidosis they also might respond to hypoxia, hypoglycemia, temperature, or blood pressure (Richerson, 2004). It is also proposed that Raphé neurons may actually act as PaCO₂ sensors, and their seemingly unrelated effects on ventilation via arousal, anxiety, cerebrovascular control and other brain functions is due to the goal of maintenance of pH homeostasis (Richerson, 2004). The contribution of medullary Raphé 5-HT neurons to ventilatory control is dual. First, they provide tonic, excitatory drive to multiple components of the respiratory network; and second they sense changes in tissue pH/CO₂ via intrinsic membrane properties, and through changes in neurotransmitter release alter the level of tonic,
excitatory drive to appropriately adjust ventilation of 5-HT neurons in the control of breathing, including eupneic ventilation and CO₂ chemoreception. (Corcoran et al., 2009; Hodges & Richerson, 2010a, b).

1.1.4. RTN neurons

Twenty years ago, Nattie showed that RTN appears to be necessary for the maintenance of eupneic phrenic activity and CO₂ sensitivity, even in decerebrated cats with intact peripheral chemoreceptors (Nattie et al., 1991). The RTN neurons are now the most completely characterized. And there is now evidence for the connections of the RTN cells within the breathing network. The RTN receives input from multiple Raphé nuclei, that serotonin, substance P, and TRH activate RTN chemoreceptors, and the excitatory effects of serotonin are mediated by distinct ionic conductances. This evidence suggests that RTN neurons may effectively mediate the respiratory stimulation of serotonergic neurons. However, serotonin does not seem to affect the cellular mechanism by which RTN neurons themselves detect pH (Mulkey et al., 2004).

RTN neurons have a high sensitivity to CO₂ in vivo presumably due to their intrinsic acid sensitivity, excitatory inputs from the carotid bodies (peripheral chemoreceptor sensing [H⁺] see next section for more details) and brain regions such as Raphé and hypothalamus, and facilitating influences from neighboring astrocytes. RTN neurons are necessary for the respiratory network to respond to CO₂ during the perinatal period and under anesthesia. In conscious adults, RTN neurons contribute to an unknown degree to the pH-dependent regulation of breathing rate, inspiratory, and expiratory activity. (Guyenet et al.) (Mulkey et al., 2007).
Research in that domain would be very important clinically as abnormal prenatal
development of the RTN contributes to congenital central hypoventilation syndrome
(CHHS). Studies have been done in animals by looking at the Phox2b transcription factor
which had been knocked out (Phox2b \(^{-/-}\), a mutation seen in CHHS). We do know that
chronic brain lesions around the RTN lead to breathing impairments (Amiel \textit{et al.}, 2003)
(Stornetta \textit{et al.}, 2006). Takakura (Takakura \textit{et al.}, 2008) later showed in adult rats that RTN
neurons play a role in the pH-regulated excitatory drive of the CPG, and that Phox2b\(^{+}\) TH \(^{-}\) neurons are responsible for the function of the RTN. Very recently, galanin has been shown
as a specific marker for the Phox2b\(^{+}\) TH \(^{-}\) neurons in the RTN (which was lacking up to now, limiting studies), which offers great potential for future studies (Stornetta \textit{et al.}, 2009).
Glutamatergic RTN neurons (ccRTN) are activated by acidification and seem to be a critical
connection for the regulation of CO\(_2\) via breathing. They are known to regulate pace of
breathing and were shown recently to have pacemaker properties, which vanish after birth to
be replaced by synaptic drives. The neonatal parafacial respiratory group (pfRG) may
represent a transitional phase during which ccRTN neurons lose their group pacemaker
properties. (Guyenet & Mulkey, 2010)

\subsection*{1.1.5. Orexin neurons}

The orexin neurons form a small cluster located in the lateral hypothalamus. They project
into the forebrain and the hindbrain and participate in various physiological functions
including sleep-wake regulation and feeding and are CO\(_2\) sensitive. Abnormality of orexin
neurons induces reduced CO\(_2\) response, especially in wakefulness state. In the other hand,
administration of an antagonist orexin known to enhance sleep is shown to reduce CO\(_2\)
response during wakefulness in the dark. Orexin neurons do participate importantly in
chemoreception, perhaps by augmenting responses during wakefulness at lower hindbrain chemoreceptor sites like the RTN (Fortuna et al., 2009) and medullary Raphé. Orexin neurons may be a source of the sleep-wake difference in CO₂ sensitivity (Nattie & Li, 2010; Nattie, 2010).

1.1.4. Anatomical neurons connection

Is there a connection between candidate respiratory neurons? Pilowsky hypothesized that [H⁺] sensitive neurons on the ventral surface are synaptically connected to dendrites from the respiratory neurons of the VRG. The VRG neurons dendritic trees extend to the surface of the medulla and receive a transmitter drive. Moreover, neuronal activity increased following CO₂ stimulation and the neurons are activated without synaptic input, meaning that these VRG neurons are sensors (Okada et al., 2002; Oshima et al., 2006).

There is a network connection between the RTN and the Raphé. The RTN receives input from multiple Raphé nuclei. Serotonin, substance P, and TRH activate RTN chemoreceptors, and the excitatory effects of serotonin are mediated by distinct ionic conductances. This evidence suggests that RTN neurons may effectively mediate the respiratory stimulation of serotonergic neurons. However, serotonin does not seem to affect the cellular mechanism by which RTN neurons themselves detect pH (Mulkey et al., 2004).

1.1.5. Acid-base balance and central chemoreflex

Not only does CO₂ itself affect [H⁺], other independent acid-base factors determine the relationship between [H⁺] and CO₂. Acid base balance affects the central chemoreflex via the extracellular fluid (ECF) pH, which is dependent on both PaCO₂ and the environmental ECF or the cerebrospinal fluid (CSF) [HCO₃⁻] (Sato et al., 1992). At altitude, the peripheral drive increases ventilation, leading to a decrease in PaCO₂, an increase in central chemoreceptor
ECF pH, and a decrease in the central ventilatory drive. The acute adaptation to altitude that occurs over hours and days leads to a decrease in CSF $[\text{HCO}_3^-]$ and $\text{PaCO}_2$, and the CSF pH stays alkaline and increases over time.

The investigations of the effects of strongly dissociated ions on the control of breathing as well as on the cerebrospinal fluid flow (CSF) indicate that the strong ion difference concentration ($[\text{SID}]$: $[\text{SID}] = [\text{Na}^+] + [\text{K}^+] + [\text{Ca}^{2+}] + [\text{MG}^{2+}] - [\text{CL}^-] - [\text{Other Strong Anions}]$) in brain fluids ($[\text{SID}]$ CSF) could stimulate the central chemoreceptors. $[\text{SID}]$ CSF consistently predicts ventilatory regulation of $\text{PCO}_2$, whereas $[\text{H}^+]$ CSF does not. $\text{PCO}_2$ acts as a ventilatory stimulus independent of $[\text{SID}]$ CSF and possibly at higher as well as lower centers of the nervous system. The concept of $[\text{SID}]$ regulation of arterial $\text{PCO}_2$ is related to the alphastat hypothesis of protein function, respiratory control, and $[\text{H}^+]$ homeostasis. Also, Angiotensin II acts centrally to stimulate ventilation. There exists evidence for the roles of both the renal and brain renin-angiotensin systems in respiratory control, and the modulation of respiratory control by vasopressin. They probably act via circumventricular organs of the brain to affect respiratory control and / or by changing the concentration of strong ions in the brain fluids (Jennings, 1994).

1.2. Peripheral Chemoreflexes

1.2.1. Anatomical location

The peripheral chemoreceptors are located in the carotid body. The carotid bodies are located bilaterally at the bifurcation of the common carotid arteries. The carotid bodies are formed by thousands of type I cells arranged in glomeruli (3-5 cells clustered). Each cluster is associated with one type II cell, blood vessels, and an afferent nerve supply.
1.2.2. Role of the carotid body

The first questions of relevance to this organ are “what is the exact role of the carotid bodies?” and “are they only oxygen sensors?” In 2007, Kumar (Kumar, 2007; Kumar & Bin-Jaliah, 2007; Kumar & Prabhakar, 2007) addressed these questions and suggested that carotid bodies are polymodal receptors, since they are able to not only detect \( \text{PO}_2 \) but also transduce \( \text{PCO}_2 \), pH, blood [K], temperature, and blood glucose. Moreover, these stimuli are sensed independently of the hypoxia transduction process, and the carotid bodies cannot distinguish between all of them. They respond to stress only by intensity gradients, inducing a cardio-respiratory and/or endocrine reflex(es). The multiple stimuli interact to provide additive and greater than additive effects.

Type I cells are the oxygen-sensing element and release a variety of neurotransmitters, such as acetylcholine, ATP, and dopamine, which trigger excitatory postsynaptic potential (EPSPs) in synapsed neurons that innervate the respiratory center. They are connected to each other via chemical and electrical gap junctions. By contrast, Type II cells act as supporting cells and do not make synaptic contact with afferent nerve fibers. The carotid bodies (CB) increase their firing rate in response to increased \( \text{PaCO}_2 \), decreased \( \text{PaO}_2 \), or decreased arterial pH. However, the exact mechanisms by which the chemoreceptors function remain uncertain. Also, the mechanism by which \( \text{O}_2 \) (and specifically, hypoxia) is sensed is not completely understood.

The total blood flow of the carotid bodies at mean blood pressures of 120 to 130 mm Hg is 40\( \mu \text{l/min} \). As the weight of the carotid body is 2 mg, this means that the flow is as high as 2000 ml 100g\(^{-1}\) min\(^{-1} \) (Joels & Neil, 1963; Kumar, 2007). These organs are highly vascularized, on the order of 5 to 6 times that of the brain. This vascularization allows the
carotid bodies to sense changes in the arterial blood and react very quickly. Also, the carotid body resting $O_2$ consumption in normoxia conditions is high; 1-2 ml per g$^{-1}$ min$^{-1}$. The range of values reported varies depending on the type of study (in vivo or in vitro), ranging from 0.6 ml 100g$^{-1}$ min$^{-1}$ to 9 ml 100g$^{-1}$ min$^{-1}$ (Purves, 1970; Keller & Lubbers, 1972).

1.2.3. Sensing neurotransmitters and ions channels

Hypoxic hypoxia induces a Ca$^{++}$-dependent increased neurosecretion from the type I cells of the glomus and an elevated action potential frequency in the postsynaptic carotid sinus nerve afferents. The membrane depolarisation in response to hypoxia, and acidosis, appears to be primarily mediated via the inhibition of a background K$^{+}$-current (Nattie & Prabhakar, 2001; Buckler, 2007; Kumar & Bin-Jaliah, 2007).

The chemoafferent discharge from the carotid body in response to hypoxic hypoxia is < 1 second with a peak at 1 - 5 seconds after subtraction of the circulatory transit delay. However, this response does not adapt and remains sustained for the whole hypoxic period. The discharge begins to rise gradually from 400-140 mmHg of PaO$_2$ until 100 mmHg, when the slope increases. Then it increases abruptly when the PaO$_2$ is 70-75 mmHg and reaches its maximum activity at a PaO$_2$ of 20-30 mmHg. Below a PaO$_2$ value of 20 mmHg, the discharge is reduced. The PaO$_2$-response curve can be modeled as a sigmoid function, the inverse of the hemoglobin oxygen-dissociation curve.

The steady state carotid sinus nerve afferent discharge increases with increasing hypoxia, in an isocapnic condition, and may be described by a single exponential function with an offset $> 0$. This offset is important since while a reduction in PaO$_2$ triggers the oxygen chemoreception process, oxygen is needed to provide the necessary energy to sustain the
chemosensory responses. As a result, while the hypoxia increases, it may fail to sustain aerobic metabolism and support the response.

Importantly, the mechanism of oxygen sensing may differ for different tissues and cells. It is not really hypoxia that is sensed by the carotid body, but rather the lack of oxygen as a consequence, which leads to a change in intracellular physiology. Up to now, no single sensor for a lack of oxygen has been defined. It is likely that different sensors are involved and react to oxygen with different affinities and have different downstream targets (Nattie & Prabhakar, 2001; Buckler, 2007; Kumar & Bin-Jaliah, 2007).

Several hypotheses on the nature of neurotransmitters and ion channels have been tested to better understand the reflex mechanism of the carotid body, particularly the oxygen sensors. According to Kumar (Kumar & Bin-Jaliah, 2007), the two major ones are the Adenosine monophosphate-activated protein kinase (AMPK, enzyme involved in cellular energy homeostasis) and the plasma-bound membrane hemooxygenase-2 (HO-2). The first increases during hypoxia, and its activation can inhibit both calcium-activated potassium (BK) and TASK-like potassium channels. The second (HO-2) uses oxygen as a substrate, and acts to gate an associated BK channel. Both sensors have been associated with potassium channel inactivation during hypoxia. Also, O$_2$ interaction with maxiK channels does not require cytoplasmic mediators. Such interaction could be mediated by a membrane hemoprotein that, as an O$_2$ sensor, would modulate channel activity (Riesco-Fagundo et al., 2001). And long term dissociated culture of the type I cells have shown they retain the expression of O$_2$-sensitive, TASK-like, and Ca$^{++}$-dependent (BK) K$^+$ channels (Nurse & Fearon, 2002; Nurse, 2005). Moreover, the parasympathetic efferent pathway plays a role and the autonomic neurons are embedded with glossopharyngeal (GPN) and carotid sinus (CSN) nerves. Also, hypoxia may activate GPN neurons by inhibition of background K$^+$ channels, leading to an
increased firing, voltage-gated Ca$^{++}$ entry, neuronal nitric oxide synthesis (nNOS) activation and NO release. Activation of GPN neurons by hypoxia and/or ATP released by hypoxic stress would lead to efferent inhibition of the carotid bodies, providing a dual mechanism for negative feedback control of respiration via the same neuronal pathway (Campanucci & Nurse, 2007).

Other hypotheses such as the mitochondrial and membrane models for potassium have shown that carotid bodies express a large number of inhibitory transmitters, that they are slowly-adapting sensory receptors, and that the increase in sensory discharge due to hypoxia is maintained during the whole duration of the stimulus (Lahiri et al., 2006). However, if transmission is due to an excitatory transmitter alone, only a brief excitation will follow, with a rapid return to the baseline discharge level, despite persistence of the hypoxic stimulus. Co-release of inhibitory messengers will provide sustained excitation by preventing over-excitation due to excitatory transmitters. The co-activity of excitatory and inhibitory messengers describes a "push-pull" mechanism (Prabhakar et al., 2007).

Reactive oxygen species (ROS) also contribute to the chemoreflex. ROS are oxygen-containing molecular entities that were considered as undesirable byproducts of cell metabolism until now. However, ROS have been considered important intracellular signaling molecules, possibly acting as mediators or second messengers in many cell functions. The oxygen-sensing role of ROS is proposed in 3 different cells such as carotid body chemoreceptor cells, pulmonary artery smooth muscle cells, and erythropoietin-producing cells. These cells are unique and have essential parts of homeostatic loops directed to maintain oxygen levels in multicellular organisms in situations of hypoxia. However, in none of the three cell types do ROS satisfy investigation, and thus it appears that alternative
mechanisms are responsible for the transduction cascades linking hypoxia to the release of neurotransmitters (Gonzalez-Garcia et al., 2004).

From an experimental point of view, it is also important to know that PaO₂ seems to not be sensed by the carotid body of all species. And anaemic hypoxia without alteration of the PaO₂ is not a principal stimulus of the carotid body. It does not stimulate ventilation. However, it seems that it does for rodent species. Also, the effects of the neurotransmitters may be completely different depending on the species under investigation. For example, dopamine and acetylcholine have different effects on cats versus rabbits.

1.2.3. CO₂ but O₂ sensor…

All these transmitters and possible stimuli are very important but miss a major player: the effect of PaCO₂ and [H⁺]. It has been shown that actually the peripheral chemoreflex responds to hypoxia not via oxygen sensors but via an increased sensitivity to PaCO₂ and [H⁺]. (Torrance, 1996; Mohan & Duffin, 1997; Rapanos & Duffin, 1997; Kumar & Bin-Jaliah, 2007)

This response may be modulated depending on the level activity of the carotid bodies and on the altitude. At sea level, the increase in CO₂ sensitivity dominates even with a small increase of the carotid bodies activity. In adapted altitude residents, the response of CO₂ sensitivity to hypoxia does not exist anymore and is replaced by a decrease in ventilatory recruitment threshold due to an increase of the carotid body activity (Smith et al., 2010).

In conclusion, a lot of hypotheses with potential ion channels have been tested but none of them exactly could answer the question" "how does it work? " However, it seems that the carotid bodies are CO₂ receptor regulated by O₂.
Also, we should keep in mind that there is discrepancy depending on the species used to make the investigation since O₂ sensing may be completely opposite and single-fibre afferent frequency may be very different.

1.2.4. Interaction of central and peripheral chemoreceptors

Both Central and Peripheral chemoreceptors respond to changes in [H⁺] but with a different time constant such as central chemoreceptor, located at the medullar brain responds slower than the peripheral chemoreceptor. In a hypoxic condition, ventilation responds by an initial contribution from the peripheral chemoreflex, followed by a slower response from the central chemoreflex. Indeed, medullary tissue PCO₂ responds slowly to PaCO₂ alterations. The time constant for the central chemoreceptor response to changes in P_{ACO₂} is about 100 sec, and it takes 3 time constants for the central chemoreceptor stimulus to catch up to changes in inspired CO₂. Hence, a sudden change in PCO₂ will lead to an initial peripheral (fast) contribution, followed by a central (later) contribution of the breathing drive (Torrance, 1996; Smith et al., 2010).

How are the peripheral and central chemoreceptors connected, are they independent or is there an interaction? If so, what is the nature of it? Up to today, the debate about the interaction between peripheral and central chemoreceptor is still open. Several studies have been conducted in animals and in humans leading to several different results. Three kinds of interactions have been suggested: additive interaction (summation), hypoadditive interaction (lower than summation), or hyperadditive interaction (higher than summation).

Animal experiments:

Studies in anesthetized and decerebrate cats (Heeringa et al., 1979; van Beek et al., 1983) support an additive interaction. These authors measured the ventilatory response to PaCO₂ at
three constant levels of central PCO\(_2\) and concluded that central and peripheral chemoreceptors take up two-third and one-third, respectively, to the whole CO\(_2\) sensitivity. They also suggested that the interaction of central and peripheral chemoreceptors could be ignored. Study conducted in awake goats (Smith et al., 2010) and decerebrated rats (Day & Wilson, 2007, 2008) supported hypoadditive interaction, by using perfusion techniques to separate the stimuli to central and peripheral chemoreceptors.

Recently, the effect of CB inhibition on eupneic ventilation in the resting, awake, intact dog has shown that CB chemoreceptors contribute more than one half to the total eupneic drive to breathe. This contribution consists of both the normal tonic sensory input from the CB to medullary respiratory controllers as well as a strong modulatory effect on central chemoreceptor responsiveness to CO\(_2\). Hence, the interaction can be hyperadditive (Blain et al., 2009) or multiplicative (Smith et al., 2010).

Three speculations would have major clinical implications in case they were confirmed. First, central and peripheral chemoreceptors do not act independently of one another. Second, interaction of central and peripheral chemosensory inputs to the ventilatory control system would not be simply additive but would likely be more complex than currently known. Third, as suggest by Guyenet et al. (Guyenet et al., 2005; Guyenet et al., 2008) the chemosensitive neurons of the retrotrapezoid nucleus (RTN) also possess integrating properties for several sensor inputs (pulmonary stretch receptors, baroreceptors, central locomotor areas), leading to a possibility that there is complex interactions between chemosensory and other non-chemosensory reflexes.

The latest dog study (Blain et al., 2009) has demonstrated a hyperadditive interaction between central and peripheral chemoreceptors during CB hypoxia. However, interaction during CB hypercapnia has not yet been done.
Humans’ studies:

However, in humans, the interaction of central and peripheral chemoreceptors has been shown as additive (independent) (Clement et al., 1992; Clement et al., 1995; St Croix et al., 1996). Hypoxia is sensed by changing the response to H⁺. This fact should highlight an argument against all the studies made from cell cultures and in vitro preparations that ignore H⁺ sensors. Nielson & Smith (Nielsen & Smith, 1952) originally showed that the sensitivity to CO₂ increases as the hypoxia increases. They also found an independent response to hypoxia. However, in 1997, Mohan et al. as well as Rapanos et al. could not repeat the observation (Mohan & Duffin, 1997; Rapanos & Duffin, 1997). By contrast, they showed that below a threshold value of PCO₂ (40 mmHg), hypoxia does not have an impact on ventilation. Two major points arise from these studies. First, the sensitivity of the response to PCO₂ is controlled by the PO₂. Second, there is a threshold PCO₂ below which hypoxia does not stimulate ventilation. The response to hypoxia may therefore be reduced or abolished by lowering the PaCO₂. That's why hypoxia without hypercapnia may be considered as a silent killer.

A recent study in human after bilateral CB resection (bCBR, for CB tumor) has suggested that the CB exert a tonic drive or tonic facilitation on the central chemoreflex loop. (Dahan et al., 2007; Dahan et al., 2008).

The debate between multiplicative or additive interaction is still open at the moment but no other studies has been conducted on that regard.

3. The effect of hypoxia on ventilation

This chapter will review the literature in regard to the hypoxic ventilatory response and how advanced technologies led to new knowledge. The full characterization of the HVR and the
adaptive response has been recently described and will be reviewed briefly. The continuation of studies investigating the mechanism of the ventilatory decline will be presented. Finally, I will discuss the important aspects to take into account in order to generate physiologically meaningful measures of HVR.

3.1. Hypoxic ventilatory response (HVR)

The hypoxic ventilatory response can be defined as the change in ventilation from a hyperoxic condition to a fixed hypoxic condition, at a specified level of PCO$_2$. The traditional method used refers to applying an inspired concentration of O$_2$ and characterizing the ventilatory response over time. The study of HVR is undertaken to better understand the physiological adaptations to hypoxia due to altitude or disease. Additionally, studies applying rebreathing technique have been also conducted in order to understand the physiology underlying the detection and response to hypoxia.

Initial studies of the ventilatory response to hypoxia described an initial increase in ventilation, driven by the peripheral chemoreceptors. However, as a result of the increase in ventilation, there was a reduction in the PaCO$_2$ and an increase in the PaO$_2$. These changes were commensurate with the change in ventilation and in turn, affected the ventilatory response. Therefore the ventilatory response could not be attributed back to the initial hypoxic stimulus (Weiskoff & Gabel, 1975) (Weil & Zwillich, 1976) (Weil & Zwillich, 1976).

The capability of maintaining isocapnia in the laboratory has led to better characterization of HVR. The first investigation regarding the effect of isocapnic hypoxia on ventilation was completed by Easton (Easton et al., 1986) who found that the hypoxic ventilatory response is time dependent showing a biphasic ventilatory response pattern to hypoxia. However, the
techniques used to keep isoxia and isocapnia were not ideal and so it was not possible to be certain of the “true” ventilatory response to a sustained hypoxic stimulus. It is now known that HVR is mediated by the peripheral chemoreflex, increasing their sensitivity to CO₂ by changes in [H⁺] at the carotid body level (Cunningham, 1987; Torrance, 1996; Kumar & Bin-Jaliah, 2007). The characterization of the full ventilatory response to hypoxia has been completed recently (Steinback & Poulin, 2007) using end-tidal forcing to administer a steady state isocapnic hypoxic ventilatory stimulus. They found an initial increase of ventilation, over the first 5 minutes, followed by a decline lasting 5 to 20 minutes. Investigators have used both isocapnia and poikilocapnia (CO₂ is not controlled) in order to differentiate the effect of respiratory alkalosis, simulating exposure to high altitude. With isocapnia, the acute hypoxic response is due to increase in both tidal volume (VT) and respiratory rate (RR), with VT responding earlier. With poikilocapnia, hypocapnia develops in tandem to increases in ventilation (which are less compared to when isocapnia is maintained) and composed predominantly in VT. Nevertheless, the relative magnitude of the HVD was similar in isocapnia and poikilocapnia, and the magnitude of the decrease was highly correlated with the initial ventilatory increase in both conditions.

Mediation of the hypoxic ventilatory response by higher brain centre adaptations has been considered, and was the reason that further study including cerebo and cardiovascular parameters was conducted (Steinback & Poulin, 2008). The middle cerebral artery velocity (MCAv) increased at the onset of hypoxia but did not adapt overtime. They showed a modest increase of the MAP, delayed during poikilocapnia, which did not adapt over the hypoxic stimulus. At the termination of the hypoxic stimulus, MAP returned to baseline during isocapnia but stayed elevated during poikilocapnia. The heart rate (HR) was found to increase at the onset of hypoxia and to adapt with the same pattern of response as the minute
ventilation, with an initial increase followed by a progressive decline suggesting that the HR response to hypoxia may be linked to the HVD. Hence, the adaptive response to hypoxia might be mediated by a cardio-pulmonary reaction associated with the HVD. However a drawback of that study was that a unique isocapnic level was applied and that it was chosen quite low (only +2 mmHg above resting level), and it is known that the isocapnic level will change the HVR itself (Mohan & Duffin, 1997; Mateika et al., 2004), as described in the next section of this chapter.

Rebreathing tests have been used in order to differentiate the contribution of peripheral and central chemoreflexes during hyperoxic and hypoxic conditions. This kind of test measures the response to a linear increase of PCO$_2$, where alveolar, arterial, mixed venous PCO$_2$ are in equilibrium, while hyperoxic or hypoxic background. During hypoxic rebreathing, the carotid body activity is higher as indicated by the ventilatory recruitment threshold (VRT, level of carbon dioxide at which ventilation starts to increase) compared to hyperoxic condition; there is a left shift of the VRT (PCO$_2$ value) and the ventilatory sensitivity to CO$_2$ is increased. Also, the peripheral chemoreceptors contribute at the initial increase of ventilation, and the central chemoreceptors contribute in the later aspects (Mohan & Duffin, 1997). With respect to ventilatory components, VT is the first to contribute to the increase of ventilation, and RR is recruited later as PCO$_2$ rises (Vovk et al., 2002). Studies performed under resting and exercise conditions have shown that the VRT value mediated by the peripheral contribution is situated approximately at 39 mmHg while the recruitment of the central chemoreceptor is at approximately 45 mmHg. These VRT values do not change with exercise (Duffin & McAvoy, 1988).
3.2. Methods and protocols used for measuring HVR

There are five types of techniques used to measure HVR in humans (Teppema & Dahan, 2010). First, steady state by using fixed inspired [O$_2$] and [CO$_2$]. This protocol does not provide a constant and repeatable stimulus. Also, it includes the hypoxic ventilatory decline (HVD) so care must be applied in interpreting results. Second, single or double breath tests involving transient hypoxic or hyperoxia for no more than a few breaths, developed by Dejours (Dejours et al., 1957). The change in ventilation within the next few breaths is a measure of hypoxic sensitivity. An advantage of this approach is that there is no “contamination” by HVD. The disadvantage is that it does not allow the full development of the HVR, and isocapnia is not maintained during the test. Moreover, repeated tests have to be done because there is a random variation effect of the background ventilation. Third, progressive hypoxic tests involving rebreathing of a gas mixture from a rebreathing bag or circuit with CO$_2$ absorption allowing some control of end tidal PCO$_2$. Fourth, step hypoxic or steady state tests with rapid decrease of PO$_2$ while maintaining isocapnia. Fifth, techniques using isoxic hypoxic and hyperoxic ventilatory CO$_2$ responses. The two last tests are more widely accepted.

3.2.1. Progressive hypoxic tests

The progressive hypoxic tests vary in terms of depth of hypoxia, or the hypoxic ramp (speed to which hypoxia is applied) and the duration. Attempts are made to maintain isocapnia by adding CO$_2$ to the circuit. Nevertheless, PCO$_2$ seems to still increase, making it impossible to distinguish if the cause of the increase of ventilation is due to HVR or to the subsequent hypercapnia. Moreover, if the test lasts more than 5 minutes, there is a contaminaton by HVD.
3.2.2. Step hypoxic tests

Two techniques allowing precise control of PetO₂ and PetCO₂, regardless of ventilation have been developed. The oldest one is the Dynamic End Tidal Forcing (DEF), developed by Swanson and Belleville late 1960s, early 1970s originally in order to measure the ventilatory response to CO₂ and to quantify the contribution of the peripheral and the central chemoreflexes to ventilation (Swanson et al., 1971; Swanson & Bellville, 1974, 1975). Later the technique has been used to study step and sinusoidal hypoxic responses (van Beek et al., 1983; Cunningham & Robbins, 1984; Ward, 1984; Steinback & Poulin, 2007, 2008; Steinback et al., 2008). The control of isocapnia and isoxia with DEF is accurate, with a standard deviation < 0.6 Torr. However, this technique is relatively complex, expensive and inherently less safe as not all gas tanks contain oxygen. It is difficult to apply to a large cohort of subjects.

A recently developed technique is the prospective end tidal targeting, with the RespirAct™ apparatus (Thornhill Research Incorporate, Canada) (Somogyi et al., 2005b; Slessarev et al., 2007; Ito et al., 2008). It allows precise control of end tidal gases, regardless of the minute ventilation of the subjects. It is also less complex, less expensive, and more inherently safe than the DEF resulting in greater applicability in research.

3.2.3. Measuring HVR via ventilatory response to CO₂

Rebreathing techniques have been developed by Read in 1967 (Read, 1967) and can be used to measure hypoxic ventilatory response as the increase in ventilation in response to progressively rising PetCO₂ at hyperoxia and hypoxia (Sato et al., 1992). Hypoxic response can be also measured as the difference in slope of the CO₂ response curve, the ratio between the two slopes, or the change in the position of the curve (Rebuck et al., 1977; Duffin, 2007).
3.2.4. Methodological aspects of the HVR

When measuring HVR, several important aspects need to be taken into consideration, as they will affect the response itself. First the issue of PCO$_2$ handling must be chosen according to the goal of the study. If the research question is regarding the effect of environmental hypoxia, the investigator could apply either poikilocapnia as in the case of measuring the ventilatory response to hypoxia of high altitude, or perform a rebreathing testing to characterize the chemoreflexes during high altitude acclimatization. If the research question involves the hypoxic response and its adaptation, isocapnia (steady state method, involving a sustained and controlled stimulus in order to elicit the dynamic response) should be used. In this case, the level at which isocapnia is maintained is critical as HVR varies with the PCO$_2$ (Mohan & Duffin, 1997; Mateika et al., 2004). For example, PCO$_2$ clamping should be above the VRT, otherwise, the HVR will be underestimated, or result in no ventilatory response, as, by definition, VRT is not reached. (Mohan & Duffin, 1997; Mahamed & Duffin, 2001a).

Also, the acid-base balance will affect the HVR by right shifting it due to metabolic alkalosis, and left shift it in the event of metabolic acidosis (Somogyi et al., 2005a). Lastly, the effect of the state of arousal will affect the HVR as the wakefulness drive is abolished during sleep, as well as under anesthesia, but increased with exercise (Neubauer et al., 1990).

In order to study the true peripheral chemoreflex response, the central drive should be kept constant through all experiments (Somogyi et al., 2005a). This is complicated, as all the conditions mentioned above will affect the central drive. Determining the value of the central drive and maintaining it constant while changing PO$_2$ levels is impossible in steady state methods due to changes in cerebral blood flow and therefore the central PCO$_2$ (Duffin et al., 2000; Somogyi et al., 2005a; Duffin, 2007). The solution is to use a rebreathing method.
where iso-oxia is maintained, and after an initial hyperventilation phase, CO₂ rises linearly. Using this method the wakefullness drive, VRT and sensitivity can be detected, and if the test is repeated at hyperoxia and hypoxia then the specific contributions of the central and peripheral drives can be determined (Mohan & Duffin, 1997; Duffin et al., 2000; Duffin, 2007). Indeed, hyperoxic rebreathing will relate the contribution of the central chemoreceptor as hyperoxic condition does silent the peripheral chemoreceptor. Hypoxic rebreathing includes contribution of both peripheral and central chemoreceptors. However, if using a steady state method, rather than keeping the central drive equal to assess the response to hypoxia, the solution would be to use two iso-oxic levels and measure the ventilatory response to several levels of isocapnia (Duffin, 2007). In this way, several CO₂ sensitivity points will be obtained. The two oxygen levels to be used may be debatable, but the hyperoxic sequence will allow the activity of the central drive to be measured, as the peripheral drive is silenced by hyperoxia (Duffin, 2007; Teppema & Dahan, 2010).

3.2.5. Proposals for the measure of HVR

A lack of comparibility between results obtained by the various groups indicated the necessity to reach a consensus about “How to measure HVR?” The Hypoxia conference held in 2005 at Lake Louise led to several proposals (see below).
### Table 6. Proposed methods to measure the hypoxic ventilatory response

#### Proposed Methods to Measure Hypoxic Ventilatory Response

**Note:** These methods are proposed for clinical testing.

<table>
<thead>
<tr>
<th>Test 1: Invasive HVIR (HVIR) to assess the hypoxic sensitivity of the arterial chemoreceptors</th>
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<td><strong>Baseline oxygen level</strong></td>
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#### Notes

- The HVIR is calculated as the change in minute ventilation (AV$_{E}$) divided by the change in arterial P$_{aCO_2}$ (AV$_{pCO_2}$).
- The CHEVR is calculated similarly as the change in minute ventilation (AV$_{E}$) divided by the change in arterial P$_{aCO_2}$ (AV$_{pCO_2}$).
- These methods are designed to assess the hypoxic response in a controlled environment.

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*An alternative is the measurement of a three-point steady-state V$_{E}$-CO$_2$ responses performed at hypoxia and normoxia. Two:** A non-invasive test in an isocapnic 25% step hypoxic test as suggested by Bersten and colleagues (2003). **I**HVR, invasive hypoxic ventilatory response; **CHEVR**, chemoreceptor hypoxic ventilatory response; **CHEVR**, chemoreceptor hypoxic ventilatory response; **CHEVR**, chemoreceptor hypoxic ventilatory response; **CHEVR**, chemoreceptor hypoxic ventilatory response; **CHEVR**, chemoreceptor hypoxic ventilatory response. **CHEVR** responses are performed at constant arterial P$_{aCO_2}$ levels according to manufacturer protocols. **CHEVR** responses are performed at constant arterial P$_{aCO_2}$ levels according to manufacturer protocols. **CHEVR** responses are performed at constant arterial P$_{aCO_2}$ levels according to manufacturer protocols. **CHEVR** responses are performed at constant arterial P$_{aCO_2}$ levels according to manufacturer protocols. **CHEVR** responses are performed at constant arterial P$_{aCO_2}$ levels according to manufacturer protocols.

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*Teppema et al., 2010.*
3.4. Hypoxic Ventilatory Decline (HVD)

The second phase of the hypoxic ventilatory response is adaptive and described as “decline” because it decreases to a new level, lower than the peak response (HVR) but still higher than the hyperoxic ventilatory response. The mechanism of hypoxic ventilatory decline has elicited a lot of interest, however it is still not fully understood (see also the Results section for new insights).

A first notion and an obvious one, was that decline is a central effect. Hypoxia leads to a known increase in CBF, which could washout the central PCO\(_2\), leading to a decline in ventilation. However, this hypothesis has not been supported by studies (see CBF chapter).

It has been demonstrated that HVD is due (Neubauer et al., 1990) to a desensitization of the peripheral chemoreflex (Sato et al., 1992; Mahamed & Duffin, 2001b) and the amplitude HVD depends on the initial HVR. It is also supported by results from rebreathing method before and after steady state eucapnic hypoxia. Only the ventilatory recruitment threshold changed (an indicator of the CB activity) over days of hypoxic exposure, confirming an effect on the carotid bodies.

Central depression has also been shown as a contributor to the decline as lactic acid generated during hypoxia within medullary chemosensor cells decrease their activity, but activity is restored if PaCO\(_2\) increases (Severinghaus, 1995). The decline is an accommodation of the response mediated by the general level of activity of the peripheral chemoreflex, or by the effectiveness of the transmission of the afferent signals (Mahamed & Duffin, 2001b). In a different approach, the effect of the time course of HVR in high altitude (HA) has been investigated. During isocapnic hypoxia, there is a total reduction of ventilation.
and an insignificant reduced sensitivity of the HVR. The authors suggested that HVD is an alteration of the central CO₂ chemosensitivity set point (Sato et al., 1994).

To complete this section, I will quote the 6 characteristic of the HVD in awake adult humans and mammals presented by Teppema as it outlines these mechanisms quite well (Teppema & Dahan, 2010):

1. The magnitude of HVD is proportionally related to the size of the initial ventilatory response to acute hypoxia.

2. Bilateral carotid body resection or chemodenervation results in the loss of the ventilatory response to acute hypoxia and absence of HVD.

3. HVD persists beyond the initial period of hypoxic exposure. The ventilatory response to acute hypoxia following 20 min of moderate isocapnic hypoxia and 5 min of air breathing is depressed by 50%. This delayed recovery persists for up to 1 h.

4. Suppression of the peripheral drive with drug (low-dose dopamine) does not lead to HVD during 20-min hypoxia. Despite the presence of initial central hypoxia, a subsequent ventilatory response to acute hypoxia develops fully.

5. The magnitude of the fall in ventilation at the relief of hypoxia is smaller than the ventilatory response generated at the onset of hypoxia.

6. Awake humans and awake cats display similar response characteristics with respect to characteristics 1–3 and 5

These observations suggest that in awake mammals the peripheral chemoreceptors play a pivotal role in the development of HVD. However, we still don’t know exactly if the HVD is
due to adaptation of the peripheral chemoreceptors (i.e., an exclusive peripheral mechanism) or to the way the Central Nervous System (CNS) processes the afferent input from the carotid bodies. Basing those two questions upon the characteristics displayed before, we can argue that the hypothesis that HVD is related to peripheral chemoreceptor adaptation is supported by characteristics 1, 2, and 5 and is further supported by the observation that hypoxic sensitivity declines during the development of HVD in isocapnic hypoxia. However, in the anesthetized cat, the peripheral chemoreceptors do not adapt during hypoxic exposure in terms of ventilation and afferent nerve activity, indicating that an exclusive peripheral mechanism is unlikely.

The actual mechanism of the decline is therefore still not completely understood. Although many studies have been conducted in order to examine the phenomenon, no standardized procedure exists.

4. Steady state method

4.1. Definition

The steady state method uses a sustained and constant stimulus in order to assess its effect on various parameters over time. Specifically for the hypoxic effect on ventilation, the typical HVR protocol uses three periods: a first 5 minutes of euoxic (targeting the resting value of the subject) or a hyperoxic phase, followed by a 20-minutes hypoxic phase, and ending by a 5-minutes euoxic or hyperoxic phase. Other protocols with steps up and/or down of oxygen or carbon dioxide, lasting between 2-3 minutes have been used as well.

The steady state method allows measuring HVR and HVD and also measuring the chemosensitivity to CO$_2$ under various isoxic levels. The Oxford Group (Severinghaus, Steinback, Poulin) in particular has used it for the purpose of descriptive analysis of
ventilatory responses. It can also be used for determining chemoreflex sensitivity. The goals of the study determine the technique best suited to achieve them.

4.2. Characteristics

As mentioned in the first chapter of this thesis, it takes 3 time-constants to allow central equilibration of CO\(_2\) so that each time response should last at least 5 minutes. On the other hand, HVD appears 5 -10 minutes after the onset of the hypoxic stimulus, so that care should be taken in order to avoid HVD. One level of CO\(_2\) is not enough to determine chemosensitivity for two reasons. First, we do know the effect of PCO\(_2\) on the HVR. This cannot be determined with a test at a single PCO\(_2\) level. Second, a minimum of three points is needed in order to define a linear relationship and so three levels of isocapnia are needed. The PO\(_2\) level is also relevant, especially if the goal is not only measuring HVR but also the chemosensitivity to CO\(_2\). Using a background of hyperoxia will shut down the peripheral chemoreceptor input and enable the researcher to use the ventilatory response to various isocapnic levels during hyperoxia to measure the contribution of the central chemoreflex drive to breathe. The ventilatory response to various isocapnic levels during hypoxia will then allow the determination of the contribution of both central and the peripheral drives to breathe. Moreover, as the steady state does not specifically measure the VRT, the difference between the hyperoxic and hypoxic responses at resting ventilation can be substituted in order to measure the peripheral drive to breathe.

The drawback of the method is that it measures the response relative to arterial PCO\(_2\). An increase on P\text{ET}CO\(_2\) will lead to an increase in CBF; and any increase in CBF will washout central CO\(_2\) and reduce the central chemoreceptor stimulus so that the steady-state ventilatory response to CO\(_2\) will be affected, underestimating the sensitivity (Ainslie & Duffin, 2009,
Xie 2006). Indeed, as there is an arterio-venous delta PCO$_2$ ($\Delta$av), the stimulus applied to the lung (in equilibrium with the arterial PCO$_2$) is different than the venous and tissue PCO$_2$ tension. Moreover, as the PCO$_2$ level increases, this ($\Delta$av) will decrease (Duffin, 2007). On the same point, this might become problematic in case of measuring specifically the CBF response to CO$_2$ by using PetCO$_2$ as a reflection of brain PCO$_2$ ($P_B$CO$_2$) (Vovk et al., 2002).

5. Clinical implications

Understand better the effect of hypoxia and hypercapnia will not only be important for physiology and for altitude medicine but also for pathophysiological advance in common diseases such as chronic obstructive pulmonary disease (COPD), obstructive sleep apnea (OSA), or acute respiratory distress syndrome (ARDS). Those diseases combine both symptoms of hypoxia and hypercapnia due to poor gas exchange or physical obstruction to ventilation.
II Introduction: Cerebral Blood Flow (CBF)

1. Control of cerebral blood flow

The control of the cerebral perfusion is closely linked to the regulation of the intracranial volume, which includes the arterial cerebrovascular bed, the large cerebral veins, and the cerebrospinal fluid (CSF). According to Poiseuille’ law, CBF is determined by the cerebral perfusion pressure (CPP) and the cerebrovascular resistance. The CPP itself is the difference between blood pressure at the level of the arteries supplying blood to the brain, and intracranial pressure (ICP). The arteries that feed the brain feed into, and originate from connections that are arranged in a circular shape providing many potential routs for distribution of the source blood supply. They are named after their discoverer, Thomas Willis, English physician from the XVII century, as “Circle of Willis”. CBF is adjusted dynamically to changes in the perfusion pressure, the metabolic activity of the brain, humoral factors, and autonomic nerve activity.

The cerebral vasculature is strongly affected by the arterial partial pressure of carbon dioxide. This sensitivity is a vital function that maintains central pH, and therefore affects the respiratory central chemoreceptor stimulus. Changes in PCO$_2$ cause vasodilation or vasoconstriction that occurs at the arterioles and precapillary sphincters. However, the two phenomena are different in the sense that vasodilation, due to the relaxation of the vascular smooth muscle of all cerebral vessels, is more prominent in the small vessels as opposed to vasoconstriction which, is unaffected by the size of the vessel (Wei et al., 1980).

The exact mechanism of how PCO$_2$ affects cerebrovascular tone is not fully understood. Elevation in PCO$_2$ leads to change in pH, which activates potassium channels in the vascular
smooth muscle. Cerebral endothelial cells express four classes of potassium channels: inward rectifying potassium ion (K$^+$) channels, calcium activated K$^+$ channels, ATP-sensitive K$^+$ channels and voltage gated K$^+$ channels. The last ones are activated by a reduction in pH. Hyperpolarization of the endothelial cells leads to a reduction in intracellular calcium, which will lead to vascular relaxation and hence vasodilation (Kitazono et al., 1995; Nelson & Quayle, 1995; Jackson, 2005). On the other hand, vasoactive factors can be the mediators of CO$_2$/pH alteration–induced vasodilation. An increased in shear stress due to increased flow velocity may mediate release of vasodilatory agents such as nitric oxide (NO) and prostaglandins. It has been shown that there is a differential change of NO across the brain during hypercapnia and hypoxia, and that C-natriuretic peptide (CNP) may play a complementary (but lesser) role on CO$_2$-induced CBF changes (Peebles et al., 2008).

Current estimates on the effect of CO$_2$ show that during rest (as opposed to exercise condition), each millimeter of mercury (mmHg) decrease of PaCO$_2$ leads to a 2-3% decrease of CBF (Brugniaux et al., 2007), limited by vasoconstriction capacity, and it seems that the lowest CBF (with then the highest vascular resistance) occurs at an alveolar carbon dioxide pressure (P$_A$CO$_2$) of 10-15 mmHg. On the other hand, CBF increases by 3-4% per unit mmHg increase of PaCO$_2$, reaching its highest level when PaCO$_2$ is elevated by 10-20 mmHg above normal resting value (Brugniaux et al., 2007).

2. Cerebral blood flow response to CO$_2$

The CBF response to step changes in CO$_2$ is almost instantaneous, with a delay of only 6 seconds (Poulin et al., 1996, 1998). This response is also referred to as the cerebrovascular reactivity (CVR), as it indicates the ability of the cerebrovascular bed to dilate or constrict in response to a change in PCO$_2$. When a broad range of PCO$_2$ is used, from 35-55mmHg, the
global CVR is 3.8%/mmHg (Reivich, 1964; Harper & Glass, 1965; Grubb et al., 1974). However, new studies have shown that CVR varies differently over hypercapnic or hypocapnic ranges, with a higher CVR at higher CO$_2$ values (Ide et al., 2003; Xie et al., 2005; Xie et al., 2006; Cummings et al., 2007). The lower reactivity might be a protective effect in order to prevent cerebral ischemia during transient drop of PaCO$_2$ occurring in daily activity (postural change, exercise) but also pathological situations (syncope, anxiety attacks).

Cerebrovascular reactivity to CO$_2$ is also dependent on a number of state factors for example, sleeping, awake, exercising, and on neuronal activity. At high altitude, the CVR is maintained over hypercapnic range but is reduced over hypocapnic ranges. Moreover, during sleep at high altitude, CVR, dynamic autoregulation (DA, see paragraph below), and values of MCAv are reduced, contributing to breathing instability. This may explain the high incidence of central sleep apnea (CSA) episodes at high altitude (Ainslie et al., 2007).

The role of cerebrovascular CO$_2$ reactivity in the ventilatory response to PaCO$_2$ has been investigated by using pharmacological agent such as Indomethacin. This agent is potent reversible cyclooxygenase inhibitor, decreasing effectively the CBF by attenuating the cerebrovascular sensitivity to CO$_2$, without affecting metabolic rate or plasma catecholamines as well as without affecting ventilation or carotid bodies activity. Studies have shown an increase in ventilatory sensitivity under steady state (SS) conditions with Indomethacin. The results show that that there is a direct role for CVR in regulating the ventilatory response to CO$_2$.

In addition, it is suggested that a higher ventilatory response will affect CVR by limiting the increase in both arterial and brain PCO$_2$ (Ainslie & Duffin, 2009). On the other hand,
Chapman (Chapman et al., 1979) has demonstrated that in unanesthetised goats, when the CBF is reduced by 30% the CVR is attenuated while the ventilatory sensitivity is increased. If the CBF is further reduced (50%), respiratory sensitivity is markedly blunted. Thus changes in CBF and CVR can have different effects on ventilatory sensitivity and therefore ventilatory threshold.

3. Cerebral blood flow response to hypoxia

The response of CBF to alteration in PO$_2$ has a slower dynamic autoregulation, and on a day-to-day level, regulation is minor. As for ventilation regulation, the level of PaCO$_2$ will affect the CBF response to PO$_2$. Moreover, there is a threshold for PO$_2$ levels (<40 mmHg), which is required to activate a CBF response (Gupta et al., 1997). Hypoxia per se is a vasodilator, however it also causes hyperventilation, which itself leads to vasoconstriction by hypocapnia. The cerebrovascular bed then receives conflicting messages during exposure to acute hypoxia. The role of PaO$_2$ is very important on ascent to high altitude (Ainslie et al., 2007; Ainslie & Burgess, 2008) and in patients with chronic lung disease such as chronic obstructive pulmonary disease (COPD) due to associated hypoxemia (Bernardi et al., 2008). As mentioned previously (HVD chapter), early investigations examined the effects of CBF on the ventilatory response to steady state hypoxia with respect to the hypoxic ventilatory decline. However, it does not seem to be responsible for the HVD (Shapiro et al., 1966b, a; Neubauer et al., 1985; Suzuki et al., 1989; Robbins, 1995) neither does it adapt in steady state isocapnic hypoxic tests (Poulin et al., 1996; Steinback & Poulin, 2007, 2008). However, over a long period of poikilocapnic hypoxia, it has been shown that CBF decreases over time (Poulin et al., 1998; Brugniaux et al., 2007). In addition, the CBF response to hypoxia is greater in isocapnia than in poikilocapnia (Steinback & Poulin, 2008). Moreover,
measurement of HVR, HVD, and cerebral blood flow (with transcranial Doppler) has been
used in order to predict the HVD if the central depression due to CO$_2$ washout would be true.
Theu found that the predicted HVD was much less than the measured HVD, ruling out
therefore the hypothetical CO$_2$ washout (Poulin & Robbins, 1998). Rebreathing testings that
included CBF have shown that the CBF sensitivity ($S$) to CO$_2$ in hyperoxia and hypoxia are
similar. However, the threshold is lower in hypoxia. Mean arterial pressure (MAP) has a
linear response to CO$_2$, in both isoxic conditions. Interestingly, the ventilatory response was
shown to have a second threshold ($T2$) in some cases, which corresponds to a sudden
increase of CBF. Therefore, it has been hypothetised from this correspondence that CBF and
$\dot{V}E$ have the same neural linkage (Vovk et al., 2002). However, there is no satisfactory
explanation of the second CBF threshold. Recent data from our laboratory has been able to
demonstrate that a change in brain perfusion pressure coincides with second increase of CBF.
(Battisti et al, data not yet published).

Similar rebreathing experiments have been conducted with the addition of sympathetic
nervous system measurements (Muscle Sympathetic Nerve Activity, MSNA in the peroneal
nerve), cardiac index determined by measuring stroke volume (SV), aortic dimensions
measured via US Doppler, ventilation and MAP (Shoemaker et al., 2002). It was shown that
MSNA threshold is lower during hypoxia; cardiac output ($Q$) was higher in hypoxia than
hyperoxia, for a same PCO$_2$ value. As well, at a normal 40 mmHg of PCO$_2$, MAP during
hypoxia was higher than during hyperoxia, however, the slope of MAP response to PCO$_2$ was
the same during the 2-isoxic conditions. So it seems that the peripheral chemoreceptor
contributes to sympathetic and cardiovascular response to hypoxic hypercapnia. One
limitation of this study was that CBF was not monitored.
In terms of integration, Ainslie (Ainslie & Poulin, 2004b, a) investigated the cardiovascular response to acute hypoxia. High acute hypoxic ventilatory response (AHVR) resulted in a smaller increase in CBF because of the associated hypocapnia from the hyperventilation. However with hypercapnia, hypoxia leads to high HVR, but also a high hypoxic cerebral blood flow response, as well as a higher MAP, suggesting a linkage of MAP and CBF to the increased $\dot{V}E$ response to hypoxia when not obscured by hypocapnia (Ainslie & Poulin, 2004b).

4. Measuring the cerebral blood flow

Cerebral blood flow can be measured non-invasively using ultrasound in the form of a transcranial Doppler device (TCD). The middle cerebral artery (MCA) carries about 85 percent of the cerebral blood flow (CBF), and can be insonated by a small ultrasound emitter/receiver held in the temple region of the skull. The velocity of the blood heading to the probe is measured from the Doppler shift of the emitted signal. Although the TCD-measured MCA velocity (MCAv) is not a reliable indicator of absolute CBF, it does accurately track changes in flow (Bishop et al., 1986), assuming that the diameter of the MCA does not change in response to changes in PCO$_2$ (Bishop et al., 1986; Poulin et al., 1996).

During steady state measurements, there are partial pressure gradients between end-tidal, arterial, and tissue PCO$_2$, with $P_{ET}CO_2$ being lower than brain tissue CO$_2$ tension (PbCO$_2$). As $P_{ET}CO_2$ increases, the CBF will increase and the gradient between $P_{ET}CO_2$ and PbCO$_2$ will decrease. If $P_{ET}CO_2$ is used as a reflection of PbCO$_2$, using steady state will underestimate the cerebrovascular reactivity to CO$_2$, as the gradient $P_{ET}CO_2$ -PbCO$_2$ is lower, showing a smaller sensitivity (slope CBF versus $P_{ET}CO_2$, compared to slope CBF vs PbCO$_2$). Secondly, steady state takes several minutes so fewer points can be generated to
make the relationship (or it will need more testing) and non-linear events, such as
breakpoints) can be missed (Vovk et al., 2002; Duffin, 2007).

During rebreathing, the gradient is abolished, and as the respiratory central chemoreceptor
responds to medullary \([H^+]\), set by medullary \([PCO_2]\), rebreathing will exclude the effects of
cerebrovascular reactivity from the ventilatory response to \(PCO_2\). As it measures the response
relative to venous \(CO_2\), reducing the \(\Delta av\) sufficiently to negate any effect of CBF change. For
CVR measurement, the same reasoning can be applied. However there are still uncertainties
regarding the exact location of the stimulus. If the stimulus is arterial and medullary, then
Rebreathing technique applies the same stimulus at both locations.

If there is a delay in the vascular response to \(CO_2\), then the full response for a given \(P_{ET}CO_2\)
might not be reached with rebreathing (Ainslie & Duffin, 2009). It is also known that during
hypercapnia, the correlation between changes in CBF and \(PCO_2\) is higher with the brain
tissue value than with the arterial value. Thus, brain \(PCO_2\) better reflect the physiological
stimulus (Shapiro et al., 1965). Conversely, Severinghaus has shown that during hypocapnia,
\(PaCO_2\) has a more important role than jugular \(PCO_2\) (which reflects \(PbCO_2\)) (Severinghaus &
Lassen, 1967). Ainslie has also shown that CVR is higher in both hyper- and hypocapnic
range when jugular \(PCO_2\) is used compared to \(PaCO_2\).

Comparison between the two methods has been made only twice before (Pandit et al., 2003,
2007) and has shown contradictory results. Those studies have unexpectedly shown that the
slope (sensitivity) is less steep during rebreathing, compared to steady state, which is
puzzling, as we know that rebreathing reflects brain \(PCO_2\), being higher, hence leading to
higher cerebral sensitivity to \(CO_2\). Their explanation was that CBF mirrors \(PaCO_2\) instead of
\(PvCO_2\), suggesting that CBF responds to PetCO2 rather than PbCO2, as also found by
(Severinghaus & Lassen, 1967). They also suggested that hyperventilation has a long lasting effect on the CBF as rebreathing results are closer to steady state than modified rebreathing.

5. Dynamic autoregulation

I have decided to include this section in order to keep in mind the effect of regulation of CBF and BP concomitantly, as I have measured both; and also, because dynamic autoregulation (DA) is impaired during exposure to environmental hypoxia. This section will be short and not extensive, as DA is not reported on the results of this thesis.

The cerebral autoregulation (CA) adjusts the caliber of the cerebral arterioles, or the cerebrovascular resistance in order to ensure that the CBF matches the metabolic needs. It contains two elements: static and dynamic. The static CA role is to adjust the CBF over gradual and progressives changes in cerebral perfusion. The DA acts as a rapid flow regulator, over changes in blood pressure occurring in a few seconds (Zhang et al., 2002).

Sustained mild hypoxia reduces CBF and continuously impairs dynamic CA, which might increase the risk of shortage of oxygen supply to the brain (Nishimura et al., 2007; Nishimura et al., 2010).

Static and dynamic CA are impaired in newcomers to High Altitude (HA), especially in the presence of acute mountains sickness (AMS) (Levine et al., 1999; Van Osta et al., 2005; Ainslie et al., 2007). Moreover, highlanders living above 3440m have also an impaired CA. This specific altitude level is a transitional zone, above which cerebral autoregulation becomes critically impaired (Jansen et al., 2007). Recently, it has been shown that hyperoxia at HA can partially improve CA impairment, with little effects on CBF (Ainslie et al., 2008).
III. Project

1. Rationale

The effect of hypoxia is of great importance to those at altitude in order to better understand the phenomenon of acute and chronic mountain sickness (AMS/CMS), as well as those in chronic disease states due to long-term hypoxic exposure (Launay et al.; Sato et al., 1992; Sato et al., 1994; Somogyi et al., 2005a; Brugniaux et al., 2007; Claydon et al., 2008; Powell & Fu, 2008; Richalet et al., 2008a; Richalet et al., 2008b; Rivera-Ch et al., 2008; Xing et al., 2008; Powell et al., 2009; Richalet et al., 2009; Slessarev et al., 2010). Moreover, a better understanding of the effect of hypoxia on ventilation as well as on blood flow under physiological conditions will inform clinical decisions for a whole range of diseases (COPD, ARDS, HPV, etc.), where not only hypoxia but also change in PCO$_2$ occur.

As mentioned at chapter one, investigations of the hypoxic ventilatory response have been carried out over the last 35 or more years, however, at the moment, no standardized method has been agreed upon. Variability due to differing methodologies and sequences used to date has prevented comparison of data gathered by different methods. Consequently, there is a need for a standardized steady state procedure to investigate the peripheral chemoreceptors during a hypoxic ventilatory response test. Despite a meeting for that purpose at the Hypoxia conference held at Lake Louise, AB, in 2005, no consensus could be reached. In 2007, Duffin published a review of the measurements of the hypoxic ventilatory response (HVR) and proposed a standardized method that is more extensive and allows comparisons between subjects and within the same subjects under different conditions (Duffin, 2007). This point is very important since it will put all researchers in the field on the same experimental basis and
will provide a more extensive knowledge of respiratory physiology. In order to evaluate the proposed method, I have conducted experiments based on Duffin’s proposal. The specific aims were to measure the effect of hypoxia on the isoxic rebreathing ventilatory response to CO$_2$ in terms of sensitivity, ventilatory recruitment threshold and wakefulness ventilation; as well as the effect of hypercapnia on the isocapnic steady state ventilatory response to hypoxia (HVR). It is the latter experiments that I report in this thesis.

There are two novel aspects to this investigation. First the methodology: referring to the paper by Duffin, (Duffin, 2007) a standardized approach for respiratory chemoreflex characterization will employ three isocapnic hypoxic steady state tests in order to characterize the full ventilatory response to hypoxia. Second is the tool used: the RespirAct$^\text{TM}$, which gives us the ability to control carbon dioxide and/or oxygen separately, independently of each other and of the ventilation of the subject. This ability allows the researcher to apply a constant and repeatable stimulus. On that point, I would like to note that the extensive review by Teppema (Teppema & Dahan, 2010) mentions all types of techniques going from simple bag with fixed gases concentration technique to more complex techniques such as dynamic end tidal forcing (DEF) but does not mention the prospective end tidal targeting (PETT). In a pilot study, I tested whether the PETT was capable of executing the HVR protocol to compare steady state and rebreathing methods for investigation of the respiratory chemoreflex characterization. Those preliminary data have been subjects of poster presentation at local (Respirology Research Day, University of Toronto, Medicine, Department of Respirology June 2009) and at international conference (XI Oxford Conference, Japan, July 2009) and appended as Appendixes 1 and 2.
Lastly, this investigation includes not only the ventilatory, but also cerebrovascular and hemodynamic responses, which have been studied in the past in combination, but again, the methodological aspect prevents comparisons between studies. My studies were designed to provide a more complete picture of the mechanisms involved in the responses to hypoxia. I had the opportunity to present preliminary data in local (Respirology Research Day, University of Toronto, Medicine, Department of Respirology June 2009; BRAIN platform Research Day, University of Toronto) and international conference (Experimental Biology, Anaheim, California, April 2009) (Appendixes 3 and 4).

In this thesis I propose to study (a) the steady state assessment and the dynamic characterization of ventilatory and cerebral blood flow responses to a sustained isocapnic hypoxic stimulus, and (b) the sensitivity of these responses to changes in PCO$_2$.

2. Aim of the study

Characterize the dynamic response of ventilation, cerebral blood flow, blood pressure, and heart rate, to hypoxia using a steady state method.

3. Study objectives

As mentioned just above, statements from the literature describe HVR as mediated by peripheral chemoreceptors, time dependent and biphasic, and linked with heart rate response to hypoxia. However, humans’ studies accurately controlling isocapnia for measurements of HVR did use only single isocapnic background, and was quite low (+1mmHg above resting value) (Easton et al., 1986; Steinback & Poulin, 2007, 2008). Previous study measuring cerebral response to sustained hypoxia had shown an increase but sustained cerebral (MCAv) response (Poulin et al., 1996). However, values of MCAv were averaged over 15 seconds period such as variations of the cerebral resposne could have been missed. Another study
measuring cerebral blood flow response to hypoxia has shown a correlation between the acute hypoxic ventilatory response and the acute hypoxic cerebral blood flow response. However, this study was no designed to measure HVR, and was completed by short step changes in oxygen (90 seconds), which does not allow a full development of the response (Ainslie & Poulin, 2004b). Therefore, the effect of PCO₂ level on the measurement of HVR, as well as the mechanism behind HVD, and the correlation with cerebral blood flow are not fully understood yet. Also, those studies included males essentially such as the results can or cannot (we do not know at this point) be applied to females.

In order to have a better and more complete picture of hypoxic responses, I will use a standardized method, as well as an appropriate apparatus to control P_{ET}CO₂ and P_{ET}O₂. The objectives are the following ones

1. Describe the dynamic characteristics of the hypoxic response of ventilation, cerebral blood flow, blood pressure and heart rate at three levels of P_{ET}CO₂. For that, I will use a standardized breathing method, but also, analysed the data in a standardized way.

2. Measure the sensitivity of ventilation and cerebral blood flow response to CO₂.

3. Investigate correlation between ventilation and brain blood flow responses to hypoxia.

4. Hypotheses

1. Increased PCO₂ increases the magnitude of the ventilatory response to hypoxia.
2. Increased PCO₂ increases the magnitude of the cerebrovascular response to hypoxia.
3. Increased PCO₂ will not increase the adaptive response of CBF to hypoxia (no decline).
4. Respiratory chemoreflex sensitivity to CO₂ can be characterized by using this method.
5. Cerebrovascular sensitivity to CO₂ can be characterized by using this method.
IV Methodology

1. Measurements

1. Respiratory variables
   - End-tidal PCO₂ (P₄ETCO₂) mmHg
   - End-tidal PO₂ (P₄ETO₂) mmHg
   - Ventilation (V̇E) L/min
   - Tidal Volume (VT) ml
   - Respiratory Rate (RR) breaths/min
   - Oxygen Saturation via finger pulsed oximeter (SpO₂) %

2. Cardiovascular variables
   - Heart Rate (HR) beats/min
   - Systolic Blood Pressure (SBP) mmHg
   - Diastolic Blood Pressure (DBP) mmHg
   - Mean Blood Pressure (MAP) mmHg

3. Cerebrovascular Variables
   - Middle cerebral artery velocity cm/s
   - Cerebral Blood Flow (CBF) ml/min

2. Apparatus and monitor

2.1. Apparatus

2.1.1. Breathing circuit: Sequential gas delivery circuit (Fig. 1)

The sequential gas delivery (SGD) allows us to control the end tidal values of carbon dioxide
and oxygen and to keep the targeting of $P_{ET}CO_2$ and $P_{ET}O_2$ under perfect control (Slessarev et al., 2007; Ito et al., 2008). The system is based on a sequential gas delivery circuit, which includes three valves arranged to provide non-rebreathing valve and a cross-over valve from the expiratory reservoir to the inspiratory reservoir. The circuit contains an inspiratory gas reservoir (G1) and the expiratory gas reservoir (G2). The key aspect of sequential gas delivery is the control of alveolar ventilation ($\dot{V}_A$), the ventilation that actually takes part in gas exchange. $\dot{V}_A$ is equal to $\dot{V}_E$ ($\dot{V}_E$, equal to tidal volume times respiratory rate) minus the dead space ventilation ($\dot{V}_D$):

$$\dot{V}_E = \dot{V}_A + \dot{V}_D$$

Ordinarily, we measure $\dot{V}_E$ but do not know $\dot{V}_D$ so we do not know, $\dot{V}_A$, much less control it. With the SGD circuit the RespirAct™ gas flow (RGF) to the circuit becomes $\dot{V}_A$. Thus, for a given CO$_2$ production and O$_2$ consumption, by controlling the RGF and the CO$_2$ and O$_2$ concentrations in the flow, we can accurately target PaCO$_2$ and PaO$_2$. This is independent of ventilation because any extra ventilation is composed of previously exhaled gas that has equilibrated with the blood and thus provides no gradient for gas diffusion, in other words, is “neutral”. Whereas $\dot{V}_E$ is ordinarily defined as the sum of $\dot{V}_A$ and $\dot{V}_D$, with SGD, $\dot{V}_E$ is the sum of RGF and the minute ventilation of the “neutral gas”. Practically, $\dot{V}_A$ is set via setting the flow and concentrations of RGF. The RGF fills the inspiratory bag. Inspiratory gas goes into the lungs via the inspiratory valve (low resistance valve). If during inhalation, the G1 empties, the cross over valve, which has a higher opening pressure than the inspiratory valve, opens, and the subject starts to inhale some exhaled gases from the expiratory reservoir.
During expiration, the subject exhales into the respiratory reservoir through the expiratory valve (low resistance valve). If the RGF is set lower than $\dot{V}E$, the RGF will reach the alveoli and the RGF will be equal to the $\dot{V}A$. This setting means that the concentrations of CO$_2$ and O$_2$ in the alveolar volume will be the targetted values and so arterial PCO$_2$ and PO$_2$ can be controlled. If the subject needs to breathe more, then they will simply breathe more from the G2 bag, and this won’t affect the end tidal or arterial values of O$_2$ and CO$_2$.

![RespirAct™ apparatus & breathing circuit](image)

**Fig-1 RespirAct™ apparatus & breathing circuit**

### 2.1.2. Gase tanks

We use 4 different tanks:

1. Medical Air,

2. 100% O$_2$,

3. 6% O$_2$, 94% N$_2$,

4. 20% CO$_2$, 6% O$_2$, 74% N$_2$. 
Every tank has O₂, so no hypoxic mixture can be administered regardless of the pattern of flow control failure.

2.1.3.RespirAct™

The RespirAct™ (Thornhill Research Inc., Toronto, Canada) (RA) is a gas blender designed to enable prospective end tidal targeting of CO₂ and O₂. The user enters physiological parameters (Age, Height, Weight, Gender), functional residual capacity (FRC). Carbon dioxide production (VCO₂) and O₂ consumption (VO₂) are calculated from tables of normal values or can be measured directly by RA. The user also inputs strategically estimated initial resting value of P_{ET}CO₂ and P_{ET}O₂. In addition, the resting P_{ET}CO₂ and P_{ET}O₂ values may be identified by reducing the RGF of room air to approximately \( \dot{V}A \) (identified by the RGF where a slight amount of rebreathing is noted on the capnograph). Then, a value of P_{ET}CO₂ and P_{ET}O₂ is targeted to be maintained constant, and this sequence is run. Depending on the target values reached and the stability of the response (presence or absence of drift), estimates of FRC, VCO₂, or VO₂ may be adjusted.

For this specific testing, I have been using the RespirActPlus Ventilation software, which is customized software, allowing SGD but with a variable and adaptive fresh gas flow (Labview, National Instrument, Texas). The regular software uses a fixed RGF, and the subject is coached to breathe at a level that exceeds this value. For this specific project, I am measuring the ventilatory response, so it has to be able to increase or decrease depending on the subject needs and yet still be the \( \dot{V}A \). We achieved this by specifying a RGF that will adjust automatically to match 0.7*\( \dot{V}E \), but dynamically adjust the PCO₂ and PO₂ of the inspired gas such that the desired \( \dot{V}A \) is maintained for CO₂ and O₂.
2.2. Experimental monitors

2.2.1. Trans-cranial Doppler

A transcranial Doppler (ST³, Spencer Technologies, Seattle, United States of America) was used to measure beat-to-beat MCAv in the middle cerebral artery (MCA). The MCAv data was collected through the temporal window above the zygomatic arch using 2 MHz probes. Ultrasound gel was applied to the probes before it was secured to an adjustable head fixture and applied to the skin and hair of the subject. Both the left and right sides of the head were insonated, as long as there were temporal windows present. In this study we insonated the MCAv, found at a depth of approximately 40 – 60mm. We limited the depth to 45-55 mm to ensure our signal came from the MCAv. The angle of probe insonation was varied until the strongest power motion mode signals were identified. The probe depth was then set within the power motion mode signal. The ST³ provides both a 150Hz envelope and mean values are displayed as a running 4 sec average, updated every second.

2.2.2. Flowmeter

A flowmeter (AWM720P1, Honeywell Freeport, Illinois) was inserted at the mouthpiece of the breathing circuit. Flow signal was recorded at 20Hz and are used to calculate VT, VE, and RR.

2.2.3. Gas sampling

A flow of gas is drawn from the mask (350ml/min) to monitor of the end tidal gases from the breathing circuit (RespirAct™ TRI Canada). PCO₂ and PO₂ signals are recorded at 20Hz. I used the tracing from the phase of ventilation (inspiration and expiration).
2.2.4. Non-invasive blood pressure and heart rate sensor

I used a finger plethysmograph (Nexfin Bmeye, Amsterdam, Netherlands) to measure beat-by-beat blood pressure. The sampling rate is 200Hz.

2.2.5. Pulse oximeter

Pulse oxymetry (Onyx II model 9550, Nonin Medical Inc Hudiksvall, Sweden) was recorded manually in order to monitor pulsed oxygen saturation.

3. Outcomes variables

1. Acute response
   - Peak response referred as the peak response to hypoxia
   - Time to peak (sec): from the onset of the hypoxic stimulus to the peak response

2. Adaptive response: after the peak response
   - Average value of the two last minutes of the hypoxic stimulus

3. Ventilatory sensitivity to CO\textsubscript{2} by establishing the relation between ventilation to \( P_{\text{ET}}\text{CO}_{2} \)

4. Cerebral sensitivity to CO\textsubscript{2} by establishing the relation between MCA to \( P_{\text{ET}}\text{CO}_{2} \)

The details of those measured values are described in the data analysis section.

4. Experimental protocol

This experiment is based on a single visit to the lab for each subject. However, 3 subjects have repeated the tests twice in order to measure the reproducibility of the results.

4.1. Subjects

I included healthy young subjects from 18-45 years, of either sex with no reported impairment of exercise capacity, or respiratory or cardiovascular diseases.
Subjects were instructed not to use alcohol, or caffeine for at least 12 hours before the testing day and not to use these substances during the course of the testing day until the day’s testing was completed. Also, they were instructed not to engage in heavy exercise or strenuous physical activity 12h before the testing period. Female subjects were analyzed during the first 5 days of the menstrual cycle/follicular phase in order to avoid any hormonal effects (progesterone) due to luteal phase or due to birth control pill. This aspect is important for any experiment, but even more in regard of HVR as it is known and shown that hormones affect HVR (White et al., 1983).

4.2. Testing conditions

The subjects were seated on a chair in a quiet room and were fitted with a facemask (SGD circuit, TRI, Toronto, Canada). During the testing, gas was supplied to the facemask by RA.

A standard commercial head harness was used to hold the TCD probes in place during testing. The MCA window was identified using an insonation pathway through the temporal window, just above the zygomatic arch. Both right and left MCA were insonated.

In addition, a non-invasive automatic blood pressure cuff and pulse oximeter (finger probe) was used.

Resting values for HR, BP, SpO₂, MCA velocity, and $P_{ET}CO_2$ and $P_{ET}O_2$ were obtained over a 5-minute period as baseline measurements prior to each of the 3 steady state tests.

All the devices were properly calibrated before each tested subject. Gases were calibrated by using 100% O₂ and a calibration gas with 9% CO₂, balance N₂. The gas concentrations in the tanks were verified by using the appropriate program (RespirActUser™), which also verify
the gas flows. The volume, measured by a flowmeter, was calibrated by using a 1L calibration syringe (series 5530, Hans Rudolph Inc., Kansas City, MO, USA).

4.3. Breathing protocol

Subjects took part in three tests separated by at least 30 minutes of rest in order to allow a resetting of the chemo and baroreflexes. The order was randomized by choosing three cards in an envelope.

Using prospective end-tidal targeting of PCO$_2$ and PO$_2$, the target P$_{ET}$CO$_2$ was raised by 4, 7, or 10 mmHg above resting and kept constant (isocapnia) while the P$_{ET}$O$_2$ was manipulated between hyperoxic and hypoxic levels. The subjects were instructed to breathe normally throughout the testing (20 minutes in duration). The following end tidal PO$_2$ values were targeted as shown in Figure 2:

![Fig-2 Breathing protocol: divided in three segments: 1) P$_{ET}$O$_2$ is kept for 5 minutes at 150 mmHg, then 2) for 10 minutes at 50 mmHg, and again 3) at 150 mmHg for 5 minutes. During the whole sequence, P$_{ET}$CO$_2$ is kept constant at 4, 7, and 10 mmHg above the resting value.](image)

This sequence was followed by a 30-minutes period of rest. The test was completed two more times with the same sequence, but with different levels of isocapnia. For each sequence, the recording from all devices was synchronized and an event marker was recorded.
5. Data processing

End tidal values for CO₂ and O₂ were analysed automatically and confirmed manually by using a customized end tidal picker program.

Tracings from the RespirAct™, the Spencer, and the Nexfin were realigned according to the event marker by using a customized software (Align & Fit Rebreathing Rev7B, Labview, National Intrument, Texas).

Beat-by-beat values for the Spencer and the Nexfin were selected and saved to a file by using a customized software (Align & Fit Rebreathing Rev7B, Labview, National Intrument, Texas).

6. Data analysis

6.1. Description of the response to hypoxia

Initially I intended to characterize the HVR and HVD as described in the literature (Duffin, 2007).

![Fig-3 Hypoxic ventilatory response and calculation of the decline (Duffin 2007)](image)

The peak response (HVR) is the variation of the ventilation from the first segment of the protocol to the peak response (ΔVi); and the HVD measured as a percent change for the HVR value as:

\[ \%\text{HVD} = 100 \times \frac{\Delta \text{Vi}}{(\Delta \text{Vi} - \Delta \text{f})} \]

Where -Δf corresponds to the difference between the ventilation value at the plateau of the decline and the initial ventilation (see figure 3).
Steady-state test data at each isocapnic level were analysed by fitting in order to obtain the better model for each of the 3 periods (Hyperoxia 1, Hypoxia, hyperoxia 2). The first period was fitted by a mean, as the values were steady. Mean values at the Hyperoxic1 phase were used as the “rest” value in order to calculate a percent change for the hypoxic data. The second and third periods were fitted according to the curve by mean (if no change) or exponential (see below). Times constants (incremental for the HVR) and decremental for the HVD) are calculated by using a dual exponential equation.

Fig-4 Fitting during the three periods: 1. Mean; 2 dual exponential; 3 mean.

However I had to revise the analysis, because in some responses ventilation did not just decline but exhibited three different patterns: I designated them as Decline, Plateau, or Double (see results for more details). The initial analysis led to improper values, the interpretation of which was difficult. The realization of the existence of these patterns also alerted me to look for these patterns in the responses of the other variables (MCAv, HR, and MAP). I therefore standardized the method for the analysis of these variables as follows:
6.1.1. Acute response

The peak value is taken as the maximum response to hypoxia expressed as absolute values for ventilation, heart rate and blood pressure. For MCAv, I expressed the values in % to a reference. The choice for the reference could have been the resting value (pre-sequence) but this fluctuates with time. I found in a related study that a consistent MCAv occurred in each subject during the 5 min hyperventilation phase of a Duffin rebreathing test. As $P_{ETCO_2}$ decreases, MCAv decreases too; however, MCAv reaches a minimal value at which vasoconstriction has reached its maximum capacity. A further decrease of $P_{ETCO_2}$ did not affect MCAv. As this minimum value is consistent between the two rebreathing tests, I used this minimum MCAv as the reference value for calculating the percent changes in MCAv. The time to reach this peak is $T_{pk}$, from the time of the onset of hypoxia up to the peak response as follows:

Fig-5 Data analysis: Acute and adaptive hypoxic response measurements,
6.1.2. Adaptive response

An average value of the measures for the last two minutes of hypoxia was calculated and expressed as an absolute value as well as in percent change from the peak response. The percent change in MCAv from the reference value was also calculated (Figure 4). This new calculation should give values similar to the initial method proposed for the “Decline” and “Plateau” categories, but importantly, should be more accurate for the “Double” response category (see results for more details). From this point, I will use the term “decline” only with the reference to that specific category and instead of referring to HVD I will use the term “adaptive response”.

I also calculated for each measure the linear slope from the peak response to the end of the hypoxic phase, that included the standard deviation (SD) of this linear fit in order to identify the Double pattern of response, where the SD would be > than 3. A Plateau response should have a slope close to zero and a small SD, in contrast to a Double response where the slope is negative and the SD should be >3. This fitting procedure will provide a specific measure on which to base the choice of the pattern. Correlation between the acute and the adaptive response was tested by using a linear regression.

6.1.3. Categories

I looked at the clustering of parameters according to their category in order to see if there were correlations between parameters in the same category.

6.1.4. Gender effect

I also tested whether there was a gender effect on the acute and adaptive hypoxic responses and if there is a response specifically associated with sex.
6.1.5. Reproducibility

Subjects in particular categories were asked to return for another visit and repeat the tests in order to determine the variability of the responses in terms of amplitude and category.

6.2. Chemosensitivity testing

The concept can be explained via the following figure:

![Figure 6: Chemosensitivity measurements](image)

*Fig-6 Chemosensitivity measurements: using the mean value of the hyperoxic phase of the three tests and plot each value against $P_{ET\text{CO}_2}$ values; and using the mean value of the hypoxic phase of the three tests and plot each value against $P_{ET\text{CO}_2}$ values*

The steady state data provides 6 points on the ventilation/CBF vs. $P_{CO_2}$ plot so that steady-state CO$_2$ sensitivities can be calculated. Mean value of the hyperoxic period for the three tests gives 3-hyperoxic points at the 3-isocapnic levels were used in order to have the central chemoreflex contribution to sensitivity to CO$_2$. And the 3 points at the peak response to hypoxia were used in order to have the central and peripheral chemoreflexes contributions. Additionnally, a 60 seconds average around the peak value is measured. In those experiments, it is correct to calculate the contribution of the peripheral contribution only by subtracting hyperoxic and hypoxic slopes. The values for MCAv are expressed in percent change from the minimal value as described earlier.
V results

1. Subjects

18 healthy subjects participated in this study: 10 males, 26 ± 5 years old, BMI at 22±2.5 kg/m² (see Table-1 for more details). All subjects provided informed written consent after receiving verbal and written instructions outlining the experimental procedures. Participants were not taking any medications, were all non-smokers, and none had any history of cardiovascular or respiratory disease that would preclude their participation in the study. This study was approved by the University Health Network Research Ethics Board (Toronto, Ontario, Canada) and conformed to the Declaration of Helsinki. Three subjects repeated the protocol on another visit in order to determine the reproducibility of the data.

Table 1: Demography and subjects information. “Subject trained” indicate if the subject had the test already in the past.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI (m/k2)</th>
<th>Handwriting</th>
<th>Gender</th>
<th>Time of day</th>
<th>Subject trained</th>
</tr>
</thead>
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<td>72</td>
<td>24.91</td>
<td>right</td>
<td>M</td>
<td>morning (9AM)</td>
<td>no</td>
</tr>
</tbody>
</table>
2. The challenges

In order to assure that isocapnia and isoxia were maintained constant accurately, I have calculated the standard deviation for all three segments (Hyperoxia 1, Hypoxia, and Hyperoxia 2) of each sequence, for all tests, all subjects. Table 2 shows the average of the standard deviation of P_{ET}CO_2 for all segments, all tests, as well as the standard deviation of this value. As the range of error of the CO_2 sensor is 2 mmHg, values shown in this table indicate that isocapnia was well controlled and therefore, any variation of hypoxic response will not be due to inaccurate isocapnia.

\textit{Table-2 Quality of isocapnia: Average of the standard deviation of P_{ET}CO_2 for all segments, all tests; standard deviation of this value.}

\begin{tabular}{|c|c|c|}
\hline
All subjects & Average SD P_{ET}CO_2 & SD \\
\hline
All segments & 0.62 & 0.27 \\
\hline
\end{tabular}

3. Characterization of the response

I classified the pattern of response to hypoxia into four categories that were applied to all measures. The first was the classical decline (Figure 7), pattern seen not only for ventilation but also for HR and MCAv. The figure shows the three segments of the sequence, and the two vertical lines indicate the hypoxic period.
Fig-7 Decline category example: graph representing one subject during one test. All variables follow the same pattern of hypoxic response. The hypoxic response is represented between the 2 vertical lines.

The second category was the Plateau (Figure 8), with a rapid rise to a plateau.

Fig-8 Plateau category example: graph representing one subject during one test. All variables follow the same pattern of hypoxic response. The hypoxic response is represented between the 2 vertical lines.

The third category was the “double” response (Figure 9), with a peak followed by a decline and a subsequent peak with a second decline. The fourth category is “No response”.
Fig-9 Double category example: graph representing one subject during one test. All variables follow the same pattern of hypoxic response. The hypoxic response is represented between the 2 vertical lines.

Table 3 shows a distribution of the ventilatory response in those categories and indicates that the occurrence of different response patterns is not a sporadic phenomenon, but actually exists.

Table-3 Distribution of the categories over the adaptive hypoxic ventilatory response: left column indicates how many subjects fall into each category, all subjects and tests included.

<table>
<thead>
<tr>
<th>Category</th>
<th>( V_E )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decline</td>
<td>19</td>
</tr>
<tr>
<td>Plateau</td>
<td>15</td>
</tr>
<tr>
<td>Double</td>
<td>16</td>
</tr>
<tr>
<td>No response</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4 displays the characterization of ventilation, left MCAv, right MCAv, HR, and MAP over hypoxia, as well as the category assigned to each test, and the order of the tests (as they were randomized). This table will be used in the results description for each parameter analysed and is also placed in the Appendix 5.
<table>
<thead>
<tr>
<th>VE Category</th>
<th>Left MCA Category</th>
<th>Right MCA Category</th>
<th>HR Category</th>
<th>MAP Category</th>
<th>Test order</th>
</tr>
</thead>
<tbody>
<tr>
<td>#03 SS+4</td>
<td>No response</td>
<td>--</td>
<td>Decline</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#03 SS+7</td>
<td>Double</td>
<td>--</td>
<td>Decline</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#03 SS+10</td>
<td>Decline</td>
<td>--</td>
<td>Double</td>
<td>Plateau</td>
<td>decline</td>
</tr>
<tr>
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<td>Plateau</td>
<td>--</td>
<td>Plateau</td>
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<td>double</td>
</tr>
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<td>Decline</td>
<td>Decline</td>
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<td>Double</td>
<td>Double</td>
<td>plateau</td>
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<td>no response</td>
<td>3</td>
</tr>
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<td>--</td>
<td>Double</td>
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<td>no response</td>
</tr>
<tr>
<td>#06 SS+10</td>
<td>Decline</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
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<td>#08 SS+4</td>
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<td>No response</td>
<td>Decline</td>
<td>no response</td>
<td>3</td>
</tr>
<tr>
<td>#08 SS+7</td>
<td>Decline</td>
<td>--</td>
<td>No response</td>
<td>Decline</td>
<td>2</td>
</tr>
<tr>
<td>#08 SS+10</td>
<td>Decline</td>
<td>--</td>
<td>Plateau</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#08 SS+4</td>
<td>increasing</td>
<td>plateau</td>
<td>no response</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#05 SS+10</td>
<td>plateau</td>
<td>no response</td>
<td>plateau</td>
<td>decline</td>
<td>2</td>
</tr>
<tr>
<td>#09 SS+4</td>
<td>Decline</td>
<td>No response</td>
<td>--</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#09 SS+7</td>
<td>Decline</td>
<td>Double</td>
<td>--</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#09 SS+10</td>
<td>Double</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#10 SS+4</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#10 SS+7</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#10 SS+10</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#11 SS+4</td>
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<td>No response</td>
<td>No response</td>
<td>Decline</td>
<td>double</td>
</tr>
<tr>
<td>#11 SS+7</td>
<td>Slow rise</td>
<td>No response</td>
<td>No response</td>
<td>Plateau</td>
<td>double</td>
</tr>
<tr>
<td>#11 SS+10</td>
<td>Decline</td>
<td>no response</td>
<td>Decline</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#12 SS+4</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>Plateau</td>
<td>double</td>
</tr>
<tr>
<td>#12 SS+7</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#12 SS+10</td>
<td>Plateau</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#13 SS+4</td>
<td>Decline</td>
<td>No response</td>
<td>No response</td>
<td>Decline</td>
<td>plateau</td>
</tr>
<tr>
<td>#13 SS+7</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#13 SS+10</td>
<td>Decline</td>
<td>No response</td>
<td>No response</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
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<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>no response</td>
</tr>
<tr>
<td>#14 SS+7</td>
<td>Decline</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#14 SS+10</td>
<td>Plateau</td>
<td>Double</td>
<td>Decline</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#15 SS+4</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#15 SS+7</td>
<td>Double</td>
<td>fast Pk rising</td>
<td>fast Pk rising</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#15 SS+10</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#16 SS+4</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>double</td>
</tr>
<tr>
<td>#16 SS+7</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#16 SS+10</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#17 SS+4</td>
<td>Double</td>
<td>No response</td>
<td>Plateau</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#17 SS+7</td>
<td>Plateau</td>
<td>Decline</td>
<td>Decline</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#17 SS+10</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#18 SS+4</td>
<td>Plateau</td>
<td>Decline</td>
<td>Plateau</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#18 SS+7</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>plateau</td>
</tr>
<tr>
<td>#18 SS+10</td>
<td>Plateau</td>
<td>Double</td>
<td>Plateau</td>
<td>Double</td>
<td>double</td>
</tr>
</tbody>
</table>
4. Ventilatory response to hypoxia

Figures 10-12 show a global view of the dynamic responses for the three tests, for all subjects. The first point represents the mean value of the first segment (hyperoxic phase) at isocapnic level elevated at 4, 7, or 10 mmHg above the resting value, which already increases ventilation if compared to resting value; the second point corresponds to the acute response to hypoxia, the third point to the 60 second average value around the peak hypoxic response; and the last point represent the adaptive response (average of the value during the two last minutes of hypoxia).

*Fig-10 Dynamic ventilatory response at isocapnic level at 4 mmHg above the resting value at the four time points of the sequence: hyperoxic segment, hypoxic acute response, and the 60 seconds average around the peak response, and last point: average of the two last minutes of the hypoxic stimulus.*
Fig-11 Dynamic ventilatory response at isocapnic level at 7 mmHg above the resting value at the four time points of the sequence: hyperoxic segment, hypoxic acute response, and the 60 seconds average around the peak response, and last point: average of the two last minutes of the hypoxic stimulus.

Fig-12 Dynamic ventilatory response at isocapnic level at 10 mmHg above the resting value at the four time points of the sequence: hyperoxic segment, hypoxic acute response, and the 60 seconds average around the peak response, and last point: average of the two last minutes of the hypoxic stimulus.

As expected, during the hyperoxic phase, ventilation increases with the increase of PCO$_2$.

Figure 13 shows the mean value of ventilation during the first hyperoxic phase during the
sequence at isocapnic level at + 4mmHg above the resting value, for each subjects. The mean of all subjects is represented by the black tracing.

Fig-13 Hyperoxic ventilatory response: All subjects across the three isocapnic levels. The black line represents the mean value. Rm ANOVA shows statistical significance between the three tests: $p < 0.0001$

Indeed, even at the lowest isocapnic level ventilation is still higher than the baseline, which has a ventilatory and cerebral effect from the level of PCO$_2$ (See Figure 14 as an example).

Fig-14 Effect of Hypercapnia and Hypoxia separately on Minute Ventilation
The difference of the hypoxic value at the thress isocapnic level is statistically significant with a p<0.0001 (repeated measure ANOVA)

4.1. Acute ventilatory response to hypoxia

The hypoxic ventilatory response increases with the isocapnic level targeted, as shown in Figure 15 shows the peak value of ventilation during the first hypoxic phase during the sequence at isocapnic level at +4mmHg above the resting value, for each subjects. The mean of all subjects is represented by the black tracing. This figure demonstrates that the highest isocapnic level produced the highest hypoxic ventilatory response.

Fig-15 Acute hypoxic ventilatory response: All subjects across the three isocapnic levels. The black line represents the mean value. Rm ANOVA shows statistical significance between the three tests: p < 0.0001

The difference is statistically significant with a p < 0.0001 (repeated measure ANOVA)

Figure 16 displays the time to peak for all subjects at the three isocapnic levels. One outlier (#8.2) shows a continuous increase of ventilation during hypoxia. The increase of response with increase of PCO2 was not observed; and no statistically significant differences were shown in the all group. If only the subject who show a trend to decrease are analysed (#3, #4,
#5, #6, #7, #8, #17, #18) then there is a statistical difference between the three tests (rm ANOVA, p< 0.0001).

Fig-16 Time to Peak values for all subjects, across the three isocapnic levels. The black line represents the mean value. P<0.001 for subjects #3, #4, #5, #6, #7, #8, #17, #18.

4.2 Categorization of the response

The adaptation of the ventilatory response varies in terms of the patterns. The distribution of the patterns is indicated in Table 4. Seven subjects (38%) had a consistent pattern of response over the three tests; four subjects (33%) showed both Decline and Double; 6 subjects (22%) showed both Plateau and Double or Decline categories; and two subjects (11.2 and 8.2) (11%) had different categories across the three tests.

Table 5 lists the total number of subjects falling into each category, showing an even distribution between the categories, with however a slight higher incidence of the Decline category.
Table 5: Distribution of the subjects in the categories

<table>
<thead>
<tr>
<th></th>
<th>VE</th>
<th>Left MCAv</th>
<th>Right MCAv</th>
<th>HR</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N decline</td>
<td>19</td>
<td>8</td>
<td>12</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>N plateau</td>
<td>16</td>
<td>9</td>
<td>13</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>N double</td>
<td>16</td>
<td>12</td>
<td>15</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>N none</td>
<td>4</td>
<td>11</td>
<td>12</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

4.3. Adaptive response to hypoxia

Figure 17 shows the adaptive hypoxic value for each subject at the three isocapnic levels, pointing out that the adaptive hypoxic ventilatory response does change also with the isocapnic level. Rm ANOVA shows significant statistical differences (p = 0.0003).

By looking at the components of the ventilation (tidal volume - VT and respiratory rate - RR), and the way they respond, I categorized the ventilatory pattern response into three classes: a) response via increase in VT, then later RR; b) increase by RR mainly, slightly or no increase in VT, c) increase in VT only. Each subject had a different way to increase ventilation, which has been described previously (Mohan et al., 1998; Mohan et al., 1999). Figures 18-20 show examples.
Fig-18 Examples of Respiratory Rate Responder

Fig-19 Example of VT & RR Responder

Fig-20 Example of VT Responder only
The following table shows the distribution of the three classes between the three isocapnic levels and shows that class c) is the most represented.

Table-6 Distribution of the ventilatory components over the three tests:

<table>
<thead>
<tr>
<th></th>
<th>VT &amp; RR</th>
<th>RR only</th>
<th>VT only</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS+4</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>SS+7</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>SS+10</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

Also, females and males are distributed evenly across the three classes with 8 females in class a), 9 females in class b), and 10 females in class c).

4.4. Correlation between the acute and the adaptive response

The correlation between the acute and the adaptive ventilatory response was high within the decline category with a coefficient of regression \(r^2\) of 0.65 (Figure 21), and lower within the Double category (Figure 22), which might be due the variability of the pattern itself, as the two-peak responses/decline can be different. There was a strong correlation for the Plateau category \(r^2:0.9\), resulting from the definition of the Plateau category (Figure 23).
Fig-21 Correlation between acute and adaptive ventilatory response to hypoxia for the results falling into the category “Decline”. The x value (here 95%) expresses the amplitude of the adaptation of ventilation at the end of hypoxia.

Fig-22 Correlation between acute and adaptive ventilatory response to hypoxia for the results falling into the category “Double”.
Fig-23 Correlation between acute and adaptive ventilatory response to hypoxia for the results falling into the category “Plateau”.

This parameter yielded the amplitude of the adaptation, in % from the acute response.

4.5. Speed of the response and acute response correlation

rmANOVA showed that within category, there was no significant correlation between the acute response to hypoxia and the the time to peak, for each variable measured.

4.6. Correlation among variables

Tables 7-9 show the distribution of the variables at the different levels of PCO₂.

The ventilatory patterns the most represented during the three isocapnic tests are the Decline (19), the Double (16), and the Plateau suggesting that in 63% there is an adaptation of the response, which was already known. However, in 29% of the time, the increase of ventilation is maintained toward the hypoxic stimulus such as there is no adaptive response, which could be due to the absence of desensitization of the peripheral chemoreflex for these subjects. To support this, I have looked at the analysis of the ventilatory chemosensitivity (see the section
result 5). A typical example is the subjects #18 & #17. Indeed, #18 have Plateau pattern for ventilation, at the three tests. As a result, the slope of the Hypoxic sensitivity (representing the contribution of the central and the peripheral chemoreflexes) is low. Also, the Double pattern was not yet described until now and represent one of the dominant patterns of response.

Table-7 Pattern of response across variables at isocapnic level at 4mmHg above the resting value

<table>
<thead>
<tr>
<th></th>
<th>VE Category</th>
<th>Left MCAv Category</th>
<th>Right MCAv Category</th>
<th>HR Category</th>
<th>MAP Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>#03 SS+4</td>
<td>No response</td>
<td>--</td>
<td>Decline</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#04 SS+4</td>
<td>Plateau</td>
<td>--</td>
<td>Plateau</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#05 SS+4</td>
<td>Decline</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#06 SS+4</td>
<td>No response</td>
<td>--</td>
<td>Slow rise</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#07 SS+4</td>
<td>Decline</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Decline</td>
<td>plateau</td>
</tr>
<tr>
<td>#08 SS+4</td>
<td>Decline</td>
<td>--</td>
<td>No response</td>
<td>Decline</td>
<td>no response</td>
</tr>
<tr>
<td>#08.2 SS+4</td>
<td>double</td>
<td>double</td>
<td>double</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#09 SS+4</td>
<td>Decline</td>
<td>no response</td>
<td>--</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#10 SS+4</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
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<td>No response</td>
<td>No response</td>
<td>No response</td>
<td>no response</td>
</tr>
<tr>
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<td>no response</td>
<td>No response</td>
<td>Decline</td>
<td>double</td>
</tr>
<tr>
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<td>Double</td>
<td>Double</td>
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<td>double</td>
</tr>
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<td>Plateau</td>
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</tr>
<tr>
<td>#13 SS+4</td>
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<td>No response</td>
<td>Decline</td>
<td>plateau</td>
</tr>
<tr>
<td>#14 SS+4</td>
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<td>Decline</td>
<td>Decline</td>
<td>no response</td>
</tr>
<tr>
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<td>Double</td>
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<td>Double</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#16 SS+4</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
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<td>Plateau</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#18 SS+4</td>
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<td>Plateau</td>
<td>Plateau</td>
<td>no response</td>
</tr>
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</table>
Table 8: Pattern of response across variables at isocapnic level at 7mmHg above the resting value

<table>
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<th>VE Category</th>
<th>Left MCAv Category</th>
<th>Right MCAv Category</th>
<th>HR Category</th>
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</tr>
</thead>
<tbody>
<tr>
<td>#03 SS+7</td>
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<td>--</td>
<td>Decline</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#04 SS+7</td>
<td>Plateau</td>
<td>--</td>
<td>No response</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#05 SS+7</td>
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<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#06 SS+7</td>
<td>Decline</td>
<td>--</td>
<td>Double</td>
<td>Decline</td>
<td>no response</td>
</tr>
<tr>
<td>#07 SS+7</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>decline</td>
</tr>
<tr>
<td>#08 SS+7</td>
<td>Decline</td>
<td>--</td>
<td>No response</td>
<td>Decline</td>
<td></td>
</tr>
<tr>
<td>#08.2 SS+7</td>
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<td>plateau</td>
<td>no response</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#09 SS+7</td>
<td>Decline</td>
<td>Double</td>
<td>--</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#10 SS+7</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#11 SS+7</td>
<td>Double</td>
<td>No response</td>
<td>No response</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#11.2 SS+7</td>
<td>slow rise</td>
<td>no response</td>
<td>No response</td>
<td>Plateau</td>
<td>double</td>
</tr>
<tr>
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<td>No response</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#12 SS+7</td>
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<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#13 SS+7</td>
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<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#14 SS+7</td>
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<td>Double</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#15 SS+7</td>
<td>Double</td>
<td>fast Pk rising</td>
<td>fast Pk rising</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#16 SS+7</td>
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<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#17 SS+7</td>
<td>Plateau</td>
<td>Decline</td>
<td>Decline</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#18 SS+7</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>plateau</td>
</tr>
</tbody>
</table>
Table-9 Pattern of response across variables at isocapnic level at 10mmHg above the resting value

<table>
<thead>
<tr>
<th></th>
<th>VE Category</th>
<th>Left MCA\v Category</th>
<th>Right MCA\v Category</th>
<th>HR Category</th>
<th>MAP Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>#03 SS+10</td>
<td>Decline</td>
<td>--</td>
<td>Double</td>
<td>Plateau</td>
<td>decline</td>
</tr>
<tr>
<td>#04 SS+10</td>
<td>Plateau</td>
<td>--</td>
<td>No response</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#05 SS+10</td>
<td>Double</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>plateau</td>
</tr>
<tr>
<td>#06 SS+10</td>
<td>Decline</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#07 SS+10</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#08 SS+10</td>
<td>Decline</td>
<td>--</td>
<td>Plateau</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#08.2 SS+10</td>
<td>plateau</td>
<td>no response</td>
<td>decline</td>
<td>Plateau</td>
<td>decline</td>
</tr>
<tr>
<td>#09 SS+10</td>
<td>Double</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#10 SS+10</td>
<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#11 SS+10</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>plateau</td>
</tr>
<tr>
<td>#11.2 SS+10</td>
<td>Decline</td>
<td>no response</td>
<td>Decline</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#11.3 SS+10</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>plateau</td>
</tr>
<tr>
<td>#12 SS+10</td>
<td>Plateau</td>
<td>Double</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
<td>#13 SS+10</td>
<td>Decline</td>
<td>no response</td>
<td>No response</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#14 SS+10</td>
<td>Plateau</td>
<td>Double</td>
<td>Decline</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
<td>#15 SS+10</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#16 SS+10</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
<td>#17 SS+10</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Plateau</td>
<td>Double</td>
<td>no response</td>
</tr>
<tr>
<td>#18 SS+10</td>
<td>Plateau</td>
<td>Double</td>
<td>Double</td>
<td>Plateau</td>
<td>double</td>
</tr>
</tbody>
</table>
4.7. PCO₂ effect on the pattern of response

As one of the hypothesis was to test the effect of PCO₂ on the hypoxic response, I have divided the distribution of the pattern of response according to the isocapnic level used as shown in Table 10. Ventilation is more often a Decline pattern at the lowest isocapnic level; while it is more often a Plateau pattern at highest level of PCO₂.

Table-10 Distribution of the ventilatory pattern response: number of subjects falling in each category, for each isocapnic level tested.

<table>
<thead>
<tr>
<th>VE</th>
<th>SS+4</th>
<th>SS+7</th>
<th>SS+10</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Decline</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>N Double</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>N Plateau</td>
<td>2</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>N None</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

4.8. Gender effect

The Table 11 shows the distribution between males and females. It shows that in general, gender differences exist for the categorization. In females, the response is more likely to be a decline pattern (n=12, 46%), while for males the response is more likely to be a Double pattern (n=12, 40%).
### Table-11 Gender effect on the hypoxic pattern of response

<table>
<thead>
<tr>
<th>Total</th>
<th>Ve</th>
<th>Left MCAv</th>
<th>Right MCAv</th>
<th>HR</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N decline</td>
<td>19</td>
<td>8</td>
<td>12</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>N plateau</td>
<td>16</td>
<td>9</td>
<td>13</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>N double</td>
<td>16</td>
<td>12</td>
<td>15</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>N none</td>
<td>4</td>
<td>11</td>
<td>12</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Males</th>
<th>Ve</th>
<th>Left MCAv</th>
<th>Right MCAv</th>
<th>HR</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N decline</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>N plateau</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>N double</td>
<td>12</td>
<td>9</td>
<td>11</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>N none</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>17</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Females</th>
<th>Ve</th>
<th>Left MCAv</th>
<th>Right MCAv</th>
<th>HR</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N decline</td>
<td>12</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>N plateau</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>N double</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>N none</td>
<td>3</td>
<td>8</td>
<td>9</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>
5. Respiratory chemoreflex characterization by using the Steady State method

As mentioned in the protocol, I assessed the sensitivity of the ventilatory chemoreflex to carbon dioxide. For that purpose, I took the values of ventilation during the 3 periods: during hyperoxic phases and during hypoxic phases. Those 3 (x2) points permitted using the data from the HVR testing to draw 2 different response lines: the ventilatory response to CO₂ during hyperoxia, (orange circles in Figure 6) and the ventilatory response to CO₂ during hypoxia (red circles in Figure 6).

![Figure 6 Chemosensitivity measurements](image)

*Fig-6 Chemosensitivity measurements: using the mean value of the hyperoxic phase of the three tests and plot each value against $P_{ET}CO_2$ values; and using the mean value of the hypoxic phase of the three tests and plot each value against $P_{ET}CO_2$ values*

These response lines indicated information regarding the contribution of the respiratory chemoreflex(e)s involved in the response. The hyperoxic response reflects the activity of the central chemoreflex, and the hypoxic response, both the central and the peripheral chemoreflexes activity. The difference between the two responses represents the ventilatory contribution of the peripheral chemoreflex. It was hypothesised that the hypoxic values will lead to an increased slope (higher sensitivity). The steady state method delivers only an
indirect estimation of the ventilatory recruitment threshold (VRT). The data is shown in Figure 24. The black diamonds represent the hyperoxic value of $\dot{V}E$ while the white squares represent the hypoxic value of $\dot{V}E$. Because ventilation during hypoxia peaks and then either declines or plateau’s, I have specified the peak minute ventilation value as well as the mean value at the peak, over a 60 sec period of time (white circles). As expected, chemosenstivity during hypoxia is increased and left shifted.

The substraction of the two plots shows the contribution of the peripheral drive to breathe itself (grey squares) and shows that it contributes as much as the central drive. A disadvantage of the steady state method is that there are only 3 points per response line, and if one is incorrect, the entire relation is affected. Aslo, breakpoints or thresholds indicating non-linearities can be missed.
Fig-24 Respiratory responsiveness
6. Characterization of middle cerebral artery velocity response to hypoxia

As described in the protocol I have insonated both right and left middle cerebral arteries, initially as a redundant measurement. However, during additional testing I have noticed that the response can differ from one side to the other. Rather than showing not the “best” signal, I show both signals. The eighteen subjects underwent three tests, leading to 54 tests in total. For 36 tests (66%), both MCAv signals were present, and 18 tests (33%) were made with only one side, mainly the right (dominant) side (15 tests, 27%).

As mentioned during the data processing section, all percent changes in MCAv are expressed to a reference, which is the minimum MCAv value during hyperventilation.

Figures 35-36 display the general dynamic of the right and left MCAv responses and adaptation (in absolute values) for all subjects at each isocapnic level.

Fig-35 Dynamic Right MCAv hypoxic response at the three isocapnic levels at the 3 times points: mean value of the first hyperoxic phase; acute hypoxic value; and average value of the 2 last minutes of hypoxia.

Fig-36 Dynamic Left MCAv hypoxic response at three isocapnic levels at the 3 times points: mean value of the first hyperoxic phase; acute hypoxic value; and average value of the 2 last minutes of hypoxia.
6.1. Acute response of the MCAv

As the case for presenting data for ventilation, I first represented the MCAv response to hypercapnia only, from the hyperoxic phase (Figures 25-26). The response shows the expected increase of both right and left MCAv during the first hyperoxic isocapnic phase. Rm ANOVA analysis shows a statistical significant difference between the three tests with p<0.0001. However, two subjects (#16 & #18) showed a limitation of the left MCAv increase at the highest level of isocapnia; and one subject (#13) showed no further increase of the Left MCAv response between the isocapnic level at +7 to +10 mmHg above resting. As well subject #16 shows a limitation as well of the right MCAv increase at the highest level of isocapnia, (similar of what subject #16 shows for the left MCAv).

Fig-25 Hyperoxic Hypercapnic Left MCAv response at the 3 isocapnic levels. The black line represents the mean value. P <0.0001
As for ventilation, the MCAv response to hypoxia increases with the level of isocapnia (Figures 27-28). Rm ANOVA shows significant statistical difference (p < 0.0001). Also, the hypoxic cerebral response is offset compared to the hyperoxic response with significant difference between hyperoxic and hypoxic periods (rmANOVA, p < 0.0001). However the two subjects (#16 & #18) showing limitation of the left MCAv response to hyperoxic hypercapnia showed also a limitation MCAv response at the highest level of isocapnia during hypoxia. As well, the subject showing a Plateau of the left MCAv response after the second level of isocapnia (#13) showed the same limitation during hypoxia. For the right MCAv response, the same phenomenon appears for the same two subjects (#16 & #18).
Fig-27 Acute Left MCAv response to hypoxia at 3 levels of isocapnia. The black line represents the mean value. There is significant difference between the three tests, with $p < 0.001$

Fig-28 Acute Right MCAv response to hypoxia at 3 levels of isocapnia. The black line represents the mean value. There is significant difference between the three tests, with $p < 0.001$

As a result of these observed limitations, I have graphed 2 sets of “typical” subjects with a limitation (Figure 29) and without a limitation (Figure 30) of the MCAv in response to
hypoaxia, and have displayed it with the corresponding mean arterial pressure (MAP) response.

Fig. 29 MCAv and MAP coupling: limitation of the MCA reactivity

Fig. 30 MCAv and MAP coupling: continuous cerebral reactivity
Figures 31 & 32 display the time to peak of left MCAv and right MCAv over the 3-isocapnic levels, for each subject. It shows variability of the speed of the response. Correlation between the speed of the response and the amplitude of the response is not statistically different.

**Fig-31 Time to Peak: Left MCAv response to hypoxia at 3 levels of isocapnia**

**Fig-32 Time to Peak: Right MCAv response to hypoxia at 3 levels of isocapnia**

### 6.2. Categorization of the response

So far, the previous investigations have shown that the cerebral response to hypoxia is an increase of the MCAv, which stays constant over time, resulting in a square wave “plateau” pattern response to hypoxia. However, such is not such a case in my study, and the response can be divided into four categories.

Table 5 shows the general distribution of the patterns for all the tests and shows that in 30% of the tests, left MCAv responds with a Double pattern, and in 27% does not respond. For the right MCAv there is an even distribution, with dominant Double pattern (28%).

<table>
<thead>
<tr>
<th></th>
<th>VE</th>
<th>Left MCAv</th>
<th>Right MCAv</th>
<th>HR</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N decline</td>
<td>19</td>
<td>8</td>
<td>12</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>N plateau</td>
<td>16</td>
<td>9</td>
<td>13</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>N double</td>
<td>16</td>
<td>12</td>
<td>15</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>N none</td>
<td>4</td>
<td>11</td>
<td>12</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>
The distribution of the subjects’ response across the three tests (Table 4) shows that subjects do vary and some do not vary in terms of the category. For left MCAv (13 subjects), 2 subjects (15%) have consistency between the three tests (No response, and Decline). However, from the eleven other subjects, 6 have consistency in two of the three tests. For right MCAv (total 17 subjects), only 2 subjects (11%) have consistency of the pattern response among the three tests. However, 14 subjects (82%) have consistency in two of the three tests.

6.3. Adaptive response of the MCAv

Figures 33-34 display the adaptive hypoxic value for each subject at the three isocapnic levels and shows that adaptive response also depends on the isocapnic level, with a significant statistical difference for the tight MCAv (rmANOVA, p < 0.0001), but not for the left MCAv (p = 0.9).

Fig-33 Adaptive left MCAv response to hypoxia at 3 levels of isocapnia. The black line represents the mean value. No statistical significance.
6.4. Correlation between acute and adaptive response

The correlation between the acute and the adaptive cerebral responses was high in the Decline category with a coefficient of regression \( r^2 \) of 0.88 (Figure 37), as well as for the “no response” category \( r^2: 0.89 \) (Figure 38) and the Plateau category \( r^2: 0.89 \) for left MCAv. For right MCAv, results are similar with a \( r^2 \) value of 0.89 for the Decline category, of 0.88 for the Double category, 0.91 for the Plateau category, and 0.98 for the “no response” category (Figure 39) Interestingly, the correlation among the Double category is also very strong, \( r^2: 0.9 \) (Figure 40). The correlation between timing and the response measures was not conclusive, as for ventilation.
Fig-37 Correlation between Acute and Adaptive Hypoxic cerebral response : Decline Category

Fig-38 Correlation between Acute and Adaptive Hypoxic cerebral response : Plateau Category

Fig-39 Correlation between Acute and Adaptive Hypoxic cerebral response : Double Category
6.5. PCO₂ effect on the pattern of response

The distribution of the pattern of response according to the isocapnic level used as shown in Table 12. At lowest PCO₂ values, MCAv does not change or stay constant through the hypoxic stimulus. At higher levels of PCO₂, MCAv does adapt more frequently by either a Decline or Double response, or stays constant.

**Table-12** Distribution of the pattern of response between the three tests, for left and right MCAv. The numbers indicate how many subjects followed the category.

<table>
<thead>
<tr>
<th>R MCAv</th>
<th>SS+4</th>
<th>SS+7</th>
<th>SS+10</th>
<th>L MCAv</th>
<th>SS+4</th>
<th>SS+7</th>
<th>SS+10</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Decline</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>N Decline</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>N Double</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>N Double</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>N Plateau</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>N Plateau</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>N None</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>N None</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

6.6. Gender effect

The table 11 shows the distribution of the parameters between males and female. It shows that in general, the distribution is even among the categories. However, in females, the response is more likely to be negative, or a Decline pattern; while for males the response is more frequently a Plateau or Double pattern. No response pattern occurs is 38% for left MCAv, 34% for right MCAv in females compared to 15% for left MCAv and 7% for right MCAv in males; Decline occurs in 28% for left MCAv, 30% for right MCAv in female
compared to 10% for left MCAv and 15% for right MCAv in ales. Males have predominantly a Double pattern: 49% for left MCAv and 68% for right MCAv or a Plateau pattern: 56% for the right MCAv and 47% for left MCAv.

7. Cerebral blood flow sensitivity to CO₂

There is a variable increase of CBF with hypoxia; however, there was in general no change in cerebral blood flow sensitivity to CO₂ (slope) from hyperoxia to hypoxia (Figure 41), showing an offset of the response. However the sensitivity varied greatly between subjects, which will be discussed on the “Discussion” chapter.
8. Cardiovascular response to hypoxia

Ventilation and heart rate responses were correlated with respect to the pattern response, for all four categories (figures 7-9).

Figure 42-44 show the general dynamic HR response over the three periods, for each isocapnic tests. It shows that there is an acute hypoxic response, for each isocapnic level.

---

Fig-41 Cerebral responsiveness

Fig-42 Heart rate dynamic response at isocapnic level of 4 mmHg above resting value at 4 time points: mean value of the hypoxic phase; peak acute hypoxic value, and the 60 sec average around the peak; average of the 2 last minutes of hypoxia.
Fig-43 Heart rate dynamic response at isocapnic level of 7 mmHg above resting value at 4 time points: mean value of the hyperoxic phase; peak acute hypoxic value, and the 60 sec average around the peak; average of the 2 last minutes of hypoxia.

Fig-44 Heart rate dynamic response at isocapnic level of 10 mmHg above resting value at 4 time points: mean value of the hyperoxic phase; peak acute hypoxic value, and the 60 sec average around the peak; average of the 2 last minutes of hypoxia.
8.1 Acute heart rate response

Figure 45 shows the HR acute response to hypoxia for the three isocapnic levels. The acute HR response to hypoxia is similar for the 3 levels of hypercapnia, and there is no statistical difference across the three tests. Then, this variable is not affected by the magnitude of hypercapnia, as opposed to ventilation and MCAv.

![Graph showing acute hypoxic response](image)

*Fig-45 HR: Acute Hypoxic response across the three levels of isocapnia. The black line represents the mean value.*

8.2. Categorization of the heart rate response to hypoxia

Table 4 displays the categorization of the hypoxic response for HR. Seven subjects (38%) have a consistent pattern response among the three tests; and 9 subjects (50%) have consistent pattern in two tests. Only 2 subjects (11%) had different categories among the three tests. Again the pattern of HR response to hypoxia is related to $\dot{V_E}$ or/and MCAv in most tests (see comparative tables 7-9).
8.3 Adaptive response of heart rate to hypoxia

The adaptive response does not change between the three tests, except for two subjects (#12, #15) (Figure 46).

Fig-46 HR: Adaptive Hypoxic response across the three levels of isocapnia. The black line represents the mean value.

8.4. PCO$_2$ effect on heart rate pattern of response to hypoxia

The distribution of the pattern of response according to the isocapnic level used as shown in Table 13. Heart rate pattern of response is not affected by the pattern of response and is mainly Double.

Table-13 Distribution of the pattern of response between the three tests, for heart rate. The numbers indicate how many subjects followed the category.
8.5. Gender effect

The table 11 shows the distribution of parameters between males and females. It shows a general dominant Double category (56%); Plateau pattern occurs in 25% of the tests and Decline pattern in 18% of the tests; and this observation is similar for males and females, as opposed to ventilation and MCAv where differences exist between males and females.

8.5. Acute mean arterial pressure response

Figure 47 - 49 display the dynamic MAP responses to hypoxia at each isocapnic level at four time points: 1) mean value of the hyperoxic phase; 2) peak acute hypoxic value; 3) 60 seconds average around the peak; 4) average of the two last minutes of hypoxia.
Figure 50 shows the MAP acute response to hypoxia for the three-isocapnic levels. The MAP response to hypoxia is generally the same for among the 3 tests, as no statistical difference can be shown. However, three subjects (#8.2, #11.2, #12) have a statistical difference (p = 0.0015) between isocapnic level + 7 mmHg test and isocapnic level at +10 mmHg test, correlated with the increased MCAv as mentioned previously.

**Fig-50 Acute Hypoxic MAP response at three levels of isocapnia. The black line represents the mean value.**
8.6. Categorization of the MAP response to hypoxia

Table 4 displays the characterization of the hypoxic response for MAP. Only two subjects (11%) have consistent pattern of response among the three tests. However, 12 subjects (66%) have consistency between two tests. Only three (16%) had different categories among the three tests.

Again the pattern of HR response to hypoxia is related to $\dot{V}_E$ or/and MCAv in most cases (see comparative tables 7-9).

8.7. Adaptive hypoxic response of the MAP

As for the acute hypoxic response, MAP adaptive response is not affected by the isocapnic level, except for three subjects (p=0.001).

Fig-50' Acute Hypoxic MAP response at three levels of isoapnia. The black line represents the mean value.
8.8. Effect of PCO₂ on MAP pattern of response to hypoxia

The distribution of the pattern of response according to the isocapnic level used as shown in Table 14. Blood pressure pattern of response is not affected by the pattern of response and is either Double or negative.

*Table-14 Distribution of the pattern of response between the three tests, for Blood Pressure. The numbers indicate how many subjects followed the category.*

<table>
<thead>
<tr>
<th>MAP</th>
<th>SS+4</th>
<th>SS+7</th>
<th>SS+10</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Decline</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>N Double</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>N Plateau</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>N None</td>
<td>11</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

9. Slope and standard deviation during hypoxic response

I used the Acqknowledge software (Biopac System, Goleta, CA), to measure the linear slope from the peak response, to the end of the hypoxic stimulus, as well as to measure the SD over that period of time, for each variable.

The idea was to see the differences of those values between categories, and parameters. However, the value of the SD can be compared between categories only within the same parameter values. Indeed, a SD of “5” is insignificant if it refers to MAP, but will be highly significant if it refers to $\dot{V}_E$. To avoid this statistical mistake, separation of the values per parameter and per category has been made for the comparison. Comparison of the slope itself across parameters has been added to the results. It confirms the description of the categories.

For a Plateau category, the slope is approximatively 0 and the standard deviation is very low; however, a slope close to 0 associated with a greater SD (3) will correspond to a Double category. For a Decline and Double categories the SD is greater, however, the slope in the Double category is sometimes artefactually equal to 0 due to the fact that the negative and the
positive parts of the slope cancel out, as opposed to Decline category that shows negative slope. (Tables 15-18). These values describe the categories themselves and confirm the label given.

Table-15 Slope & SD of Ventilation during adaptive hypoxic response

<table>
<thead>
<tr>
<th>Category</th>
<th>Decline</th>
<th>Plateau</th>
<th>Double</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>21</td>
<td>10</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>mean slope</td>
<td>-0,01</td>
<td>0</td>
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<tr>
<td>SD slope</td>
<td>2,98</td>
<td>1,36</td>
<td>5,13</td>
<td>1,11</td>
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</table>

Table-16 Slope & SD of HR during adaptive hypoxic response

<table>
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<th>Double</th>
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</tr>
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<td>11</td>
<td>14</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>mean slope</td>
<td>-0,02</td>
<td>0</td>
<td>-0,01</td>
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</tr>
<tr>
<td>SD slope</td>
<td>4,25</td>
<td>3,08</td>
<td>5,16</td>
<td></td>
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</table>

Table-17 Slope & SD of left MCAv during adaptive hypoxic response

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<td>12</td>
<td>10</td>
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<tr>
<td>mean slope</td>
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<td>0</td>
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</tr>
<tr>
<td>SD slope</td>
<td>3,33</td>
<td>2,39</td>
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<td>1,72</td>
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Table-18 Slope & SD of right MCAv during adaptive hypoxic response

<table>
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<td>3,05</td>
<td>2,4</td>
<td>3,21</td>
<td>1,71</td>
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</tbody>
</table>
10. Correlation among parameters

Tables 7-9 show how the parameters fall into the categories, per subject, among the variables, per test. For the SS+4 mmHg test: 2 subjects had the same pattern of response for the four variables ($V_E$, MCAv, HR, MAP); 4 subjects had $V_E$-MCAv corresponding pattern, four for $V_E$-HR corresponding pattern, three for MCA-MAP corresponding pattern, two for $V_E$-MAP corresponding pattern, and four subjects had three parameters with corresponding pattern of response.

For the SS+7 mmHg test, 5 subjects had the same pattern of response for the four variables; 0 subjects had $V_E$-MCA corresponding pattern, five for $V_E$-HR corresponding pattern, two for MCA-MAP corresponding pattern, 0 for $V_E$-MAP corresponding pattern, and two subjects had three parameters with corresponding pattern of response. Two subjects had no corresponding pattern among the variables.

For the SS+10 mmHg test, 4 subjects had the same pattern of response for the four variables; 3 subjects had $V_E$-MCA corresponding pattern, 2 for $V_E$-HR corresponding pattern, 5 for MCA-MAP corresponding pattern, 2 for $V_E$-MAP corresponding pattern, and two subjects had two parameters with corresponding pattern of response. One subject had no corresponding pattern among the variables.

This indicates that there is always parity between two variables.

Table 4 shows how the parameters fall into the categories and shows that even few subjects have consistency of the pattern response among the three tests, at least two of the three tests show consistent pattern response for 50-66%, as described in each section of the characterization of the response for ventilation, MCA, HR, and BP.
11. Reproducibility

In order to see if my findings were repeatables over different days, three subjects repeated the full protocol on a second visit. Figures 51-56 show the acute and adaptive responses for ventilation, MCAv, and HR.

Fig-51 Reproducibility: Acute ventilatory response to hypoxia: 3 subjects repeated

Fig-52 Reproducibility: Adaptive ventilatory response to hypoxia: 3 subjects repeated

Fig-53 Reproducibility: Acute cerebral response to hypoxia: 3 subjects repeated

Fig-54 Reproducibility: Adaptive cerebral response to hypoxia: 3 subjects repeated
Fig-55 Reproducibility: Acute HR response to hypoxia: 3 subjects repeated

Fig-56 Reproducibility: Adaptive HR response to hypoxia: 3 subjects repeated

The trends were similar for $\dot{V}E$ and CBF for the acute and adaptive responses. However, Table 19 shows that the pattern of response varies.

Table 19: Reproducibility of the pattern of response

<table>
<thead>
<tr>
<th>VE Category</th>
<th>Left MLN Category</th>
<th>Right MLN Category</th>
<th>HR Category</th>
<th>MAP Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}E$</td>
<td>$\dot{V}E$</td>
<td>$\dot{V}E$</td>
<td>$\dot{V}E$</td>
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<td>$\dot{V}E$</td>
<td>$\dot{V}E$</td>
<td>$\dot{V}E$</td>
</tr>
</tbody>
</table>
VI Discussion

1. Main findings

In this study I investigated responses to hypoxia with respect to ventilation, MCAv, HR, and BP. The new and important findings are the followings. First, I can categorize the pattern of response among four categories, applicable for the four variables measured. Some of those categories were surprising and contradicting the current literature. However, my study has included various and greater level of PCO$_2$ while measuring HVR, which might account for that, as distribution of the pattern of response between the three isocapnic tests show that lowest isocapnic tests were similar to what the literature shows. However, pattern of response at highest levels of PCO$_2$ corresponded to the unexpected pattern of response. Therefore, the various and higher levels of PCO$_2$ I have used might account for the novelties. I have also included a greater cohort as compared to the literature. In addition, we do know that the carotid bodies, mediating the HVR, will be more sensitized at higher level of PCO$_2$ (Torrance, 1996).

Second, related to my former statement, the PCO$_2$ level affects the acute hypoxic response in terms of value, for both ventilation and cerebral blood flow, but not for heart rate or blood pressure. As only single level of PCO$_2$ was used in the past measure of HVR studies, (Easton et al., 1986; Severinghaus, 1995; Steinback & Poulin, 2007, 2008), I am the first one showing it, and this substantiate my two first hypotheses.

Third, the cerebrovascular response to hypoxia does adapt after the peak response. This is a first and novel finding as so far studies had shown MCAv response to hypoxia elevated but sustained (Poulin et al., 1996; Poulin & Robbins, 1998; Steinback & Poulin, 2008) and is therefore rejecting hypothesis three. My study used various and higher level of PCO$_2$. 

therefore MCAv response to hypoxia might be amplified by a higher PCO₂. Also, I have included more subjects. From another point of view, we do assume that the diameter of the MCA does not change during the experiment, therefore, can be used as a surrogate for measuring cerebral blood flow (Poulin et al., 1996). However, small changes in the diameter could explain the adaptation of the MCAv during hypoxia?

Four, the acute and adaptive response to hypoxia do correlate, and so for all variables, within each category. We did know that HVR and HVD (Sato et al., 1992; Mahamed & Duffin, 2001b) do correlate but it was not shown/studied for the other variables. This might had account by the fact that so far MCAv and blood pressure had been shown as sustained and constant during hypoxia, therefore not adapting (Steinback & Poulin, 2008).

Five, the results in terms of acute response (value) are repeatable as the three subjects who have repeated the protocol on a second visit did have the same trend of response.

Six, in the same direction, the pattern seem to be repeatble on the same days, on the same subject, over the three tests, as 38% of the subjects had the variable behaving the same way; and for 2 of the 3 tests, 50-70% of the 38% of the subjects have the same pattern of response. Again, measuring HVR at various levels of PCO₂ had not been realized before, so we are the first to show it.

Seven, within each test, the variables seem to share the same pattern of response. There is a consistent pattern of response for at least 2 variables in 85% of tests and full consistency of the pattern of response for all variables in 20% tests. Ventilation is correlated in terms of pattern of response with another variable in 94% of the tests. This observation suggests that there are links between these variables, and seems possible as all the variables measure are controlled within the nucleus tractus solitarii. However, I cannot exclude that this is due to the hazard.
My fourth hypothesis was that “the respiratory chemoreflex sensitivity to carbon dioxide can be characterized by using this steady state method”. My findings support this hypothesis, as there is an increase in ventilatory response sensitivity with hypoxia, as well as a left shift of the response with hypoxia as shown in previous studies using rebreathing tests (Berkenbosch et al., 1989; Mohan & Duffin, 1997; Duffin, 2007; Slessarev et al., 2010). However, in order for the testing to succeed in this aim care has to be taken with respect of the isocapnic levels chosen. First, at least three tests are needed. Second, the isocapnic levels used have to be higher than the VRT, which is not determined by the steady state method and has to be estimated, otherwise HVR will be underestimated and the test points will lead to an erroneous sensitivity. Indeed, a ventilatory response at a CO$_2$ level below the VRT does not belong to chemosensitivity but to basal ventilation.

My last hypothesis was that “cerebrovascular sensitivity to CO$_2$ can be characterized by using this method”. My findings did support this hypothesis in the sense that I was able to show that there is an offset between hyperoxic and hypoxic responses, with an equal sensitivity between the two tests. However, there is a complication; the sensitivity is variable between subjects and perfusion pressure becomes involved in cerebral blood flow increase at higher levels of PCO$_2$. Also, as mentioned in the “CBF” background section, the steady state may not be ideal for measuring CBF response, because hypercapnia increases CBF, which in turn, decreases any central stimulus due to the CO$_2$ washout (Vovk et al., 2002; Ainslie & Duffin, 2009, Xie 2006).

2. Data analysis

I would like to point out that the way the data are analyzed/processed could affect the results. I expressed the results for ventilation, heart rate and blood pressure in absolute values.
Expression in percent change would require a reference point such as resting values. However, resting values can change over time and therefore are not an ideal reference.

Although using resting values as reference points would demonstrate the response to hypoxia itself but could also underestimate the response. For example, at the highest isocapnic level, ventilation and MCAv are already increased, and a further increase during hypoxia would look relatively small compared to that at a lower PCO$_2$. For that reason, I have kept the values for ventilation, heart rate and MAP in absolute values.

For MCAv, as mentioned in the methods section, I have expressed it as the % change from the minimal MCAv during voluntary hyperventilation. In addition, I did not average any of the data: raw and beat-to-beat data were used. This latter difference in analysis may explain some differences with other studies that averaged resultsover 15 seconds for MCAv response (Poulin et al., 1998); or for the whole group, where the graphs is shown as the mean of all subjects (Steinback & Poulin, 2007, 2008). Those last two studies aimed to describe the hypoxic response by showing mean values at 5, 10, 15, and 20 minutes of hypoxia, and were not aiming on describing the dynamic response with analysis of the tracing itself.

3. Categorization of the hypoxic response

I found that not only does ventilation adapt after the initial/acute response but all ventilatory and hemodynamic parameters do as well; to my knowledge this is the first demonstration of such a phenomenon. Steinback (Steinback & Poulin, 2008) also showed that HR declines and MCAv increases with hypoxia, but did not show that these parameters adapt over time.

Indeed, it is interesting to recall that Steinback et al. (Steinback & Poulin, 2008) predicted that the response of CBF as well as other hemodynamic parameters would adapt over time, but they were not able to demonstrate it. In my study, all parameters measured showed a
tendency to adapt in at least some subjects. One difference that may be important is that in my study, rather than increasing $P_{ET}CO_2$ by only 1mmHg from resting, I performed repeated isocapnic tests at different PCO$_2$ levels. This increase in PCO$_2$ may account for my observation of the decline in all parameters because, as also shown in this study, the increase in PaCO$_2$ amplifies the responses of ventilation and MCAv to hypoxia. The higher PCO$_2$ provides a greater central chemoreflex drive to breathe during hyperoxia and also sensitizes the peripheral chemoreflex response to CO$_2$ during hypoxia. In addition, my mode of data analysis may have increased my likelihood of observing these changes. Unlike previous studies (Poulin et al., 1996, 1998; Poulin & Robbins, 1998; Steinback & Poulin, 2007, 2008), I did not average the results for all subjects. Furthermore, I completed 3 steady state tests in 18 subjects, many more than previous studies. As a result, I am confident of the observation I made.

Initially, the discovery of the Double hypoxic ventilatory response was unexpected as it had not been previously observed. However, the observation that the other measures also exhibited a Double type response, and that this response type occurred in a number of subjects, increased my confidence that the observation was not artefactual. Moreover, as shown by the tables, the number of variables categorized as "Double" is high and in fact is a dominant characteristic of the responses. The slope and standard deviation measurements gave me an objective means of categorization, and so while a visual inspection of the response might initially suggest that the response was a Plateau, statistical analysis of mean square difference from a linear fit, which typically results in SD of 3, separated the Double category from an actual Plateau, which typically has a SD $\leq$ 1.5. This form of analysis therefore increases the sensitivity of detection of Double responses whereas plotting on a compressed Y scale would obscure the pattern.
The mechanisms underlying these novel observations is not known and here I will develop hypotheses about the possible mechanisms involved. With regard to the Decline pattern, peripheral chemoreceptors desensitization may account for the decline of ventilation, including a central depression due to washout of CO$_2$ by a hypoxic-induced increased cerebral blood flow (Berkenbosch et al., 1989; Neubauer et al., 1990; Xie et al., 2006). Furthermore, depletion of the neurotransmitters within the carotid body could explain the fact that ventilation decreases and adapts overtime. Type I cells of the CB act as chemotransducers, with synapses between type I cells and the afferent terminals of the carotid sinus nerve, indicating the essential role of neurotransmitters in chemosensory signalling. These neurotransmitters are packaged into synaptic vesicles clustered beneath the membrane on the presynaptic side of a synapse, and are synthesized from precursors, such as amino acids. The excitatory neurotransmitters involved in the carotid body are Acetylcholine (Ach), ATP via purinergic P2X receptors and dopamine. Inhibitory neurotransmitters include GABA and excitatory modulators include serotonin via G-protein-coupled receptors (Kumar, 2007; Teppema & Dahan, 2010; Nurse, 2010). How these neurotransmitters interact and whether they are depleted during HVD is presently unknown.

Another similar hypothesis would be the correlation with the amount of energy available (energy depletion). Indeed. Increase of carotid body blood flow in response to hypoxia will lead to great increase in its glucose consumption and oxidation. Oxygen disappearance is positively correlated to sinus nerve afferent discharge. Hence oxygen is needed to provide enough energy to sustain the CB response to hypoxia (Kumar, 2007). However, at some point, failure might occur, leading to the decrease of response to hypoxia? Reserve in ATP and speed of restocking could be then put in question. In addition, ATP is one of the neurotransmitter responding to hypoxia in the CB.
The decline in MCAv shows that the cerebrovascular system adapts as well. This decline in the CBF response was not observed by (Steinback & Poulin, 2008), and they hypothesized that the hypoxic response was due to an increase in perfusion pressure rather than cerebral vasodilation induced by hypoxia, which they observed did not decline. My observations therefore agree with previous studies that show a decrease in vascular brain resistance during hypoxia (Cohen et al., 1967; Kogure et al., 1970; Shapiro et al., 1970). My study shows an adaptative hypoxic response of MCAv, as opposed to Steinback (Steinback & Poulin, 2008) and Poulin (Poulin et al., 1996) findings that show no decline. I hypothesize that there is a vasodilation of the cerebral blood vessels, mediated and modulated by neurotransmitters and neuromodulators such as amines (Acetylcholine, Dopamine, serotonin) and neuropeptides (substance P, endothelin, angiotensin II) and amino acids (GABA, purine ATP), or NO. Because ventilation, CBF, heart rate and MAP are all autonomic responses controlled from the brainstem via such neurotransmitters and neuromodulators, I hypothesize that depletion and restoration of neurotransmitters can explain the variety of patterns of response among the variables measured as well as their commonality. In the case of a decline for example, there is a depletion of the neurotransmitters, and the rate of depletion will determine the rate of the decline. This argument is supported by the observation of a correlation between the amplitude of HVR and the amplitude of HVD (Easton et al., 1986; Steinback & Poulin, 2007). I also suggest that the correlation between ventilation and HR pattern in the Decline category can be explained in part by a hyperventilation-induced sympatho-excitation (Halliwill et al., 2003; Ainslie & Poulin, 2004b, a; Steinback & Poulin, 2008); these experimenters found a decline pattern of response for HR, corresponding to a decline in the response of ventilation, during steady state hypoxia.
If the neurotransmitters involved in the responses have either late release properties or quick restoration properties, these properties could explain the Double pattern in which the decline of the response is followed by a second increase. However this second increase is not maintained and is followed by a second decline of the response. Hypoxia induces intracellular calcium elevation with neurosecretion from type I cells calcium dependent. This elevation of intracellular calcium is accomplished mainly via membrane channels, and in the absence in external calcium, the response to hypoxia is attenuated (Kumar, 2007). Could the depletion of external calcium available for the membrane depolarization account for the decline in the response to hypoxia? Alternatively could the decline be due to the effect of inhibitory neurotransmitters such as GABA, also released during hypoxic stimulation? This latter hypothesis receives support from studies in young piglets suggesting that most of the decline in the ventilatory response to hypoxia is mediated by inhibitory amino acids neurotransmitters, GABA, glycine, and taurine, in the NTS (Neubauer et al., 1990; Tabata et al., 2001; Hehre et al., 2008).

Could the same arguments be applied to explain the Plateau pattern? A large storage of neurotransmitters, allowing continuous discharging and activation of the relevant neurons could explain the plateau response to hypoxia. Alternatively, could the plateau pattern result from a competition between neurotransmitters? If excitatory and inhibitory transmitter release was in balance, the response may be maintained. This latter hypothesis could be applied to account for variations in the patterns of response among variables within the same test/subject. Poulin (Poulin et al., 1996) showed a sustained elevated MCAv response to isocapnic hypoxia, but did not give any explanation for this observation. Steinback (Steinback & Poulin, 2008) suggested mediation via changes in perfusion pressure, rather than hypoxic vasodilation in the brain. However, I did not always find correlation of a
Plateau response for MCAv with a plateau response for MAP. If perfusion pressure
determined MCAv, then in cases where MAP had No response or a Plateau response to
hypoxia, perfusion pressure cannot account for the observation of an increasing/adaptating
MCAv response to hypoxia.
These arguments raise the question of how neurotransmitters are allocated to efferent
neurons; do different transmitters dominate specific responses? Also, we do not know if there
is a cascade relay of the firing of the type I cell, releasing different neurotransmitters at
different time points… however, the neuromodulation and pathway were not part of this
project and these suggestions stay hypothetical.
Another hypothesis with respect of the pattern of response for ventilation would be the
negative feedback part of the loop of the respiratory chemoreceptors, driven by hydrogen ion
concentration and acid base balance. As negative regulator, an increase of ventilation would
lead to a decrease of it. Could the Plateau response for ventilation be explained as so?
However, this will account only for ventilation. As the patterns are occurring in all variables,
this explanation is less likely to be.
Last hypothesis would be that one variable drives the others. All variables are controlled fro
input to the Nuclear Tractus Solitarii (NTS), in the medulla, so it might not be surprising that
they share common aspects in terms of control. However, which variable will be the “driver”
is unknown and could it be variable between subjects is unknown too. Also, if it is the hazard
that link the variables, then pattern of response is unpredictable, which was shown in the fact
that repeated subjects did not repeated their pattern of response, despite the repetitivity in
terms of acute and adaptive responses (values).
4. Hypoxic response and effect of PCO$_2$

I have shown that PCO$_2$ level does affect the hypoxic response for ventilation and MCAv. However, this was not the case for HR and MAP. While hypoxia does induce increases in HR and MAP, they were not significantly different among the three tests.

5. MCAv response

During the hyperoxic period MCAv increases as the level of isocapnia increases for 80% of the subjects. The 20% of remaining subjects (Subjects 8, 11, 16, 18) showed a limitation of the MCAv response: MCAv did not increase further even at the highest level of PCO$_2$. With induction of hypoxia, 80% of the subjects responded, increasing the amplitude of their response in an additive interaction. As for the hyperoxic period, the same 20% of the subjects showed a limitation of the acute hypoxic MCAv response: MCAv did not increase further despite the highest level of PCO$_2$. By contrast, subjects who did not have a limitation of the MCAv response also exhibited an increase in MAP. It therefore appears that there is correlation between MCAv response and MAP increase. Data from the other part of this project gave us some insight about this phenomenon. Using Duffin-type isoxic rebreathing tests we measured the responses of both MCAv and MAP to CO$_2$. Comparisons of isoxic hyperoxic with isoxic hypoxic tests enabled the effect of oxygen tension to be determined. During rebreathing the MCAv response to CO$_2$ was sigmoid below a discernible threshold CO$_2$ tension, increasing from a hypocapnic minimum to a hypercapnic maximum. In most subjects this threshold corresponded with the CO$_2$ tension at which MAP began to increase. Above this threshold both MCAv and MAP increased linearly with carbon dioxide tension.
The sigmoid MCAv response was centered at a CO$_2$ tension close to normal resting values. While hypoxia increased the hypercapnic maximum MCAv it did not affect other sigmoid parameters as at higher level of PCO$_2$, MCAv increase might be due to increase in perfusion pressure. Also, only in the MCAv response range below the threshold for the increase of MAP with CO$_2$ does the MCAv measurement reflect vascular reactivity to CO$_2$ alone (Submitted to Journal of Physiology January 2011).

6. Chemoreflex sensitivity

With the repeated isocapnia protocol I was able to calculate the sensitivity of the ventilatory and the MCAv responses to CO$_2$ at 2 isoxic levels, and thereby separate the central and peripheral chemoreflex responses. In three tests $\dot{V}E$ did not increase at the isocapnic level 4 mmHg above the resting value. I believe that this lack of response occurred because this CO$_2$ level was lower than the VRT. This condition would artefactually reduce the slope of the ventilatory response (chemoreflex sensitivity) because this point is actually a measure of basal ventilation and not part of the chemoreflex response.

The MCAv response to CO$_2$ was highly variable between subjects. I categorized the sensitivity into three patterns of response based on the assumption of a sigmoid relationship between the vasoreactivity of the brain resistance vessels and CO$_2$ (Figure 57). This assumption is based on the consideration that there are limitations in the ability of MCAv to respond to CO$_2$ because the vessels cannot expand or constrict beyond their physical limits. In order words, at some range of CO$_2$, the blood vessels have reached maximal dilation and the MCAv cannot increase anymore (see ***). For the hypocapnic part of the curve (see *), the vessels become minimally dilated at a threshold PCO$_2$ and the MCAv reaches a minimum. Between the maximum and minimum there is a sigmoid relationship between
PCO$_2$ and vessel diameter that appears roughly linear (see **). As the maximum and minimum vessel resistance is approached the rate of change of resistance with respect to change in PCO$_2$ is reduced, resulting in a sigmoid relationship between PCO$_2$ and MCAv (**)(Claassen et al., 2007). Ide et al. (Ide et al., 2003) showed a curvilinear relationship of MCAv and CO$_2$ for the lower range of PCO$_2$, but the upper plateau range has not been well studied.

![Fig-57 Sigmoid model for the cerebral reactivity: Illustration of specific cases: At the low part of the curve (*), the vessels velocity is increased but constant for a specific range of $\text{PETCO}_2$, which varies with subject. When the threshold is reached the vessel velocity increases in a linear way (**), until it reaches a maximum such as if the reactivity is situated on the high part of the curve, it will be maxed out, due to an incapacity to expand more. (***)](image)

7. Gender effect

There is a gender effect with respect to the response patterns, especially for ventilation and MCAv. Male responses were mainly of the Double and Plateau for both $\dot{V}E$ and MCAv type.
whereas female responses were mainly of the Decline ($\dot{V}E$ & MCAv) and No response type (MCAv). The patterns of response for HR were similar for males and females, with the Double pattern dominating. The patterns of response for MAP were also similar between males and females, with the “No response” dominating. I concluded that males and females should be studied separately while measuring hypoxic responses.

Literature has shown that HVR, as well as hypercapnic ventilatory response (HCVR) in males in superior than in females, which could explain our findings (White et al., 1983). In addition, if ventilation and MCAv are related, a higher HVR will lead to a higher MCAv response to hypoxia. This aspect is already known (Ainslie & Duffin, 2009). With respect to cerebral response, it is known that response to hypercapnia is depending on hormones, that estrogen increases the cerebral blood flow and that postmenopause females have lower CVR than premenopause females and than men (Matteis et al., 1998; Nevo et al., 2007). However, literature is not extensive and data with respect to effect of gender cerebral response to hypoxia are poor.
VII Conclusion

The main goal of this project was to characterize the hypoxic response by using a standardized protocol, which would allow comparison among subjects and within subjects. I have demonstrated that the patterns of response did not correspond to those predicted by reference to previous studies. Thus, I am the first to show that hypoxic responses vary from a pattern perspective, and furthermore, that this patterning is applicable for all of the physiological variables studied. I described four patterns of response (categories), and was able to demonstrate that the different measures correlated in terms of their category. Which of the measured variables controls the others or what external factor determines them all cannot be resolved from these findings.

The isocapnic CO₂ tension affects the ventilation and CBF responses to hypoxia, but does not affect the HR and MAP responses to hypoxia. In addition, I showed that high levels of PCO₂ can reduce the CBF responsiveness to CO₂ for both hyperoxic and hypoxic conditions, and that further CBF increase is accompanied by an increase in MAP. I was also able to demonstrate that within the same pattern of response category, there was a correlation between the acute and adaptative response.

I also concluded that respiratory chemosensitivity can be measured by these steady state tests as long as the appropriate isocapnic levels are used (in order to make sure to trespass the VRT). However for measuring CBF responses to CO₂ and hypoxia, rebreathing techniques are more appropriate.

Finally, I concluded that there are differences between males and females, especially for the ventilatory and cerebral responses to hypoxia. Females are more likely to have Decline patterns for ventilation and No response patterns for cerebral blood flow; while
males are more likely to have Double patterns for ventilation and Double or Plateau patterns for cerebral blood flow. Hence, they should be separated for extensive comparisons and conclusions.

VII Further directions

These novelties will hopefully open doors to other studies. Indeed, the mechanism behind the adaptation to hypoxia seems complex and is for sure not understood yet. Contribution of neuroscience focused in signaling/pathway will be very interesting in order to better understand neurotransmission and efferent responses. Collaboration with animal experiments studying vascular tone in brain vessels would also indicate if small change in MCA diameter would be correlated to our pattern of response.

Also, the use of specific drugs such as beta-blockers or nitrate would delineate the effect of blood pressure and heart rate.
VIII Appendixes

Appendix-1: Respirology Research Day 2009, University of Toronto: poster

The idea: A set of standardised procedures (1) allows comparisons of the chemoreflex control system parameters between individuals and between states.

Procedure One

For the modified rebreathing procedure (2):
- In a 10 Liter rebreathing chamber the subject breathes a mixture of 5% CO₂ in N₂, for 1,5 minutes.

Procedure Two

This figure demonstrates the response of the hypoxic ventilatory response (HVR) to a stepwise decrease in inspiratory oxygen saturation (O₂). The subjects were divided into two groups: Group A and Group B.

Procedure Three

The response to the hypoxic ventilatory response (HVR) is shown in this figure. The responses are depicted as changes in ventilation (liters/min) over time.

References:

Introduction:
To provide a detailed assessment of the peripheral chemoreflex response to hypoxia that allows comparisons between individuals and between different physiological and environmental conditions Duffin has proposed a regime to measure HVR consisting of 3 procedures (2): The first measures the peripheral chemoreflex responsiveness to both hypoxia and CO\textsubscript{2} in terms of hypoxia’s effects on the sensitivity and ventilatory recruitment threshold of the peripheral chemoreflex response to CO\textsubscript{2}. The second procedure measures the time course of the decline in the isocapnic ventilatory response to hypoxia over a 20-minute period. The third procedure measures the time course of the poikilocapnic ventilatory response to hypoxia over a period of 20 minutes.

While the first procedure can use several methods, including rebreathing and steady state, the latter two methods require steady state techniques that maintain isoxia and isocapnia independent of ventilation — methodology currently requiring complex end-tidal forcing methods (6). Our first aim was to demonstrate a method for steady state control of end-tidal PCO\textsubscript{2} and PO\textsubscript{2} for the latter two procedures using the new technique of prospective end-tidal targeting (3).

While comparisons are problematic for data from a single isocapnic hypoxia test (procedure 2) as pointed out in the proposal article (2), if data at 3 or more levels of isocapnia are obtained then they can be used to construct steady state isoxic hypoxic and hyperoxic responses to CO\textsubscript{2} (procedure 1) that can be compared between subjects and conditions. Our second aim was to show that the data obtained with the second procedure, i.e. measuring the ventilatory response to isocapnic hypoxia, can also be used to obtain a steady state isoxic hypoxic and hyperoxic response to CO\textsubscript{2} as provided by the first procedure.

Methods:
After receiving institutional Research Ethics Board approval, we obtained a signed informed consent from the test subject (female; age 26 years, height 173 cm, weight 60 kg) to participate. All procedures conformed to the Declaration of Helsinki. The test subject was seated comfortably in a chair and a face mask applied using adhesive tape (Tegaderm, 3M Health Care, St Paul, MN, USA) was used to seal any leaks. The mask was connected to a sequential gas delivery circuit via a flow transducer (AWM720P1; Honeywell Freeport, Illinois). End-tidal PO\textsubscript{2} and PCO\textsubscript{2} were controlled by providing pre-calculated flows of gas mixtures (RespirAct™, TRI, Toronto, Canada). In this circuit, when subject ventilation exceeds the flow supplied, the remainder is provided by rebreathing previously exhaled gas; this circuit thus forms a self-regulating system where flows of rebreathed gas are proportional to increases in ventilation, thereby maintaining PetO\textsubscript{2} and PetCO\textsubscript{2} constant (3,5). The RespirAct also measures end-tidal PCO2 and PO2 from a sample drawn continuously from the mask.

To implement the first procedure, measuring the peripheral chemoreflex responsiveness to both hypoxia and CO\textsubscript{2} in terms of hypoxia’s effects on the sensitivity and ventilatory recruitment threshold of the peripheral chemoreflex response to CO\textsubscript{2}, we used a modified rebreathing technique (4) implemented via specially-written software to control the RespirAct™ operation as follows. A high flow of air was supplied and the subject asked to voluntarily empty the inspiratory reservoir bag every breath to achieve hyperventilation for a
5-minute period to lower CO\textsubscript{2} stores (end-tidal PCO\textsubscript{2} 20-25 mmHg). While the subject continued to empty the inspiratory reservoir every breath, the FICO\textsubscript{2} and FIO\textsubscript{2} were changed to target the mixed venous PCO\textsubscript{2} and desired isoxic PO\textsubscript{2} for 8 breaths and then the subject was instructed to return to relaxed breathing. In this way the PCO\textsubscript{2} of the expiratory reservoir, alveolar compartment, arterial blood and mixed venous blood were equalized at the start of rebreathing. During relaxed rebreathing, the FICO\textsubscript{2} was set to zero and the FIO\textsubscript{2} to 100% and flow was reduced to equal oxygen uptake to maintain isoxia as the subject rebreathed from the expiratory reservoir.

To implement the second and third procedures prospective targeting was used to control end tidal PCO\textsubscript{2} and PO\textsubscript{2} such that isocapnia (PCO\textsubscript{2} = 44, 47 & 49 mmHg; procedure 2) or poikilocapnia (PCO\textsubscript{2} uncontrolled; procedure 3) was maintained during a sequence of a 5-minute period of hyperoxia (PO\textsubscript{2} = 150 mmHg), a 10-minute period of isoxic hypoxia (PO\textsubscript{2} = 50 mmHg) and a 5-minute period of hyperoxia (PO\textsubscript{2} = 150 mmHg).

The measured breath-by-breath ventilatory responses were fitted using custom software (LabVIEW, National Instruments). From the isocapnic hypoxic ventilatory responses we obtained ventilation during hyperoxia and hypoxia for each isocapnic test and plotted these data on the same graph as the rebreathing responses to obtain steady state isoxic responses to CO\textsubscript{2}.

Results:

![Graph showing modified rebreathing response in hypoxia (PO\textsubscript{2} 50 mmHg).](image)

Figure 1: the modified rebreathing response in hypoxia (PO\textsubscript{2} 50 mmHg).
Figure 2: the steady-state hypoxic ventilatory response at an isocapnia of 47 mmHg. During hyperoxia ventilation was 21.3 L/min, rising to a peak of 40.5 L/min during hypoxia, with an exponential decline to 57% of the peak with a time constant of 83 s.
Figure 3: the modified rebreathing tests results plotted with the steady state responses obtained from the steady state isocapnic ventilatory responses to hypoxia (Figure 1).

Note that the steady-state responses are left-shifted because they are obtained relative to arterial PCO$_2$ whereas the rebreathing responses are relative to mixed venous PCO$_2$. This difference is affected by cerebral blood flow, which itself responds to CO$_2$ (1). In subjects with high cerebrovascular reactivity the steady state and rebreathing responses converge at elevated PCO$_2$ so that the slopes may differ, with the steady state response reflecting the effects of both ventilatory chemoreflex responses and cerebrovascular reactivity and the modified rebreathing response reflecting only the respiratory chemoreflex response.

Discussion:

The results for this example subject show that:

1) Prospective end-tidal control using a sequential gas delivery circuit provides the necessary steady state control of PCO$_2$ and PO$_2$ to implement the steady state procedures for measuring the ventilatory response to hypoxia.

2) Three steady state isocapnic ventilatory responses to hypoxia can provide hypoxic and hyperoxic ventilatory responses to CO$_2$ that can be compared between individuals and between different physiological and environmental conditions as well as to modified rebreathing responses.

Acknowledgement: This work was supported by Thornhill Research Inc.

References:

Appendix-3. BRAIN platform research day 2009, University of Toronto: posters

Cerebral and ventilatory responses to hypercapnia and hypoxia
A. Battler*, J. Fisher & S. Quirke
Department of Physiology & Anesthesiology, University Health Network, University of Toronto

Objectives
1. Compare 2 basic conditions with those isocapnic levels regarding for VE & MCAo
2. Standardized methods
3. Compare results under and heter subjects

Protocol
1. Three isocapnic steady-state hypocapnic tests

The Apparatus

Measurements
1. Ventilation (VCO2, mmHg)
2. PaO2, PaCO2, and pH
3. MCAo via transcranial doppler (TCD)
4. Blood pressure & heart rate

Results
1. N: 10 subjects (7 males, 29 ± 5 years old)
2. UVF (left panel) & MCAo (right panel) hypocapnic responses at a time and 1.5 times

Conclusion
1. The method allows comparison between conditions and subjects for analysis of ventilatory and cerebral response to CO2 and hypoxia by using several isocapnic hypocapnic responses.
2. Whereas UVF, ventilation, increases hypoxia increases sensitivity to CO2 (increase in slope of VE vs. PO2), hypoxia causes an overall increase in CBF with no change in sensitivity (same slopes).

Chemoreflexes characterization by two methods: application to ventilation and cerebral blood flow
A. Battler*, J. Fisher & S. Quirke
Departments of Physiology & Anesthesiology, University Health Network, University of Toronto

Objectives
1. Apply the Duffin standardization method to characterize the respiratory response
2. Apply the Duffin standardization method to characterize the cerebral response
3. Compare the Duffin standardization method to characterize the cerebral response

The Apparatus

Graphing results
1. Relationship between ventilation and CO2 for the two methods

Protocol
1. Rebreathing tests
2. Steady state tests

Measurements
1. Ventilation (VCO2, mmHg)
2. PaO2, PaCO2, and pH
3. MCAo via transcranial doppler (TCD)
4. Blood pressure & heart rate

Results
1. N: 10 subjects (7 males, 29 ± 5 years old)
2. The two methods gave the sensitivity of both VE and MCAo to both isocapnic and hypocapnic
3. The VE response slope will differ between the two methods, because the VE method includes the cerebral response response to CO2, which will increase the contribution of the cerebral chemosensitive response.
4. However, the MCAo response will be faithfully fit by the arterial response (PCO2, because the cerebral response to PO2, measured via transcranial doppler, is referenced to arterial tension.
5. Therefore, the TCD response, which tracks the PCO2 of arterial blood should be similar for the two methods.

Conclusion
The two methods yield the same trends. However, the inter-individual variability for MCAo is larger than expected, and some subjects may have a limited MCAo response. Further analysis still need to be done.
Appendix-4. Experimental Biology 2010

The effect of hypoxia on the ventilatory and cerebral blood flow (CBF) responses to CO₂
A. Battist i, J. Fisher & J. Duffin
Deparments of Physiology & Anesthesiology, University Health Network, University of Toronto

Objectives
1. Characterize the respiratory \( P_{aCO_2} \) responses using 3 steady state (SS) hypoxic ventilatory responses (HVR) tests and 2 Duffin rebreathing tests.
2. Apply the same methods to characterize cerebral blood flow (CBF) responses to hypoxia.
3. Enable comparison independent of the method used.

Protocols
Study site: Response in hypoxia at Oregon O2 Institute.

Measurements
Measurements of ventilation, arterial blood gases, and cerebral blood flow.

Observations
1. 10 subjects (7 males, 29 ± 5 years)
2. Thresholds were detected in the rebreathing experiments.
3. The study site P_{aCO_2} responses were more robustly ventilatory, while cerebral blood flow responses were more robustly hypoxic.

Apparatus

Conclusions
1. Both methods measured the sensitivity of the ventilatory response to CO₂, but the steady state method included the effect of the cerebral hypercapnic response, and did not detect thresholds.
2. The cerebral hypercapnic response differs from the methods in some subjects but not in others.
3. Further research is needed regarding the influence of thresholds between the ventilation and cerebral hypercapnic responses in same subjects.

References

This work was supported by Thornhill Research Inc.
### Appendix-5 Comparative tables

**Table-4 Comparative stratification of the variables among the categories, all subjects**

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<tr>
<th></th>
<th>VE Category</th>
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Table 5: Distribution of the subjects into the categories

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Table 7: Pattern of response across variables at isocapnic level at 4mmHg above the resting value

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<td>Double</td>
<td>double</td>
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</tr>
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<td>No response</td>
<td>Decline</td>
<td>plateau</td>
<td></td>
</tr>
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<td>Decline</td>
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<td></td>
</tr>
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<td></td>
</tr>
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<td></td>
</tr>
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<td>#18 SS+4</td>
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Table-8 Pattern of response across variables at isocapnic level at 7mmHg above the resting value

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<tr>
<th>#</th>
<th>VE Category</th>
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<th>MAP Category</th>
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<tr>
<td>#03 SS+7</td>
<td>Double</td>
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<td>Double</td>
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</tr>
<tr>
<td>#04 SS+7</td>
<td>Plateau</td>
<td>--</td>
<td>No response</td>
<td>Double</td>
<td>double</td>
</tr>
<tr>
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<td>Decline</td>
<td>Decline</td>
<td>Decline</td>
<td>decline</td>
</tr>
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<td>#06 SS+7</td>
<td>Decline</td>
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<td>Double</td>
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<td>no response</td>
</tr>
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<td>Double</td>
<td>Double</td>
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</tr>
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<td>Decline</td>
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<td>Decline</td>
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</tr>
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<td>increasing</td>
<td>plateau</td>
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<td>Plateau</td>
<td>no response</td>
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<tr>
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<td>Decline</td>
<td>Double</td>
<td>--</td>
<td>Plateau</td>
<td>no response</td>
</tr>
<tr>
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<td>Double</td>
<td>--</td>
<td>Double</td>
<td>Double</td>
<td>double</td>
</tr>
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<td>No response</td>
<td>Plateau</td>
<td>no response</td>
</tr>
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<td>Plateau</td>
<td>no response</td>
</tr>
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<td>Decline</td>
<td>Double</td>
<td>Decline</td>
<td>decline</td>
</tr>
<tr>
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<tr>
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<td>Decline</td>
<td>Double</td>
<td>double</td>
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<td>Plateau</td>
<td>Plateau</td>
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Table-9 Pattern of response across variables at isocapnic level at 10mmHg above the resting value

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</tr>
<tr>
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<td>Plateau</td>
<td>Double</td>
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<td>#06 SS+10</td>
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<td>Double</td>
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Table-11 Gender effect on the hypoxic pattern of response

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<th>HR</th>
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IX References


Guyenet PG & Mulkey DK. (2010). Retrotrapezoid nucleus and parafacial respiratory group. Respir Physiol Neurobiol 173, 244-255.


